Arun D. Singh · Bertil Damato
Editors

Clinical Ophthalmic Oncology

Basic Principles and Diagnostic Techniques

Second Edition

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ISBN 978-3-642-40488-7 ISBN 978-3-642-40489-4 (eBook)
DOI 10.1007/978-3-642-40489-4
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013954969

To my parents who educated me beyond their means, my wife Annapurna, and my children, Nakul and Rahul, who make all my efforts worthwhile. ADS

To my family, Frankanne, Erika, and Stephen. BED

Preface

The management of patients with an ophthalmic tumor presents particular challenges. Ophthalmic tumors are rare and diverse so that their diagnosis can be quite complex. Treatment usually requires special expertise and equipment and, in many instances, is controversial. The field is advancing rapidly, because of accelerating progress in tumor biology, pharmacology, and instrumentation. Increasingly, the care of patients with an ocular or adnexal tumor is provided by a multidisciplinary team, comprising ocular oncologists, general oncologists, radiotherapists, pathologists, psychologists, and other specialists. For all these reasons, we feel that there was a continued need for a textbook of ophthalmic oncology, which would amalgamate knowledge from several different disciplines, thereby helping the various specialists to understand each other better and to cooperate more efficiently eventually moving ophthalmic oncology in the realm of evidence-based medicine.

As several important studies have been published in recent years, the purpose of *Clinical Ophthalmic Oncology* (2nd edition) is to provide up-to-date information on the whole spectrum of eyelid, conjunctival, intraocular, and orbital tumors, including basic principles of chemotherapy, radiation therapy, cancer epidemiology, angiogenesis, and cancer genetics. Several chapters authored by radiation oncologists, medical physicists, pediatric oncologists, hematologist-oncologists, and medical geneticists have been included to provide a broader perspective.

Although each section of *Clinical Ophthalmic Oncology* now represents a stand-alone volume, each chapter has a similar layout with boxes that highlight the key features, tables that provide comparison, and flow diagrams that outline therapeutic approaches. Each chapter has been edited (with author's approval) to present a balanced view of current clinical practice, and special attention has been paid to make the text easily readable.

The authors followed a tight timeline to keep the contents of the book current. As we undertook this ambitious task of editing a multiauthor, multivolume textbook, we were supported and guided by the staff at Springer: Sverre Klemp, Ulrike Huesken, Ellen Blasig, and the staff at SPi Global, India. Jennifer Brown kept the seemingly chaotic process under control.

It is our sincere hope that readers will find as much pleasure reading this volume as we had in writing and editing it. If you find *Clinical Ophthalmic Oncology* informative, it is because (paraphrasing Isaac Newton), "we have seen further, by standing on the shoulders of the giants."

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Contents

Principles of Clinical Epidemiology

1

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1.1 Introduction

During the last decade evidence-based medicine (EBM) has become a dominant approach in many medical fields, including ophthalmology [1, 2]. Clinical epidemiological studies provide evidence that can aid the decision-making processes. An overwhelming amount of clinical epidemiological papers are being published every year, and critical appraisal of the findings can be challenging, especially for the busy clinician who is not formally trained in the field of clinical epidemiology. Therefore the available evidence

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_1,

is increasingly bundled in clinical guidelines. The aim of this chapter is to provide the readers with some basic knowledge to allow them to judge the value of clinical epidemiological papers and thus of the pillars of evidence-based clinical guidelines. Examples from ocular oncology will be used to illustrate the methodological principles.

1.2 Research Question

A clinical epidemiological study should always start with a well-defined research question. Similarly, when reading a paper, one should always first identify the question(s) the authors wish to address (Fig. 1.1). Research questions can be aimed at explanation or description. Explanatory research examines causal relationships, while descriptive research is merely descriptive. In addition, research questions are also often being categorized as etiological, diagnostic, or prognostic (Table 1.1). For example, an explanatory research question related to etiology in the field of ocular oncology is as follows: are children born after in vitro fertilization at higher risk of developing retinoblastoma as compared to children born after natural conception? [3] A correct explanatory research question should contain information on the *p*atients, *i*nterventions, *c*ontrast, and *o*utcomes (*PICO*) at issue.

1.3 Outcome Measures

Traditionally, prevalence, incidence, and mortality (survival) have been the outcome measures in clinical cancer epidemiology studies. More recently, quality of life measures have become increasingly popular. In ophthalmic oncology, visual acuity is also an important outcome measure.

1.3.1 Prevalence

Prevalence refers to the proportion of the study population with the condition of interest. Usually prevalence is given for a specific moment in time (point prevalence), but sometimes it is estimated for a period of time (e.g., 1-year or lifetime prevalences). For example, the lifetime prevalence of uveal melanoma in a Caucasian population with oculo(dermal) melanocytosis is estimated to be 0.26 % [4].

1.3.2 Incidence

Whereas prevalence relates to existing cases, incidence relates to the proportion of new cases in the study population. It is important that the population under investigation is *at risk* of developing the condition. For example, persons with bilateral enucleation are no longer *at risk* of developing uveal melanoma. There are two different measures of incidence: cumulative incidence (CI) and incidence density (ID). CI is the proportion of new cases in a population at risk over a specified period of time. For example, the CI of second malignant neoplasms in hereditary retinoblastoma patients is 17 % at the age of 35 years [5]. ID refers to the rate of developing the condition during follow-up, usually expressed as a proportion per person-year at risk.

1.3.3 Mortality

Mortality refers to the incidence of death. The mortality rates can be all cause, indicating all deaths or disease specific, for instance, mortality caused by melanoma or retinoblastoma. Case fatality rate refers to the proportion of patients with a given disease who will die from that disease and thus reflects the seriousness of the condition. More formally put, it concerns the cumulative incidence of death among the diseased. Often the cumulative incidence of survival is presented, typically labelled as survival rate. For example, the 2-year survival rate after breast cancer metastases to the choroid is 30 % [6]. This means that of all the patients diagnosed with choroidal metastases from breast cancer, 30 % are still alive 2 years after diagnosis. It is important to realize that these mortality figures will be highly dependent on certain characteristics of the population, such as age, stage of cancer, and comorbidity.

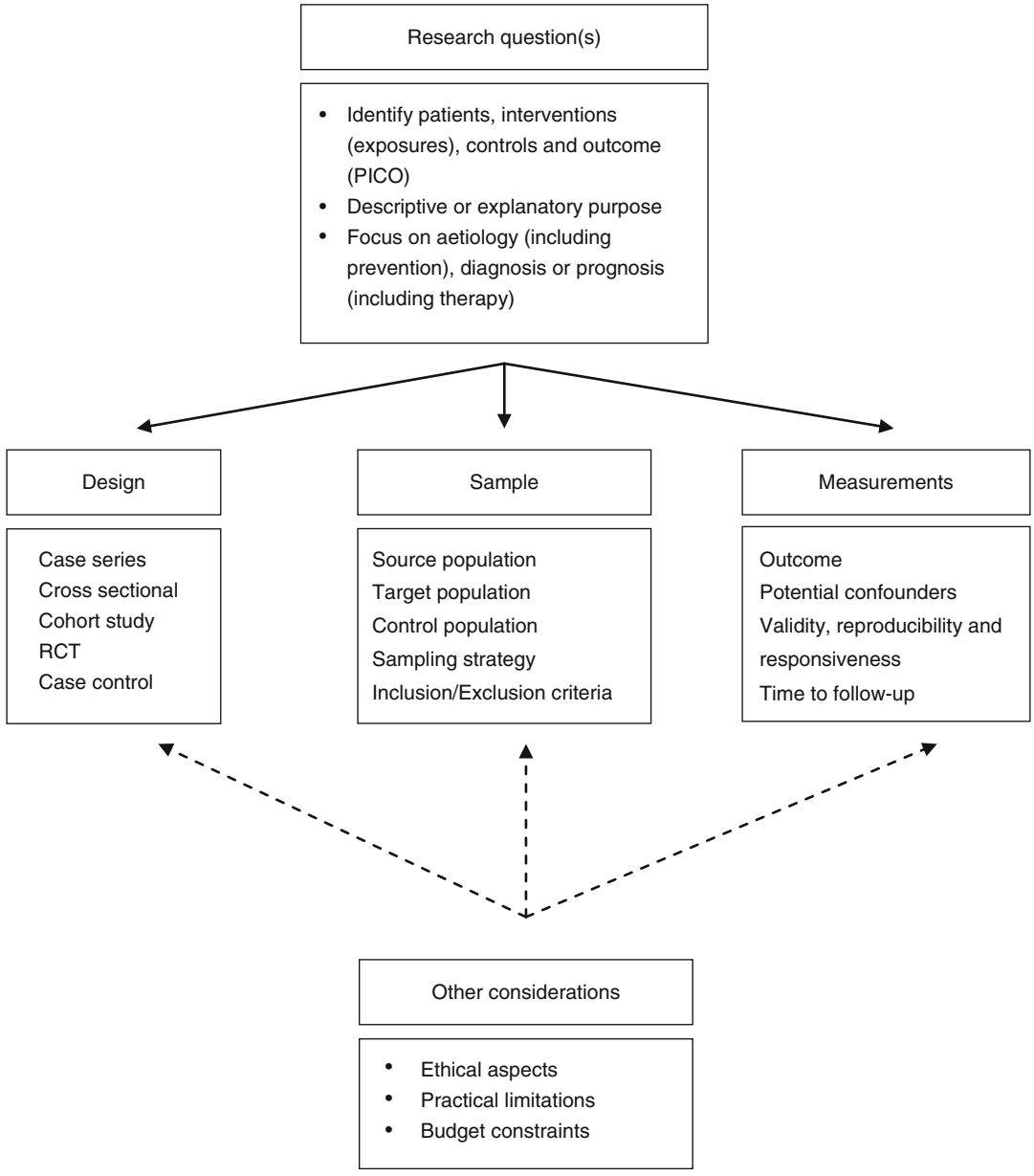

Fig. 1.1 Steps in designing a clinical epidemiological research

Table 1.1 Types of epidemiological research

Type of research	Purpose	Example
Etiology (including prevention)	To examine possible etiological factors for the occurrence of a disease	Association between ultraviolet radiation and uveal melanoma
Diagnosis	To examine the usefulness of diagnostic tests for the disease	Accuracy of magnetic resonance imaging in determining choroidal invasion of retinoblastoma
Prognosis (including interventions)	To examine possible prognostic factors for the disease	Association between external beam therapy for retinoblastoma and the incidence of second malignant neoplasms

1.3.4 Quality of Life

With the increasing survival rate and the severe side effects of some treatment modalities, quality of life measures have become increasingly important in ophthalmic oncology. These measures encompass symptoms and physical, social, and psychological functioning from a patient's perspective. Usually quality of life is assessed with a structured questionnaire, and scores are summarized assuming an interval scale. Several questionnaires have recently been developed for patients with ocular diseases, such as the measure of outcome in ocular disease [MOOD]) [7].

1.4 Measures of Association

In epidemiological research we are usually interested in associations between certain interventions or exposures and the outcomes, e.g., is there an association between paternal age and retinoblastoma in the offspring? [8] There are several statistical approaches which can be used to quantify associations, either as a ratio or as a difference, depending upon the study design and statistical method used (Table 1.2).

1.4.1 Relative Risk

The ratio of cumulative incidences of exposed and unexposed individuals (or between treated and untreated patients) is the relative risk (RR). For example, in the Netherlands, the RR of retinoblastoma in children conceived by in vitro fertilization is between 4.9 and 7.2. This implies that the risk of getting retinoblastoma is between 4.9 and 7.2 times higher for children conceived after IVF than naturally conceived children.

1.4.2 Hazard Ratio

The ratio of incidence densities of unexposed and exposed patients (or between treated and

Table 1.2 The relation between outcome, measures of association, study designs, and statistical methods

Outcome	Measure of association	Computation	Study designs	Statistical methods
Prevalence	Prevalence rate	P_1/P_2	Cross-sectional	Chi-square test
				Logistic regression analysis
	Prevalence difference	$P_1 - P_2$	Cross-sectional	Chi-square test
Odds of exposure	Odds ratio	Odds of exposure group 1/odds of exposure group 2	Case-control study (cohort study, RCT)	Chi-square test
				Logistic regression
Cumulative incidence (CI)	Relative risk	CI_1/CI_2	Cohort study/RCT	Chi-square test
	Risk difference	$CI_1 - CI_2$	RCT	
Incidence density (ID)	Hazard ratio	ID_1/ID_2	Cohort study/RCT	Kaplan-Meier
				Cox regression
	Risk difference	$ID_1 - ID_2$	RCT	Kaplan-Meier
	O/E ratio	Observed ID/ Expected ID in general population	Cohort study/registry study	
Quality of life	Difference in mean score	$X_1 - X_2$	Cohort study/RCT	Independent *t*-test
				Linear regression analyses

P_1 prevalence group 1, P_2 prevalence group 2, *CI* cumulative incidence, CI_1 CI group 1, CI_2 CI group 2, *ID* incidence density, ID_1 ID group 1, ID_2 ID group 2, *O/E ratio* observed to expected ratio, *RCT* randomized controlled trial
X_1 = mean score group 1; X_2 = mean score group 2

untreated patients) is the hazard ratio (HR), which has a similar interpretation as the RR. This measure is often used in relation to mortality, because we are generally interested not only in the proportion of patients that die but also in the time from baseline (diagnosis or start of treatment) until death. A special application of the HR is the ratio of the observed to the expected number of cases (O/E ratio). In this case the observed incidence density is calculated for the study population, and this is compared to the expected incidence density derived from a population registry (e.g., cancer registration). For example, in a study of lifetime risks of common cancers among 144 hereditary retinoblastoma survivors, 41 cancer deaths were observed, whereas only 7.58 deaths due to cancer were expected. This data can be expressed as standardized mortality ratio of 5.41 [9].

1.4.3 Odds Ratio

The odds ratio (OR) is the most commonly reported measure of association in the literature, due to the fact that this is the statistic that can be derived from the popular logistic regression analysis. The OR is the ratio of the odds of outcome of interest between the exposed and the unexposed. Generally speaking the OR is a good approximation of the RR or HR.

1.4.4 Differences in Risk

Differences in risks (RD) are preferably reported as outcome of randomized controlled trials. The RD is easy to interpret and can be used to calculate the number of patients needed to treat (NNT) to prevent one extra event (e.g., death) compared to the standard treatment or placebo. The NNT can be calculated as inverse of RD (1/RD). A related concept is that of the number needed to screen (NNS). This refers to the number of patients needed to screen to prevent one extra event compared to the situation without a screening program. The NNS thus depends on the predictive probability of the screening test as well as on the efficacy of treatment for people that are diagnosed with that screening test. The value of routine neuroimaging screening of pineoblastoma in retinoblastoma patients is uncertain and a point of discussion [10].

1.4.5 Differences in Mean Score

For scores on interval scales, such as quality of life, differences in mean score between exposed and unexposed participants are the most important measure of interest. These can be derived from independent samples *t*-test of general linear models (e.g., linear regression analysis).

1.5 Precision of the Estimate

When interpreting an outcome, we do not only want to know the numerical value of the point estimate, but also the precision with which it has been assessed. In other words, can we be confident that the outcome is not just a chance finding? The usual standard for accepting an outcome as being beyond chance is p (probability) <0.05. A more informative description is provided by the 95 % confidence interval (CI). The rough interpretation of the 95 % CI is that there is a 95 % probability that the real value lies within the confidence interval.

Statistical significance and the width of confidence intervals are strongly dependent on the sample size of a study. This means that in very large samples, weak (and potentially unimportant) associations can be statistically significant. In contrast, in small samples, strong (and potentially important) associations are sometimes not statistically significant. Such findings should of course not be dismissed as being irrelevant. Instead they should be replicated in larger study populations. The associations, although statistically significant, need not be clinically important. Therefore, interpretation of findings should never solely rely on statistical significance.

1.6 Bias

An estimate can be very precise, but still not be accurate because of bias. Three main sources of bias exist: confounding, selection, and information bias.

1.6.1 Confounding

Confounding occurs when the association between exposure and outcome is influenced by a third variable that is both related to the exposure and the outcome (Fig. 1.2). A recent study found an association between cooking (as occupation) and the incidence of ocular melanoma [11]. It could be argued that as many cooks work at night, it is possible that they could have relatively high exposures to sunlight due to daytime leisure activities compared to people working during the daytime. It is implied that the association between cooking and ocular melanoma could potentially (in part) be explained by a higher exposure to sunlight by cooks.

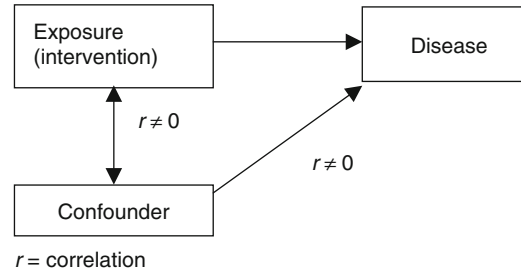

Fig. 1.2 Schematic representation of confounding

1.6.2 Selection Bias

Selection bias may occur when the chance of being included in the study population is not random for all members of the source population. For example, patients with advanced tumor stage are more likely to be referred to a special cancer center than patients with a less advanced tumor stage. This form of selection bias is called referral bias. Selection bias could also be introduced in a study by choosing the wrong control group, especially if controls are selected from hospital patients.

1.6.3 Information Bias

Information bias occurs when outcome or exposure variables are not accurately assessed. This is especially problematic when this occurs differently for exposed versus nonexposed or for cases versus controls. A well-known type of information bias is recall bias. This refers to the phenomenon that patients tend to remember more details about exposures that are possibly related to their disease than controls. For example, the patients with uveal melanoma are probably more aware of the fact that their disease could be related to sunlight exposure. In turn they reflect upon their own past exposure to sunlight in much more detail than healthy controls. This can lead to a relative underestimation of exposure in controls and hence an overestimation of the association with sunlight exposure.

1.7 Study Designs

There are several research designs, such as case series, cross-sectional, cohort, randomized control trial, and case-control study that can be adopted in order to address a research question. Each of the study designs has its advantages and disadvantages (Table 1.3).

1.7.1 Case Series

In case series the authors present the clinical data regarding a group of patients, e.g., tumor response to chemotherapy combined with diode laser in retinoblastoma patients. The major disadvantage is that this kind of study does not have a comparative design and does not permit an answer to a question such as “there is a good response, but compared to what, as there is no control group? [12]

Table 1.3 Advantages and disadvantages of different study designs.

Considerations		Type of study			
Methodological		Cross-sectional	Cohort	RCT	Case control
	Confounding	–	–	+	–
	Selection bias	–	±	±	–
	Information bias	±	±	±	–
	Prior exposure	–	+	+	–
	Incident cases	–	+	+	+
Practical	Length of study	+	–	±	+
	Organization	+	±	–	±
	Expenses	+	–	–	+

RCT randomized controlled trial
Negative score (–) indicates disadvantage compared to other study designs
Positive score (+) indicates advantage compared to other study designs
Equivocal score (±) indicates neither advantage nor disadvantage as compared to other study designs

1.7.2 Cross-Sectional Study

In a cross-sectional study, the outcome (and exposure) is assessed at one point in time. In prevalence studies only the outcome is measured (e.g., prevalence of retinoblastoma in Denmark). In addition, the outcome between exposed and unexposed study participants can be compared in order to explore etiological questions. In a cross-sectional study on the association between iris color and posterior uveal melanoma, melanoma patients ($N=65$) with light iris color were significantly more likely to have darker choroidal pigmentation than controls ($N=218$) ($p=0.005$). In addition, darker choroidal pigmentation was associated histologically with increased density of choroidal melanocytes ($p=0.005$). The authors concluded that increased choroidal pigmentation, as a result of an increase in the density of pigmented choroidal melanocytes, is not protective but may actually be a risk factor for the development of posterior uveal melanoma in white patients [13].

The cross-sectional study design has the advantage that it is relatively easy to plan, that only one measurement is needed, and that it is inexpensive and quick to perform. From a methodological point of view, however, the design has some disadvantages. As both exposure and outcome are measured at the same time, we cannot be sure that the exposure preceded the outcome (the most important criterion for causality). Moreover, the outcome is always measured in terms of prevalent cases, and prevalent cases may have a relatively better prognosis (they are still alive) than the incident cases. Therefore, the associations found in a cross-sectional study can only be interpreted as being causal in rare instances.

1.7.3 Cohort Study

Some of the problems listed above can be overcome by conducting a (prospective) cohort study. At baseline, one starts with a cohort of people free from the outcome of interest. During or at the end of follow-up, incident cases in both the unexposed and the exposed groups are identified, and RRs or HRs can be calculated. Despite theoretical advantages of a cohort design, there are some practical disadvantages. The cohort studies often need large sample sizes and/or long follow-up to accumulate enough incident cases for meaningful analyses. These studies are often expensive. From a methodological point of view, the potential bias of (residual) confounding can never be totally excluded.

1.7.4 Randomized Controlled Trial

Randomized controlled trials are a specific type of cohort study. At the start of the study,

participants are randomly assigned to the intervention group (treatment under investigation) or a control group (no treatment, placebo, or standard treatment). After the start of a treatment, patients often get better. This may be due to the treatment or to other circumstances such as spontaneous resolution, effective co-interventions, and placebo effects. Only a sufficiently large randomized, blinded trial is useful to estimate the efficacy of drugs and other treatments. The best comparison is often between the new treatment and the best available one, not the sham treatment [14]. The randomization, if successful, ensures that confounding factors are evenly distributed between the intervention and control groups. As with the cohort studies, incident cases in both groups are determined during or at the end of follow-up, allowing for the risk estimates to be calculated.

For clinicians interested in evidence pertaining most directly to a particular class of patients, subgroup analyses can be very informative. The strength of evidence for subgroup effects depends on the question whether hypotheses have been defined prior to analysis, whether potential problems regarding multiple comparisons have been considered, and whether there is biological plausibility of the effects found. Using these guidelines, the reader of a trial report should be able to decide if presented subgroup effects are of clinical importance or if the overall result is a better estimate of treatment effect [15].

1.7.5 Case-Control Study

In contrast to cohort studies, the starting point in case-control studies is not to assess the exposure status, but the disease status. People with the disease of interest are selected, and a control group of people without the disease is subsequently recruited. The control group should include people from the same source population as the cases implying that if any of the controls had developed the disease, they would have been eligible for inclusion in the study as a case.

The selection of a valid control group is important in case-control studies and has therefore generated a fair amount of discussion in the epidemiological literature. It is possible to select population controls, hospital controls, friends or relatives of patients, or any combination of these [16]. Case-control studies have the advantage of being relatively quick and inexpensive to conduct and are especially appealing in rare diseases. A disadvantage is the large potential for selection bias, especially in the recruitment of controls. In addition there is also a real danger for information (recall) bias. Similar to cohort studies, bias by confounding can never be totally ruled out.

1.7.6 Pilot Study

A pilot study is often performed before the start of a large study. Its aim is to improve the methodological quality and evaluate the feasibility. The estimate of the effect of an intervention in a pilot study is determined to a large extent by chance and therefore cannot be considered as conclusive. However, inclusion of such results in a later cumulative meta-analysis may lead to sufficient power so as to assess the efficacy of an experimental intervention [17].

1.7.7 Systematic Review

In a systematic review all available evidence (literature) on a certain topic is reviewed in a systematic, transparent, and reproducible manner. These studies can be especially useful when results from single studies are contradictory and/or have large confidence intervals due to small sample size. When the studies in a systematic review are reasonably homogenous, their results can be pooled in meta-analyses. This results in one effect size for all the studies together, with a much smaller confidence interval than the individual studies. An example is a systematic review on the survival of patients with uveal melanoma treated with brachytherapy. The result of this meta-analysis showed that the 5-year melanoma-related mortality rate was 6 % for small and medium tumors and 26 % for large tumors [18].

1.8 Analysis of Microarray Data

Microarrays are complex and sophisticated assays, and so the methods for interpreting microarray data are similarly complex. The purpose of this section is to give a brief introduction to the analysis of microarray data with a focus on understanding the main principles, and not on the technical or theoretical details. There are a number of software options for analyzing microarray data. Software packages are available commercially, such as GeneSight (BioDiscovery), GeneSpring (Agilent Technologies), and Affymetrix [19]. Some are available for free, such as Bioconductor [20], D-Chip [21], and TM4 [22]. Other microarray analysis tools are even available online, such as ArrayMining.org [23] and WebArray [24]. The ideal software to use depends on the goals and constraints of the experiment. Steps in analysis of gene expression microarray data are listed below.

1.8.1 Image Processing

After the assay is completed, the microarray slide is scanned with a confocal laser. The resulting image file is saved digitally. An algorithm converts fluorescence of each probe to relative abundance; higher intensity fluorescence is the result of more frequent hybridization with the probes, suggesting higher levels of gene expression. Different software packages approach this problem in different ways [25].

1.8.2 Scaling and Normalization

After the raw intensity data are collected, they must be adjusted for background noise. Additionally, the data from different samples must be normalized in order to make direct comparisons. Otherwise, the differences in experimental procedure will overwhelm any biological differences. Methods for normalization differ depending on the specific assay and experimental objectives [19, 26–29].

1.8.3 Strategies for Analyzing Gene Expression Data

The end product of the data preprocessing is a gene expression matrix, where each row has the expression for a gene across all samples and each column has the expression for a sample for all genes. The researcher now has many options in terms of moving forward with the analysis. The following is a brief discussion of three of the most common approaches to analyzing microarray data.

1.8.3.1 Unsupervised Analysis

In an unsupervised strategy, the researcher does not assign any information about the samples in the analysis (Fig. 1.3). Rather, they assess the natural groupings of the samples by assessing the similarity between samples measured by a number of different metrics. The two most popular methods are Euclidean distance and Pearson's correlation-coefficient distances [30]. Once the number of groups in the sample is assessed, correlations between the groups and certain phenotypes can be explored. This strategy is helpful for exploratory data analysis, but interpreting the results might not be so straightforward. Classes may be defined not on any biological basis, but perhaps by experimental artifact. For example, a researcher might find that her cancer samples neatly separate into two classes but that these classes correspond to experimental batch, as opposed to any phenotype of interest.

1.8.3.2 Supervised

In a supervised strategy, the investigator has already defined the groups of interest and wants to determine the transcriptomic differences between the groups. For example, a researcher might be interested in finding the gene expression differences between metastatic and nonmetastatic tumors. Depending on the design of the experiment, platform used, and goal of researcher, there are a number of different algorithms that can be used to identify these differences [23]. When searching for these differences, special statistical techniques must be employed, since the number of features analyzed is far greater than

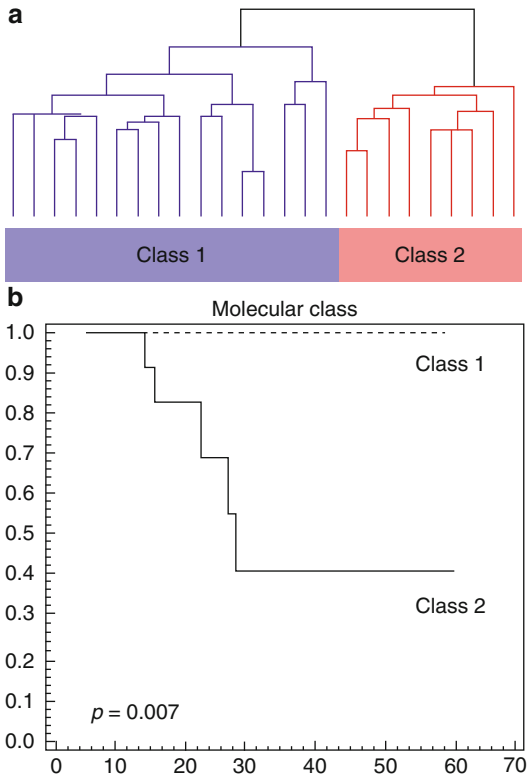

Fig. 1.3 Unsupervised hierarchical cluster analysis of gene expression microarray data from 25 primary uveal melanomas, showing the natural segregation of tumors into two groups, class 1 and class 2 (**a**). Kaplan-Meier survival analysis showing no deaths among class 1 patients and five metastatic deaths among class 2 patients. This difference in survival was highly significant (**b**) (Reproduced with permission from Singh et al. [31])

the number of samples. Usually, these techniques employ some form of correction for multiple testing, which is a method to reduce the number of false-positive results.

A supervised strategy is helpful in identification of novel biomarkers that might aid in diagnosis, prognosis, or treatment stratification. But, this technique may be unreliable as identified groups within study sample might not correspond to any true biologically distinct groups.

1.8.3.3 Pathway Analysis

For improved detection of biologically relevant significance, there is a trend towards analyzing the differences in expression of genes belonging to a biological pathway rather than single genes. The argument goes that gene-level expression is too granular to understand how the difference in biology between phenotypes. Pathway-level expression is calculated as a function of the genes that comprise the pathway [32].

Conclusions

In general, ophthalmic tumors are rare compared to other ophthalmic diseases. Therefore, it is difficult to conduct large studies with enough power to get statistically significant and clinically relevant results. A lot of studies are published each year, most of them are descriptive and concern retrospective patient series. To conduct randomized clinical trials, international collaboration is necessary to include enough patients in the different treatment arms of the study. Furthermore, uniform definitions and study methodology are very important to compare the different studies in the literature and to be able to perform systematic reviews and meta-analyses. Microarrays generate large datasets that need special statistical methods of analysis.

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Etiology of Cancer

2

Brian T. Hill

Contents

2.1 Introduction

Cancer is a pathologic accumulation of clonally expanded cells derived from a common precursor. The fundamental cause of all cancers is genetic damage that is usually acquired but is sometimes congenital. In general, the genetic dysregulation that gives rise to uncontrolled cell proliferation results from either activation of growth-promoting oncogenes and/or deletion/inactivation of growth-inhibiting tumor suppressor genes. Additional contributions to carcinogenesis come from genes that regulate programmed cell death (apoptosis) and genes involved in DNA repair. The most widely accepted theory of cancer development is the Knudson "2-hit" hypothesis, which posits that a mutation in one predisposing gene is necessary but not sufficient for malignancy and that only after development of a second mutation will invasive cancer develop (Fig. 2.1) [1]. In this chapter, we discuss the major categories of carcinogens and their role in the etiology of cancers with emphasis on common ophthalmic cancers.

2.2 Carcinogenic Agents

Although a clearly defined cause is not apparent in the majority of *de novo* malignancies, it is well documented that carcinogenic agents contribute to many human cancers. These fall into several broad groups that can be categorized as infections (such as viruses), chemical (including

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_2,

occupational, environmental, and therapeutic), electromagnetic radiation [i.e., ultraviolet (UV), X-ray, gamma-ray], and immunosuppressive (HIV, immunosuppressive medications) (Table 2.1).

2.3 Chemical Carcinogens

Several hundred chemicals have been shown to be carcinogenic in humans. Exposure to these chemical carcinogens can be a result of occupation (asbestos, aniline dyes), environmental (alcohol, tobacco), or iatrogenic (chemotherapy). While many carcinogens are directly mutagenic to DNA, the majority of carcinogens undergo activation after exposure to reactive metabolites that are responsible for genetic damage.

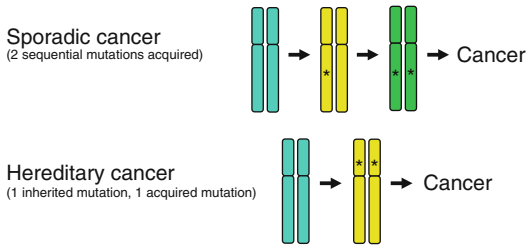

Fig. 2.1 Schematic representation of Knudson 2-hit hypothesis in which a normal cell requires two successive mutations to delete both functional copies of a given gene to develop cancer. In contrast, cells with germline mutations only require a single genetic event to develop malignancy

2.3.1 Environmental Exposure

Of the known environmental carcinogens, perhaps the most well-documented agent known to cause cancer is asbestos. Asbestos is a naturally occurring mineral that has a broad range of industrial and commercial applications, but aerosolized fibers of asbestos are known to lodge in small airways and lead to tissue damage and significantly predispose exposed individuals to both lung cancer as well as mesothelioma [2].

In addition to asbestos, a number of other agents have been associated an elevated risks of various cancers through epidemiologic studies. For example, several agents have been implicated in leading to lung cancer including heavy metals (arsenic, cadmium, chromium, and nickel), as well as BCME (bis chloromethyl ether) [3]. Aromatic amines (such as 4-AMP, 4-aminobiphenyl, naphthylamine, and benzidine) are associated with an increased risk of bladder cancer [3]. Aflatoxin and vinyl chloride are associated with liver cancers. An exhaustive list of known environmental carcinogens is beyond the scope of this text but should be considered when

Table 2.1 Various types of carcinogens

Chemicals	Radiation	Infectious	Dietary	Iatrogenic
Occupational	UV light	Virus	Fat	Radiation
Arsenic	X-ray	HPV	Calorie	Immunosuppression
Asbestos	Gamma-ray	EBV	Low fiber	Chemotherapy
Coal tars	Nuclear radiation	KSHV		
Soot		HTLV-1		
Benzene		HEP B/C		
Vinyl chloride				
Behavioral		Bacteria		
		H. pylori		
		C. psittaci		
Tobacco		Fungus		
		A. flavus		
Alcohol		Parasite		
		Schistosoma		
		Clonorchis		

obtaining the occupational and social history of patients with newly diagnosed cancer.

2.3.2 Behavioral Exposure

2.3.2.1 Tobacco

Tobacco is responsible for over 30 % of the cancer deaths and represents the single greatest cause of preventable cases of cancer. Cigarettes as well as smokeless tobacco are associated with cancers of multiple organs including the oral cavity, pharynx, larynx, lung, esophagus, stomach, pancreas, colon, rectum, kidney, bladder, ureter, and cervix. Over 60 carcinogens have been identified in tobacco, with the highest carcinogenic potency attributed to polycyclic aromatic hydrocarbons, nitrosamines, and aromatic amines [4]. The risk of tobacco-associated cancer is proportional to total lifetime cigarette smoke exposure. The risk of lung cancer among long-term heavy smokers is approximately 10–20 times greater than nonsmokers.

2.3.2.2 Alcohol

Excessive and repeated alcohol intake has been is associated with liver, rectal, and breast cancers. Interestingly, simultaneous exposure to alcohol and cigarette smoke results in synergistic increased risk of oral cancer, with a relative risk relative to nonsmoker, nondrinkers of over 35-fold for the heaviest consumers. Similar tobacco-alcohol interactions are also known for esophageal cancer.

2.4 Electromagnetic Radiation

2.4.1 Ultraviolet Light

Ultraviolet light B (UV-B) has a wavelength between 280 and 320 nm and is the primary oncogenic form of UV light, whereas ultraviolet A (UV-A, wavelength 320–400 nm) has insufficient energy to induce DNA damage and ultraviolet light C (UV-C, 200–280 nm) is absorbed by the atmospheric ozone and is not a significant source of environmental cancer.

UV light induces cancer by formation of pyrimidine dimers in affected DNA which can result in inactivation of tumor suppressor genes such as p53. Patients with the autosomal recessive disorder xeroderma pigmentosum (XP) lack the ability to repair DNA damage caused by UV light owing to a defect in the nucleotide excision repair pathway (NER), resulting in an increased risk of skin cancer at least 2,000-fold relative to unaffected individuals [5, 6].

UV light from sun exposure is associated with increased incidence of several types of skin cancer including squamous cell carcinoma, basal cell carcinoma, and melanoma [7]. In the eye, UV exposure increased the risk of ocular surface squamous neoplasia (OSSN), which includes dysplasia, carcinoma in situ, and squamous cell carcinoma of the conjunctiva or cornea. In the case of uveal melanoma, sunlight exposure has not been implicated as a cause for this condition, whereas known risk factors include Caucasian race, light skin color, blond hair, and blue eyes [8].

2.4.2 Ionizing Radiation (X-Rays and Gamma-Rays)

In the early twentieth century, it was first recognized that radiation could cause tissue damage and subsequently cancer including acute leukemia and skin cancer. Ionizing radiation causes cell death by DNA damage leading to the induction of apoptosis and cell cycle arrest. In general, a dose response relationship is well described, with both duration and intensity of radiation affecting relative risk of developing cancer.

Sources of ionizing radiation include occupational exposure such as X-rays or exposure to nuclear power or atomic weapons. Iatrogenic sources of radiation for cancer treatment are also a significant source of secondary malignancy. The best described data on radiation-induced cancers comes from the study of the survivors of the Chernobyl nuclear power plant disaster and Hiroshima and Nagasaki atomic bomb events of World War II [9, 10]. In the latter case, the relative risk of all cancers was 1.53 and included multiple solid tumors including breast, lung, esophagus, stomach, and GU cancers. All of

these solid tumors had a relative risk between 1.2 and 2.4. A notable outlier was a relative risk of leukemia in this patient population, which had a relative risk of 5.6 [9].

2.5 Infections Agents

A number of infectious agents are carcinogenic. The mechanisms by which each pathogen leads to the development of cancer are diverse and include induction of genomic instability as a result of chronic inflammation, impairment of host immunity, and in some cases, a modulation of the balance between proliferation and antiproliferation signals.

2.5.1 Viruses

A significant proportion of human cancers are caused by DNA and RNA viruses. Human papilloma virus (HPV), Epstein-Barr virus (EBV), hepatitis viruses B and C, and Kaposi sarcoma herpes virus (KSHV) are examples of the most well-described oncogenic viruses.

2.5.1.1 Human Papillomavirus (HPV)

HPV was one of the first viruses to be identified as causing human cancer. Almost all of the 200+ genotypes of HPV infect epithelial cells, causing benign papillomas (warts) or squamous cell carcinoma of the oral, laryngeal, cervical, and anogenital region. The strongest association between invasive cervical carcinoma is with HPV subtypes 16 and 18, which can be identified in approximately 85 % of cases. HPV causes uncontrolled cellular proliferation by inactivating the tumor suppressor proteins p53 and pRB. DNA of HPV with high oncogenic potential has been identified in dysplastic and malignant lesions of the conjunctiva and cornea suggesting that HPV may contribute to the development of OSSN [11, 12].

2.5.1.2 Epstein-Barr Virus

Epstein-Barr virus (EBV) has been implicated in the pathogenesis of a number of cancers including the African form of Burkitt lymphoma, B-cell lymphomas in immunosuppressed individuals such as posttransplant lymphoproliferative disorder (PTLD), and nasopharyngeal carcinomas. The mechanism by which EBV transforms B lymphocytes is not clearly defined but most likely involves viral protein expression that leads to a viral latency program associated with proliferation. This is particularly relevant to the pathogenesis in the absence of a host antiviral response as in the case of HIV-associated lymphoma and/or PTLD. EBV infection is not clearly linked to ophthalmic cancer. Although EBV viral DNA has been identified in both primary intraocular as well as ocular adnexal lymphoma, this is of uncertain significance [13].

2.5.1.3 Kaposi Sarcoma Herpesvirus

Kaposi sarcoma herpesvirus (KSHV), also known as human herpesvirus 8, is the causative agent of Kaposi sarcoma (KS). KS can affect the skin of any site but also can involve the eyelid and the conjunctiva. Uncommon in immunocompetent individuals, the incidence of KS increased significantly during the AIDS epidemic of the 1980s. Since the introduction of highly active antiretroviral therapy (HAART), the incidence of KS lesions including those of the eyelid and conjunctiva has decreased significantly.

2.5.1.4 Human T-Cell Lymphotropic Virus-1

Human T-cell lymphotropic virus type 1 (HTLV-1) infection is endemic in Asia, Africa, and much of Latin America. The most well-described cancer associated with HTLV-1 is T-cell leukemia/lymphoma. Known ophthalmic manifestations of HTLV-1 include direct ocular infiltrates in patients with adult T-cell leukemia/lymphoma, but it can also be associated with nonmalignant ocular complications including retinal degeneration, neuro-ophthalmic disorders, uveitis, necrotizing retinal vasculitis, and keratoconjunctivitis sicca [14].

2.5.1.5 Hepatitis B and C Virus

Chronic infection by hepatitis B or hepatitis C viruses is a well-defined risk factor for hepatocellular carcinoma. In addition, extranodal marginal zone lymphoma is increased in patients with hepatitis C. Importantly, from the standpoint of ophthalmic cancers, there have been conflicting reports of

an increased incidence of hepatitis C in patients with ocular adnexal lymphoma [15, 16].

2.5.2 Bacteria

2.5.2.1 *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) infection is associated with peptic ulcer disease, gastric carcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach [17, 18]. A prevailing theory on the pathogenesis of *H. pylori*-associated malignancy is that chronic inflammation from the infection leads to atrophic gastritis and consequent achlorhydria, which in turn favors bacterial growth. Excessive gastrointestinal flora can result in excess reduction of nitrates to nitrites, which have been associated with DNA damage and, thus, carcinogenesis of bystander tissues including the gastrointestinal epithelium as well as mucosa-associated lymphoid tissue. In the case of isolated gastric MALT lymphoma, treatment with *H. pylori* eradication can lead to complete and durable remission, thereby obviating the need for chemotherapy or radiation.

2.5.2.2 *Chlamydia psittaci*

A number of human diseases including cancers are strongly associated with infection by *Chlamydia*. *C. trachomatis* and *C. pneumoniae* have been associated with cervical carcinoma and lung cancer. In addition, *C. psittaci* has been associated with ocular adnexal lymphomas, although there appears to be geographic variation in the strength of this association [19].

2.5.3 Fungus

Aspergillus flavus is a fungus found in grains and peanuts. It produces aflatoxins that cause a large proportion of liver cancer in the developing world China and South Africa.

2.5.4 Parasites

Schistosoma, *Clonorchis*, and *Opisthorchis* cause chronic inflammation, thereby leading to the development of cancer. The best described association of a cancer with parasitic infection is that of bladder cancer with schistosomiasis.

2.6 Dietary Factors

A significant proportion of cancer deaths can be attributed at least partially to dietary factors. Diets with excessive calories from saturated fat are known to increase the risk of hormone-dependent cancers such as breast, ovarian, endometrial and prostate cancers. In contrast, a low-fiber diet is associated with an increased risk of colorectal cancer, possibly causing increased exposure of colonic mucosa to potential mutagens.

2.7 Genetic Susceptibility

There are a number of well-defined cancer syndromes, including Li-Fraumeni, hereditary non-polyposis colon cancer, and polyposis colon cancer syndromes; mutations in the BRCA-1 and BRCA-2 oncogenes; and others. In the case of breast cancer, however, only approximately 10 % of patients have an identifiable germline mutation. Many of the inherited cancer predisposition syndromes such as BRCA-1 and BRCA-2 involve defects in DNA repair pathways.

In the case of ophthalmic cancers, inheritance of the retinoblastoma gene is particularly relevant because it shows an autosomal dominant inheritance pattern. In contrast, xeroderma pigmentosum displays an autosomal recessive inheritance pattern. A more comprehensive discussion of cancer genetic factors is discussed in Chap. 6.

2.8 Iatrogenic Cancers

2.8.1 Immunosuppression

Immunosuppression increases the risk of cancer, particularly lymphoma. After solid organ transplantation, lifelong immunosuppression with calcineurin inhibitors is well known to increase

the risk of PTLD, which is commonly EBV-positive. Inhibitors of tumor necrosis factor-alpha (TNF-α) such as infliximab also increase the risk of lymphoma. In addition, solid tumors are also more commonly observed after organ transplant relative to the general population.

2.8.2 Chemotherapy

Treatment-related cancer can occur after both radiation and chemotherapy, but for patients who have received chemotherapy alone, the rate of secondary malignancy may be as high as 10 %. In general, diseases with higher cure rates are more at risk, due to the longer duration of risk – most notably Hodgkin lymphoma, testicular cancer, and small-cell lung cancer. The most commonly implicated agents for causing secondary malignancies are alkylating agents such as cyclophosphamide and topoisomerase inhibitors such as etoposide.

2.8.3 Radiation Therapy

Therapeutic radiation causes DNA damage and can result in solid tumors including sarcoma and breast cancer, depending on the anatomic site. Hematologic malignancies including MDS and acute leukemia are also well described. In the case of patients with retinoblastoma, an increased risk of sarcomas after radiation is well documented [20].

2.9 Summary

The development of cancer can rarely be attributed to as single cause. Genetic predisposition confers significant risks for developing cancer in a minority of patients. Rather, risk can be modified by carcinogen exposure, immunosuppression, and environmental/iatrogenic causes. It is difficult to quantitate the individual contribution of several potential risk factors for most cases (Fig. 2.2). Furthermore, it is noteworthy that with few exceptions, ultimately most cancer treatment is independent of its etiology.

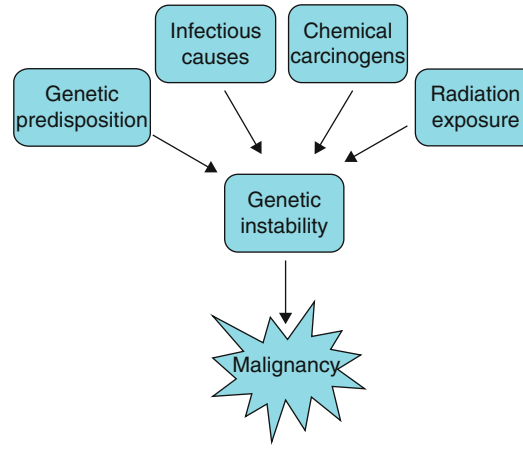

Fig. 2.2 Schematic representation of multiple factors potentially involved in malignant transformation

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Cancer Pathology

3

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3.1 Introduction

This text is intended as an overview of cancer pathology with particular reference to classification, tumorigenesis, microscopic features, sampling, and diagnostic techniques for ocular cancer.

3.2 Classification of Neoplasia

Tumors may be either benign or malignant, where benign tumors may be precursors to malignant tumors. The most significant difference between benign and malignant tumors is that the malignant tumors have metastatic potential, whereas benign tumors do not metastasize. However, any benign or malignant tumor may cause death if inappropriately located. Characteristic differences between benign and malignant tumors are summarized (Table 3.1). Below, different benign and malignant tumors are exemplified.

3.2.1 Benign Tumors

Benign tumors are usually labeled by the suffix -oma. Some exceptions tend to cause confusion; lymphoma and melanoma are by definition malignant, irrespective of the suffix -oma. To emphasize this, terms like malignant lymphoma and malignant melanoma are sometimes used:

- Adenoma is composed of cells originating from glandular epithelium.
- Hamartoma is composed of physiologic cells originating at the affected site.

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_3,

Table 3.1 Characteristic differences between benign and malignant tumors

Feature	Benign	Malignant
Cellular pleomorphism	None or mild	Mild to severe
Cellular dedifferentiation	None or mild	Mild to severe
Necrosis	Rare	Occasionally
Basement membrane invasion	Never	Frequent
Metastatic spread	Never	Occasionally

- Choristoma is composed of cells not normally occurring at the affected site, but otherwise histologically physiologic.
- Teratoma is composed of pluripotent cells forming different types of tissue originating from one or more of the three germ cell layers. A teratoma may be either benign or malignant.

3.2.2 Malignant Tumors

- Carcinoma is a neoplasm of epithelial origin. For example, an adenocarcinoma of the lacrimal gland is a cancer derived from the glandular epithelium of the lacrimal gland.
- Sarcoma is derived from mesenchymal tissue.
- Blastoma is a malignant tumor of embryonic origin. For example, retinoblastoma is derived from retinoblasts in the developing retina.
- Leukemia is a malignancy of blood cells that arises from the bone marrow precursor cells and is present in the peripheral blood.
- Lymphoma is a malignancy derived from lymph nodes, but may occasionally be present in other organs such as the lacrimal gland or in peripheral blood.
- Melanoma is a malignant tumor that originates from melanocytes, i.e., cells containing intracytoplasmic pigment lodged in specific organelles, melanosomes. These cells appear in the skin, uvea, conjunctiva, and a variety of other tissues.

3.3 Tumorigenesis

Tumorigenesis is a multistep process in which normal cells progressively evolve to a neoplastic state. Advances in techniques for studying cancer genetics have revolutionized our knowledge of tumorigenesis [1]. The rapidly accumulating information on the genetic and epigenetic constitution of malignancies has made it possible to tailor novel therapeutic agents, several of which are in clinical use. Hanahan et al. structured a succession of "hallmarks" of tumor cells which mark the steps of tumorigenesis [1]. These hallmarks include *genomic instability and mutations*, *evading immune destruction*, *proliferative signaling and reprogramming energy metabolism*, *resisting growth suppressors*, *escaping cell death*, *replicative immortality*, *angiogenesis*, *invasion and metastasis*, and *the tumor microenvironment*. The hallmarks are briefly outlined below; the genes involved in tumorigenesis are discussed in greater detail elsewhere (Chap. 6).

3.3.1 Genomic Instability and Mutations

Believed to underlie many of the hallmark capabilities in neoplastic cells, genomic instability generates random mutations including chromosomal rearrangements (Fig. 3.1). Tumors progress by stepwise accumulation of enabling genetic mutations. Random mutations occur in genomic regions that drive malignant progression. Non-tumorigenic regions may also harbor "passenger" mutations that are not pathogenic but may serve as biomarkers [2]. Recurrent genetic aberrations and mutations across several different tumor types indicate that some genetic regions are important drivers of tumorigenesis [3]. The recent application of deep sequencing of entire cancer cell genomes will increase our knowledge of these apparently random mutations [4]. Studies have shown that mutations of telomerase and telomeres are required to achieve endless replication and survival [5]. However, recent evidence suggests that cells that survive the telomere erosion enter breakage-fusion-bridge cycles with resulting gross genetic aberrations. Tumor cells with these complex genetic alterations will have an accelerated acquisition of mutations and rapidly progress to more malignant states [6].

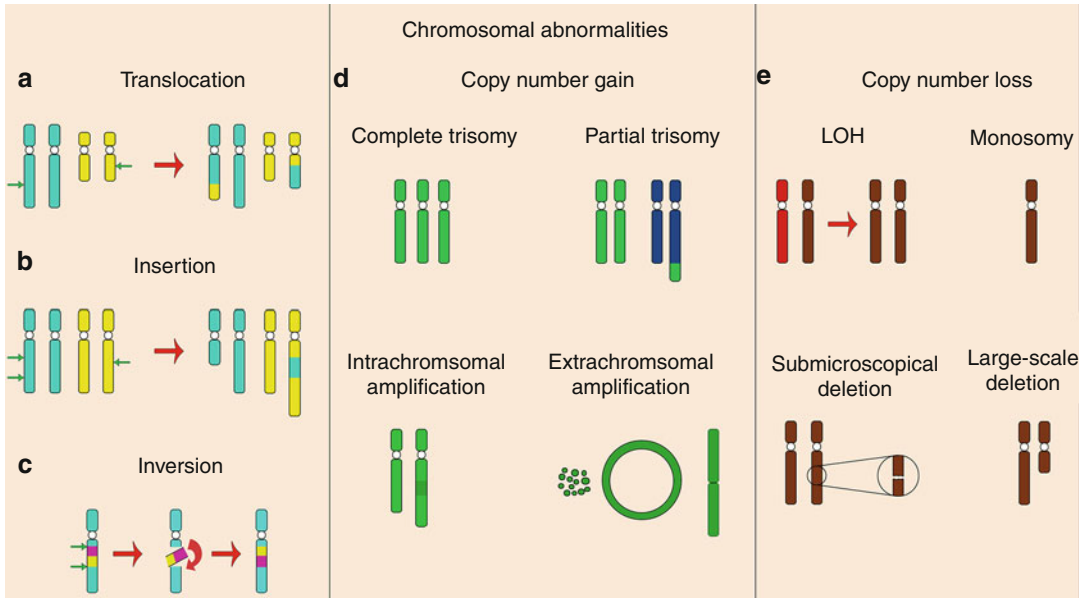

Fig. 3.1 Chromosomal abnormalities in human cancer. A multitude of different chromosomal aberrations underlie tumorigenesis. These may lead to the formation of fusion gene or over- or underexpression of structurally normal gene. The most common are included in this figure. (**a**) Translocation: one chromosomal segment is translocated from one chromosome to another and vice versa. (**b**) Insertion: a chromosomal segment is inserted into another chromosome. (**c**) Inversion: a 180° rotation of a segment. (**d**) Copy number gain can occur through, e.g., complete or partial trisomy, intrachromosomal, and extrachromosomal amplifications. (**e**) Copy number loss includes copy number neutral loss of heterozygosity (LOH), submicroscopic, or large-scale deletions

3.3.2 Evading Immune Destruction

The immune system plays an important role in eliminating both micrometastases and late stage tumors [1]. Studies on mouse models have revealed that deficiencies of NK and T cells result in a higher risk of developing cancer, which suggests that both the adaptive and innate immune systems serve as barriers to tumor progression [7, 8]. Tumor cells must therefore be able to evade immunological killing in order to thrive. This can be achieved, e.g., by altering immunological reactions by secreting immunosuppressive factors such as TGF-β [9].

3.3.3 Proliferative Signaling and Reprogramming Energy Metabolism

Proliferative signaling has been recognized as one of the earliest and most important steps in tumorigenesis [1]. It is mediated in large by growth factor ligands that bind to cell-surface receptors (frequently tyrosine kinase domains), which mediate proliferative signals, such as progression through the cell cycle, growth, survival, and energy metabolism [1]. By altering the proliferative signaling network, neoplastic cells become independent of their environment and are placed in a continuous state of "on." The genes involved in growth signaling are referred to as oncogenes. Recently, new light has been shed on the adjustments of energy metabolism to suit the rapid growth of cancer cells [1]. The energy metabolism of cancer cells has been demonstrated to be different compared to normal cells; by using aerobic glycolysis tumor cells can increase their uptake and utilization of glucose, which has been documented in many tumor types and is utilized to visualize tumor dissemination in PET-scan examination [10].

3.3.4 Resisting Growth Suppressors

Apart from activating proliferation, tumor cells must also be able to avoid signaling pathways that negatively regulate cell proliferation. The

genes controlling progression in the cell cycle and inhibitory signaling are commonly referred to as tumor suppressor genes. One of the most common suppressor genes is the *RB1* gene. This gene is mutated in a wide range of tumors [11]. Individuals with germline mutations of *RB1* suffer a great risk of developing bilateral retinoblastoma at young age, as well as several other tumor types. In normal cells, cell-to-cell contact mediates suppression of growth [1]. If normal cells lose the cell-to-cell contact, they undergo programmed cell death. This inhibitory signal is often lost in tumor cells. A set of cell-surface adhesion molecules, most notably the cadherins (E-cadherin and N-cadherin), are commonly altered in tumor cells enabling the tumor cell to let go and then reattach, thus serving as an important step in metastatic spread [12, 13].

3.3.5 Escaping Cell Death

Apoptosis is triggered by, e.g., environmental stress, elevated levels of oncogene signaling, and loss of attachment to other cells and functions as a guardian against malignant genetic change [14]. By escaping programmed cell death, tumor cells manage to survive even though they harbor multiple genetic changes.

3.3.6 Replicative Immortality

Normal cells are only able to replicate a limited number of times before they enter either senescence or cell death. Telomeres and telomerase protect the ends of chromosomes and are intimately involved in replicative immortality [1]. While telomerase is barely expressed in normal cells, its overexpression in malignancies allows the cells to escape destruction [4, 15].

3.3.7 Angiogenesis

Tumors must acquire new vasculature by angiogenesis to be able to grow beyond a size of approximately 2 mm^2 (Chap. 4).

3.3.8 Invasion and Metastasis

Tumor metastases account for approximately 90 % of tumor-related death [16]. By pinpointing the processes underlying metastasis, greater understanding and treatment options will emerge [17]. The metastatic process is thought to start with individual tumor cells dislodging from the primary tumor and spreading by blood or lymphatic vessels. The timing of tumor dissemination remains a subject of debate. There are two major models: the linear progression model where a cell acquires a set of characteristics through stepwise alteration before dissemination and the parallel progression model where cells that are not yet fully neoplastic are circulating, suggesting that they are disseminated from an early malignant lesion [18, 19]. The invasion-metastasis cascade is a complex, multistep process where tumor cells must be able to invade locally through the extracellular matrix and stromal cell layers. The precisely organized architecture of surrounding normal epithelium serves as an effective barrier, which is overcome by invading tumor cells when the metastatic process starts [17]. The matrix metalloproteinases secreted by macrophages at the tumor periphery contribute to the loss of the basement membrane by proteolysis, which facilitates invasion of the stromal compartment [17, 20]. Invading cells must also become motile to escape the primary tumor; this is achieved by alterations in cell-surface proteins that promote migration. Alteration of the cytoskeleton allows for movement along the extracellular matrix and surface of other cells. Further, the Ras family of GTPases alters actin and myosin activity, which promotes movement [1].

After leaving the primary tumor and invading the stroma, the tumor cells must intravasate into blood or lymph vessels. Intravasation into blood vessels is enabled because malignant blood vessels have insufficient pericyte coverage and the interaction is weak between adjacent endothelial cells [17]. Acquiring an intrinsic vasculature is important for primary tumors in order to metastasize and for metastases to grow beyond a certain size. Indirect evidence for this has been obtained

in uveal melanoma (and several other tumors) as tumor vessel counts have been associated with a poor prognosis [21]. Following intravasation, the tumor cells must survive the detachment from the supporting tumor matrix, the hemodynamic forces in the circulation, as well as the hostile immune system. The process of extravasation begins when the cell attaches to the vessel wall in the target tissue. This is achieved either by a tumor forming in the vessel wall, which eventually ruptures the vessel wall, or by penetrating the endothelial cells and pericyte layers and thereby establishing micrometastasis (Box 3.1) [22]. Tumors originating within the eye undergo hematogenous dissemination, because there is no lymphatic drainage. Tumors originating in the orbit or eyelids may undergo either hematogenous or lymphatic spread, depending on the tumor type.

Box 3.1: Steps in Metastatic Process

- Tumor invasion of the vasculature or lymph vessels
- Tumor cell survival in the circulation
- Cellular extravasation
- Establish a metastasis at a distant site
- Acquiring an intrinsic vasculature

Little is known about the predilection for particular metastatic targets, but two major theories have emerged: the mechanistic theory where tumor cells arrest within capillary beds due to size restrictions of the capillary vessels. The other theory describes receptor-ligand binding between tumor cells and capillaries, also known as the "seed and soil" theory: the provision of a fertile environment in which compatible tumor cells can grow [23]. Most likely the combination of both theories is true for the majority of metastatic malignancies. Some microenvironments seem to be more hospitable than others, exemplified by preferential dissemination by a wide range of different tumor types to the liver, bone marrow, and lung tissue. Extravasation of tumor cells is more challenging than intravasation since intravasation occurs at the primary tumor site where the vasculature is already quite leaky and therefore easy to traverse [17]. After intravasation the cancer cells must be able to adapt to a very different environment from that of the primary tumor. The process of metastasis is very inefficient. Indeed, it has been suggested that only <0.01 % of tumor cells that enter the circulation go through the metastatic process to establish clinical metastasis [24]. Even if the tumor cells initially survive in the new microenvironment, it is not certain that they will grow rapidly. On the contrary, most micrometastasis seems to go through a state of dormancy. This may be because of incompatibilities with the foreign environment; it may also be that the cells proliferate, though a net increase does not occur because of the counteracting effects of a high apoptotic rate [17]. An explanation of the high cell turnover and dormancy may be the failure to form new vessels [24].

3.3.9 The Tumor Microenvironment

Tumors are rarely isolated masses of homogenous proliferating cells, but rather a makeup of different tumor subpopulations as well as tumor-associated stroma, normal tissue, and infiltrating lymphocytes. Recent studies have shed light on the importance of recruitment of normal cells, which play a distinct role in the development of neoplasms, and also have been implicated in processes such as drug resistance [25]. The stroma surrounding the tumor is frequently reactive and shares similar upregulated pathways, e.g., chronic inflammation or wound healing tissue [26]. The tumor-associated stroma is induced by the neoplasm, and in turn, the stromal cells may increase the aggressive behavior of the tumor by releasing growth stimulating factors [17, 27]. Other incidents in the tumor microenvironment may increase the reactivity of the tumor surrounding stroma further. As an example, necrotic cell death releases pro-inflammatory signals into the surrounding microenvironment, which recruits inflammatory cells [26]. These cells may promote angiogenesis, cancer cell proliferation, and invasiveness [28]. Necrosis may

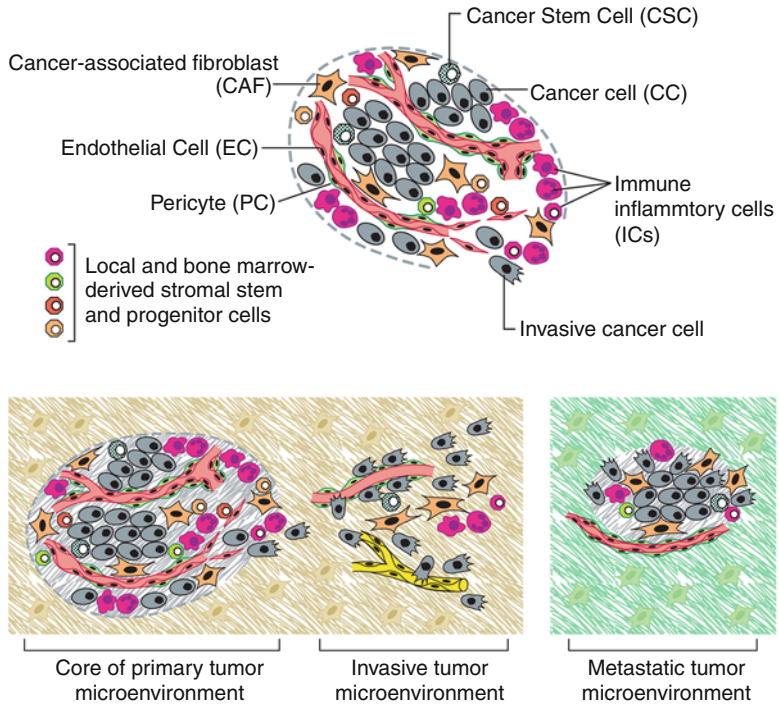

Fig. 3.2 The cells of the tumor microenvironment. *Upper:* An assemblage of distinct cell types constitutes most solid tumors. Both the parenchyma and stroma of tumors contain distinct cell types and subtypes that collectively enable tumor growth and progression. Notably, the immune inflammatory cells present in tumors can include both tumor-promoting as well as tumor-killing subclasses. *Lower*: The distinctive microenvironments of tumors. The multiple stromal cell types create a succession of tumor microenvironments that change as tumors invade normal tissue and thereafter seed and colonize distant tissues. The abundance, histologic organization, and phenotypic characteristics of the stromal cell types, as well as of the extracellular matrix (*hatched background*), evolve during progression, thereby enabling primary, invasive, and then metastatic growth. The surrounding normal cells of the primary and metastatic sites, shown only schematically, likely also affect the character of the various neoplastic microenvironments (Reprinted from Hallmarks of Cancer: The Next Generation, 144, Douglas Hanahan, Robert A. Weinberg, Page No 662, Copyright (2012), with permission from Elsevier [1])

therefore act tumor promoting; indeed tumors with necrosis are associated with worse clinical prognosis.

Solid tumors constitute a mixture of different tumor clones at different stages of development, which lead to genetic heterogeneity [29]. The existence of cancer stem cells as an important composition of the proliferating neoplasm has been a source of ongoing discussion (Fig. 3.2). Cancer stem cells are defined by their ability to seed new tumors when inoculated in host mice [30]. They have been suggested as the culprits of acquired chemotherapy resistance as well as disease recurrence after successful debulking of primary disease where no residual disease can be detected [31].

3.4 Microscopic Features of Neoplasia

Certain histopathologic features differentiate benign and malignant tumors from surrounding normal tissues. In general, the differences from the normal tissue are more marked in malignant tumors than in benign tumors.

3.4.1 Cellular Proliferation

Many malignant tumors feature a large number of dividing cells with abnormal mitotic patterns (Fig. 3.3a). Cell proliferation markers like

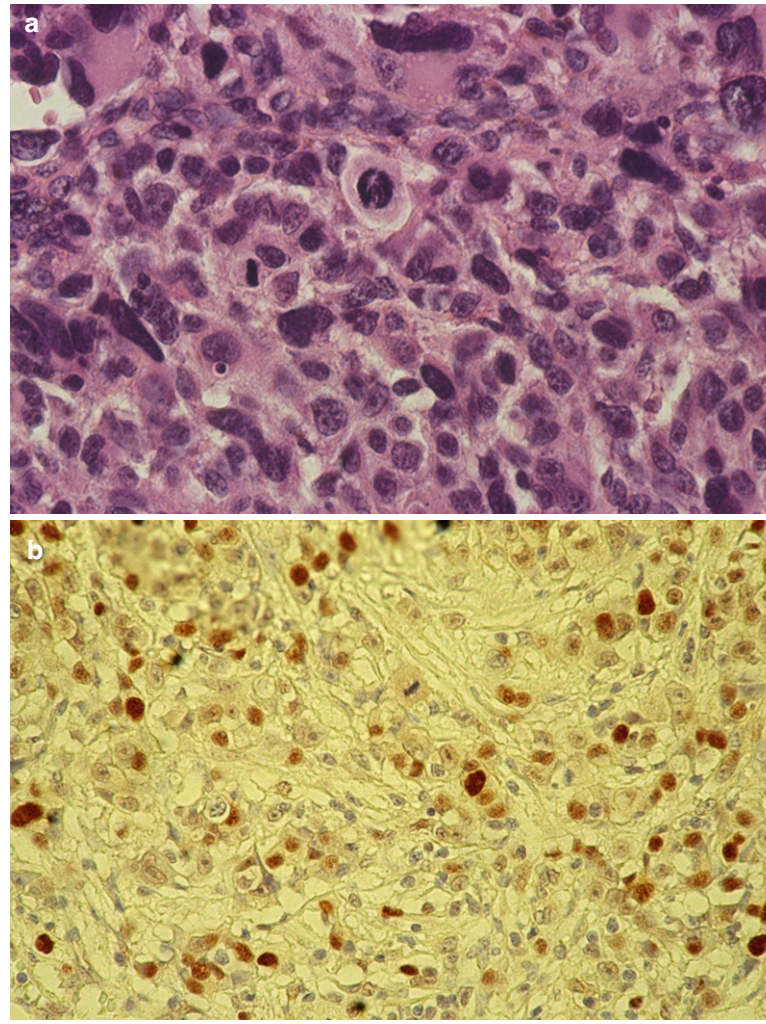

Fig. 3.3 Many malignant tumors feature a large number of dividing cells with abnormal. mitotic figures (**a**). Cell proliferation markers like proliferating cell nucleolar antigen and Ki-67 often reveal a larger proportion of proliferating cancer cells than detected by mitotic counts alone (**b**)

proliferating cell nucleolar antigen (PCNA) and Ki-67 often reveal a larger proportion of proliferating cancer cells than detected by mitotic counts alone (Fig. 3.3b). A high mitotic index (mitotic count per unit of microscopic area) is often found in rapidly growing tumors although this is usually balanced by the presence of many apoptotic cells. Not all cancers show high cell proliferation rates; typically uveal melanoma features comparatively low counts, but nonetheless may metastasize. However, in many cancers, including uveal melanoma, a high cell proliferation rate is associated with poor prognosis [32, 33].

3.4.2 Cellular Pleomorphism

When tissue and cellular architecture is distorted, the tissue is classified as dysplastic. Epithelial dysplasia may be divided into mild, moderate, and severe dysplasia. Severe dysplasia is characterized by full thickness dysplasia with prominent cellular atypia and is synonymous with carcinoma in situ. It is debatable whether a slight or even moderate degree of dysplasia is precancerous, a condition inevitably leading to cancer. When tissue architecture is considerably distorted with individual cells showing a significant degree of variation in shape and size including abnormal nuclei sometimes

featuring binucleate or multinucleate forms, this is referred to as pleomorphism at the cellular level. Cellular pleomorphism, including the extreme state when the tissue of origin is no longer recognizable (anaplasia), is a hallmark of cancer.

3.4.3 Cellular Differentiation

Typically, cancers recapitulate the architecture of the tissue of their origin to some extent. This recapitulation may closely resemble the original structure and such cancers are highly differentiated, whereas others are poorly differentiated or even anaplastic. Usually, poorly differentiated cancers carry a worse prognosis than cancers more closely mimicking the original tissue appearance. In retinoblastoma, the rosettes appearing in moderately and highly differentiated retinoblastoma are believed to be an attempt to recapitulate the original retinal structure (Fig. 3.4a). In uveal melanoma, the tumor spindle cell morphology resembles the original melanocytes, and the presence of less differentiated epithelioid cells is associated with an adverse outcome (Fig. 3.4b, c) [34].

3.4.4 Nuclear Cytoplasmic Ratio

Most neoplastic cells have a relatively large nucleus in relation to the amount of cytoplasm. However, this varies significantly with the type of neoplasia. Clear cell carcinoma of the kidney features large cells with abundant cytoplasm, whereas the retinoblastoma typically is composed of cells with relatively large nuclei and small amounts of cytoplasm.

3.4.5 Invasion of Surrounding Tissues

Tumor cells invade surrounding tissue by direct infiltration or by dissemination along blood or lymphatic vessels. In carcinoma, breakdown of the basement membrane and stromal invasion signify the progression of an in situ carcinoma to invasive carcinoma. Cancers with minimally invasive features are sometimes referred to as microinvasive.

Some malignant tumors show a particular affinity for spread along peripheral nerves extending a considerable distance away from the primary site (e.g., perineural growth in adenocystic carcinoma of the lacrimal gland). In some of these tumors, severe pain due to invasion or compression of sensory nerve fibers may be the first clinical presentation.

3.4.6 Tumor Infiltration by Normal Cells

In some malignant tumors, infiltration by macrophages or lymphocytes is a characteristic feature reflecting recruitment of the immune system as well as physiologic cells by neoplastic tissue. Macrophage infiltration in uveal melanoma is associated with a poor prognosis [35].

3.5 Tissue Sampling and Processing

3.5.1 Cytological Sampling

Sampling typically includes a number of individual cells disrupted from their original tissue. Diagnosis is therefore usually made on the morphological appearance of individual cells because the relationship to surrounding cells is lost. Cytological samples may be used for immunocytochemistry, which is conducted to characterize protein expression, and for auxiliary techniques such as flow cytometry. Two basic techniques are used for sampling.

3.5.1.1 Exfoliative Cytology

Exfoliative cytology involves sampling of cells that are dispersed in fluids (e.g., cerebrospinal fluid sampled by lumbar puncture) or forcibly removed by a spatula, brush, or some other instrument or filter paper. Cells may also be dislodged from a surface by a touch preparation, for example, imprint cytology for conjunctival tumors. The exfoliative sample is then spread on a glass slide and stained. Vitrectomy samples may be filtered though a membrane (e.g., Millipore filter), centrifugated in a pellet (cytospin preparation) or paraffin embedded as a cell block.

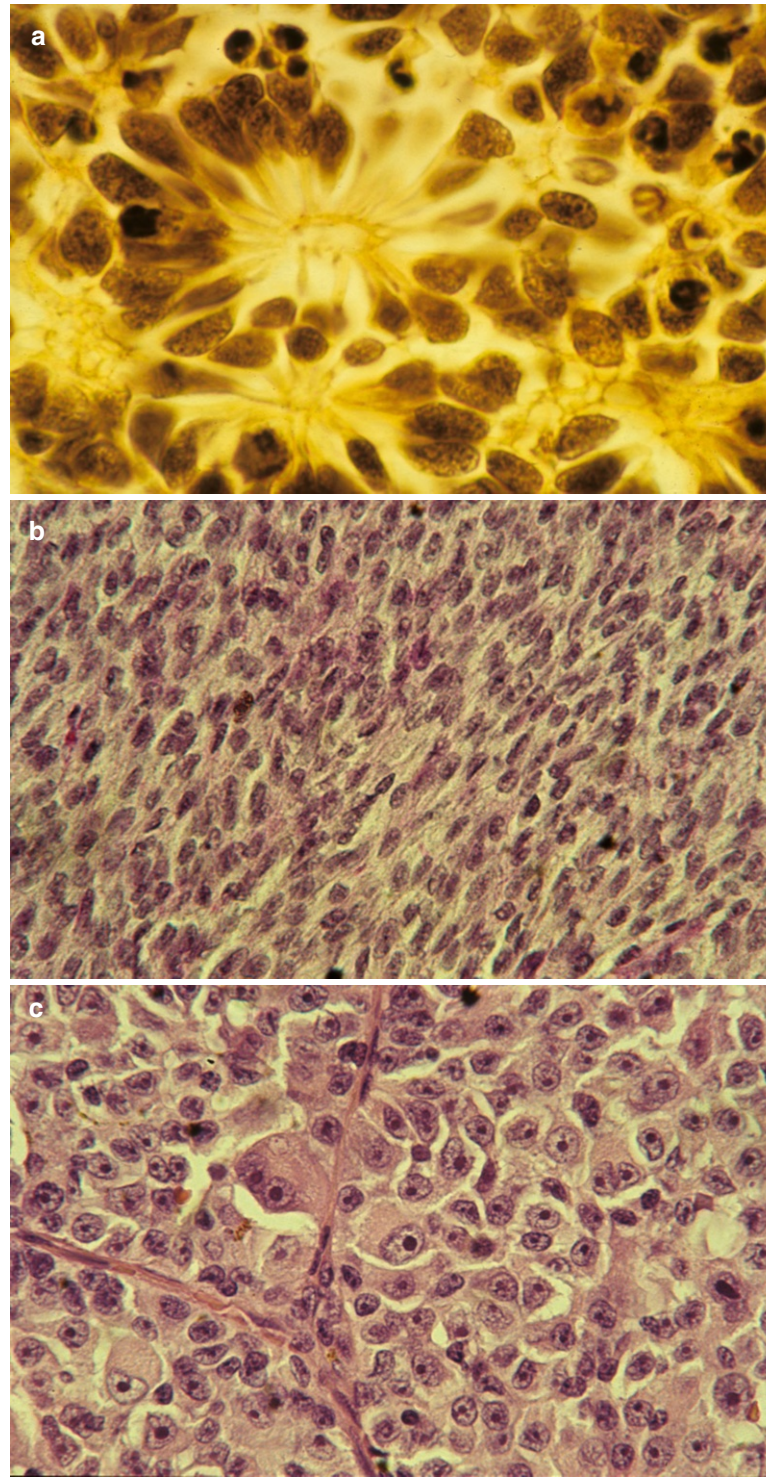

Fig. 3.4 Highly differentiated retinoblastoma with tumor cells arranged in a radiating, flowerlike pattern referred to as Flexner-Wintersteiner rosettes (**a**). Uveal melanoma specimens featuring spindle-type tumor cells (**b**) and cells with epithelioid appearance (**c**)

3.5.1.2 Aspiration Cytology

Aspiration cytology may be applied to palpable lesions, guided by ultrasound or computerized tomography imaging. Intraocular fine-needle aspiration biopsy (FNAB) is performed with needles between 21 and 25 gauges [36]. A pars plana approach guided by indirect ophthalmoscopy may be used, but clear cornea and transscleral routes have also been advocated [37–39]. The various techniques of intraocular biopsy are discussed in detail under uveal tumors. Local tumor spread using FNAB in loosely cohesive tumors is a concern. For this reason, FNAB should only be used with extreme caution and almost never in suspected cases of retinoblastoma. The requirements for tissue handling vary between laboratories; therefore, it is advisable to contact the local cytopathologist before sampling.

3.5.2 Histopathologic Sampling and Processing

Tissue is obtained by incisional or excisional biopsy of the lesion. Caution not to coagulate or otherwise maltreat the tissue sample is recommended. Incisional biopsies or core needle biopsies should be avoided in tumors prone to local recurrence, e.g., the pleomorphic adenoma of the lacrimal gland. In such tumors, primary complete excision or possibly diagnostic FNAB followed by complete excision is recommended. The optimal fixative for the surgical biopsy varies depending on the local setting and the technique used for histopathologic examination, but for most cases, formaldehyde is sufficient.

When biopsying tissues like the conjunctiva or iris, care should be taken to orientate the specimen and to make sure the tissue is maintained flat and does not curl. This can be achieved by attaching the tissue to a piece of filter paper and annotating the specimen mount before immersion in the fixative. Assessment of the surgical margins is pivotal in any excisional tumor biopsy. To facilitate this, the surgical margins may be marked by the pathologist before gross sectioning and paraffin embedding. Also, special techniques like Mohs technique using cryosectioning or modifications using vertical paraffin-embedded sections have been advocated for eyelid and skin tumors [40, 41]. Cryosectioning allows for a rapid assessment (within 30 min) of margins or malignancy and can be used as a preoperative procedure; however, paraffin sections allow a more reliable microscopic assessment. Specific guidelines for histopathology reports on cancer specimens from the eye and adnexa have been discussed elsewhere [42].

3.6 Diagnostic Techniques

Traditionally, cancer diagnosis was made using light microscopic examination, but recent advances in molecular diagnostics have created a completely new set of tools with which tumors may be more accurately diagnosed and better characterized. Some of these techniques are outlined below.

3.6.1 Light Microscopy

When processed for routine light microscopy, samples are usually fixed in formaldehyde and then embedded in paraffin. This allows for the cutting of 3–4 μm sections. Paraffin sections are usually routinely stained with hematoxylin and eosin, although this may differ depending on the local routine (Fig. 3.5a). Other stains like the periodic acid-Schiff dye preferentially stain mucopolysaccharides and glycogen and are appropriate for study of basement membrane material like the lens capsule.

3.6.2 Immunohistochemistry

This technique has rapidly evolved from a research tool to a diagnostic technique and has revolutionized diagnostic pathology. Several commercially available antibodies may be used in combination with routine fixatives like formaldehyde and staining may be enhanced by antigen retrieval techniques (Fig. 3.5b). Fixation over

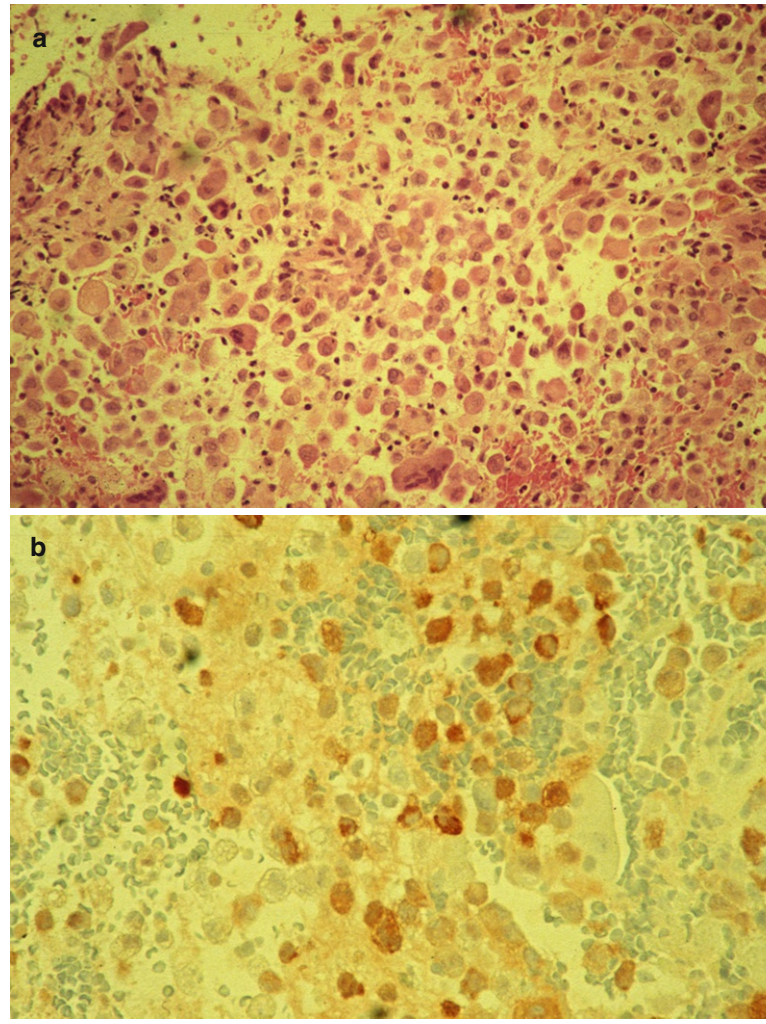

Fig. 3.5 Langerhans' cell histiocytosis. Light microscopy (**a**) and immunohistochemical stain recognizing S-100 protein (**b**)

prolonged periods of time may cause reduced staining and the wary pathologist uses negative and positive controls.

3.6.3 Additional Techniques

Recent advances in molecular pathology have generated numerous techniques for the genetic and epigenetic study of ocular tumors. One of the most important laboratory techniques is the polymerase chain reaction (PCR). Several different types of PCR have been developed, including competitive or real-time PCR, which allows for sensitive assays of gene expression at the RNA level. Further, several experimental laboratory techniques have been translated into clinical use, e.g., flow cytometry in lymphoma and tissue imprints for gene rearrangements in rhabdomyosarcoma. In several cases, tissue needs to be submitted fresh, which requires close collaboration with the examining laboratory for optimal results. Microarrays have revolutionized our understanding of the tumor genome and transcriptome by making it possible to study thousands of genes in one experiment at a single gene resolution. Distinct cancer biomarkers and gene expression profiles have been identified through microarray studies. After vigorous evaluation, several assays have been developed and are now in clinical use. Two of the earliest assays developed include the MammaPrint® and Oncotype DX® assays

[43, 44]. These assays predict prognosis and assess the likely benefit from treatment by analyzing signatures of multiple genes. In uveal melanoma, genome-wide expression profiling has identified two subsets of tumors: nonmetastasizing low-grade tumors and metastasizing high-grade tumors [45]. From these results, a 15-gene assay has been developed, which successfully separates the tumors into two distinct subgroups [12, 46]. Through fine-needle biopsies, the assay can be used to determine metastatic potential and patients can be selected for interventions like adjuvant therapies [47, 48]. Functional analysis of the genes involved in the high-grade profile has allowed identification of upregulated genes and pathways in uveal melanomas. This constitutes an important basis for novel strategies for early tumor identification and application of targeted therapies. Several antibodies have been investigated in ocular tumors, including aflibercept (VEGF-Trap) and ipilimumab (anti-CTLA4) [49, 50]. Ipilimumab is applied in routine clinical treatment, and several drugs are in various stages of clinical investigation (www.clinicaltrials.gov). In line with the findings in microarray studies, proteomics hold a similar promise and generate vast amount of data on protein expression.

Next generation sequencing of the whole genome has become a groundbreaking tool in the study of cancer cells. It is used for the characterization and identification of, e.g., DNA, RNA, exome, and transcriptome sequences of tumor cells. It enables identification of, e.g., copy number variants, sequence variants, mutations, and structural changes such as chromosomal translocations, and fusion genes. Various open source projects aim at collecting sequence variants and mutations critical in the development of human cancers. Public sharing of data has enabled inclusion in larger data sets for reanalysis and construction of databases of cancer profiles.

Described here are only a handful of the techniques developed for research and clinical study of tumors. In the near future many of the techniques used for experimental purposes will delineate central pathways in cancer cells with prognostic, diagnostic, and treatment implications in human cancer.

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Cancer Angiogenesis

4

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4.1 Introduction

Angiogenesis, the growth of new blood vessels from preexisting vasculature, is both a physiologic and pathologic process. The formation of new vessels is important during embryogenesis, wound healing, and in the uterus during the menstrual cycle and pregnancy. Interest in targeting angiogenesis for the treatment of cancer began in the early 1970s, when it was postulated that the formation of new vessels in tumors is essential for tumor growth and metastasis [1]. Subsequently, our improved understanding of the regulation of angiogenesis has revealed an important role in other diseases, such as proliferative diabetic retinopathy and age-related macular degeneration, rheumatoid arthritis, and psoriasis [2]. In this chapter we provide a brief review of mechanism(s) of tumor angiogenesis, current applications in general and more specifically in ophthalmic oncology, and experimental

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_4,

drugs with potential for clinical applications. The final section of this review deals with angiogenic aspects of a few specific ophthalmic tumors.

4.2 Tumor Angiogenesis

Tumorigenesis is a multistep process, caused by mutations in the course of time [3]. Small microscopic tumors can obtain their nutrients and oxygen by diffusion alone. However, with increasing distance from a capillary vessel, oxygen supply is insufficient for the proliferation of tumor cells (Fig. 4.1) [4]. Only those tumors that can recruit new vasculature will grow to a visible size if experimentally implanted into another location. The others remain microscopic in size, without any sign of growth, a phenomenon called "no take."

4.2.1 Cooption

At an early stage, some tumors and metastases can invade healthy vascularized tissue, forming microcylinders up to a diameter of 200 μm around preexisting healthy capillaries, via a mechanism called cooption [5].

4.2.2 Angiogenic Switch

Ultimately, all tumors rely on the formation of new blood vessels to grow to a clinically detectable size. Tumor cells implanted in a rabbit cornea show a low growth rate as long as the cornea remains avascular. With the onset of revascularization, the tumor cells switch to a higher proliferation rate, and the tumor grows rapidly [6]. The same is observed for tumors in the anterior chamber of a rabbit eye. Although revascularization of the iris develops early, the tumors do not grow to a large size until they are in touch with the iris and the newly formed vessels [7]. The ability of a tumor to induce growth of its own vessels usually develops after malignant transformation in response to hypoxia and nutrient deprivation and is called angiogenic switch [8]. However, cervical dysplasia demonstrates an angiogenic phenotype even before definite signs of malignancy are present [9].

4.3 Steps in Angiogenesis

The angiogenic response to a tumor is influenced significantly by tumor environment, which is comprised of multiple cell types [10]. In addition to sprouting angiogenesis from preexisting

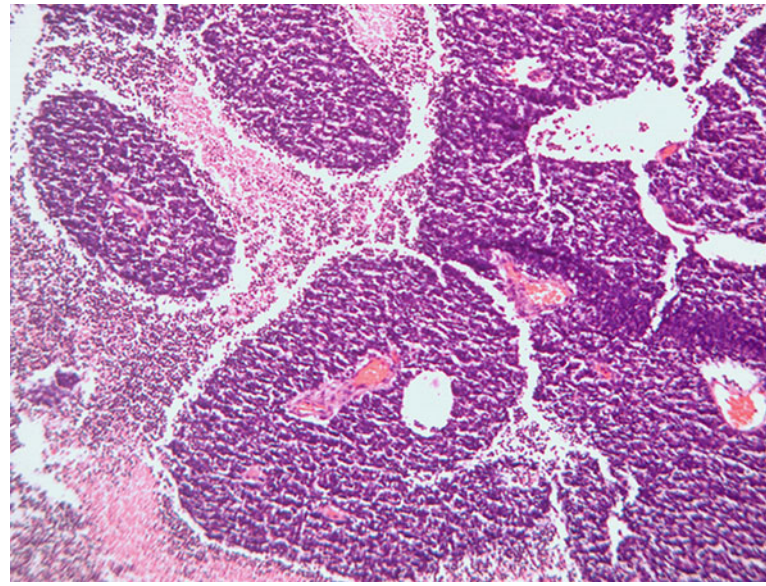

Fig. 4.1 With increasing distance from a capillary vessel, oxygen supply is insufficient for the proliferation of tumor cells. Note viable cells surrounding the central vessel. Areas of tumor necrosis are evident away from the vascular channels (untreated retinoblastoma) (hematoxylin and eosin; original magnification 10×)

vessels, it is now apparent that in tumors, new vessels can originate from cancer stem cells as well as from endothelial progenitor cells recruited from the bone marrow. Angiogenesis begins with vasodilation and an increase in vascular permeability because of dissolution of adherens junctions and detachment of perivascular pericytes with consequent vascular leakage leading to extravasation of plasma [11]. Matrix metalloproteinases degrade the basement membrane and the surrounding extracellular matrix, facilitating the vascular remodeling and amplifying the angiogenic stimulus by releasing angiogenic and inflammatory factors. Proliferating endothelial cells now migrate away from the vessel. The new sprout appears a solid column, until a lumen is formed, either by intracellular vacuolar fusion or by arrangement of cells around a central lumen [12]. The new capillary then builds anastomoses with other capillaries, and the new vessel is stabilized by a new basal membrane and pericytes. Concomitantly with this sprouting response, platelets are recruited to the site of basement membrane degradation and upon activation release angiogenic factors into the tumor microenvironment. Bone-marrow-derived endothelial progenitor cells and myeloid cells are also recruited to this site and release additional factors. In addition, cancer stem cells can differentiate to become endothelial cells or can form vascular tubelike structures without differentiation (a process called vascular mimicry) [13].

4.4 Tumor Vessels

The vascular density in a tumor can be many times the density of normal tissue [14]. However, the vessels remain functionally insufficient. They are disorganized and tortuous and show a chaotic blood flow [15]. Tumor blood vessels are generally leaky and poorly perfused. So despite high vascular density, the tumor remains hypoxic.

4.5 Metastatic Cascade

The metastatic cascade is a series of complex events that allow a tumor to disseminate and grow at a distant secondary site. Tumor cells must first access the circulation to disperse malignant cells into the blood stream (Fig. 4.2). The capillaries in the tumor often lack a proper basement membrane, making it easier for cell to invade the lumen; and the higher the density of vessels within a tumor, the more cells can escape to the circulation [16]. For uveal melanoma, a correlation between tumor cell

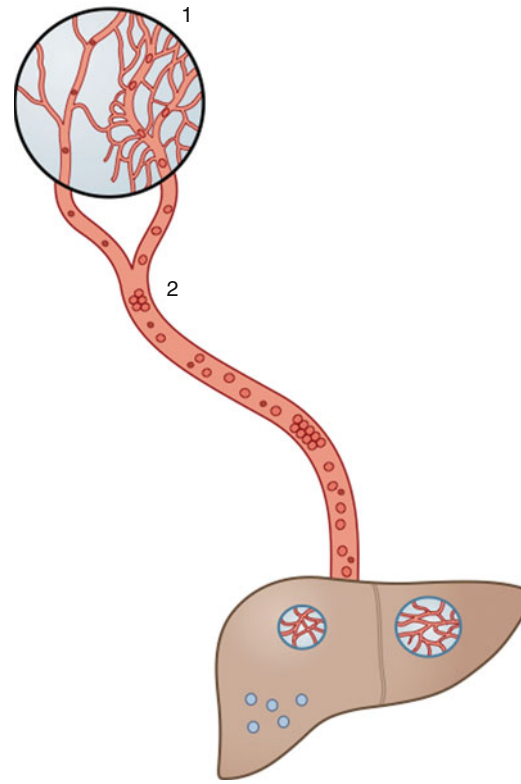

Fig. 4.2 Metastatic cascade. The metastatic cascade begins, when tumor cells escape from the primary solid tumor and enter the general circulation. It seems that this shedding of tumor cells starts at an early stage, and uveal melanoma cells have been detected in the peripheral blood long before metastasis could be seen clinically. (*1*) Different parts of the tumor can show different vascular density, a fact that might contribute to the number of the cells that can give rise to metastasis. However, only very few of those tumor cells can survive in the general circulation. (*2*) Formation of larger cell aggregates, which also include platelets, contributes to an increased survival of tumor cells. At their target site they embolize a capillary or adhere to the vessel wall, pass it, and settle in the new organ. The mechanism behind the organotropism of metastasis is not fully understood. Besides hemodynamics specific properties of the vessels, properties of the endothelium as well as characteristics of the target organ, like the expression of certain growth and chemotactic factors, might be important

ingrowth into blood vessels and the development of distant metastasis has been established [17]. Once the metastatic cells reach their target organ, they start to proliferate to form a secondary tumor under permissive conditions. However, without inducing the growth of new vessels, these secondary tumors will grow slowly and might never reach a size that is clinically detectable. In fact the proliferation rate does not differ significantly in such a dormant metastasis and in growing metastases, rather the total number of cells is balanced by a high rate of apoptosis [18].

4.6 Promoters of Angiogenesis

Angiogenesis can be induced by a variety of factor and conditions. Inflammation, hypoxia, and mechanical factors can induce the production or the release of proangiogenic factors and cytokines. It has also been shown that the products of many oncogenes are proangiogenic and that this angiogenic activity might be one of their mechanisms of action [19].

4.6.1 Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) was discovered in 1989 for its ability to increase vascular permeability and named as vascular permeability factor (VPF) [20]. Since then we have learned that it is a central player in physiologic and pathologic angiogenesis. The lack of a single VEGF gene leads to abnormal blood vessel formation that results in early embryonic death [21].

4.6.1.1 Functions

In vitro and in vivo experiments have demonstrated that VEGF is a strong mitogen and survival factor for endothelial cells, promoting endothelial cell motility and the growth of new blood vessels and lymphatic vessels [22]. The VEGF-induced vasculature consists of a primitive vascular plexus, which is highly fragile and hemorrhagic [23]. In vivo it is physiologically expressed in areas of wound healing, bone growth, and the female reproductive organs [24]. VEGF plays a role in the pathogenesis of retinopathy of prematurity (ROP), choroidal neovascularization in age-related macular degeneration, and neovascularization of the iris [25–27]. It is believed that the modulation of VEGF function would be therapeutic in these diseases.

4.6.1.2 Subtypes (VEGF-A)

The VEGF family of growth factors consists of a least six members binding to different VEGF receptors (VEGFR) on the cell surface. In general terms, when VEGF is mentioned, it is the VEGF-A that is being referred to. VEGF-A is a secreted, freely diffusible glycoprotein, with a half-life time in the circulation of only 3 min. It forms homodimers and binds to VEGFR-1 and VEGFR-2 on the surface of endothelial cells. The gene for VEGF is located on the short arm of chromosome 6 (6p21) [28], and alternative splicing results in the different isoforms of the protein, which are named after the number of their amino acids [29]. VEGF 121, 145, 165, 189, and 206 differ in their affinities to the VEGF receptors and to structures of the extracellular matrix.

4.6.1.3 VEGF Receptors

The function of VEGF is mediated by different cell surface receptors, the VEGF receptors VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4), and neuropilin (NRP-1 and NRP-2) receptors. The VEGFRs are a family of transmembrane receptor tyrosine kinases. They all have an extracellular immunoglobulin-like domain, a lipophilic transmembrane region, and an intracellular domain that includes the tyrosine kinase (TK) [30].

4.6.1.4 Regulation of VEGF

Hypoxia

An important factor for the upregulation of VEGF under physiologic and pathologic conditions is hypoxia. When tumors grow to a larger size, they usually show areas of necrosis, surrounded by underperfused regions, showing upregulation of VEGF [31]. Hypoxia in those regions with low oxygen concentration induces the expression of two hypoxia-inducible factors (HIF-1 and HIF-2) [32]. The-α subunits translocate to the nucleus,

bind to subunit-β and to hypoxia response elements. This induces the expression of VGEF and more than 60 other genes [33]. Under normoxic conditions, however, HIF undergoes rapid degradation. The von Hippel-Lindau (vHL) protein plays a key role in this process by forming an ubiquitination complex and labelling HIF for degradation by the proteasome [34]. If the vHL protein is missing, mutated, or not functional, HIF is not inactivated properly and normoxic tissue behaves like under hypoxic conditions.

Other Factors

Apart from hypoxia several other growth factors can upregulate VEGF expression in vitro and in vivo. Epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), keratinocytic growth factor (KGF), and insulin-like growth factor (IGF) can all induce VEGF in vitro or in vivo, as can prostaglandin $(PGE)_2$, interleukin (IL)-1α, thyroid-stimulating hormone (TSH), and angiotensin II.

4.6.2 Fibroblast Growth Factor

Fibroblast growth factor (FGF) belongs to a family of at least 22 proteins, of which only a few are related to angiogenesis. FGF was the first angiogenic protein to be purified. It has a strong affinity for heparin and heparan sulfate and is stored in the extracellular matrix. Proteases can release FGF, which then can bind to tyrosine kinase FGF receptors on the cell surface and lead to activation of the MAPK pathway by protein kinase C (PKC)-dependent signaling.

The basic fibroblast growth factor (bFGF or FGF-2) and the acid fibroblast growth factor (aFGF or FGF-1) have been shown to induce angiogenesis [35]. FGF is elevated in the serum of cancer patients and also seems to play a role in Kaposi's sarcoma and hemangioma [36].

4.6.3 Angiopoietins

The four members of the angiopoietin family, ang-1, ang-2, ang-3, and ang-4, all bind to another group of receptor tyrosine kinases (RTK), the Tie (Tie-1 and Tie-2) receptors. All the four angiopoietins are known to bind to the Tie-2 receptor. They do not induce proliferation but influence cell migration, sprouting, tube formation, and cell survival. Additionally, Ang-1 reduces vascular leakage induced by VEGF [37]. A ligand for the Tie-1 receptor has not yet been identified. However, Tie-1 deficiency in mice results in severe vascular defects [38]. This suggests that Tie-1 is an important inhibitor of angiogenesis.

4.7 Inhibitors of Angiogenesis

Angiogenesis inhibitors can be classified in two ways based on their mechanism of action and whether they are endogenous or synthetic (pharmaceutical). Based on their mechanism of action, angiogenesis inhibitors can be classified as indirect or direct. *Indirect inhibitors* block endothelial cell stimulation by preventing the release of angiogenic factors by tumor cells, neutralizing the angiogenic factors, or blocking the receptors to which angiogenic factors bind. *Direct inhibitors* block the response of endothelial cells directly, inhibiting their response to any proangiogenic stimulus. As direct inhibition targets the genetically stable endothelial cell instead of the tumor cells, it is less prone to drug resistance and treatment failure.

Angiogenesis inhibitors can also be classified into endogenous and pharmaceutical inhibitors.

4.7.1 Endogenous

4.7.1.1 Thrombospondin

Thrombospondin was the first naturally occurring inhibitor to be identified. It acts indirectly by inhibiting matrix metalloproteinase MMP-9 but also has a direct effect on the endothelial cells. During tumorigenesis it is downregulated by several oncogenes (ras, c-myc, v-src, c-jun), while tumor suppressor genes (p53, PTEN) induce thrombospondin.

4.7.1.2 Angiostatin

Angiostatin is a fragment of plasminogen that is generated from plasminogen by, among others, urokinase plasminogen activator (uPA) and matrix metalloproteinases (MMP). It can be found in the urine and serum, only in the presence of a tumor, and its half-life time in the circulation is approx. 2.5 days, much longer than for VEGF. The effects of angiostatin are very selective for endothelial cells including induction of cell arrest and apoptosis, and it can reduce the number of circulating precursor endothelial cells from bone marrow. This makes angiostatin an interesting candidate for antiangiogenic therapy. It has been successfully used in animal models [39] and has been transfected to uveal melanoma in dogs [40]. As angiostatin levels decrease in the serum after radiation therapy of the primary tumor, additional therapy with angiostatin could be used to prevent accelerated growth of metastatic tumors [41].

4.7.1.3 Endostatin

Like angiostatin, endostatin is a fragment of a larger molecule. Proteolytic enzymes, elastase, cathepsin L, and matrix metalloproteinases cleave an internal fragment of collagen XVIII, generating endostatin. The gene for collagen XVIII is located on chromosome 21, and patients with Down's syndrome show higher levels of endostatin and, interestingly, have a lower overall incidence of solid tumors. Like angiostatin its effects are very specific for endothelial cells, inhibiting many steps in angiogenesis. It inhibits VEGF and matrix metalloproteinases, downregulates c-myc, inhibits cyclin D-1, and promotes endothelial cell apoptosis. Another effect is the decrease of vascular permeability, and reduction of edema could enhance the effect of chemotherapy (see further down). In animal models it shows regression of tumors, sometimes to microscopic lesions, and until now no toxicity and no drug resistance has been observed. Endostatin plays also a critical role in the development of the retinal vessels. In Knobloch syndrome, a deficiency of endostatin, the hyaloid artery of the vitreous fails to regress [42], and endostatin levels are elevated in eyes with proliferative diabetic retinopathy [43]. Phase I clinical trial testing endostatin in advanced solid tumors demonstrated low toxicity and showed reduced blood flow and increased apoptosis of tumor and endothelial cells [44, 45].

4.7.2 Pharmaceutical

4.7.2.1 Anti-VEGF Agents

VEGF is one of the most important factors in angiogenesis and big efforts have been made to block VEGF effects to inhibit angiogenesis. There are direct and indirect ways to inhibit VEGF, which include blocking the VEGF at the mRNA level with small interfering RNA (siRNA); [46] targeting the soluble VEGF ligand with antibodies, antibody fragments, or decoy receptors; blocking the extracellular domain of the VEGF receptors (VEGFR-2); and inhibition of the intracellular signaling pathway by blocking the receptor tyrosine kinase domain (Fig. 4.3) [47].

Bevacizumab (Avastin®)

Bevacizumab is a monoclonal anti-VEGF antibody that binds the free soluble ligand. By doing so, bevacizumab inactivates VEGF and has been shown to reduce the VEGF level. Bevacizumab is currently being used in general oncology combination with chemotherapy and has been shown to improve outcome for previously untreated patients.

Ranibizumab (Lucentis®)

Ranibizumab is an antibody fragment binding with high affinity to VEGF and approved for treatment of age-related macular degeneration (AMD). In a monkey model, it can penetrate through the internal limiting membrane into the subretinal space [48].

Pegaptanib (Macugen®)

Pegaptanib is an aptamer, a small 28-base oligonucleotide that binds to VEGF with very high affinity, and blocks specifically the 165-amino acid isoform of VEGF from binding to its receptor. Like ranibizumab, it is approved used for treatment of AMD.

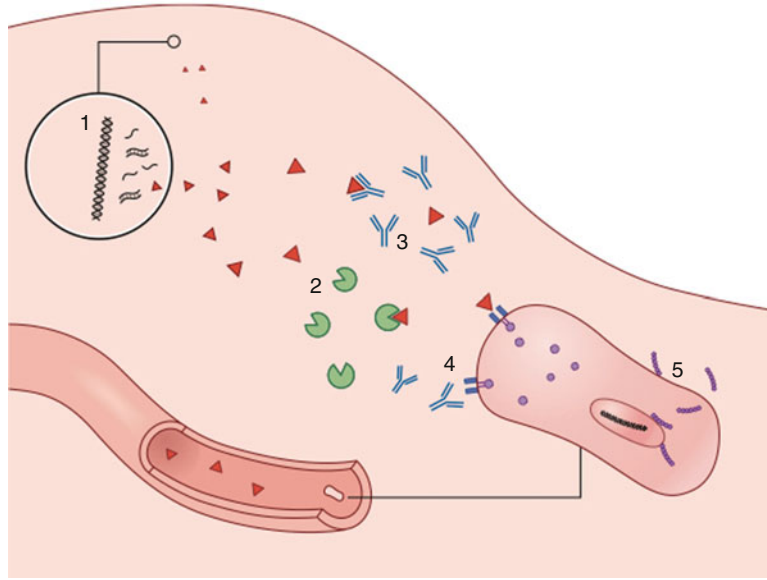

Fig. 4.3 Direct and indirect ways of inhibiting angiogenesis. Antiangiogenic therapy can interfere with proangiogenic stimuli on different levels. (*1*) Antisense oligonucleotides bind to mRNA of proangiogenic factors, thus inhibiting translation by the ribosome and inducing degradation. (*2*) Small molecules, oligonucleotides, and (*3*) antibodies against diffusible circulating angiogenic factors bind and inactivate those factors, before they reach the endothelial cell. (*4*) Antibodies can also bind to the cell surface/transmembrane receptor and prevent stimulation by angiogenic factors. (*5*) Direct inhibitors bind to the cell surface or get internalized, exerting an inhibiting function on their target cells

Aflibercept (VEGF Trap®)

Aflibercept is a fusion protein, consisting of the extracellular parts of the VEGF receptors (1 and 2) bound to the Fc portion of the human immunoglobulin G. It has been approved for neovascular age-related macular degeneration, and macular edema following central retinal vein occlusion, and has shown some beneficial effect in inoperable metastatic cutaneous and uveal melanoma [49].

SU5416, Semaxanib

Semaxanib, SU5416, is a small molecule inhibitor of receptor tyrosine kinases (RTK). As its effect targets more than one receptor, it is called a multitargeted tyrosine kinase inhibitor (TKI). It inhibits the intracellular domain of the VEGF receptor 2 (KDR) but also KiT and Flt-3, two other RTKs that are important in hematopoiesis [50]. SU5416 has no direct cytotoxicity; however, it shows inhibition of tumor growth in animal models for many types of tumors. It has been used with promising results for treatment of central nervous and retinal hemangioma in von Hippel-Lindau disease and also for suppression of choroidal neovascularization [47].

Celecoxib

Celecoxib is a cyclooxygenase 2 (COX 2) inhibitor showing antiangiogenic effects, probably due to a downregulation of endostatin. In a murine model of retinoblastoma and in retinoblastoma cell lines, it has failed to inhibit cell proliferation [51].

Trastuzumab (Herceptin®)

Trastuzumab is a recombinant monoclonal antibody against an epidermal growth factor receptor (EGFR)-related tyrosine kinase,exerting - besides others - some anti-angiogenic effects. The corresponding gene HER-2 (ERBB2/NEU) is over expressed in 20–25 % of breast cancers (primary tumor and metastasis). Though many tumors show progression after 1 year of treatment, Herceptin® could be an alternative to radiation treatment for choroidal metastases.

Interferon α2a

Interferon (INF) α2a was the first angiogenesis inhibitor to be tested in clinical trials. As it works in part by downregulation of bFGF, it shows the best results for tumors that express bFGF. It is used for life- or vision-threatening hemangioma, but because of its neurologic toxicity, it has to be used carefully, and improvements often cannot be seen until 6 weeks of treatment [52]. Angioblastomas and giant-cell tumor of the mandible have also shown to improve after prolonged treatment up to 1 year [53]. Used as an adjuvant treatment for uveal melanoma however, interferon α2b failed to improve survival [54, 55].

4.8 Antiangiogenic Therapy in Clinical Practice

A detailed review of the clinical applications of several commercially available antiangiogenic agents is beyond the scope of this chapter. Moreover, excellent reviews on this topic have been published [56]. Nevertheless, it is important to point out that despite the effects of anti-VEGF therapy on tumor vessels [57], anti-VEGF monotherapy (i.e., without chemotherapy or radiation therapy) has failed to produce improvements in overall patient survival [56]. However, the beneficial effects of prolonging overall survival are apparent when bevacizumab (Avastin®) is added to standard chemotherapy for patients with colorectal and lung cancer and progression-free survival in breast cancer patients [56]. Multitargeted tyrosine kinase inhibitors today are standard first-line and second-line treatment in patients with renal cell carcinoma [58, 59].

The relationship between anti-VEGF therapy and the usual methods of treatment, such as radiation therapy and chemotherapy, is complex and perhaps best explained by the concept of "vascular normalization." [60] It is suggested that anti-VEGF therapy does not only reduce vascular density but induces structural and functional changes in the tumor vasculature that improve cellular oxygenation and perfusion ("normalization") [60]. Improved oxygenation consequently increases the efficacy of radiation therapy and normalized perfusion improves the delivery of chemotherapy. However, bevacizumab has also been shown to increase VEGF expression in vitro and in animal models ("pseudohypoxic phenomenon"), presumably an adaptive tumor response [61]. Theoretical considerations of combining novel anti-VEGF therapies with standard forms of therapy are discussed in detail elsewhere [56].

4.9 Angiogenic Aspects of Specific Ophthalmic Tumors

4.9.1 Retinoblastoma

4.9.1.1 Introduction

Retinoblastoma is the most common primary intraocular malignant tumor in children. The average annual incidence of retinoblastoma in the United States is 10.9 per million for children younger than 5 years [62]. Leukocoria or white pupil and strabismus are the most common presenting signs.

4.9.1.2 Pathogenesis

Retinoblastoma is a genetic disorder with an autosomal dominant inheritance and can be classified as familial or sporadic, bilateral or unilateral, and heritable or nonheritable. The human retinoblastoma susceptibility gene (RB1), a tumor suppressor gene, is located on the long arm of chromosome 13 (region 13–14) [63].

Histopathologic examination shows a highly vascular tumor in which the vessels are cuffed by a layer of viable cells having a mean thickness of 90–100 μm and surrounded by necrotic debris (Fig. 4.4) [64]. The extent of angiogenesis in retinoblastoma tumors may be a predictor of metastatic potential [65]. In a pilot study of patients with unilateral retinoblastoma treated solely by enucleation, it was observed that a tumor's relative vascular area was a better predictor of disease dissemination than either choroidal or optic nerve invasion [65]. Retinoblastoma expresses

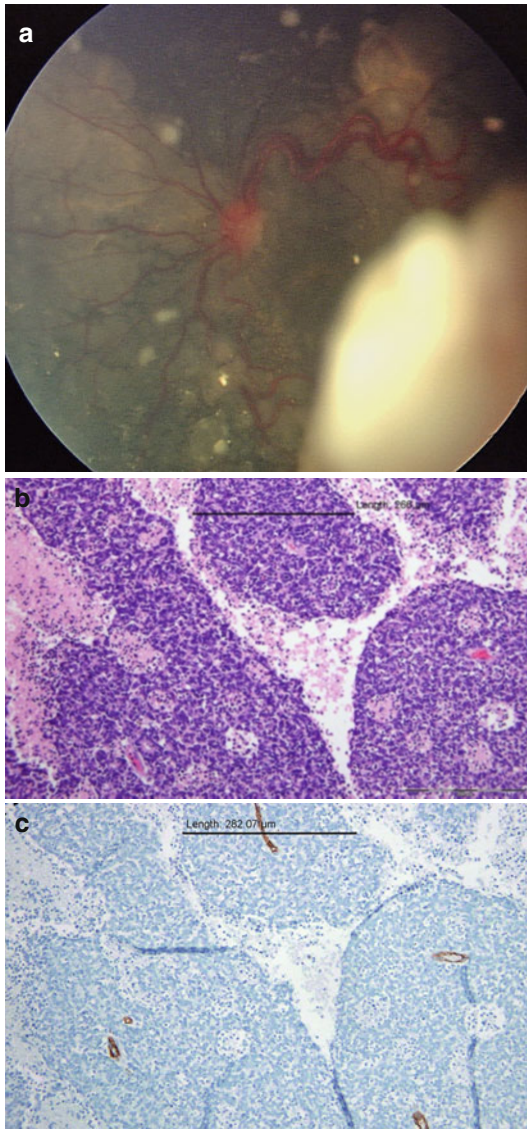

Fig. 4.4 Retinoblastoma. Note prominent feeder vessel (**a**). Vitreous seeds are about 200 µm in diameter, which is the maximum attainable size in absence of intrinsic circulation. Islands of viable tumor cells surrounding a central blood vessel (**b**; hematoxylin and eosin stain; original magnification 10×) and with endothelial stain (**c**; CD 34 stain; original magnification 10×)

large amounts of VEGF [66]. Moreover, retinoblastoma cell lines, which express low levels of VEGF under normal culture conditions, show a strong increase in VEGF secretion under hypoxia, suggesting that focal hypoxia may act as a stimulus for VEGF production in retinoblastoma, which in turn contributes to tumor growth by stimulating angiogenesis [66].

4.9.1.3 Treatment

In recent years there has been a trend away from enucleation, with the increased use of alternative globe conserving methods of treatment including laser photocoagulation, cryotherapy, transpupillary thermotherapy, plaque radiotherapy, external beam radiotherapy, and chemotherapy.

Specific antiangiogenic treatment modalities have not been applied in the clinical routine until now. However, several antiangiogenic drugs have been evaluated for their effects on retinoblastoma in cell cultures and animal models. Combretastatin A-4 phosphate (CA-4P) induces dose-dependent decrease in microvessel density and consequent tumor volume reduction in an animal model of hereditary retinoblastoma [67, 68]. Similarly, anecortave acetate (AA) could induce apoptosis and reduce tumor burden used alone or in combination with glycolytic inhibitors [69, 70]. Bevacizumab has also shown an inhibitory effect on angiogenesis and growth of retinoblastoma [71]. However, the clinical challenge in the management of advanced retinoblastoma is the presence of vitreous seeding, which is not vasculature dependent (Fig. 4.4). Effects on the differentiation of retinoblastoma cells have been demonstrated, however, without direct cell toxicity [72]. Therefore, solely on theoretical grounds, it is unlikely that antiangiogenic therapies will play a major role in management of advanced intraocular disease associated with vitreous seeding.

4.9.2 Uveal Melanoma

4.9.2.1 Introduction

Uveal melanoma is the most common primary intraocular malignant tumor [73]. Even so, melanomas of the ocular and adnexal structures only comprise approximately 5 % of all melanomas [74]. The majority (85 %) of ocular

melanomas are uveal in origin whereas primary eyelid, conjunctival, and orbital melanomas are very rare [73, 74]. Based on anatomic location, uveal melanoma has three types: iris melanoma, ciliary body melanoma, and choroidal melanoma. The diagnosis of uveal melanoma is essentially clinical, based on indirect ophthalmoscopy, angiographic studies, and ultrasonographic pattern. Survival in patients with uveal melanoma is compromised by its tendency to undergo hepatic metastasis.

4.9.2.2 Pathogenesis

Primary Uveal Melanoma

Primary uveal melanoma is a malignant tumor arising from uveal melanocytes. Although, uveal melanocytes and cutaneous melanocytes share a common embryologic origin and some morphologic properties, several significant differences exist between cutaneous and uveal melanomas in terms of the prognostic factors, sites of distant metastasis, and the response of metastatic disease to chemotherapy.

Pseudovascular Channels (Vasculogenic Mimicry)

Among other factors, tumor size, tumor cell type, pseudovascular channels, and presence of cytogenetic changes are significant prognostic factors in uveal melanoma [75, 76]. The pseudovascular channels are identified as PAS-positive loops or networks in histological preparations as well as in vitro studies (Fig. 4.5) [75]. It is now believed that pseudovascular channels are laminin-rich extravascular matrix patterns that conduct plasma [77]. As these channels lack endothelial cells, are independent of angiogenesis, and do not conduct blood cells, the term "vasculogenic mimicry" is used to describe their origin. The angiogenesis-independent supply of the tumor with nutrients and oxygen via those channels might limit the effect of antiangiogenic therapy in the treatment of primary uveal melanoma.

Microvessel Density

There are contradictory reports about the presence [78, 79] or absence [80, 81] of a relationship between microvessel density and prognosis in uveal melanoma. Furthermore, the relationship between microvessel density and pseudovascular patterns is not clearly established [79, 82]. Such conflicting results may be due to variations in the methodology used by different investigators [83].

Tumor Ingrowth into Blood Vessels

Getting access to the systemic circulation is a crucial step at the beginning of the metastatic cascade. Choroidal melanomas arise in a highly vascularized location and show a dense intrinsic vascularity. Intratumoral vessels can be seen on angiography ("double circulation") [84], and their presence has been used as a diagnostic factor in standardized echography [85].

Indocyanine Green Angiographic Patterns

In addition to the prominent intrinsic tumor vessels visible on angiography ("double circulation"), small microcirculatory patterns have been identified and shown to be correlated with the risk of growth (malignant transformation) of a small choroidal melanocytic lesion [86, 87]. However, there are different interpretations of the nature of the microcirculatory patterns observed with ICG. One group believes that these represent pseudovascular channels [86, 88], whereas the other believes that they represent microcirculatory patterns derived from true vessels [87]. The latter view is compatible with the concept of "angiogenic switch" outlined above.

VEGF Expression

Despite an initial study indicating a lack of over expression of VEGF in uveal melanoma [66], subsequent studies have indicated significant over expression of not only VEGF [89–91] but also of βFGF in uveal melanoma [90]. However, there seems to be no correlation between level of VEGF expression and tumor microvascular density [91], 6p21 region copy number (locus of the VEGF gene) [92], and metastasis [91]. Tumor necrosis [91] and previous radiotherapy [93] correlate with the level of VEGF expression in uveal melanoma. Overall, βFGF expression is more frequently observed than VEGF expression in uveal melanoma [90]. VEGF is also found in the vitreous and aqueous humor of eyes harboring

choroidal melanoma [94], and vitreous level correlates with tumor size [95].

Metastatic Uveal Melanoma

Besides the growth of the primary tumor, angiogenesis also plays an important role in the growth of metastasis. Of the four clinical patterns of presentation of metastases (early metastasis, simultaneous metastasis, occult primary, long-term dormancy) [2] uveal melanomas seem typically to manifest a pattern of long-term dormancy [96]. Different explanations for the different patterns have been given including accumulating genetic mutations over time, changes in immunologic surveillance, and hormonal stimuli. However, with growing insight into the mechanisms of angiogenesis, an explanation based on a balance of proangiogenic and antiangiogenic factors becomes persuasive [18]. The lack of angiogenic switch can explain the long-term dormancy of micrometastasis. Production of antiangiogenic factors such as thrombospondin, angiostatin, and

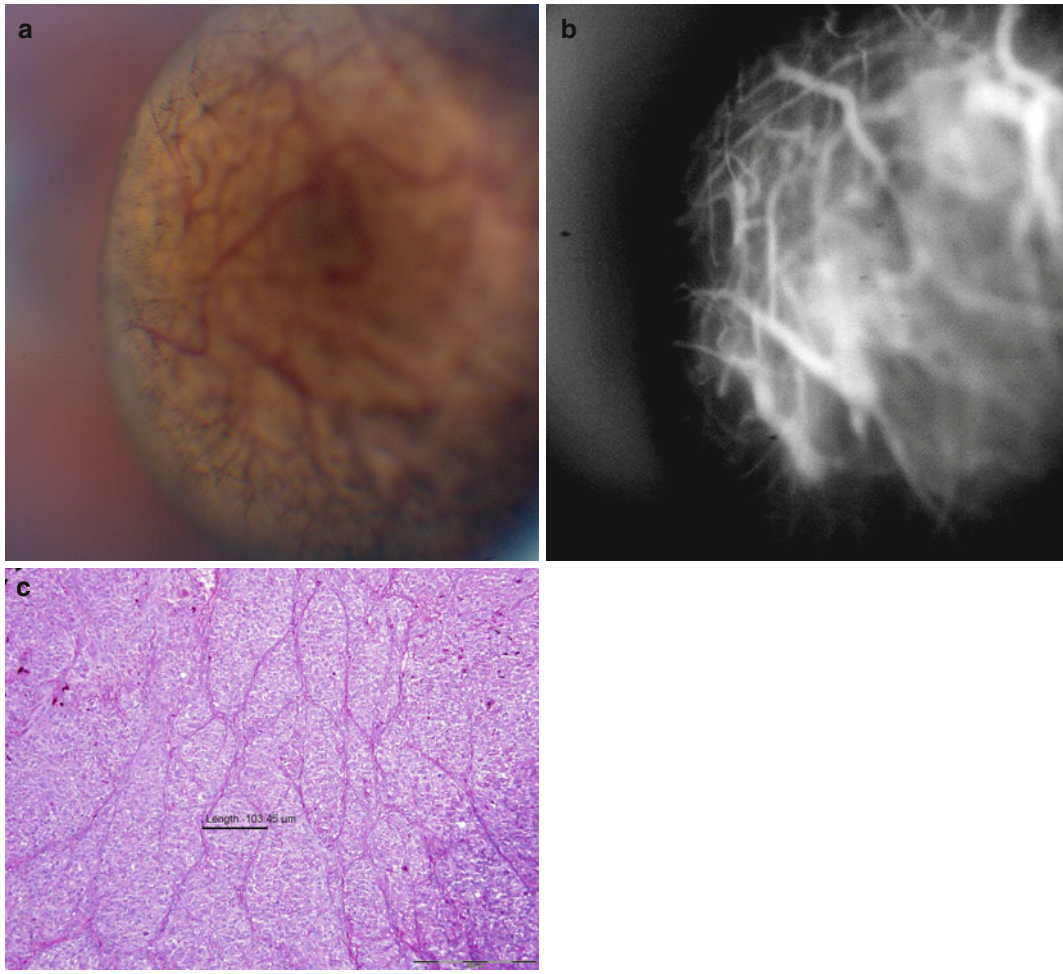

Fig. 4.5 Uveal melanoma. Intrinsic vessels within an amelanotic tumor (**a**). The abnormal choroidal vessels are best visualized with indocyanine angiography (**b**). Pseudovascular patterns are discernible (**c**; PAS stain; original magnification 20×). These "channels" are not lined by endothelium as shown by absence of staining with CD 34 (**d**; original magnification 20×). Note the presence of true tumor vessels that are stained brown. The exact nature and role of these "channels" is still investigational but they do not contain collagen (**e**; Masson trichrome stain; original magnification 20×)

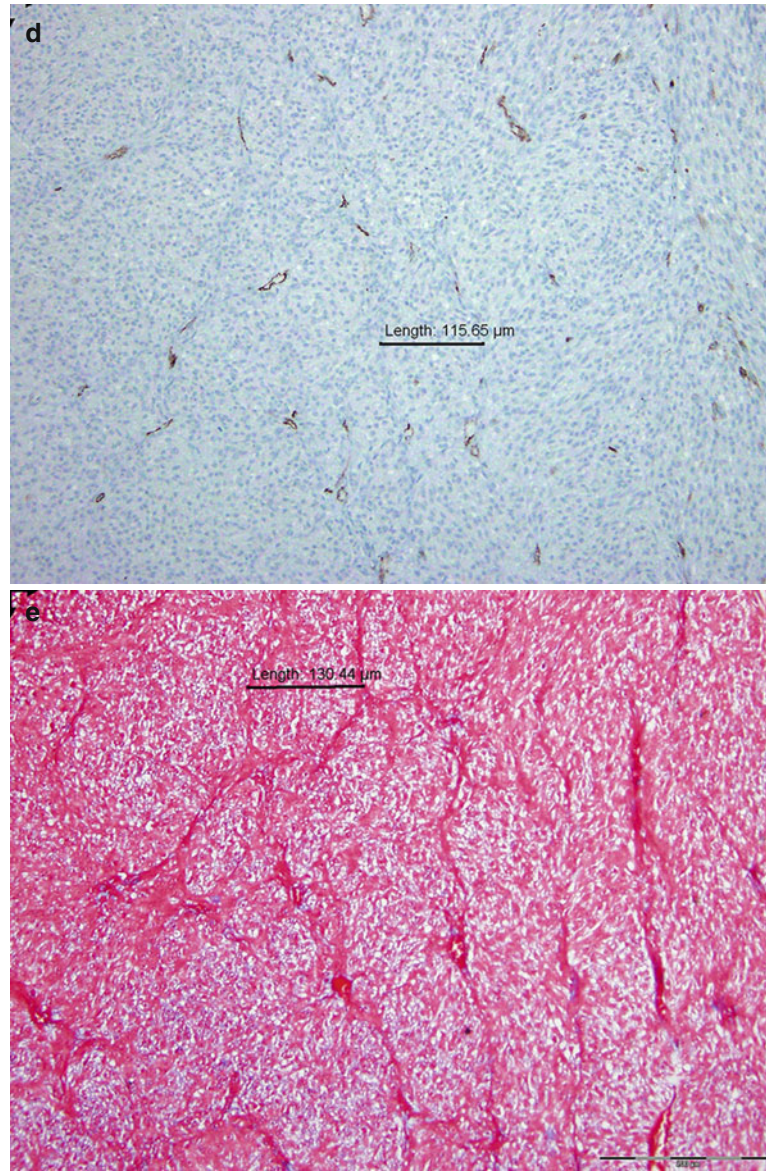

Fig. 4.5 (continued)

endostatin by the primary tumor keeps metastatic tumors at a small size [97, 98]. Because these antiangiogenic factors have a longer half-life time than the proangiogenic stimuli, their effects on metastases are stronger. With the removal of the primary tumor, the proangiogenic stimuli predominate, resulting in growth of metastasis [99].

Although such a pattern of post-enucleation rise in mortality with uveal melanoma was attributed to the shedding of cells (Zimmerman-Mclean-Foster Hypothesis) [100], recent critical appraisal of the published evidence confirms such a trend in mortality but does not attribute it to mechanical shedding of the cells [101]. Rather, alternate explanations based on cell-doubling models have been put forward [101–103]. Additional investigations are necessary to explain clinically observed relationship between primary and metastatic uveal melanoma.

4.9.2.3 Treatment

Primary Uveal Melanoma

The treatment of primary uveal melanoma is essentially surgical or radiational. In a murine model of uveal melanoma, topical application of 1 % anecortave acetate (angiostatic agent) significantly slowed the tumor growth [104]. However, accidental use of bevacizumab in three reported cases did not lead to growth arrest [105]. More so, an animal model even showed acceleration of tumor growth after addition of bevacizumab [61].

Radiation Retinopathy

Another potential application of antiangiogenic therapy is in the treatment of radiation retinopathy, which today is essentially untreatable over the long term (Chap. 11). Recent reports suggest encouraging responses of radiation-induced iris neovascularization and radiation retinopathy to intravitreal injections of bevacizumab (Avastin®) and ranibizumab (Lucentis®), which however need to be repeated over long term [106–108].

Metastatic Uveal Melanoma

The COMS data indicate approximate melanoma-related mortality at 5 years of 1, 10, and 25 % for small, medium, and large choroidal melanoma [109]. The chemotherapy regimens currently used in the treatment of metastatic cutaneous melanoma are ineffective against metastatic uveal melanoma [110]. Sorafenib, a tyrosine kinase inhibitor targeting multiple receptors (VEGF and PDGF receptors, c-Kit and Flt-3), is currently being evaluated alone and in combination with fotemustine. Sunitinib, another tyrosine kinase inhibitor, has also shown clinical benefit as is evaluated in clinical trials [111]. Intravenous aflibercept (VEGF Trap) also improved survival in metastatic cutaneous and uveal melanoma; however, it showed significant toxicity [49].

4.9.3 Von Hippel-Lindau Disease

4.9.3.1 Introduction

Clinical, statistical, and genetic studies of von Hippel-Lindau disease (VHL) have suggested that tumor formation in VHL disease follows the "two-hit model" initially hypothesized by Knudson for retinoblastoma (Chap. 6) [112, 113]. According to this model, in inheritable cases, one allele is constitutionally inactivated (first hit) and the second allele is inactivated in specific tissues, (second hit) whereas in acquired cases, both alleles are inactivated in the specific tissues.

4.9.3.2 Pathogenesis

The *VHL* gene is represented in 3 exons contained within a 20 kb region at chromosome 3p25-26 [114]. Patients with VHL disease show an overexpression of hypoxia-inducible genes. The role of VEGF, as one of the upregulated genes, in retinal hemangioma, CNS hemangioblastoma, renal cell carcinoma, and pheochromocytoma in vHL syndrome, is well established (Fig. 4.6) [115].

4.9.3.3 Treatment

Retinal capillary hemangioma is usually treated with laser photocoagulation and cryotherapy. In cases not amenable to usual therapies, systemic use of SU5416 (Semaxanib) and bevacizumab (Avastin®) has been described [116–118]. In the reported cases, there was stabilization of the lesion, reduction of retinal edema, and improved visual function. SU5416 has also been used for CNS hemangioblastomas in small series of patients [119]. For renal cell carcinoma in VHL, bevacizumab has been replaced by sunitinib (Sutent®) and sorafenib (Nexavar®) as standard first-line and second-line treatment [59].

4.9.4 Hemangioma of the Eyelid

4.9.4.1 Introduction

Infantile hemangiomas are common and are present in 1 % of neonates. Eyelid and orbit are frequent sites of involvement and because of their location they may cause amblyopia and astigmatism (Fig. 4.7).

4.9.4.2 Pathogenesis

These tumors rapidly grow in the first year of life, slowly grow over the next 5 years, and eventually regress by age 10–15 years. The tumors are composed of endothelial cells, endothelial progenitor

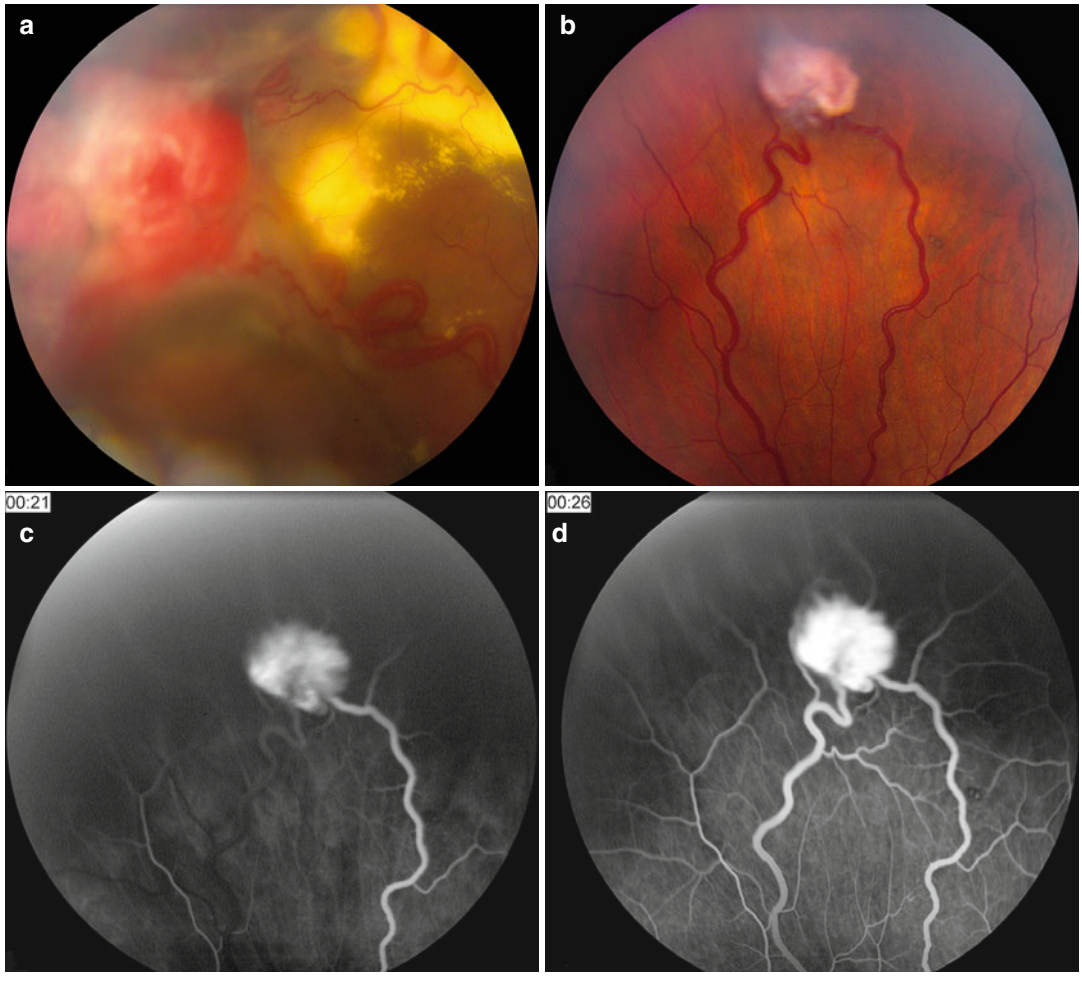

Fig. 4.6 Retinal capillary hemangioma. Ophthalmoscopic appearance of a large hemangioma with retinal exudation and detachment (**a**). Note prominent feeder vessels (**b**). Fluorescein angiogram initially fills the supplying artery (**c**) and the draining vein fluoresces few seconds later (**d**)

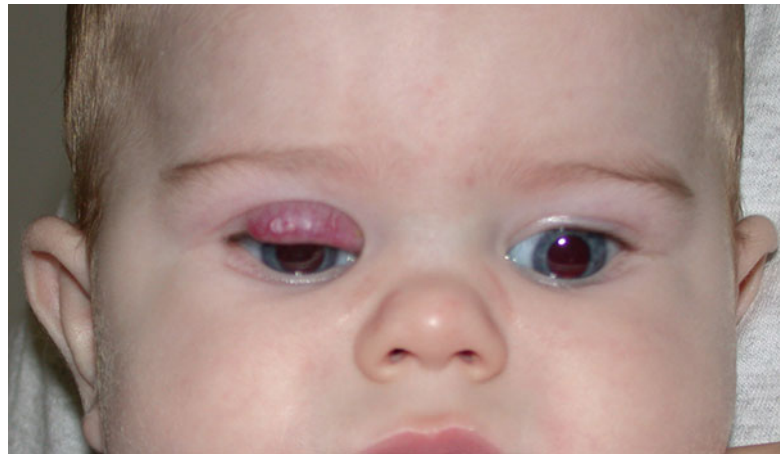

Fig. 4.7 Capillary hemangioma of the eyelid. Courtesy: Elias Traboulsi, MD, Cleveland, Ohio

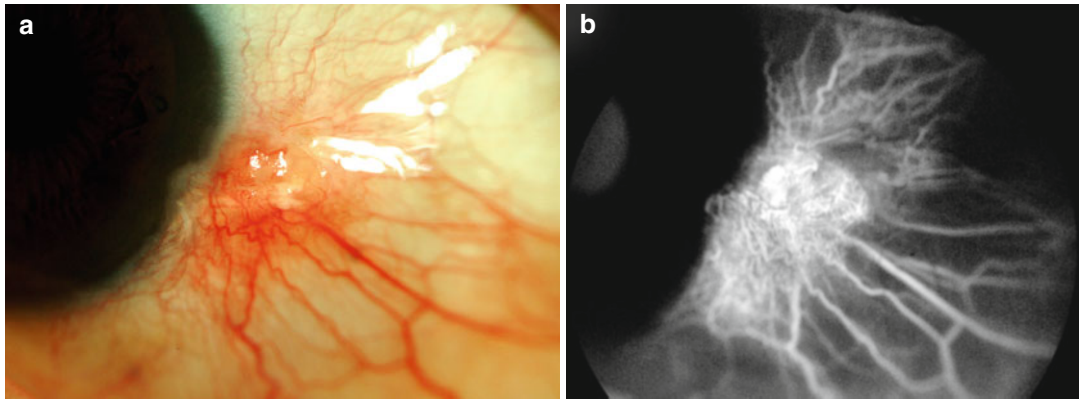

Fig. 4.8 Squamous cell carcinoma of the conjunctiva. Clinical appearance with prominent feeder and intrinsic vessels (**a**). The vessels are best visualized with fluorescein angiography (**b**)

cells, and perivascular and hematopoietic cells that respond to various proangiogenic and antiangiogenic factors [120].

4.9.4.3 Treatment

Steroids (oral or intralesional) are the most frequently prescribed method of treatment [121]. Intralesional bevacizumab has successfully been used to shrink a cutaneous capillary hemangioma of the lower lid after unsuccessful steroid injection in an adult [122]. Life-threatening hemangiomas have responded well to interferon α2a therapy [123, 124]. In a series of 15 children with sight-threatening hemangioma unresponsive to steroid that were treated with antiangiogenic interferon alfa-2b (3 million units/m^2 subcutaneously daily for 3 months), there was a significant regression of the hemangioma in all patients that had completed treatment [52]. Moreover, interferon alfa-2b was well tolerated by all children. Similar observations for capillary hemangioma extending into the orbit and pharynx have also been reported [125]. More recently, regression of infantile hemangioma with propranolol has been observed [126–128]. However, the mechanism of this regression, possibly through blocking of proangiogenic signals (VEGF, basic fibroblast growth factor, matrix metalloproteinase), is not well understood [127].

4.9.5 Conjunctival Squamous Cell Carcinoma

4.9.5.1 Introduction

Squamous cell carcinoma (SCC) is the most common malignancy of the conjunctiva in the United States (Fig. 4.8) [129]. Most squamous tumors originate from the interpalpebral limbus and involve both the conjunctiva and the cornea.

4.9.5.2 Pathogenesis

Ultraviolet-B light exposure has consistently emerged as a major etiologic factor [130]. Human papillomavirus infection (subtypes 16 and 18) has been associated with SCC but is probably not an independent cause of conjunctival neoplasia [131]. Systemic immunosuppression, as seen in acquired immune deficiency syndrome, is also a risk factor [132].

4.9.5.3 Treatment

Management is influenced primarily by the extent of the lesion. Surgical excision with 2–3 mm margins has been the preferred approach for localized disease. Application of intraoperative supplemental cryotherapy (double freeze-thaw cycles) to conjunctival margins reduces the risk of tumor recurrence [133]. Topical chemotherapy has been advocated as an adjuvant for incomplete excision

of large and diffuse primary or recurrent tumors [134]. While mitomycin-C is the best-studied of the chemotherapeutic agents, other topical agents including interferon alpha-2b have also been investigated [135, 136]. The antineoplastic effects of interferon alpha-2b are due to direct antiproliferative effect, indirect antiangiogenic effects, and immune-mediated effects [137]. In recurrent multifocal conjunctival carcinoma, repeated subconjunctival injections of ranibizumab induced (partial) regression in the majority of cases over a mean follow-up of 19 months [138].

Conclusions

Advances in technology offer possibilities for designing pharmaceutical agents that specifically target cellular pathways. Angiogenesis represents one important such pathway. Optimal clinical application and the efficacy of angiogenic inhibitors are currently under investigation. Treatment with angiogenic inhibitors already has a broad spectrum of applications in the management of intraocular and adnexal tumors, which is expected to expand.

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Immunology of Ocular Tumors

5

Martine J. Jager and Inge H.G. Bronkhorst

Contents

5.1 Introduction

Understanding the fundamental immunological processes that play a role in tumor development will help us to better understand how to develop safe and efficient immune intervention strategies against ocular tumors and their metastases. Antitumor immune responses by T cells and antibodies may limit tumor growth, while on the other hand, the presence of tumor-infiltrating leukocytes may lead to the production of cytokines that stimulate tumor growth. In addition, environmental factors may have an influence on the tumor, as may aging, because aging is associated with a phenomenon known as para-inflammation, which is a local increase in infiltrating leukocytes and cytokines.

5.2 Innate and Specific Immune Responses

One can differentiate between basic general immune responses and induced specific ones. The most primitive and direct defense against microbes, trauma, or abnormal cells is the innate immune response. As soon as "danger" is encountered, granulocytes and macrophages are alerted and will try to inactivate the danger. If this response is inadequate, antigen-presenting cells will pick up parts of the microbes or abnormal cells and take them to the regional lymph node. Proteins of the microbe or the abnormal cells are degraded into peptides, which will be presented

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_5, © Springer-Verlag Berlin Heidelberg 2014

by the antigen-presenting cell to T lymphocytes. If the appropriate T cell encounters its own antigen, it will proliferate and become an activated T cell. There are different kinds of T cells, such as T cells that can kill (cytotoxic T cells, CTLs), those that can cause local inflammation (delayed-type hypersensitivity T cells), and regulatory T cells that are able to suppress other T cells.

Furthermore, T cells are necessary to stimulate B cells to begin the production of specific antibodies. Finally, the different T and B cells will try to deal with the aggressor, and the end result is usually that the body takes care of the "danger". If not, the lack of an effective response may lead to the death of the host.

5.3 Immunological Escape

It appears that the immune responses that do exist are limited in their effectiveness inside the eye, which is known to be an immunologically privileged site. This implies that, similar to the brain and the placenta, many immunological reactions are actively or passively downregulated, leading to the suppression of effective immunity. The development of effective antitumor antibodies or cytotoxic T lymphocytes (CTLs) is halted, as well as their activity. It is even possible that the presence of the tumor in the eye will induce systemic tolerance, thereby inhibiting effective immune responses against metastases. The presence of regulatory T cells may suppress an effective immune response. This is observed when antigens are injected into the anterior chamber of the eye of a mouse, which leads to the induction of an unusual immune response known as anterior chamber-associated immune deviation (ACAID) [1]. ACAID is characterized by the presence of regulatory splenic T cells that prevent the development of delayed-type hypersensitivity and prevent the maturation and differentiation of precursor CTLs [2]. In experimental animal models, it has been shown that tumor cells that are rejected when placed in the skin enjoy an immune-privileged ocular environment when placed inside the eye.

5.4 Immunological Aspects of Uveal Melanoma

As much is known about the immunology of uveal melanoma (UM), this chapter will illustrate the role of immune responses specifically in that malignancy. For UM, an age-related influence can clearly be identified, as this malignancy is typically a disease of middle-aged people. One of the early changes that leads to the development of UM is a mutation in one of the GNAQ or GNA11 G proteins, which stimulates cell proliferation [3]. In addition to the occurrence of a mutation in a melanocyte stem cell, the soil for growth should be fertile, and one of the important parameters may be the local inflammation that occurs in the eye and other parts of the body during aging [4]. An increased availability of blood vessel formation may be the environmental factor that helps in the development of the intraocular tumor, leading to a clinically significant lesion. Finally, a specific change is associated with metastasis formation, which is loss of one chromosome 3. This genetic aberration together with a mutation in the BAP1 gene on the remaining chromosome 3 leads to a significant change in gene expression, which is associated with metastatic spread [5].

5.4.1 Immune Responses Against UM in Humans

The eye is known as one of the immune-privileged organs. Even though intraocular tumors are partly shielded from immunological attack due to this immune-privileged environment, this does not prevent their detection by the immune system. However, intraocular tumors, just like tumors in other body sites, try to circumvent immune responses that endanger their survival, but this evasion is not always successful: UM contains all kinds of tumor-infiltrating leukocytes (TIL), as demonstrated by immunohistochemical analysis (see below).

Clinical observations support the fact that an active immune system may be able to attack the intraocular tumor. Spontaneous eradication of UM has been described in some patients [6, 7]. Serum antibodies against melanoma-associated antigens

were detected in 16 out of 22 patients [8]. Also, positive cutaneous delayed-type hypersensitivity reactions against melanoma-associated antigens occur in patients with UM, and the blood of UM patients often contains lymphocytes that are cytotoxic to uveal and skin melanoma cells [9]. Melanoma-specific $CD8^+$ T cells from patients with UM were able to recognize autologous and allogeneic uvea melanoma cells, indicating that tumor-associated antigens are shared between the various uvea melanoma cell lines [10, 11]. Unfortunately, these immune cells are unable to reject the intraocular tumor in vivo.

A variety of cutaneous melanoma antigens recognized by anti-melanoma CTL have been identified [12]. Similar to cutaneous melanomas, UMs have been shown to be antigenic: UM express tyrosinase, MART-1, and GP100 [13], while discussion exists regarding the expression of MAGE antigens in primary and metastatic UM [14]. UM patients have precursor lymphocytes that can recognize and kill target cells presenting peptides of these antigens [15].

5.4.2 HLA Antigen Expression

Tumor antigens can only be recognized by CD8+ T cells in an MHC class I-restricted manner. In humans, the MHC complex is referred to as human leukocyte antigen (HLA) system. Alterations in HLA expression levels will have serious consequences for the host defense against tumor growth. In many tumors, including cutaneous and UM, expression of HLA class I varies between tumors [16–18]. Absence of allele-specific HLA class I molecules may prevent recognition of the tumor cells by cytotoxic T cells. While HLA class I loss is associated with a worse patient survival in cutaneous melanoma, in contrast, in UM, a low HLA class I expression is correlated with a better patient survival [16, 19, 20]. Similarly, absence of HLA class II is correlated with a better survival of patients with UM [19, 20]. A lack of expression of HLA class I on UM causes the cells to become more susceptible to natural killer (NK) cell-mediated lysis in vitro [21]. TGFβ has been shown to decrease HLA expression [22]. Although NK cell-mediated lysis of UM is inhibited in the eye by macrophage migration inhibitory factor (MIF), it has been suggested that melanoma cells that disseminate from the eye into the liver are at risk for surveillance by NK cells [23, 24]. Progressive intraocular tumor growth and an increase in hepatic metastases were observed in experimental animal models deficient of NK cells [21, 25].

5.4.3 Leukocyte Infiltrate

Various tumor-infiltrating leukocytes such as $CD3^+$, $CD8^+$, and $CD4^+$ T cells are present in UM [17, 26]. Previous studies did not always clearly reveal the relevance of these TIL in UM, as the presence of these cells was considered either favorable or unfavorable for the survival of patients [27, 28]. Recent studies analyzed the presence of different cell subtypes including Foxp3+ regulatory T cells (Tregs); the majority of T cells were CD8 positive, but $CD4^+$ and $Foxp3^+$ cells were usually also present and it was noticed that the presence of TILs was highly variable between tumors. A high number of Tregs was linked to a bad prognosis [29, 30]. The presence of any of the T-cell types was increased in tumors with loss of one chromosome 3.

In addition to T cells, infiltrating leukocytes may be macrophages: the presence of high numbers of tumor-infiltrating macrophages within the primary ocular melanoma is associated with loss of one chromosome 3 and an increased mortality [31, 32]. Macrophages can belong to different subtypes: the M1 macrophages that play a role in antigen presentation and the M2 macrophages that have a pro-angiogenic function. The majority of intra-tumoral macrophages in UM are of the pro-angiogenic M2 type, which supports the prior finding of an association between the number of infiltrating macrophages and the presence of blood vessels in UM [33, 34]. The presence of different T-cell types was correlated with the number of infiltrating macrophages, especially the number of M2 macrophages [33]. It has been suggested that Tregs may support the differentiation of monocytes to tumor-promoting M2 macrophages [35]. The strong correlation between tumor cell type and amount of infiltrate

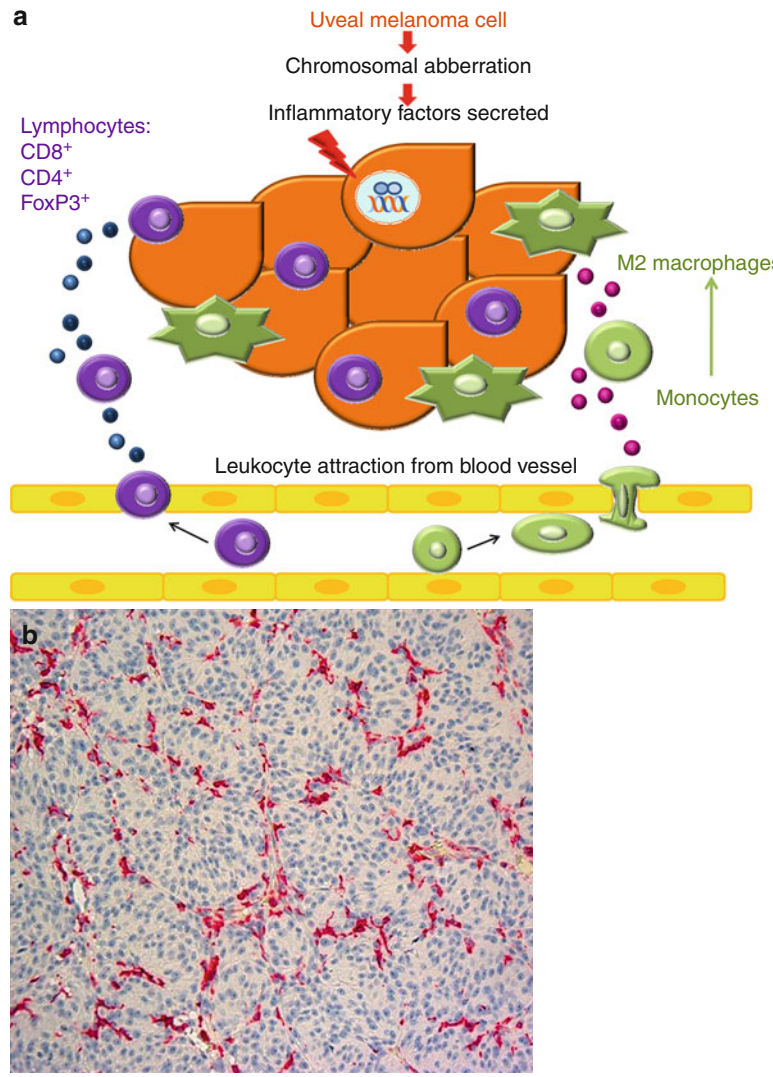

Fig. 5.1 Cancer-related inflammation in uveal melanoma. Inflammation is proposed to drive some cancers to more aggressive phenotypes. Studies show the presence of $CD8^+$, $CD4^+$, and $FoxP3^+$ Treg cells in uveal melanoma, suggesting that they can induce a local immune response at the tumor site (**a**). Tumor-associated macrophages could also play an important role in inhibiting immune responses and angiogenesis, which has been linked to cancer development and progression (**b**)

indicates that basic tumor characteristics determine the influx of immune cells (Fig. 5.1) [36]. The amounts of different types of TIL were also correlated to the HLA class I and II expression levels, in keeping with the idea of a tumor-encompassing inflammatory phenotype [31].

5.4.4 Previously Irradiated Tumors

As irradiation of a tumor may affect tumor characteristics and potentially induce an increased immune cell infiltration, immunohistochemistry to determine the amount of infiltrate was performed on a series of 46 UMs [37]. These UMs were enucleated after previous irradiation had taken place, and a complication that necessitated enucleation had occurred. This could be lack of responsiveness, tumor recurrence, or, e.g., the development of untreatable glaucoma. Similar to previous studies, on nonirradiated UM-containing eyes, a wide variation in the numbers of TIL was observed. Irradiated tumors did not contain more macrophages than nonirradiated UM, but the numbers of lymphocytes were significantly higher in previously irradiated eyes. This was true for CD3, CD4, CD8, as well as $Foxp3^+$ cells and independent of the reason for enucleation. Tumors containing

epithelioid cells showed significantly more lymphoid infiltration.

These observations show an association between the presence of an infiltrate and a bad prognosis in irradiated and nonirradiated tumors. How the innate immune response is related to a worse prognosis is as yet poorly understood, but it may be that the influence of the intraocular environment is such that it leads to systemic immunosuppression instead of immune stimulation; this may have important implications for prospective immunotherapy, as discussed below.

5.4.5 Immunotherapy

Tumors that do activate innate immune signals and do recruit T cells also show high expression of selected immune suppressive pathways. This suggests that promoting better innate immune activation, facilitating T-cell trafficking, and blocking specific negative regulatory pathways could support improved immune-mediated rejection of UM. At present no useful adjuvant or systemic treatment is available. Encouragingly, many drugs that target cancer-related inflammation are currently in clinical trials for other diseases. Obstacles to such studies include the rarity of UM, and clinical trials have only attracted limited numbers of patients with different characteristics (often unresectable or metastatic UM patients) in phase I or II trials [38]. Immune-based therapies have a long history in the treatment of cutaneous melanoma [39], and sometimes also UM patients are included. Some new immunotherapeutic approaches in (ongoing) trials in UM patients include fully human monoclonal anti-CTLA4 antibody (ipilimumab), anti-PD-L1, IFN-alpha 2b, and a dendritic cell vaccine therapy program.

5.4.5.1 Ipilimumab

The monoclonal antibody ipilimumab activates the body's immune system to fight cancer by blocking a protein known as fully human anticytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 is a molecule on T cells, known to play a critical role in regulating immune responses. CTLA-4 suppresses the immune system's response to disease, so blocking its activity stimulates the immune system to fight the melanoma. Ipilimumab was one of the first agents to show a survival benefit of patients with advanced cutaneous melanoma in a phase 3 trial; however, UM patients were excluded in the first trials. The assessment of the activity and safety of ipilimumab in a small group of 13 patients with UM showed overall no objective response [40]; the median survival was 36 weeks (1 year up to 172 weeks), with two patients developing stable disease, and with a third patient achieving stable disease after initial progressive disease. Another small group of five patients was recently reported [41]. Except for two disease stabilizations, the three other patients developed progressive disease. Despite initial stabilization, one maintained disease control at 11 months, and the second patient progressed after 15 months. Furthermore, despite its different biology, the tumor kinetics and response patterns resembled that of ipilimumab in cutaneous melanoma. Therefore, ipilimumab may potentially benefit patients with advanced UM, which will be further investigated in clinical trials.

5.4.5.2 Programmed Death-Ligand 1

A cell membrane-bound molecule, programmed death-ligand 1 (PD-L1) is a component of the immune suppression by the tumor microenvironment. It may contribute to the tumor's capacity to evade immune elimination, induced by IFN-γ and by suppressing IL-2 production [42]. Interaction of PD-1 with PD-L1 downregulates T-cell proliferation and cytokine production and induces T-cell apoptosis, hereby promoting UM's escape from T-cell-mediated immune surveillance. The immunological approach by selective blockade of the immunological synapse anti-PD-L1 is also being studied in ongoing trials (NCT 00729664).

5.4.5.3 Interferon-Alpha 2b

Another experience was reported with adjuvant IFN-alpha 2b [43]. Among 39 patients, serious side effects (e.g., leucopenia, thrombopenia) were seen, and the initial dose had to be reduced or therapy had to be withdrawn. Neither a univariate approach nor a multivariate approach could show a protective effect of interferon treatment on survival.

5.4.5.4 Dendritic Cell Therapy

A currently ongoing nonrandomized phase II intervention study (NCT00929019) uses dendritic cell therapy as an immunological adjuvant following local therapy (enucleation or irradiation) in UM patients. This immune vaccine trial has already shown promising results in cutaneous melanoma patients [44]. Based on tumor-specific genetic changes in loss of chromosome 3, high-risk patients can easily be selected at an early stage in order to eliminate circulating tumor cells and micrometastatic lesions. Patients are immunized by their own dendritic cells, which stimulates T cells to detect remaining tumor cells and metastases. Tumor cell destruction is initiated by presenting tumor particles to cytotoxic T cells. In this way, one hopes to induce a specific and long-lasting immunity to prevent recurrent disease.

5.4.6 Immune-Related Response to Therapy

In those studies, besides survival, there is particular focus on the immune-related responses and adverse events, and sometimes, absolute lymphocyte counts are also evaluated. For example, a regressing lesion in one cutaneous melanoma patient revealed a significantly increased and selective $CD8^+$ T-cell infiltrate and an acute decline in $CD3^+$ T cells following anti-PD-1 therapy [45]. In light of these observations, it is interesting to speculate that the therapy may cause redistribution of lymphocyte subsets from the blood into tumor and tissue sites (of course, validation has to be performed on larger numbers).

In other UM studies, which were initiated to inhibit tumor cell growth, not much is documented about the immunological effects. Contrary to primary UM, the immunological characteristics of its metastases are not well established. The presence of mononuclear inflammatory infiltrates in UM metastases has been reported. One important consideration that should be made when designing clinical trials testing effectiveness of various therapies of metastatic UM, is the fact that, in metastatic UM, monosomy 3 is associated with highly aggressive, rapidly progressive disease, while a prominent mononuclear inflammatory infiltrate in the metastases is associated with a better prognosis [46]. It is in contrast to what had been found in primary tumors. This recent study of exclusive metastasis material (and only studying 11 cases) indicates that host factors are relevant in the development and progression of metastatic disease and implies that immunological therapies to treat metastatic UM may have the potential to impact patient survival. In addition, one group found that hepatic metastases had a significantly more intermediate and dendritic types of CD68-immunopositive macrophages than round ones [34]. The difference in the type of inflammatory cellular infiltrate between primary and metastatic tumors might have to be taken into account. In addition to a combination of anti-inflammatory approaches that target the tumor microenvironment with more knowledge-based selective tumoricidal drugs, future therapies should also take notice of the natural genetic variation (e.g., monosomy 3 or gene expression class 2) that affects inflammation and immunity. A still open question is whether leukocyte infiltration predicts the response to therapy, as suggested by a previous study [47]. As reviewed [48], pre-therapeutic immune response can determine the efficacy of conventional chemotherapies, and therefore anticancer immune responses may be indispensable for the achievement of optimal therapeutic results.

Conclusions

Immune cells often infiltrate in and around uveal melanoma, and it is suggested that the cancer cells can modulate and regulate these immune cells. The nature of the uveal melanoma microenvironment offers therapeutic opportunities. Preclinical studies have supported this premise and clinical translation of many of these strategies is ongoing. Ultimately, by combining selective impairment of the neoplastic cell populations residing in the eye with immunotherapy – as this may be more effective than single therapy regimes – it may be possible to bring UM growth, invasion, and metastasis to a halt.

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6.1 Introduction

A fundamental characteristic of cancer cells is their ability to proliferate and survive outside of their normal physiologic context. This ability is acquired through genetic and epigenetic alterations that modify the cell's interaction with its environment. As such, some of the most common cancer-related genes are involved in cell cycle, differentiation, apoptosis, and angiogenesis. These genes are often referred to as oncogenes and tumor suppressor genes, depending on whether cancer-causing mutations result in gain or loss of function, respectively. Here we will review some of the genes and pathways that commonly are deregulated in cancer.

6.2 Proliferation and the Cell Cycle

6.2.1 Growth Factor Signaling Pathways

Since a fundamental characteristic of cancer cells is their uncontrolled proliferation, it is not surprising that many of the cancer-related genes are involved in cell cycle regulation. Normal cells are limited in their proliferative capacity by the availability of mitogenic (growth-stimulating) signals. Cancer cells often overcome this limitation and become independent of growth signals by acquiring gain-of-function mutations in growth factor receptors such as the epithelial growth factor receptor and the c-Kit receptor. Such mutations

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_6,

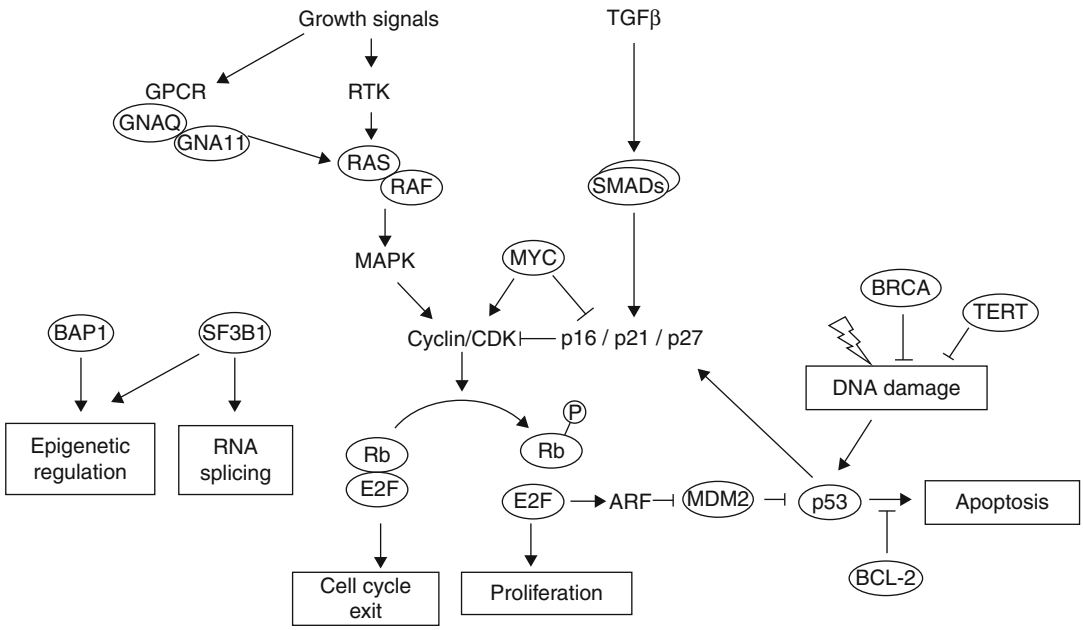

Fig. 6.1 This diagram illustrates some of the major components of proliferative and apoptotic pathways that are linked through a complex, interdependent network of interactions that pose a formidable obstacle to neoplastic transformation and cancer progression. Rb and p53 pathways are interconnected and form a cornerstone for the cellular strategy against neoplastic transformation. When Rb is hypophosphorylated and able to bind E2F transcription factors, it inhibits proliferation and promotes cell cycle exit (differentiation or senescence). However, when Rb is phosphorylated as a result of upstream signaling through the Ras-Raf-MAPK, TGF-**β** or signaling pathways, cell cycle progression is permitted. If this cell proliferation is deregulated, for example, when an oncogene such as Ras is mutated, excessive levels of E2F are released from inhibition by Rb and trigger apoptosis through activation of p53. Likewise, DNA damage and other cellular abnormalities can activate p53, which serves to arrest or sacrifice the cell if potentially neoplastic aberrations are encountered. Cancer cells overcome these tumor suppressor mechanisms by many different strategies that aim to disrupt the Rb-p53 network. Mutations in the G-coupled protein receptor alpha-q subunits, GNAQ or GNA11, are mutually exclusive and occur in almost all uveal melanomas. These mutations lead to constitutive MAPK activation. Mutations in BAP1 and SF3B1 are mutually exclusive in uveal melanoma, suggesting that they may function in overlapping pathways. BAP1 functions at least in part as an epigenetic regulator. SF3B1 is an RNA splicing factor but may also participate in epigenetic regulation

circumvent the requirement for growth factors and can lead to constitutive growth signaling and autocrine stimulation. Alternatively, growth factor signaling can be disrupted by mutations downstream of the receptor. For example, mutations in GNAQ or GNA11, G protein subunits involved in downstream signal transduction of G protein-coupled receptors, have been found in 85 % of uveal melanomas [1, 2]. These mutations result in constitutive activation of their cognate G protein-coupled receptor, leading to activation of signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway, a pathway that is frequently disrupted in cancer [3]. Components of the MAPK pathway include the RAS and RAF families of oncogenes [4, 5]. GNAQ/GNA11 mutations also are found in blue nevi, a benign melanocytic lesion, and are not prognostic of clinical outcome in uveal melanoma [6]. Thus, mutations in GNAQ/GNA11 likely represent early and perhaps initiating events in uveal melanoma, but other molecular events are likely necessary for metastasis to occur (Fig. 6.1).

The tumor growth factor (TGF)-β pathway acts on its receptors to regulate a broad range of physiologic events and is a frequent target of cancer mutations [7]. One of the key activities of TGF- β is to induce nuclear localization of the transcription

factor Smad4, which activates cell cycle inhibitory genes such as p15Ink4b, p21CIP, and p27KIP. Cancer cells can disrupt this pathway through various mechanisms, including mutation of the TGF- β receptor or Smad4. Uveal melanomas frequently exhibit defects in the TGF- β pathway, although specific mutations are yet to be identified [8].

6.2.2 Rb-p16Ink4a-Cyclin D Pathway

A major downstream target of most growth signaling pathways is the retinoblastoma (Rb) pathway, which is disrupted in virtually all cancers [9]. While the Rb gene itself is mutated in only a small subset of cancers, mutations in other members of the Rb pathway are common. Typically, the effect of these other pathway mutations is to maintain Rb in a hyperphosphorylated (inactive) state where it is incapable of inhibiting cell proliferation. In uveal melanomas, for example, Rb is inappropriately phosphorylated as a result of cyclin D overexpression or p16Ink4a inactivation, both of which result in excessive Rb kinase activity [10]. Not surprisingly, cyclin D overexpression is associated with poor prognosis [11, 12].

6.2.3 MYC Family of Oncogenes

The MYC family of oncogenes are often upregulated or amplified in cancer [13]. The Myc proteins regulate many cellular processes, including proliferation and apoptosis. The c-Myc gene is located at chromosome 8q24 within the region that is frequently amplified in uveal melanoma [14], and many uveal melanomas overexpress high levels of the c-Myc protein [15].

6.3 Apoptosis and Senescence

Normal cells undergo apoptosis (programmed cell death) or senescence (permanent cell cycle withdrawal) if they stray from their normal environmental restraints [16]. The success of cancer cells depends on their ability not only to proliferate but also to avoid apoptosis and senescence. As such, the rate of tumor growth represents a balance between cell proliferation and cell attrition. Mutations in apoptosis regulators are ubiquitous in cancer cells, indicative of their importance in tumor suppression.

6.3.1 p53 and MDM2

The p53 tumor suppressor can activate senescence and apoptotic programs in response to abnormalities associated with neoplastic transformation, such as excessive proliferation and DNA damage [17]. Over half of all cancers contain loss-of-function mutations in the p53 gene, and the p53 protein is functionally inhibited in most other cancers. In uveal melanoma, p53 is rarely mutated and exhibits normal activation in response to DNA damage [11, 18]. However, the p53 pathway is functionally impaired, and this is explained at least in part by overexpression of the p53 inhibitor MDM2 [11, 18]. Similarly, p53 mutations are rare in retinoblastoma [19], but these cancer cells are exquisitely sensitive to MDM2 inhibition, suggesting that p53 is functionally inhibited by MDM2 in this cancer as well [20].

6.3.2 Bcl-2

Bcl-2 is an antiapoptotic factor and the namesake of a family of pro- and antiapoptotic proteins that interact in a complex manner to regulate apoptosis by the intrinsic mitochondrial pathway [21]. Bcl-2 is overexpressed in many cancer types as a mechanism for inhibiting apoptosis. The majority of uveal melanomas express high levels of Bcl-2 [20], and inhibition of Bcl-2 causes tumor cell apoptosis [20], indicating that Bcl-2 is important to uveal melanoma cell survival.

6.3.3 Telomere Maintenance

Cell cycling is normally accompanied by shortening of the telomeres until they are reduced to a critical length that triggers a DNA damage response and p53-mediated senescence or apoptosis [22]. Telomerase is an enzyme capable of extending the

length of telomeres [22]. Consequently, cancer cells often hijack this enzyme by upregulating its catalytic subunit, TERT, to maintain telomere length. Telomerase is highly active in uveal melanomas [23].

6.3.4 BRCA and BAP1

BRCA-1 and BRCA-2 are DNA damage repair proteins, and mutation of these genes leads to an accumulation of DNA damage and an increased likelihood of acquiring cancerous mutations [24]. The BRCA genes were originally identified as common mutations associated with familial breast cancer, but they have since been linked to many other cancers. For instance, germline BRCA-2 mutations have been found in up to 3 % of uveal melanoma patients [25, 26].

Recently, mutations in BRCA-1 associated protein 1 (BAP1) have been found in metastasizing class 2 uveal melanomas [27]. Thus, BAP1 appears to act not only as a tumor suppressor but, more specifically, as a metastasis suppressor in uveal melanoma. Somatic and germline mutations in BAP1 have subsequently been identified in cutaneous melanoma, mesothelioma, renal cell carcinoma, and other cancers, revealing a newly described BAP1 familial cancer syndrome [28–31].

6.4 Splicing Factor Mutations

Gene transcription requires a number of processing steps, including the removal of intronic sequences and joining of exonic sequences to create mature mRNAs. Alternative splicing of introns can result in multiple mRNAs and proteins from the same transcript, resulting in a large diversity of proteins from a single transcript. Aberrant regulation of RNA splicing can confer dramatically different properties to the protein and can significantly affect growth and differentiation pathways of cells which may lead to cancer [32]. Mutations in splicing factors are rapidly being reported in an increasing number of cancer types [32]. Recently, mutations in SF3B1, a subunit of an RNA splicing complex, were reported in less aggressive class 1 uveal melanomas and, as such, are associated with better prognosis [33]. SF3B1 mutations appear to be mutually exclusive with BAP1 mutations, suggesting that the two genes may function in an overlapping pathway. In addition to its role in RNA splicing, SF3B1 may also interact with epigenetic regulators such as Polycomb proteins [34]. The precise role of SF3B1 mutations in uveal melanoma is yet to be determined.

6.5 Angiogenesis

Cancer cells require a supply of nutrients and oxygen to survive. Passive diffusion of nutrients is limited to about a distance of 3 mm [35]. Thus, for tumors to grow beyond this size, they must develop a blood supply. Tumor angiogenesis can involve many mechanisms, the most familiar of which are discussed here.

6.5.1 VEGF

Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are two important angiogenic factors that are often involved in tumor angiogenesis [35]. Retinoblastomas are highly vascular tumors that express VEGF [36]. Recent work suggests that targeting the tumor vasculature may be an effective treatment for retinoblastoma [37]. Uveal melanomas also express VEGF, but it remains unclear whether VEGF alone accounts for the complex tumor vasculature in these tumors [38].

6.5.2 HIF/VHL

Hypoxia-inducible factor (HIF) is a sensor of hypoxia and regulator of the cellular hypoxic response. HIF is a heterodimeric complex comprised of an α-subunit, usually HIF-1α, and a β-subunit, usually the aryl hydrocarbon receptor nuclear translocator (ARNT). Under normoxic conditions, HIF-1α is polyubiquitylated and targeted for proteasomal degradation. However, under hypoxic conditions, HIF-1 α is no longer polyubiquitylated and accumulates, allowing it to

dimerize with ARNT and activate transcription of target genes. HIF appears to be able to function as an oncogene, since it is overexpressed in some cervical, breast, ovarian, endometrial, and stomach cancers, and is associated with poor prognosis [39].

Von Hippel-Lindau syndrome (VHL) is a phakomatosis that features retinal and cerebellar hemangioblastomas, as well as renal cell carcinomas and other tumors. The VHL gene encodes a tumor suppressor that binds to HIF-1α and targets it for degradation via the ubiquitin pathway, thereby maintaining HIF in an inactive state. VHL binds a domain of HIF-1α that must be hydroxylated for binding to occur. This hydroxylation in turn requires molecular oxygen as a substrate. Thus, when oxygen levels are low, VHL binding to HIF-1α is impaired, thereby freeing HIF-1α to activate genes involved in angiogenesis, such as VEGF [40].

Conclusions

All cancers face a similar set of obstacles that they must overcome to survive and proliferate. The tissue-specific regulatory environment determines the specific details of how these obstacles must be overcome, but there is a common theme throughout most cancers: circumvention of cell cycle control, suppression of apoptosis, avoidance of senescence, and induction of angiogenesis. Ocular cancers are no exception to this rule, and in fact, uveal melanoma and retinoblastoma are ideal models for studying some of these mechanisms, with multiple genetic mutations now identified in uveal melanoma. As we gain further understanding of these molecular processes, it will become increasingly possible to design targeted therapies to delay or prevent cancer progression.

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Cancer Staging

7

Paul T. Finger

Contents

7.1 Introduction

Informatics is transforming medicine. As electronic medical records (EMR) become ubiquitous throughout the world, government committees hope to sift through scientific data in efforts to prove or disprove the efficacy of medical practice. In this new world, eye cancer specialists will be required to prove efficacy for their methods and "statistical significance" will challenge "the art" of medicine.

We can see the early changes in our meetings, publications, and textbooks [1–3]. Presentations are transitioning from single-center case series to large multicenter retrospective studies [1–9]. Cooperative clinical and eye cancer research organizations are being created [2, 4, 5]. Our journals' instructions for authors are becoming increasingly complex, including more standardization of terms, increased prominence of and requirements for clinical trials, and a greater emphasis on evidence-based medicine.

Staging systems have long served as an important building block for the development of medical evidence. For example, past uveal melanoma staging systems have included Callender's prognostic classification related to histopathology and the clinically driven Collaborative Ocular Melanoma Study (COMS) [6, 7]. Using COMS-staged definitions of "medium" tumor size, plaque radiation was found equivalent to enucleation for the prevention of metastasis [8]. COMS also found no survival benefit to pre-enucleation external beam radiation therapy

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_7,

prior to enucleation surgery for "COMS-large" melanoma [9, 10]. Radiation retinopathy has also been staged for risk of vision loss [11].

Retinoblastoma was initially staged for response to radiation therapy by Reese and Ellsworth [12]. Then, as clinical practice evolved away from radiation, others devised classifications to predict retinoblastoma response to chemotherapy [13–15]. Lommatzsch evaluated published TpNM staging to predict mortality [16]. Staging also helped the Intergroup Rhabdomyosarcoma Study succeed in evaluation of treatment [17–19].

Thus, ophthalmic oncology has already benefited from staging to predict metastasis, response the therapy and the evolution of side effects. However, clinical staging also offers an opportunity to define epidemiological factors, biometric findings, as well as results of treatment and their side effects. In sum, staging systems serve as a standardized language for research, clinical practice and interdisciplinary communication. However, only universal staging can serve as a foundation for data collection, sharing for meta-analysis, and determinations of statistical significance.

7.2 Universal Staging and the AJCC–UICC Ophthalmic Oncology Task Force

The American Joint Committee on Cancer (AJCC) together with the Union International for Cancer Control (UICC) prompted the creation of The AJCC–UICC Ophthalmic Oncology Task Force (OOTF) [4, 5]. Its mission was to create a universally accepted staging system for all eye cancers [1]. Though these staging systems would continue to be anatomically based, the OOTF was also charged to provide practice guidelines for clinical data collection as well as pathology. For the first time, evidence-based genetic and molecular biomarkers were to be included for prospective data collection. Most importantly, the OOTF was to be made more robust and "internationalized" with members of the UICC.

7.2.1 The Community

Though past AJCC staging systems were comprehensive, eye cancer specialists did not typically use them. Even when the 6th edition made uveal melanoma staging roughly equivalent to the COMS classification, it failed to attract clinical use. Therefore, it was clear that the OOTF had to be reinvented. Rules, structure, and new concepts were required to guide the 7th edition toward universal acceptance (Table. 7.1).

The 7th edition, AJCC–UICC Ophthalmic Oncology Task Force was expanded to include 46 eye cancer specialists from 11 countries (France, England, Sweden, Finland, the United States of America, Canada, India, Japan, the Netherlands, Hungary, and Germany). Though weighted toward ophthalmic oncologists, orbital adnexal surgeons, and ophthalmic pathologists, the OOTF included input from radiation, medical, and pediatric oncologists as well as nonphysician specialists involved in the care of eye cancer patients [20].

7.2.2 The Process

Starting in 2004, the OOTF embarked on a 5-year peer-review process. Each tumor section (uveal melanoma, retinoblastoma, eyelid carcinoma, conjunctival squamous, conjunctival melanoma, lacrimal gland carcinoma, and orbital sarcoma) was reviewed for clinical methods and pathology techniques. The 7th edition staging system included but was not to be limited to *t*umor, *n*ode, and *m*etastatic (*TNM*) grading. There was general agreement that all changes were to be based on the "best data."

Table 7.1 Basic concepts: there is power in numbers!

What is made by the *community* will be used by the community
Sating systems will function as "*common language*" to collect patient data
Eye tumor specific *electronic medical records* (ETS-EMR) will facilitate prospective, multicenter data collection
Statistically *significant evidence* will be used as threshold to determine the allocation of resources
Database analysis will *improve patient care*

There was an emphasis on statistical significance. As available, evidence-based medicine was incorporated to achieve the highest possible standards.

Each 6th edition AJCC section was reviewed by a committee. Then a team consisting of a clinician and pathologist were tasked with writing a first draft revised staging system. In peer-review fashion, that draft was reviewed by a second, internal clinician pathologist team. Once found acceptable, the text was sent for multiple outside reviewers leading to secondary internal revisions and (in some cases) further external review for internal adjudication of conflicts. Lastly, prior to publication, each section was vetted by the AJCC executive committee, the publisher, and tumor registry representatives.

Despite all our efforts to create community consensus, the OOTF, the AJCC, and UICC all realize that tumor classification will be an ever-evolving process. For example, new biologic factors may help predict cancer outcomes and response to treatment, and they will have to be included in future revisions. As evidence-based and statistically significant data becomes available, it will be evaluated for incorporation into subsequent iterations.

7.2.3 Acceptance

So far, the 7th edition AJCC–UICC staging system has been accepted and used by ophthalmic oncologists, ophthalmic plastic surgeons, radiation oncologists, ophthalmic pathologists, tumor registries, and many related eye cancer specialties. This is in part due to their participation in its creation as well as its general acceptance by the College of American Pathology (CAP), the American Society of Ophthalmic Plastic and Reconstructive Surgeon (ASOPRS), medical journals, associated ophthalmic societies, and organizations [21–23]. To date, over 12 ophthalmic journals now either require or suggest that manuscripts use the 7th edition AJCC staging system in manuscript preparation. Further, over 130 world cancer agencies use the AJCC–UICC staging. This brings ophthalmic oncology into the mainstream of world cancer care.

7.2.4 Impact

7.2.4.1 Language: Do You Speak Ocular Tumor?

Language is defined as the human use of spoken or written words as a communication system. Sharing a common language allows us to communicate our ideas and thus enables progress. In order for the staging system to function as a universal language, it needed to be accepted by the entire community [1]. From this measure, the 7th edition AJCC–UICC staging system has indeed become "universal."

7.2.4.2 Patient Care

This is not a trivial point, because the use of the 7th edition staging system will save lives. For example, when a researcher publishes staged results of a new treatment for conjunctival melanoma, he or she will not only better understand the relative size, location, and distribution of the tumors treated, but can better compare those results against other published clinical studies. Staging will allow the clinician to better understand if the results of two differing treatments are confirmative or dissimilar.

Our clinical decisions are primarily based on ideas and studies and what we have heard in lectures or read in the literature, coupled with our medical experience. Therefore, the quality of those life and death decisions is only as good as the information we acquire. Universal staging will allow us to better categorize and understand that information. Future clinical decisions will be increasingly based on a foundation of defined tumor sizes and distribution.

Speaking "ocular tumor" will also affect informed consent. Much of our time as clinicians is spent explaining the current knowledge and ophthalmic practice to patients. A typical explanation of the risks and benefits includes what has been proven by statistics-based research and what is offered as traditional practice. Universal staging allows us to tell our patients that tumors of certain sizes and locations are more or less likely to respond to specific treatments or exhibit particular side effects [24, 25].

A common eye tumor language will change the way we practice medicine and help us cope with the current medical information explosion. From a time when there was only surgery, radiation, and chemotherapy, we currently have to widen our mindset to incorporate immunotherapy, epidemiology, genetics, and molecular biology (Table. 7.2). All these disciplines and their associated specialists must be linked to determine evidence-based best medical practice. This is particularly difficult for rare diseases like eye cancer, where research funds and statistically significant evidence are scarce. Excellence in ophthalmic oncology will increasingly require a multifaceted approach to individualize and maximize of patient care.

7.2.4.3 Eye Tumor-Specific EMR-Based Database

The evolution of staging systems will generate data fields that will be used to form intelligently structured, eye tumor-specific electronic medical records (ETS-EMR). In turn, this ETS-EMR will be used to link eye cancer specialists throughout the world. A form of this approach called "e-cancer Care" is currently being implemented for retinoblastoma linking Canada, India, and Kenya [26].

To accomplish this, each center will have an overlay ETS-EMR that virtually sits on top of their clinic-specific medical record. Each of these two programs will work symbiotically. That is, each EMR will be able to both withdraw and distribute what information is required to accomplish their individual functions.

Table 7.2 The evolution of cancer care

Since 1500 BC	1903	1940s	Present
Surgery	Surgery	Surgery	Surgery
	Radiation	Radiation	Radiation
		Chemotherapy	Chemotherapy
			Genetics
			Immunotherapy
			Molecular biology
			Epidemiology

Once installed, multiple international centers will be empowered to collect large amounts of equivalent data that can be added to derive statistical significance [20, 21]. This will enable further refinements in staging systems, incorporation of valid genetic and molecular biomarkers, comparisons of commonly used diagnostic and therapeutic methods, as well as monitoring of newly devised interventions.

7.2.4.4 Multicenter Collaboration

Multicenter collaboration will allow recruitment of larger numbers of patients and/or clinical samples in shorter periods of time. They will prevent duplication of effort, loss of resources, and encourage cooperation. In comparison to single-center studies that are particularly sensitive to selection bias, poor quality data collection and analysis; prospective multicenter studies are more likely to be better funded, organized, and thereby higher quality.

Universal staging and multicenter collaboration will accelerate the development of new methods of eye cancer diagnosis and treatment. In this case, speed will save lives. For example, what if by the year 2023, 20 ophthalmic oncology centers had been collecting standardized ETS-EMR data from each and every choroidal melanoma patient encounter for 10 years. This would be the best quality data, derived at the time of the specialist–patient interaction using the community-designed data fields and standards embedded within ETS-EMR. Given each center was able to enter 100 cases per year for 10 years, these 20 centers could draw on a database of 20,000 cases of choroidal melanoma. By the year 2033, it would be 40,000 cases or more. These are unprecedented numbers of choroidal melanoma patients from which we could discover statistically significant epidemiological features as well as differences in efficacy of our methods of diagnosis and treatment. There would also be opportunities to examine large numbers of less common tumors (e.g., adenoid cystic carcinoma of the lacrimal gland, conjunctival melanoma). In addition, consider the value of a database collection of rare therapeutic side effects and their treatments.

What if a future researcher thinks he or she has discovered "a cure" for retinoblastoma? But that researcher's single megacenter treats only 40 new patients per year and they require 1,000 patients for a statistically significant prospective randomized clinical trial. If that researcher were to employ that "new treatment" for each and every patient in their center, it would take 25 years to enroll patients for that one study and may take another 5 years to follow the last patient for local recurrence or metastasis (Table. 7.3). Simply put, it would take an academic career or 30 years to properly study this one treatment. On the other hand, with a multicenter cooperation of 10 centers each recruiting 40 patients per year, prospective recruitment would take just 2.5 years. Even with the additional 5 years of follow-up, multicenter cooperation would reduce the total project time from 30 to 7.5 (a reduction of 22.5 years). Plus, those 25 centers can go on to evaluate the next diagnostic method or treatment after just 2.5 years of recruitment (Table. 7.3). Multicenter cooperation would enable recruitment for up to 12 studies during that same researcher's 30-year career.

Multicenter cooperation could also function as an early detection system for failed treatments. A large database could anonymously collect the few cases from here and there where a treatment failed but was not published. Rather than allowing failed treatments to proliferate and fade away, recording the outcomes of unsuccessfully treated patients will spare future patients to repetition of those suboptimal or failed treatments and keep us from wasting precious resources.

Table 7.3 Testing a new cancer treatment

Single center	Multi-center
40 patients per year ×25 years =1,000 patients	40 patients per year × 10 centers × 2.5 years =1,000 patients
5 years follow-up	5 years follow-up
One study = 30 years	One study = 7.5 years
A lifetimes work testing one treatment hypothesis	Plus another study every 2.5 years or 10 completed studies within 30 years

Lastly, consider the wealth of patient information that can be permanently embedded in an electronic database. A functioning eye cancer bioinformatics grid will not lose data when physicians retire or move elsewhere. Quite the contrary, a functioning bioinformatics grid will gift opportunities for retrospective data analysis for future generations of eye cancer specialists.

7.3 Summary

The OOTF composed of members of the AJCC, UICC, representatives of our journals, and societies have developed universal 7th edition AJCC–UICC staging, a foundational element for an eye cancer bioinformatics grid (http://eyecancerbig.com). Our next step is to provide evidence that these data points and tumor stages are valid. Therefore, the 7th edition system is currently being tested within the framework of large multicenter retrospective studies that will be used to evolve eye cancer staging in the 8th edition, improve universal staging, and serve as a foundational element for the multicenter ETS-EMR eye cancer-specific bioinformatics grid.

Our generation is standing at the forefront of a new medical information age. Many of the advances in medicine will stem from or be enabled by multicenter cooperative data analysis. It is time for ophthalmic oncology to evolve from megacenter to multicenter in the best interest of our patients. Bioinformatics can be used as a tool to improve the standards of our medical practice by supporting evidence-based preferred (the proven) diagnostic techniques and therapeutic options. Cooperation will allow us to speed the wheels of progress, shape the destiny of ophthalmic oncology and, most importantly, save the vision and lives of our patients.

Acknowledgements The members of the 7th Edition, AJCC–UICC Ophthalmic Oncology Task Force include the following:

Col. Darryl J. Ainbinder, MD; Daniel M. Albert, MD, MS; James O. Armitage, MD; James J. Augsburger, MD; Nikolas E. Bechrakis, MD; Major John H. Boden, MD; Patricia Che'vez-Barrios, MD; Sarah E. Coupland, MBBS, PhD, FRCPath; Bertil Damato, MD, PhD; Laurence Desjardins, MD; Ralph C. Eagle, MD; Deepak P. Edward, MD; Bita Esmaeli, MD; Paul T. Finger, MD (Chair); James C. Fleming,

MD; Brenda L. Gallie, MD; Dan S. Gombos, MD; Jean-Daniel Grange, MD; Hans E. Grossniklaus, MD, MBA; Barrett G. Haik, MD; Col. John B. Halligan, MD; Santosh Hanovar, MD; J. William Harbour, MD; George J. Harocopos, MD; Leonard M. Holbach, MD; John L. Hungerford, MD; Martine J. Jager, MD, PhD; Zeynel A. Karcioglu, MD; Tero Kivela, MD; Emma Kujala, MD; Ashwin C. Mallipatna, MBBS; Col Robert A. Mazzoli, MD; Hugh McGowan, MD; Tatyana Milman, MD; A. Linn Murphree, MD; Tim G. Murray, MD, MBA; Jack Rootman, MD, FRCS; Didi de Wolff-Rouendaal, MD, PhD; Andrew P. Schachat, MD; Stefan Seregard, MD; E. Rand Simpson, MD; Arun D. Singh, MD; Valerie A. White, MD, MHSc; Matthew W. Wilson, MD; Christian W. Wittekind, MD; and Guopei Yu, MD, MPH.

The AJCC–UICC Ophthalmic Oncology Task Force was supported by the American College of Surgeons, the American Joint Committee on Cancer (AJCC), the International Union Against Cancer (UICC), Springer, and The Eye Cancer Foundation.

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Principles of Cryotherapy

8

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8.1 Introduction

Cryotherapy (or cryosurgery) is the technique of precise freezing and thawing of undesirable tissue resulting in cell death and regression. It is a highly effective technique available to the ophthalmologist for local control and eradication of various intra- and periocular tumors and can serve as an alternative or adjunct to other methods such as surgery or radiotherapy.

Arnott described crushed ice and salt (NaCl) used to freeze advanced breast and uterine malignancies in the mid-nineteenth century [1]. By the beginning of the twentieth century, solid CO_2 was being used to treat various skin and gynecologic cancers [1]. During this period, most freezing devices were crude and were only able to penetrate superficial layers of tissue, which limited its clinical application.

The commercial availability of liquid nitrogen and the introduction of the cryoprobe by Cooper and Lee in the 1960s heralded significant advancements in the field [1, 2]. For the ophthalmologist, modification of the cryoprobe led to significant surgical advances in cataract extraction, glaucoma management, and repair of retinal tears. Subsequent pioneering work by Lincoff [3], Fraunfelder [4], and Jackobeic [5] led to application of cryotherapy in the management of various intra- and periocular tumors.

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_8,

8.2 Mechanism of Tissue Injury

Initially, the cryoprobe begins cooling the tissues with removal of heat. Over time, tissue in contact with the probe freezes. Subsequently, the freezing interface progresses in an outward direction resulting in a temperature distribution that is coldest at the point of contact with the probe. Once freezing is complete, thawing is facilitated by heat from the adjacent tissues [1, 6].

8.2.1 Direct Effects

Microscopically, the initial decline in temperature in the extracellular space forms crystals, leading to a hyperosmotic environment, extracting water from the cells, and causing them to shrink. As the temperature lowers, intracellular crystals form, leading to disruption of organelles and cell membranes. This affects the ability of membrane proteins to control intracellular ionic content. During the thawing phase, as the frozen water crystals dissolve, the extracellular space becomes hypotonic. Limited only by a defective cell membrane, extracellular water enters the cell and disrupts it [7]. In addition, the cold temperature physically disrupts the cellular cytoskeleton and denatures proteins [1, 6].

8.2.2 Indirect Effects

Freezing temperatures are also associated with vascular stasis and cellular anoxia. Initially, the cold temperatures lead to vasoconstriction followed by vasodilation, increased vascular permeability, and edema during the thawing process. Endothelial damage leads to stagnation of blood and thrombus formation. The resultant hypoxia promotes tissue necrosis. Some experiments suggest that this mechanism is more important in the death of tumor cells than direct injury from freezing.

8.3 Technical Aspects

Certain technical factors such as tissue temperature, cooling rate, freeze-thaw cycle, and the number of repetitions influence the efficacy of cryotherapy [8, 9].

8.3.1 Tissue Temperature

Tissue temperature is the most important factor with cell death occurring at temperatures between −20 and −50 °C [10].

8.3.2 Cooling Rate

A rapid cooling rate is more effective in causing cell death.

8.3.3 Freeze-Thaw Cycle

Studies indicate that a slow thaw is among the most important variables contributing to cell death.

8.3.4 Number of Repetitions

Multiple freeze-thaw cycles further increase cell damage and death [11].

8.4 Indications

Over the past 40 years, the indications for cryotherapy have expanded to a number of intra- and periocular tumors. In some instances, it serves as

primary treatment, while in others it functions in an adjuvant setting.

8.4.1 Eyelid Tumors

Eyelid lesions including actinic and seborrheic keratosis are generally amenable to cryotherapy sometimes combined with surgical excision. Basal cell carcinoma particularly lesions less than 1 cm in diameter can be cured with this approach. Select cases of squamous cell and meibomian gland carcinoma of the lid have also been treated with cryotherapy as an alternative to surgery and/or radiotherapy [12–15].

8.4.2 Conjunctival Tumors

In the adjuvant setting, cryotherapy plays an important role in the management of conjunctival lesions. Conjunctival intraepithelial neoplasia and squamous cell carcinoma [16], conjunctival melanoma, and primary acquired melanosis with atypia [5] are amenable to adjuvant cryotherapy [17]. It has also been described in the management of large bulky lesions where complete excision was challenging including papillomas and lymphomas [18].

8.4.3 Intraocular Tumors

Certain intraocular tumors (particularly those anterior to the equator) are amenable to cryotherapy as the treatment of choice. Small peripheral retinal capillary hemangiomas [19], Coats's disease, and retinoblastoma foci (those less than 2 mm in thickness) respond well to cryotherapy [20].

8.4.4 Orbital Tumors

The cryoprobe can be used intraoperatively to assist in the excision of orbital lesions such as cavernous hemangiomas and dermoid cysts. The probe is applied directly to the lesion allowing for traction and careful dissection from adjacent structures. The technique of freezing uveal melanoma, prior to transection of the optic nerve ("no touch" enucleation) is currently used infrequently [21, 22].

8.5 Techniques of Cryotherapy

Cryotherapy can be applied in various fashions depending upon the indication and location of the tumor.

8.5.1 Eyelid Tumors

Eyelid tumors can be treated with a liquid nitrogen spray. Following local anesthesia, the area is draped and an ocular protector is placed over the eye to prevent freezing of the globe or adjacent structures. A thermocouple is inserted into the center of the lesion to monitor its temperature (recommended target temperature −50 °C). Freezing is applied to the lesion and a margin of adjacent normal appearing skin. A double freeze cycle is usually administered.

8.5.2 Conjunctival Tumors

Conjunctival tumors are generally treated with a hammerhead-shaped cryoprobe. In most instances, cryotherapy is performed as an adjunct to surgical excision. Following removal of the tumor, double or triple cryotherapy is administered to the underside of the conjunctiva (adjacent to the

resection) and the scleral bed [17]. Cryotherapy as primary therapy can also be performed with newly designed cryoprobes [23].

Fig. 8.1 A retinal cryoprobe. Note the ice ball on the tip of the probe

8.5.3 Intraocular Tumors

Intraocular lesions (such as retinoblastoma and retinal capillary hemangioma) are generally treated using a nitrous oxide retinal cryoprobe (Fig. 8.1). Local (retrobulbar) or general anesthesia (for children) is indicated. Using indirect ophthalmoscopy and scleral indentation, the lesion is isolated (Fig. 8.2). It is frozen under direct visualization such that the resulting ice ball completely encompasses the entire tumor (usually to a temperature of −70 °C). The lesion is allowed to slowly thaw, and the freeze-thaw cycle is repeated twice [20]. The tumor is reexamined in 3–4 weeks and may require additional therapy (Fig. 8.3). Most lesions can be treated transconjunctivally; however, those significantly posterior to the equator may require a conjunctival incision [20].

8.6 Complications

While cryotherapy is generally safe and effective, there are numerous complications that must be considered [24]. In most instances, transient edema and injection occur at the site of treatment. Lesions on the eyelid and close to the lash margin can develop ptosis, trichiasis, and ectropion.

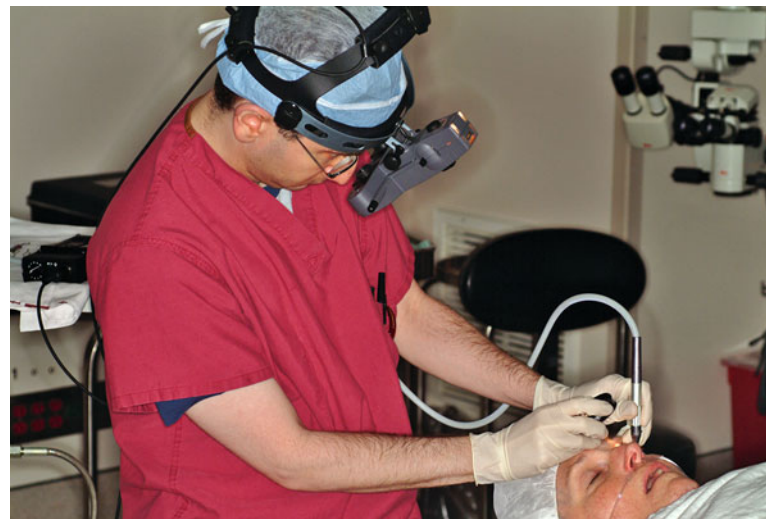

Fig. 8.2 Technique for transcleral cryotherapy of an intraocular tumor under indirect ophthalmoscopic visualization

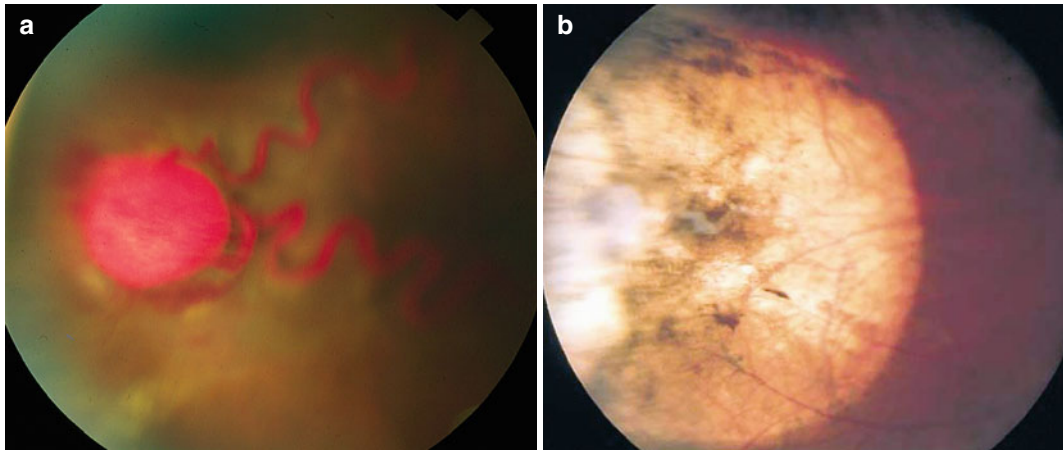

Fig. 8.3 (**a**) Retinal capillary hemangioma treated with cryotherapy. (**b**) Ten months later, the hemangioma appears as a gliotic nodule in an area of chorioretinal atrophy (Reproduced with permission from Singh et al. [25])

Hypertrophic scarring can occur as can skin depigmentation in darker patients.

The conjunctiva generally tolerates freezing well. However, repeated application can lead to limbal stem cell failure, dry eye, and symblepharon formation. Periocular edema and pain are not uncommon following cryotherapy of intraocular lesions. Uveitis may develop requiring the use of topical steroids. Cryotherapy can increase the risk of exudative and rhegmatogenous retinal detachments as well as vitreous hemorrhage. Muscle paresis and changes in pupillary response have also been described.

Conclusions

Cryotherapy is an excellent means of treating certain intra- and periocular tumors. In ophthalmic oncology, this generally translates to high cure rates and minimal ocular morbidity. Cryotherapy is effective in treating small and well-defined tumors. Good technique is critical, with a rapid freeze and slow thawing being most effective in causing the cell death. Multiple freeze-thaw cycles further increase treatment effectiveness. When indicated, cryotherapy can serve as an alternative to more destructive treatment modalities such as surgical excision, chemotherapy, and radiation therapy.

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Principles of Laser Therapy

9

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9.1 Introduction

The theoretic principles behind the laser were developed as early as 1917 when Einstein laid the groundwork for stimulated emission in his treatise "On the Quantum Theory of Radiation." In 1949, Meyer-Schwickerath created chorioretinal burns around retinal holes using the sun as the light source. A variety of different light sources were investigated in the prototype instruments before Carl Zeiss developed the first commercial model using a Xenon lamp in 1956 [1].

Maiman and Gould (1960) invented the ruby laser considered to be the first successful laser. The ruby laser was followed by the argon (L'Esperance, Zweng, and Little, 1968–1969) and krypton (Tek, 1978) lasers.

Further innovations such as Nd:YAG (neodymium:yttrium-aluminum-garnet) laser (Frankhauser and Aron Rosa, 1981), excimer laser (Trokel, 1983), and dye laser (L'Esperance, 1986) followed.

Clinical trials of laser photocoagulation were first initiated by Campbell, Noyori, and Zweng. More recently, photodynamic therapy for the treatment of intraocular tumors (Murphree, 1987) and of age-related macular degeneration (Schmidt-Erfurth and Miller, 1999) has vastly expanded the range of clinical laser applications [2, 3].

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_9,

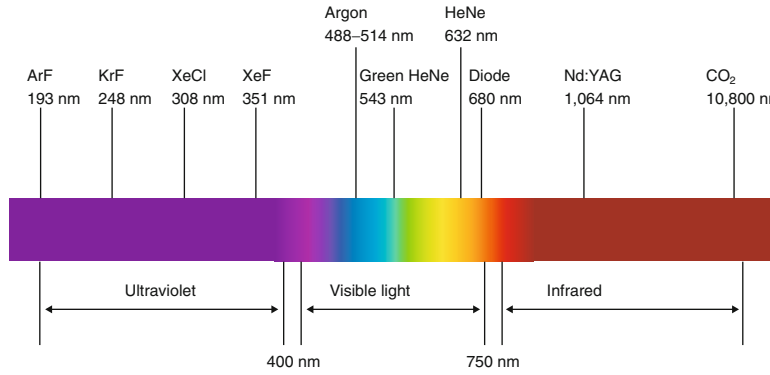

Fig. 9.1 Electromagnetic spectrum of lasers used in ophthalmology

9.2 Basic Considerations

9.2.1 Laser Properties

Laser is an acronym for light amplification by the stimulated emission of radiation. A laser beam is a monochromatic (single wavelength), coherent, and parallel beam of light, usually of high energy. The range of laser radiation extends from the ultraviolet through the visible to the infrared regions of the optical spectrum (Fig. 9.1) [4].

9.2.2 Laser Output

The output of a laser can be continuous wave or pulsed. Retinal photocoagulation is usually performed with a continuous wave laser. The output for retinal photocoagulation is usually delivered over a time interval of from 0.1 to 1.0 s.

9.2.3 Tissue Effects

There are two forms of interaction between light and ocular tissue: absorption and ionization. Ionization is primarily used to incise tissues of the eye, e.g., in YAG capsulotomy. Absorption interaction is used in laser photocoagulation, thermotherapy, and photodynamic therapy.

9.2.4 Treatment Variables

Optical clarity of ocular tissues, the degree of absorption by ocular pigments, specific wavelengths used, spot size, power applied, and the exposure time are some of the important variables that influence the treatment effects.

9.2.4.1 Clarity of the Media

It is important to take opacities of the media into consideration as scattering and absorption of energy may occur while the laser travels toward the target tissue [5]. Longer wavelengths have less scattering and are more efficient in delivering energy to the retina.

9.2.4.2 Tissue Absorption

The absorption spectrum of the three ocular pigments (melanin, hemoglobin, and xanthophyll) varies and can be used to achieve tissue selectivity of laser effects. Melanin has a maximum absorption between 400 and 600 nm (blue, green, yellow, and red) with greater absorption for shorter than longer wavelengths (Fig. 9.2) [6]. Hemoglobin has a narrower range of maximum absorption that includes blue, green, and yellow wavelengths but excludes red wavelengths. Xanthophyll has maximal absorption limited only to the blue wavelengths [3].

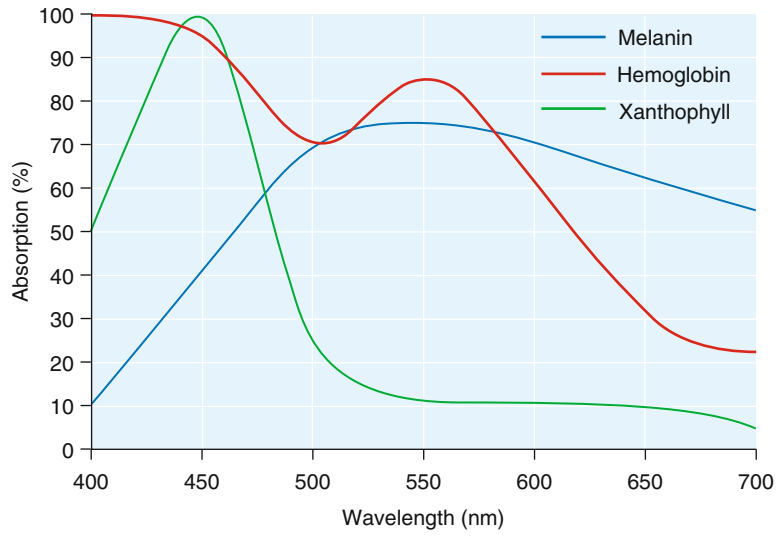

Fig. 9.2 Absorption of visible wavelengths by three ocular pigments (melanin, hemoglobin, and xanthophyll) (Modified with permission from Peyman et al. [6])

Table 9.1 Multiplication factors for retinal spot size in an emmetropic eye

Type of lens	Multiplication factor
60-Diopter	0.92
Area Centralis	1.01
Three-mirror Goldmann	1.08
Transequatorial	1.43
Quadraspheric	1.92

9.2.4.3 Spot Size

The laser burn is round and proportional to the square of the radius. Many more small spots are required to ablate the same area of retina as a few large burns. This is an issue in panretinal photocoagulation. Decreasing a spot size requires a decrease in power level, while increasing a spot size requires a power increase.

9.2.4.4 Magnification Factor

The actual spot size on the retina and, therefore, laser irradiance is influenced by the magnification induced by the type of contact lens used (Table 9.1) [7, 8]. With area centralis of the three-mirror Goldmann lens, the spot size setting on the slit lamp is about the same size as the actual burn on the retina.

9.3 Types of Lasers

9.3.1 Ruby Laser

A solid-state laser based on a pulsed ruby laser was the first commercially available ophthalmic laser photocoagulator and operated at a constant coagulation or exposure time of about 500 μs. The drawback of the ruby laser was its pulsed and uneven output.

9.3.2 Argon Laser

The argon laser was the first laser system to enjoy broad acceptance. It is a continuous wave laser and emits two wavelengths: 514 nm (green) and 488 nm (blue). It is ideally suited for retinal use, since there is excellent absorption at the level of the retinal pigment epithelium and the hemoglobin. Blue light-induced photochemical damage to the macula (due to xanthophyll) can be reduced by incorporating a green filter.

9.3.3 Krypton Laser

Krypton laser sources emitting 647 nm as a continuous wave overcomes the absorption difficulties

of the argon laser. Krypton is poorly absorbed by hemoglobin because it is a red source, so accidental coagulation of blood vessels can be avoided. The disadvantage of the argon laser and krypton laser is a low efficiency of laser production [9].

9.3.4 Dye Laser

Dye lasers have the same disadvantages of an argon or krypton laser. Additionally, the dye (rhodamine) is carcinogenic and requires special handling. Therefore, dye lasers are infrequently used today [10].

9.3.5 Diode Laser

Diode lasers are compact and portable due to their small size. Despite their low input power, diode lasers may represent a significant hazard to vision, especially when the output is collimated, invisible, and of higher power (>3–5 mW) [7, 11, 12].

9.3.6 Holmium Laser

The holmium laser has a CO_2-laser-like action. The holmium laser crystal is similar to the Nd:YAG laser in that the holmium atoms are distributed throughout a YAG host.

9.3.7 Excimer Laser

The excimer laser is a gas laser, which generates a powerful ultraviolet beam. This technique can be used to ablate cornea to any depth. The intensity is graded from the center to the periphery of the circular field so as to control the depth of ablation.

9.4 Techniques of Laser Therapy

9.4.1 Laser Photocoagulation

Laser photocoagulation is the thermal denaturation of tissues using a high-intensity laser of the wavelength range that is intensively absorbed by hemoglobin or other ocular pigments.

9.4.1.1 Indications

Laser photocoagulation is used for a variety of chorioretinal diseases such as diabetic retinopathy, retinal tears or holes, age-related macular degeneration, and chorioretinal tumors such as retinal capillary hemangioma, choroidal hemangioma, and retinoblastoma [3].

9.4.1.2 Complications

With proper attention to detail, complications are infrequent. During laser treatment, focal breaks in Bruch's membrane and retinal hemorrhage may occur. Following photocoagulation, exacerbation of diabetic macular edema, retinal pigment epithelial metaplasia, subretinal fibrosis, and absolute scotomas are seen infrequently. Patients may develop choroidal effusion if a large number of burns are applied during a single treatment session. Laser photocoagulation applied over extensive intraretinal hemorrhage can cause damage to the nerve fiber layer [2, 7, 12, 13].

9.4.2 Transpupillary Thermotherapy

Similar to laser photocoagulation, transpupillary thermotherapy (TTT) is based on the concept of tissue hyperthermia [3, 14]. However, TTT delivers less thermal energy compared with traditional laser and is based on the absorption of near infrared light (810 nm) by the melanin-filled pigment epithelium. In contrast to laser photocoagulation (argon and krypton lasers), TTT uses a lower irradiance with a prolonged exposure and is designed to gently heat the choroidal lesion (sub-photocoagulation level), thus limiting damage to the overlying photoreceptors [15]. Variations in blood flow and chromophore concentrations can strongly influence the treatment effect of TTT. The most common use is the treatment of age-related macular degeneration and small choroidal melanocytic lesions including small choroidal melanoma [14, 15].

9.4.3 Photodynamic Therapy

In PDT, light and light absorbing agents such as verteporfin are combined in an oxygen-rich environment. The chemical reactions follow three main pathways, which start from the common metastable triplet state of the photosensitizer. During the first pathway, a hydrogen atom exchange between the photosensitizer and the substrate molecule leads to the formation of peroxide radicals and oxidized substrate molecules. The second pathway includes an electron transfer between photosensitizer and substrate molecules leading to the formation of radical ions (superoxide radical anion, H_2O_2, and hydroxyl radical). Processes 2 and 3 together are called type I reactions. The third pathway is an energy transfer, which is called type II reaction, leading to bleaching of the photosensitizer itself ("cage" reaction) or oxidization of the adjacent biomolecular target [16].

9.4.3.1 Practical Considerations

PDT is a two-step process. During the first step, the patient receives a verteporfin infusion over 10 min through a cubital vein. Verteporfin is a special dye with a light absorption peak at 692 nm. It is prepared in a 30-ml glucose solution at a dose of 6 mg per m^2 of the body surface area.

The second step implies the exposure of the lesion with laser light, i.e., the photoirradiation. Fifteen minutes after the start of the infusion, the fundus lesion receives a diode laser application via *a* slit-lamp delivery system and a handheld contact lens. A light dose of 50 J/cm^2 is delivered at an intensity of 600 mW/cm^2 for 83 s as one spot covering the lesion in its greatest linear diameter plus a safety zone of 500 µm. The currently recommended treatment protocol has been proven to be safe and effective in the TAP and VIP trials [17].

9.4.3.2 Indications

In ophthalmology, PDT is used in the treatment of choroidal neovascularization due to AMD and secondary CNV in high myopia, following presumed ocular histoplasmosis syndrome (POHS), central serous retinopathy, and other conditions leading to ingrowth of choroidal vessels following an alteration of Bruch's membrane [16], as well as retinal capillary hemangioma [18, 19] and circumscribed choroidal hemangioma [3, 20, 21].

9.4.3.3 Complications

One of the most important problems with current PDT regimen is the high rate of recurrence of choroidal neovascularization in the setting of AMD. Recurrence is triggered by a transient occlusion of the adjacent choriocapillary layer leading to an increased expression of vascular endothelial growth factor (VEGF) [22]. In occult lesions with RPE detachment, a severe loss in vision may occur in 2–4 % of treated eyes. RPE rips were described early after PDT due to enhanced exudation or later during follow-up as a result of a reactive fibrosis of the membrane. Excessive and multiple additive treatments can cause atrophy of the choriocapillaris and retinal pigment epithelium [21, 23].

9.5 Summary

Laser is an acronym for light amplification by the stimulated emission of radiation. There are several diagnostic and therapeutic applications of lasers in ophthalmology. Laser radiation is a parallel, high energy, coherent, and monochromatic beam of light. Tissue effects of laser can be either due to absorption or ionization. The ionization is primarily used to incise tissues of the eye, e.g., in YAG capsulotomy. Absorption interaction is used in laser photocoagulation, thermotherapy, and photodynamic therapy. Laser photocoagulation is the thermal denaturation of tissues by using laser wavelengths that are absorbed by ocular pigments. In contrast to laser photocoagulation, thermotherapy uses a lower irradiance with a prolonged exposure and is designed to gently heat the choroidal lesion. Photodynamic therapy offers selective tissue laser effects by using light absorbing agents (such as verteporfin).

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Principles of Radiation Therapy

10

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10.1 Introduction

Radioactivity was first described by Henri Becquerel and Pierre and Marie Curie in the late 1890s. Wilhelm Roentgen discovered x-rays in 1895, and subsequent physics and biology research revealed the therapeutic properties of radiation. X-rays were first used to treat cancer in 1897. Soon after, the concept of brachytherapy was developed when radium was implanted into tumors for therapeutic effect. Low-voltage x-ray machines were built in the 1920s for the external treatment of superficial tumors. The first cyclotron (used to accelerate heavy particles such as protons, neutrons, and deuterons) was invented in 1932 in California. In 1951, the first clinical cobalt-60 unit was built in London, Ontario, Canada. It created a gamma ray photon beam from the emissions of a cobalt-60 source as it went through nuclear decay. External beam radiation therapy was further refined in 1953 with the development of linear accelerators (linacs) that could produce megavoltage electron and x-ray photon beams using pulsed microwaves and an electron gun.

With improvements in radiographic imaging techniques such as CT and MRI, conformal radiation therapy has been developed.

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_10, © Springer-Verlag Berlin Heidelberg 2014

Three-dimensional conformal radiation therapy (3D-CRT), intensity-modulated radiation therapy (IMRT), stereotactic radiosurgery, and charged particle therapy focus therapeutic dose while minimizing damage to surrounding normal structures. In this chapter we will review the basic principles of radiation therapy and its application as the definitive, adjuvant, salvage, and palliative management of a variety of ophthalmic cancers.

10.2 Basic Principles

The unique characteristics of an individual element are determined by its atomic structure – the number and configuration of electrons, protons, and neutrons. Radiation therapy takes advantage of the energy created by interaction of electrons, protons, and neutrons with each other. This energy can break chemical bonds and create ions such as oxygen radicals.

10.2.1 Dual Nature of Radiation

Radiation can be in the form of electromagnetic waves, particles, or both.

10.2.1.1 Electromagnetic Radiation

Electromagnetic or photon radiation has a broad spectrum of wavelengths ranging from 10^{7} m (radio waves) to 10^{-13} m (ultrahigh energy x-rays) (Fig. 10.1). Energy is propagated at the speed of light (c) with the frequency (ν) and wavelength (λ) being inversely related: $c = \nu\lambda$. Linear accelerators produce photon beams with wavelengths in the range of 10^{-11} to 10^{-13} m.

10.2.1.2 Particle Radiation

Particle radiation can be neutral (neutrons) or charged (protons, electrons). As the particles travel through space, they interact with matter and produce varying degrees of energy transfer to the medium. Linear accelerators and cyclotrons are used to produce this type of radiation.

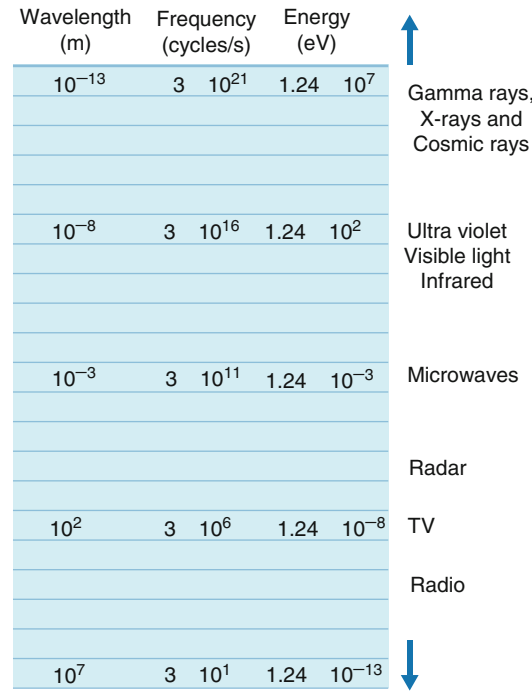

Fig. 10.1 The electromagnetic spectrum. Therapeutic x-rays and gamma rays are in the high frequency, high energy range. Ranges are approximate

10.2.2 Radioactive Decay

Radioactive elements are in an unstable, high-energy state and emit radiation to return to a stable, low-energy state. This process of returning to stability is called decay. Three different types of radiation can be emitted from the nucleus during this process: alpha particles with a positive electrical charge (helium nucleus), beta particles with a negative charge (electrons), and gamma rays with no electrical charge. Radioactive decay is the process utilized in cobalt-60 machines and Gamma Knife radiotherapy.

10.2.3 Ionizing and Nonionizing Radiation

Radiation has both ionizing and nonionizing effects on tissues. Ions are created when an atomic particle or photon hits another atom resulting in loss of an electron, proton, or neutron. Ions interact with DNA

resulting in single-strand breaks, double-strand breaks, or base-pair alterations, impairing a cell's ability to regenerate and duplicate. Nonionizing effects cause excitation of the outermost electrons of an atom. This is of less clinical significance.

10.3 Teletherapy Sources

Teletherapy is the process of delivering radiation from a remote distance. In modern clinical practice, a cobalt-60 unit, linear accelerator, or cyclotron is used to generate and deliver external beam photon therapy and particle radiotherapy.

10.3.1 Cobalt-60 Unit

A cobalt-60 unit holds a radioactive cobalt source that is emitting gamma radiation as it decays to nickel-60. The average energy of the gamma photon beam is 1.25 million electron volts (MeV), with the maximum dose being delivered to a depth of 0.5–1 cm.

10.3.2 Linear Accelerator

A linear accelerator uses high-frequency electromagnetic waves to accelerate electrons to high energies through a linear vacuum tube. The monoenergetic electron beam can be used to treat superficial tumors. Typical energies used range from 6 to 18 (MeV), with 80 % of the maximum dose delivered to a depth of 2–6 cm and a relatively steep dose drop-off beyond (Fig. 10.2).

When deeper tumors need to be treated, or the skin needs to be spared, the linear accelerator electron beam is directed at a target (usually tungsten). The resultant atomic interactions produce a range of high-energy x-rays, also called photons. Photons are characteristically more penetrating than electrons. As an example, a 6 MeV electron beam creates a photon beam with a maximum energy of 6 megavolts (MV). Typical energies of photon beams range from 6 to 18 MV, with the depth of maximum dose ranging from 1.5 to 3.5 cm and a more gradual dose drop-off beyond (Fig. 10.3).

10.3.3 Cyclotron

A cyclotron is a heavy particle accelerator capable of producing neutron and proton beams. Neutrons and protons have a higher linear energy transfer than photons, meaning they cause more damage as they pass through tissue. They cause direct damage to the nucleus of an atom, making them potentially more effective at treating hypoxic tumor cells because there is no dependence on the production of oxygen radicals.

Proton beams have a unique dose distribution characteristic called the Bragg peak. There is a

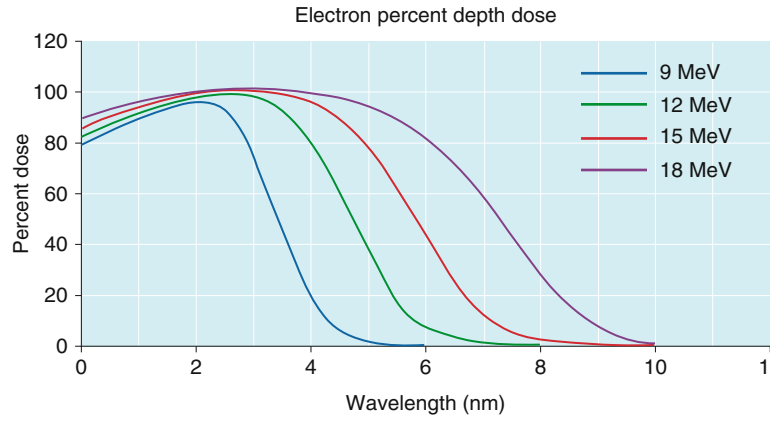

Fig. 10.2 Percent depth dose curves for commonly used electron beams. Divide the beam energy by 3 to estimate the depth in tissue of 80 % of the maximum dose. Divide the beam energy by 2 to estimate the range of penetration

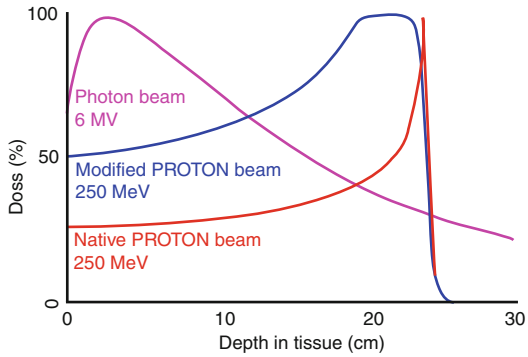

Fig. 10.3 The Bragg peak allows for low energy deposition along the entrance pathway, very high dose delivery over a narrow depth, with minimal exit dose. The spread out Bragg peak allows for dose deposition over a greater distance for treatment of a larger volume of tissue. Available at http://upload.wikimedia.org/wikipedia/commons/1/12/BraggPeak.png

steep peak of maximal dose deposit with sharp distal drop-off (Fig. 10.3). This Bragg peak can be directed accurately and precisely onto the tumor [1]. The sharp distal drop-off and minimal scatter from proton beams translate into less dose to surrounding normal tissues. Proton beam radiotherapy is used for treatment of uveal melanoma and retinoblastoma [2, 3]. Proton beam radiotherapy of uveal melanoma is discussed under uveal tumors.

10.4 Radiation Parameters

10.4.1 Radiation Dose

Radiation absorbed dose is defined in gray (Gy), which represents one joule of energy absorbed per kilogram of mass. Centigray (cGy) is also commonly used and it is 1/100th of a gray. The previous convention was to define dose in 100 ergs absorbed per gram or rad (1 centigray = 1 rad).

10.4.2 Relative Biological Effectiveness

Relative biological effectiveness (RBE) is a measure of the efficiency of a specific radiation in producing a specific biological response. This can be expressed in the following equation: $RBE = D_s/D_r$, where D_s and D_r are the doses of standard radiation (250 kVp x-rays) and a test radiation (r) needed to produce an equivalent biological response (Table 10.1). Protons and neutrons have greater biological effectiveness than photons and electrons.

Table 10.1 Relative biological effectiveness (RBE) values of commonly used radiations

Radiation	RBE
Standard (250 kVp x-rays)	1.0
Linac (6–15 MeV)	~0.8
Cobalt-60	0.8–0.9
Protons	~1.1
Neutrons (19 MeV)	1–2

10.4.3 Cobalt Gray Equivalents

The amount of absorbed dose from neutron and proton beams is higher than with x-ray or gamma ray beams. In order to compare to standard doses, the term cobalt gray equivalents (CGE) was developed: CGE = dose in proton or neutron gray x corresponding RBE value.

10.5 Treatment Parameters

10.5.1 Target Volume

Several target tissue volumes are considered when determining the prescription dose. Gross target volume (GTV) is the visible tumor extension or the surgical bed. Clinical target volume (CTV) is the GTV plus margin to cover microscopic tumor extension. Planning target volume (PTV), the volume ultimately treated, is CTV plus a safety margin accounting for set-up variations and organ motion. The treatment margin beyond the GTV typically ranges from 0.5 to 2 cm, depending on the accuracy of the treatment machine, immobilization device, and the tumor type.

Table 10.2 External beam radiation therapy dose/fractionation schedules for common ophthalmic cancers

Disease	Total dose	Dose per fraction	Number of fractions
Uveal melanoma	60–70 CGE (protons)	14–15 CGE	4–5
Retinoblastoma	40–50 Gy	2–2.5 Gy	20–25
Uveal/orbital metastasis	40 Gy	2 Gy	20
Orbital lymphoma	30 Gy	2 Gy	15
Basal or squamous cell carcinoma of the eyelid	35–42.5 Gy	4.25–7 Gy	5–10
Palliation	30 Gy	3 Gy	10

CGE Cobalt gray equivalent

Table 10.3 Normal tissue tolerance dose to external beam radiation (cGy) [5]

	Complication rate at 5 years		
Organ	5 %	50 %	Clinical endpoint
Brain	6,000	7,500	Necrosis, infarction
Optic nerve	5,000	6,500	Visual acuity <20/200
Optic chiasm	5,000	6,500	Visual acuity <20/200
Lens	1,000	1,800	Symptomatic cataract
Retina	4,500	6,500	Visual acuity <20/200

10.5.2 Total Dose

The total given dose depends on the tumor responsiveness, gross versus microscopic disease, and purpose of the radiation (curative or palliative).

10.5.3 Fractionation

In general, total dose is broken into fractions delivered over many weeks. Fractionation is used to minimize late radiation side effects. The larger fraction size (>200 cGy) is associated with greater tendency for late side effects such as severe dry eye, cataract, and optic neuropathy. On the other hand, reducing the fraction size diminishes the therapeutic effects of radiation (tumor kill). Conventional fractionation uses one treatment per day, at a dose of 180–200 cGy/fraction, for 5 days a week. Recent data suggests that hyperfractionation (1.10–1.20 Gy/fraction; twice daily) may reduce the risk of radiation retinopathy in patients treated for head and neck cancer [4]. Palliative radiation therapy often utilizes larger fraction sizes given over a shorter period of time, with the assumption that the natural history of the disease precludes development of the late radiation effects. Some common dose/fractionation schedules are listed in Table 10.2.

10.5.4 Tissue Tolerance

In treating ophthalmic tumors with external beam radiation, radiation exposure to several critical structures such as lens, optic nerve, opposite orbit, pituitary, and brain must be taken into account. Normal tissue tolerance to external beam radiation (180–200 cGy/fraction) and the total doses that result in 5 and 50 % complication rates 5 years after treatment are listed in Table 10.3 [5]. Ophthalmic complications of radiation therapy are discussed in detail elsewhere (Chap. 11).

10.6 Teletherapy Techniques

10.6.1 External Beam Therapy

When a patient is scheduled for external beam therapy, the first appointment is for simulation. During simulation, the patient is placed in the treatment position, including immobilization devices such as a mask. An MRI scanner, CT

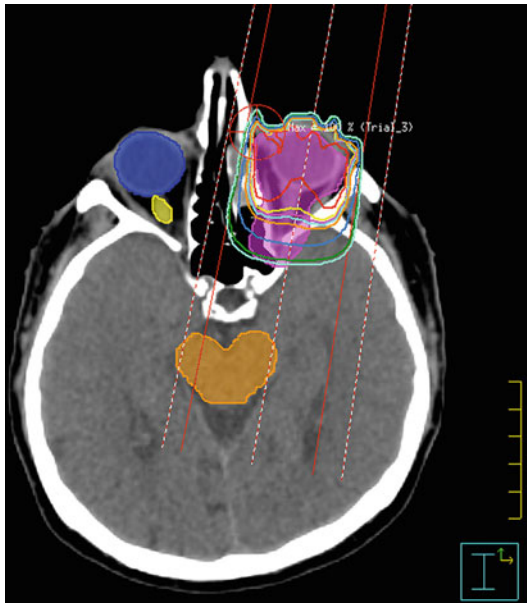

Fig. 10.4 Axial CT with *en face* photon beam demonstrating coverage of the planning target volume (PTV) covered by the 95 % isodose line (*aqua*)

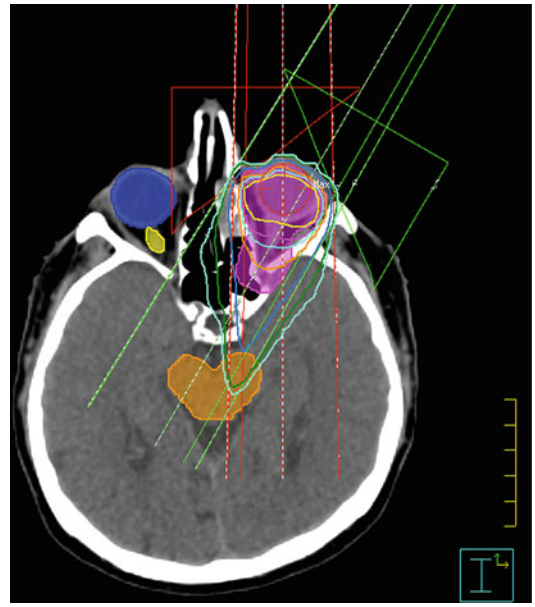

Fig. 10.5 Axial CT with "wedged pair" photon beam demonstrating coverage of the planning target volume (PTV) covered by the 95 % isodose line

scanner, or fluoroscope is used to take images of the patient's anatomy in the treatment position. The tumor area and the surrounding normal structures are identified and contoured on the simulation images. The beam arrangement may be set at the time of simulation, or a dosimetrist may later create several plans to determine the best beam arrangement, which may include a simple technique of one electron beam *en face* (Fig. 10.4) two photon beams delivered at oblique, perpendicular "wedged pair" (Fig. 10.5), opposing angles (Fig. 10.6), or other beam arrangements (Fig. 10.7). Several techniques of beam design and arrangements, so-called lens-sparing radiation therapy, have been developed in order to minimize radiation exposure to the lens and avoid radiation-induced cataracts [1, 6–12].

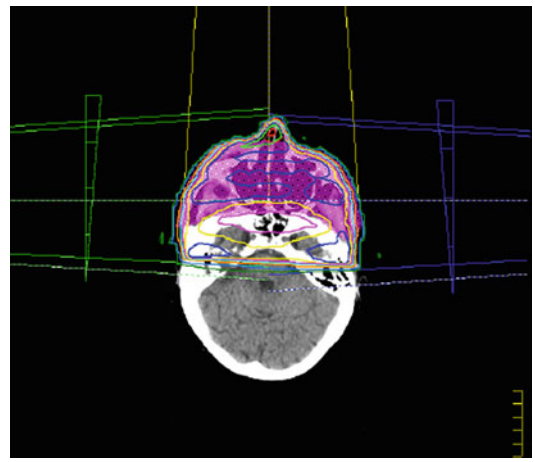

Fig. 10.6 Opposed lateral photon beams demonstrating coverage of the bilateral orbits (100 % isodose line in *yellow*)

10.6.2 Conformal Radiation Therapy

Standard or conventional radiation therapy uses bony landmarks on fluoroscopy or external landmarks on physical examination to determine gross anatomical boundaries for field shapes and sizes. With simulation images from MRI and CT scanners, a radiation oncologist can precisely identify tumor and critical structures, calculate the dose they will receive, and design beam arrangements that will minimize long-term damage. This is termed conformal therapy.

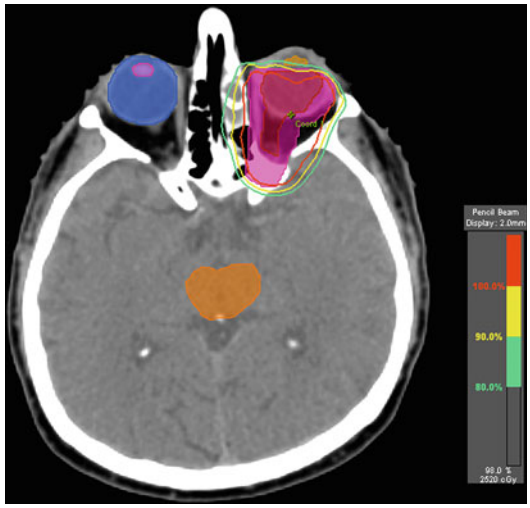

Fig. 10.7 Radiotherapy plan demonstrating isodose distributions with intensity modulated radiotherapy (IMRT), which conform to the planning treatment volume (*pink*)

10.6.3 Three-Dimensional Conformal Therapy

Three-dimensional conformal therapy or 3D-CRT is the technique of using CT or MRI simulation images to create a three-dimensional target. Various beam arrangements are entered into the planning computer and altered until an acceptable dose distribution is reached for both the tumor and normal structures. This process of first entering the beam arrangements and then looking at the dosimetric outcomes is termed forward planning.

10.6.4 Intensity-Modulated Radiation Therapy

In intensity-modulated radiation therapy (IMRT), a process of inverse planning is often used, which means that the dose criteria are set before beam arrangements are designed. The computer generates a plan that best fits the specified criteria. During treatment, a multi-leaf collimator moves dynamically to create hot and cold spots within the volume of treated tissue. The intention is to place the majority of the hot spots within the tumor volume rather than the surrounding normal tissue, while minimizing delivery of high doses of radiation to nearby critical structures, including the lens.

10.6.5 Stereotactic Radiosurgery

Stereotactic radiosurgery uses highly focused, precisely aimed radiation (usually within 0.5 mm of the specified isocenter) to treat small tumors (≤4 cm) at very high doses per fraction. A stereotactic frame fixed to the patient's skull is used for precise localization and positioning. Planning CT and MRI images are taken with the frame in place, so that accurate coordinates can be established for the target. Two forms of stereotactic radiosurgery exist: linac based and Gamma Knife.

10.6.5.1 Linac-Based Stereotactic Radiosurgery

In linac-based stereotactic radiosurgery, specialized cones are used to target the tumor during multi-arc therapy [13]. Stereotactic radiosurgery for uveal melanoma is discussed under uveal tumors.

10.6.5.2 Treatment Delivery

Advances in technology have allowed for the development of highly conformal treatment plans through the ability to fuse MRI with CT for target delineation, development of complex treatment machines, and progress in the capabilities of treatment planning systems. However, with increasing treatment complexity and smaller amounts of surrounding normal tissue included in treatment targets, accuracy of reproducibility of the simulation set-up becomes increasingly important. Historically, physical frames were applied to patients to ensure precise treatment delivery. Verification of treatment set-up, or image guidance, for large treatment fields and simple fields is achieved with portal images. Patients treated with complex treatment plans, small set-up margins, and small numbers of fractions undergo daily image guidance with more advanced systems including low-resolution CT scans (cone-beam CT), orthogonal x-rays, fiducials, and motion-based gating systems being developed [14–19].

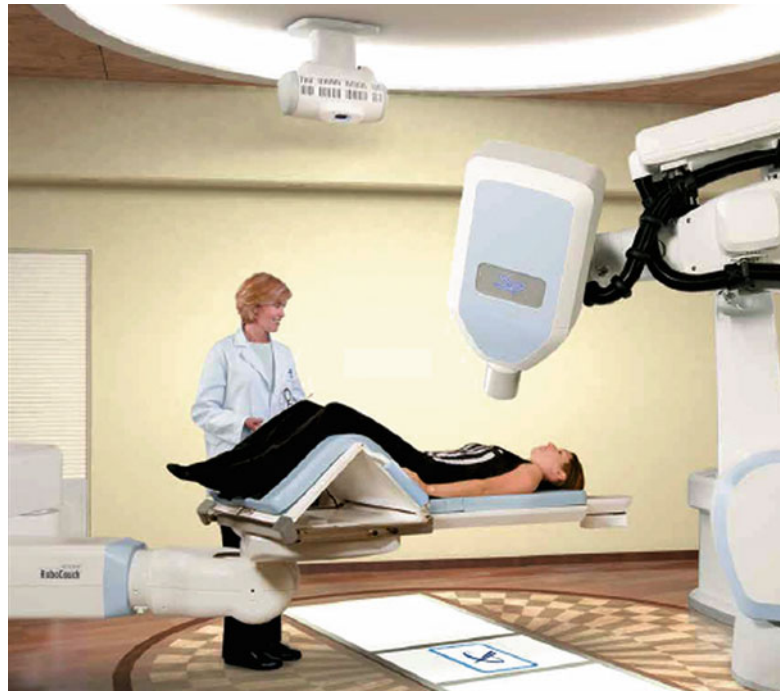

Fig. 10.8 A small, single energy linear accelerator mounted on an industrial robotic arm may be utilized for delivery of stereotactic radiotherapy (CyberKnife Robotic Radiosurgery System, Accuray, Sunnyvale CA). The robotic arm allows for greater freedom of movement. Image guidance is obtained through orthogonal planar radiographs. Reproduced with permission from http://www.cyberknife.com/uploaded-Files/CyberKnife_Overview/500929.A_CyberKnife_Patient_Brochure_FINAL.pdf. Accessed 24 Dec 2012

Patients treated with IMRT, fractionated stereotactic radiotherapy, or SRS may be treated with the Cyberknife® system [20–22]. This consists of a small, single energy linear accelerator mounted on a robotic arm (Fig. 10.8). Advantages of the Cyberknife® system include nearly unlimited treatment angles, whereas traditional linear accelerators rotate around a point in space and the treatment table must be moved to accomplish similar treatment angles. However, image guidance is limited to orthogonal x-rays, whereas confirmation of patient positioning may be achieved with pretreatment cone-beam CT imaging prior to treatment with standard linear accelerators. Also, standard linear accelerators allow for treatment with either electrons or low-energy photons.

10.6.5.3 Gamma Knife Radiosurgery

With Gamma Knife, gamma ray beams from 201 cobalt-60 sources are collimated to focus on a single point. The patient's head is positioned to place the tumor at that isocentric point [23]. Gamma Knife radiosurgery for uveal melanoma is discussed under uveal tumors.

10.7 Charged Particle Therapy

The use of charged particles for the treatment of ophthalmologic tumors is well studied. Charged particles are well suited for the treatment of ophthalmologic tumors as the treatment target is often in proximity to several nearby critical structures including the uninvolved orbital contents, optic nerves, and brain. The physical dose distribution characteristics of charged particle beams allow for treatment of small structures to high doses while sparing nearby critical structures. Charged particles deliver a high concentration of energy over a discrete range, known as the Bragg peak, with a dramatic drop in dose-deposition beyond the depth of maximum. The range of the Bragg peak may be widened, known as the "spread out Bragg peak," to allow for treatment of tumors of any depth and diameter [14, 24].

The very sharp dose gradients offer a dosimetric advantage by delivering high doses of radiation to the treatment target while minimizing treatment of nearby normal structures. To maximize the benefits of high dose gradients, accuracy of delivery is paramount. Treatment frames, thermoplastic

mesh masks molded to the anatomy of the individual, fiducial placement, video-based systems, orthogonal x-rays, and cone-beam CTs are used to ensure accurate set-up prior to, and in some cases during, treatment delivery. This therapy offers dosimetric advantages, but is not widely available. At the writing of this chapter, there are 10 centers offering proton radiotherapy in the United States.

10.8 Brachytherapy

Brachytherapy is the process of placing a radioactive source next to or within a tumor. The dose characteristics of the isotopes used in brachytherapy are such that a very high dose of radiation is given within millimeters of the source and there is a steep dose drop-off outside of that range, thus protecting surrounding normal structures. Isotopes that have been employed include cobalt-60, iridium-192, ruthenium-106, gold-198, palladium-103, and iodine-125 [25]. Brachytherapy is used for the treatment of intraocular tumors such as choroidal metastasis [26], uveal melanoma [25, 27], and retinoblastoma [28]. Brachytherapy for uveal melanoma is discussed under uveal tumors.

10.9 Summary

Radiation therapy takes advantage of the energy created when electrons, protons, and neutrons interact with each other. Radiation can be in the form of particles and electromagnetic waves. Linear accelerators and cyclotrons are used to produce radiation. In addition, radioactive decay of an isotope can generate radiation. Radioactive decay is the process utilized in cobalt-60 machines and Gamma Knife radiotherapy. Radiation interacts with DNA, resulting in single-strand breaks, double-strand breaks, or base-pair alterations, impairing a cell's ability to regenerate and duplicate. Protons and neutrons have greater biological effectiveness than photons and electrons. Several treatment parameters such as target volume, total dose, and fractionation influence tissue effects. The larger fraction size (>200 cGy) is associated with greater tendency for late side effects such as severe dry eye, cataract, and optic neuropathy. Normal tissues vary greatly in their tolerance to external beam radiation. Teletherapy techniques include conventional external beam therapy, three-dimensional conformal therapy, intensity-modulated radiation therapy, stereotactic radiosurgery, and Gamma Knife radiosurgery. Brachytherapy is the process of placing a radioactive source next to or within a tumor. Ruthenium-106, palladium-103, and iodine-125 are commonly used isotopes for the treatment of ophthalmic tumors. With continued improvements in radiographic imaging techniques and better understanding of radiation tissue effects at the molecular level, it will be possible to precisely focus therapeutic dose while minimizing damage to surrounding normal structures.

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Ocular Complications of Radiotherapy

11

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11.1 Introduction

Although radiotherapy is the standard treatment for most intraocular malignancies, various ocular complications may occur, including radiation-induced dry eye, cataract, secondary glaucoma from neovascularization of the iris, scleral necrosis, retinopathy, and optic neuropathy. In this chapter we review the ocular side effects of radiation administered from brachytherapy, proton beam radiotherapy, and external beam radiotherapy and their potential treatments. Particular emphasis will be placed on radiation retinopathy and optic neuropathy, the two most visually significant complications of radiotherapy.

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_11,

The ideal method to deliver radiation to malignant tissue is to reduce the dose to critical normal structures without decreasing dose to the tumor. Within the realm of brachytherapy, these methods can be broadly separated into two categories: low-penetrating brachytherapy isotopes and intraocular radiation blocking. The radioisotope iodine-125 was chosen for the Collaborative Ocular Melanoma Study because it emits relatively low-energy gamma ray photons and is readily commercially available [1]. Its low energy results in ease of shielding, which reduces dose to the patient outside the eye and protects medical personnel involved in the treatment. Theoretically, lower-energy gamma radiation results in lower penetration through the eye itself due to increased photoelectric interactions, which would lead to sparing of critical ocular structures. For that reason, the isotope palladium-103 with a steeper dose falloff in the eye than iodine-125 results in a modest decrease in predicted dose to critical structures [2, 3]. Although its use has been accepted by the American Brachytherapy Society [4], this isotope is not in wide use as its clinical benefit has not been demonstrated in controlled clinical trials.

In contrast to gamma radiation, which has an approximately exponential dose falloff, beta (electron) radiation is characterized by a much steeper dose falloff after the depth of maximum dose. The beta emitter ruthenium-106 has been in use for ocular melanoma for several decades, primarily in Europe. The rapid dose falloff indicates a theoretical benefit to critical structures displaced from the tumor and, however, also means that it can be safely used only with low-height tumors, up to about 5 mm [5]. Thus, in principle, ruthenium-106 therapy would be most suited to treatment of low-height tumors in the anterior portion of the eye [6].

As early as 1990, Finger et al. discussed the concept of intraocular radiation blocking [7]. Finger used a rabbit model to investigate attenuating radiation with intraocular iodinated contrast agent. Significant attenuation was obtained, but the technique was limited by the fact that the contrast agent exited too rapidly from the eye. More recently, our group demonstrated the use of silicone oil as an attenuating agent [8]. As with iodinated contrast agents, silicone oil works in this case because of the combination of an absorber with a significantly higher atomic number compared to water and a low-energy gamma radiation, leading to enhancement of the photoelectric effect. Our Monte Carlo simulation and experimental study found up to 55 % attenuation inside the human eye, relative to saline, was possible with the silicone oil. Clinical use of this technique is facilitated by the fact that vitrectomy with silicone oil endotamponade is an established surgical technique for treatment of non-oncologic ocular diseases.

11.2 Adnexa

11.2.1 Extraocular Muscles

Extraocular muscles are theoretically shielded from most of the iodine-125 radiation as only 0.1 % of radiation passes through a 0.5-mm-thick gold foil [1]. However, with the current plaque design, extraocular muscles may actually be exposed to a significant amount of laterally directed and uncollimated radiation. Kiratli et al. [9] compared biopsy specimens from radiation-exposed extraocular muscles with nonirradiated, extraocular muscles from enucleated controls and found that the radiation-exposed muscles had a focal decrease in muscular tissue with increased fibroblasts and collagen. Furthermore, on electron microscopy, a loss of sarcoplasmic reticulum with mitochondrial swelling was noted. The authors argued that the sarcoplasmic reticulum loss, vascular wall thickening, and focal muscle tissue loss suggested radiation injury rather than pure mechanical injury due to stretching and ischemia. Although these may simply represent nonspecific ultrastructural changes, they may affect extraocular muscle function.

11.2.2 Dose Relationship

Sener et al. [10] observed that 60 % of their patients (12/20) had ocular alignment and motility problems following plaque brachytherapy. However, only 10 % (2/20) of patients complained of diplopia. Dawson et al. 67 found that 1.7 % (16/929) of their patients developed persistent diplopia or strabismus following plaque brachytherapy during an 8-year follow-up. For 69 % (11/16) of these patients, the onset occurred within the first year. Whether these findings are attributable to radiation or simply mechanical injury from muscle manipulation, patients and physicians should be aware of this potential complication.

11.2.3 Treatment

Sener et al. [10] suggest using either prism correction or botulinum toxin A injections in the early postoperative period and recommend waiting at least 6 months before pursuing surgical correction of strabismus as radiation effects may be variable for some time.

In our experience, patients may require strabismus surgery in the long term to correct exotropia secondary to the loss of fixation in a poor seeing eye. We find it helpful to avoid use of an eye patch after brachytherapy in order to stimulate fixation to the greatest extent possible in the treated eye and encourage orthophoric alignment.

11.3 Periorbital Skin

11.3.1 Acute

Loss of eyelashes is one of the first and most common adverse effects to occur after radiotherapy, although they do usually grow back (Fig. 11.1). Erythema may occur within hours of radiotherapy [11]. Desquamation and scaling of the skin can follow lower-dose (10 Gy) radiation exposure, and more severe dermatitis occurs with higher doses (40 Gy) [12].

11.3.2 Late

Loss of eyelashes and eyebrows may be permanent [11, 12]. Other late sequelae of regular and high-dose radiotherapy (40 Gy or higher) to the eyelids include trichiasis, telangiectasia, hyperpigmentation, hyperkeratosis, entropion, ectropion, and punctal occlusion. Eyelid atrophy, necrosis, and frank ulceration are uncommon.

11.3.3 Management

Mild acute radiation effects can be relieved with the administration of topical corticosteroids, such as 1 % hydrocortisone. Late effects may be remedied by wound debridement, antibiotic therapy, and reconstructive surgery.

11.4 Conjunctiva

11.4.1 Acute

Conjunctivitis, chemosis, and a clear or purulent discharge may occur when radiotherapy doses of >5 Gy are used [11, 12].

11.4.2 Late

Effects of radiotherapy on the conjunctiva include telangiectasia, symblepharon, and sequelae of loss of goblet cells (keratinization and scarring). Doses of approximately 50 Gy lead to conjunctival scarring. Severe contracture occurs with doses of more than 60 Gy, and symblepharon is frequent with doses above 80–100 Gy [13].

11.4.3 Management

Topical corticosteroids are indicated for early conjunctivitis and chemosis. Artificial tears and ointment help replace the moisture lost due to damage to goblet cells and keratinization.

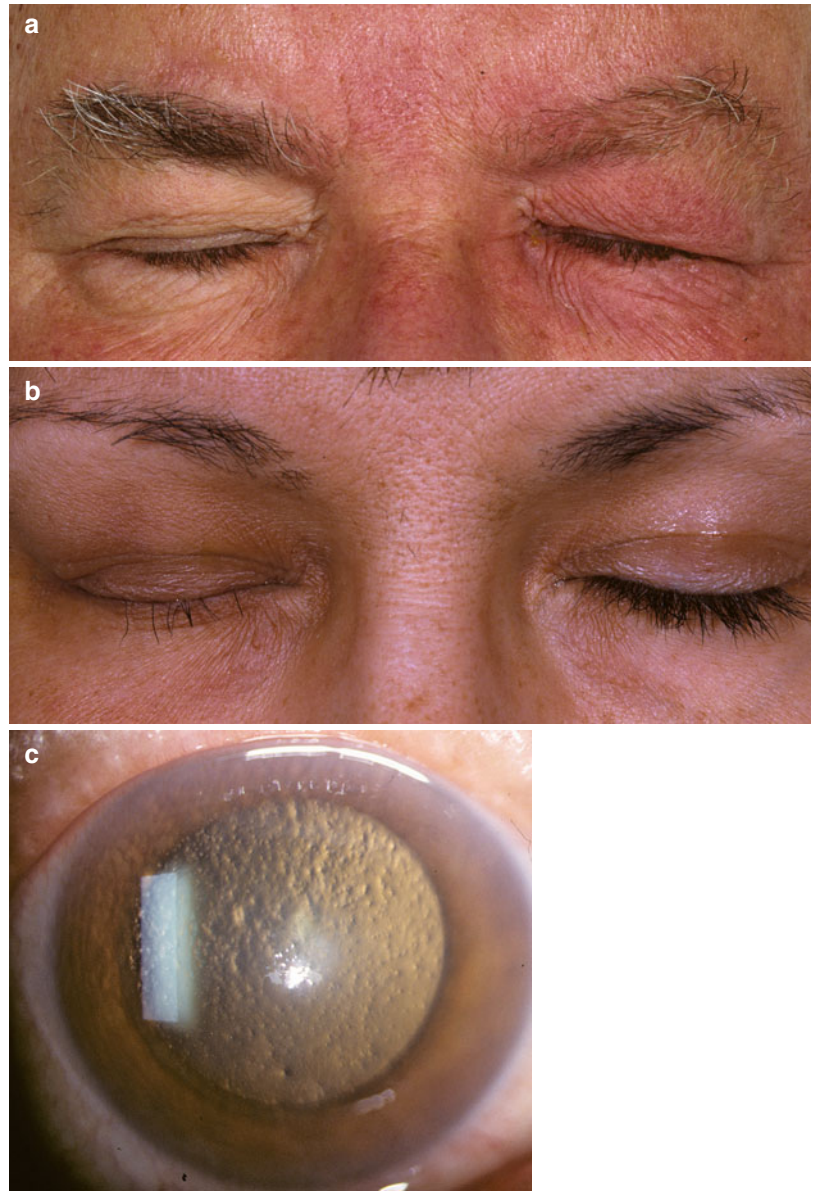

Fig. 11.1 Acute complications of radiotherapy. (**a**) Radiation dermatitis. (**b**) Loss of eyelashes. (**c**) Punctate keratitis

11.5 Cornea

11.5.1 Radiation-Induced Dry Eye and Keratitis

Dry eye and keratitis are complications frequently seen after external beam radiotherapy for uveal metastases and proton beam radiotherapy for uveal melanoma, but are infrequent following brachytherapy (Fig. 11.1). An increase in conjunctival epithelial stratification and reduction in goblet cells contribute to dry eye [14]. Tear film instability and dysfunction may cause punctate epithelial erosions [15].

At our center, dry eye after iodine-125 brachytherapy is not more frequent than other procedures that involve alteration of the conjunctiva, such as scleral buckling. Dry eye was reported in 8.3 % of patients and occurs an average of 20.7 months after treatment with iodine-125

plaque [16]. In contrast, another study found that keratitis was present in 20.9 % of patients at 2 years after treatment, and this decreased to 2.8 % of patients by 5 years after treatment [17]. Few other studies reporting on iodine-125 brachytherapy describe this complication.

There is greater literature on developing dry eye after external beam radiation therapy and in particular its relationship to lacrimal gland dose. Parsons et al. reported on the University of Florida experience of patients treated with external beam radiation that included the entire orbit for head and neck cancers and found all patients developed dry eye who received doses ≥57 Gy, whereas 19 and 0 % did at doses of 30–45 Gy and <30 Gy, respectively [18]. The dose threshold for the lacrimal gland is different for fractionated stereotactic radiotherapy with one study that treated patients with 50 Gy in 5 fractions showing a median dose of 10 Gy/fraction resulting in a 50 % probability of dry eye syndrome, while a median dose of 7 Gy/fraction causing a 50 % risk of low Schirmer results [19]. Using a single fraction approach, dose to the lacrimal gland has also been shown to significantly correlated with Schirmer test values at 24 months when comparing the treated eye to the nontreated one [20]. Ultimately the dose to the lacrimal gland is an important factor in the development of a dry eye and efforts should be made to respect its tolerance as much as possible without compromising tumor coverage.

11.5.2 Treatment

Symptomatic treatment is recommended including topical lubricants and lacrimal punctal occlusion.

11.6 Scleral Necrosis

Scleral necrosis following plaque brachytherapy has been reported, often in association with postoperative brachytherapy. Petrovich et al. described the histologic appearance of enucleated eyes with choroidal melanoma that had been treated with plaque brachytherapy. Scleral atrophy was seen in 33 % of post-plaque eyes (Fig. 11.2) [21]. However, in the studies with iodine-125 brachytherapy, few reports mention scleral atrophy or necrosis as a complication. Stack et al. [22] documented that none of their 84 patients developed scleral necrosis after iodine-125 brachytherapy. Kaliki et al. recently reported a scleral necrosis rate of 1 % following iodine-125 brachytherapy with ciliary body location being the strongest risk factor. Observation was indicated in the majority of cases [23].

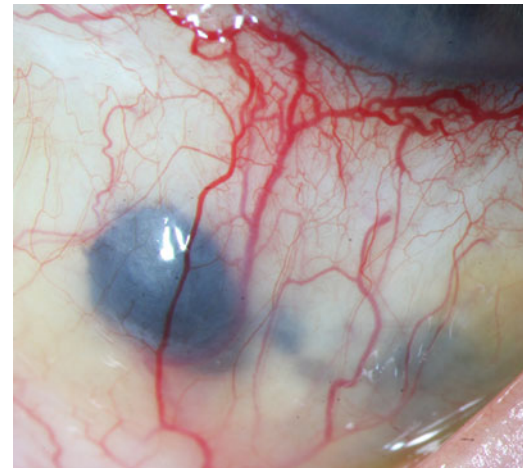

Fig. 11.2 Scleral atrophy 10 months following plaque radiotherapy for a large ciliochoroidal melanoma

11.7 Iris

11.7.1 Radiation-Induced Iris Neovascularization and Neovascular Glaucoma

Though complications of the anterior segment occur frequently with external beam radiation, they also occur with plaque brachytherapy [24]. Ischemia associated with radiation retinopathy may result in iris neovascularization. This presents clinically as rubeosis iridis and neovascular glaucoma (Fig. 11.3). A careful examination of the iris and anterior chamber angle prior to pupillary dilation may detect early signs of neovascularization.

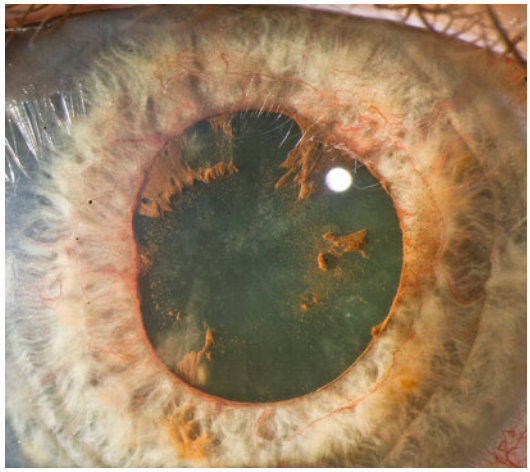

Fig. 11.3 Neovascular glaucoma following radiation therapy

Rubeosis iridis following iodine-125 plaque brachytherapy is reported at rates of 4–23 %, occurring at a mean of 26.7 months. Neovascular glaucoma rates ranged from 2 to 45 %. Numerous factors may contribute to iris neovascularization. Studies using cobalt-60 and palladium-103 have associated increased neovascularization with an anterior tumor location [25, 26]. Increased tumor thickness is associated with higher rates as well as decreased time to the development of iris neovascularization and may be related to the elevated levels of tumor-related angiogenic factors [24, 27]. Recent data on a cohort of patients treated with stereotactic radiotherapy where six underwent enucleation for neovascular glaucoma and four because of tumor progression showed a lack of conclusive anterior segment changes attributable to radiation [28]. Mechanistically it is thought that proangiogenic factors released from radiation damage to endothelial cells diffuse through the vitreous to reach the anterior segment thereby promoting the formation of neovascularization on the iris and in the angle. This proposed mechanism is similar to other retinal vascular diseases like diabetic retinopathy [29].

11.7.2 Treatment

Currently, there are few studies supporting any specific treatment for radiation-induced neovascular glaucoma or rubeosis iridis (Chap. 16). Enucleation has traditionally been indicated for eyes with neovascular glaucoma in the setting of media opacity and poor vision. The rate of enucleation secondary to neovascular glaucoma after iodine-125 brachytherapy ranges from 1 to 12 % and indicates the difficulty in managing this complication. Although controversial, tube shunt procedure with vitrectomy and endolaser in eyes with good visual prognosis may also be considered. A report by Yeung et al. suggests that intravitreal bevacizumab may be used to treat neovascular glaucoma and salvage the eye following proton beam radiotherapy (Fig. 11.4) [30].

11.8 Lens

11.8.1 Clinical Features

Ionizing radiation is known to damage the lens equatorial fibers because of their high mitotic rate [31]. The compensatory mitosis occurs with disrupted organization and leads to deposition of Wedl cells at the posterior pole. The clinical appearance of a radiation cataract is of a small dot at the posterior pole of the lens and subsequently increases to a diameter of 1–2 mm [32]. The opaque region is comprised of scattered granules and vacuoles. As the cataract continues to develop, the center of the opacity clears, and the overall appearance is that of a doughnut with a total diameter of 3–4 mm. Radiation exposure may also lead to the development of cortical cataract or exacerbate existing nuclear sclerotic cataract [33].

11.8.2 Dose Relationship

The development of cataract is associated with a dose-dependent increase in radiation to the lens. In the largest study to date, the Collaborative Ocular Melanoma Study followed the incidence of cataract development in phakic patients over the first 5 years following iodine-125 brachytherapy [34]. The study found that 68 % (362/532) of study eyes developed vision-limiting cataract or underwent cataract surgery after iodine-125 brachytherapy

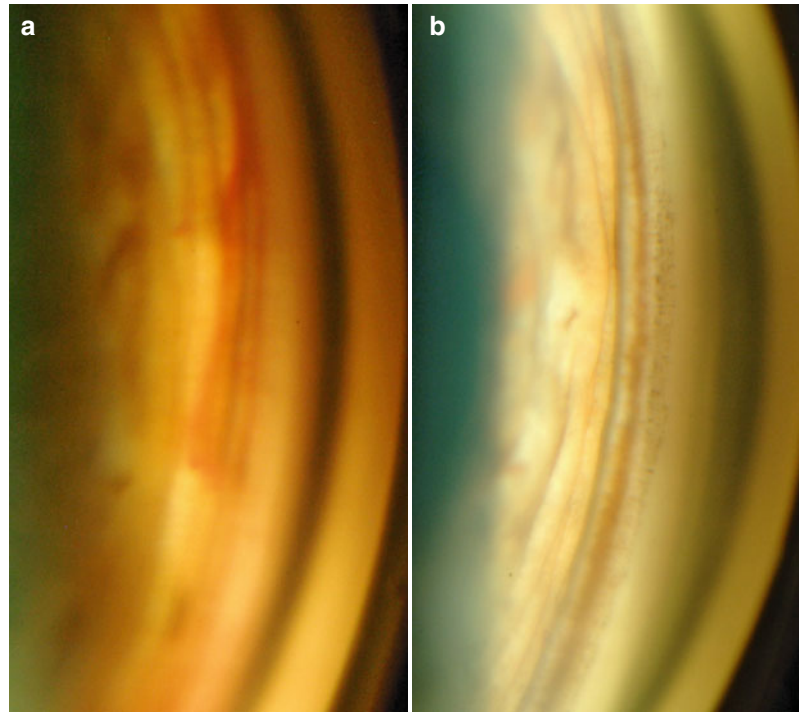

Fig. 11.4 Gonio-photograph showing neovascularization and bleeding in the anterior chamber angle (**a**) Eight weeks after treatment with pan-retinal photocoagulation and intravitreal injection of bevacizumab (1.25 mg/0.05 ml). Note that the angle neovascularization and hyphema has resolved completely (**b**)

with a greater proportion developing cataract following higher doses to the lens. With a cumulative dose to the lens of 24 Gy or more, the 5-year cumulative incidence of cataract was 92 % compared with 65 % in those with less than 12 Gy.

The radiation dose to the lens is affected by both tumor size and location. Increasing tumor height has been shown to decrease the time to cataract development and a greater tumor diameter increases the risk of cataract [17, 24]. The location of the tumor is also important as treatment of an anterior tumor exposes the lens to more radiation. Fontanesi et al. [35] found that cataract developed earlier with anterior tumors (median 11 months posttreatment) compared with posterior tumors (median 26 months posttreatment) with a greater proportion of cataract occurring in eyes with anterior tumors. Data from patients treated with stereotactic radiation (10 Gy×5 fractions) also suggests a dose response relationship with cataract formation and dose to the lens and ciliary body with a median dose of 5 Gy/fraction causing a cataract in 50 % of cases and an overall rate of CTCAE version 3 grade 3 cataracts of 10 % [36].

11.8.3 Treatment

Radiation-induced cataract may be successfully treated with standard surgical techniques with improvement in vision (Chap. 15) [34]. Patients whose vision may fail to improve frequently have comorbidities, including radiation retinopathy, vitreous hemorrhage, retinal detachment, or optic neuropathy. We have found that eyes developing cataract following iodine-125 plaque brachytherapy tolerate standard phacoemulsification with lens implantation well.

11.9 Radiation Retinopathy

11.9.1 Clinical Features

Radiation retinopathy was first described in 1933 and includes microaneurysms, telangiectases, neovascularization, vitreous hemorrhage, hard exudates, cotton wool spots, and macular edema (Fig. 11.5). The pathogenesis of radiation reti-

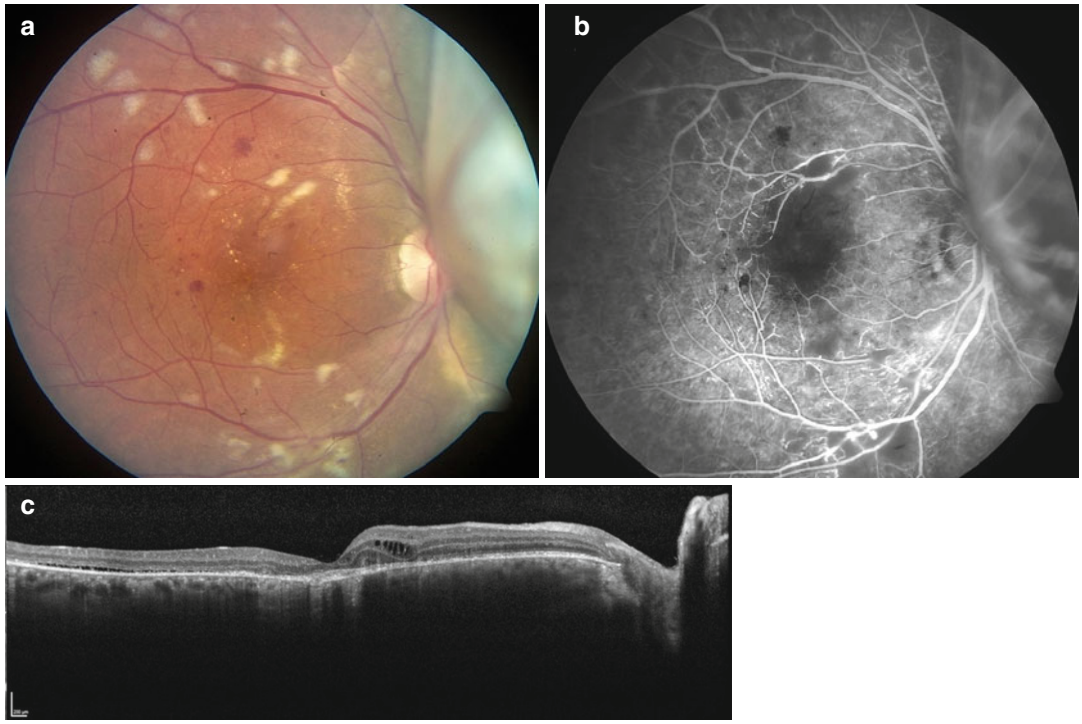

Fig. 11.5 Characteristic ophthalmoscopic features of nonproliferative radiation retinopathy, such as cotton wool spots, telangiectasia, retinal hemorrhages, and macular edema following brachytherapy for choroidal melanoma (**a**). Retinal capillary nonperfusion in the macula and microaneurysms are most evident on the fluorescein angiography (**b**). Cystoid macular edema and foveal atrophy on the optical coherent tomograph (**c**)

nopathy begins after radiation exposure with the preferential loss of vascular endothelial cells and relative sparing of pericytes [11]. It has been hypothesized that the differential sensitivity between retinal endothelial cells and pericytes may be related to the direct exposure of the endothelial cells to high ambient oxygen and iron from blood that generates free radicals and damages cell membranes [37]. In acellular, poorly supported capillaries, microaneurysms emerge and telangiectatic-like channels appear, straddling regions of nonperfusion. Ultimately, the inner retinal ischemia leads to neovascularization, vitreous hemorrhage, tractional retinal detachment, and macular edema. On fluorescein angiography, the earliest changes that appear are focal capillary closure with neighboring areas of irregular capillary dilation and microaneurysms (Fig. 11.5).

11.9.2 Dose Relationship

The rate at which radiation-induced retinopathy and maculopathy develop ranges from 10 to 63 % and 13 to 52 %, respectively. The mean time to develop maculopathy was found to be 25.6 months after treatment [26]. The risk of radiation retinopathy and maculopathy after plaque therapy is related to radiation dose and factors affecting radiation dose, such as the height and location of tumor (Fig. 11.6). Higher radiation dose and tumors with thickness greater than 4 mm increase the risk for radiation maculopathy. Stack et al. [22] found a 63 % risk for radiation maculopathy if the dose to the macula exceeded 90 Gy. With the advent of ocular coherence tomography to evaluate the macular anatomy and wide-field angiography, which detect preclinical features of altered retinal

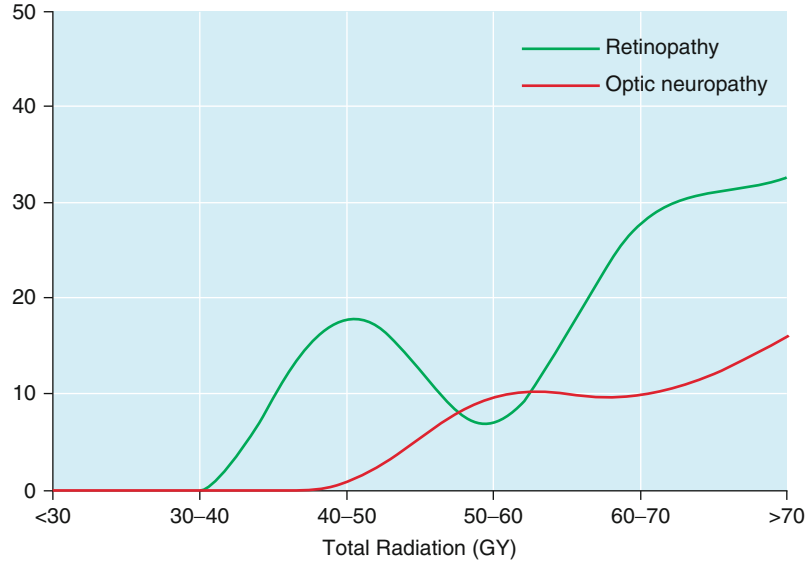

Fig. 11.6 Total dose of radiation and risk of radiation retinopathy and radiation optic neuropathy (Modified with permission from Monroe et al. [51] and Bhandare et al. [52])

anatomy and function, radiation retinopathy is likely to occur in almost every patient treated with radiation over time.

11.9.3 Treatment

Numerous treatment modalities have been utilized in the management of radiation retinopathy and maculopathy. Recent studies and their results include intravitreal injections of triamcinolone and bevacizumab, laser photocoagulation, and hyperbaric oxygen treatment (Box 11.1).

Box 11.1: Salient Features of Radiation Retinopathy

- Total dose and fraction size of radiation are the key determinants
- Presence of diabetes and history of prior chemotherapy increases the risk and severity of radiation retinopathy
- Doses of less than 45 Gy (fractions size ≤2.0 Gy) are unlikely to cause significant retinopathy in the absence of coexisting host risk factors
- Insult to vascular endothelial cell is the underlying basis of radiation retinopathy
- Discrete foci of capillary nonperfusion (cotton wool spots) and telangiectasia are the earliest features
- The incidence peaks 2–3 years after radiation exposure
- At present, there is no effective treatment of radiation retinopathy

Intravitreal triamcinolone acetonide is used to treat macular edema secondary to other retinal vascular diseases. Although the mechanism is poorly understood, triamcinolone may help to restore a compromised inner blood–retinal barrier [38]. Triamcinolone acetonide is thought to modulate cytokines and regulate capillary permeability [39]. However, steroid-induced glaucoma necessitating topical therapy and or surgery may complicate this treatment modality.

Bevacizumab has been used to treat exudative age-related macular degeneration, diabetic retinopathy, and vein occlusion. Bevacizumab is a humanized monoclonal antibody to vascular

endothelial growth factor (VEGF), and blocking VEGF is thought to decrease vascular permeability and inhibit abnormal neovascularization [40]. Twenty-one patients with radiation retinopathy following palladium-103 brachytherapy were treated with intravitreal bevacizumab (1.25 mg/0.05 ml) every 6–12 weeks. After a mean follow-up of 7.8 months, 86 % (18/21) had stable or improved visual acuity, with 14 % (3/21) regaining 2 or more Snellen lines [38].

However, some studies utilizing bevacizumab suggest that the improvement is likely temporary [41]. It is our experience that intravitreal bevacizumab in patients with recent onset visual decrease secondary to radiation maculopathy have only a transient subjective response. However, vision usually returns to pre-injection treatment levels within a year. It is generally believed that the role of bevacizumab for radiation maculopathy is limited, as the initial damage has occurred at the time the radiation was delivered.

Laser photocoagulation has also been used to treat or prevent radiation retinopathy and macular edema. Pan-retinal laser photocoagulation has been shown to successfully treat proliferative radiation retinopathy, whereas focal photocoagulation has been used to treat or prevent macular edema with a more variable degree of vision improvement [42–44]. In 19 patients with radiation-induced macular edema, focal laser therapy led to resolution of edema in 26 % (5/19) at 6 months compared with 4 % (1/19) in the untreated group [42]. However, after 2 years, there was no significant difference in visual acuity between treated and untreated eyes.

11.9.4 Preventive Strategies

As the current experience with treating radiation damage of the retina remains disappointing, recent efforts to attenuate iodine-125 with vitreous substitutes at the time of treatment have been reported. Oliver et al. were the first to demonstrate an attenuating effect of silicone oil 1,000 centistokes, silicone oil 5,000 centistokes, perfluorocarbon, and heavy liquid against iodine-125 in cadaver eyes, in an in vitro model, and with Monte Carlo modeling. The effect of silicone oil 1,000 centistokes was the most robust at approximately 55 % when compared to vitreous [8]. Therefore, performing vitrectomy with silicone oil 1,000 centistokes at the time of iodine-125 plaque surgery may be a feasible method to reduce the exposure of healthy tissues to iodine-125 radiation and is currently being offered as a treatment option for uveal melanoma at some centers.

11.10 Radiation Optic Neuropathy

11.10.1 Clinical Features

Although poorly understood, ionizing radiation is believed to damage the optic nerve through injury to both glial and endothelial cells. Over time, these injured cells accumulate and lead to demyelination and neuronal degeneration. Damage to the vascular endothelial cells leads to vascular occlusion and necrosis. Pathology specimens show a decreased number of endothelial cells and endothelial cell-lined vessels as well as fibrosis of vessel walls, reactive gliosis, ischemic demyelination, and perivascular inflammation [45, 46]. The slow cellular turnover rate of endothelial and glial cells is consistent with the delayed onset of radiation-induced optic neuropathy [47].

Clinically, radiation-induced optic neuropathy may present with sudden, painless, monocular vision loss. Radiotherapy may lead to ischemic insult anterior to the lamina cribrosa, which causes swelling of the optic nerve head [48]. Other features of optic neuropathy may include disc swelling, peripapillary hard exudates, and hemorrhages (Fig. 11.7). Optic nerve head swelling eventually resolves resulting in a pale and featureless nerve associated with limited vision [49].

11.10.2 Dose Relationship

Important risk factors for developing postradiation optic neuropathy include close proximity of the tumor to the optic disc, greater dose to the optic disc, and large tumor size (Fig. 11.6) [33, 44, 45].

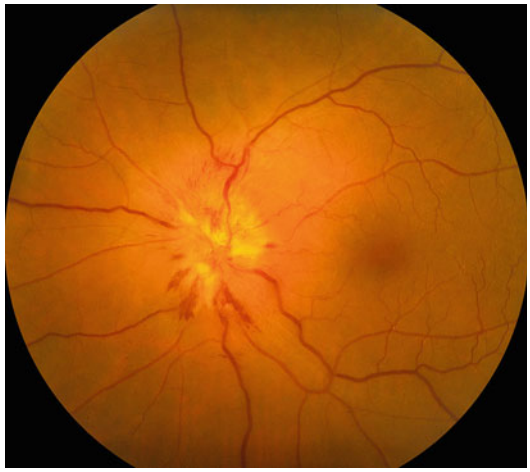

Fig. 11.7 Typical appearance of radiation optic neuropathy. Note optic disc swelling with surrounding exudates and hemorrhages

11.10.3 Treatment

There are some reports of spontaneous improvement of anterior radiation-induced optic neuropathy. However, most cases progress to severe monocular vision loss and optic atrophy. Although there are studies examining treatment for optic neuropathy secondary to external beam radiation with bevacizumab, hyperbaric oxygen, corticosteroids, and pentoxifylline and vitamin E, few reports describe successful treatment for optic neuropathy after plaque brachytherapy.

Of all the complications associated with radiotherapy, optic neuropathy is the most devastating to visual function. It is our experience that most cases of severe visual loss following treatment are the results of this unavoidable and untreatable complication.

Conclusions

Although radiation therapy has become the treatment of choice for intraocular malignancies, there are numerous posttreatment complications of relevance to the ocular oncologist and referring ophthalmologist. Anterior segment pathology occurs in 4–23 % of treated patients with enucleation rates for neovascular glaucoma found to be as high as 12 % after treatment, perhaps greater in eyes undergoing proton beam radiation. Radiation-induced cataract develops in 8–83 % by 5 years post-radiation, and radiation retinopathy may occur in at least 10–63 % of treated eyes, if not more over time. Optic neuropathy has been reported in up to 16 % of patients. All of these complications affect overall visual acuity, and 26–62 % of treated eyes experience a loss of at least 2 Snellen lines. Although cataract surgery for radiation-induced cataract may be effective in improving visual acuity, other treatment modalities, such as intravitreal triamcinolone or bevacizumab injections, hyperbaric oxygen treatments, and laser photocoagulation, for radiation-induced retinopathy, maculopathy, and optic neuropathy appear to be far less effective. Ocular complications associated with radiotherapy are well known, and the incidence of reported complications is highly variable. Complications not only depend on tumor size and location but also may be related to radiation planning and surgical technique that may vary between treatment centers.

While radiation complications are difficult to compare between brachytherapy and stereotactic fractionated and single fraction therapy because of differences in fraction size, total dose, dose rate, and treatment volumes, it is clear that the risk of many complications increases above certain dose thresholds. Future efforts may be directed toward limiting the exposure to healthy tissue at the time that the radiation is delivered [50] such as has been demonstrated with silicone oil 1,000 centistokes placement at the time of brachytherapy [8].

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Principles and Complications of Chemotherapy

12

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12.1 Introduction

The overall survival rate for cancer has improved dramatically since the institution of chemotherapy for the treatment of childhood leukemia in the 1940s [1]. The ultimate prognosis for each cancer is contingent on the histologic type, extent of disease, and several other biologic parameters [2].

Recent progress in molecular biology, biochemistry, and genetics has provided new insights into understanding of the complex molecular changes associated with malignant transformation of a cell. These discoveries are offering new classes of drugs that are being currently evaluated in clinical trials along with conventional agents [3]. This chapter will review some of the basic principles and complications of chemotherapy with examples from common ophthalmic cancers.

12.2 Basic Principles

Most conventional anticancer drugs have nonselective mechanisms of action that target DNA, RNA, or metabolic pathways in both malignant and normal cells. The latter result in undesirable and potentially severe toxic effects.

12.2.1 Mechanism of Action

Alkylating agents such as cyclophosphamide, cisplatinum, and carboplatinum damage DNA by covalently binding to and cross-linking

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_12, © Springer-Verlag Berlin Heidelberg 2014

Table 12.1 Commonly used antineoplastic agents and their mechanism of action

Group	Drug	Mechanism of action
Antimetabolites	Mercaptopurine	Arrests purine synthesis, interrupts DNA/RNA synthesis
	Methotrexate	Prevents thymidine synthesis, arrests conversion of folate to active form
	Cytosine arabinoside	Disrupts DNA by inhibiting DNA polymerase
	5 Fluorouracil	Disrupts DNA/RNA synthesis, inhibits thymidine synthesis
Alkaloids	Vinca alkaloids: vincristine, Velban	Inhibits mitosis by binding tubulin
	Etoposide (VP-16)	DNA strand breaks (topoisomerase II mediated)
	Taxanes	Mitotic arrest by stabilizing microtubules
	Camptotecans: topotecan, irinotecan	DNA strand breaks (topoisomerase I mediated)
Alkylating agents	Cyclophosphamide, melphalan, ifosfamide	DNA disruption by intrastrand and interstrand cross-linking
	Cisplatinum, carboplatinum	Intra- and interstrand cross-linking of DNA by platination
Antibiotics	Anthracyclines: Adriamycin, daunomycin	DNA strand breaking, free radical formation, interference of DNA religation (topoisomerase II mediated)
Miscellaneous	Corticosteroids	Lympholysis
	L-asparaginase	Inhibition of protein synthesis by L-asparagine
	Rituxan	Anti-CD-20 antibody
	Thalidomide	Antiangiogenesis
	Retinoic acid	Differentiating agent

nucleosides within the DNA. Antimetabolites such as methotrexate block the synthesis of nucleotide precursors or are directly incorporated directly into DNA as fraudulent bases. Topoisomerases are nuclear enzymes that maintain the three-dimensional structure of DNA, critical for DNA replication, transcription, and recombination. Etoposide (VP-16), anthracyclines (doxorubicin), and camptothecins (topotecan, irinotecan) interfere with religation of DNA, resulting in DNA strand breaks. Commonly used antineoplastic agents and their mechanisms of actions are summarized in Table 12.1.

A relatively new class of chemotherapy drugs, such as monoclonal antibody Rituxan (anti-CD 20), has been used successfully in the treatment of CD-20 positive non-Hodgkin's lymphomas. Other agents such as prednisone, cyclosporine, and interferons alter the immune system. Thalidomide, because of its antiangiogenic effects, has become an important drug in the treatment of chemotherapy-resistant brain tumors. Advances in basic research continue to offer new cancer therapies based on understandings of the pathogenesis of cancer. For example, the development of tyrosine kinase inhibitor such as Gleevac®, which allows for apoptosis of the cancer cells, is effective for the treatment of chronic myeloid leukemia.

12.2.2 The Cell Generation Cycle

Drugs affect different stages of the cell cycle (Fig. 12.1). Knowledge of the normal cell cycle and how different chemotherapeutic anticancer drugs disrupt this cycle is integral in helping to develop effective chemotherapy regimens. Agents that are cell cycle phase specific will kill a fraction of cells passing through that phase of cycle. On the other hand, agents that are not phase specific produce a continuous exponential decrease in cell survival because they affect all cells regardless of their phase in the cell cycle [4, 5].

Combining agents that enhance the disruption of vital intracellular processes through concurrent

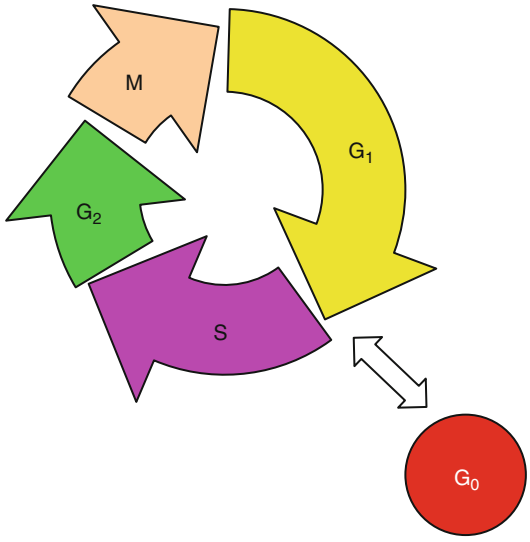

Fig. 12.1 The cell generation cycle is divided into four phases. *G1* begins immediately after mitosis. It is a period when the cell carries on its usual non-mitotic functions. It lasts until the beginning of the *S* phase. The *S* phase is the synthetic period when synthesis of DNA occurs and the genome is replicated. *G2* is the period between the end of DNA synthesis and the beginning of cell division. *M* phase is the mitotic phase during which chromosome condensation occurs, the mitotic spindle appears, sister chromosomes separate, and the process of cell division occurs. *G0* cells are those that have left the cycling pool, either temporarily or permanently. Cells that are temporarily in *G0* can be recruited back into the proliferative cycle by appropriate stimuli or growth factors. Some *G0* cells may be terminally differentiated and cannot be recruited back into the proliferative cycle

and/or sequential blockade of the cell cycle as well as inhibition of specific metabolic pathways has been a traditional approach in designing chemotherapy protocols [4].

12.2.3 Tumor Cell Kinetic Model

Exposing a tumor to a drug, at a determined concentration, over an established period of time, will result in a constant fraction of tumor cell kill, regardless of the tumor size. This fractional cell kill hypothesis forms the rationale for repeated courses of therapy at maximum tolerated doses. The chemotherapy is given in cycles as soon as blood counts recover to achieve the goal of tumor cells reduction to zero [5].

12.2.4 Combination Regimen

The importance of administering anticancer drugs in combination regimens was first recognized in the treatment of childhood cancers. In acute lymphocytic leukemia, for example, a complete remission can be achieved in 60 % of the patients treated with a single agent such as aminopterin, prednisone, vincristine, L-asparaginase, or daunomycin. However, most of these patients relapsed despite the continued use of that agent [6]. Patients retreated with the same agent at the time of relapse do not respond. These observation implied emergence of a preexisting drug-resistant subclone and/or residual subclinical disease. The use of combination chemotherapy with alternating regimens of non-cross-resistant drugs decreases the incidence of the development of drug resistance. The most successful combination chemotherapy uses agents that also have additive or synergistic mechanisms of action with nonoverlapping toxicities (if possible) to allow each agent to be used at optimal dosing [4]. This approach has contributed to the significant improvement in survival (90 % cure rate) in acute lymphocytic leukemia [2].

12.3 Drug Resistance

Resistance to anticancer drugs is the primary reason for treatment failure in cancer patients. Drug resistance can be present at diagnosis or may develop over time by exposure to chemotherapy. Cancer cells undergo spontaneous generation of drug-resistant clones by mutation, deletion, gene amplification, translocation, or chromosomal rearrangement. This is due to the inherent genetic instability of each cancer cell. Drug resistance can target a single drug or multiple drugs. For example, P-glycoprotein (P-gp) expression leads to the reduction of the intracellular concentration of drug due to increased activity of membrane bound ATP-dependent drug efflux pumps [3]. Drugs like verapamil, calcium channel blocker, and cyclosporine can act as chemosensitizers and reverse multidrug resistance caused by P-gp. Multidrug resistance is also the result of the cancer cell's enhanced ability to repair DNA,

decreased levels of the target enzyme (topoisomerase II), and suppression of apoptotic pathways. The mechanism of drug resistance is important to consider in designing treatment protocols for relapsed patients.

12.4 Drug Development

The initial critical step in anticancer drug development is to identify new candidate drugs. The National Cancer Institute's Drug Screening Program uses a panel of 60 human tumor cell lines to identify new candidate drugs. The screening program identifies more than 10,000 new chemical agents per year. New gene chip technology has now been incorporated into the NCI's drug screening program. Candidate compounds are then tested in mice and dogs to determine the maximum tolerated dose (MTD). Pharmacokinetic parameters are also studied in animals to establish a safe starting dose for human clinical trials [3, 5, 7].

12.5 Clinical Trials

12.5.1 Phase I Trials

A Phase I trial is designed to determine the MTD for a specific dosing schedule, define toxicity profile in humans, identify dose-limiting toxicities, and study the pharmacokinetics. Phase I studies are open to patients who have had relapse(s) and have exhausted standard and established therapies. Usually small numbers of patients (15–30) are enrolled, and the dose is then escalated in successive cohorts of 3–6 patients until a dose-limiting toxicity is consistently observed.

12.5.2 Phase II Trials

After the optimal dose and schedule for a new drug is determined, phase II trials are conducted to determine the spectrum of antitumor activity and the response rate of drugs against a number of different tumors. Patients who have failed standard therapy are eligible for phase II trials [3, 8].

12.5.3 Phase III Trials

New chemotherapy agents, which have successfully been tested in phase I and II trials enter into phase III trials. Phase III trials are randomized studies in which new agents are studied in previously untreated patients (standard therapy and new agent vs. standard therapy alone). Additional factors that must be considered in the development of treatment protocols include drug mechanism of action, pharmacokinetics, toxicities, potential drug interactions, and mechanisms of drug resistance [1, 6].

12.6 Multimodality Therapy

At times, other modalities of treatment such as surgery and radiation therapy are used in conjunction with chemotherapy for maximal clinical benefit.

12.6.1 Adjuvant Chemotherapy

Adjuvant chemotherapy is in a setting wherein patients receive local therapy at diagnosis (surgery and/or radiation therapy) to the primary tumor prior to chemotherapy (Table 12.2).

12.6.2 Neo-adjuvant Chemotherapy

In the neo-adjuvant setting, patients receive chemotherapy first at diagnosis to reduce cancer burden prior to local measures are undertaken. In retinoblastoma, chemotherapy is used as a chemoreductive approach (neo-adjuvant therapy). Currently six cycles of vincristine, etoposide (VP-16), and carboplatinum are given every 3–4 weeks. This is followed by local measures such as thermotherapy, cryotherapy, and plaque radiotherapy to treat intraocular tumors so as to avoid use of external beam radiotherapy and enucleation [8].

12.7 Complications

Antineoplastic drugs have the lowest therapeutic index of any class of drugs. They therefore predictably produce significant and at times

Table 12.2 Multidisciplinary treatment of common ophthalmic tumors

Tumor	Chemotherapy	Surgery	Radiotherapy
Orbital rhabdomyosarcoma (embryonal: nonmetastatic)	Vincristine actinomycin D	+	+
Optic nerve glioma	Vincristine + carboplatin Vincristine + actinomycin D Velban	±	+ (age > 5 years)
Retinoblastoma (nonmetastatic)	Vincristine + etoposide + carboplatin	+	+
Retinoblastoma (metastatic)	Vincristine + etoposide + carboplatin	+	+
Uveal melanoma (nonmetastatic)	Multiagent	+	
Uveal melanoma (metastatic)	Multiagent	+	
Conjunctival melanoma (nonmetastatic)	±	+	−
Conjunctival squamous cell carcinoma (nonmetastatic)	±	+	−

Table 12.3 Systemic side effects of commonly used antineoplastic agents[a]

Group	Drug	Toxicity
Antimetabolites	Mercaptopurine	Hepatotoxicity
	Methotrexate	Hepatotoxicity, nephrotoxicity
	Cytosine arabinoside	Hepatotoxicity, nephrotoxicity, neurotoxicity
Alkaloids	Vinca alkaloids: vincristine, Velban	Neurotoxicity, constipation
	Etoposide (VP-16)	Hepatotoxicity, nephrotoxicity
Alkylating agents	Cyclophosphamide	Hepatotoxicity, nephrotoxicity, cystitis
	Cisplatinum, carboplatinum	Nephrotoxicity, ototoxicity
Antibiotics	Anthracyclines: Adriamycin, daunomycin	Hepatotoxicity, cardiotoxicity
Miscellaneous	Corticosteroids	GI bleed, Cushing's syndrome, diabetes
	L-asparaginase	Pancreatitis
	Interferon alpha	Fever, malaise, myalgia
	Thalidomide	Teratogenicity
	Retinoic acid	Hypervitaminosis A

[a]Bone marrow depression, alopecia, nausea, and vomiting are frequent side effects of many antineoplastic agents

life-threatening toxicities (Table 12.3). The oncologist must balance the risk of toxicities against the risk of relapse as a result of inadequate treatment. Even a small reduction or delay in therapy to mitigate toxicities can result in tumor recurrence, which may lead to the death of the patient.

Systemic chemotherapy may also be associated with ocular side effects that may be acute or chronic. With the development of newer cancer therapies and the use of more aggressive regimens of already existing agents/combination of agents, the list of associated ocular side effects may well increase. This may be particularly true knowing the increased patient survival rate that many of these agents have helped achieve.

Several commonly used chemotherapeutic agents have been reported to induce ocular morbidity [9–14]. Although a comprehensive review of the literature related to ocular side effects of chemotherapeutic agents is beyond the scope of this chapter, some of the commonly used agents and their ocular toxicity are summarized (Table 12.4). Some agents affect various ocular and periocular structures as well as the optic nerve, cranial nerves, and central nervous system components serving vision and the oculomotor function. For some agents, the mechanism of a particular side effect is known, but for the most part the exact etiology behind many of the ocular side effects associated with these agents remains uncertain.

It is important to realize, however, that while ocular side effects related to these agents are

Table 12.4 Ocular side effects of commonly used chemotherapeutic agents

Chemotherapeutic agent/class	Anatomic location	Specific ocular side effect
L-asparaginase, taxans, and platinum salts	Ocular adnexal structures	Hyperpigmentation, sclerodermiform dermatitis, photosensitivity. and hypersensitivity reactions
Methotrexate	Ocular adnexal structures	Periocular edema, blepharitis
	Anterior segment	Conjunctivitis
	Posterior segment	Retinopathy with reduced b-wave amplitude on electroretinography, macular edema
	Optic nerve/oculomotor nerves	Optic neuropathy and internuclear ophthalmoplegia
5-Fluorouracil capecitabine	Ocular adnexal structure	Blepharitis, eyelid dermatitis, cicatricial ectropion, punctual/canalicular/ductal stenosis, alopecia, phlebitis, chemical cellulitis, diffuse sclerosis, sterile folliculitis, and flushing reactions
	Anterior segment	Conjunctivitis, keratitis
Interferon	Ocular adnexal structures	Alopecia, trichomegaly, and hypertrichosis
	Anterior segment	Acute corneal graft rejection, glaucoma
	Posterior segment	Retinopathy with retinal hemorrhages, cotton wool spots, macular edema, and retinal ischemia
	Optic nerve	Anterior ischemic optic neuropathy
Mitomycin-C	Anterior segment	Conjunctivitis and keratitis, worse with topical application
Carmustine	Anterior segment	Conjunctivitis and keratitis
	Posterior segment	Retinal hard exudates, hemorrhages, infarcts, and retinal artery occlusion
	Optic nerve	Optic neuropathy
Tamoxifen	Anterior segment	Corneal subepithelial deposits, whorls and linear opacities, posterior subcapsular cataract
	Posterior segment	Pigmentary retinopathy, macular crystals and drusen, macular edema
	Optic nerve	Optic neuritis
Cytarabine	Anterior segment	Conjunctivitis and keratitis
Cisplatin and etoposide	Posterior segment	Retinopathy, reduced a-wave and absent b-waves on ERG, retinal ischemia, and neovascularization
Cisplatin, vinblastine, and vincristine	Optic nerve and CNS	Optic neuritis with disc edema, transient cortical blindness or homonymous hemianopia, cranial nerve dysfunction
Intracarotid cisplatin	Ocular adnexal structures	Orbital myositis
	Posterior segment	Central retinal artery occlusion, uveal effusion
	Optic nerve	Optic neuritis
Chlorambucil	Anterior segment	Keratitis
	Posterior segment	Retinal hemorrhages
	Optic nerve	Papilledema
Cyclophosphamide	Ocular adnexal structures	Blepharitis, lacrimal duct stenosis
	Anterior segment	Kerato-conjunctivitis sicca
	Posterior segment	Retinal hemorrhages
Rituximab	Ocular adnexal structures	Transient periocular edema
	Anterior segment	Conjunctivitis
Paclitaxel	Posterior segment	Cystoid macular edema
Docetaxel	Anterior segment	Canalicular stenosis

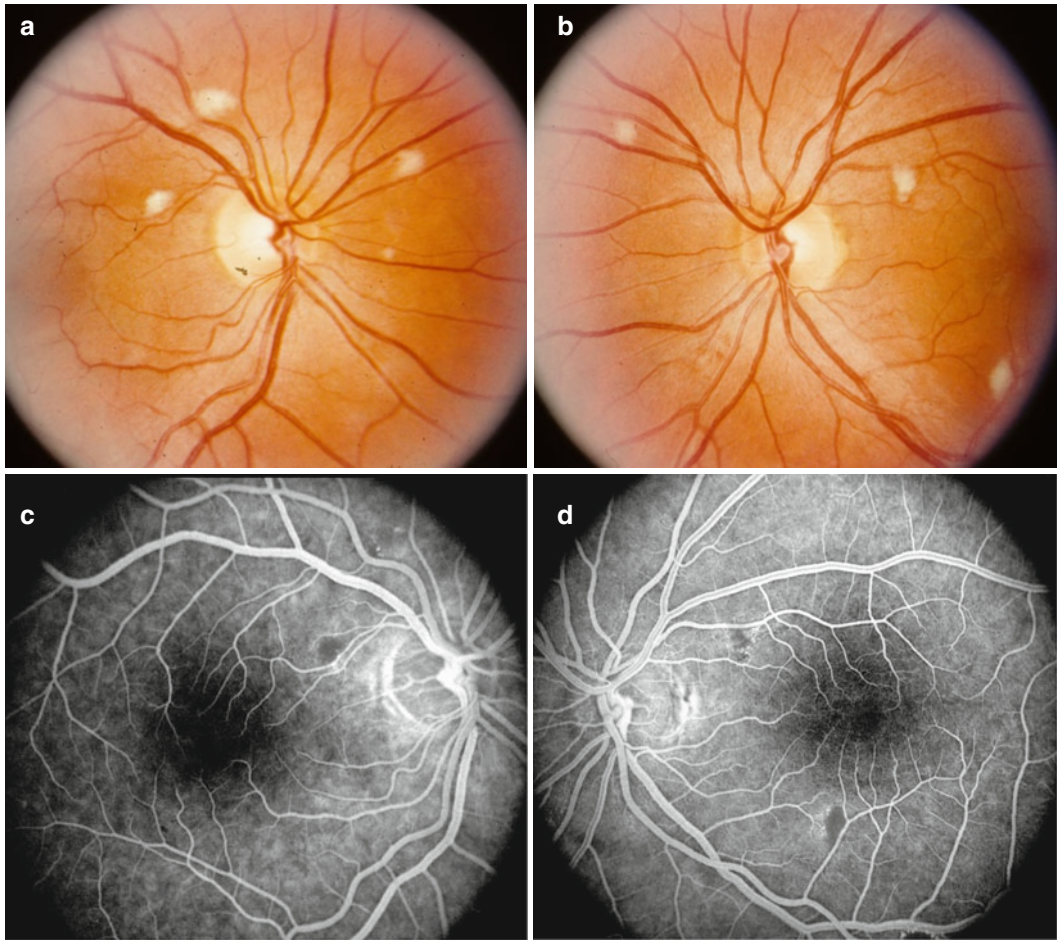

Fig. 12.2 A 30-year-old male presented with complaints of mild visual distortion in both eyes of several weeks duration. He had been in good health until 5 months prior to presentation when he was diagnosed with a malignant cutaneous melanoma. The patient underwent surgical resection, and on histopathologic review, the primary tumor was graded T4bN2aM0. There was no evidence of metastatic disease. The patient received 20 days of high-dose IV interferon alpha 2-b (30–36 million IU over 30 min) and was then switched to subcutaneous interferon injections. One week prior to presentation at the ophthalmology department, the patient was hospitalized for an acute stroke that presented with symptoms of right-sided facial droop, dysarthria, and right upper extremity weakness. MRI of the brain revealed an infarct in involving the left caudate nucleus and a portion of the left posterior limb of the internal capsule. On examination, the patient's vision measured 20/20 without correction at distance and near. Dilated fundus examination revealed scattered cotton wool spots throughout the posterior pole in both eyes (**a, b**). The rest of the ophthalmic examinations including visual field testing, color vision, and slit lamp exam were normal. Fluorescein angiogram confirmed retinal capillary non perfusion (ischemia) corresponding to the cotton wool spots (**c, d**). A recent history of stroke along with findings of focal micro-retinal infarctions prompted a complete review of coagulation and cardiovascular system, which were all reported as normal except for an ejection fraction of 30 % suggestive of cardiomyopathy. Interferon treatment was discontinued promptly, and the patient was monitored clinically. At 10-month follow-up, there was complete resolution of retinopathy and full recovery from the stroke with no residual neurological symptoms. A strong temporal relationship between the onset of symptoms and the initiation of interferon therapy and absence of any other probable cause of vasculopathy suggested that stroke, cardiomyopathy, and retinopathy observed in our patient had a shared underlying pathogenetic mechanism of interferon-induced ischemia

usually not preventable, many of them are reversible with discontinuation of the drug or dose reduction (Fig. 12.2). Thus the role of the ophthalmologist in baseline evaluation of patients receiving chemotherapy, screening for potential side effects, and early diagnosis may be critical. While the table lists the common direct ocular side effects, it is important to recognize that some of these agents

may be associated with indirect ocular side effects such as ocular opportunistic infections (Fig. 12.3) and retinopathy (Fig. 12.4) resulting from compromised immunity and hematologic disorders (such as anemia and thrombocytopenia), respectively. In some instances, it may be difficult to differentiate whether an ocular presentation is the result of a direct or an indirect effect of a chemotherapeutic agent. Therefore, a close collaboration between the ophthalmologist and the treating oncologist may be critical to reach the proper diagnosis and management.

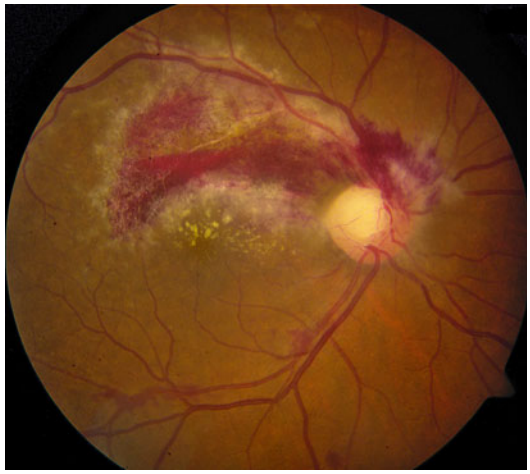

Fig. 12.3 A 58-year-old man referred for evaluation of floaters OD for past couple of weeks. He had a history of colon cancer s/p resection and had received chemotherapy. Visual acuity was 20/50 OD and 20/25 OS. Anterior segment examination was normal. Fundus examination of the right eye revealed a hemorrhagic white retinal lesion of CMV retinitis

12.8 Summary

Most conventional anticancer drugs have nonselective mechanisms of action that target DNA, RNA, or metabolic pathways in both malignant and normal cells. Recent progress in molecular biology, biochemistry, and genetics is offering new classes of targeted drugs (Chap. 13). Exposing a tumor to a drug, at a determined concentration, over an established period of time, results in a constant fraction of the tumor cell kill, regardless of the tumor size. This fractional cell kill hypothesis forms the rationale for repeated courses of therapy at maximum tolerated doses. Combining agents that work through concurrent and/or sequential blockade of the cell cycle as well as inhibition of specific metabolic pathways has been a traditional approach in designing chemotherapy protocols. The use of combination chemotherapy with alternating regimens of non-cross-resistant drugs decreases the

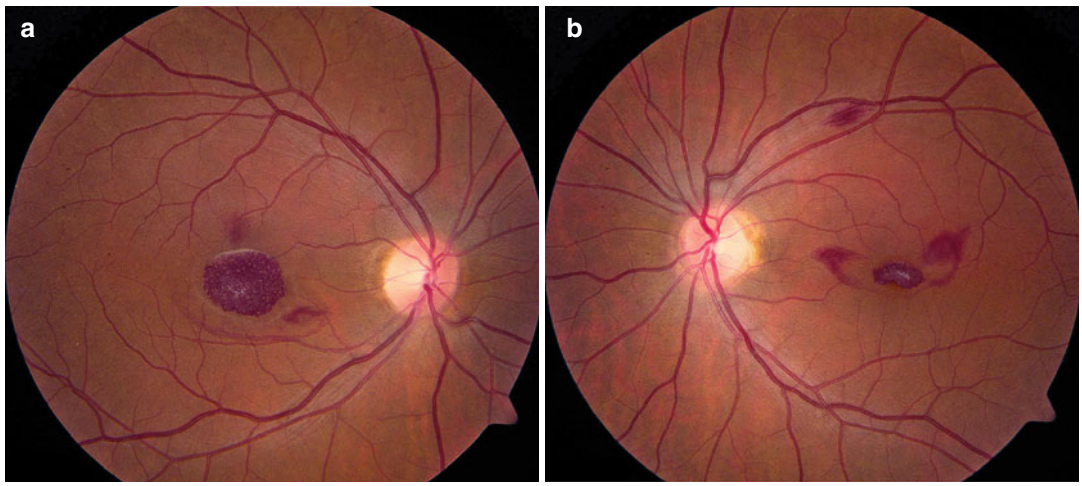

Fig. 12.4 A 30-year-old female with diffuse large B-cell lymphoma after 4 cycles of chemotherapy presented with blurred vision of 2 weeks duration. Examination revealed visual acuity of 20/70 OD and 20/40 OS. Fundus examination showed bilateral localized retinal hemorrhages (**a**, **b**). Optical coherence tomography confirmed sub-ILM location of the hemorrhage (**c**, **d**). Eight weeks later, the hemorrhage had completely resolved with full visual recovery associated with normalization of blood counts (**e**, **f**)

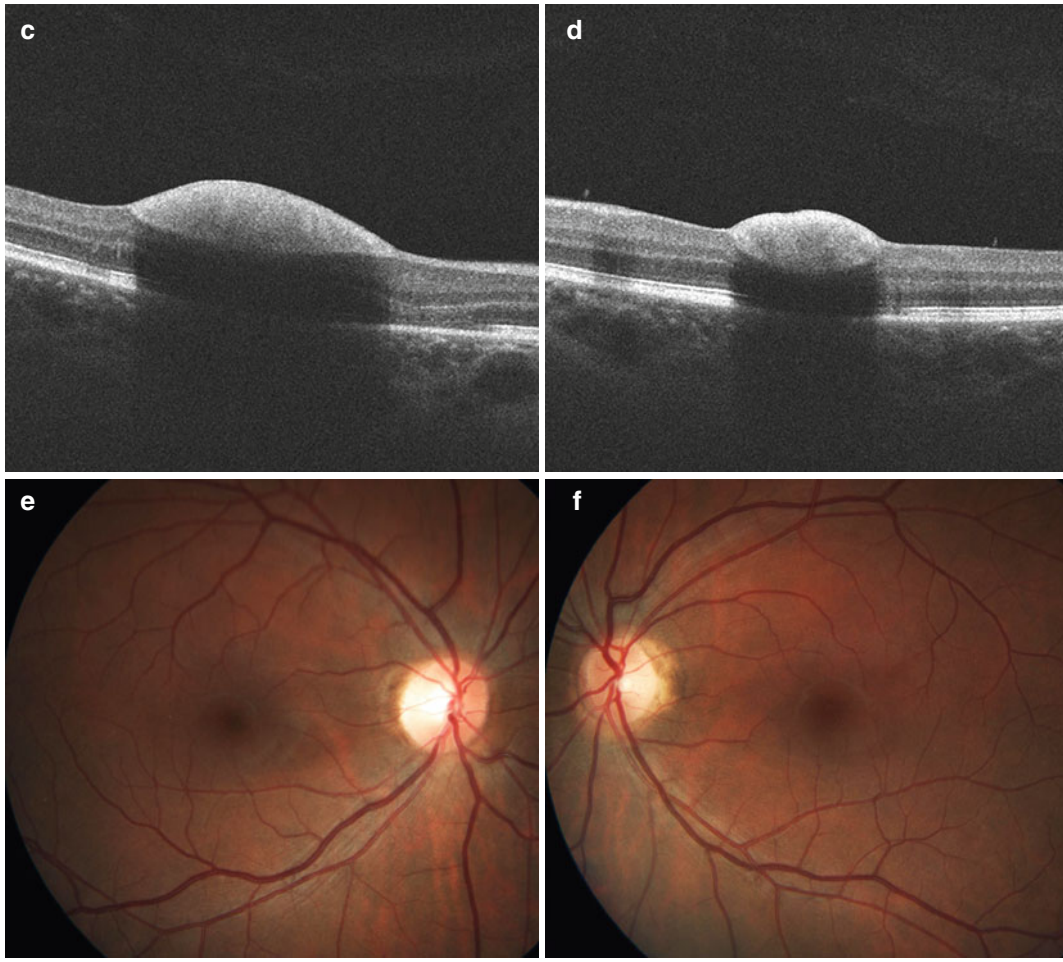

Fig. 12.4 (continued)

incidence of the development of drug resistance. Antineoplastic drugs have the lowest therapeutic index of any class of drugs. The oncologist must balance the risk of toxicities against the risk of relapse as a result of inadequate treatment.

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Targeted Therapy and Their Ocular Complications

13

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Contents

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13.1 Introduction

The rapid advancements in the field of oncology have resulted in the transition from cytotoxic chemotherapeutic agents to molecularly targeted therapies that interfere with cellular signaling pathways important for the survival and propagation of neoplastic cells. Some of these agents have changed the landscape in the management of several hematologic and solid malignancies. Molecularly targeted agents are selected and designed to provide the maximal antitumor affect with the minimal functional alteration of the normal healthy cells [1]. However, the complexity of cellular pathways proves very difficult to design therapeutic agents that do not overlap with the physiologic activities of the normal human tissues. The recognition of a myriad of distinct adverse effects related to the molecularly targeted therapies has emerged over the last decade with their increased clinical usage [2, 3].

Molecularly targeted agents act through the variety of specific mechanisms, which results in the unique side effects related to a specific agent. Clinically recognized side effects depend on the cellular pathways which are inhibited and their expression in the different ocular and adnexal tissues. Most ocular tissues have been found susceptible to the development of side effects secondary to these drugs, from the periocular skin to the central nervous system visual pathways. Familiarity of the ophthalmologists with the potential ocular side effects is important for the early recognition of ocular toxicities.

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_13, © Springer-Verlag Berlin Heidelberg 2014

13.2 Specific Agents

13.2.1 Tyrosine Kinase Inhibitors

Tyrosine kinases are enzymes involved in signal transduction pathways regulating cell growth and response to the extracellular stimuli. Human malignant cells develop mutations that can lead to constantly activated tyrosine kinase enzymes resulting in uncontrolled cellular growth and proliferation. Inhibition of aberrantly activated tyrosine kinase enzymes was the first success story of molecularly targeted therapy [4].

Imatinib (Gleevec™), also known as signal transduction inhibitor (STI)-571, was the first FDA-approved molecularly targeted agent in May 2001, which inhibits abnormally fused break point cluster-Abelson (BCR-ABL) tyrosine kinase in chronic myelogenous leukemia (CML) caused by the 9:22 translocation. Subsequently, it was discovered that imatinib also blocks the activity of tyrosine kinases associated with platelet-derived growth factor receptor (PDGFR) and c-Kit (CD117), the receptor for stem-cell factor (SCF). Blockade of mutated c-Kit results in apoptosis of gastrointestinal stromal tumor (GIST) cells and dramatic regression of neoplastic lesions [5, 6].

PDGFR and c-Kit tyrosine kinases are prevalent in dermal dendrocytes that reside in normal human skin including periorbital area. There is some evidence that PDGRF signaling pathway plays a role in the regulation of interstitial fluid pressure and its inhibition in dermal dendrocytes may lead to the extravasation of plasma into the extracellular space. Anatomical structure of the periorbital skin makes it very susceptible to the interstitial fluid changes. It is not surprising that the most common ophthalmic side effect of imatinib therapy is the development of periorbital swelling affecting around 70 % of treated patients. Peripheral edema occurs less frequently affecting about 29 % of patients. The severity of periorbital edema may range from mild to moderate, which is the most common presentation, to severe swelling resulting in the obstruction of visual axis. Edema usually develops 2–3 months after the initiation of therapy, but it can be seen as soon as 1 day or up to a year after the beginning of medication. The increased dose may be associated with the higher rates of periorbital edema. The management of periorbital edema in cancer patients treated with imatinib depends on the severity of symptoms and may include observation for mild edema, low-dose diuretics such as furosemide for moderate edema, and surgical debulking of the excessive edematous tissue in very severe cases. Periorbital edema almost never requires the cessation of cancer therapy because it can be successfully managed by medical or surgical approaches [7–9].

Epiphora is also a common complaint in patients treated with imatinib. It occurs in about 18 % of treated patients occurring almost exclusively in patients who also had concomitant periorbital edema. Clinical evaluation of these patients showed no evidence of punctual, canalicular, or nasolacrimal duct obstruction as probing or irrigation revealed patent tear outflow system. Half of the patients were noted to have conjunctival chemosis related to the fluid collection underneath the conjunctiva or conjunctivochalasis partially blocking the opening of inferior puncta. Concomitant periorbital edema may disrupt the functional force of pretarsal orbicularis oculi muscle and result in lacrimal pump dysfunction. Imatinib metabolism may also lead to by-product secretion and its accumulation in tear film, which could act as an irritant causing overproduction of tears. Most patients with mild epiphora can be observed. If symptoms are severe enough to interfere with quality of life, 40 mg of furosemide daily and prednisolone acetate (1 %) four times a day were found to be effective in alleviating symptoms [9, 10].

Increased intraocular pressure (IOP) is a possible complication from imatinib therapy in patients with CML, and the literature reports supporting it are very scarce. A report from Italy showed that 1.6 % of 250 patients treated with imatinib developed increased IOP, which required either discontinuation of therapy or reduction in dose. One patient required glaucoma surgery. The same group also reported several patients developing recurrent conjunctival hemorrhages in spite of no evidence of thrombocytopenia or coagulation

abnormalities. It is postulated that the inhibition of c-kit enzyme found on mast cells that populate the conjunctival substantia propria make the mucosa more vulnerable to mild trauma [11].

Imatinib can very rarely lead to vision-threatening complications compromising the function of retina or optic nerve. Experimental models demonstrated widespread apoptosis of retinal ganglion cells (RGCs) in the cell culture treated with imatinib for up to 48 h due to downstream disruption of PDGF receptor signaling mediators that promoted the survival of RGCs. Animal experiments indicated that high-dose (1.65 mg) intravitreal injection of imatinib resulted in extensive retinal necrosis in contrast to low-dose (165 or 825 μg) injection that showed no ocular toxicity [12]. These experimental models only underline possible retinal toxicities and in no way simulate in vivo imatinib effect from the current oral dosing of the medication. Nevertheless, there are very sparse reports of imatinib causing optic disc edema, retinal hemorrhages, retinal neovascularization, and cystoid macular edema. There was a resolution of side effects and improvement in visual acuity after the dose reduction or cessation of imatinib therapy in these reports [11, 13–16].

It is of paramount importance to rule out neoplastic infiltration of the optic nerve prior to suspecting imatinib toxicity as a culprit for the optic nerve disease.

Dasatinib and *nilotinib* are newer inhibitors of tyrosine kinases, including the BCR-ABL tyrosine kinase, that are used for the treatment of Philadelphia chromosome-positive CML that is resistant to imatinib. Their safety profile may be distinct from imatinib, but given the similar mechanism of action between these agents, the potential for development of ocular side effects will remain present [17].

13.2.2 Epidermal Growth Factor Receptor Inhibitors

The epidermal growth factor receptor family (also known as ErbB) consists of four receptors: the epidermal growth factor receptor (EGFR/ErbB1/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). Their function is essential for the normal growth and differentiation of epithelial cells. It is highly expressed in the various ocular tissues including the periocular skin, hair follicles, meibomian and lacrimal gland, conjunctiva, and cornea. It is not surprising that the most side effects of EGFR inhibitors are related to the ocular surface [18].

Erlotinib (TarcevaTM) is a potent inhibitor of EGFR tyrosine kinase competitively blocking ATP binding site at the active tyrosine kinase region and disrupting downstream cellular signaling cascade, used for the treatment of locally advanced or metastatic non-small cell lung cancer and pancreatic cancer. Ocular side effects were reported in up to 12 % of treated patients during the clinical trial mostly consisting of the ocular surface disease. Inhibition of EGFR may disrupt keratin gene expression within the eyelash follicles leading to the abnormal growth cycle and trichomegaly affecting 29 % of patients [18, 19]. The aberrant eyelashes can rub against the ocular surface resulting in corneal abrasion and ulceration. The mechanical trauma caused by trichomegaly is accentuated by the EGFR inhibition causing meibomian gland secretion abnormalities, the abnormal quality and quantity of the tear film and delayed corneal epithelial healing. The prompt recognition of these side effects is important to prevent vision-threatening corneal infections. Eyelash trimming, aggressive lubrication, and topical antibiotic instillation with careful monitoring are common therapeutic approaches. The tear film abnormality can be treated with oral doxycycline and artificial tears. Erlotinib was also reported to cause periorbital rash, blepharitis, hyperemia, and telangiectasia that can be treated with topical Maxitrol eye ointment and fluorometholone (0.1 %) ointment (Fig. 13.1) [18–20]. Cessation of therapy can lead to the healing of persistent epithelial defect, but it is rarely necessary to discontinue therapy unless severe corneal disease develops. Corneal perforation was reported in a patient treated with erlotinib for stage IV lung squamous cell carcinoma that required surgical intervention and discontinuation of therapy due to severe vision-threatening side effects [21].

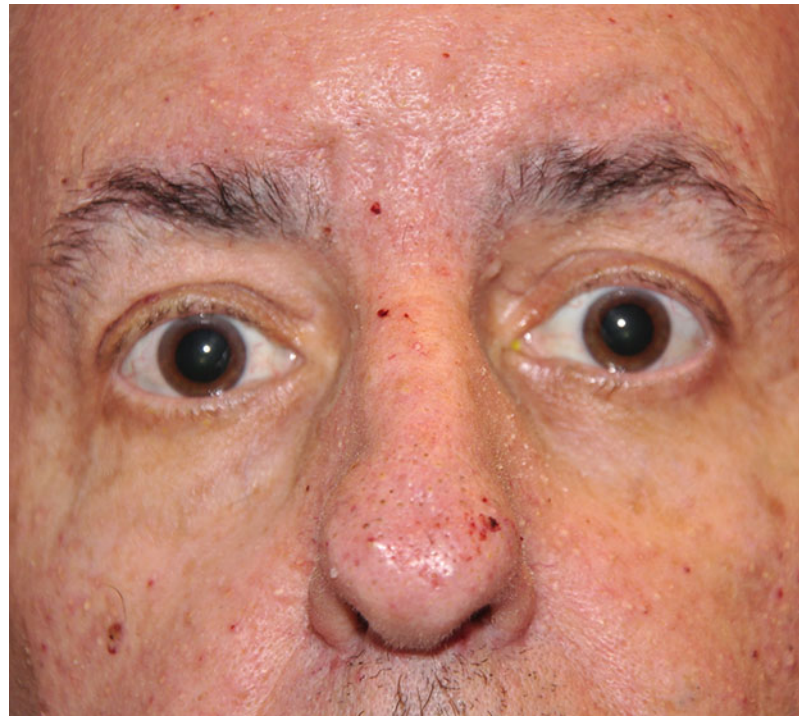

Fig. 13.1 Periorbital rash, hyperemia, and telangiectasia induced by *erlotinib (Tarceva™)*

Gefitinib (Iressa™) inhibits the EGFR tyrosine kinase activity by competitive blockade of ATP binding site, used for the treatment of non-small cell lung cancer in patients who have activating mutations in EGFR gene caused by in-frame deletions within exon 19 and the L858R point mutation, which are more prevalent in non-smokers, Asians and women [22]. The ocular side effects of gefitinib overlap with the side effects of erlotinib given its similar mechanism of action. During the clinical trial, gefitinib therapy was found to cause trichomegaly, meibomitis, tear film abnormalities, and reversible recurrent corneal erosions in 8 % of patients. The side effects can be managed by eyelash epilation, systemic doxycycline, topical corticosteroids, and antibiotic ointments [20, 23].

Cetuximab (Erbitux™) is a chimeric antibody targeting the extracellular domain of EGFR that prevents ligand-dependent signaling and receptor dimerization. Its antitumor activity differs from erlotinib and gefitinib, even though these medications target the same enzyme. It is used in conjunction with radiation therapy for regionally advanced squamous cell carcinoma of the head and neck and as a single agent for EGFR-positive metastatic colorectal cancer [24]. Ocular side effects of cetuximab are similar to the side effects noted in the patients treated with erlotinib or gefitinib. In one report, tear film abnormalities and dry eye symptoms were noted in 67 %, blepharitis (63 %), trichomegaly or trichiasis (38 %), and eyelid rash or hyperemia (38 %) of treated patients [18]. Most commonly, side effects of cetuximab are mild and can be easily managed; however, if not addressed promptly, recurrent corneal irritation may cause scarring and permanent blindness.

Panitumumab (Vectibix™) is a fully humanized, recombinant IgG2k antibody against the extracellular domain of EGFR. The binding of EGFR on cell surface by panitumumab does not mediate antibody-dependent cell-mediated cytotoxicity that is a characteristic of cetuximab. It is used for the treatment of metastatic colorectal carcinoma [25]. Ocular side effects from panitumumab are rarely reported, but they are expected to be similar to the side effects caused by other EGFR inhibitors such as cetuximab, erlotinib, and gefitinib. There is a single case report of

severe bilateral multiple epithelial defects resulting in corneal melting and perforation in one of the eyes after the infusion of panitumumab in a patient with metastatic colorectal cancer. The perforation was sealed with a conjunctival flap, and aggressive bilateral lubrication was initiated to prevent further progression of corneal disease, which resulted in significant clinical improvement. Symptoms recurred after the second panitumumab infusion, which prompted the cessation of therapy secondary to the blinding ocular side effects [21]. Evaluation of patients with ocular complaints is important to recognize early toxicity and prevent rare devastating corneal decompensation.

Trastuzumab (Herceptin™) is a humanized IgG1 kappa monoclonal antibody against the external domain of HER2 (ErbB2). It is used for the treatment of HER2-positive breast cancer and HER2-positive metastatic gastric or gastroesophageal junctional adenocarcinoma. It was the first monoclonal antibody approved for the treatment of a solid malignancy [26]. Experimental animal models showed that trastuzumab was effective in preventing corneal neovascularization by blocking EGFR upregulation of angiogenic factors, which could potentially be beneficial in patients with corneal neovascularization [27]. Ocular side effects of trastuzumab therapy are usually mild and rarely reported. In a study of 112 patients treated with trastuzumab combined with antimicrotubule agent DM1, 31.3 % of patients reported ocular problems most commonly consisting of dry eyes, increased lacrimation, conjunctivitis, and blurred vision [28]. Bilateral ischemic maculopathy was reported in a single case manifesting as the enlargement of foveal avascular zone and cystoid macular edema after trastuzumab infusion for the treatment of metastatic breast cancer. Discontinuation of therapy resulted in stabilization of the disease, and reintroduction of trastuzumab treatment caused the progression of capillary dropout and vision loss. It is postulated that trastuzumab blockade of pro-angiogenic factors by inhibition of HER2 receptors might have resulted in damage of susceptible retinal capillary network [29].

13.2.3 Inhibitors of Angiogenesis

Bevacizumab (Avastin™) is a humanized monoclonal IgG1 antibody against vascular endothelial growth factor (VEGF-A) with molecular weight of 149 kD. It binds VEGF and prevents its activation of surface receptors VEGFR-1 and VEGFR-2 found on the surface of endothelial cells. This disrupts the receptors activation and downstream molecular cascade, which is a critical step in angiogenesis. Bevacizumab has received the FDA approval as a combination therapy for metastatic colon cancer, non-small cell lung cancer, renal cell cancer, and glioblastoma multiforme [30]. It has been used as an off-label treatment of age-related macular degeneration (AMD) since 2005. Multiple studies have demonstrated the safety of intraocular bevacizumab, and the reported side effects are mostly related to the injection procedure rather than the medication [31, 32]. Ocular side effects from systemic bevacizumab therapy are rarely reported in the literature. Severe optic neuropathy has occurred in 1.2 % of 503 glioblastoma patients treated with bevacizumab and 0.2 % of 567 glioblastoma patients treated without bevacizumab suggesting its possible role in development of optic nerve disease [33]. Bevacizumab has also been implicated in the development of posterior reversible encephalopathy syndrome (PRES), which causes reversible cortical blindness by disruption of CNS visual pathways. Patients with PRES are found to have vasogenic edema primarily involving parietal and occipital lobes [34, 35]. It is hypothesized that PRES develops due to combination of increased arterial blood pressure and changes in endothelial function from bevacizumab leading to dysfunction of blood–brain barrier and resulting in posterior cerebral edema. Withdrawal of bevacizumab and blood pressure control lead to resolution of symptoms and visual recovery [36].

Sunitinib (Sutent™) is a VEGFR, EGFR, and PDGFR inhibitor used to treat kidney and liver cancers. Several cases of posterior reversible encephalopathy syndrome have been reported following sunitinib therapy. It is thought that mechanism of PRES development related to sunitinib is similar

to that of bevacizumab and includes increased systemic blood pressure and changes in endothelial cells function of cerebral vasculature [36, 37]. A single case report described development of bilateral optic disc edema in a patient treated with sunitinib for advanced renal cell carcinoma (RCC). The resolution of edema was noted 2 months after stopping sunitinib [38]. Neurosensory retinal detachment is also a potential side effect of sunitinib therapy. Bilateral serous retinal detachments developed in a patient treated for metastatic RCC. Two weeks after discontinuation of therapy, subretinal fluid was reabsorbed and the normal retinal contour was reestablished. The same clinical picture developed following re-initiation of sunitinib. Since anti-VEGF agents such as bevacizumab can be used to treat neurosensory detachment, it is thought that sunitinib inhibition of target enzymes other than VEGF is likely responsible for this side effect [39].

13.2.4 mTOR Pathway Inhibitors

Perifosine is an inhibitor of phosphoinositide-3 kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway specifically blocking Akt activity at low micromolar concentrations. It also affects other signal transduction pathways, including the c-Jun N-terminal kinase pathway, all of which are associated with programmed cell death, cell growth, cell differentiation, and cell survival. Perifosine is used to treat myeloma and neuroblastoma. It is being investigated for the treatment of metastatic colorectal carcinoma [40]. Cases of ulcerative keratitis presenting with perilimbal ring-shaped infiltrates resembling autoimmune keratitis have been reported in patients treated with combination therapy of imatinib and perifosine for gastrointestinal stromal tumors resistant to imatinib monotherapy. Withdrawal of perifosine resulted in improvement of keratitis in several patients. It has been speculated that combination therapy might have provoked this reaction; however, no similar cases of ulcerative keratitis have been reported following imatinib therapy. The exact mechanism of corneal toxicity remains poorly understood, but it is postulated that perifosine might have triggered sensitization to corneal self-antigens resulting in autoimmune-like keratitis. A combination of oral and topical corticosteroids, topical antibiotics, and lubricating agents was used to successfully treat peripheral ulcerative keratitis in the effected patients [41]. Rapidly progressive corneal ring infiltrates leading to complete corneal opacification, and peripheral corneal thinning occurred in a patient treated with perifosine monotherapy for Waldenstrom's macroglobulinemia. Aggressive systemic immunosuppressive therapy halted the progression of disease and resulted in resolution of autoimmune reaction; however, the patient ultimately required penetrating keratoplasty to clear visual axis [42]. Ocular complaints should be promptly investigated in patients treated with this agent, since early therapy might prevent serious ocular sequelae.

13.2.5 Anaplastic Lymphoma Kinase (ALK) Inhibitor

Crizotinib (Xalkori™) is a first-in-class inhibitor of the anaplastic lymphoma kinase (ALK), a protein kinase involved in carcinogenesis. It is approved for the treatment non-small cell lung cancer positive for ALK gene rearrangements. Visual complaints including blurred vision, photopsia, photophobia, floaters, and diplopia were reported in 62 % of treated patients in the phase I and phase II clinical trials. The visual disturbances (unknown mechanism) usually commenced within 2 weeks of the initiation of therapy and were transient in nature minimally impacting, the patients' quality of life. No vision-threatening or permanent ocular side effects have been reported in the studies [43].

13.2.6 Mitogen-Activated Protein Kinase RAS-RAF Signaling Pathway

Mitogen-activated protein kinase pathway is a complex cellular signaling pathway that regulates multiple essential cell functions including growth, survival, differentiation, and inflammation. Deregulation of this critical cellular

transduction cascade may lead to abnormal cell proliferation and tumorigenesis, which is found to be present in multiple human cancers. Numerous molecularly targeted agents inhabiting the RAS-RAF-MEK-ERK/MAPK signaling pathway are currently being investigated for the treatment of multiple different malignancies [44]. Ocular toxicities related to these medications have emerged. Transient blurring of vision, photopsias, and colored spots have been reported with several agents. A phase I trial of MEK inhibitor *trametinib* for the treatment of multiple different advanced solid malignancies demonstrated ocular toxicity in 31 (15 %) out of 206 patients involved in the trial, mostly including blurred vision and photophobia. Four patients developed serious ocular events with three cases of central serous retinopathy (CSR) and one case of central retinal vein obstruction (CRVO). CSR resolved upon withdrawal of trametinib [45]. Another MEK Inhibitor *RO4987655 (CH4987655)* also demonstrated ocular toxicities in 13 (27 %) of treated patients including a case each of CRVO and CSR [46]. Mitogen-activated protein kinase inhibitors are promising targets of abnormally activated transduction molecules in this critical regulatory pathway of cellular metabolism. Most of these agents are currently being investigated in phase I or phase II clinical trials, but the increased rate of ocular toxicities has been recognized necessitating careful monitoring of vision complaints in treated patients (Fig. 13.2).

Vemurafenib (Zelboraf™) received FDA approval in August 2011 for the treatment of melanoma harboring V600E mutations in the BRAF gene. The protein kinase BRAF is one of the critical enzymes in RAS-RAF cellular signaling pathway, and its deregulation leads to abnormal cellular proliferation and survival independent of extracellular growth factors. One of the most common side effects of vemurafenib therapy occurring in 26 % of treated patients was development of cutaneous neoplasms including basal-cell carcinoma, keratoacanthoma, and squamous cell carcinoma, which may potentially arise from the periocular area. Several ocular complications were reported in clinical trials. Most common ophthalmic side effect in phase III clinical trial was development of iritis which was reported in ten patients, who were managed with topical corticosteroids and mydriatic ophthalmic drops (Fig. 13.3). One case of CRVO was reported in phase II clinical trial requiring discontinuation of therapy [47, 48].

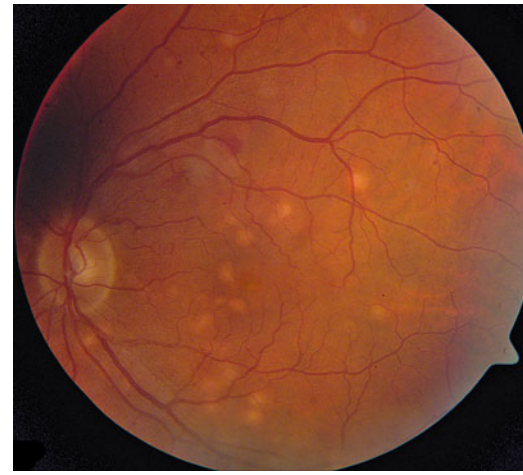

Fig. 13.2 This 50-year-old woman underwent enucleation for a large choroidal melanoma in January 2008. She was started on experimental MEKI for breast metastasis 6 months ago. Visual acuity 20/20 in the left eye. Fundus examination revealed multiple sub-RPE deposits of yellow/vitelliform material (Courtesy: Dr. Louise Mawn and Dr. Anita Agarwal, Nashville, TN)

13.2.7 Immunotherapy

Ipilimumab (Yervoy™) is a fully humanized monoclonal antibody against the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) preventing its interaction with the co-stimulatory molecules CD80 and CD86 (B7-1 and B7-2) on antigen-presenting cells. CTLA-4 acts as a regulatory molecule suppressing T-cells and its inhibition results in activation of T-cell lymphocytes enhancing immune response directed toward immunogenic tumor cells. It was FDA approved for the treatment of metastatic melanoma. Development of episcleritis and uveitis were reported in 1 % of patients in clinical trials usually occurring 2 months after initiation of therapy. Mild intraocular inflammation responded well to topical corticosteroids therapy. However, severe cases of ocular inflammation required cessation

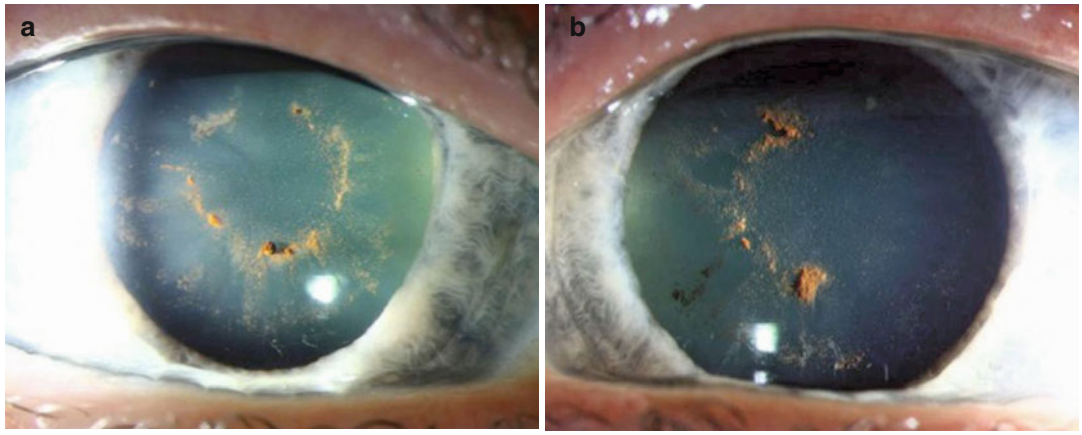

Fig. 13.3 Iritis is the most common ophthalmic side effect of *vemurafenib (Zelboraf™)*. Right eye (**a**) and left eye (**b**)

of ipilimumab therapy and initiation of systemic corticosteroids to prevent severe ocular damage [49, 50]. Abrupt development of drug-induced Graves disease was reported in a single case after a second infusion of ipilimumab presenting as severe proptosis, diplopia, and exposure keratitis. MRI demonstrated fusiform extraocular-muscle enlargement characteristic of Graves disease. The patient required cantholysis and responded well to systemic corticosteroids [51].

Conclusions

With increased clinical usage of molecularly targeted agents, distinct adverse effects have emerged. As molecularly targeted agents act through specific mechanisms, they result in unique side effects. Most ocular tissues are susceptible to the development of side effects from the periocular skin to intraocular structures (iritis) and central visual pathways. Familiarity of ophthalmologists with the potential ocular side effects is important for the early recognition of ocular toxicities.

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Counseling Patients with Cancer

14

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Contents

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14.1 Introduction

All involved in cancer care need to understand their patients' needs and fears. They must also be able to recognise and respond to patients' psychological concerns appropriately. Every oncology service should have systems for providing appropriate psychological support to patients and their relatives.

14.2 Emotions and Fears

It is common for people with cancer to experience psychological distress, which is an understandable response to a traumatic and threatening life event (Box 14.1). On receiving a diagnosis of cancer, many patients experience a range of strong emotions, such as fear, anger, sadness and many others, which may be overwhelming in their intensity. For the majority, this distress will be a short-lived experience, not causing lasting problems [1]. In such cases, it can be understood as part of the patients' normal adjustment to their diagnosis. However, for some, the diagnosis and treatment of cancer increase the risk of developing depression, anxiety and other forms of psychological morbidity such as adjustment disorder.

Uncertainty is always unsettling so that it is only natural for patients to be afraid of 'the unknown' and to worry about what side effects might occur as a result of their treatment, whether their tumour will recur and how long they might

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_14,

Box 14.1: Patients' Emotions and Fears When Informed of Their Diagnosis and Treatment

- Uncertainty about local tumor control and survival
- Sense of 'unfairness'
- Concerns about possible loss of independence
- Fears about facial deformity

live. These valid concerns make many individuals feel 'like a walking time-bomb' or 'in limbo' as if their life is 'put on hold'.

Not surprisingly, the diagnosis of eye cancer comes as a shock to most patients. Many want to know why this has happened to them 'out of the blue'. They wonder whether they have done anything to deserve their illness and can feel angry, especially if they feel they have led a healthy life so that they perceive their situation as being 'unfair'. In addition to worries about their general health and mortality, patients with ocular malignancy are concerned about visual loss and all its implications. Some fear that they will lose their ability to work and their independence, becoming a burden to others. Their self-esteem and identity are therefore threatened.

Quite reasonably, patients are also afraid that they might lose their eye and that this may cause disfigurement, which might disrupt social relationships, work and other important aspects of life.

Many patients are concerned about the potential for tumour recurrence or metastatic disease. Indeed, feelings of uncertainty have fertile ground in which to develop, and the fear of recurrence is highly relevant in these patients. Other than their ocular symptoms, if any, they are most likely to be asymptomatic with regard to metastatic disease. Furthermore, fear of recurrence has been associated with psychological morbidity in patients with cancer [2]. Some aspects of uncertainty may diminish temporarily when the objective situation can be ameliorated, for example, following a clear scan, a normal blood result or a reassuring clinic review. However, there will be further follow-up, and uncertainty about recurrence often re-emerges before those episodes.

Giving information can help reduce uncertainty but needs to be sensitive to patients' changing needs and to take into account individuals' contextual factors such as stage of disease, prior life experience, age and degree of social support. Information alone may, however, not reduce uncertainty. It is important to recognise the critically important role of caring professionals whom patients can trust. It is not just the security of the relationship with the clinician that is important; it is the hope that if disease is found, there is the medical expertise and technology available to treat it [3]. Furthermore, while it may be a relief when an intensive course of treatment ends, patients may feel insecure because they receive less attention from the clinician [4].

Some individuals can learn to live with uncertainty. For many, however, this is not possible without help and support. Professionals therefore need to be alert to the distress that can develop as a result of uncertainty at different times during the cancer trajectory.

14.3 Coping Mechanisms

Patients develop their own individual coping mechanisms, with varying degrees of success depending on their personality, background, previous experiences of challenges, illness perceptions and support from family and friends. Crying and other manifestations of distress sometimes make patients feel that they are not coping, which is not the case. It is often helpful to remind people that their emotions are quite understandable given the circumstances. In the early stages, some people reduce their distress by retreating into a form of temporary denial or avoidance of their illness. Such responses represent normal psychological defences that can be adaptive in the short term. However, a complication for patients with ocular malignancy is that some common distraction strategies, such as watching TV or reading, might be difficult if vision is impaired.

14.4 Patients' Needs

Understandably, patients desperately want to survive and resume normal life as quickly and painlessly as possible (Box 14.2). They are anxious to do everything possible to achieve these ends. They therefore want to know that they are receiving the best possible treatment. Most want to be informed about their condition and its treatment and are usually keen to be involved in decisions about their treatment and care. However, it should be noted that patients vary in the amount of information they want and that this changes over time [5]. Furthermore, while patients wish to feel involved in decision-making, this does not necessarily mean they want to take responsibility for medical decisions [6]. Above all, patients want to be treated as individuals, with dignity and respect. They wish to have their say and need to feel understood. Different patients have different needs at different stages of their illness, and psychological management needs to be responsive to this variability [7].

Box 14.2: Patients' Emotional Needs

- Reassurance that they are receiving best possible treatment
- Involvement in decision-making
- To be treated with dignity, as individuals
- To feel they are understood

14.5 Eliciting and Addressing Patients' Emotional Needs

Although the potential for psychological distress among cancer patients is recognised, in many cases, it remains undetected and untreated [8]. Eliciting and addressing patients' emotional needs should not be considered as something separate from medical care. Instead it should be recognised as routine clinical practice [7].

Many barriers inhibit this process, some attributable to patients' attitudes, beliefs and behaviour and some to clinicians [9]. It is often the case that patients normalise or somatise their feelings, and this may reduce their chances of detection. Furthermore, many feel that their concerns are unreasonable or consider it inappropriate to raise them with the clinician [9]. Some clinicians also feel that it is not their role to address patients' psychological needs [9]. They may feel uncomfortable or may consider themselves inadequately trained to respond to patients' emotional distress. As a result, clinicians might employ techniques such as changing the subject, ignoring the cues, normalising distress and/or offering false or premature reassurance, which inhibit patients' disclosure [9]. Practitioners may also be concerned that by responding to patients' distress, they may lengthen the consultation, whereas research indicates the opposite is often the case [10].

Even when clinicians feel that it is their role to address patients' psychological concerns, they sometimes lack the necessary skills to elicit symptoms [9]. It is therefore important that clinicians are trained in techniques that increase their confidence and encourage rather than discourage emotional disclosure. Such techniques might include demonstrating empathy through active listening, the use of open questions, responding to emotional cues (verbal and non-verbal), acknowledging patients' distress and the use of a patient-centred consulting style [9].

14.6 Communication

Effective communication is key to eliciting and addressing patients' psychological needs (Box 14.3). It also fosters good relationships between the patients and their carers. Patients' psychological well-being is greatly influenced by the way in which they are informed about their diagnosis and treatment. The manner in which healthcare staff respond to patients' concerns is also important [11, 12]. If information is poorly communicated and patients concerns are left undisclosed and unresolved, patients can become confused and resentful and have a high risk of developing clinical anxiety or depression [11, 13]. If done well, communication can lessen distress and assist understanding and adaptation [12, 13].

Box 14.3: Requirements for Effective Communication

- Quiet surroundings, free of interruptions and distractions
- Compassion and empathy
- Close friend or relative accompanying patient
- Respect for how much they wish to know
- Opportunity to ask questions and express opinions
- Help remembering what was said
- Chance to speak to previously treated patients

Consequently, it is important to ensure that patients receive as much information as they want and that this information is provided honestly and compassionately in simple and unambiguous language [14]. When counseling patients about their condition, it is useful to describe the ocular anatomy and the tumor briefly, using a model eye and pictures of the tumor. Next, the patient is informed of how the tumor is likely to behave and what might happen to the eye and vision if it is not treated. This is a good lead-in to a short discussion on the objectives of treatment, explaining what is and what is not achievable. The preferred treatment is introduced, together with the logistics involved (i.e. anesthesia and days in hospital). Estimates of the chances of achieving the main objectives and of developing complications are given, in terms that the patient can understand. Alternative treatments are discussed, with reasons why they are less suitable than the preferred approach. The patient is then informed of plans for early aftercare and long-term follow-up. The scope of adjuvant therapy and screening for metastasis is discussed. The impact of the patient's condition on driving and other activities is considered. Finally, any family implications are discussed, whether or not the disease is hereditary.

The information given to the patient should be summarised in the charts. The senior author (BD) has prepared a list of outcomes and complications so that any estimates given to patients are recorded. It is preferable if these discussions are conducted by a senior clinician having special skills in counseling cancer patients. Ideally, these discussions should be held in surroundings that are quiet, private and comfortable. Enough time for proper discussion should be allowed, and precautions should be taken to prevent interruption by nonurgent phone calls and distractions. If possible, a close relative or friend should be present. The physician should try to find out how much the patient wishes to know about prognosis and other sensitive issues and should respect the patient's wishes. The patient and any accompanying persons should be given plenty of opportunity to ask questions and to express their views. The physician should confirm that the patient has understood what has been said. It is often difficult for patients to remember what they were told at times of high emotion [14]. Therefore, for several years, the senior author has given each new patient an audio recording of the actual consultation on cassette tape or CD-ROM. Feedback has been positive, although a few patients have preferred not to listen to the recording [15]. Other useful aids to communication are a dedicated website (e.g. www.looc.uk.com); a guidebook to the oncology service, which can be mailed to the patient before the first appointment; information leaflets specific to the selected treatment; and an information sheet regarding aftercare, which is given to the patient on discharge from hospital.

Immediately after the consultation, it is helpful if the patient and any accompanying persons can spend some time with a specialist nurse in a quiet room. There are usually many questions that patients consider too trivial to trouble a doctor with. It is often necessary for the nurse to provide consolation, reassurance and other psychological support. Some patients find it helpful to speak to someone who has been through a similar experience, and it is useful to have a 'bank' of volunteer patients who are available on the telephone. During their stay in hospital, patients find it reassuring when they are kept informed of how their treatment is progressing. It is well known that many patients feel particularly 'low' soon after

their return home from hospital. It is therefore comforting for them to receive a telephone call from a specialist nurse at this difficult time.

Because of the rarity of ophthalmic tumors, patients find it difficult to get appropriate information and advice from their family doctor and from general ophthalmologists at their own hospital. It is therefore useful for them to be able to contact the ocular oncology service at any time if they ever have any questions or concerns. Appropriate contact information such as telephone number or e-mail is should be provided. Patients and their families should also be informed of any organisations and website that they might find helpful.

Increasingly in Britain, correspondence to the family doctor and general ophthalmologist is also sent to the patient. The senior author has followed this practice for several years, with positive feedback. However, if the results of pathological or genetic investigations might cause distress, they should be communicated in person, ideally at a hospital clinic or, if this is not possible, by means of a prearranged telephone call.

Follow-up assessments provide a good opportunity for psychological support, and the ocular oncologist can be particularly effective in this regard, for example reminding the patient of a good prognosis that was originally given or emphasising that the chances of local tumor recurrence become very small once the tumor has responded well to conservative therapy. In patients who were initially given a guarded prognosis, there might be scope for encouraging optimism once the 'danger period' has passed without incident.

At all times, the clinician should use every opportunity to encourage hope and optimism, albeit in an honest and realistic manner.

14.7 Decision-Making

In many countries, 'paternalistic' selection of treatment and other aspects of care has largely been replaced by a more 'consensual' approach. Although most patients want to have a say in their care, however, it is often difficult for them to decide what is best for them, especially if they are overwhelmed by the information provided to them and distraught as a result of their illness. Furthermore, many patients do not wish to take responsibility for major life-changing decisions.

The senior author approaches decision-making by first determining the patient's priorities, needs and fears then explaining how these are met by the various options and encouraging the patient to indicate the preferable course of action. Almost always, the patient spontaneously selects what the senior author considers to be the optimal treatment or action, whereupon the author immediately reassures the patient that the correct decision has been made. In this way, patients benefit by 'having a say' in the decision-making without being burdened by the responsibility for their decision [16]. When the patient selects what the author considers to be an inappropriate course of action, gentle probing usually reveals a misconception or misunderstanding so that the problem is resolved with further explanation. This combined 'paternalistic-consensual' approach is most successful when patients trust their clinician [17]. In an attempt to merit such trust, the senior author continuously performs a wide range of quality control studies evaluating not only objective outcomes, such as local tumor control, but also subjective measures such as the patients' long-term quality of life and satisfaction with the decision-making process.

14.8 Psychological Support

As they begin to adjust to their diagnosis and its implications, patients will each face a unique set of challenges shaped by their own personal circumstances, prior experience and beliefs [12]. Consequently, different patients are likely to require individualised psychological support, which may change at different stages of their illness [7]. Because of this variability, no single approach will meet the needs of all patients, and it is therefore best to view the patient as 'expert' in his or her own emotional adjustment [7].

The build-up of a patient's unresolved concerns has been shown to predict later distress

Table 14.1 Patients requiring additional psychological support

Failing to adjust over time
Experiencing an intense emotional reaction that compromises their mechanisms for family and social support
'Stuck' in a way that is likely to inhibit future adjustment
Experiencing emotional problems that are unlikely to resolve over time in a supportive environment

[11]. Careful assessment by interview is therefore recommended to identify and explore (1) the specific challenges faced by each patient, (2) the availability of emotional support and (3) the patient's ability to use different ways of coping. It may be helpful at this time to engage with patients in problem-solving including identifying and accessing sources of practical and emotional support and considering alternative coping responses that may reduce the emotional impact of their illness, also promoting adaptation. Eventually, in a supportive environment, most patients will adjust to their condition and its implications, in their own time [5]. However, some patients may require more specialised help (Table 14.1).

14.9 Patient-Reported Outcome Measures (PROMs)

It is not only the length of survival that is important to patients but also the quality of that survival. There are many ways in which the quality of life (QoL) and mood of patients with uveal melanoma might be impaired. Firstly, many of these patients must come to terms with a high chance of early death, even if their ocular tumor has been successfully eradicated. Secondly, most patients need to cope with some degree of visual loss, which may restrict their activities. Some may also be concerned about cosmetic deficit, caused by treatment such as iridectomy or enucleation. Assessment of patient-reported outcomes using self-report questionnaires to measure quality of life and mood can provide a better understanding of the effects on treatment over time. Clinicians can then advise patients about the functional difficulties they may experience and how they are likely to respond emotionally to their condition. Such insights can enable clinicians to anticipate problems and to adjust behavior and care accordingly. Additionally, by means of a simple tick box on the questionnaire, patients can be asked at regular intervals whether they would like any psychological support.

Unfortunately, studies investigating PROMs after treatment for uveal melanoma have produced conflicting results [18–23], possibly because of small sample sizes, and few have made comparisons with normative data. Therefore, units need to monitor these outcomes routinely so as to audit their own practice and to build up large data sets that would provide more definitive information about PROMs after uveal melanoma.

14.10 Self-Help

Every opportunity should be taken to encourage and facilitate self-help. There are many ways in which patients can improve their own well-being, and most are successful in discovering their individual ways of coping. Patients seem to do particularly well if they feel that some good has come out of their crisis, for example, discovering what is and what is not important in their life, helping others, fundraising and getting closer to their friends and family. Many patients find it helpful to write down their experiences, so that this idea should be given to new patients in case the thought of a diary does not occur to them. All patients should also be informed of any self-help groups that are available, both in their community and on the Internet. Considerations for organising effective psychological care to be available to patients with eye cancer are summarised in Table 14.2.

14.11 Family Support

Patients' well-being is greatly enhanced by any support they receive from relatives and friends. Such carers may themselves need support. It is therefore

Table 14.2 Considerations for providing effective psychological care

Appropriate training of all staff coming into contact with patients, so that distress can be recognised
Mechanisms to empower patients to recognise and manage their own psychological needs
Protocols for assessing all patients psychologically at key points in their care pathway
Protocols for providing psychological support that is appropriate to the severity of any distress, with mechanisms for organising specialist care if needed
Information on how to contact emergency psychiatric services, readily available wherever patients are at risk of self-harm
Systems enabling patients and caregivers to participate in their own care, in self-help activities and peer support
Mechanisms for ensuring that the psychological needs of staff caring for patients and caregivers are adequately met

helpful for the clinician to acknowledge carers that are present in any consultation and to provide them with recognition and encouragement.

Some patients faced with a diagnosis of cancer gain reassurance from still feeling physically well. That is, because their bodies feel normal, they can regard the cancer as less threatening. Their carers, however, do not have access to this way of coping [24]. Carers also often feel that they cannot share their concerns and fears with the patient for fear of upsetting the patient. Clinicians therefore often have a role of promoting open communication between patients and their carers and thereby circumventing the 'conspiracy of silence' that can add to the burden for each party [12].

Carers may benefit from speaking with a member of the clinical team when the patient is absent, for example, if the patient is admitted to hospital or in the operating room. It is therefore helpful to prepare for such an eventuality in advance by proactively obtaining and documenting the patient's consent for such communication.

Oncology services should also be ready to provide support to close friends and relatives at a time of bereavement. Carers will avail themselves of this support only if they know in advance that this service is provided.

14.12 Support from Patient Organisations

Patients should be informed of any organisations that can provide them with support. Examples of such organisations include Cure OM in the United States and Ocumel UK in the United Kingdom.

14.13 Children with Cancer: What Should They Be Told?

It is difficult to speak to a child about cancer and its implications. However, even very young children can sense fear and anxiety among family members [12]. If children are deprived of information about what is happening, they may generate their own ideas, causing them to fantasise or worry. Consequently, it is generally recommend that parents are as honest as possible with their child, allowing the patient to openly express fears and ask questions [26].

Parents know their children better than anyone else; therefore, their judgement of what is the right thing to do for their child and how much they should tell them should be supported and encouraged [12]. In a recent study of parents who had lost a child to cancer, it was observed that parents who talked to their dying child about death had no regrets about having done so [25]. Some parents who did not talk to their child about death subsequently wished they had done so. Clearly, the clinician's aim in such circumstances should be to help parents make the best decision for their family [26].

When providing any information to a child (as with adults), it is clearly important to use language that can be understood [7]. It should be noted that children are developing all the time, consequently information considered appropriate at one age may no longer be sufficient as the child gets older [12]. Therefore, it is important to establish the child's intellectual capacity as well as the level of understanding of the illness or treatment in question. Explanation and reassurance can then be provided in ways that make sense to the child [7].

Conclusions

Psychological support is an integral part of patient care, particularly with serious diseases such as cancer. Such support should be provided not only to the patients themselves but also to their close relatives. Any support should be tailored to each individual case.

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Cataract Associated with Intraocular Tumors

15

Carlos A. Medina, Mary E. Aronow, and Arun D. Singh

Contents

15.1 Introduction

Cataracts are cloudy or opaque areas in the lens that should otherwise be clear. They results in changes that can impair vision. Cataracts can be secondary to age and mechanical, chemical, or radiation trauma. They are the single largest cause of blindness in the world accounting for over 47 % of blindness worldwide [1, 2]. Intraocular tumors are a rare but important cause of cataract, and the presence of intraocular tumor as an underlying cause should be excluded when the cataract is unilateral, total, sectoral, or posterior subcapsular without obvious cause such as trauma, inflammation, or steroid use (Fig. 15.1). The cataract may be caused by the tumor itself or by previous interventions to diagnose (biopsy) or treat (steroids, excision, radiation, chemotherapy) the intraocular tumor. In the case of a poor view on funduscopy, the clinician must rely on thorough examination techniques and ancillary tests such as ultrasonography and ultrasound biomicroscopy to determine the presence and extent of an intraocular tumor [3]. It is important to remember that the management of patients with intraocular tumors is complex, sometimes controversial, and in some instances the tumor may have been treated with unfamiliar techniques. In this chapter we will discuss the various treatment-related causes of cataracts, specific tumor entities associated with cataracts, and special considerations for the management of cataracts in patients with intraocular tumors.

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_15, © Springer-Verlag Berlin Heidelberg 2014

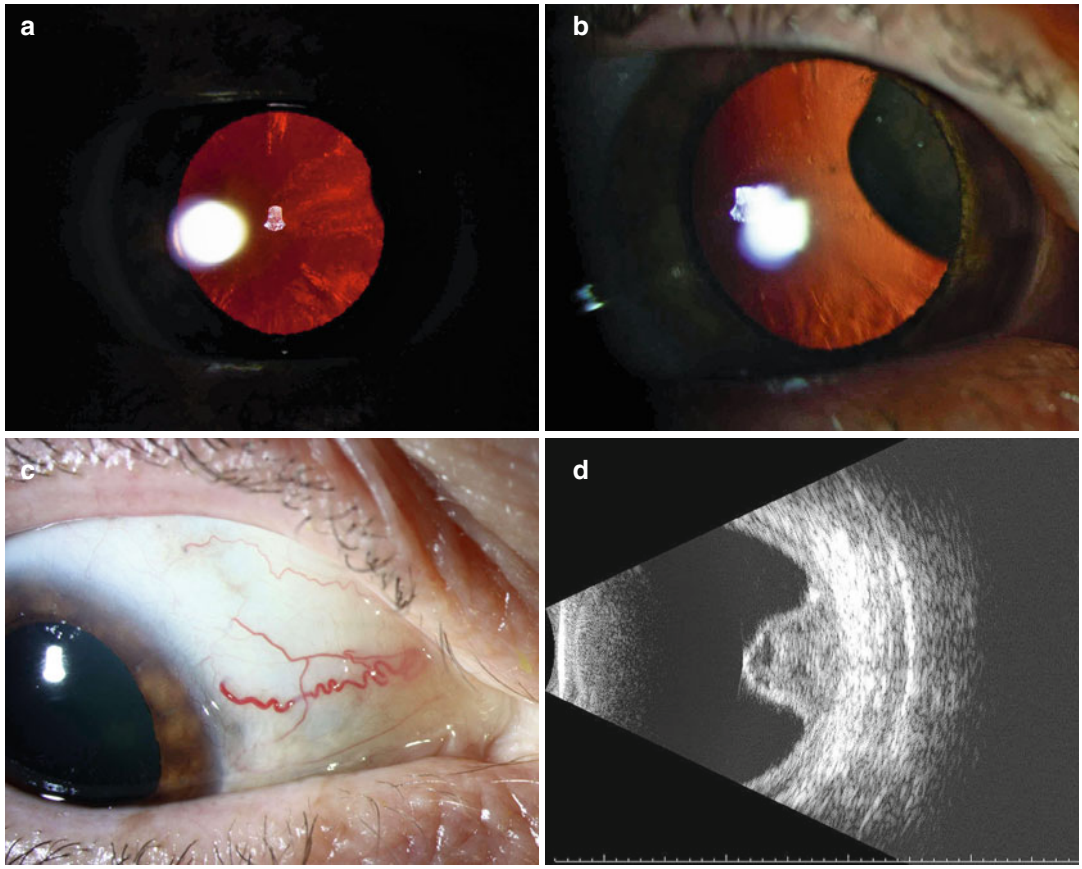

Fig. 15.1 Anterior segment photograph of the left eye. Retroillumination showing wedges of nasal posterior cortical cataract (**a**). A dark retrolental mass in the same quadrant (**b**). Prominent sentinel vessel (**c**) offers clue to the presence of a ciliary body melanoma (**d**)

15.2 Surgical Treatment-Induced Cataract

Fine-needle aspiration biopsy, performed for diagnostic or prognostic purposes, may rarely (less than 1 %) be complicated by development of cataract [4]. Transpupillary thermotherapy is also known to cause heat-induced focal cataracts in approximately 24 % of patients, and these may become visually significant [5]. Iridectomy, iridocyclectomy, choroidectomy, vitrectomy, and virtually any intraocular procedure can lead to cataract formation either due to surgical manipulation, postoperative inflammation, or the use of steroids (Fig. 15.2).

15.3 Radiation-Induced Cataract

Radiation is often used for the treatment of intraocular and periocular tumors. It is usually administered externally in the form of external beam radiation or internally in the form of brachytherapy. The crystalline lens is the most radiosensitive organ and as such is exquisitely sensitive to radiation as doses of less than 2Gy have been reported to cause cataracts [7]. Cataract removal is the only curative treatment for radiation-induced cataracts.

The incidence of cataracts after radiation exposure is high with a reported range between 45 and 100 % [8]. As part of the Collaborative Ocular Melanoma Study (COMS), phakic

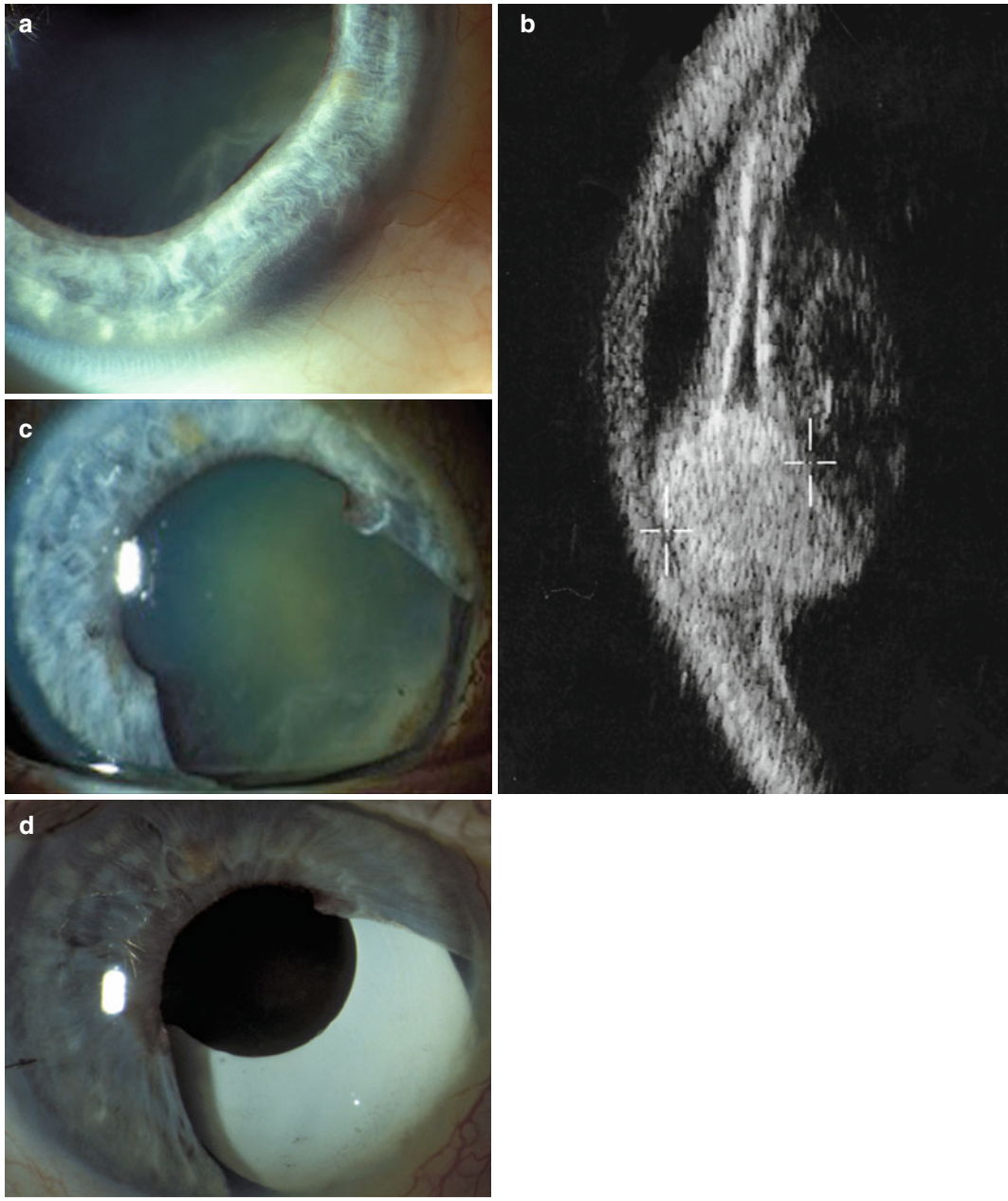

Fig. 15.2 Anterior segment photograph of the right eye. Note a dark brown lesion visible inside the pupillary margin from 4 to 5.30 o'clock position with thinning of iris stroma anteriorly and a sectoral cataract posteriorly (**a**). Anterior segment ultrasound biomicroscopy (20 MHz) revealed a circumscribed solid mass in the angle region tilting the inferior pole of the lens (**b**). Adenoma of the iris pigment epithelium was confirmed histopathologically after removal by iridocyclectomy (**c**). The cataract progressed over the next 3 months. Phacoemulsification cataract surgery with capsular bag placement of intraocular lens was performed. In addition, a blue-colored Oculaid iris prosthesis (Ophtec Inc., Boca Raton, USA) was placed in the capsular bag to mask the surgical coloboma (**d**) (Reproduced with permission from Singh et al. [6])

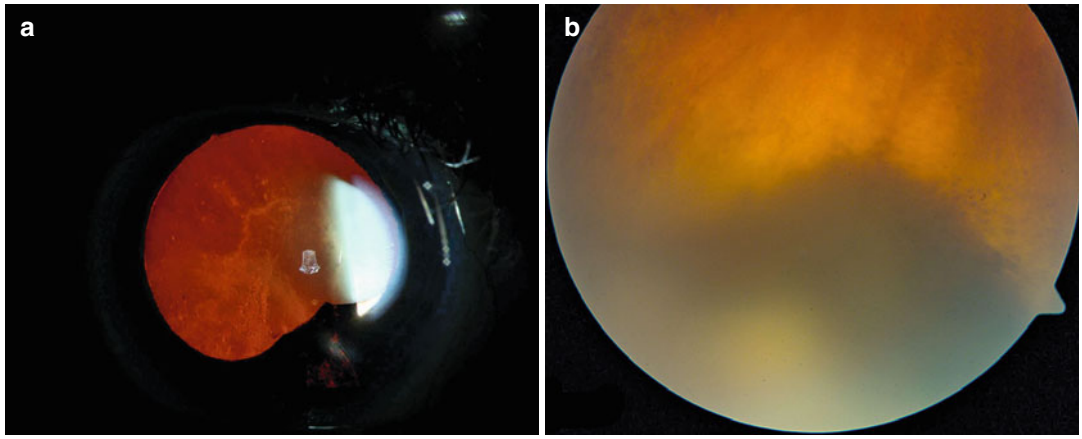

Fig. 15.3 Postradiation-induced sectoral cataract. A 65-year-old man with a ciliochoroidal melanoma in the inferior quadrant of the right eye (**a**). The tumor was treated with I-125 plaque brachytherapy. Twenty four months later, a mild posterior subcapsular cataract with posterior synechiae is present (**b**)

patients who were treated with iodine-125 brachytherapy and had no history of cataract were observed for the development of vision-limiting lenticular opacities or cataract surgery. During the first 5 years of follow-up, cataract developed in 362 (68 %) of eyes, and of these, 49 (9 %) of eyes underwent cataract surgery. The 5-year rate of reported cataract in eyes receiving a dose to the lens of ≥16 Gy was 92 %. After cataract surgery, visual acuity improved by 2 lines or more in 32 (66 %) of patients and remained stable in 12 (26 %) of patients.

The formation of cataracts may occur as early as 1 month following treatment and as late as 7 years depending upon several factors including the type of radiation, total dose, delivery portals, and the time after exposure [9]. Ionizing radiation has been primarily associated with posterior subcapsular cataracts although cortical and nuclear changes have also been reported (Fig. 15.3).

In the COMS the most common cause of lack of visual improvement after surgery was the presence of radiation retinopathy. Cataract surgery did not affect the mortality or metastasis adversely [10].

Aside from cataract formation, radiation therapy can also result in ocular surface damage leading to decreased tear production and keratitis, both of which can have a profound effect on vision and comfort. Furthermore, these factors can impact the surgeon's view during subsequently planned surgeries. Corneal anesthesia may lead to neurotrophic ulcers, and therefore, the ocular surface should be optimized prior to performing cataract surgery. Special considerations must be taken by the surgeon as these patients might have other radiation-induced changes in their anterior chamber that can make cataract removal more challenging. Iris neovascularization and neovascular glaucoma are not infrequent, and the surgeon should closely monitor the patient for any changes in the iris and intraocular pressure. Radiation retinopathy and neuropathy may limit visual recovery following cataract surgery. The use of lead shields and fractionation of the radiation dose has been shown to decrease the total dose of radiation to the lens [11, 12].

15.4 Chemotherapy- and Hormonal Therapy-Induced Cataract

A number of chemotherapeutic agents and hormones used in the treatment of cancer patients have been shown to cause cataracts. It is well known that corticosteroids (such as dexamethasone and prednisone) administered either locally or systemically can cause cataracts. Anastrozole

and busulfan are commonly used in the management of cancer patients and are also known to cause cataracts [13, 14]. In one clinical trial, 6.8 % of postmenopausal women treated with tamoxifen developed cataracts [15]. Cataract is the most common complication observed in patients receiving intravitreal methotrexate for intraocular lymphoma [16].

15.5 Specific Entities

15.5.1 Medulloepithelioma

Medulloepithelioma is a rare tumor of the ciliary pigment epithelium. It usually becomes apparent during the first decade of life and presents as an irregular, variably sized, white or gray translucent mass. Occasionally cysts may be present. Medulloepithelioma is divided into teratoid and non-teratoid types and either may have benign or malignant cytologic features. Because the tumor arises from the ciliary region, it is often hidden by the iris and difficult to diagnose until the tumor is significant in size. For this reason, thorough evaluation including a fully dilated funduscopic examination, transpupillary, and transocular transillumination, as well as ancillary testing such as ultrasonography and ultrasound biomicroscopy are helpful in determining the presence and extent of the tumor. Because most of these tumors are cytologically malignant, enucleation is usually advisable. A subset of these tumors may be managed by local resection or brachytherapy [3]. Medulloepithelioma should be suspected as an underlying cause of nontraumatic cataract in a child. The presence of a lens coloboma or iris/angle neovascularization should increase suspicion.

Prior to cataract removal, stable tumor regression and absence of recurrence must be established in consultation with the treating oncologist. Presence of comorbidities such as tractional retinal detachment, radiation optic neuropathy, or radiation retinopathy may not favor cataract removal because of limited visual potential. Published reports of cataract removal in cases of treated medulloepithelioma are not available due to the rarity of the disease.

15.5.2 Retinal Lymphoma

Primary retino-vitreal lymphoma is a variant of primary central nervous system non-Hodgkin's lymphoma with predominantly ophthalmic involvement that usually presents in elderly patients. Intraocular involvement may occur prior to, after, or at the time of central nervous system (CNS) disease.

Diagnosis may be challenging especially in cases where intraocular involvement precedes systemic symptoms, and these patients are sometimes mistakenly treated as uveitis. Once the diagnosis is made, treatment with ocular radiation is implemented and is adequate in controlling ocular involvement in the majority of cases [17]. Because of the high incidence of bilateral disease, irradiation of both eyes is usually performed. Though ocular disease is controlled, most patients usually go on to develop CNS disease. Recently a combination of intravitreal and intrathecal chemotherapeutic agents such as methotrexate has been used, yet unfortunately even with new advances in treatment, the mortality still remains high and most patients expire within 2 years of diagnosis [18, 19].

Cataracts are the most common complication observed in patients receiving intravitreal methotrexate; furthermore, most of these patients have also undergone a diagnostic vitrectomy prior to starting treatment [16]. A major goal of ocular treatment in patients diagnosed with retinal lymphoma is to maximize quality of life. Currently, there are no data to support an increase in morbidity or mortality in patients with primary retinal lymphoma following cataract surgery, and cataract surgery in these patients may be appropriate and should be considered on a case-by-case basis.

One study reported progression of cataracts in 73 % of eyes receiving intravitreal methotrexate. Cataract surgery was successful in seven of eight eyes that underwent cataract extraction with intraocular lens implantation [16]. In one case an expulsive suprachoroidal hemorrhage occurred intraoperatively and subsequently the eye became phthisical [16].

15.5.3 Retinoblastoma

Retinoblastoma is a malignant tumor arising from the retina. The initial clinical presentation is that of a young child with leukocoria, strabismus, "red eye," heterochromia, hyphema, or pseudohypopyon. The average age of diagnosis is 24 months for unilateral and 12 months for bilateral cases [3]. Although cataract is not usually observed in patients with retinoblastoma, the clinician should rule out intraocular malignancy in all pediatric cataracts. Ancillary testing such as ultrasonography is helpful in cases where the view to the fundus using ophthalmoscopy is inadequate.

Improvements in early detection and treatment have made globe-conserving therapy for retinoblastoma increasingly common. Although chemotherapy with or without cryotherapy and thermotherapy is frequently selected, plaque radiotherapy or external beam radiation continues to be used as salvage therapy. In cases where salvage therapy is used, doses in excess of 40 Gy are delivered, and cataracts are expected to occur 1–3 years following treatment [7]. Cataracts impair vision as well as the ability for the ophthalmologist to examine the retina, and amblyopia is of particular concern in the young. As previously mentioned, radiation is associated with posterior subcapsular cataract, and these occur in 22–87 % of eyes after external beam radiation and 9.7–31 % of eyes after brachytherapy [20].

Of particular concern in retinoblastoma is the possibility for tumor dissemination when intraocular surgery is performed. Although absence of association with systemic metastasis has been observed, there are reports of local tumor recurrence after cataract surgery [21, 22]. Several studies have shown that cataract extraction with or without intraocular lens placement is safe in patients who have previously received radiation treatment for retinoblastoma [7, 20, 21, 23]. Adequate treatment of the tumor as well as an appropriate quiescent interval of 12–18 months appears to be key in preventing recurrence and metastasis [22–25]. Although both clear corneal and pars plana approaches have been successfully used, a pars plana approach may be preferable as this provides more support in preventing postoperative wound leak in the child who is prone to eye rubbing. Subconjunctival spread of the tumor via the scleral tunnel has, however, been observed [21].

Pediatric cataract surgery is associated with a high likelihood of developing posterior capsular opacities. The decision to perform a primary posterior capsulotomy and anterior vitrectomy at the time of cataract surgery should be based on the patient's age, quiescent interval, location and stage of the tumor, and clarity of the visual axis once the cataract has been removed. It has been postulated that leaving the posterior capsule intact may potentially function as a barrier to tumor spread as well as reducing the risk of retinal detachment, and therefore, it may be prudent to leave the capsule intact initially and to consider capsulotomy as a second procedure only if necessary.

15.5.4 Uveal Melanoma

Uveal melanoma is the most common primary intraocular malignancy in adults. It includes primary melanocytic tumors of any of the three parts of the uvea, which is composed of the iris, ciliary body, and choroid. Iris melanoma is the least aggressive of the three types of uveal melanoma and is usually not associated with severe cataract. Presence of a sectoral cataract could imply ciliary body involvement and may be a primary feature of ciliary body melanoma which is the most aggressive of the three [3]. Management of uveal melanoma depends on the size, location of the tumor, and visual potential of the eye. Brachytherapy, proton beam radiotherapy, thermotherapy, stereotactic radiotherapy, resection, and enucleation are common methods of treatment. Resection of the tumor may include iridectomy, iridocyclectomy, and trans-scleral or trans-retinal choroidectomy.

Uveal melanoma should be suspected with any cataract having an atypical presentation, as multiple cases have been reported of unsuspected uveal melanoma diagnosed after cataract extraction [26–29]. In these cases, a complete investigation

including ultrasonography and ultrasound biomicroscopy should be performed. Some authors advocate the use of ultrasonography in all eyes with opaque media regardless of history and even in the case of bilateral senile cataracts. Transillumination can also be used to establish the diagnosis of uveal melanoma in the setting of a dense cataract.

Two hypotheses have been proposed regarding the safety of performing cataract surgery in patients treated for uveal melanoma. The first is that the tumor has received adequate irradiation/treatment and is therefore incapable of metastasis. The second is that many patients who present with uveal melanoma already have hepatic micrometastases at the time of diagnosis [30].

Outcomes after cataract extraction in the setting of treated melanoma are well documented. The Collaborative Ocular Melanoma Study demonstrated that 83 % of the study eyes (532) developed cataracts 5 years after treatment [10]. Of these, only 12 % underwent cataract surgery. Cataract surgery did not affect the mortality or metastasis adversely [10]. After cataract surgery, visual acuity increased by two lines or more in two-thirds of the patients (66 %). Vision-limiting complications were reported in 33 of 49 eyes. These included radiation retinopathy, which was present in 67 % eyes and was the most common cause of lack of visual improvement after cataract surgery. Cystoid macular edema was reported in 13 eyes and neovascular glaucoma in 6 eyes. Other less common comorbidities included diabetic retinopathy [1], vitreous hemorrhage [1], radiation optic neuropathy [1], and retinal detachment [1, 10]. Other authors have reported similar postoperative issues [31, 32]. In a large retrospective study including 72 eyes, rubeosis iridis, secondary glaucoma, and posterior synechia were present in about one-third of cases. Intraoperatively, defects of the posterior capsule occurred in 12.5 % of the eyes. Visual acuity equal to or better than preoperative vision was achieved in 95.8 % of the patients. Treatment of radiation retinopathy and tumor metastasis was described in 13.8 and 8.3 % of patients, respectively.

Recently, combined microincisional vitrectomy and phacoemulsification with intravitreal triamcinolone acetonide injection and direct endolaser tumor ablation has been reported to be safe and effective in patients treated for uveal melanoma with complications from I-125 brachytherapy [33]. The results of these studies show a relatively low complication rate and unchanged metastatic rates suggesting that cataract surgery in adequately treated patients is a safe procedure that improves visual acuity in the majority of patients.

15.5.5 Uveal Metastases

Metastatic disease is the most common intraocular tumor. Metastatic breast cancer is the most commonly diagnosed metastasis constituting about half of all the patients diagnosed with metastatic disease to the uvea. This is followed by lung cancer which is present in about one-quarter of all patients with uveal metastatic disease. Other less common primary sites include prostate carcinoma, gastrointestinal carcinoma, renal cell carcinoma, and cutaneous melanoma. Up to one-quarter of the patients are found to have bilateral metastatic disease at the time of presentation, and multifocal infiltrates are common. The surgeon should consider several aspects to determine the most appropriate therapeutic approach. Ocular treatment is sometimes based on improving quality of life, and the main goal of treatment is to preserve or improve vision.

Cataracts are common in patients with uveal metastasis as external beam radiation is the most commonly used modality for treatment [34]. Cataract surgery should be considered in patients with significant visual impairment due to cataracts. Potential benefit, current quality of life, associated comorbidities, and life expectancy are important considerations.

15.6 Summary

Cataracts are commonly found in patients with intraocular tumors. There are several special considerations such as monocular status, associated comorbidities, and potential benefit of surgical

Table 15.1 Special considerations for management of tumor-associated cataract

Factor	Comment
Dry eyes	Surface issues, lubrication, punctal plugs
Astigmatism	Irregular or regular, suboptimal visual outcome
Iridectomy	Photophobia, pupilloplasty, iris prosthesis
Zonular support	Gentle manipulations, capsular rings
Associated glaucoma	Combined surgery
Radiation retinopathy	Combined PRP and intravitreal anti-VEGF
Radiation optic neuropathy	Poor visual outcome
Monocular status	Choice of anesthesia
Risk of tumor dissemination	Document tumor stability
Immunocompromised status	Risk of infection
Shortened life expectancy	Careful assessment of benefit

PRP pan retinal photocoagulation, *VEGF* vascular endothelial growth factor

intervention in a setting of shortened life expectancy that must be assessed when managing a patient with tumor-related cataract (Table 15.1). The risk factors, management, and visual outcome of tumor-associated cataract depend on the location, size, type of intraocular tumor, and previous interventions. Management of tumor-related cataracts should be done in conjunction with an ocular oncologist or an ophthalmologist that is well versed in dealing with intraocular tumors.

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Tumor-Associated Glaucoma

16

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16.1 Introduction

Glaucoma secondary to intraocular tumors is not uncommon, and intraocular tumor type and location determines the prevalence of secondary glaucoma and ocular hypertension. A review of 2,597 patients with intraocular tumors found elevated intraocular pressure [1] in 5 % of tumor-containing eyes at the time of diagnosis. Of those eyes, 2,111 had uveal melanoma, and secondary IOP elevation was present in 3 % of these eyes. Seventeen percent of the melanoma eyes involved the ciliary body, and there were 7 % with iris involvement, and 2 % with choroidal involvement. Two hundred and fifty-six of the 2,597 eyes had uveal metastases, and glaucoma occurred in 5 % of these patients [1]. Another survey of 227 cases of metastatic carcinoma to the eye and orbit found that glaucoma was present in 7.5 % of the patients [2].

Direct invasion of the angle and ciliary body as well as pigmentary dispersion associated with uveal melanomas commonly leads to elevated IOP. Tumors that are metastatic to the eye can infiltrate the angle or close the angle by mass effect and are associated with higher rates of glaucoma. Angle neovascularization associated with retinoblastomas can lead to secondary glaucoma. A histopathologic and clinical review of 149 eyes with retinoblastoma revealed histologic evidence of glaucoma in 50 % and increased IOP in 23 % of these eyes [3]. Tumors such as lymphoma, leukemia, benign reactive hyperplasia, medulloepitheliomas, iris adenomas, and iris melanocytomas account for a small percentage of intraocular

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_16,

tumors and are infrequently associated with secondary glaucoma. These tumors rarely present with elevated IOP as the initial manifestation.

16.2 Pathogenesis

Intraocular tumors can present with both open-angle and angle-closure glaucoma through various mechanisms.

16.2.1 Open-Angle Glaucoma

There are three principal mechanisms by which intraocular tumors can cause open-angle glaucoma: direct closure of the anterior chamber angle by the tumor mass, tumor cells seeding the anterior chamber angle, and necrotic tumor cells engulfed by macrophages that obstruct the outflow pathways in the trabecular meshwork.

Closure of the anterior chamber angle by mass effect can be caused by many types of tumors, including melanoma, nevus, metastasis, and medulloepithelioma. Extension of melanoma and nevus across the trabecular meshwork can result in increased IOP [1].

Tumor cells can seed the anterior chamber angle and lead to open-angle glaucoma. Metastatic tumors typically directly infiltrate the trabecular meshwork and lead to increased IOP and secondary glaucoma (Fig. 16.1) [1, 4]. Metastases of systemic melanoma can also cause pigmentary dispersion leading to glaucoma. In cases of intraocular melanoma, pigmented cells are dispersed into the anterior chamber and can become lodged in the spaces of the trabecular meshwork, hindering the outflow of aqueous humor (Fig. 16.2) [5]. Leukemias and lymphomas can result in a pseudohypopyon in the anterior chamber composed of layered tumor cells deposited within the angle resulting in open-angle glaucoma.

The third mechanism is specific to melanotic tumors. Necrotic tumor cells are engulfed by macrophages within the angle that occlude the spaces within the trabecular meshwork and raise intraocular pressure, causing melanomalytic glaucoma [5, 6].

16.2.2 Angle-Closure Glaucoma

Angle closure is commonly seen in patients with intraocular tumors. This occurs through three main mechanisms: forward movement of the lens-iris diaphragm, peripheral anterior synechiae formation, and neovascular glaucoma.

Tumor mass effect can cause forward rotation of the lens-iris diaphragm, leading to closure of the anterior chamber angle. Patients can present with manifestations of acute or chronic angle-closure glaucoma. This is more commonly seen with posterior segment tumors and is frequently accompanied by retinal detachment. Therefore, any patient with unilateral serous retinal detachment and glaucoma should raise the suspicion for underlying malignancy.

Forward movement of the iris by anterior uveal tumors such as ciliary body melanoma can result in occlusion of the trabecular meshwork and PAS formation. Massive subretinal hemorrhage can also result in forward movement of the iris and PAS formation and is seen in patients with leukemia or myelodysplastic syndrome [7, 8].

Neovascular glaucoma is a common cause of angle-closure glaucoma in intraocular melanomas [1, 5]. Intraocular metastasis, retinoblastoma, and medulloepithelioma can also be associated with neovascularization and secondary glaucoma. Radiation-induced retinal ischemia can lead to neovascularization and is an important cause of glaucoma as this is a common treatment modality for intraocular tumors (Fig. 16.3).

16.3 Clinical Features

Patients with glaucoma are typically asymptomatic; however, glaucoma related to intraocular tumors is frequently associated with presenting ocular symptoms. Iris heterochromia, poor response to IOP-lowering therapy, and serous retinal detachment in a patient with unilateral or very asymmetric glaucoma should alert the clinician to the possibility of an intraocular tumor [4, 9].

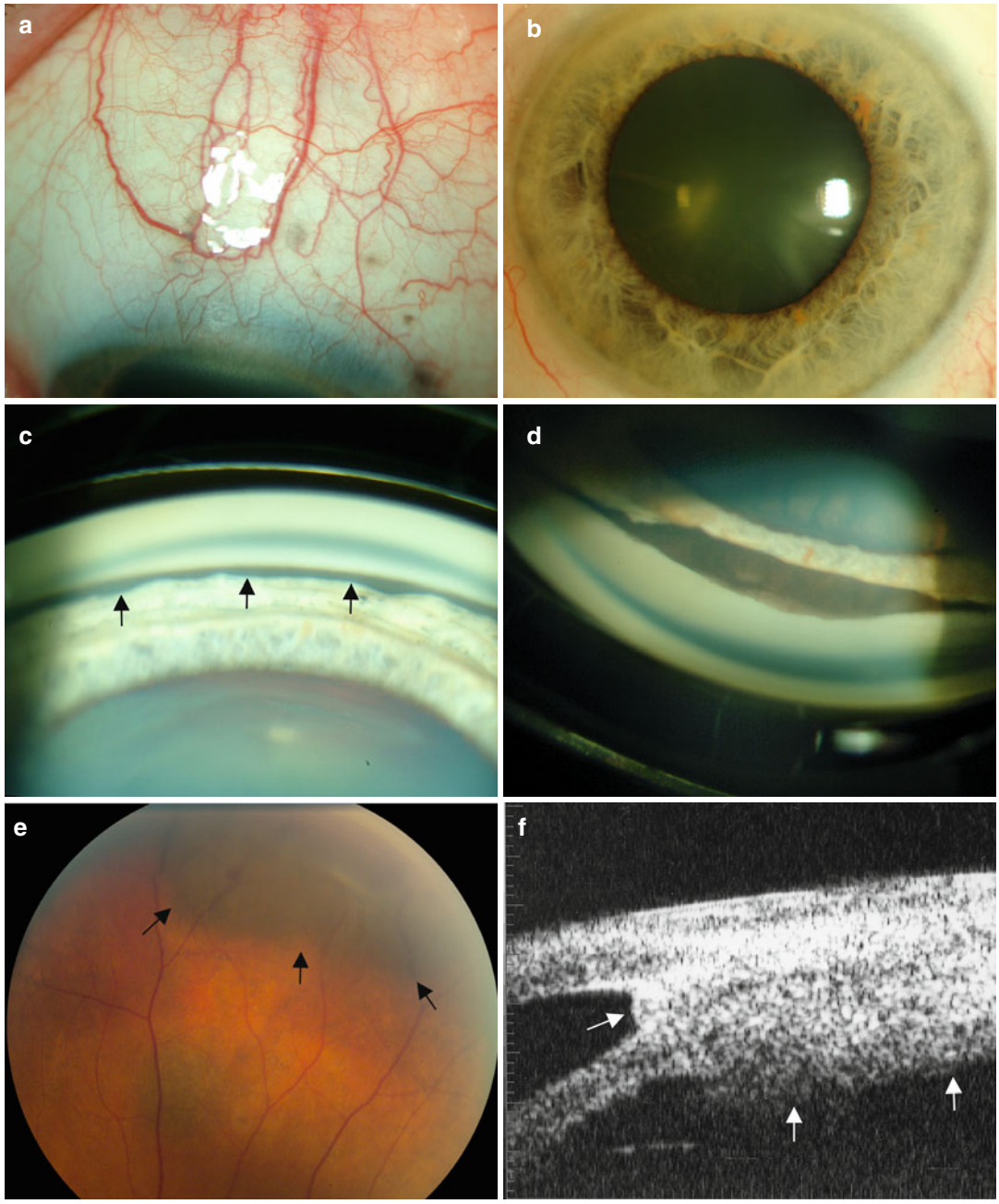

Fig. 16.1 A 64-year-old woman was diagnosed with ocular hypertension OS. The IOP was 35 mmHg. On external examination prominent sentinel vessels with episcleral pigmentation was noted superiorly (**a**). The anterior segment appeared normal (**b**). On gonioscopy there was diffuse pigment seeding in the angle (**c**, *arrows*) with area of tumor extension from 12 to 1 O'clock meridian (**d**). Ophthalmoscopy revealed peripheral choroidal melanoma (**e**, *arrows*). Ciliary body and angle extension were confirmed by ultrasound biomicroscopy (**f**, *arrows*)

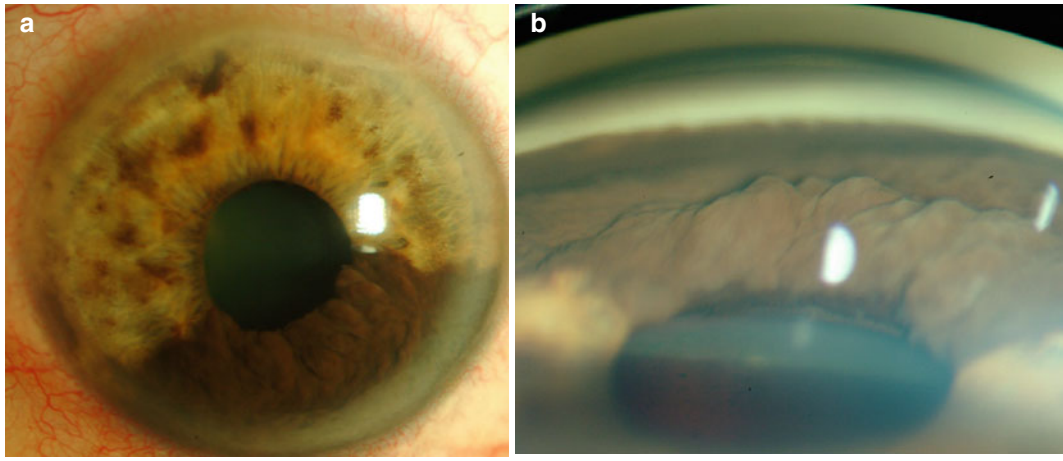

Fig. 16.2 Diffuse iris melanoma of the lower half of the iris causing secondary glaucoma (IOP = 26) (**a**). Gonioscopy confirmed pigment seeding in the angle (**b**)

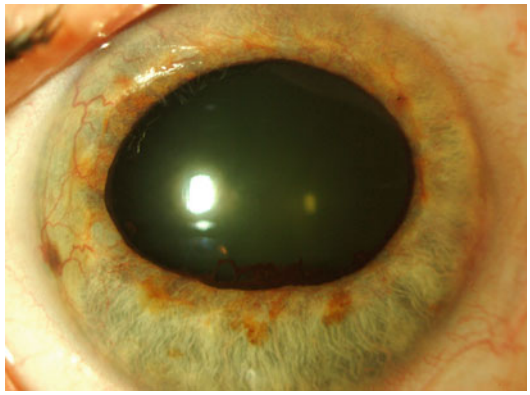

Fig. 16.3 Neovascular glaucoma 13 months following Iodine-125 plaque radiotherapy for a medium-sized choroidal melanoma

16.4 Diagnostic Evaluation

A thorough ophthalmic examination including slit lamp biomicroscopy, gonioscopy, transillumination of the globe, and posterior segment examination is critical. On slit lamp examination, the iris may reveal a melanotic or nonmelanotic lesion, and pigmented cells may be present in the anterior chamber indicative of an anterior or posterior melanoma [5, 6]. The iris may be displaced and may have neovascularization, or the lens can be subluxed or focally opacified.

Direct tumor invasion of the anterior chamber angle may be seen on gonioscopy as well as angle seeding by pigmented or nonpigmented tumor cells and neovascularization of the angle [1, 5, 10].

Malignant melanomas can often show transillumination defects; as shown in a report of 23 ring melanomas, in which 100 % of patients had transillumination defects [11].

Dilated fundus examination may reveal a choroidal tumor, retinal detachment, or vitreous seeding with tumor cells. In cases of angle closure or occludable angles, dilation should be postponed until after peripheral iridotomy or iridectomy.

Clinical examination alone may not be adequate to provide definitive diagnosis. For example, clinically, iris nevi and melanomas are indistinguishable. Fluorescein angiography of the iris, ultrasound biomicroscopy, and anterior segment OCT can be helpful in identifying malignant lesions [12]. Ultrasound biomicroscopy is useful in detecting ciliary body and choroidal tumors, but cannot reliably distinguish between a benign and a malignant lesion [13, 14]. It can accurately detect changes in tumor size, growth, and extension, useful for monitoring of suspicious lesions, and helpful when contemplating surgical tumor resection [13, 15]. When these modalities are inconclusive, fine-needle aspiration biopsy of the tumor is crucial to arrive at a definitive diagnosis [16, 17]. In cases of suspected primary retinal lymphoma, vitrectomy can provide samples for cytologic and immunohistochemical analysis [18].

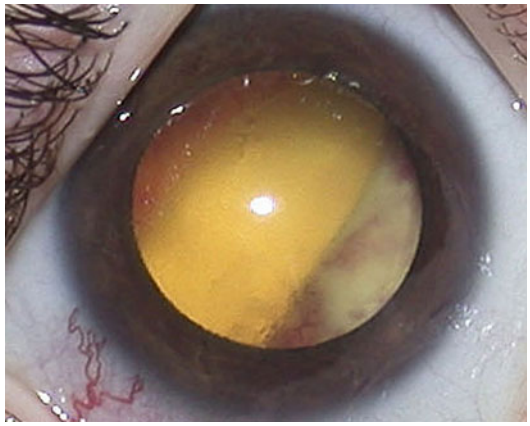

Fig. 16.4 Neovascular glaucoma associated with Stage E (international classification) retinoblastoma. Note ectropion iridis

16.5 Specific Entities

16.5.1 Childhood Glaucoma

Glaucoma in childhood is typically associated with congenital and developmental anomalies, which can be easily distinguished from secondary glaucoma due to intraocular tumors. In cases of neovascular angle closure, one must consider retinoblastoma, as this is the most common cause of glaucoma in these patients (Fig. 16.4). Retinoblastoma associated glaucoma with iris neovascularization as the initial presenting sign has been reported in 7 % of cases [19]. Any child with neovascular glaucoma should be evaluated for retinoblastoma. Other conditions that present with neovascular glaucoma include retinopathy of prematurity, persistent hyperplastic primary vitreous, retinal dysplasia, Coats' disease, toxocariasis, and infantile retinal detachment [20]. Additionally, some rare childhood conditions associated with glaucoma include phakomatoses, juvenile xanthogranuloma, and medulloepithelioma.

16.5.1.1 Sturge-Weber Syndrome

Cutaneous vascular hamartomas or hemangiomas along the distribution of the 5th cranial nerve are the hallmark of Sturge-Weber syndrome, and most patients present with a cutaneous hemangioma, especially of the upper eyelid. Central nervous system involvement can be seen with manifestations including leptomeningeal angioma and cortical calcifications. Dilated and tortuous episcleral veins and telangiectatic conjunctival vessels are commonly seen on ophthalmic examinations [21]. Approximately half of the patients are affected by glaucoma and commonly is secondary to elevated episcleral venous pressure but can also be the result of anomalous development of the anterior chamber angle [22].

16.5.1.2 Neurofibromatosis Type 1

Hyperpigmented lesions, or café-au-lait spots and neurofibromas within the peripheral nervous system, are characteristic of neurofibromatosis type 1. Lisch nodules can be seen on anterior segment examination, and elevated IOP can result from iris neovascularization secondary to retinal vasoproliferative tumor or developmental angle anomalies [23]. Neurofibromas of the upper eyelid have been associated with higher rates of glaucoma. Glaucoma occurs more commonly in those with orbitofacial involvement, as revealed by a retrospective chart review of 95 patients diagnosed with NF1 in a 15-year period. Of these patients with facial involvement, glaucoma occurred only on the ipsilateral side at a rate of 23 %, always with ipsilateral globe enlargement, required glaucoma surgery, and was associated with a poor prognosis [24].

16.5.1.3 Von Hippel-Lindau Disease

Neovascular glaucoma can be seen in these patients with neglected or advanced cases of retinal capillary hemangioma.

16.5.1.4 Juvenile Xanthogranuloma

Ocular manifestations of juvenile xanthogranuloma include lightly pigmented iris lesions, histologically composed of foamy histiocytes and Touton giant cells, and can cause spontaneous hyphema, resulting in secondary glaucoma. Direct invasion of the anterior chamber angle by histiocytes can also lead to elevated IOP and glaucoma. Patients with this condition also have yellow papular skin lesions on the head and neck [25].

16.5.1.5 Medulloepithelioma

Tumors that arise from the nonpigmented epithelium of the ciliary body are called medulloepithelioma. They can be benign or

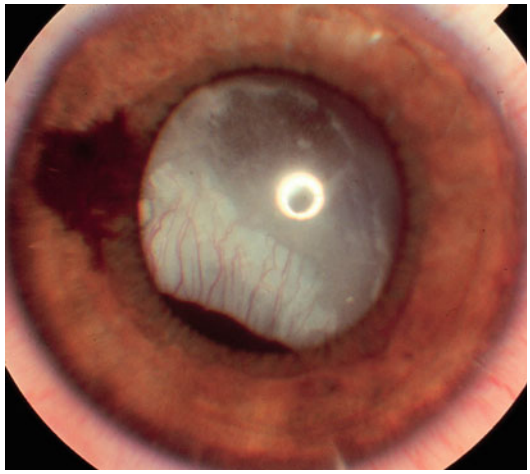

Fig. 16.5 Anterior segment photograph showing lens coloboma and a vascularized opaque cyclitic membrane. Note a pigmented mass in the ciliary body region (Reproduced with permission from Singh et al. [27])

malignant and appear as a whitish gray mass of the ciliary body or iris. Neovascularization of the anterior chamber angle and forward displacement of the iris and PAS formation are common causes of glaucoma in these patients (Fig. 16.5) [26, 27].

16.5.2 Adult Glaucoma

16.5.2.1 Iris Stromal Cysts

Both angle-closure and open-angle mechanisms can cause glaucoma in cases of iris stromal cysts whether spontaneously occurring or following surgery or trauma to the eye [28]. Ultrasound biomicroscopy can differentiate iris cysts from malignant lesions [29].

16.5.2.2 Melanocytoma

Melanocytomas are benign pigmented lesions that most frequently affect the optic nerve but can occur within the iris or ciliary body. These tumors can cause glaucoma by extension into the trabecular meshwork or by pigment dispersion or necrotic melanocytoma cells [30].

16.5.2.3 Fuchs Adenoma

Fuchs adenoma is a benign tumor of the ciliary body that can involve the iris and cause glaucoma through pigment dispersion [31].

16.5.2.4 Uveal Melanoma

On slit lamp examination, iris melanoma typically appears as an elevated brown mass. In cases of ciliary body involvement, early detection can be more difficult. A dome-shaped elevation of the iris is the typical presentation and can be seen between the iris and lens on dilated gonioscopic examination. In cases of ring melanoma, the patient may present with unilateral glaucoma, and a mass may extend circumferentially around the trabecular meshwork. This type of tumor can arise from the iris, the ciliary body, or the trabecular meshwork [10]. Anterior uveal melanomas with a pale, nodular appearance that resembles tapioca pudding are termed tapioca melanomas, and glaucoma has been reported in one third of these patients [32, 33]. When choroidal melanomas are associated with glaucoma, frequently a large mass that pushes the lens/iris diaphragm forward is seen on exam. Additionally, retinal detachment or vitreous hemorrhage may present with pigment dispersion into the vitreous and melanomalytic glaucoma. Neovascular glaucoma can be a late sequela.

16.5.2.5 Metastatic Tumors

Intraocular metastases most commonly arise from carcinoma of the lung or breast, and the choroid is the most frequent intraocular metastatic site [34]. Glaucoma is more commonly associated with anterior segment metastases [4]. On exam, a metastasis to the iris or ciliary body appears as a translucent and gelatinous mass and may be indistinguishable from an amelanotic melanoma. Frequently, these tumors are associated with iris neovascularization or hyphema (Fig. 16.6).

16.5.2.6 Leukemia/Lymphoma

Massive subretinal hemorrhage and acute angle-closure glaucoma can be a result of choroidal infiltration by leukemia [7]. Anterior segment exam may reveal hypopyon or hyphema with resultant open-angle glaucoma [35]. Lymphoma can affect the anterior segment and present as iritis with elevated IOP, although this is rare [36]. Investigation for recurrence of leukemia or lymphoma is essential in these patients presenting with iritis.

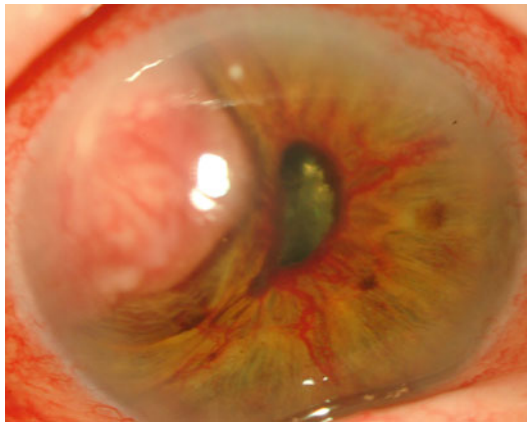

Fig. 16.6 Iris metastasis from endometrial carcinoma causing neovascular glaucoma

16.5.2.7 Myelodysplastic Syndrome/ Multiple Myeloma

A few cases of myelodysplastic syndrome presenting with serous retinal detachment and angle-closure glaucoma have been reported [8]. A case of multiple myeloma with initial manifestation as iritis has been reported, and a cytologic analysis of the aqueous sample revealed neoplastic plasma cells [37].

16.6 Treatment

Treatment of the tumor results in improvement in intraocular pressure in most cases. In patients whose glaucoma does not respond to tumor ablation, management can be difficult [38, 39]. In cases of uveal melanoma, and retinoblastoma, filtering surgery carries the risk of iatrogenic dissemination of the tumor and should be avoided, and medical therapy should be attempted first [39, 40]. Laser trabeculoplasty can be considered in these patients, taking care to avoid areas of the anterior chamber angle that involve the tumor. Those tumors that are associated with pigment dispersion and secondary glaucoma may respond well to laser trabeculoplasty [40].

In some situations of uncontrolled IOP, a cyclodestructive procedure can be considered, as the integrity of the anterior chamber is preserved and the procedure can be titrated to some degree [41]. Techniques that do not penetrate the anterior chamber including canaloplasty and deep sclerotomy have shown promising results in these patients [42, 43]. One case report describes successful treatment of glaucoma secondary to iris melanoma with canaloplasty [44]. In many cases of ciliary body or choroidal melanoma and retinoblastoma associated with glaucoma, the treatment of choice is enucleation.

When non-penetrating surgery is unsuccessful, penetrating surgery can be considered. Radiotherapy can result in local conjunctival inflammation and scarring, making trabeculectomy difficult [45]. In a case series of 31 patients with elevated IOP, following proton beam radiotherapy for anterior uveal melanoma with documented tumor regression, that were treated with Baerveldt shunt implantation, 86 % of patients treated with glaucoma medications achieved adequate IOP control. On follow-up exam, none of these patients had local or systemic dissemination of their tumors [46].

Patients with Sturge-Weber syndrome-associated glaucoma require special consideration. Those with elevated IOP caused by elevated episcleral venous pressure, surgical therapy is frequently required, although these patients have an increased risk of intraoperative choroidal effusion and expulsive hemorrhage [47]. Goniotomy has been successful in some patients and carries a decreased risk of complications [48].

16.7 Summary

Glaucoma is a manifestation of intraocular neoplasia that not only affects the health of the eye but may also affect the patient's systemic prognosis. Open-angle and angle-closure mechanisms can be associated with glaucoma in intraocular tumors. Patients presenting with unilateral glaucoma should be evaluated for associated intraocular tumors. The management of glaucoma associated with intraocular tumors should be adjusted depending on the mechanism of elevated intraocular pressure and should be individualized given the complexities of these cases (Table 16.1).

Table 16.1 Special considerations for management of tumor-associated glaucoma

Factor	Comment
Dry eyes	Surface issues, lubrication, punctal plugs
Conjunctival scarring	Choice of surgery
Iris abnormalities	Synechiae, iridectomy
Associated cataract	Combined surgery
Radiation retinopathy	Combined PRP and intravitreal anti-VEGF
Radiation optic neuropathy	Poor visual outcome
Risk of tumor dissemination	Document tumor stability
Immunocompromised status	Risk of infection
Shortened life expectancy	Careful assessment of benefit

PRP Pan retinal photocoagulation, *VEGF* Vascular endothelial growth factor

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Graft-Versus-Host Disease

17

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17.1 Introduction

Allogeneic (genetically different, same species) hematopoietic stem cell transplantation (HSCT) is a curative therapy for a variety of hematological malignancies, autoimmune diseases, inherited disorders of metabolism, histiocytic disorders, and other malignant solid tumors [1–3]. The number and indications for HSCT continue to increase, with more than 30,000 procedures performed annually across the world [2]. The number of unrelated donor transplants, the most commonly performed transplant, is expected to double within the next 5 years due to improvements in techniques including donor leukocyte infusions and isolation of umbilical cord stem cells [2, 3].

The major histocompatibility complex contains the genes that encode tissue antigens, which is referred to as the human leukocyte antigen (HLA) region in humans [4]. Syngeneic

Relevant financial disclosures: no relevant financial interests.

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_17, © Springer-Verlag Berlin Heidelberg 2014

transplantation, between identical twins, represents the optimal form of HSCT and, unlike other allogeneic donors, does not carry risk of GVHD [5]. Even with sibling donors, which are more likely than unrelated donors to be HLA-matched, 25–35 % of recipients develop GVHD [2, 5]. Despite HLA matching between a patient and donor (sibling or unrelated), substantial numbers of patients still develop GVHD because of differences in minor histocompatibility antigens that lie outside the matched HLA loci [2, 5].

GVHD remains the most frequent and serious complication limiting broader application of HSCT. Given the increasing number of transplant recipients, larger numbers of patients affected with GVHD are expected in the near future. As many recipients of HSCT become long-term survivors, their quality of life becomes increasingly important.

17.2 Etiology

Billingham formulated three requirements for the development of GVHD in 1966: the graft must contain immunologically competent cells, the recipient must express tissue antigens that are not present in the transplant donor, and the recipient must be incapable of mounting an effective response to eliminate the transplanted cells [6, 7].

GVHD develops when donor T cells respond to recipient tissue antigens secondary to mismatches between major or minor histocompatibility antigens between the donor and recipient [8]. Allogeneic HSCT is the most common setting for the development of GVHD.

17.3 Pathogenesis

Four important factors influence the pathogenesis of GVHD:

(A) *Source of hematopoietic stem cells*: Peripheral blood stem cells (PBSC) have largely replaced marrow for autologous and most allogeneic transplantations. Peripheral blood stem cells also contain T cells that increase the incidence of GVHD. A process called apheresis or leukapheresis is used to obtain PBSCs for transplantation [2, 3]. A more recent technique to obtain hematopoietic stem cells is the preparation of umbilical cord blood [2, 9]. In cases of urgent transplantation or if donors cannot be found, umbilical cord blood becomes an alternative. The establishment of a worldwide network for umbilical cord blood cell procurement, typing, and cryopreservation has resulted in a large collection and facilitated more than 7,000 unrelated transplants. Cord blood as a source of stem cells has several advantages: its transplantation requires less-stringent HLA matching than is required for that of peripheral blood or marrow, and mismatched cord blood cells are less likely to cause GVHD [10, 11].

(B) *Preparation of donor*: For 4 or 5 days before apheresis, the donor may be given granulocyte colony-stimulating factor (GCSF) to increase the number of stem cells in circulation. The stem cells are isolated from circulation based on the cell membrane expression of CD34+, a hematopoietic stem cell marker [12]. These peripheral blood CD34+ stem cells are capable of forming colonies of granulocytes/macrophages, erythrocytes, and other multipotential or immature progenitors. The CD34+ stem cells are frozen until they are infused to the recipient.

(C) *Preparation of recipient*: The recipient first receives a conditioning regimen consisting of chemotherapy, which is often combined with radiotherapy and T-cell-depleting antibody designed to immunosuppress the host in order to decrease the possibility of graft rejection and, when used to treat cancer, to reduce the number of malignant cells. This is followed by the infusion of donor cells [2].

(D) *Depletion of T cells*: Whereas bone marrow cells and GCSF-mobilized PBSCs are both enriched with hematopoietic progenitors, they also contain mature T cells that are responsible for graft rejection [2, 6, 7]. Three main strategies to deplete T cells and

decrease the incidence of GVHD have been proposed:

1. Selection of T cells ex vivo before transplantation
2. Positive selection of CD34+ stem cells ex vivo by immunomagnetic separation [12]
3. Antibodies against T cells in vivo [13]

These approaches showed substantial reduction of both acute and chronic GVHD. However, reduced frequency of severe GVHD is offset by high rates of graft failure, relapse of malignant disease, infections, and Epstein-Barr virus-associated lymphoproliferative disorders. Moreover, overall survival has not significantly improved when compared with use of non-T-cell-depleted bone marrow [12, 13].

17.4 Classification of GVHD

Graft-versus-host disease presents in an acute or chronic form. Historically, the acute and chronic forms were arbitrarily defined based on the time of onset since transplant (less than or more than 100 days, respectively) [2]. A clear distinction between acute and chronic forms of GVHD as originally described can no longer be delineated, given the alterations in the recipient's immunosuppression [2, 14]. In 2005, the National Institutes of Health working group sought to standardize the definitions of acute and chronic GVHD (Table 17.1). Currently, the diagnosis of chronic GVHD is based on specific signs, degree of organ involvement (mild, moderate, severe), laboratory data, or histopathological confirmation rather than time of onset since transplant (Table 17.2) [15].

17.4.1 Acute GVHD

Despite prophylactic measures, the incidence of aGVHD is estimated to be 40–60 % among patients receiving transplants from HLA-

Table 17.1 Categories of GVHD

Category	Time of symptoms after HSCT	Presence of acute GVHD features	Presence of chronic GVHD features
Acute GVHD			
Classic acute GVHD	≤100 days	Yes	No
Persistent, recurrent, or late onset acute GVHD	>100 days	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

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Table 17.2 Signs of chronic GVHD

Skin	Poikiloderma, lichen planus-like features, sclerotic features, morphea-like features, lichen sclerosus-like features, often areas of depigmentation: hypopigmentation or hyperpigmentation
Nails	Nails dystrophy or loss
Hair	Alopecia, scaling
Mouth	Xerostomia, restriction of mouth opening from sclerosis, mucosal atrophy, pseudomembranes, and ulcers
Muscle, fascia, joints	Fasciitis, myositis, or joint contractures
Gastrointestinal/liver	Anorexia, weight loss, esophageal web or strictures, elevation of total bilirubin and liver enzymes
Lungs	Restrictive or obstructive defects on pulmonary function tests, bronchiolitis obliterans, pleural effusions
Kidneys	Nephrotic syndrome
Heart	Pericarditis
Bone marrow	Thrombocytopenia, anemia, neutropenia

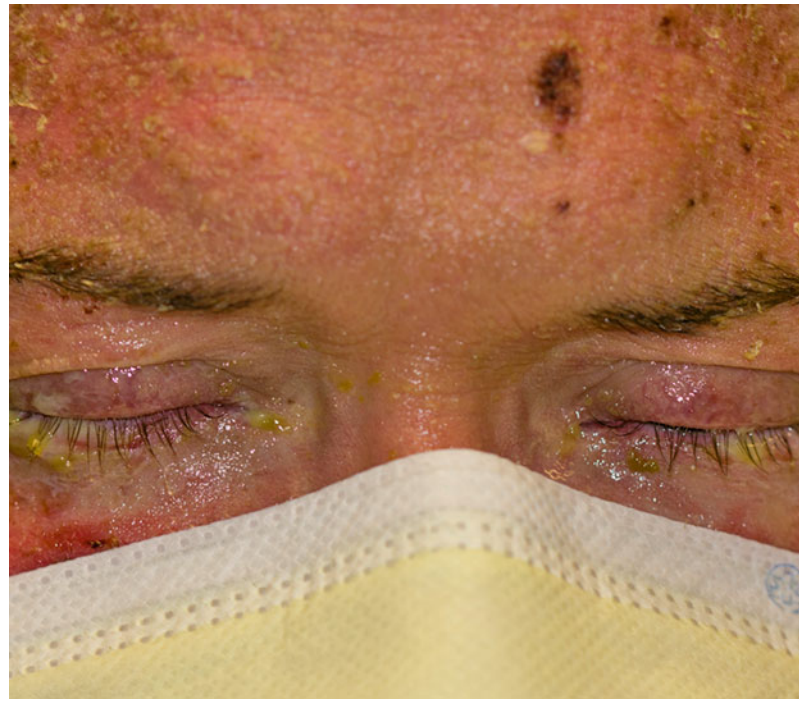

Fig. 17.1 Acute GVHD characteristically affects epithelial cells in the body. Periocular skin involvement is common as well as conjunctival chemosis and epithelial erosions in the lid margin epithelium

identical sibling donors and 75 % in patients receiving HLA-matched unrelated donors [16]. Acute GVHD is characterized by selective epithelial damage of target organs [17] such as skin, liver, and gastrointestinal tract within 14–42 days of infusion [14]. A "hyperacute" form of GVHD may occur within 14 days of infusion, in patients with severe HLA-mismatched donor or in those that have received inadequate GVHD prophylaxis [14]. Hyperacute GVHD is manifested by high fever and severe cutaneous component (generalized erythema with desquamation), in addition to hepatitis and intestinal symptoms; this form of GVHD may be rapidly fatal [15].

In ocular graft-versus-host disease, the histopathological changes are mainly seen in the conjunctiva and lacrimal gland tissue [18, 19]. As mentioned previously, aGVHD is primarily a T-cell-mediated process, and in the conjunctival tissue of patients with aGVHD-related pseudomembranous conjunctivitis, donor-derived mononuclear T lymphocytes have been detected [20] (Fig. 17.1).

17.4.2 Chronic GVHD

Patients who have received stem cells/bone marrow from an HLA-mismatched related donor or from an HLA-matched unrelated donor are at an increased risk of cGVHD [2]. Other factors that increase the risk of cGVHD include older recipients and those who have already experienced aGVHD [2]. The chronic form of graft-versus-host disease (cGVHD) has features resembling autoimmune disorders such as scleroderma, Sjögren syndrome, primary biliary cirrhosis, bronchiolitis obliterans, immune cytopenias, and chronic immunodeficiency. Symptoms usually present within 3 years after allogeneic HSCT and are often preceded by a history of acute GVHD [2, 5]. Manifestations of cGVHD may be restricted to a single organ or tissue or may be widespread (Table 17.3).

Dry eye is the most frequent ocular complication usually occurring approximately 6 months posttransplantation [21]. In chronic ocular GVHD, inflammatory destruction of the conjunctiva and lacrimal gland with fibrosis occurs

resulting in aqueous tear deficiency and damaged ocular surface [22] (Fig. 17.2). Extensive tissue destruction, tissue atrophy, and fibrosis of the tubuloalveolar glands and ducts in the lacrimal gland have been shown on histology [17].Chronic GVHD can lead to debilitating sequelae such as joint contractures, loss of sight, end-stage lung disease, or mortality from profound chronic immune suppression [2, 5].

17.5 Clinical Features: Ocular Surface Manifestations

17.5.1 Symptoms and Signs

Ocular cGVHD mimics other immunologically mediated inflammatory diseases of the ocular surface and there are no specific symptoms or clinical signs. Ocular manifestations, present in 60–90 % of patients with cGVHD, primarily affect structures of the anterior segment, mainly the lacrimal gland, meibomian glands, and conjunctiva [23]. Typical symptoms of cGVHD are dry eye, photophobia, foreign body sensation, irritation, burning, epiphora, redness, and blurriness [24].

Table 17.3 Diagnostic criteria for cGVHD

Distinction from acute GVHD
Presence of at least 1 diagnostic clinical sign of cGVHD
Presence of at least 1 distinctive manifestation confirmed by biopsy or other relevant tests
Exclusion of other possible diagnoses

17.5.2 Target Tissues

17.5.2.1 Lacrimal Gland

The lacrimal gland is an important ocular target for the pathogenesis of GVHD [21, 23, 25]. Fibrotic processes often affect the lacrimal gland reducing its secretory capacity or causing complete stasis with distended ductules and obliteration of ducts lumen, similar to bile duct damage seen in liver cGVHD [25]. Histological studies also showed extensive destruction, tissue atrophy, and fibrosis of the tubuloalveolar glands and ducts in the lacrimal gland with an increase in CD34+ stromal fibroblasts accompanied by mild lymphocytic infiltration [21].

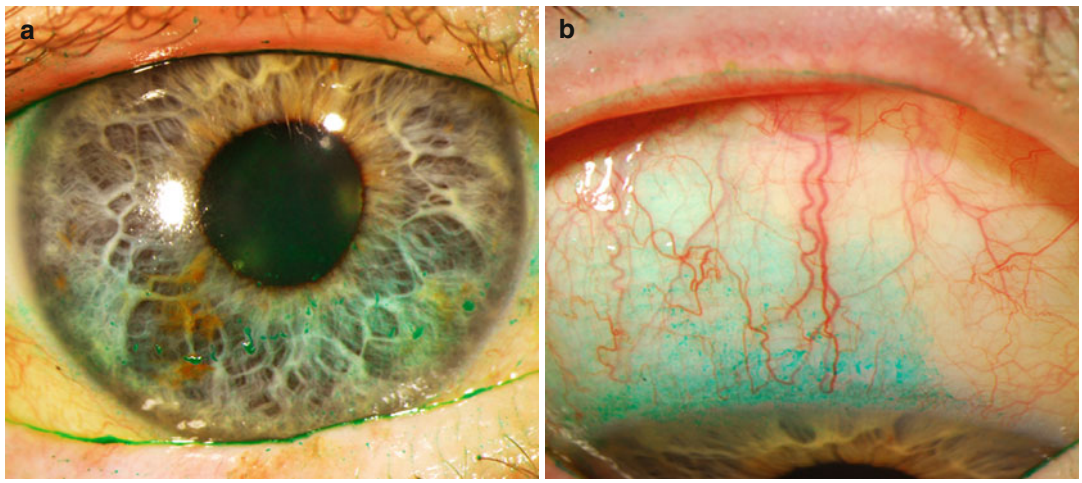

Fig. 17.2 Ocular GVHD manifests with different degrees of severity in the ocular surface. Lissamine green staining in the interpalpebral area of this patient affected with chronic ocular GVHD (**a**). The same patient with superior limbal keratitis shown by lissamine green staining in the superior limbal region (**b**)

17.5.3 Meibomian Glands

Besides aqueous tear deficiency, progressive decline of conjunctival goblet cells and the dysfunction of meibomian glands contribute to the overall breakdown of the ocular tear film causing severe keratoconjunctivitis sicca [23, 26]. In vivo confocal microscopy shows destruction of the ductal epithelia due to lymphocyte infiltration, sloughing of epithelial cells, pseudomembrane formation, and subsequent excessive fibrosis around the orifice, ducts, ductules, and acini of the meibomian gland; all these findings together may explain the development of meibomian gland disease [27].

17.5.4 Conjunctiva

Pseudomembranous conjunctivitis can rarely occur in cGVHD and is partly considered as an acute variant of GVHD [26]. Sterile inflammatory conjunctival involvement is a common finding, which can be accompanied by formation of pseudomembranes, loss of lashes, and stenosis or closure of the lacrimal punctum. Palpebral and subtarsal conjunctival scarring is seen in a number of patients, sometimes resulting in the formation of cicatricial lagophthalmos [26, 28].

17.5.5 Cornea

Corneal findings include punctate keratopathy, formation of mucus filaments, painful erosions, and eventually secondary corneal infections [26, 28]. Less frequently, sterile corneal stromal necrosis and perforations may occur [29]. Superior limbic keratoconjunctivitis in the setting of ocular chronic GVHD is believed to be more common in peripheral stem cell transplantation patients compared with bone marrow recipients [23].

17.6 Diagnostic Evaluation

Given the different target tissues and possible sequelae involved in ocular GVHD, the potential list of differential diagnoses is extensive. It is essential to evaluate the patient's medical history since the diagnosis of ocular GVHD is more likely in the setting of severe refractory dry eye disease, a decrease in Schirmer scores, and worsening ocular symptoms [23]. A full ophthalmological examination including best-corrected visual acuity and slit-lamp examination using vital dyes such as lissamine green, rose bengal stain, or fluorescein is needed to evaluate punctate keratopathy, corneal erosions, or ulcers. Additionally, a thorough subtarsal and meibomian gland inspection, evaluation of tear film breakup time, and a Schirmer test are essential in patients suspected of ocular GVHD [24]. According to Filipovich et al. [15], a diagnosis of chronic GVHD can be made by low Schirmer test values (without anesthesia) with a mean value of both eyes <5 mm at 5 min or a new onset of keratoconjunctivitis sicca by slit-lamp examination with mean values of 6–10 mm on the Schirmer test accompanied by distinctive manifestations in at least 1 other organ. Special diagnostics such as tear film osmolarity, corneal sensitivity testing, in vivo confocal microscopy for inflammatory cells, and photodocumentation can assist in therapeutic decisions and follow-up.

Slit-lamp examination of the lens should also be performed to assess for posterior subcapsular cataract, which commonly occurs secondary to steroid and/or radiation treatment. Systemic steroid therapy and topical steroid therapy also necessitate assessment for glaucoma including intraocular pressure measurements and visual field testing.

Posterior segment involvement such as CMV retinitis occurring in the setting of immunosuppression should be excluded [24]. Posterior scleritis, choroidal thickening, and serous detachments have rarely been observed in the acute or hyperacute GVHD [23].

It may also be beneficial to use quality of life questionnaires such as the Ocular Surface Disease Index to evaluate the extent of disease and the response to treatment [24].

17.7 Differential Diagnosis

The most common ocular manifestation of GVHD is keratoconjunctivitis sicca, which usually develops in conjunction with inflammatory

signs of the conjunctiva (conjunctival edema, chemosis, pseudomembrane formation) and chronic blepharitis [23, 26, 28]. Keratoconjunctivitis sicca primarily results from either aqueous tear deficiency or abnormal tear composition. The differential diagnosis of aqueous tear deficiency consists of autoimmune diseases such as Sjögrens, a multitude of medications such as diuretics and antihistamines, and any process that results in infiltration of the lacrimal gland including sarcoidosis, tumors, or postradiation fibrosis.

Cicatricial meibomian gland dysfunction along with the other changes described above can also occur in trachoma, ocular pemphigoid, and erythema multiforme. Other causes of meibomian gland dysfunction include seborrheic dermatitis, atopy, acne rosacea, and psoriasis.

17.8 Treatment of Systemic GVHD

17.8.1 Prevention of GVHD

Prevention of acute GVHD by the use of pharmacologic prophylaxis is an integral component to the management of patients undergoing allogeneic HSCT [5, 14, 26]. A regimen based on methotrexate with a calcineurin inhibitor, a cytoplasmic enzyme important for activation of T cells, is standard practice and is recommended in different studies [14, 16, 26]. The most widely used regimen includes a combination of either cyclosporine or tacrolimus with a brief course of methotrexate [14, 26]. For higher-risk groups (such as mismatched donors, older patients), more intensive immunosuppression is often required [2, 5, 26].

17.8.2 Treatment of Established GVHD

Although many therapeutic options have been used in the management of ocular GVHD, adequate treatment remains a challenge. The management is guided by a multidisciplinary approach, including adjustment of immunosuppression and aggressive supportive care. The treatment approach should include multiple strategies (topical and oral medications, surgery, environmental control, and systemic immunosuppression). Communication with the transplantation team is crucial in the optimal management of GVHD patients. Symptomatic mild cGVHD may often be treated with local therapies alone (e.g., artificial tears, topical steroid, serum drops). However, in patients with cGVHD that involves three or more organs or severe damage in any single organ, systemic immunosuppressive therapy may be considered.

17.8.3 Treatment of Ocular GVHD

Patients with ocular symptoms need close ocular supportive care focused on improving ocular surface moisture and decreasing ocular surface inflammation by using multiple available treatments. However, treatment of severe ocular cGVHD is challenging.

17.8.3.1 Topical Lubricants

The traditional treatment for dry eye symptoms consists of topical lubricants [30]. Data is not available on the efficacy of specific artificial tear medications in ocular GVHD. Although artificial tears and lubricants are needed to lubricate the ocular surface, ocular cGVHD is a complex problem of the ocular surface with multiple dysfunctional tear components. Punctal plugs can also be considered to improve the benefit of topical lubricants.

17.8.3.2 Topical Corticosteroids

Corticosteroids remain essential for controlling active chronic graft-versus-host disease. Systemic steroids represent the mainstay in the treatment of acute (exacerbations of) cGVHD but not enough data are available on the efficacy of topical steroids in ocular GVHD. In a small study of seven patients with conjunctival GVHD, resolution or improvement of the conjunctival signs was achieved using topical corticosteroids but the signs of KCS remained unchanged [31]. Patients receiving topical corticosteroids should be monitored for adverse effects. In presence of corneal epithelial defects, stromal thinning, or infiltrates, topical corticosteroids are contraindicated.

17.8.3.3 Topical Cyclosporine A

Cyclosporine A is a cyclic polypeptide produced by the fungus Tolypocladium inflatum Gams. Cyclosporine 0.05 % ophthalmic emulsion (Restasis, Allergan, Inc., Irvine, CA) has been FDA-approved for treatment of dry eye disease since 2003. Cyclosporine A emulsion has shown to decrease the number of activated T cells in the ocular surface, increase the goblet cell density of the conjunctiva, decrease epithelial cell apoptosis, and reduce proinflammatory cytokines [32]. In a small study of eight cGVHD patients treated with cyclosporine 0.05 % ophthalmic suspension twice a day for at least 3 months, researchers noted mean Schirmer score increases, tear breakup time improvement, and subjective symptoms improvement [33]. In another study of only 16 patients (32 eyes) with GVHD, dry eye symptoms improved in 62.5 % of patients and corneal fluorescein staining improved in all eyes after a mean follow-up of 90 days [34]. Baptista Malta reported a retrospective study with 105 patients of whom 43 patients developed cGVHD. All patients were initially started on topical cyclosporine before the HSCT. They conclude that cyclosporine is helpful in decreasing the incidence and severity of dry eyes in patients who are under topical cyclosporine before the HSCT [35].

Although beneficial effect of topical cyclosporine on ocular GVHD has been documented in several studies comprised of small number of cases, there is, at present, no large randomized study that clearly suggests its usefulness.

17.8.3.4 Tacrolimus

FK506 (tacrolimus) is a macrolide antibiotic extracted from the soil fungus Streptomyces tsukubaensis and its mechanisms of action and pharmacokinetics are similar to cyclosporine, although the immunosuppressive potency of tacrolimus in vitro is 50–200 times greater. Although the beneficial effect of systemic tacrolimus on ocular GVHD has been observed [16, 36], topical administration might be a better treatment option because of the adverse effects associated with its long-term systemic administration. Except for two case reports in which ocular GVHD was successfully treated, not enough data are available on the use of topical tacrolimus [37, 38].

17.8.3.5 Autologous Serum Eyedrops

Fox et al. described serum drops as a tear substitute free of preservatives in 1984 [39]. Autologous serum contains vitamin A, epidermal growth factor, fibronectin, and transforming growth factor beta, which all are needed for a healthy ocular surface epithelium [40, 41]. The efficacy and safety of autologous serum drops were investigated in a small study of 14 patients with ocular GVHD and severe cGVHD not responsive to conventional artificial tears therapy. After 4 weeks of treatment, significant improvement was observed in dryness symptoms and fluorescein scores and also in rose bengal staining and tear breakup time [41]. The improvement was noted in all patients at the 4-week follow-up [41]. The risk of contamination and subsequent infection forms a possible complication of autologous serum drops.

17.8.3.6 Contact Scleral Lenses

In patients with cGVHD affecting the ocular surface, two different types of lenses can be used, the bandage soft lens and the scleral rigid lens. The fluid-ventilated, gas-permeable scleral lens has been effective in mitigating symptoms and resurfacing corneal erosions in the treatment of moderate and severe ocular surface disorders of multiple etiologies. The fluid-filled reservoir shields the cornea from blink trauma, noxious environmental stimuli, and inflammatory mediators in the tears. The body-temperature saline reservoir also prevents corneal cooling and nerve firing that occurs during the inter-blink intervals [42].

One of the scleral lenses used (Boston Scleral Lens Prosthetic Device) was approved for the management of corneal disorders by the Food and Drug Administration in 1994. Takahide published a retrospective review on 9 patients fitted for refractory ocular surface disease secondary to cGVHD [43]. Contact lens fitting was prompted by debilitating ocular discomfort, visual impairment, or keratopathy. Some of the

patients evaluated were using artificial tears, cyclosporine eyedrops, punctal plugs, autologous serum tears, and moisture chamber eyewear. All patients reported improvement of ocular symptoms, reduced use of topical lubricants after fitting, and improvement in the ocular surface disease index [43].

The same group published results of 33 consecutive patients with severe dry eye from cGVHD, unresponsive to conventional therapy. Ninety-four percent of patients reported improvement in photophobia in the worse eye. Ninety-seven percent of patients reported improvement in life quality with no complications noted during the follow-up period [44]. Schornack reported the successful use of the Jupiter scleral contact lens (Medlens Innovations, Front Royal, VA or Essilor Contact Lens, Inc., Dallas, TX) in the management of 10 eyes of 5 patients with cGVHD. All patients had improvement in symptoms and some improved visual acuity. Jupiter lenses are commercially available in the US and may therefore be more accessible and affordable to patients who could potentially benefit from this treatment [45].

Although scleral lenses are maybe the most important tool in the armamentarium, their use is not widespread. Published data suggest that scleral rigid gas-permeable lenses are an important therapeutic option for patients with recalcitrant ocular surface compromise and debilitating symptoms. High cost, inadequate fitting, poor tolerance by some patients, and discomfort with blinking in the presence of severe meibomian gland disease and keratinization are some of the drawbacks. To our knowledge, no comparative prospective study has evaluated the different types of scleral lenses available.

17.9 Follow-Up

According to the participants in the German/Austrian/Swiss Consensus Conference on clinical practice in chronic GVHD, recommendations for monitoring patients are as follows: (1) a baseline ophthalmological workup including the Schirmer test before HSCT, (2) a screening examination at day 100–200 after HSCT, and (3) an ophthalmological assessment in case of ocular symptoms or any other manifestation of GVHD. An ophthalmologist should complete these examinations. This protocol allows for (1) a baseline examination to detect progressive KCS earlier, (2) diagnosis of ocular cGVHD earlier, and (3) an early start with treatment to prevent excessive scarring and inflammation processes that may lead to serious complications ultimately improving symptoms and quality of life.

17.10 Prognosis of Ocular GVHD

A few reports have studied the long-term prognosis in patients affected with ocular cGVHD. Sales et al. in a case series of 49 patients reports that in the long term, many patients with cGVHD may experience improved dry eye symptoms as a result of effective treatment. Although only nine patients completed this 3-year prospective case series, stable visual acuity, tear production, and lissamine green staining and a statistically insignificant improvement in dry eye symptoms were reported [46]. A recent study has reported that the incidence of dry eye was significantly higher in the recipients of peripheral blood stem cells than those receiving bone marrow or cord blood.

Another retrospective cohort study reported that of 56 patients with cGVHD, 39 % developed symptoms of photophobia, irritation, and foreign body sensation [47]. Over time, patients required fewer topical medications to control their symptoms; only 5 % of patients required more than two medications for dry eye disease management at the end of follow-up [47].

In contrast, Ogawa et al. series of 53 patients suggested that the symptoms of dry eye, including ocular fatigue, foreign body sensation, pain, blurring, photophobia, and epiphora, were almost universally worse among 22 participants at 30 months after HSCT in comparison to before HSCT [48].

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Uveal Tumors: Diagnostic Techniques: Angiography

18

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Contents

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18.1 Introduction

Fluorescein (FA) and indocyanine green angiography (ICGA) are usually not necessary to diagnose ocular tumors except for retinal tumors, when these ancillary tests assist in establishing the correct diagnosis. Among choroidal tumors, FA and ICGA are particularly useful in establishing the diagnosis of choroidal hemangioma and for revealing choroidal folds (Table 18.1). In addition, over the past few decades, these diagnostic procedures have played a pivotal role in expanding our understanding of retinal and choroidal anatomy, pathology, and pathophysiology. These tools are also used to assess disease progression and treatment side effects (radiation retinopathy).

18.2 Basic Principles

Both forms of angiography are based upon fluorescence. Fluorescence involves the use of shorter wavelengths (higher frequency and thus higher

A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_18, © Springer-Verlag Berlin Heidelberg 2014

Table 18.1 Fluorescein and indocyanine green angiographic findings of some common retinal and choroidal tumors

Lesion	FA (early)	FA (mid)	FA (late)	ICGA (early)	ICGA (late)
Retinoblastoma	Hyper	Hyper	Hyper, leakage	NA	NA
Retinal capillary Hemangioblastoma	Hyper	Hyper	Hyper, leakage	Hyper	Hyper, leakage
Vasoproliferative tumor of the retina	Hyper	Hyper	Hyper/leakage	Hyper	Hyper/leakage
RPE adenoma	Hypo	Hyper	Hyper/leakage	Hyper	Hyper/leakage
Choroidal nevus	Hypo	Hypo	Hypo	Hypo	Hypo
Choroidal melanoma	Hypo, hyper	Hyper	Hyper, leakage	Hypo, iso	Hypo, iso
Choroidal hemangioma	Hyper	Hyper	Hyper, leakage	Hyper	Hypo
Choroidal metastasis	Hypo	Hyper	Hyper	Hypo	Hypo
Vitreoretinal lymphoma	Hypo	Hyper	Hyper	Hypo	Hyper/hypo
Choroidal lymphoma	Hypo	Hypo	Hypo	Hypo	Hyper/hypo

Hypo hypofluorescence, *Iso* isofluorescence, *Hyper* hyperfluorescence, *NA* not enough data available

energy) which is absorbed by a molecule and which then excites the molecule to a higher energy state. As the molecule returns to a lower energy state, it releases a packet of higher wavelength (lower energy). Fluorescein angiography (FA) uses fluorescein sodium, which absorbs around 490 nm (blue wavelength) and emits at 514 nm (green). Indocyanine green absorbs around 790 nm (near infrared) and emits around 810 nm. The camera uses a filter that only allows the lower wavelength to enter the eye and another filter that only allows the higher wavelengths back into the detector or film [1].

Light entering the eye is scattered, reflected, or absorbed. Higher wavelengths tend to be scattered less than lower wavelengths, and therefore, ICG is superior for deeper choroidal lesions. Conversely, lower wavelengths have greater resolution so that smaller anterior lesions are better visualized by fluorescein than with ICG.

Sodium fluorescein is usually injected as a bolus. Though it is rare to be allergic to this dye, severe cases of anaphylaxis have occurred. Occasionally, some patients have developed hives, and in those cases, it is best not to use it again. More commonly, some patients develop nausea from the injection. This is not a true allergy, and slowing the infusion rate can decrease the recurrence of this event. The sodium in the fluorophore can cause worsening of heart failure or hypertension in patients with severe renal insufficiency so care should be used in that situation. Its use is probably not indicated during pregnancy.

Indocyanine green (ICG) is a molecule used to image the choroid layer of the eye. ICG utilizes water-soluble tricarbocyanine dye that binds tightly to plasma proteins confined within blood vessels. This dye fluoresces in the infrared frequencies allowing visualization through the retinal and thin subretinal hemorrhages to better image the choroidal abnormalities. There is iodine in ICG so those that are truly allergic to iodine should not use ICG. True iodine allergy is rare and those that have seafood allergy most often have an allergy to proteins in the seafood and not to iodine [2].

18.3 Anterior Segment Angiography

18.3.1 Iris Tumors

Tumors and granulomas of the iris tend to be vascularized, and so with FA, these vessels can be visualized (Fig. 18.1). This helps distinguish the tumors from cysts, which are not vascularized though with ultrasound biomicroscopy the role of FA has become limited. Melanomas of the iris also demonstrate intrinsic vascularity (Fig. 18.1).

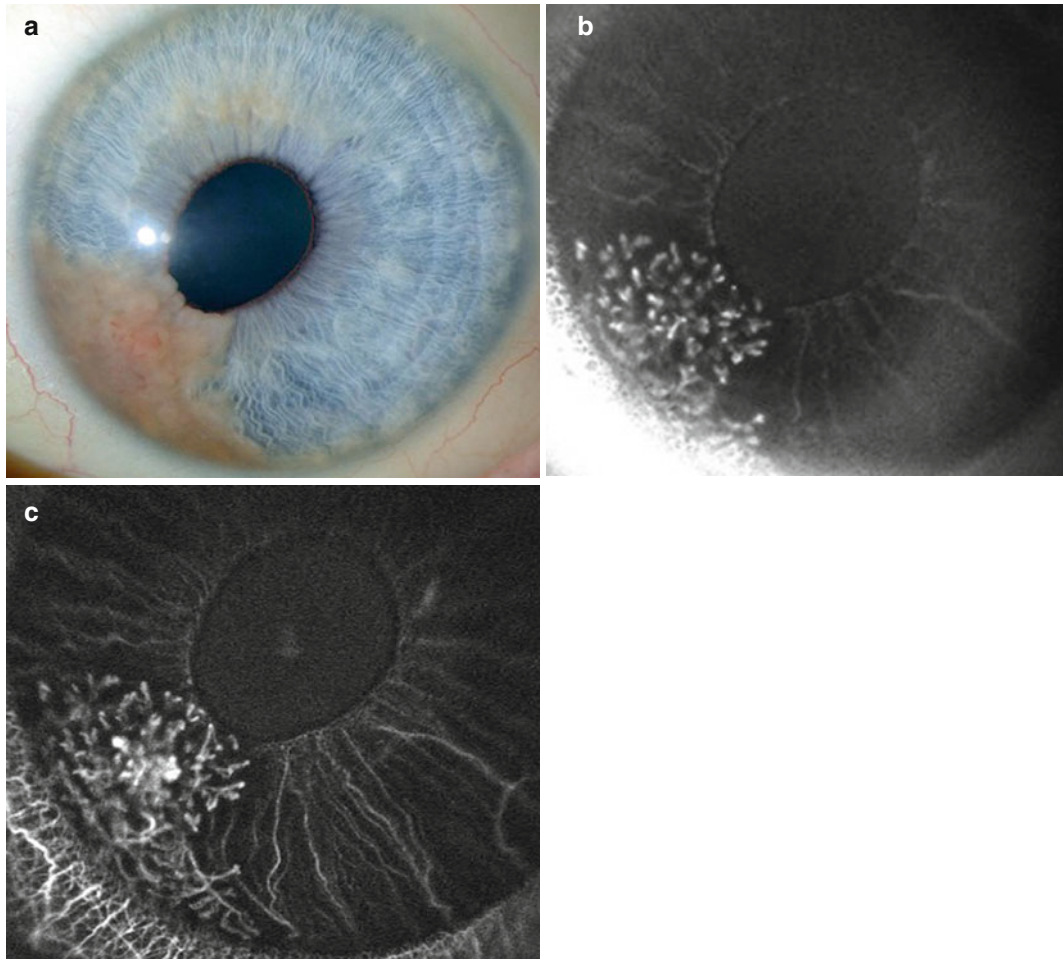

Fig. 18.1 Iris melanoma (**a**). FA (**b**) and ICG (**c**) showing the intrinsic vascularity of the iris melanoma

18.3.2 Iris Hemangioma

Vascular anomalies of the iris are rare. They are classified into five distinct clinical entities based on their clinicopathologic features: racemose hemangioma or iris arteriovenous malformation (AVM), capillary hemangioma, cavernous hemangioma, microhemangiomatosis, and iris varix [3–5]. Four of the five types are clearly documented on FA with the exception of cavernous hemangioma. For iris lesions, FA is similar to ICG.

Iris AVM is the most common vascular tumor of the iris and can be subdivided into simple (single tortuous vessel) and complex (multiple convoluted vessel involvement). FA of an iris AVM shows uniform hyperfluorescence in early phases and rapid transit in the affected iris vessels with no leakage in the late phase [5]. In between the AVM vessels are areas of nonperfusion of the iris. Iris capillary hemangioma on FA appears as hypofluorescent mass without prominent feeder vessels or intrinsic vasculature throughout all phases of the angiogram. With FA, microhemangiomatosis is hyperfluorescent with leakage of dye into the aqueous humor [4]. Iris varix reveals an early focal hyperfluorescent leakage surrounded by an area of hypofluorescence on FA [3].

18.4 Retinal Tumors

In general, indocyanine green is not needed or used for retinal tumors since the tumors are usually well visualized by FA. Occasionally, exophytic tumors that are somewhat masked by the overlying retina might be better visualized with ICGA.

18.4.1 Retinoblastoma

FA shows a vascular tumor that fills rapidly with fluorescein and shows late hyperfluorescence [6]. During the vascular filling phase, the feeding arteries fill rapidly, and fine capillary ramifying blood vessels typically are often seen in the superficial portions of the tumor. These vessels leak profusely in the later angiograms, and it is important to distinguish them from neovascularization. Leakage into the vitreous and subretinal space is seen in more advanced tumors. After successful treatment of the tumor, the blood supply is diminished or absent, and the lesion is more hypofluorescent [7]. Spontaneously arrested or regressed retinoblastomas also show variable degrees of hyperfluorescence even though they are clinically inactive.

18.4.2 Retinal Vascular Tumors

18.4.2.1 Retinal Hemangioblastoma (Retinal Capillary Hemangioma)

When considering the angiographic imaging of retinal hemangioblastoma, three types of retinal hemangioblastoma need to be considered, namely, papillary, exophytic or tumors growing into the subretinal space, and endophytic tumors which grow into the vitreous cavity.

- Endophytic Hemangioblastoma: The diagnosis can usually be based upon the clinical findings in cases of endophytic hemangioblastomas especially for hemangioblastomas that are not papillary. FA, however, is helpful in distinguishing the artery from the vein (Fig. 18.2). This determination is important if there is consideration of laser photocoagulation or even photodynamic therapy of the hemangioblastoma. By identifying the feeding artery, which would have shown fluorescence earlier in the study than the tumor, laser photocoagulation is first applied to the artery to slow flow to the tumor, allowing better uptake in the vasculature of the tumor. In addition, the high flow also inhibits the formation of occlusion by platelets or white cells in the vasculature [1].
- Exophytic hemangioblastoma of the retina is less common than endophytic hemangioblastoma and papillary hemangioblastoma. The dilated feeding and draining vessels can be seen diving through the retina into the tumor, and fluorescein angiography can delineate these vessels. In these cases as well as in exophytic papillary tumors, ICGA can reveal the capillaries under the retina. This is probably the only time that ICGA is useful in diagnosing retinal tumors of uncertain diagnosis.
- Papillary hemangioblastoma can be both endophytic and exophytic. Endophytic hemangioblastoma is relatively easy to diagnose. Fluorescein angiography will show the capillaries within the tumor as well as the feeding and draining vessels. Exophytic papillary tumor is harder to diagnose because the tumor is usually not directly visualized (Fig. 18.2). FA may show dilated vessels entering into the disc or at the edge of the tumor. ICGA is helpful in the cases of exophytic tumors by demonstrating the leakage from the deeper aspects of the optic nerve or surrounding retina.

18.4.2.2 Vasoproliferative Tumor of the Retina

FA is of variable quality owing to the extreme peripheral location of most of these tumors. The angiograms in general show rapid filling of the tumor in the arterial phase through a nondilated or mildly dilated retinal feeder arteriole [8, 9]. A capillary network may appear on the tumor surface. In the venous phase, diffuse leakage and staining of the mass and subretinal fluid are usually demonstrated. As opposed to retinal hemangioblastoma, this benign reactive vascular tumor does not have dilated draining

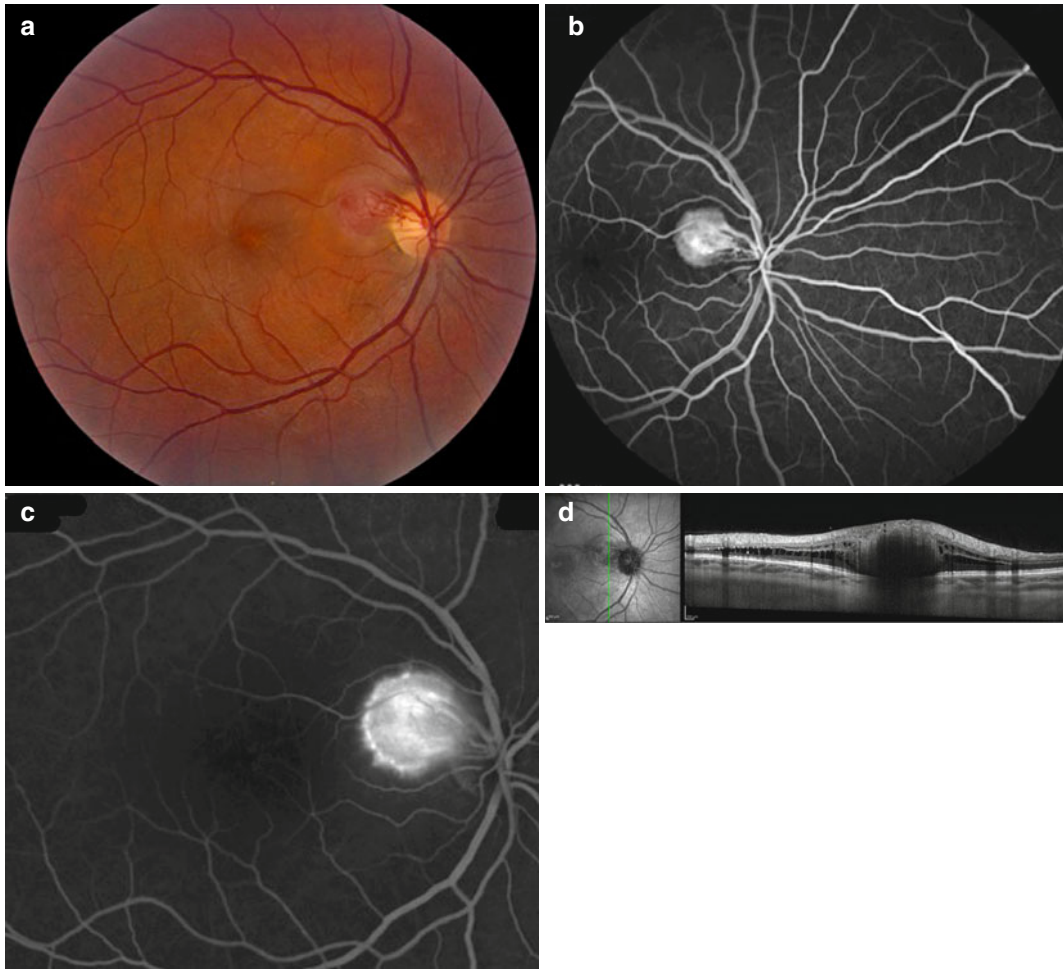

Fig. 18.2 Color photograph of a peripapillary exophytic hemangioblastoma (**a**). Venous phase showing the feeding arterioles and the large draining vein (**b**). Late phase showing leakage from the hemangioblastoma (**c**). OCT showing that the tumor is exophytic (**d**)

and feeding vessels. The filling with fluorescein is not as rapid as that seen with retinal hemangioblastoma.

18.4.3 Retinal Astrocytic Hamartoma

The tumor in the arterial phase is relatively hypofluorescent in most cases. Tortuous or corkscrew retinal blood vessels are generally apparent in and around the tumor. In the venous phase, a network of fine blood vessels typically becomes apparent in the superficial portion of the tumor with fairly intense late staining from leakage [10]. There is limited data on ICGA characteristics of retinal astrocytic hamartoma.

18.4.4 Retinal Pigment Epithelial Tumors

Retinal pigment adenoma/adenocarcinoma and simple hamartoma of the retinal pigment epithelium tend to be pigmented tumors. Since they are anterior to Bruch's membrane, they have dilated feeding and draining retinal vessels. FA shows leakage in the later phases of the angiogram, and ICG shows the internal vascularity (Fig. 18.3).

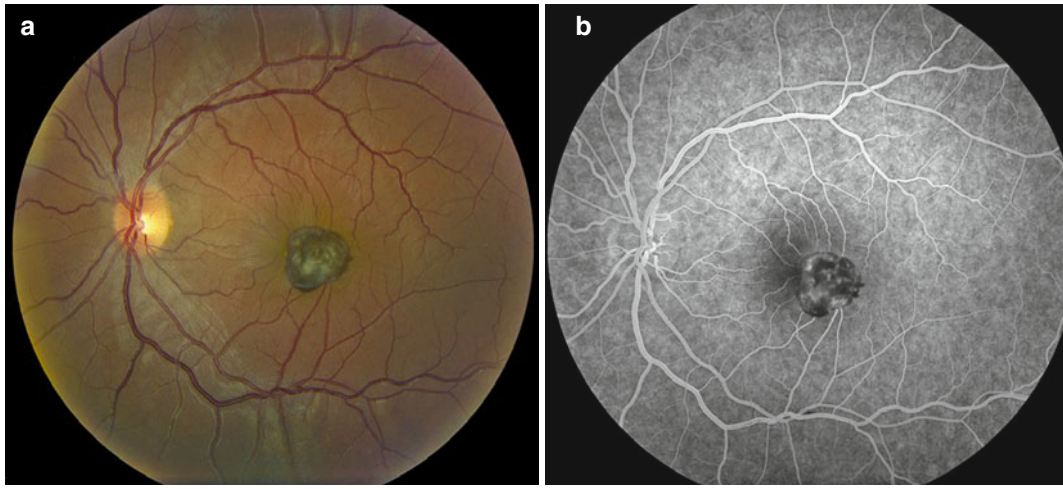

Fig. 18.3 Simple hamartoma of the retinal pigment epithelium (**a**). Notice the jet black color. Fluorescein angiogram highlights the feeding and draining retinal vessels (**b**)

18.5 Choroidal Tumors

18.5.1 Choroidal Nevus

Fluorescein angiography is not a routine test to be performed for most small asymptomatic choroidal nevi if the diagnosis can be established ophthalmoscopically. Fluorescein angiographic features of a choroidal nevus depend mainly on 3 factors: the degree of pigmentation within the tumor, its location within the choroid, and the alterations in the overlying RPE [11–13]. Deeply pigmented choroidal nevi tend to be relatively hypofluorescent, whereas those that are less pigmented tend to be more hyperfluorescent (Fig. 18.4). A deep-setting nevus, confined to the outer choroid and sparing the choriocapillaris, gives a relatively normal fluorescein pattern because the fluorescein enters the patent choriocapillaris. If the nevus is thicker and compresses or replaces the choriocapillaris, FA may demonstrate relative hypofluorescence because there is less fluorescein in the choroidal vessels over the tumor. Late staining from overlying drusen can lead to hyperfluorescence. FA shows that areas of orange pigment remain hypofluorescent throughout the study. The hypofluorescence of orange pigment appears to be due to blocking of choroidal fluorescence transmitted through the melanocytic choroidal lesion.

Choroidal nevi rarely display choroidal neovascularization, pigment epithelial detachment, or subretinal fluid over the lesion. The occurrence of a choroidal neovascular membrane can be suspected based on the ophthalmoscopic presence of subretinal hemorrhage or subretinal fluid over the lesion. Fluorescein angiography in these cases usually reveals a choroidal neovascular membrane that appears brightly in the early frames of the study and leaks progressively by the late frames. Retinal pigment epithelial detachment usually presents as late hyperfluorescence from pooling of the dye under the pigment epithelial detachment. In cases with subretinal fluid, RPE hyperfluorescent leak sites can be seen in the early frames of the study and gradually increase in prominence as the study continues. Fluorescein usually leaks into an ophthalmoscopically evident localized blister of subretinal fluid.

On ICGA, most pigmented choroidal nevi are hypofluorescent. A nonpigmented choroidal nevus demonstrates isofluorescence. However, some nonpigmented nevi are still hypofluorescent because of the compression of the choroidal vessels by the choroidal mass [13].

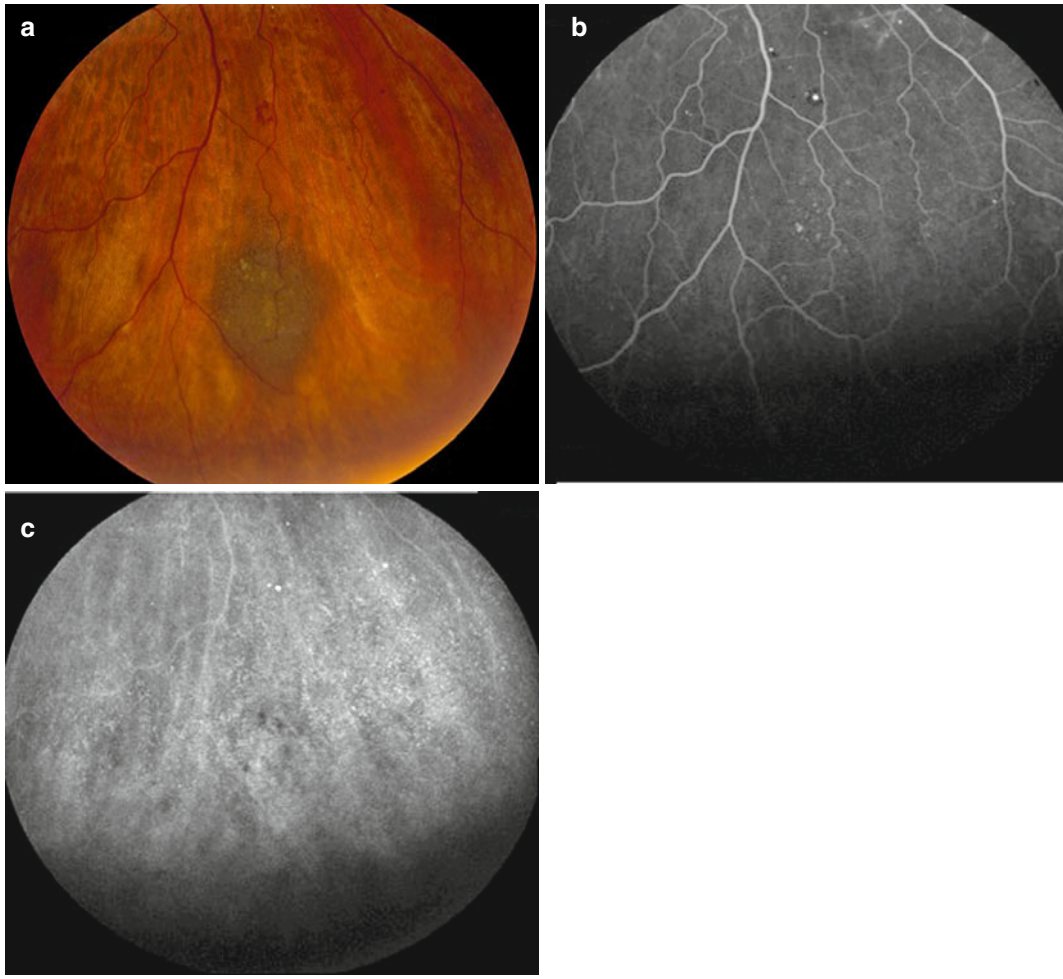

Fig. 18.4 Choroidal nevus with overlying drusen in a patient that has nonproliferative diabetic retinopathy (**a**). FA (**b**) and ICG (**c**) show only some staining of the drusen

18.5.2 Choroidal Melanoma

In most cases, fluorescein angiography does not provide findings that are pathognomonic of choroidal melanoma. The fluorescein findings can be described as compatible or incompatible with the diagnosis of a choroidal melanoma [11]. Fluorescein angiographic patterns of choroidal melanoma usually reflect the degree of pigmentation of the tumor and its effect on adjacent ocular structures, particularly the RPE. A small choroidal melanoma with an intact overlying RPE can demonstrate no appreciable abnormality on FA [14].

For most choroidal melanomas, FA in the arterial or early venous phase usually shows hypofluorescence with mottled hyperfluorescence [15–18]. This mottled hyperfluorescence is due to several ill-defined large-caliber deep intralesional blood vessels that usually become identifiable against the hypofluorescent background through atrophy of the overlying retinal pigment epithelium. As the study continues, the large intralesional blood vessels leak progressively, so that the surface of the lesion commonly appears hyperfluorescent in the late frames. If prominent clumps of orange lipofuscin pigment are present

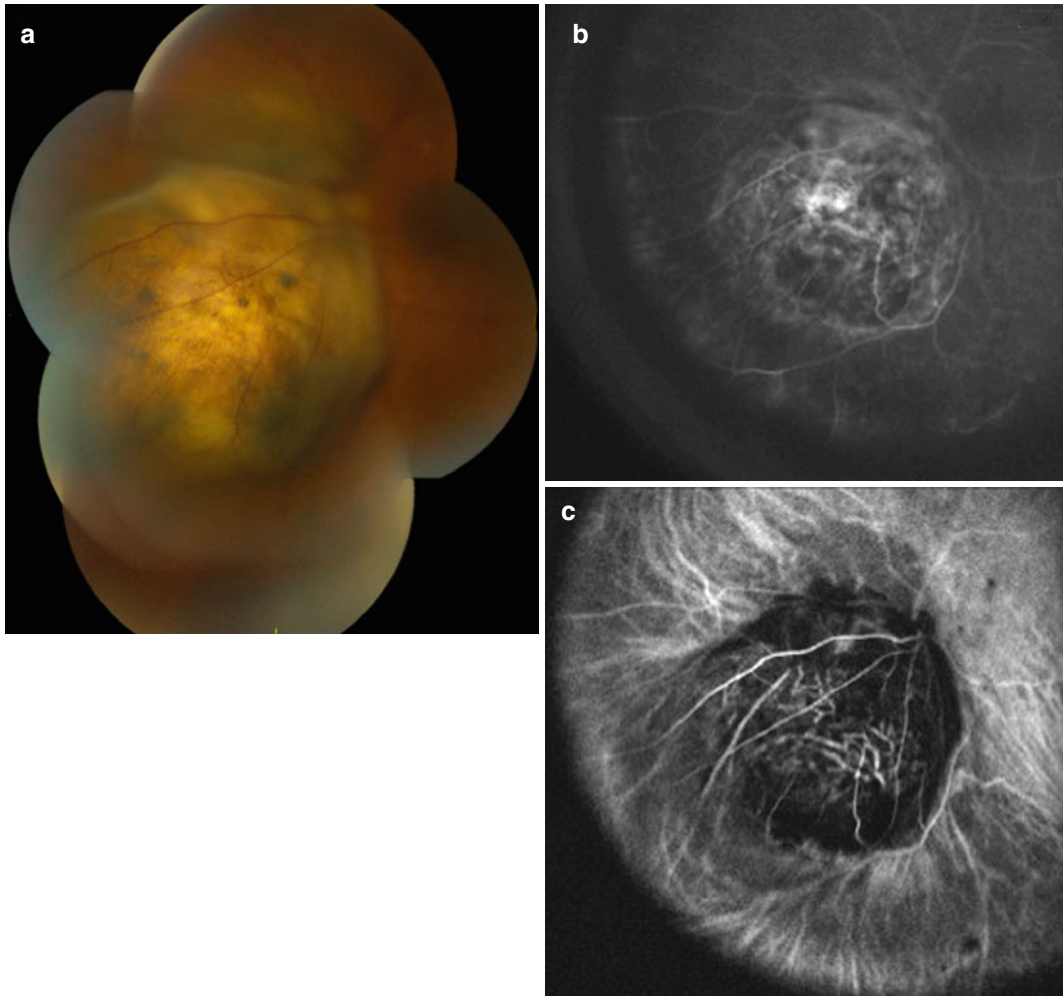

Fig. 18.5 Amelanotic peripapillary choroidal melanoma (**a**). FA (**b**) and ICGA (**c**) showing internal vascularity

on the surface of the tumor, they appear intensely hypofluorescent throughout the entire study because of blockage of the underlying choroidal and tumor vascular fluorescence. If there is associated serous retinal detachment, the fluorescein can also accumulate in the surrounding and overlying subretinal fluid by the late frames leading to hyperfluorescence. In choroidal melanoma that is clinically relatively amelanotic, the early frames of the angiogram show less lesional hypofluorescence and more prominent intralesional blood vessels. The remainder of the angiogram is similar to that seen in more typically pigmented melanoma.

A proportion of the melanoma can erupt through the overlying Bruch's membrane, giving the lesion a mushroom shape in cross section. In most cases of mushroom-shaped melanoma, the portion of the tumor that has broken through Bruch's membrane appears to be less darkly pigmented compared to the base of the tumor [15, 18]. Many large-caliber intralesional blood vessels can often be observed ophthalmoscopically within the apical portion of the mass (Fig. 18.5). These correspond mostly with the tumor veins that are congested in response to the constricting effect of the intact portion of Bruch's membrane. Fluorescein angiography in such cases shows

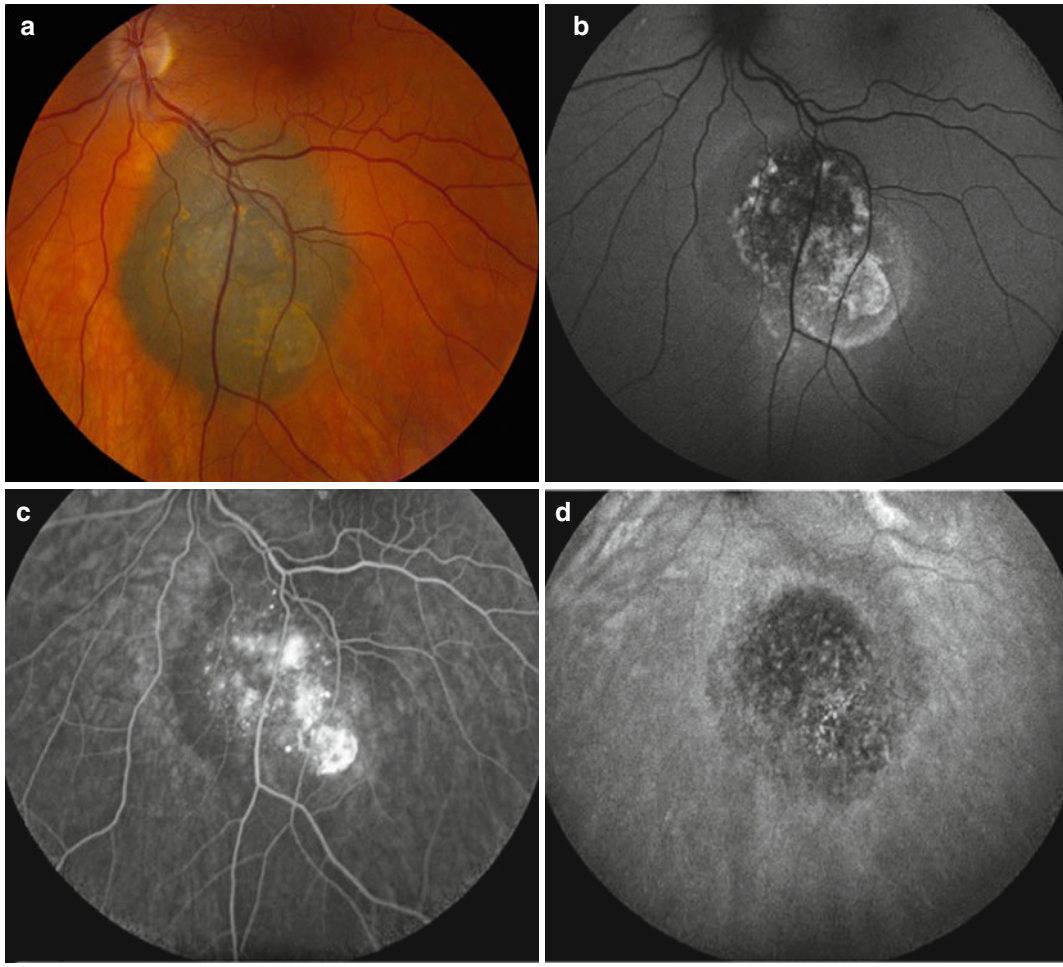

Fig. 18.6 Symptomatic small posterior choroidal melanoma with orange pigment and subretinal fluid (**a**). The autofluorescence shows leakage and autofluorescence from the orange pigment (**b**). The late-stage angiograph shows focal spots of leakage by fluorescein angiography (**c**) and also from ICG (**d**)

progressive hyperfluorescence of the apical blood vessels of the lesion starting usually in the early venous frames with intense late staining in the late-phase frames. The tumor typically shows hypofluorescence during the early frames with progressive mottled hyperfluorescence of the extravascular portions of the tumor becoming apparent in the late frames. This hyperfluorescence usually masks most of the retinal and choroidal blood vessels.

Some choroidal melanomas especially those breaking through Bruch's membrane invade the overlying sensory retina. Areas of tumor invasion of the overlying sensory retina are relatively hypofluorescent throughout the entire angiogram sequence [19]. The retinal blood vessels at the margins of the melanoma with retinal invasion are often abnormal and leaky, as might be expected on the basis of the associated retinal invasion. Neovascularization over a melanoma is rare [20].

On ICGA, most choroidal melanomas remain hypofluorescent/isofluorescent with respect to the surrounding tissues during the entire angiogram. This is because pigmented choroidal melanoma blocks ICG fluorescence because of the absorption of the near-infrared light by the melanin-containing lesion (Fig. 18.6).

Microvascularization patterns can be imaged by ICGA using a confocal scanning laser ophthalmoscope. On ICGA, the presence of arcs, loops, cross-links between parallel vessels, irregular or tortuous vessels, abnormal branching patterns, and networks are considered significant for intrinsic tumor vessels [21, 22]. ICGA can assist in visualizing the typical pattern of choroidal blood vessels in the tumor when there is overlying blood [2]. Prominent early choroidal filling is a feature more typical of circumscribed choroidal hemangioma [23].

18.5.3 Choroidal Hemangioma

Choroidal hemangioma is a benign abnormal collection of blood vessels in the choroid, which is better documented on ICGA than FA. It can be found in either a discrete, circumscribed form or a diffuse form associated with Sturge-Weber syndrome. In Sturge-Weber syndrome, there can be discrete hemangioma overlying the diffuse tumor. FA of circumscribed choroidal hemangioma typically shows early irregular hyperfluorescence of the choroidal vessels during the choroidal phase prior to filling of the retinal arterioles. Subsequently, the fluorescence increases and develops a more confluent pattern during the arteriovenous phase of the angiogram. The later phases display accumulation of dye in multiple cavities in the outer retina [24]. This typical angiographic appearance is characteristic of choroidal hemangioma, but not pathognomonic for this tumor because other choroidal disorders have been noted to have a similar appearance especially in the choroidal phase [23]. FA is often useful in making the diagnosis of choroidal hemangioma; however, ICGA is superior in documenting this tumor (Fig. 18.7).

ICGA of choroidal hemangioma shows a well-defined area of early, intense, and fairly even hyperfluorescence with a late rapid loss of fluorescence. On ICGA, the intrinsic vessels of the choroidal hemangioma are observed in the majority of patients, and the late multifocal spots of dye accumulation without leakage are seen in about 50 % of the tumors [23].

18.5.4 Choroidal Osteoma

FA of choroidal osteoma typically shows early patchy hyperfluorescence with late diffuse intense staining [25, 26]. The fluorescein traverses through the Haversian canals of bone, and due to the bony nature of the tumor, the fluorescein dye is slow to penetrate the mass corresponding with early patchy hyperfluorescence. The dye also has difficulty exiting the tumor, and thus the typical fluorescein pattern of choroidal osteoma is of slow uptake and persistent late fluorescence. The early hyperfluorescence of the capillary network inside the bony tumor corresponds to the yellow-white areas of the tumor (Fig. 18.8).

The presence of choroidal neovascularization has a lacelike pattern of early hyperfluorescence with early leakage of dye followed by late staining of the surrounding tissues. Meanwhile, subretinal hemorrhage or clumps of pigment in the retinal pigment epithelium are seen as areas of persistent hypofluorescence. ICGA of choroidal osteoma shows an early hypofluorescent mass, which later appears as a fine diffuse fluorescence from multiple focal areas within the mass and gradually becomes confluent in late frames [27].

18.5.5 Choroidal Granuloma

Choroidal granuloma is an uncommon ocular manifestation of inflammatory diseases such as sarcoidosis, tuberculosis, and secondary syphilis. On FA, the granulomas show early choroidal hypofluorescence from blockage of the choroidal vessels followed by late subretinal fluorescein leakage and staining [28]. There can be choroidal-retinal anastomosis which is rare in other choroidal tumors. ICGA is not typically performed, but if it is, it does not add much more to the findings than does the FA.

A secondary choroidal neovascular membrane can occur in which case there can be lacy fluorescence sometimes better seen with ICGA. There is then late leakage and areas of hypofluorescence if there is subretinal blood.

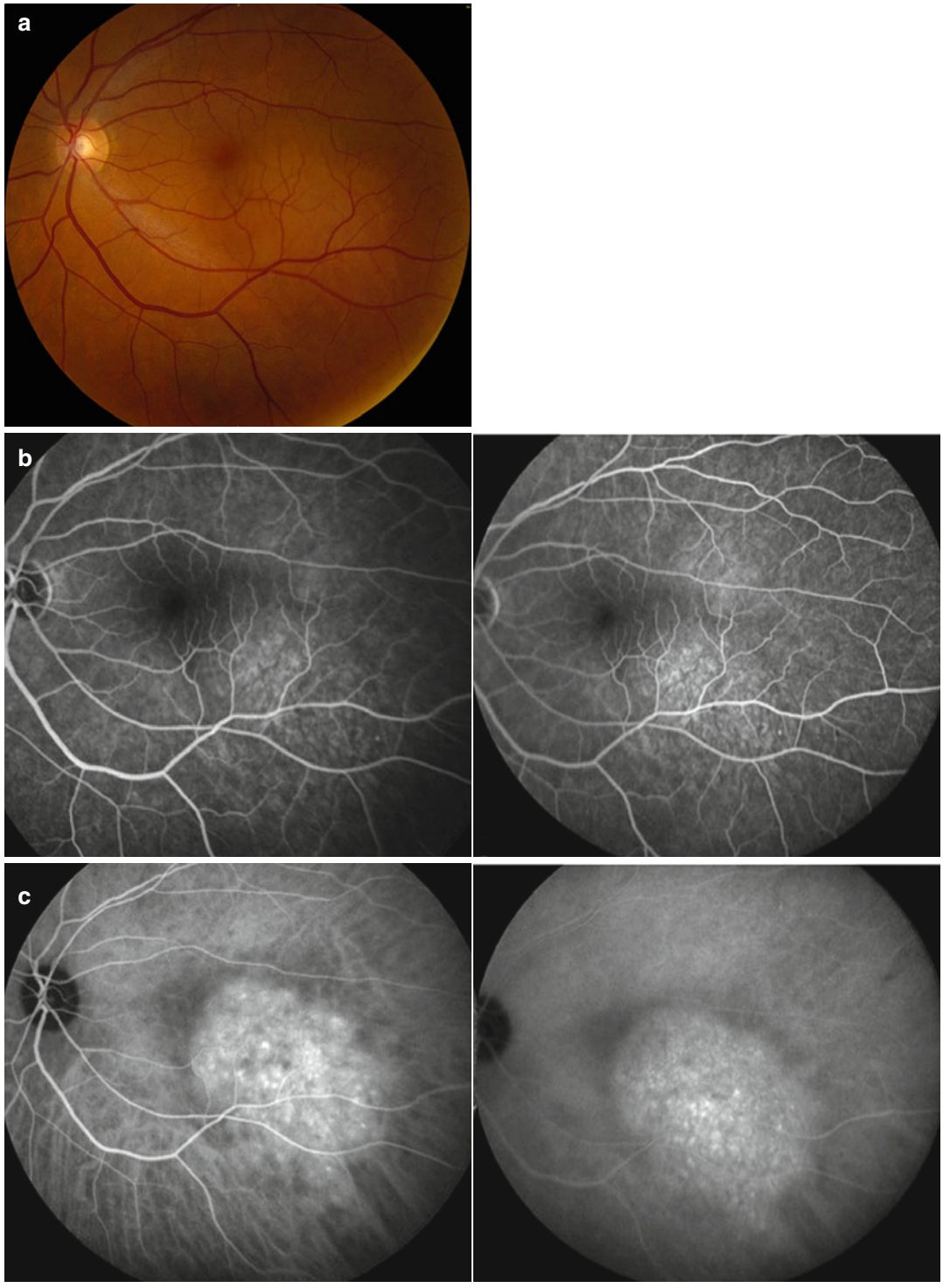

Fig. 18.7 Asymptomatic choroidal hemangioma (**a**). Fluorescein angiography (**b1**) and ICG (**c1**) in the venous phase showing fluorescence from both modalities. In the late phase, there is actually less fluorescence (**b2** and **c2**)

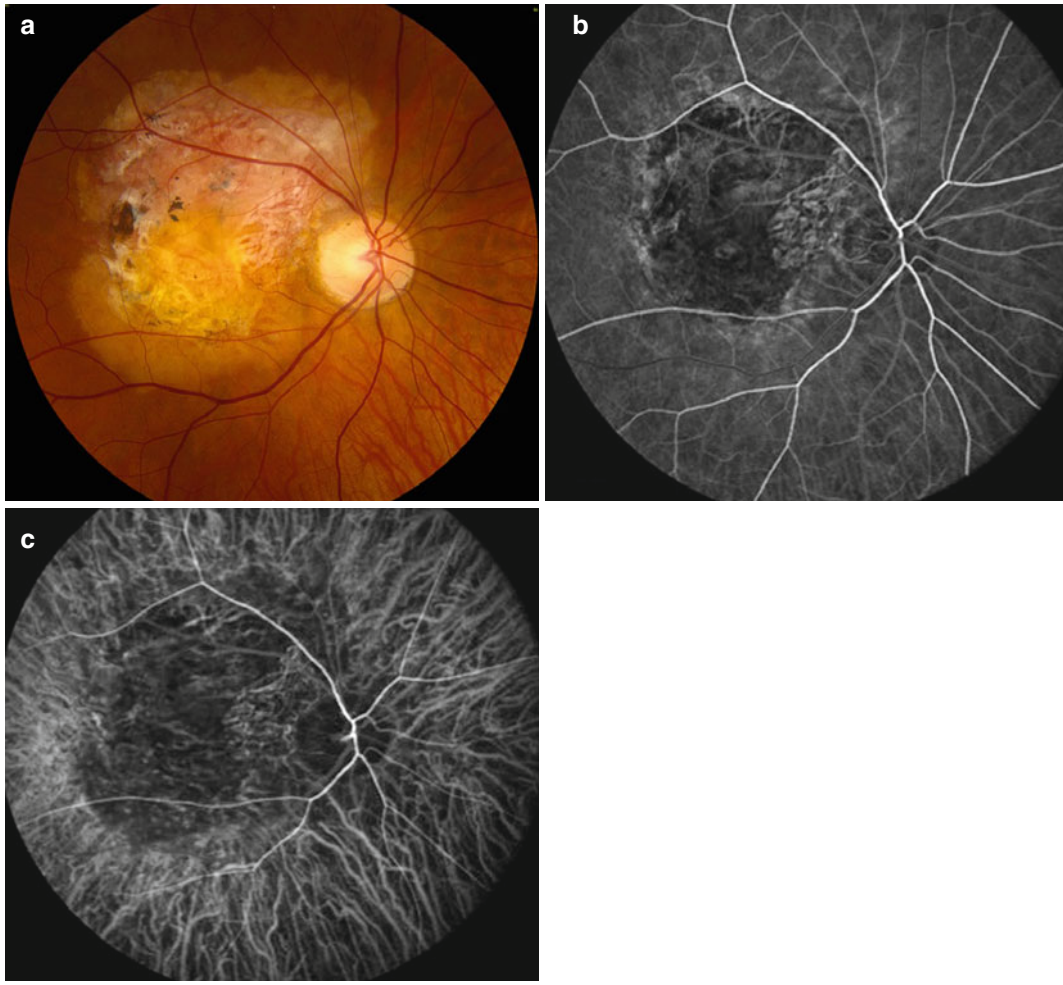

Fig. 18.8 (**a**) Choroidal osteoma that was partially treated with photodynamic therapy. ICG early (**b**) and late phase (**c**). Note the small penetrating vessels through the bone in the inferior aspect of the tumor

18.5.6 Choroidal Metastatic Tumors

Choroidal metastases tend to affect the posterior pole. They are often associated with exudative retinal detachment leading to metamorphopsia and vision loss, which are the most common ocular symptoms. Metastatic tumors to the optic nerve and retina are much less common.

In contrast to choroidal hemangioma and melanoma, metastatic carcinoma is usually hypofluorescent in the arterial and early venous phases and shows progressive hyperfluorescence in the subsequent phases [9, 29–31]. Pinpoint foci of hyperfluorescence appear over the tumor in the late phase of the study. This appearance is typical of choroidal metastatic lesions and is attributed to microcytic retinal pigment epithelial degeneration. These pinpoint foci of hyperfluorescence may enlarge to form globular areas of hyperfluorescence over the tumor. The late angiograms also demonstrate marked hypofluorescence of the brown pigment clumping on the tumor surface, similar to the pattern seen with the orange pigment over melanotic choroidal melanomas. There may be moderate late hyperfluorescence of the serous subretinal fluid overlying and adjacent to the metastatic tumor.

There is noted to be diffuse and multifocal hypofluorescence and hyperfluorescence at the RPE level overlying the RPE as a result of the

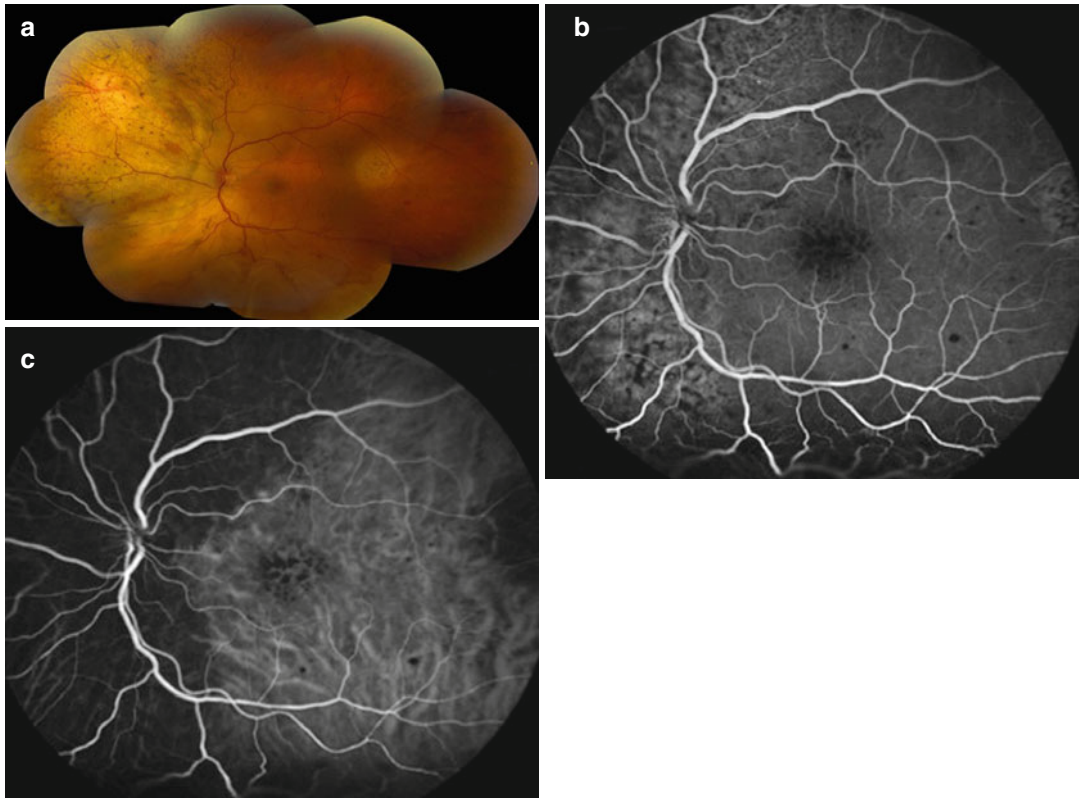

Fig. 18.9 Breast metastases with leopard spotting (**a**). The fluorescein accentuates the confluence of the leopard spots (**b**). ICG shows an infiltrative process that has segmentally abolished the inner choroidal vessels (**c**)

damaging effects of the choroidal tumor on the overlying RPE. The large-caliber vessels and double circulation pattern that are often seen with choroidal melanomas and the late multiloculated diffuse staining pattern often seen with choroidal hemangioma are rarely present in the FA of metastatic tumors (Fig. 18.9).

ICGA is rarely of significant help in diagnosing uveal metastatic lesions [1]. Choroidal metastasis usually shows homogeneous hypofluorescence in comparison with the surrounding choroid. The normal choroidal pattern can often be visualized underlying or within the metastasis as if the tumor were acting as a relative filter of the fluorescence of the underlying choroid. This filtering effect extends for the entire base of the metastasis and stops abruptly at the margin of the tumor. ICGA may reveal subtle occult lesions separate from the symptomatic lesion manifesting as hypofluorescent areas of choroid [3]. In thick choroidal metastatic lesions, intrinsic tumor vessels might be visible on ICGA (Fig. 18.10).

18.5.7 Vitreoretinal Lymphoma and Choroidal Lymphoma

It is important to distinguish the different forms of intraocular lymphoma. Choroidal lymphoma tends to be a low-grade B-cell lymphoma, while vitreoretinal lymphoma is a form of diffuse large B-cell lymphoma in 95 % of cases, while in 5 %, it is a form of T-cell lymphomas. Both forms of vitreoretinal lymphomas are much more aggressive than choroidal lymphomas. As a rule, choroidal lymphoma does not extend anterior to Bruch's membrane, and vitreoretinal lymphoma does not extend posterior to Bruch's membrane. Vitreoretinal lymphoma may involve only the

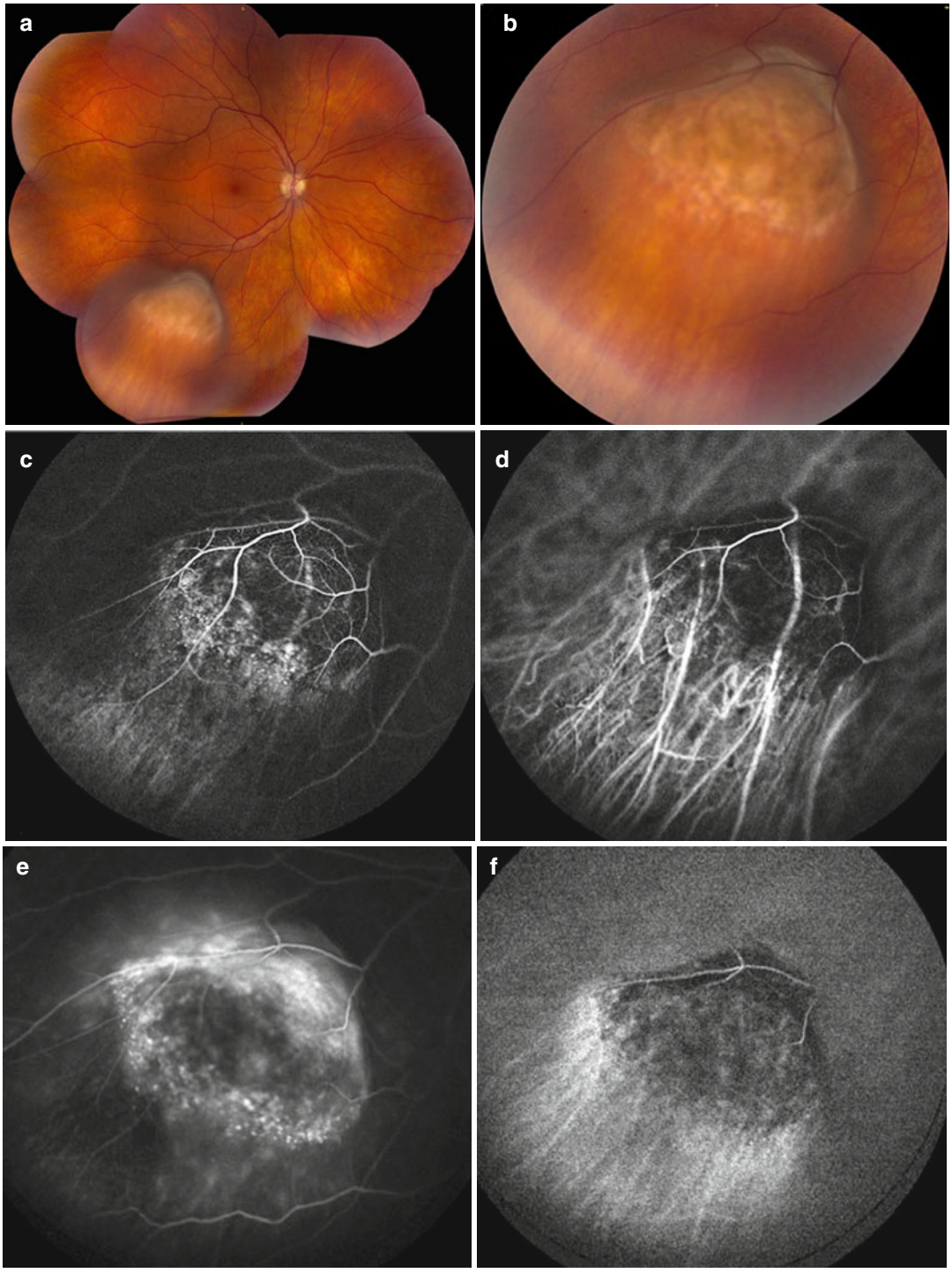

Fig. 18.10 Metastatic neuroendocrine tumor (**a**, **b**). The fluorescein (**c**) and ICG (**d**) angiogram venous phase show a few intrinsic small vessels. In the late phase of FA (**e**) and ICGA (**f**), there are a few mild punctate leakages

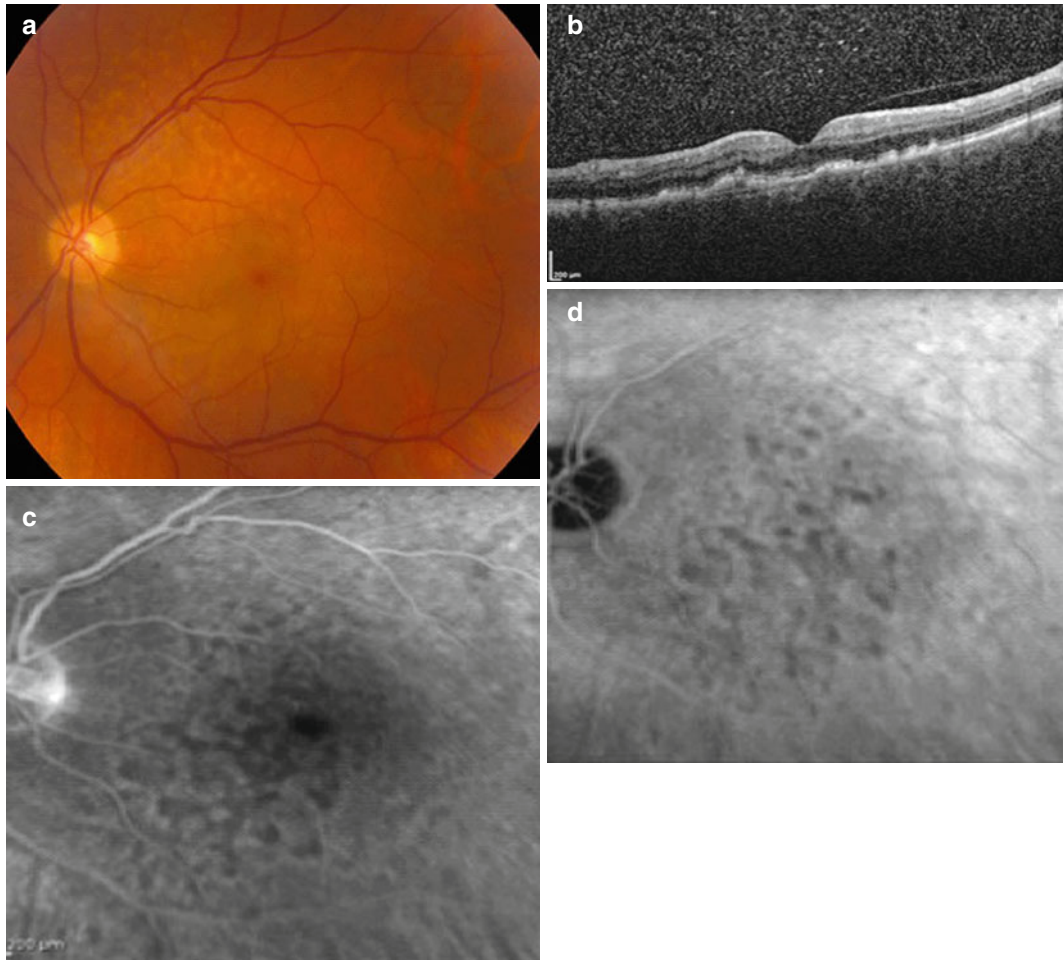

Fig. 18.11 Color fundus photograph of a case of primary vitreoretinal lymphoma showing whitish subretinal pigment deposits of the lymphoma (**a**). This is confirmed by OCT (**b**). Fluorescein (**c**) and ICG (**d**) angiography show hypofluorescent spots because of blockage by the subretinal deposits

vitreous, the vitreous and the retina, or just the retina alone. Though cases that involve the retina usually involve the subretinal pigment epithelial space, some cases involve full thickness retina and have to be distinguished from a severe infectious retinitis.

FA in the majority of cases of diffuse large B-cell vitreoretinal lymphoma does not show leakage from macular edema, so even in the presence of a vast amount of cells, there is very little if any leakage of fluorescein in the macula and in the disc. In about 15 % of cases, there is leakage of the retinal vessels [32, 33]. Another common finding in vitreoretinal lymphoma is the accumulation of cells under the retinal pigment epithelium. These can be small aggregates or very large clumps. The effect of these cells is that they cause blocked fluorescence in the areas involved giving a leopard spot appearance in the early phase (Fig. 18.11). There is hyperfluorescence sometimes with leakage in the late phase. Unfortunately, a leopard spot appearance also occurs in choroidal lymphoma, but in addition, choroidal lymphoma causes choroidal folds which would appear as alternating areas of hyper- and hypofluorescence. Choroidal lymphoma can present as initial hypofluorescent spots in the choroid by both FA and ICGA (Fig. 18.12). They can stay

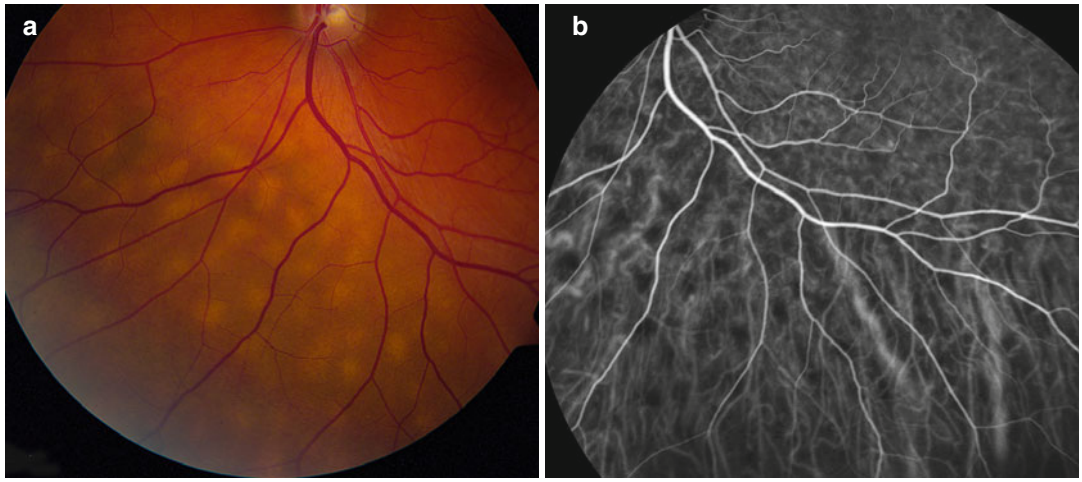

Fig. 18.12 Fundus photograph of an eye with choroidal lymphoma demonstrating focal choroidal infiltrates in the mid-periphery (**a**). ICGA demonstrating areas of hypofluorescence corresponding to focal choroidal infiltrates (**b**)

hypofluorescent especially by fluorescein, but occasionally, they can become hyperfluorescent by ICGA.

18.6 Simulating Conditions

18.6.1 Coats' Disease

The typical fluorescein angiographic finding of Coats' disease is of unilateral, multiple localized vascular anomalies in the retinal vessels ranging from telangiectasia, aneurysmal dilatations, and beading of vessel walls to vascular communicating channels in larger vessels. On fluorescein angiography, the telangiectatic vessels display early hyperfluorescence and persistent fluorescein leakage, while exudation shows hypofluorescence [34]. Microvascular involvement is reflected by areas of diffuse loss of the capillary bed or areas of complete capillary nonperfusion (Fig. 18.13).

18.6.2 Persistent Hyperplastic Primary Vitreous/Persistent Fetal Vasculature

Persistent hyperplastic primary vitreous (PHPV), also known as persistent fetal vasculature (PFV), usually involves only one eye. Anteriorly, there can be a prominent persistent tunica vasculosa lentis, and posteriorly, there is a prominent canal which can extend between the disc and the posterior aspect of the lens. This canal contracts over time because of fibrosis. This can cause a tractional retinal detachment of the retina in severe cases. In milder cases, the posterior lens capsule contracts posteriorly causing a cataract while the retina may contract anteriorly forming retinal folds. There can also be persistence of the iris vasculature and vascularity over the ciliary body [35].

FA can show leakage from the vessels under traction in the retina. Anterior segment fluorescein angiography can demonstrate the persistent vasculature and leakage from it. In dense cataracts, there can still be evidence of the vascularity on the posterior aspect of the lens.

Familial exudative vitreoretinopathy can show similar leakage from the retinal vessels on the tractionally detached retina. In milder cases there can be evidence of peripheral avascularity. Similar findings can be seen in incontinentia pigmenti.

18.6.3 Scleritis

Scleritis is in the differential diagnosis of patients with choroidal masses. Usually the patients have pain. The scleritis causes decreased

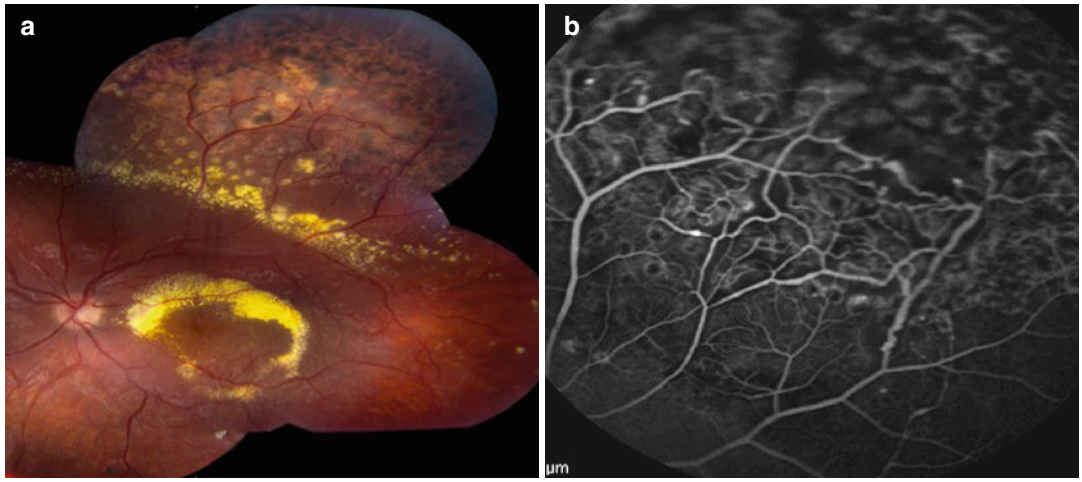

Fig. 18.13 Fundus photography showing partially treated Coats' disease (**a**). Fluorescein angiography shows telangiectatic vessels in both the posterior pole and the periphery (**b**)

outflow from the choroid, and there is also thickening of the sclera, together causing a pseudomass of the choroid. FA can show the presence of the choroidal folds as discussed in the section on choroidal lymphoma. In addition, with chronicity, the leopard spotting occurs as well as leakage from the choroid. There can also be leakage from the disc if the scleritis is near the posterior pole.

18.6.4 Central Serous Chorioretinopathy (CSCR)

Central serous chorioretinopathy (CSCR) has been historically diagnosed using FA; however, the availability of ICGA has increased the knowledge of this disease. On FA, CSCR typically appears as one or several hyperfluorescent leaks at the level of the retinal pigment epithelium. In most cases, the fluorescein dye symmetrically spreads, slowly staining the subretinal detachment area evenly, but does not stain the retina beyond the edges of the detachment.

On ICGA, CSCR is displayed as a localized vascular hyperpermeability of the choroid best visualized in the mid-phase of the angiogram. The leaked dye disperses into the deeper layers of the choroid producing a characteristic hyperfluorescent patch in the choroid that enlarges away from the center during the later stages of ICGA [36]. In comparison to tumors, there are very small areas of leakage, and the fellow eye could have evidence of staining. In addition, enhanced depth OCT helps in determining the status of the choroid in the areas that are involved and helps to show that the underlying choroid is thick but not infiltrated.

18.6.5 Polypoidal Choroidal Vasculopathy (PCV)

The FA findings of polypoidal choroidal vasculopathy (PCV) and CSCR are very similar, and thus ICGA plays an important role in differentiating these two diseases. PCV is typically diagnosed using ICGA, at which the early stages show early filling of larger vessels within the PCV network followed by filling of retinal vessels; however, the areas surrounding the lesion are hypofluorescent compared to the normal choroid. ICGA also displays small hyperfluorescent outpouches arising from the larger choroidal vessels, which can be easily identified in the choroid (Fig. 18.14). The late phase of ICGA has a reversal in the pattern of fluorescence observed in the earlier stages in that the area surrounding the lesion becomes hyperfluorescent and the center of the lesion hypofluorescence [37].

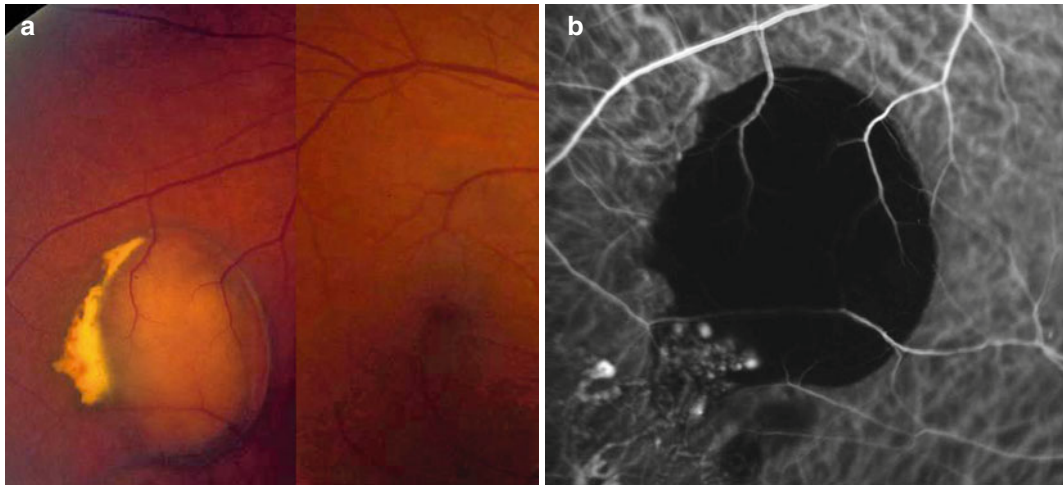

Fig. 18.14 The patient presented with this elevated subretinal lesion temporal to the macula with associated exudate (**a**). ICGA revealed characteristic hyperfluorescent "polyps" (**b**)

18.6.6 Uveal Effusion Syndrome

In this syndrome, there appears to be thickening of the sclera causing decreased outflow from the choroid. Again, leopard spotting is noted because of chronic leakage similar to patients with scleritis or choroidal lymphoma.

18.6.7 Paraneoplastic Disorders

There are several paraneoplastic disorders that involve the retina or choroid:

1. Carcinoma-associated retinopathy is the most common. The retina shows some disc atrophy and attenuation of the retinal vessels, occasional spicules might be seen, and cystoid macular edema may be noticed in some cases, but otherwise, the fundus is nondescript. Fluorescein angiography does not help very much in making the diagnosis.
2. Acute exudative polymorphous vitelliform maculopathy is seen in idiopathic cases but also in cancers especially skin melanoma. Fluorescein angiography in these cases shows initial blockage by the vitelliform material followed by slow staining. ICG does not add any further information.
3. Bilateral diffuse uveal melanocytic proliferation is associated systemically mainly with small-cell lung cancers and gynecologic cancers, but again other cancers are associated as well. It has been associated with a factor termed CMEP factor (cultured melanocyte elongation and proliferation factor) which is present in the IgG fraction of serum. Fluorescein and ICG angiography show a reticular pattern of staining where the RPE has atrophied secondary to the loss of the choriocapillaris resulting from infiltration of the uvea by the proliferating melanocytes (Fig. 18.15). There is also hypofluorescence from the areas of orange lipofuscin accumulations.

18.6.8 Radiation Retinopathy

Radiation retinopathy can be either nonproliferative or proliferative. Fluorescein angiography is helpful in quantitating the amount of ischemia as well as the amount of leakage from the disc and macula. OCT is useful for determining treatment response, but the initial determination of where to treat with laser photocoagulation is best done with widefield fluorescein angiography (Chap. 11).

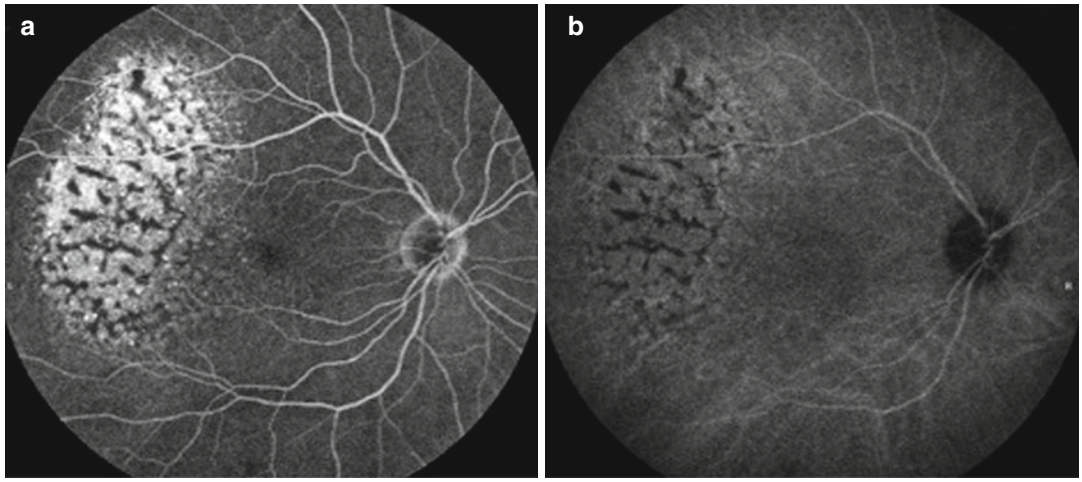

Fig. 18.15 Bilateral diffuse uveal melanocytic proliferation in the right eye. Fluorescein angiogram (**a**) and ICGA (**b**) showing the blockage by the orange pigment and the window defects

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Diagnostic Techniques: Optical Coherence Tomography

19

Rubens Belfort, Andre Romano, Eduardo Rodrigues, and Arun D. Singh

Contents

19.1 Introduction

Optical coherence tomography (OCT) is a powerful imaging technique that provides cross-sectional imaging of the internal microstructures of biological tissues [1]. Among several implementations of OCT, spectral-domain OCT (SD-OCT) provides high-resolution, high-sensitivity, and high-speed imaging. Such implementations enable visualization of the microstructures of the human eye by noninvasive and noncontact measurement. High sensitivity of OCT detection enables imaging of structures with very low optical scattering, such as the retina. The high axial and lateral resolution enables the precise morphological investigation of various pathological conditions. Recently, hardware and software upgrades have improved the ability to better evaluate intraocular tumors [2, 3].

19.2 Conjunctival and Corneal Tumors

OCT can provide valuable information in the diagnosis of various tumors of the cornea and conjunctiva. Conjunctival nevi are one of the most common benign tumors of the ocular surface. On histopathology, a nevus typically consists of nests of nevus cells that are present in the junctional and subepithelial area of the conjunctiva. Conjunctival inclusion cysts are characteristic for nevi and may be observed clinically. Anterior segment OCT enables high-quality

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_19,

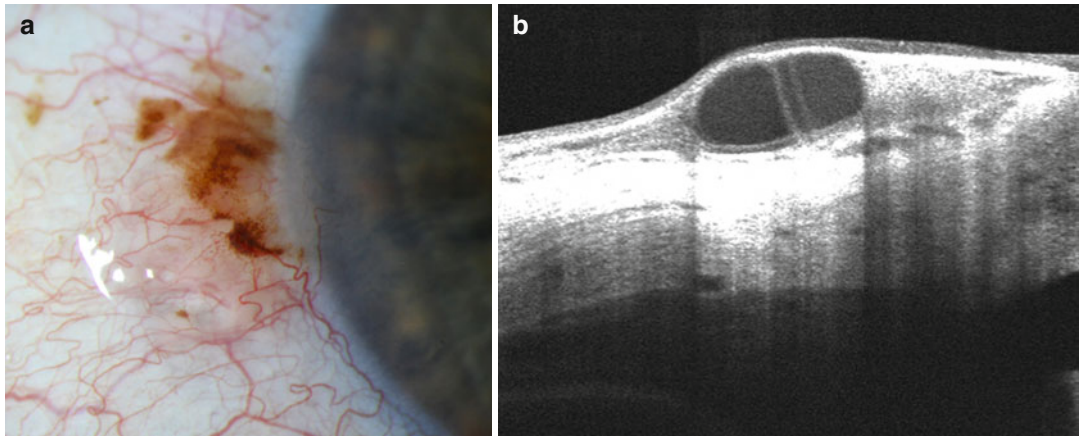

Fig. 19.1 Conjunctival nevus with cyst. Clinical appearance (**a**). Anterior segment OCT confirms presence of an intralesional cyst (**b**)

imaging of conjunctival nevi to reveal all margins and to provide information on the presence of the typical intralesional cysts (Fig. 19.1). OCT allows identification of the margins of the tumors and additional features that include thickening of the conjunctiva. One of the main disadvantages of OCT is the optical shadowing of deeper structures from pigment in conjunctival nevi [4].

Ocular surface squamous neoplasia can involve the conjunctiva, the cornea, or both and consist the spectrum of neoplasia of intraepithelial neoplasia and squamous cell carcinoma. A small pilot study involving the uterine cervix demonstrated the ability of OCT to distinguish abnormal epithelium from normal epithelium in the examination of cervical tissue [5]. Kieval et al. investigated the use of an ultrahigh-resolution OCT as an adjuvant diagnostic tool in distinguishing ocular surface squamous neoplasia and pterygia (Fig. 19.2) [6]. Preoperative ultrahigh-resolution OCT images demonstrated similarities to the histopathologic specimens. Both optical and pathologic specimens showed a thickened layer of epithelium, often with an abrupt transition from normal to neoplastic tissue in eyes with squamous cell carcinoma. Differences in the measured epithelial thickness on ultrahigh-resolution OCT between squamous cell carcinoma and pterygia were statistically significant. Scleral lesions can be visualized partially by OCT (Fig. 19.3).

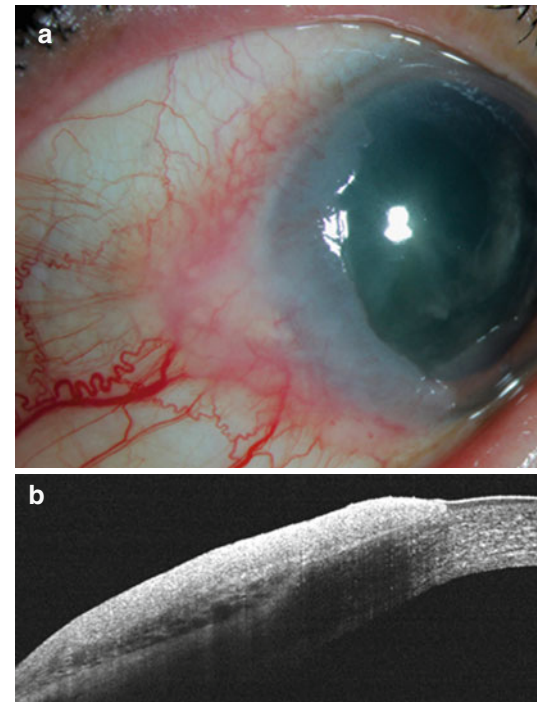

Fig. 19.2 Ocular surface squamous neoplasia. Clinical appearance (**a**). Anterior segment OCT reveals thickened epithelium crossing the limbus from conjunctiva to cornea (**b**)

19.3 Anterior Uveal Tumors

Imaging technologies for anterior segment tumor evaluation provide useful information with regard to tumor size, shape, internal features, and

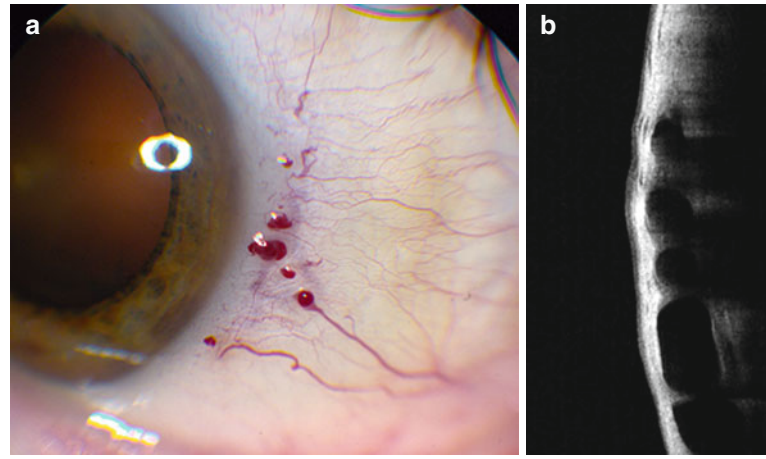

Fig. 19.3 Scleral vascular anomaly. Clinical appearance (**a**). Anterior segment OCT showing partial view of several intrascleral clear spaces (**b**)

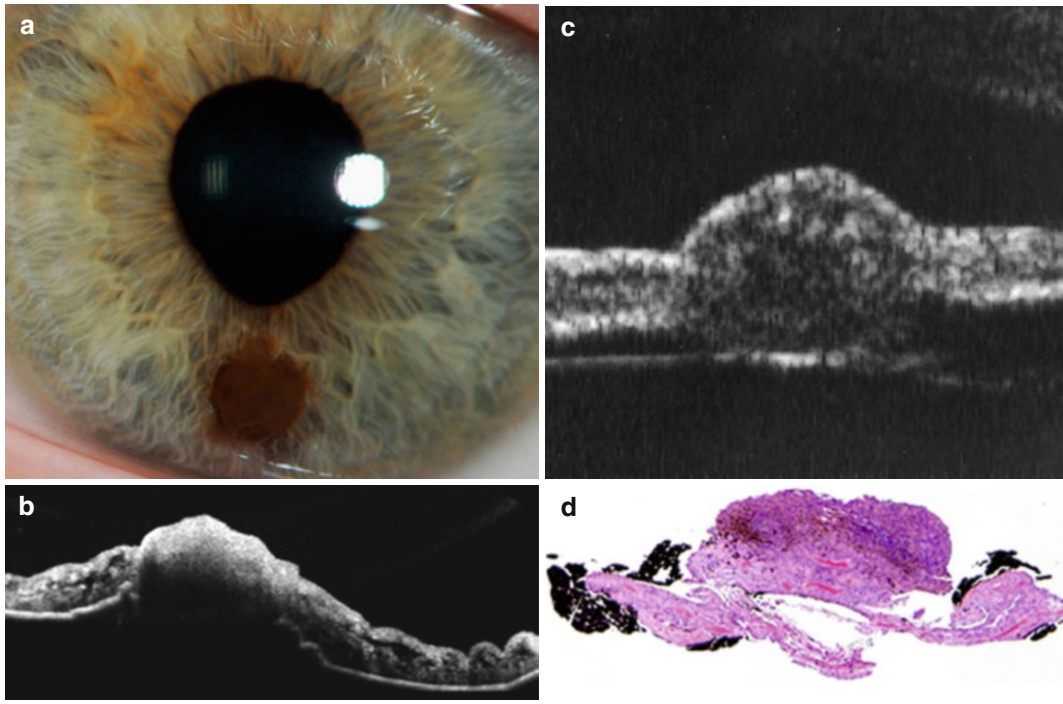

Fig. 19.4 Pigmented iris melanoma. Clinical appearance (**a**). Anterior segment OCT shows hyperreflectivity with shadowing and obscuration of the posterior margin (**b**). Ultrasound biomicroscopy revealed isolated iris involvement with lesion thickness of 1.3 mm (**c**). The tumor was classified as a mixed cell-type melanoma (**d**, hematoxylin and eosin; 40× magnification) (Reproduced with permission from Hood et al. [30])

extension. Given that some of these conditions can threaten vision and life, such as iris melanoma, a precise and complete image of the lesion is of extreme relevance.

Few clinical studies compared the usefulness of OCT and UBM in the evaluation of ciliary body and iris tumors (Fig. 19.4). Siahmed et al. described the use of UBM and AS-OCT for imaging 61 cases of iris tumors, observing that only UBM provided a precise measurement and regular surveillance capabilities compared with OCT [7]. Pavlin et al. published a series of patients with iris and ciliary body tumors that were evaluated with both UBM and OCT [8].

They observed that OCT was able to visualize all margins of only small hypopigmented tumors, whereas UBM had a superior ability to penetrate larger, highly pigmented tumors and those located in the ciliary body. Bianciotto and coworkers also reported UBM as a superior method for imaging of anterior segment tumors, such as ciliary body and iris melanoma [9].

19.4 Intraocular Tumors

19.4.1 Retinal and Retinal Pigment Epithelial Tumors

19.4.1.1 Congenital Hypertrophy and Combined Hamartoma of the Retina and Retinal Pigment Epithelium

A typical appearance of congenital hypertrophy of the retinal pigment epithelium (RPE) is of thickened RPE layer by OCT (Fig. 19.5). Combined hamartoma of the retina and RPE is of an elevated grey mass of the retina blending imperceptibly with surrounding retina and RPE without retinal detachment, or vitreous inflammation. Spectral-domain OCT reveals an elevated hyperreflective mass in the retina with mild attenuation of the retinal pigment epithelium and photoreceptor inner segment/outer segment junction and may be accompanied by a hyperreflective epiretinal membrane [3, 10, 11].

19.4.1.2 Retinal Astrocytic Hamartoma

Retinal astrocytic hamartoma (astrocytoma) is a vascularized, benign, glial tumor of the retina that can be acquired or congenital. SD-OCT depicts a thickened inner retinal with epiretinal membrane, some disorganization of the inner and outer retinal layers, and an intact RPE underlying the tumor (Fig. 19.6) [12, 13].

19.4.1.3 Retinal Cavernous Hemangioma

Cavernous hemangioma is a benign retinal vascular tumor that appears as dark-red saccular aneurysms. It is often associated with vitreous hemorrhage, preretinal fibrosis, or vascular occlusion. Descriptions of retinal cavernous hemangioma on OCT are a disorganized epiretinal membrane with cystic spaces within inner and outer layers that may represent the saccular aneurysms (Fig. 19.7) [3, 14].

19.4.1.4 Retinal Capillary Hemangioma

Retinal hemangioblastoma (capillary hemangioma) is an orange circumscribed vascular lesion with dilated feeding artery and draining vein. They can be associated with Von Hippel-Lindau disease. OCT evaluation of these lesions shows a hyperreflective lesion with little inner tumor

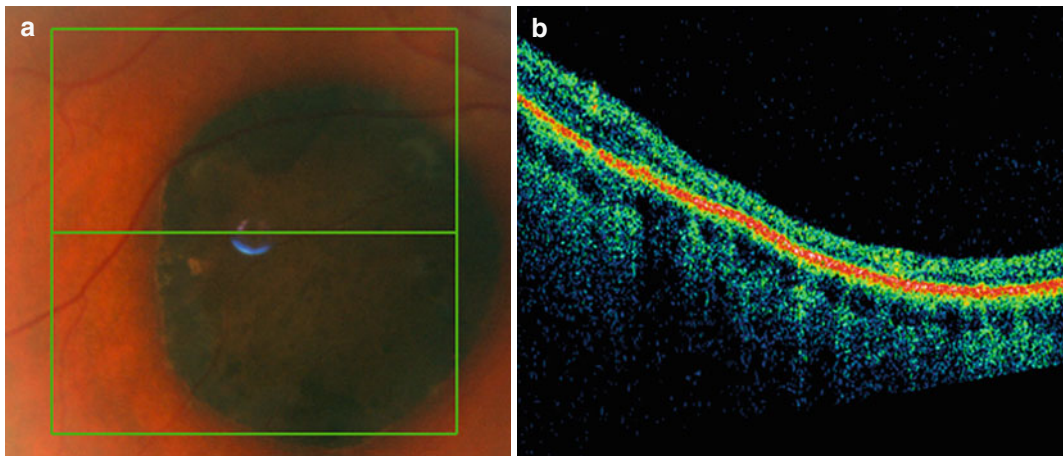

Fig. 19.5 A typical fundus appearance of congenital hypertrophy of the retinal pigment epithelium (*RPE*) (**a**) is of thickened RPE layer by OCT (**b**)

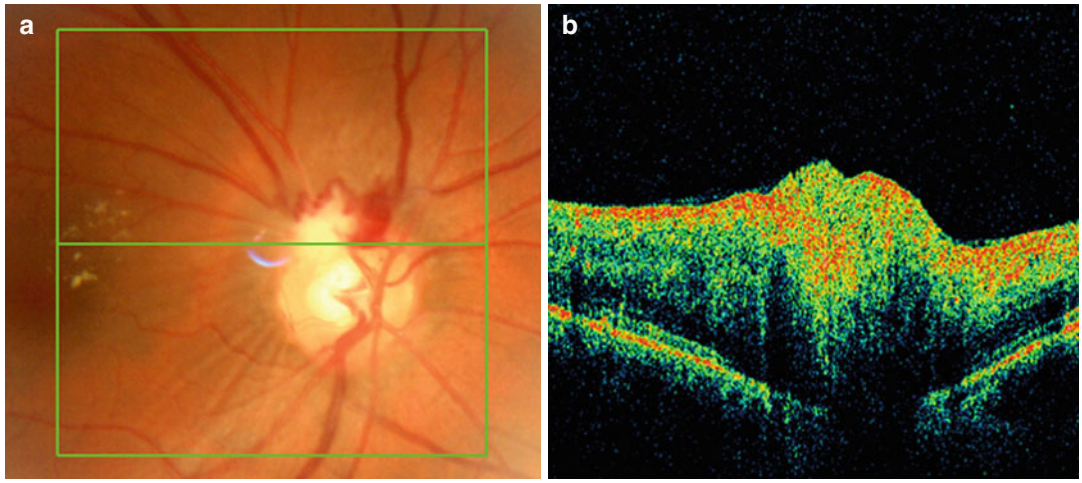

Fig. 19.6 Retinal astrocytic hamartoma. Clinical appearance (**a**). Note thickened inner retina with disorganization of the inner and outer retinal layers (**b**)

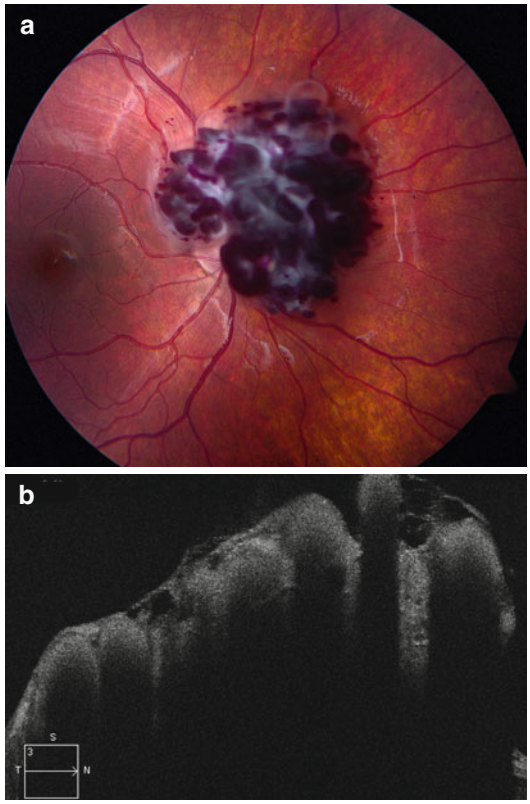

Fig. 19.7 Retina cavernous hemangioma. Clinical appearance (**a**). Epiretinal membrane bridging across multiple cystic spaces within inner and outer retinal layers (**b**)

detail, but macular evaluation by OCT is useful to detect associated macular edema, epiretinal membrane, and subretinal fluid [3].

19.4.1.5 Retinal Vasoproliferative Tumor

The vasoproliferative tumor of the retina is usually a unilateral, yellow-red retinal lesion located in the inferotemporal periphery with minimally dilated or nondilated feeding artery and draining vein in contrast to hemangioblastoma. Its peripheral location makes it difficult to image with OCT, requiring advanced changes in the OCT scan length. Disorganization of the inner retinal layer and posterior shadowing are features of vasoproliferative tumors on OCT [15]. Vision loss is usually due to epiretinal membrane or exudative macular detachment, and OCT is useful to identify these changes and follow-up during treatment.

19.4.1.6 Optic Disc Melanocytoma

Melanocytoma of the optic nerve head is a benign melanocytic lesion that usually occurs in the inferotemporal area of the optic nerve head. It is a dome-shaped lesion with a gradual slope at the tumor margin. Areas of irregular hyperreflectivity were observed in the area of melanocytoma, but with little internal detail of the tumor (Fig. 19.8) [16].

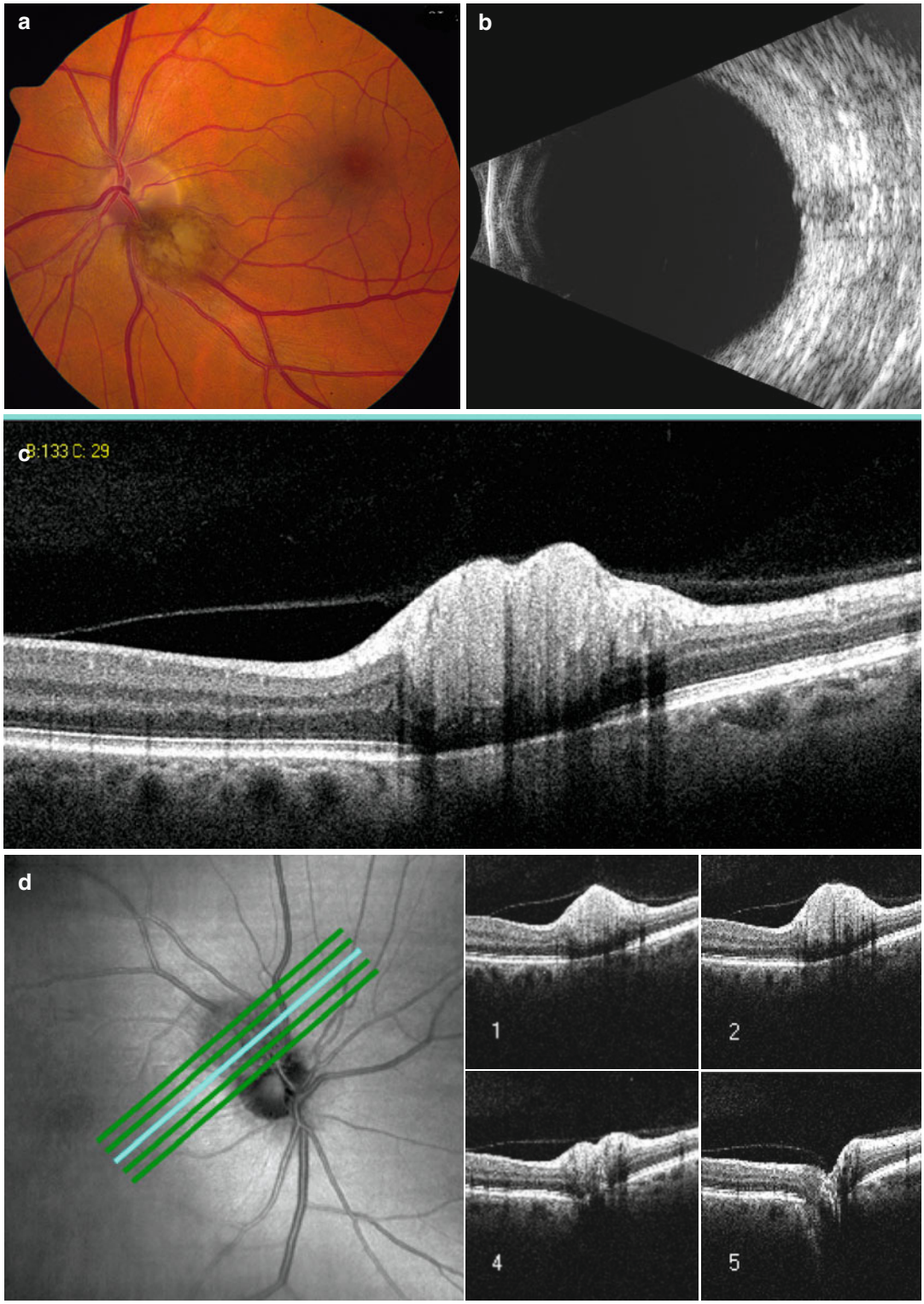

Fig. 19.8 Optic disc melanocytoma. Clinical appearance (**a**). The lesion is barely detectable by conventional B-scan ultrasonography (**b**). The inner layers of retinal appear to be thickened with disorganization of outer layers. The RPE is intact with normal underlying choroid placing the tumor on the retina rather than the choroid (**c**). Location of OCT scans (**d**)

19.4.2 Choroidal Tumors

19.4.2.1 Choroidal Nevus

Choroidal nevus is the most common intraocular tumor. Nevi are round pigmented subretinal lesions, with smooth margins and frequently present with overlying drusen, measuring less than 5 mm in basal diameter and 1 mm in thickness. OCT is able to detect retinal and anterior choroidal changes that include retinal thinning, retinal edema, subretinal fluid, RPE detachment, and drusen. OCT evaluation is more sensitive than clinical examination to detect retinal edema, subretinal fluid, retinal thinning, and RPE detachment. Also, it helps to determine the status of photoreceptor and to characterize retinal edema (cystoid versus non-cystoid). Drusen that are clinically visible are evidenced by OCT as small dome-shaped elevations at the level of Bruch's membrane (Fig. 19.9) [17, 18].

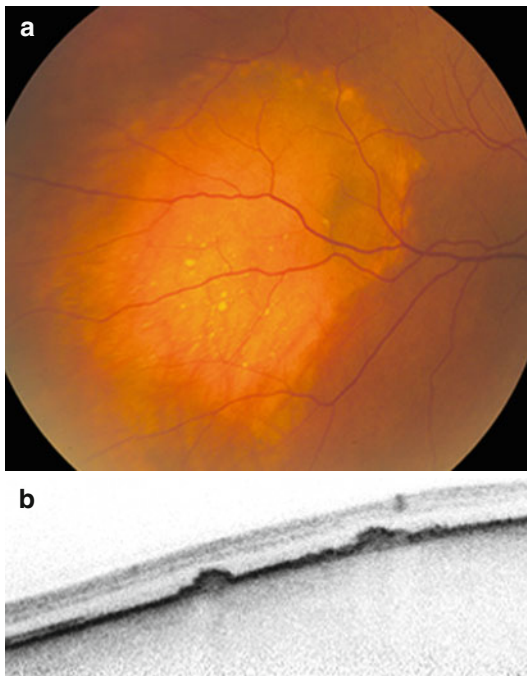

Fig. 19.9 Indeterminate choroidal melanocytic lesion (small choroidal melanoma). Fundus photograph (**a**). Drusen are visible as small dome-shaped elevations at the level of Bruch's membrane (**b**) (Reproduced with permission from Singh et al. [19])

19.4.2.2 Indeterminate Melanocytic Lesions

Indeterminate melanocytic lesions (IML) are pigmented choroidal lesions that could be a large nevus or small melanoma (between 1 and 3 mm in thickness). In contrast to medium- and large-sized choroidal melanomas that can be reliably diagnosed on the basis of ophthalmoscopic, angiographic, and ultrasonographic features with more than 99 % accuracy, IML can present a diagnostic challenge. Some of the features predictive of growth (or the risk of the lesion being melanoma) include thickness greater than 2 mm, presence of orange pigment and subretinal fluid, and absence of drusen. OCT is able to detect some of these changes. Singh and associates used spectral-domain OCT to describe accumulation of subretinal deposits corresponding to orange pigment over a small IML that had not been observed with time-domain OCT [19]. Chronic retinal changes observed by OCT as thinned retina with intraretinal cysts and RPE thickening were associated with lesions less likely to grow. Hyper-autofluorescence (see below) correlates to OCT findings of lipofuscin or subretinal fluid (Fig. 19.10) [19].

19.4.2.3 Choroidal Melanoma

Uveal melanoma is the most common primary intraocular malignant tumor, and 85 % develop in the choroid. They often present as a pigmented, elevated, choroidal mass. In medium- to large-sized melanomas, OCT analysis is limited to superficial changes in the retina and anterior surface of the tumor. Subretinal fluid is an important characteristic related to underlying choroidal melanoma, may be detectable by OCT in almost all cases [20].

Sayanagi and coworkers used 3D spectral-domain OCT and found a significantly higher prevalence of subretinal fluid (91 % versus 14 %), retinal edema (61 % versus 14 %), and subretinal deposits (61 % versus 11 %) in choroidal melanoma compared with nevi [2]. The limitation of OCT for choroidal melanoma lies in the inability to image past the anterior choroidal surface (Fig. 19.11) [21]. Reflectivity of the anterior choroid in melanoma is variable even with spectral-domain OCT [19].

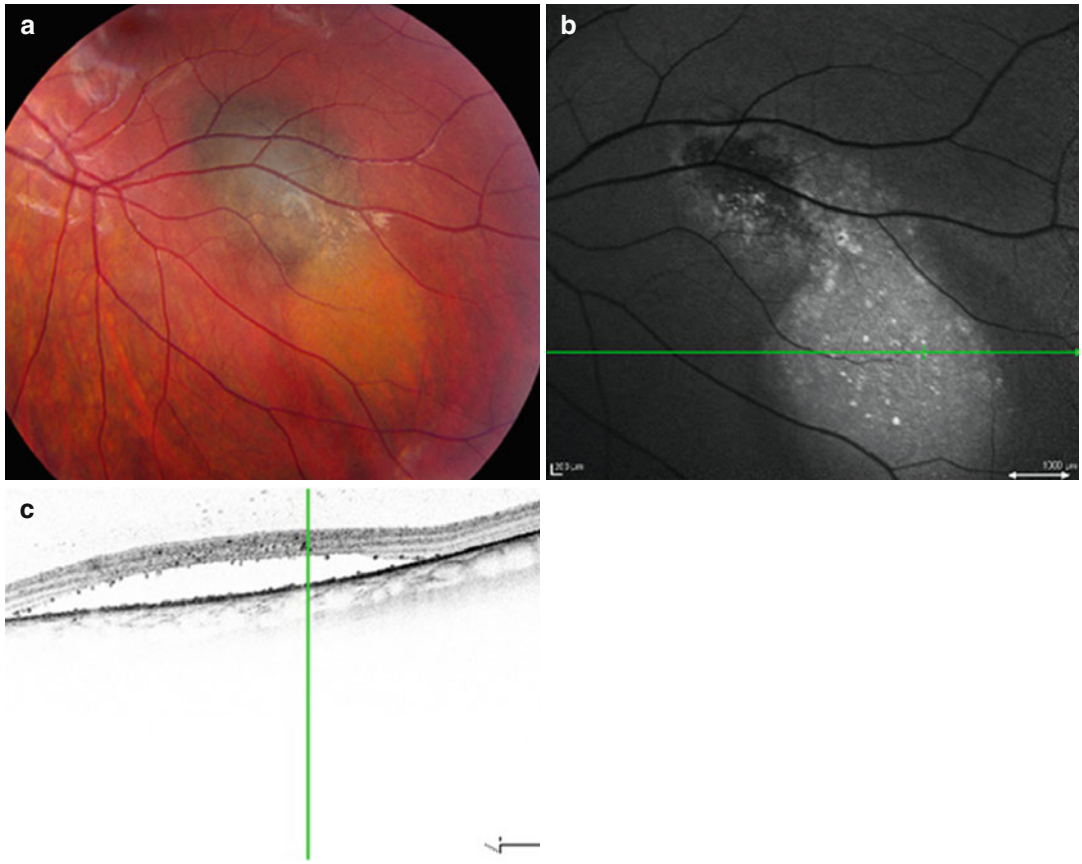

Fig. 19.10 Indeterminate choroidal melanocytic lesion (small choroidal melanoma). Orange pigmentation (lipofuscin) on fundus photograph (**a**) corresponds with hyperautofluorescent spots on fundus autofluorescence image (**b**) and deposition of hyperreflective material both near the retinal pigment epithelium and in the outer retina on the spectral-domain OCT (**c**) (Reproduced with permission from Singh et al. [19])

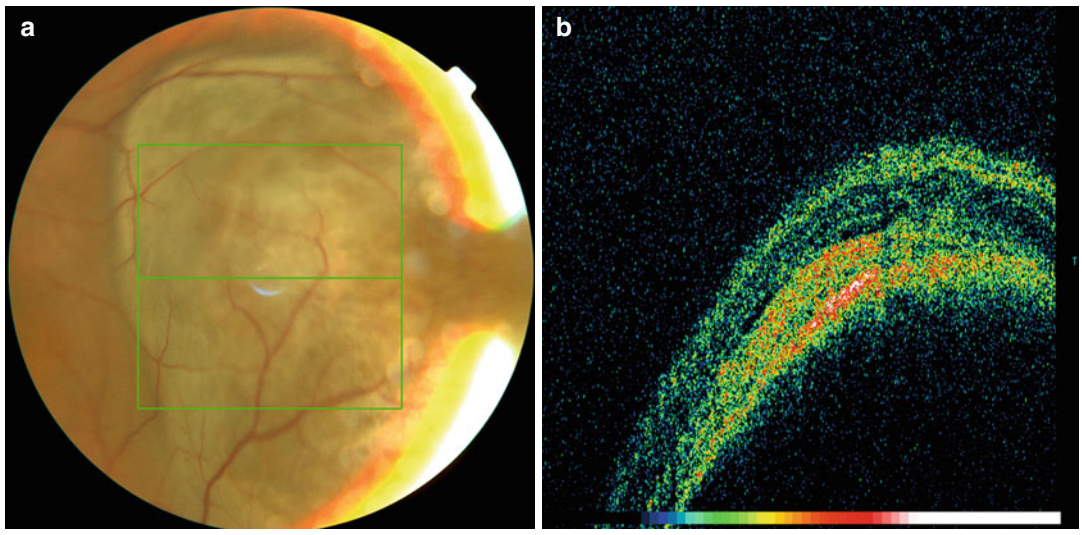

Fig. 19.11 Malignant melanoma. Fundus appearance (**a**). OCT image notes irregularity of retinal pigment epithelium, subretinal fluid, intraretinal edema, and cyst (**b**) (Reproduced with permission from Sayanagi et al. [2])

OCT has been used to monitor retinal changes related to complications of radiotherapy like macular edema, which can be present in 61 % of the cases, 24 months after treatment [22].

19.4.2.4 Choroidal Osteoma

Choroidal osteoma is an osseous tumor reported in young females. The tumor is located in the juxtapapillary region or macula and presents an orange-yellow color when calcified and as a white tumor when decalcified. Complications of choroidal osteoma that cause vision loss are related to the tumor decalcification and include neovascular membranes and retinal changes (subretinal fluid or photoreceptor loss), both identifiable by OCT imaging.

Shields and coworkers reported on the OCT features of choroidal osteoma that included heterogeneity that depended upon the amount of tumor calcification [23]. Calcified portions of the tumor reveal mostly intact inner (100 %) and outer (95 %) retinal layers, a distinct RPE (57 %), and mild transmission of light (95 %) (Fig. 19.12). In contrast, decalcified portions of the tumor reveal intact inner retinal layers (90 %), thinned outer retinal layers (100 %), an indistinct RPE (90 %), and marked light transmission into the tumor (70 %). The anterior tumor surface was hyperreflective in 48 % and isoreflective in 52 % if calcified, but was mostly hyperreflective (90 %) when decalcified [23].

19.4.2.5 Choroidal Metastasis

On OCT examination, choroidal metastases demonstrate a dome-shaped elevation of the RPE and retina with adjacent subretinal fluid. OCT imaging of choroidal lesions is limited to retinal changes and anterior choroid evaluation. Retinal changes include retinal edema, intraretinal cysts, thickening, and detachment of the RPE. Arevalo and colleagues found highly reflective points within neurosensory detachment in 14.2 % of cases and concluded that these points "may correspond to retinal compromise by cancer cells or macrophages containing lipofuscin and melanin granules" [24].

19.4.2.6 Choroidal Hemangioma

Circumscribed choroidal hemangioma is usually orange colored, round, and located in the posterior pole. It usually exhibits overlying retinal edema, subretinal fluid, and RPE alterations [25].

Ramasubramanian and colleagues described OCT findings in circumscribed choroidal hemangioma and found subretinal fluid (19 %), retinal edema (42 %), retinal schisis (12 %), macular edema (24 %), and localized photoreceptor loss (35 %) [26]. Retinal changes are secondary to tumor leakage. OCT shows preserved photoreceptors and normal retinal thickness if the leakage is acute and degenerated photoreceptors,

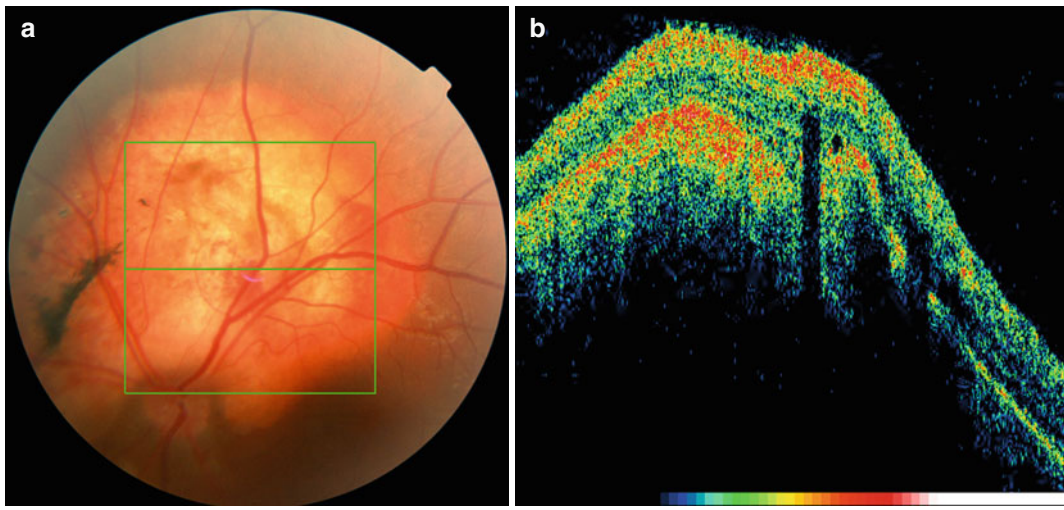

Fig. 19.12 Choroidal osteoma. Fundus appearance (**a**). OCT image reveals irregularity of retinal pigment epithelium/choriocapillaris (**b**) (Reproduced with permission from Sayanagi et al. [2])

retinoschisis, and intraretinal edema if the leakage is chronic [3]. OCT has been used to monitor response to treatment by photodynamic therapy, transpupillary thermotherapy, plaque radiation, or laser photocoagulation.

19.5 Enhanced Depth Imaging

Recently, Spaide and associates have described a simple method using commercially available SD-OCT devices to image the choroid called enhanced depth imaging (EDI) [27]. In an exploratory study, Torres et al. described the intrinsic optical characteristics of choroidal tumors imaged with EDI SD-OCT technique and correlated tumor measurements with ultrasonography [28]. In their study, all the cases that were too small (<1.0 mm in thickness) to be adequately identified by ultrasonography (such as choroidal nevi) were adequately imaged and objectively measurable by EDI SD-OCT techniques. On the other hand, tumors that were >1.0 mm in height and/or >9.0 mm in diameter were not suitable to be measured by EDI SD-OCT. Consequently, they suggested that EDI SD-OCT and ultrasound may be considered as complimentary techniques for tumor thickness measurement [28].

Specific EDI SD-OCT findings are influenced by cellular composition, compactness of tissues, tumor vascularity, presence of light reflection/scattering, melanin, and other factors. Circumscribed choroidal hemangioma showed low/medium reflectivity and a homogenous signal with large intrinsic spaces (Fig. 19.13). In contrast, choroidal metastasis revealed a low reflective band in

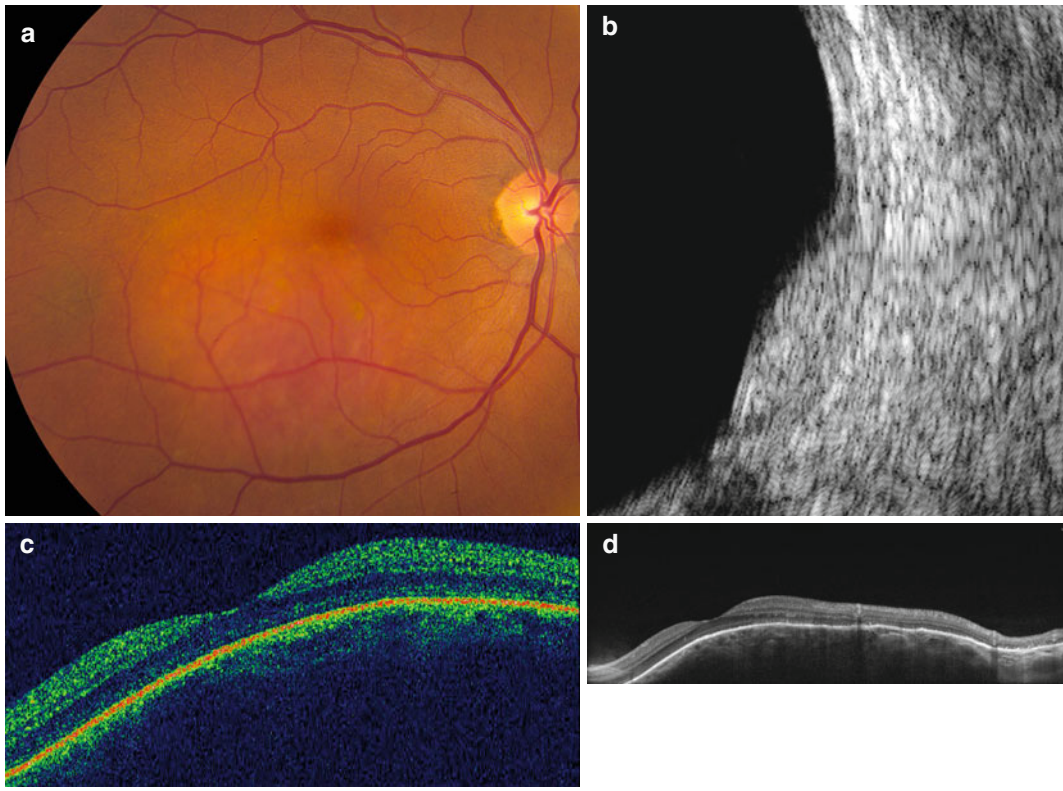

Fig. 19.13 Circumscribed choroidal hemangioma. Fundus appearance (**a**). B-scan ultrasound shows a dome-shaped solid lesion, with high and homogenous reflectivity (**b**). Spectral-domain OCT (Topcon-1000). There is elevation of retina/choroid complex with nonspecific signals from the underlying choroid (**c**). Spectral-domain OCT EDI technique (inverted). Note a low to medium reflective signal from the lesion. Small and large spaces (possibly vascular) are also identified within the tumor (**d**) (Reproduced with permission from Torres et al. [28])

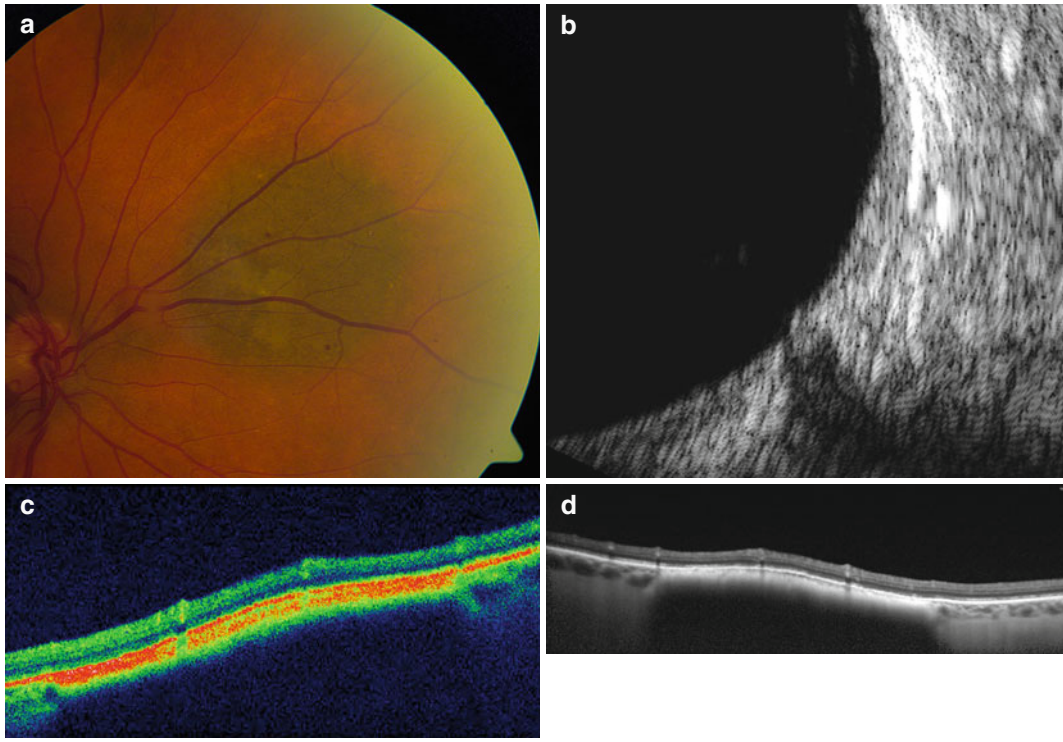

Fig. 19.14 Choroidal melanocytic nevus. Fundus appearance (**a**). B-scan ultrasound is unable to detect the lesion (**b**). Spectral-domain OCT (Topcon-1000). The lesion appears as a sharply highly reflective band at the Bruch's/RPE/choriocapillaris layer (**c**). Spectral-domain OCT EDI technique (inverted). The lesion is well distinguished from surrounding normal choroid as a highly reflective band with posterior shadowing. A thin hyporeflective line separates the RPE and the anterior tumor surface (**d**) (Reproduced with permission from Torres et al. [28])

the deeper choroid with enlargement of the suprachoroidal space. All melanocytic tumors (nevus and melanoma) demonstrated a highly reflective band within the choriocapillaris layer with posterior shadowing (Fig. 19.14) [29].

Conclusions

OCT enables identification of anterior and posterior tumor margins, alterations or thickening of the overlying epithelium, and recognition of secondary features like conjunctival cysts. OCT is also a useful tool for evaluation of small anterior iris tumors. It enables identification of tumor margins and tumor surface. In cases of posterior iris and ciliary body tumors, OCT tends to be inferior to UBM. Novel developments in OCT technology may enable better visualization of deep anterior segment tumors and choroidal tumors.

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Diagnostic Techniques: Autofluorescence

20

Edoardo Midena, Elisabetta Pilotto, and Raffaele Parrozzani

Contents

20.1 Introduction

Autofluorescence is a relatively new, noninvasive diagnostic tool recently introduced in the armamentarium of intraocular tumor diagnosis and characterization. This technique may reveal the effects of choroidal tumors on the overlying retinal pigment epithelium and may also provide specific information on intrinsic pigments that compose and characterize benign and malignant, congenital and acquired chorioretinal lesions. Lipofuscin, melanin, and melanolipofuscin strongly influence the different autofluorescence patterns of chorioretinal tumors, but other pigments may theoretically influence the autofluorescence pattern of these lesions. In this chapter we review the role of autofluorescence in the diagnosis of benign and malignant chorioretinal tumors.

20.2 The Origin of Autofluorescence

The retinal pigment epithelium (RPE) digests the tips of the outer photoreceptor segments by phagocytosis on a daily basis [1]. The digestion process is physiologically efficient, but a tiny fraction of this material is incompatible for degradation and accumulates in RPE lysosomes by age. This undigested fraction is called lipofuscin (LF). LF is a pigment that exhibits a characteristic autofluorescence (AF) (fluorophore) when excited in ultraviolet (UV) or blue light. Above the age of

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_20,

70, as much as 20 % of the free cytoplasmic space of the RPE cells is occupied by LF granules but also by melanolipofuscin granules (MLF), a compound product of LF and melanin [2]. Also MLF acts as a fluorophore, needing specific wavelengths of excitation and cutoff filters for emitting light (different from LF) to obtain the MLF-related emission peak [3]. MLF is present in RPE in physiologic conditions, but increases in different pathologic conditions as well as LF. Roughly all disorders causing RPE changes by direct or indirect involvement, as well as any disease affecting RPE metabolism, may cause a change in physiologic fundus AF by changing the amount of RPE-related fluorophores [3].

20.3 Types of Autofluorescence

20.3.1 Short-Wavelength AF

Short-wavelength AF (SW-AF), also called standard AF, mainly reflects the amount and the topographic distribution of LF deposits in the RPE [1–3]. Ex vivo studies have shown that the amount of RPE LF increases gradually from the fovea to a maximum of about 10° from the fovea and then decreases toward the periphery [4]. The topographic distribution of fundus AF, measured with an excitation at 550 nm, confirmed in vivo these findings, revealing that SW-AF is maximal at an eccentricity of 7–13°, where AF is 1.7 times higher than in the fovea [5]. Fundus SW-AF is also not symmetrically distributed around the fovea and is maximal about 12° temporally and superiorly, lower inferiorly and nasally, roughly matching the distribution of rod photoreceptors [1].

Despite intensive research, the composition of LF is still not completely understood. RPE LF is proposed to contain highly modified, undegradable remnants of phagocytosed photoreceptor outer segments (e.g., retinoids and carotenoids), and the best-characterized fluorescent component of RPE LF is the Schiff base N-retinyl-N-retinylidene-ethanolamine (A2E), a degradation product of retinal photoreceptors [1]. The emission spectra of SW-AF are broad and maximal in the 600–640 nm region of the spectrum, shifting slightly toward longer wavelengths with increasing excitation wavelength. This "red shift" occurs for excitation at the long-wavelength end of the absorption spectrum for some fluorophores in viscous or polar environments [1]. This red shift may explain the variability of emission spectra reported in literature; with excitation wavelength ranging from 364 to 580 nm, the emission peak varies between 590 and 660 nm. The excitation spectra have their maxima between 490 and 530 nm. In contrast to the spectra measured in extra-foveal areas (usually 7° temporal to the fovea), the fovea spectra are attenuated. This is due to the lower amount of LF in the foveal area and to the absorption of the excitation light by macular pigments [1].

Other fluorophores may theoretically influence SW-AF, mainly in young patients because of the low amount of LF deposits in the RPE of these subjects [6, 7]. Therefore, secondary fluorophores emitting in the 500–540 nm spectral range are under investigation. The macular pigment itself, flavins, collagen, microglia, and hyaluronic acid in the vitreous have specific AF characteristics, similar to those described for LF, although weaker than LF itself. Finally, AF contributions from the choroid and sclera have been estimated in patients with geographic atrophy to be 6–15 % of the total fundus SW-AF, for dark- and light-pigmented subject respectively [1].

20.3.2 Near-Infrared AF

Near-infrared AF (NIR-AF), which uses the same excitation light and cutoff filters employed for indocyanine green angiography (ICG), was originally described as pseudo-fluorescence, commonly detected prior to standard ICG angiography [8].

Because different fluorophores are present within the retina and RPE, a specific selection of the wavelength for AF excitation (and the wavelength of the cutoff filter for emitting light) could provide additional possibilities (theoretically a wide range of possibilities) to analyze specific RPE substances [1, 3]. NIR-AF was first reported in 2006 and has been applied in several recently published studies on age-related

macular degeneration. As the intensity of emitted AF is about 60–100 times lower compared to that of SW-AF, imaging of NIR-AF is usually obtained using a confocal scanning laser ophthalmoscope [1]. Diode laser light (787 nm) is used to excite NIR-AF, whereas a band-pass filter with a cutoff at 800 nm is inserted in front of the detector. Because of the lower intensity, images are averaged to obtain the final image, and optimal imaging is obtained with dilated pupils.

Physiologic NIR-AF is characterized by the dark appearance of optic disc and retinal vessels [3]. The NIR-AF signal is maximal at the posterior pole, with a peak at the fovea and a marked decline toward the peripheral regions [1]. The major fluorophores contributing to NIR-AF are most likely melanin and its related compounds (melanolipofuscin (MLF), melanolysosomes, and oxidized melanin) [1, 3]. The notion that the NIR-AF signal derives from melanin is based on the following evidence: the NIR-AF distribution corresponds to that of RPE melanin; the severely reduced signal of NIR-AF in areas of RPE loss indicates that the major source of NIR-AF is the RPE; and the marked increase of NIR-AF in flat choroidal nevi [9]. Melanin AF is increased by oxidation. MLF (melanin with a cortex of LF) and melanolysosomes (melanin with a cortex of enzyme-reactive material) accumulate with age (and RPE disease) and represent melanin in the process of repair, modification, or degradation [9]. The AF of melanin, oxidized melanin, and compound granules containing melanin contributes to the NIR-AF signal. An increase in NIR-AF could be caused by melanogenesis, formation of melanolysosomes, or MLF or altered characteristics of melanin in disease process [1, 9]. The possible contribution of other fluorophores to the NIR-AF signal is under investigation [9]. Although the possibility that LF contributes to NIR-AF signal cannot be ruled out, this is unlikely because of the different AF distributions observed in normal subjects and in patients with a variety of retinal diseases. Possible fluorophores include collagen, elastin, and porphyrin [1, 9].

20.4 Choroidal Melanocytic Tumors

AF patterns in small, medium, and large choroidal melanomas share common characteristics, such as a homogeneous increased AF signal and a confluent plaque-like configuration [1]. Shields et al. have demonstrated, since 1976, that LF accumulates in RPE cells and macrophages overlying choroidal melanomas [10]. This phenomenon is clinically evident by biomicroscopy, described as "orange pigment" over the lesions. However, depending on whether the tumor is melanotic or amelanotic, the color may vary from orange to brown, respectively. Shields et al. also reported that the presence of orange pigment over small indeterminate choroidal pigmented lesions is a specific risk factor for malignant transformation [11, 12]. The exact mechanism by which choroidal melanoma induces lipofuscinogenesis remains unclear. Font et al. reported that sheets of proliferated RPE cells and clusters of pigment-laden macrophages are present under the degenerated neurosensory retina, corresponding to the position of the clinically observed orange pigment [12]. Histochemical analysis indicates that orange pigment of tumors stains positive for PAS, Sudan Black B, and long Ziehl-Neelsen; reduces silver salts with the Fontana-Masson method; bleaches partially with potassium permanganate; and is acid fast and oil red O positive in paraffin sections. It also exhibits a golden-yellow AF when examined using UV light or a fluorescence microscope, confirming that this pigment is in fact LF [1].

20.4.1 Choroidal Nevus

SW-AF imaging of choroidal nevus usually shows a normal pattern of background fundus AF, with no area of increased or decreased AF signal (Fig. 20.1). In a minority of cases, nevi may also reveal areas of mild decreased or increased SW-AF signal (Fig. 20.2) [1]. Drusen, commonly overlying choroidal nevi, when large or coalescent may appear as areas of localized increased SW-AF (Fig. 20.3).

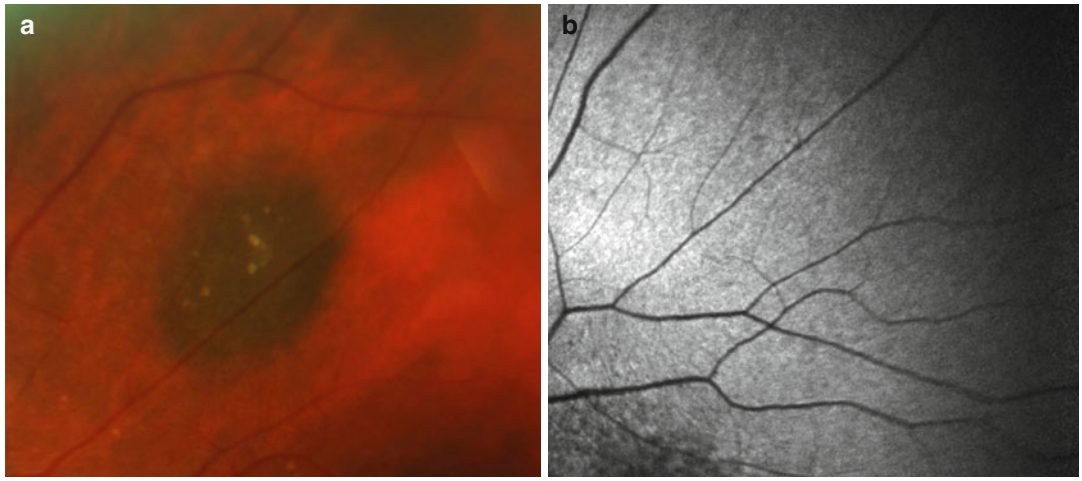

Fig. 20.1 Choroidal nevus (**a**, fundus appearance) showing normal pattern of background fundus SW-AF (**b**). Note absence of hyper- or hypofluorescence despite the presence of drusen over the lesion

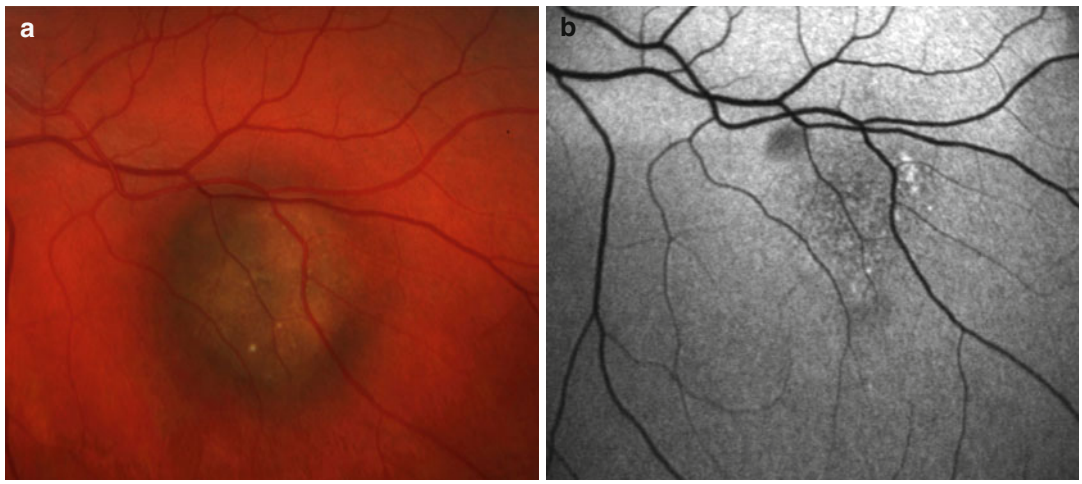

Fig. 20.2 Choroidal nevus (**a**, fundus appearance) showing mild hypo-AF with bright hyper-AF spots in SW-AF (**b**)

However, the increased AF signal over these areas is not a sign of LF accumulation, as it occurs in choroidal melanoma or small indeterminate choroidal pigmented lesions with risk factors for growth [1]. Recently, Shields et al. reported that choroidal nevi can show faint intrinsic AF [13]. Nevertheless, overlying RPE alterations (quite common in choroidal nevi) may show different AF pattern, ranging from normal (Fig. 20.1) to mild or dark hypo-AF due to RPE atrophy (Fig. 20.2) to bright hyper-AF (Fig. 20.3). Common causes of increased SW-AF in nevi include hyperpigmentation, drusen, and fibrous metaplasia (probably because they also cause LF accumulation in the overlying RPE).

We recently investigated SW-AF in 30 choroidal pigmented lesions, analyzing the role of this technique in the diagnosis and characterization of these lesions (unpublished data). In this study, AF imaging was performed using a confocal system (Heidelberg Retinal Angiograph, HRA 2; Heidelberg Engineering, Heidelberg, Germany). SW-AF images were recorded at 488 nm wavelength using a barrier filter for the detection of emitted light above 500 nm (SW-AF). At SW-AF,

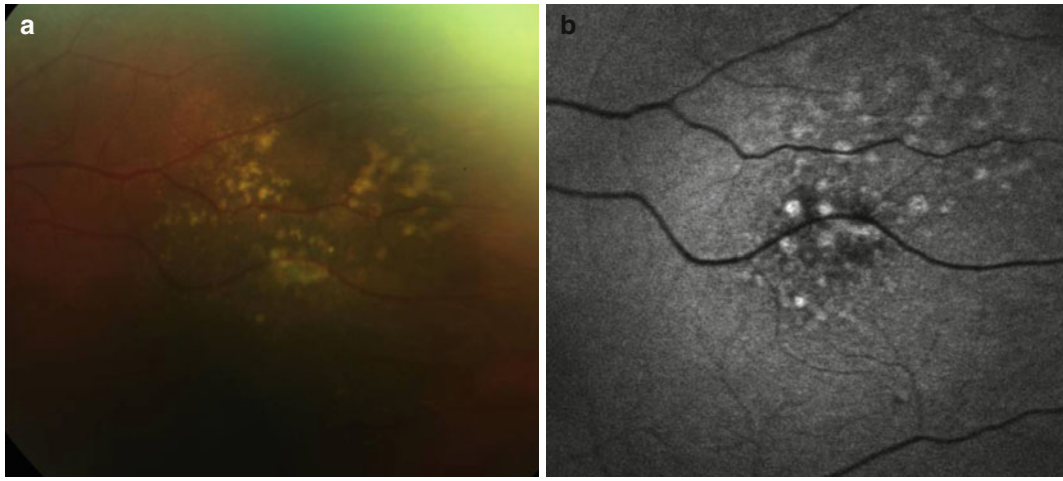

Fig. 20.3 Choroidal nevus (**a**, fundus appearance) showing mild hyper-AF with bright hyper-AF spots in SW-AF, mainly corresponding to overlying drusen (**b**)

choroidal nevi showed a normal pattern of background AF with no corresponding areas of hyperfluorescence or hypo-AF over the nevi in 50 % of the cases. In the remaining 50 % of cases, the lesion showed mild hypo-AF (in 25 %) or hyper-AF (in 25 %), with faint localized hyper-AF small areas in all cases. Nevi with normal background fundus AF were flat, while nevi with a different pattern of fundus fluorescence were elevated with drusen or LF.

20.4.2 Indeterminate Choroidal Melanocytic Tumor (Small Choroidal Melanoma)

Materin et al. investigated small choroidal melanomas by SW-AF, reporting that all tumors with orange pigment had an increased AF signal, although of different intensities, and the majority of lesions showed diffuse mild increased SW-AF [14]. Subretinal fluid associated with choroidal melanoma showed an increased SW-AF signal in 60 % of the cases.

Gunduz et al. recently reviewed the SW-AF characteristics of a variety of choroidal melanocytic lesions [15]. The AF patterns of these lesions were classified as patchy or diffuse. The patchy pattern was defined as the presence of distinct areas of increased AF between areas of normal AF. The diffuse pattern was characterized by the presence of increased AF with indistinct borders over a larger part (>50 %) of the lesion in the absence of such intervening areas. Choroidal melanomas presented with either a diffuse or patchy pattern, whereas choroidal nevi demonstrated only the patchy pattern (Fig. 20.4). The diffuse AF pattern was more often associated with larger choroidal melanomas, as well as with early venous and late hyperfluorescence on fluorescein angiography

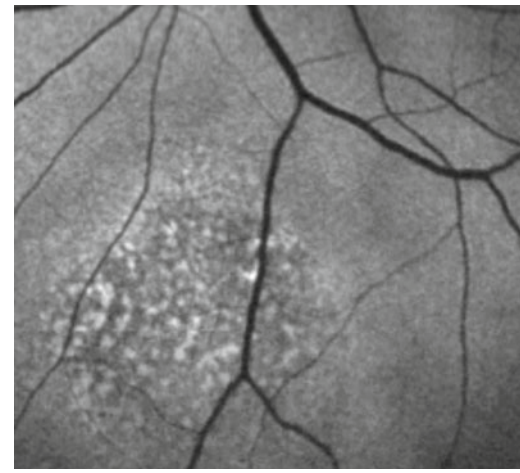

Fig. 20.4 Patchy SW-AF pattern in indeterminate choroidal melanocytic lesion. This pattern is more frequently observed among nevi

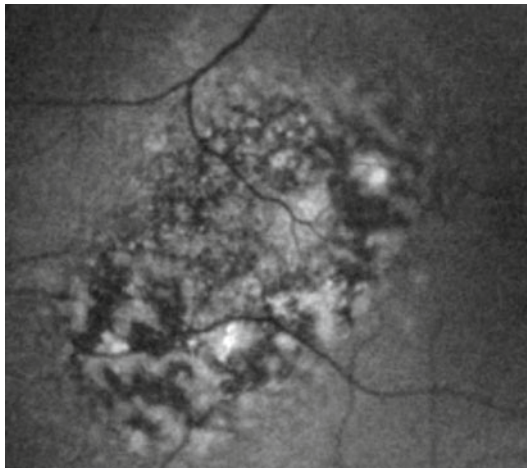

Fig. 20.5 Diffuse SW-AF pattern in an indeterminate choroidal melanocytic lesion. This pattern is more frequently observed in melanomas compared to nevi

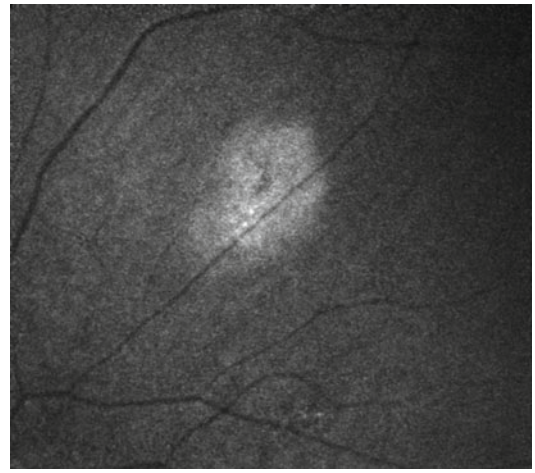

Fig. 20.6 NIR-AF of the same lesion reported in Fig. 20.1. Note the marked hyper-AF of this lesion in the NIR-AF despite the normal pattern of background fundus in SW-AF

(Fig. 20.5) [15]. Limitations of these observations depend on the field of depth of confocal scanning laser ophthalmoscopy; thus, AF from other planes could not be detected. Increased retinal thickness, intraretinal edema, or presence of subretinal fluid may also affect the AF signal. Nevertheless, the same authors reported a total or partial correlation between AF and the foci of LF and hyperpigmentation in about 90 % of the cases [15].

20.4.3 Choroidal Melanoma

Gunduz et al. reported that nearly 90 % of choroidal melanomas show at least one focus of increased SW-AF, corresponding to the location of LF or hyperpigmentation of the lesion [15]. Moreover, in 75 % of amelanotic choroidal melanoma, increased SW-AF was observed in areas of hyperpigmentation. Medium and large choroidal melanomas also appear to have increased SW-AF. Superficial fibrosis and subretinal fluid have been reported to have an increased SW-AF also in large tumors. However, in highly elevated tumors, artifacts related to the technique may limit the analysis [15].

It has been suggested that longer and deeper wavelengths, such as NIR, could provide more information about the pigment composition of choroidal melanocytic lesions [3]. With NIR-AF, the melanin distribution can be explored, as oxidized melanin or compounds closely associated with melanin. We recently investigated SW-AF and NIR-AF in 30 choroidal pigmented lesions, analyzing the role of these techniques in the diagnosis and characterization of these lesions (unpublished data). In this study, AF imaging was performed using a confocal system (Heidelberg Retinal Angiograph, HRA 2; Heidelberg Engineering, Heidelberg, Germany). The NIR-AF images were recorded at 780 nm with a barrier filter at 820 nm. Using NIR-AF, flat nevi appeared as bright hyper-AF areas (Fig. 20.6). Elevated nevi with mild hyper-AF or hypo-AF in SW-AF were hypo-AF in NIR-AF. In our study on NIR-AF vs. SW-AF in choroidal-pigmented lesions, at NIR-AF imaging, choroidal melanomas showed hypo- and hyper-AF areas in all cases, with a hyper-AF halo in 62 %, due to retinal detachment around the tumor. The hyper-AF areas at NIR-AF corresponded to the pigmented areas of the tumor. Comparing SW-AF and NIR-AF images of choroidal melanomas, we were unable to detect significant differences (Fig. 20.7).

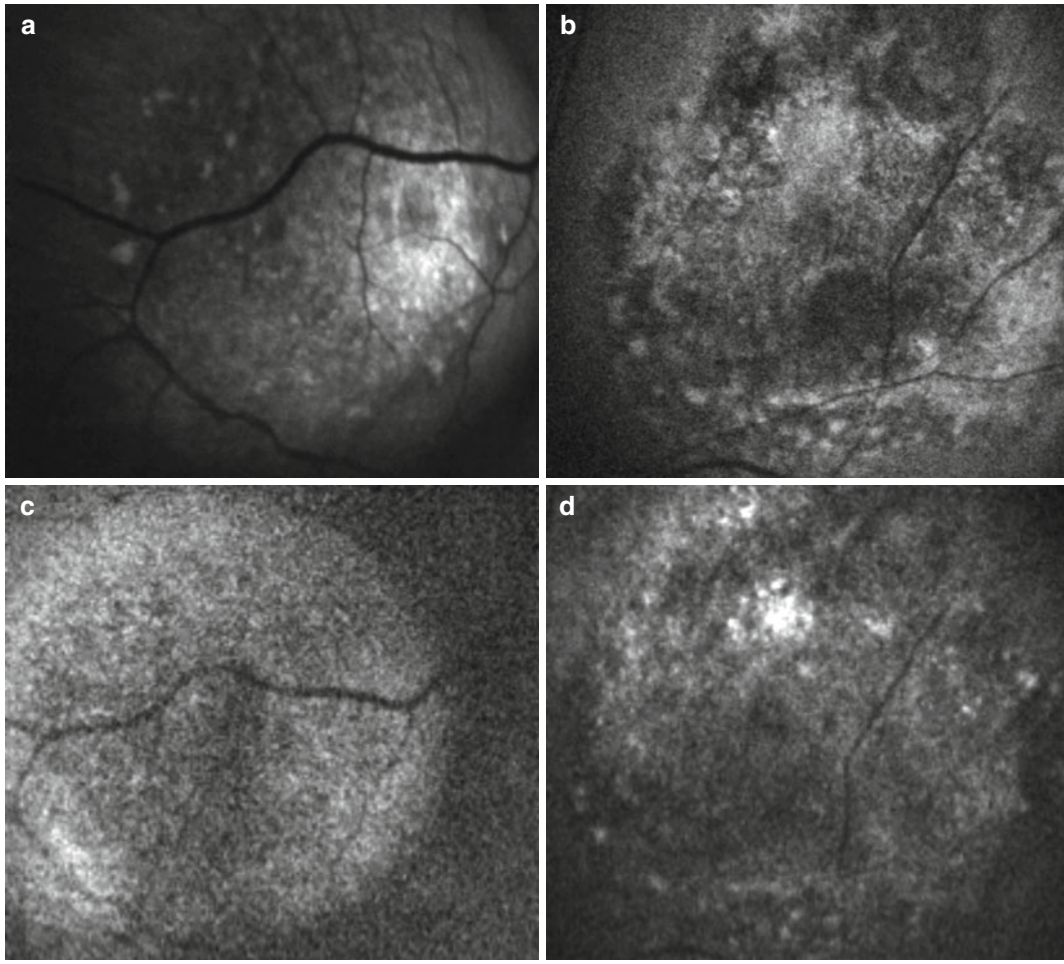

Fig. 20.7 SW-AF (**a**, **b**) and NIR-AF (**c**, **d**) of two choroidal melanomas. Comparing SW-AF and NIR-AF of these lesions, only small and nonsignificant differences in AF patterns are present

20.5 Choroidal Hemangioma

Shields et al. described the intrinsic SW-AF properties of choroidal hemangioma (CH) and the extrinsic findings of the overlying retinal/RPE alterations [16, 17]. They reported that both circumscribed and diffuse CH (untreated) showed general intrinsic iso-SW-AF or hypo-SW-AF (Fig. 20.8) [16, 17]. In contrast, treated choroidal hemangioma appeared more hypo-SW-AF. The intrinsic AF pattern did not correlate with tumor location, size, or thickness. The extrinsic SW-AF features of choroidal hemangioma were more dramatic. The most brilliant hyper-SW-AF was from overlying orange pigment notable in some eyes. Other extrinsic findings of RPE hyperplasia, RPE atrophy, and RPE fibrous metaplasia generally were seen as hypo-SW-AF in both circumscribed and diffuse CH. Retinal pigment epithelium atrophy (found in 25 % of untreated circumscribed CH and 87 % of treated circumscribed CH) appeared moderately to markedly hypo-AF in SW. Retinal pigment epithelium hyperplasia, commonly found in treated choroidal hemangioma, appeared moderately to markedly hypo-AF in SW. The intrinsic and extrinsic AF patterns were most often altered after therapy. Most tumors showed intrinsic hypo-AF after therapy and most showed extrinsic hypo-AF from RPE hyperplasia or atrophy. Moreover,

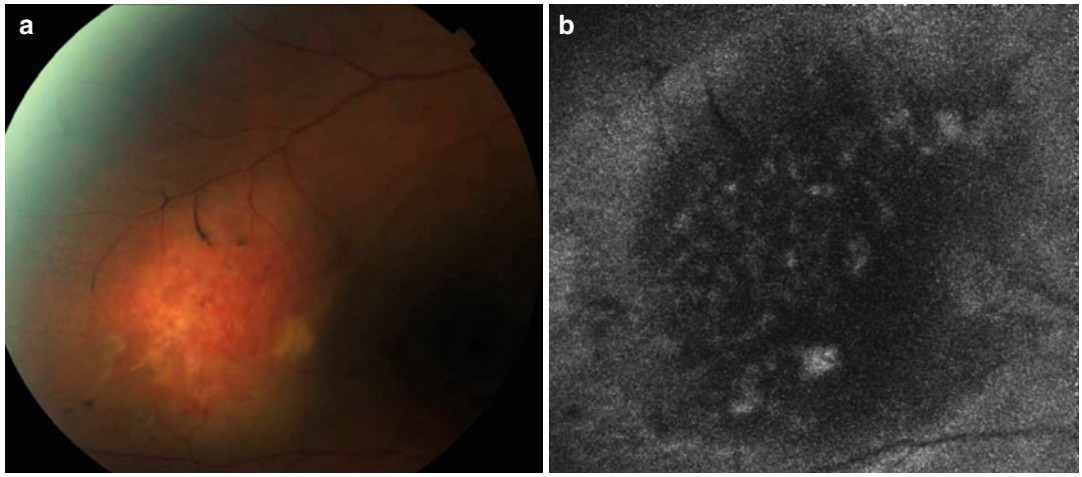

Fig. 20.8 Choroidal hemangioma. Fundus appearance (**a**). Hypo-AF area with hyper-AF spots in a case of untreated circumscribed choroidal hemangioma (SW-AF) (**b**)

subretinal fluid showed varying SW-AF patterns depending on the chronicity. Fresh subretinal fluid with intact RPE appeared moderately hyper-AF, whereas chronic subretinal fluid with RPE hyperplasia and atrophy showed moderate to marked hypo-AF in SW mode [16]. To the best of our knowledge, no data are actually published on NIR-AF in CH. In our cohort of patients, circumscribed or diffuse hemangioma shows a specific NIR-AF pattern, usually depending on RPE status overlying the lesions and commonly characterized by hypo-NIR-AF with bright hyper-NIR-AF spots.

20.6 Choroidal Metastasis

Limited data are reported on SW-AF of choroidal metastatic lesions. Collet et al. reported 15 metastatic tumors in 14 eyes of 11 consecutive patients concluding that amelanotic choroidal metastases with hyperpigmented foci are associated with increased SW-AF in most of the cases and that subretinal fluid overlying choroidal metastasis may cause increases in SW-AF as well [18]. Ishida et al. reported two cases of metastatic choroidal tumors, concluding that the AF pattern of these cases was approximately the reverse picture of fluorescein angiography pattern [19]. Natesh et al. investigated the correlation between SW-AF imaging and clinical, OCT, and fluorescein angiographic findings in ten choroidal metastases concluding that hyper-AF in SW-AF mode correlates with focal hyperpigmentation, subretinal fluid, and advancing tumor edges. Moreover, SW-AF appears to better define surface characteristics and tumor margins compared to standard biomicroscopy [20]. In our experience, SW-AF of choroidal metastasis is usually characterized by hypo-AF with hyper-AF spots and hyper-AF halo (Fig. 20.9). To the best of our knowledge, no data are actually published on NIR-AF in choroidal metastases. In our cohort of patients, NIR-AF of choroidal metastases is extremely variable. Therefore AF needs to be investigated in a larger series to be included in the diagnostic process.

20.7 Congenital Hypertrophy of the Retinal Pigment Epithelium

Shields et al. investigated with SW-AF 13 consecutive eyes with congenital hypertrophy of the retinal pigment epithelium (CHRPE) reporting that CHRPE showed hypo-AF in all cases [21]. Compared to the central portion of CHRPE, the margins showed iso-AF ($n=8$) or trace hyper-AF ($n=5$). There were eight lesions with intralesional lacunae, and this feature generally showed

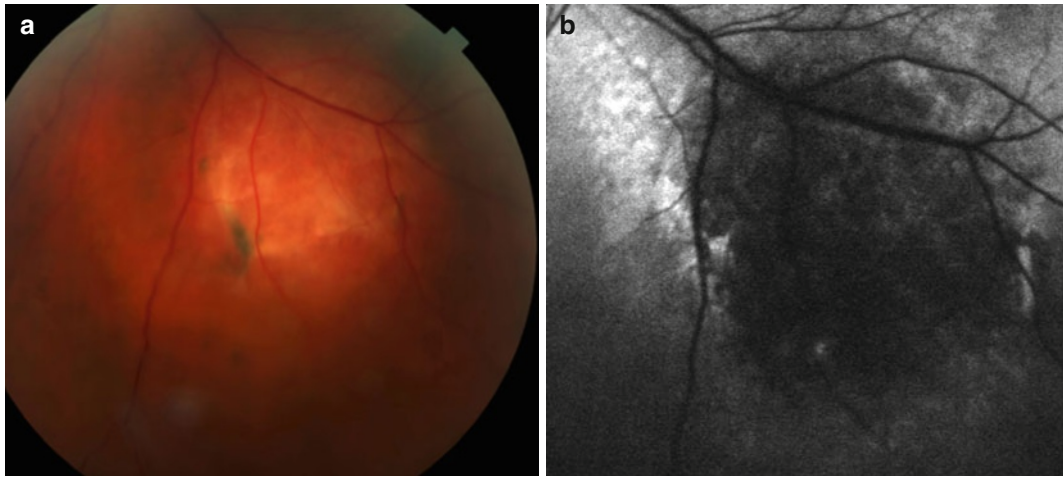

Fig. 20.9 Choroidal metastases. Fundus appearance (**a**). Hypo-AF area with hyper-AF spots and hyper-AF halo (SW-AF) in a case of choroidal metastases from lung adenocarcinoma (**b**)

trace to moderate hyper-AF ($n=6$). A nonpigmented halo was present around six lesions, showing trace hyper-AF ($n=3$). A pigmented halo surrounded eight lesions, usually showing iso-AF ($n=6$). The authors concluded that CHRPE shows striking hypo-AF, and this correlates with known histopathologic evidence of lack of LF in the retinal pigment epithelium of CHRPE.

Conclusions

AF is a noninvasive, valuable diagnostic tool for assessing intraocular tumors, mainly revealing the effects of these lesions on the overlying retinal pigment epithelium. SW-AF and NIR-AF may also provide specific information on intrinsic pigments that compose and characterize benign and malignant, congenital, and acquired chorioretinal lesions. LP, melanin, and MLF play the leader role in the different AF patterns of chorioretinal tumors, but other pigments (such as porphyrin) may influence AF imaging of these lesions. Some findings are strongly characteristic of specific lesions, mainly the bright hyper-AF overlying choroidal melanoma and the dark hypo-AF of congenital hypertrophy of the RPE. Moreover, SW-AF enhances the visualization of orange pigment located over small indeterminate choroidal-pigmented tumors, allowing better stratification of the risk of growth of these lesions. The use of AF may contribute to the diagnosis and characterization of choroidal tumors.

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Diagnostic Techniques: Ophthalmic Ultrasonography

21

Brandy Hayden Lorek, Mary E. Aronow, and Arun D. Singh

Contents

21.1 Introduction

Ophthalmic ultrasonography is essential for documentation, measurement, and differentiation of intraocular tumors. It is a safe, noninvasive diagnostic technique providing instant images for interpretation. This chapter provides a description of instrumentation and special examination techniques necessary for the documentation and measurement of intraocular tumors. Additionally, ultrasonographic findings of common intraocular lesions including iris and ciliary body melanoma, choroidal melanoma, choroidal hemangioma, metastatic and disciform lesions, and retinoblastoma are included.

21.2 Basic Physics

Ultrasound is as an acoustic wave with frequency above the audible range (20 kHz). When ultrasound waves encounter an interface with unequal acoustic impedance, echoes are generated. Modern ultrasound machines produce, detect, process, and amplify the returning echoes. A piezoelectric crystal is used to generate a mechanical short acoustic pulse that acts as a transducer to convert electric

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A.D. Singh, B. Damato (eds.), *Clinical Ophthalmic Oncology*,
DOI 10.1007/978-3-642-40489-4_21, © Springer-Verlag Berlin Heidelberg 2014

energy into ultrasound. When the probe receives the reflected echoes, they are converted back into an electrical signal and processed. Each ultrasound receiver is designed slightly differently, and thus, the images produced must be evaluated based on the model and calibration needed for each machine and probe combination. The parameters for processing usually include amplification, compensation, compression, demodulation, and rejection. A variety of amplification curves are preset on most ultrasound machines; however, it is essential that they are also adjusted manually by the examiner. The adjustment of this relative amplification is measured in decibels (dB) and referred to as the gain.

The physical laws of acoustic energy are dependent on reflection, refraction, and absorption [1]. The strength of echo returning to the probe is largely dependent on the angle of incidence. The sound beam with an angle of incidence of 90°, i.e., perpendicular to the structures being examined, is necessary to accurately assess these structures based on the intensity of the echoes. If the sound beam is misdirected in an oblique angle, the resulting signal is weaker than it should be due to the reflection of some of the sound beams away from the probe. Differences in the shape and size of the acoustic interface can also result in scattering of the sound beams. Additionally, irregular surface can lead to scattering of reflected echoes, resulting in significant loss of echo strength.

Ophthalmic ultrasound machines currently use frequencies ranging from 8–80 MHz. These frequencies are higher than those most commonly used in other fields of diagnostic ultrasound which are in the range of 2–6 MHz. In order to evaluate small ocular structures, a high-frequency device with a directly proportional high resolution is essential. The superficial location of the eye and its primarily aqueous composition allows the use of high frequencies [2]. The limitations of physics make it difficult to assemble array elements with the necessary half-wavelength spacing, therefore necessitating use of mechanical scanning by single-element focused transducers [3]. However, movement of the eye and positioning of the transducer allow the sound beam in ophthalmic devices to reach all areas of the eye in a close to optimal perpendicular orientation.

21.3 Instrumentation

21.3.1 Diagnostic A-Scan

A-scan is echo strength over time displayed in one dimension. Spikes in the vertical orientation correspond to echo intensity and are displayed along the horizontal axis as a function of time. Standardized A-scan was developed by Ossoinig as a special diagnostic instrument for differentiation of ocular structures [4, 5]. The A-scan probe has an operating frequency of 8 MHz and an S-shaped amplification curve for post processing [6]. The S-shaped curve is beneficial in the differentiation of tissues with acoustic impedances that are close to one another. It provides the benefit of the wide range of logarithmic amplification and the high sensitivity of linear amplification. Standardized A-scan has a standardized decibel setting, referred to as tissue sensitivity. At the tissue sensitivity setting, standardized A-scan is designed to display an echo spike for retina that is 100 % on the echo intensity scale when the sound beam is directed perpendicular to the retina (Fig. 21.1). All ocular structures that are equal to or more dense than retina will also produce 100 % echo spikes. Intraocular structures that are lower in density than retina including the diffuse vitreous hemorrhage and thin vitreous membranes will produce echoes of less than 100 % intensity. The varying reflectivity of the A-scan spike at tissue sensitivity also allows intraocular and orbital tumor structure to be evaluated and differentiated. In combination with B-scan, diagnostic A-scan is essential in the differentiation of intraocular tumors.

21.3.2 B-Scan

The foundation for diagnostic ultrasound in ophthalmology is based on B-scan images. Contact B-scan displays shape, location, and extension of structures by using horizontal and vertical orientation in two dimensions. The strength of the echo is determined by the brightness of dots on the screen representing each echo. Logarithmic or S-shaped amplification curves are most commonly used at a frequency range of 10 MHz [7].

The two-dimensional, high-resolution images provide accurate representation of ocular structures [8, 9]. Three basic B-scan probe orientations are used in ophthalmic ultrasonography:

axial, longitudinal, and transverse (Figs. 21.2, 21.3, and 21.4) [10]. B-scan evaluation is a dynamic process requiring sweeping motions of the probe and specific attention to the mobility of echoes. The evaluation of static B-scan images in isolation can lead to misinterpretation [10].

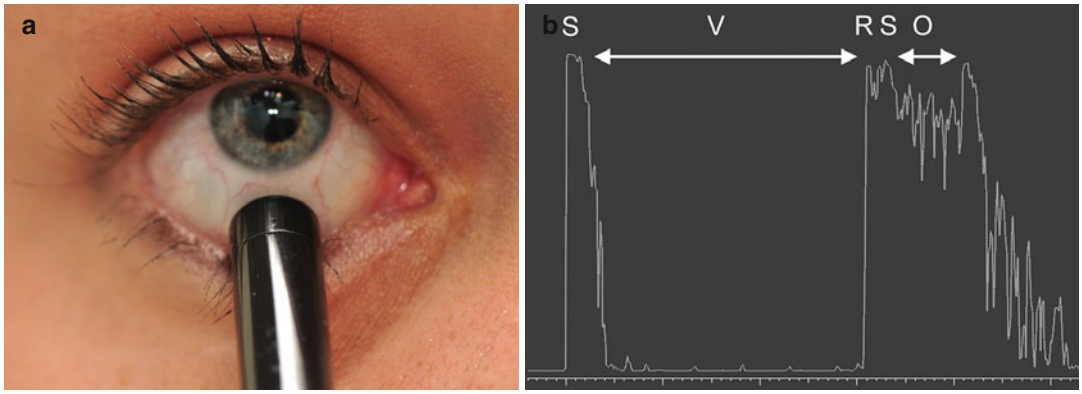

Fig. 21.1 Diagnostic A-scan. A-scan probe tip placed near the limbus with a sound beam directed at 12 o'clock posterior to the equator (**a**). Resulting A-scan image shows a linear display with echo spikes representing each tissue interface (**b**, *V* vitreous, *R* retina, *S* sclera, *O* orbital tissue) (Reproduced with permission from Hayden and Singh [31])

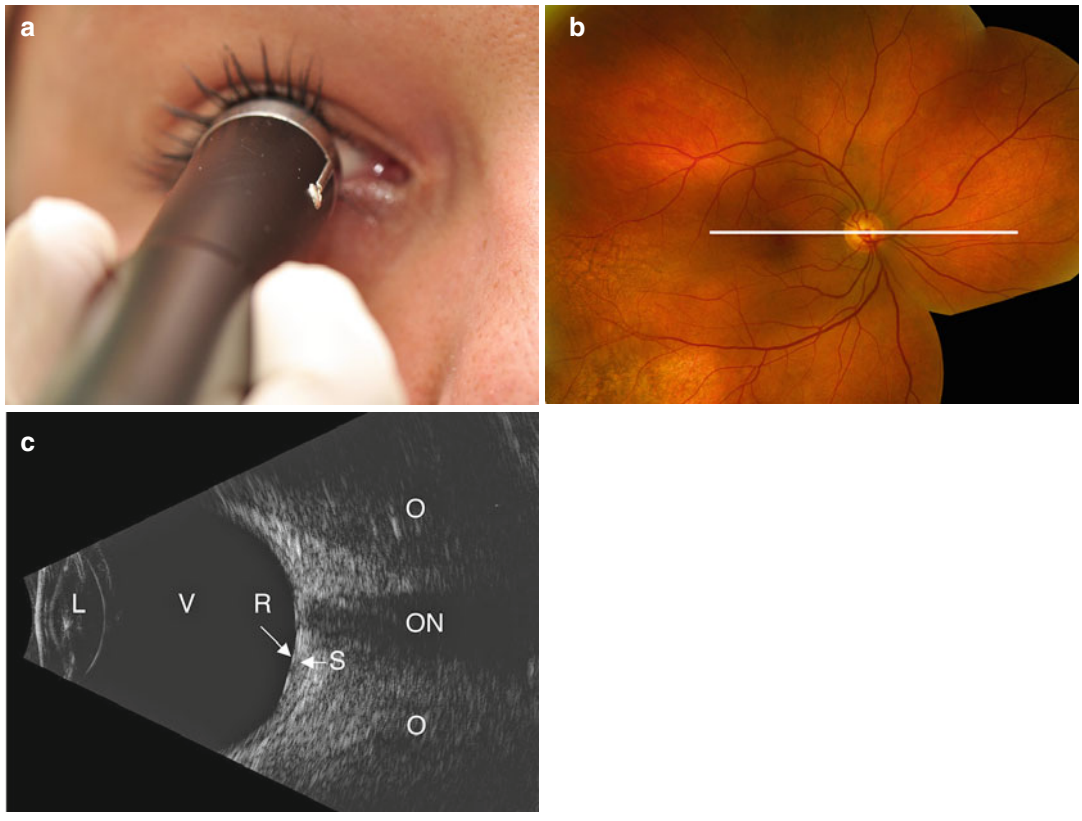

Fig. 21.2 Axial probe position. B-scan probe tip placed directly over the cornea (**a**). Corresponding ultrasonographed fundus (**b**, *line*). Resulting B-scan image showing the centered crystalline lens and optic nerve (**c**, *L* lens, *V* vitreous, *R* retina, *S* sclera, *ON* optic nerve, *O* orbital tissue) (Reproduced with permission from Hayden et al. [32])

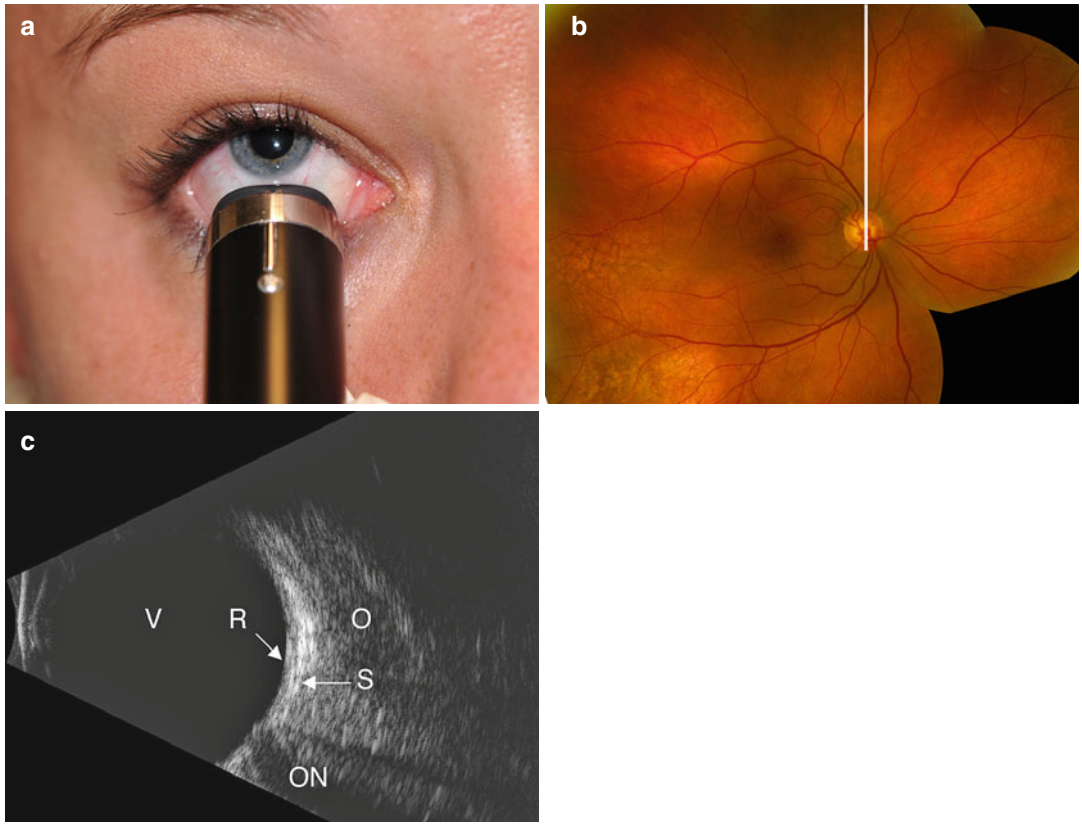

Fig. 21.3 Longitudinal B-scan position. B-scan probe tip placed on the conjunctiva near the limbus with the marker pointed superiorly (**a**). Corresponding ultrasonographed fundus (**b**, *line*). Resulting B-scan image showing a radial plane superiorly (**c**, *V* vitreous, *R* retina, *S* sclera, *ON* optic nerve, *O* orbital tissue) (Reproduced with permission from Hayden et al. [32])

21.3.3 Ultrasound Biomicroscopy (UBM)

UBM utilizes frequencies from 35–80 MHz for the acoustic evaluation of anterior segment of the eye [17]. UBM can be of paramount importance when examining an eye with a lesion that is too far anterior to be imaged with trans-scleral B-scan. Lesions of the conjunctiva, iris, ciliary body, and pars plana regions are best imaged with UBM. A typical high-resolution 50 MHz UBM probe has a penetration depth of approximately 5.0 mm and a resolution of 37 μm. During a UBM examination, the probe is placed directly over the anterior ocular structures to be imaged, not transocularly as in contact B-scan. Additionally, a fluid immersion technique is necessary to provide the standoff distance required to view the anterior structures (Fig. 21.5). Similar to B-scans, axial, radial (longitudinal), and transverse scans are used to best document anterior lesions. The radial scan is best suited to access the anterior to posterior extent of mass lesions of the iris and ciliary body (Fig. 21.6).

21.4 Methods for Differentiation of Intraocular Tumors

Standardized echography refers to the combined use of contact B-scan and standardized A-scan. It provides a reliable method to evaluate ocular lesions based on the topographic, quantitative, and kinetic properties of the echo intensities and patterns [5, 11–13]. Topographic, quantitative,

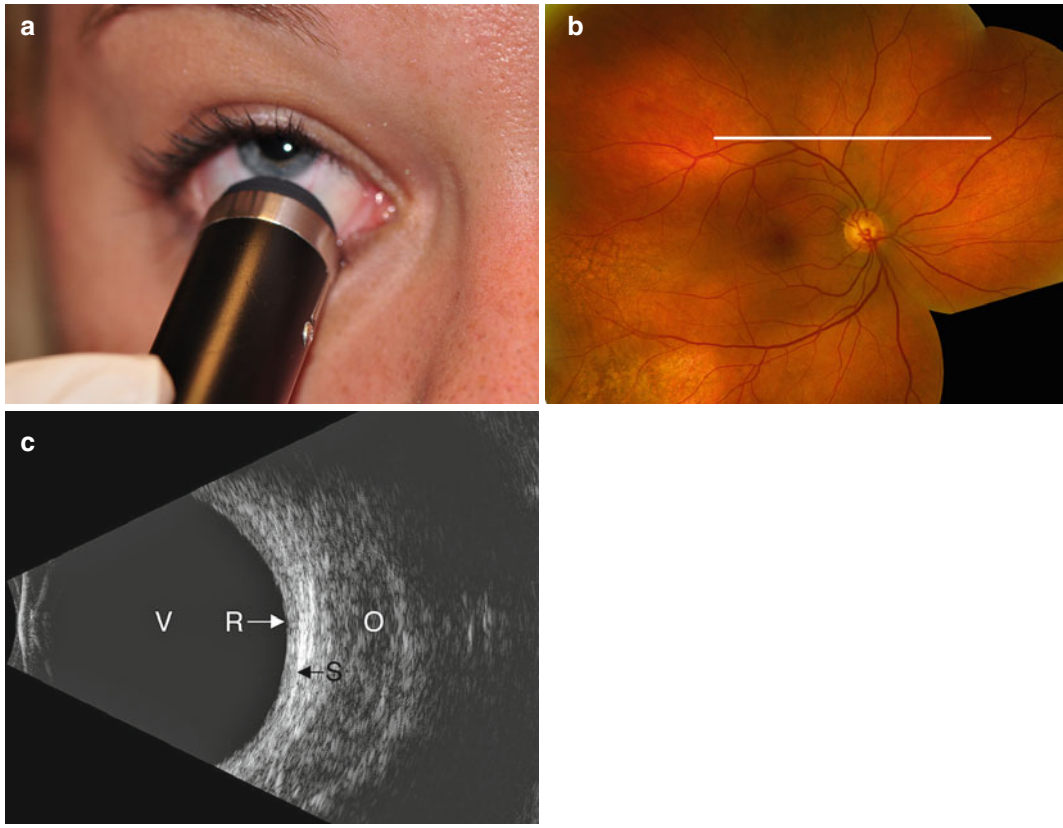

Fig. 21.4 Transverse B-scan position. B-scan probe tip placed on the conjunctiva near the limbus (**a**). Corresponding ultrasonographed fundus (**b**, *line*). Resulting B-scan image showing the superior quadrant (**c**, *V* vitreous, *R* retina, *S* sclera, *O* orbital tissue) (Reproduced with permission from Hayden et al. [32])

and kinetic features of many intraocular tumors are well established in the literature [14–16]. B-scan and UBM examination yield topographic information including shape, size, and location as well as vascularity, a kinetic feature. Diagnostic A-scan examination yields quantitative information including structure and reflectivity and in some instances vascularity. Utilizing these combined techniques (standardized echography), malignant intraocular tumors can be differentiated from benign lesions whose appearance on visual examination may be similar (Table 21.1).

21.4.1 Topographic Ultrasonography

Topographic evaluation includes determination of location, shape, and extension of the lesion. A transverse B-scan of the lesion should be performed first to display the lateral extent of the lesion and the gross shape. A sweeping motion through the solid intraocular mass is important in order to determine the maximal height and lateral basal dimensions (Fig. 21.7) [14, 15]. A longitudinal B-scan is then performed to evaluate the anterior to posterior topographic features of the lesion. This scan displays the shape and anterior to posterior extent of the lesion. The probe does not need to be shifted dramatically as required during the transverse scan. However, subtle shifts around the clock hour of interest will aid in the identification of the maximal dimensions of a solid intraocular lesion. Axial scans are not usually necessary in the evaluation of topographic features. However, in very posterior lesions, the relationship to the optic nerve and the maximal height can be assessed with axial scans.

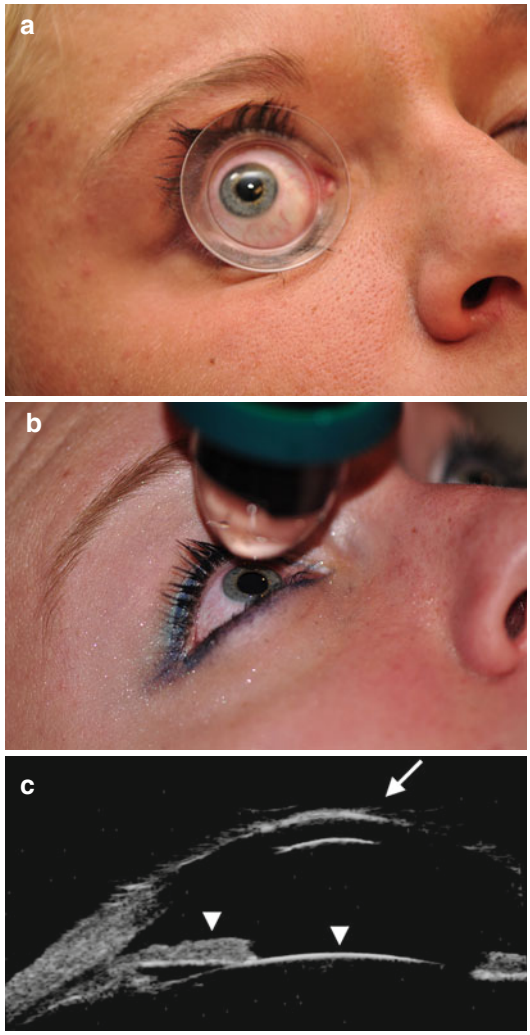

Fig. 21.5 UBM. UBM immersion shell designed to hold the eyelids open and provide a reservoir for the coupling agent (**a**). ClearScan® acoustically invisible film that can be filled with water and placed over the probe tip is placed directly over the anterior ocular structures to be imaged (**b**). Resulting B-scan image showing the cornea at the *top of the screen*, closet to the probe tip (*arrow*) and the anterior portion of the crystalline lens and iris are at the *bottom of the screen* (*arrowheads*, **c**) (**b**, **c**, Reproduced with permission from Quantel Medical, Bozeman, MT, USA)

21.4.2 Quantitative Ultrasonography

Quantitative ultrasonography utilizing diagnostic A-scan can determine reflectivity, internal structure, and sound attenuation.

21.4.2.1 Reflectivity

The height of the spike on A-scan corresponds to grades of reflectivity. Reflectivity of a lesion can only be graded when both the A-scan probe is calibrated for tissue sensitivity and the sound beam is directed perpendicular to the lesion (Fig. 21.8). Evaluating the reflectivity of a lesion from the contact B-scan signal intensity echoes is only an estimate of reflectivity and should not be relied upon.

21.4.2.2 Internal Structure

Quantitative ultrasonography can further assess the internal structure that reflects the histologic composition [18]. Homogenous tissue architecture within the lesion results in little variation in the height and length of the spikes on A-scan, whereas a heterogeneous tissue architecture results in marked variation.

21.4.2.3 Sound Attenuation

The last component of quantitative ultrasonography is sound attenuation or acoustic shadowing. It can be caused by many different echo-dense structures including calcified lesions, foreign bodies, and bone (Fig. 21.9). Sound attenuation from or within a lesion can be detected on both contact B-scan and diagnostic A-scan. It is delineated by a marked, progressive decrease in the strength of the echoes within or behind the lesion. On B-scan, this results in reduction of the intensity of echoes or complete lack of echoes behind the lesion termed shadowing. On A-scan, a linear decrease in the height of the spikes is evaluated as an angular measurement (angle kappa) [5]. Angle kappa is directly proportional to the amount of sound attenuation; the greater the attenuation of sound, the greater the angle kappa. Large choroidal melanomas often present with a positive angle kappa (Fig. 21.10).

21.4.3 Kinetic Ultrasonography

Ocular lesions can be further defined in near real time by dynamically observing and documenting mobility, vascularity, and convection movement.

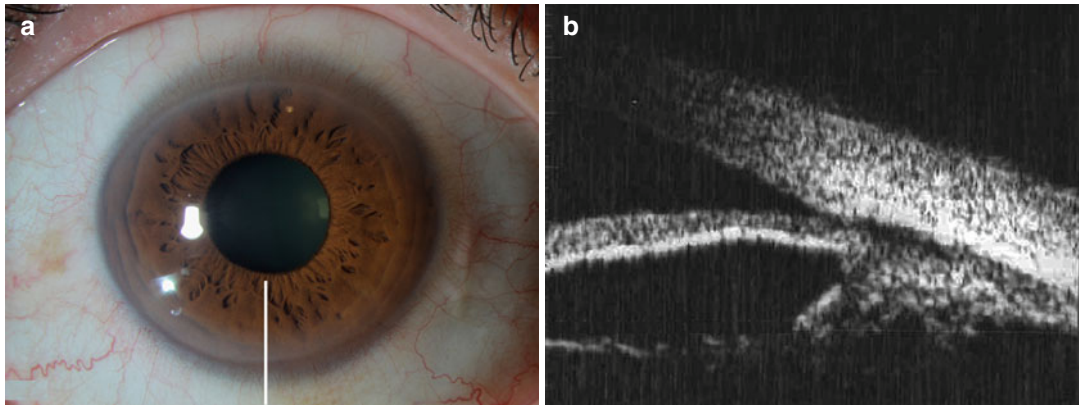

Fig. 21.6 Radial (longitudinal) scan. Slit lamp photography of the anterior segment of the eye showing the plane of a radial scan (**a**). Corresponding UBM image showing the cornea and the central iris on the left side of the display and the limbus, ciliary body, and sclera on the right (**b**) (Reproduced with permission from Hayden and Singh [33])

21.4.3.1 Mobility

The mobility of a lesion is most easily evaluated with B-scan. It is determined by after movement of a membrane or opacity following a change in gaze. Depending on the location of the intraocular lesion, either a transverse or a longitudinal B-scan is performed, and the patient is instructed to move their eyes. Truly solid intraocular lesions will not exhibit any subretinal or subchoroidal movement. Assessing the mobility of presumed solid intraocular lesions is often essential to differentiate choroidal melanoma from hemorrhagic choroidal detachment.

21.4.3.2 Vascularity

Vascularity is the detection of blood flow within an ocular lesion and can be detected by both A-scan and B-scan. It is defined as fast, low amplitude flickering consistent with blood flow. The probe and gaze are held stationary, while the internal echo flickering is observed over time. The intensity of the echo flickering is documented as mild, moderate, or marked corresponding to grades of vascularity.

21.4.3.3 Convection

Convection movement is the slow, continuous movement of intraocular contents that occurs secondary to convection currents best detected with B-scan while holding both the probe and the eye fixed. The moving echoes most often represent blood, layered inflammatory cells, or cholesterol debris. This slow movement is most often seen in eyes with long-standing vitreous hemorrhage that has settled beneath a tight funnel-shaped retinal detachment. Solid intraocular mass lesions can be differentiated from layered, debris with specific attention to this minor movement.

Table 21.1 Diagnostic features for assessing intraocular lesions

Topographic	Quantitative	Kinetic
Location	Reflectivity	Mobility
Shape	Internal structure	Vascularity
Extent	Sound attenuation	Convection movement

21.5 Differentiation of Anterior Segment Tumors

21.5.1 Iridociliary Pigment Epithelial Cyst (IPE Cyst)

IPE cysts frequently appear as anterior bulging of the iris stroma, most commonly near the iris root [19]. On UBM, the lesions are round to oval in shape and bow the iris forward while conforming to the structures of the posterior segment. IPE cysts demonstrate a thin wall with echolucent

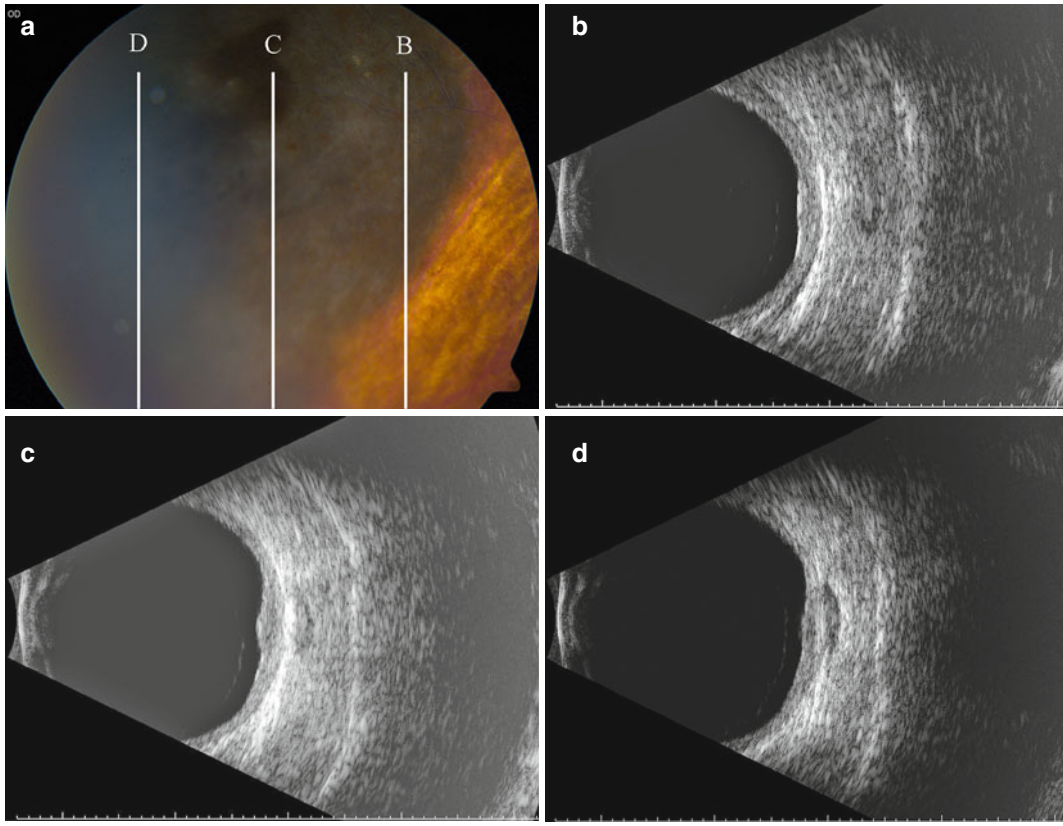

Fig. 21.7 Topographic ultrasonography. Transverse B-scan of a solid intraocular mass at 8:30 posterior to the equator. Fundus photograph (**a**). *Lines* (*B*, *C*, and *D*) represent ultrasonography of fundus as shown (**b**, **c**, **d**). Resulting B-scan image shows a *dome-shaped* elevated fundus lesion (**b**). Slight shifting of the probe anteriorly centers the sound beam on the thickest portion of the mass lesion (**c**). Continued shifting of the probe assesses the peripheral aspect of the mass lesion (**d**) (Reproduced with permission from Hayden and Singh [31])

internal reflectivity, differentiating them from mass lesions of the iris (Fig. 21.11). Eyes presenting with a single large cyst are frequently found to have additional small cysts, when other quadrants are explored. It is also important to note that IPE cysts may be present adjacent to solid lesions of the iris and ciliary body. Thus it is essential to thoroughly examine all surrounding structures.

21.5.2 Iris and Ciliary Body Melanoma

Melanomas of the iris and ciliary body can vary in pigmentation, size, and patterns of growth. They can present as circumscribed or diffuse lesions, and iris melanoma has a predilection for the inferior half of the iris (Fig. 21.12). Due to their anterior location, UBM is the preferred modality of ultrasonographic examination. Circumscribed lesions on UBM usually appear as dome shaped or lobulated. Diffuse iris and ciliary body lesions are irregular in shape and can span from a few clock hours to 360° [20, 21]. Careful examination with UBM of all tumor margins including the ciliary body is necessary to rule out ring patterns and ciliary body involvement not readily seen clinically. Likewise, the posterior borders of a ciliary body melanoma should be examined to rule out further posterior extension (Fig. 21.13). When the posterior boundary of the ciliary body lesion cannot be imaged with UBM, a posterior B-scan examination should be performed. Both circumscribed and diffuse lesions are mainly

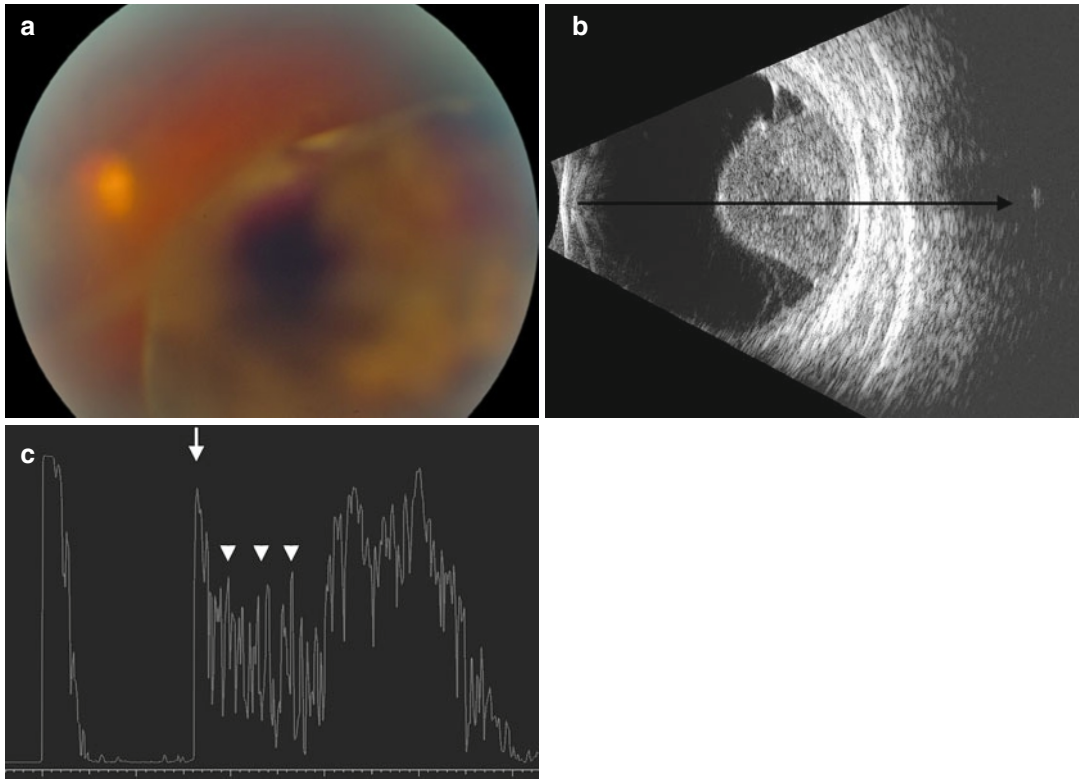

Fig. 21.8 Quantitative ultrasonography (reflectivity). Fundus photo showing choroidal melanoma with vitreous hemorrhage (**a**). Corresponding B-scan (**b**). *Arrow* represents the path of sound beam used to generate the A-scan. Diagnostic A-scan of the solid fundus mass shows high reflectivity of the retina (**c**, *arrow*) and low to medium internal reflectivity of the mass lesion (**c**, *arrowheads*) (Reproduced with permission from Hayden and Singh [31])

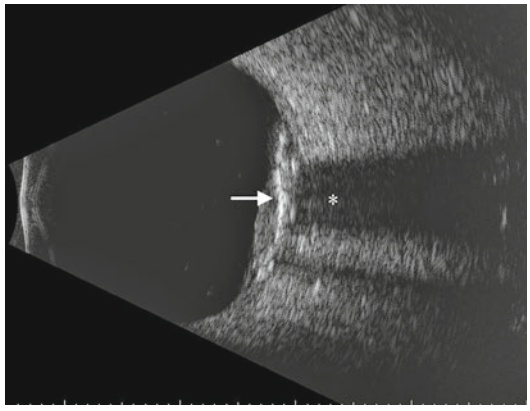

Fig. 21.9 Quantitative ultrasonography (sound attenuation, B-scan). Transverse B-scan shows an irregularly shaped fundus lesion with highly reflective areas (*arrow*) causing shadowing of the orbital structures (*asterisk*) (Reproduced with permission from Hayden and Singh [31])

regular in structure and echo dense with no sound attenuation. Iris and ciliary body melanomas are usually too shallow to be evaluated with diagnostic A-scan or assessed for vascularity.

21.5.3 Ciliary Body Melanocytoma

Uveal melanocytoma is a benign, darkly pigmented, circumscribed mass with minimal elevation involving the uveal tract or optic disc. When present in the ciliary body, these lesions can be confused with melanomas. On both UBM and B-scan, these lesions appear as a dome-shaped solid mass with regular structure, very echo-dense reflectivity, and marked sound attenuation (Fig. 21.14). The tightly packed, heavily pigmented structure of an iridociliary

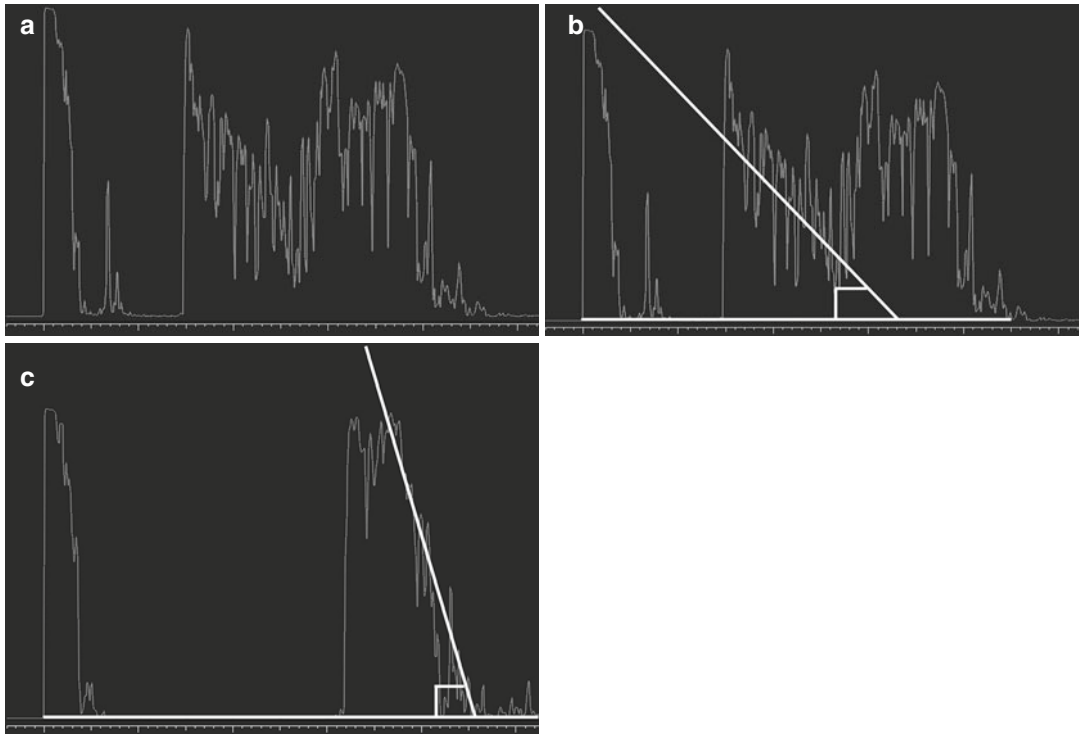

Fig. 21.10 Quantitative ultrasonography (sound attenuation, A-scan, angle Kappa). Progressive decrease in the height of the spikes can be depicted as an angular measurement (**a**, **b**). Angle kappa is proportional to the extent of sound attenuation; the greater the attenuation of sound, the larger the angle kappa (**b**, **c**) (Reproduced with permission from Hayden and Singh [31])

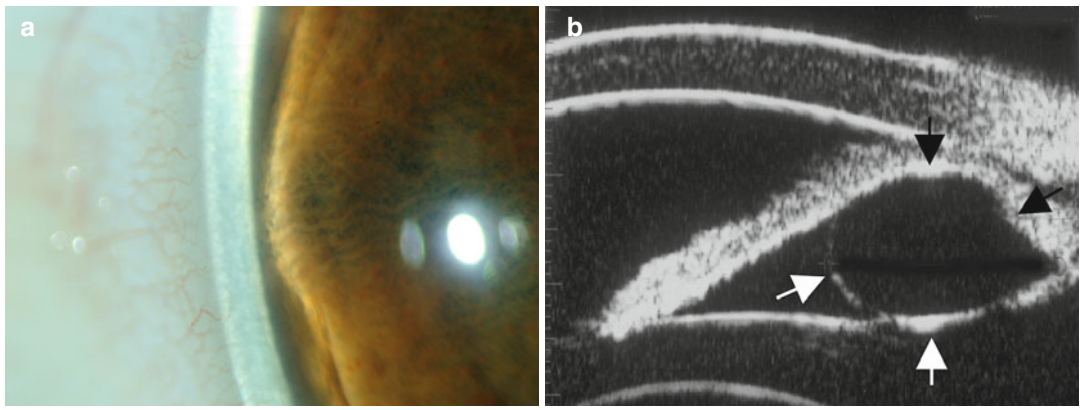

Fig. 21.11 Iridociliary pigment epithelial cyst. Anterior segment photograph with a slit beam (**a**). Note anterior bulging of the iris. UBM revealed echolucent thin-walled cyst (**b**, *arrows*) (Reproduced with permission from Rockwood et al. [34])

melanocytoma is responsible for the sound attenuation often leading to shadowing of the internal portions of the lesion on UBM. This finding is rare in UBM evaluation of melanomas and therefore may be helpful in diagnosis.

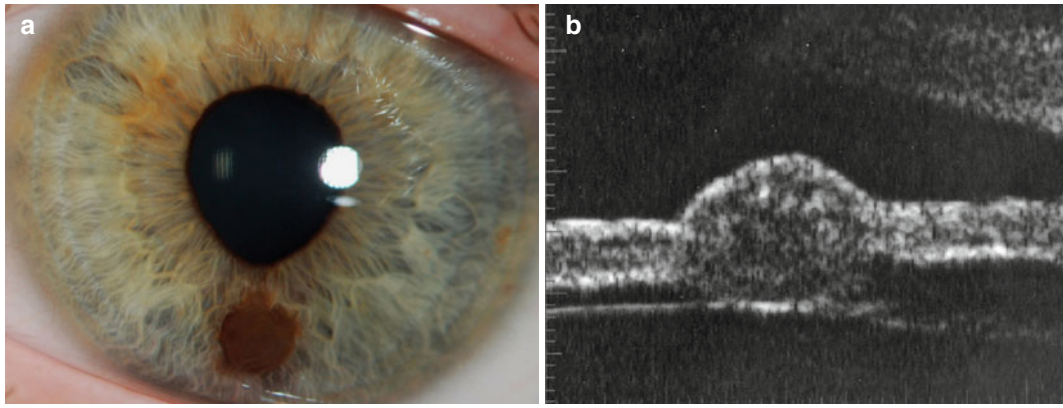

Fig. 21.12 Iris melanoma. Clinical photograph demonstrating pigmented iris lesion (**a**). UBM revealed isolated iris involvement (**b**) (Reproduced with permission from Hood et al. [35])

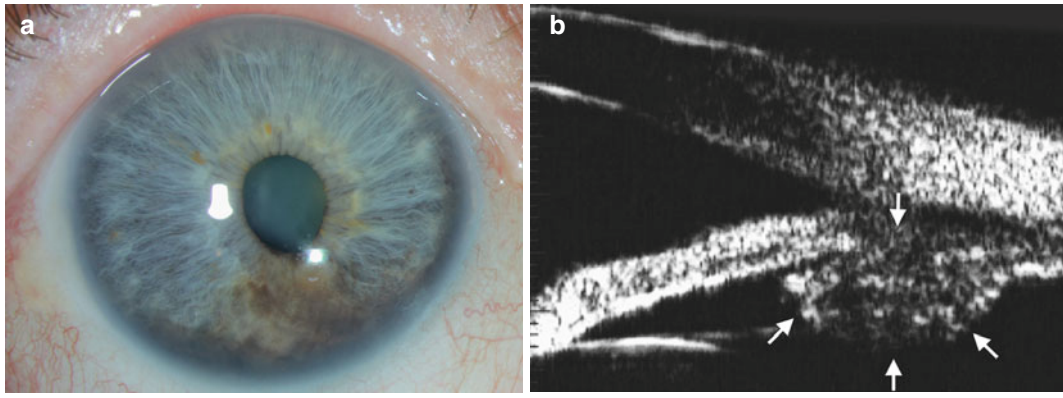

Fig. 21.13 Radial (longitudinal) scan for the evaluation of a mass lesion of the peripheral iris and ciliary body. Slit lamp photograph showing iris melanoma (**a**). UBM inferiorly reveals a mass in the peripheral iris and ciliary body (**b**) (Reproduced with permission from Hayden and Singh [33])

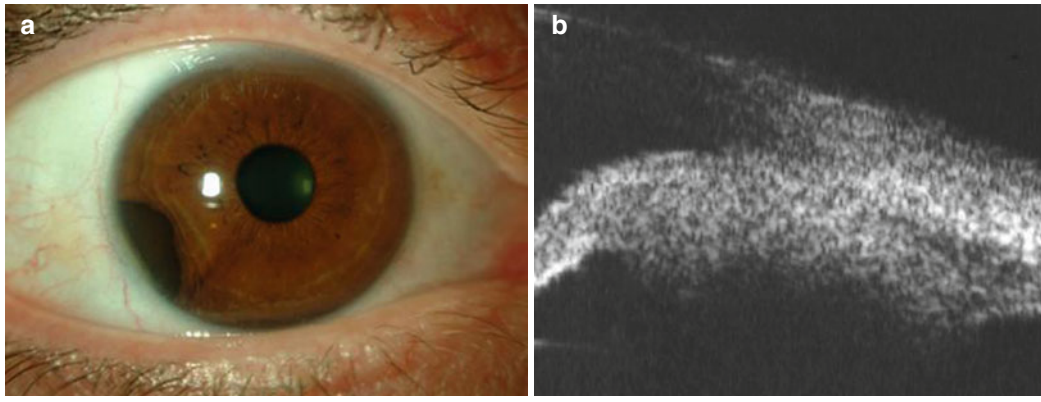

Fig. 21.14 Ciliary body melanocytoma. Slit lamp photograph demonstrating pigmented peripheral iris mass (**a**). UBM showing a dense ciliary body mass with sound attenuation (**b**) (Reproduced with permission from Turell et al. [36])

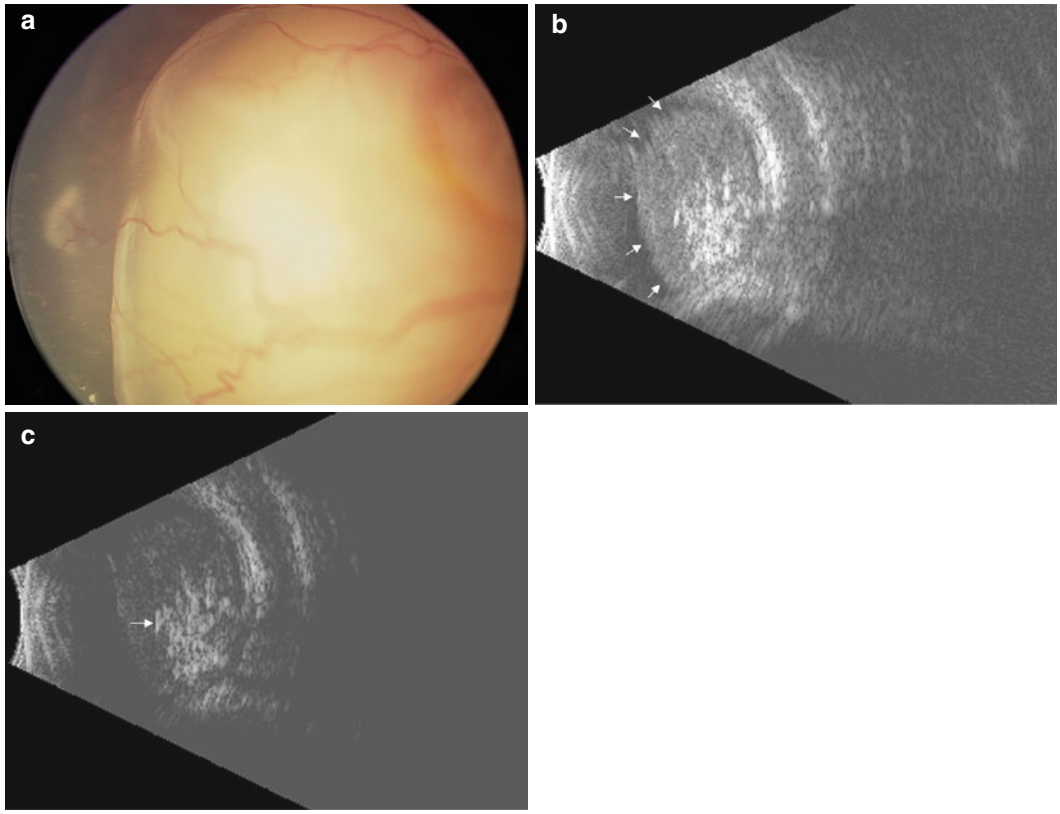

Fig. 21.15 Classic presentation of retinoblastoma. Retinoblastoma with calcification. Fundus photograph (**a**). Transverse B-scans demonstrates a large, dome-shaped lesion with marked internal calcification. High gain showing the lesional boundaries (**b**, *arrows*). Low gain showing the internal calcification causing shadowing of the orbit (**c**, *arrow*) (Reproduced with permission from Fu et al. [37])

21.6 Differentiation of Retinal Tumors

21.6.1 Retinoblastoma

21.6.1.1 Topographic

Ophthalmic ultrasonography plays an integral role in the differentiation of retinoblastoma from other causes of leukocoria, particularly in the presence of an extensive retinal detachment. Retinoblastoma can be unilateral or bilateral and varies somewhat in appearance based on the stage at presentation. On B-scan examination, retinoblastoma appears most commonly as an irregularly surfaced, round or dome-shaped lesion. Mildly elevated and diffuse lesions have been reported in the literature but are rare [22, 23]. Most retinoblastomas exhibit marked internal calcification that is readily detected with B-scan if examined at a low to medium gain. At these gain settings, internal calcification of the lesion remains very highly reflective and causes marked shadowing of the intraocular and orbital structures behind the lesion (Fig. 21.15). B-scan may also reveal associated retinal detachment and vitreous opacities frequently indicative of vitreous seeding. Extraocular extension of retinoblastoma most often involves the retrobulbar optic nerve. B-scan ultrasonography is helpful in the gross determination of enlargement of the nerve; however, shadowing from calcification often limits its diagnostic use.

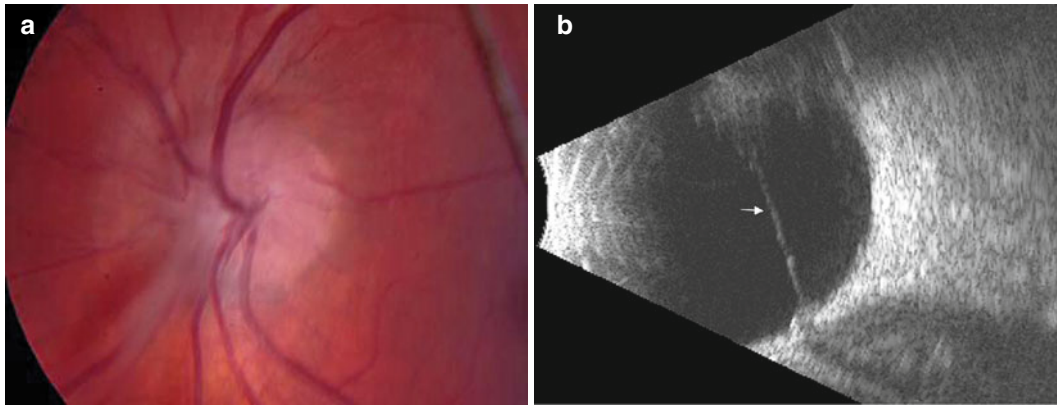

Fig. 21.16 Persistent fetal vasculature (PFV). Fundus photograph (**a**). Longitudinal B-scan demonstrates taunt, thickened vitreous band adherent to the slightly elevated optic disc (**b**, *arrow*) (Reproduced with permission from Fu et al. [37])

21.6.1.2 Quantitative and Kinetic

The structure and reflectivity profiles in retinoblastoma vary greatly depending upon the degree of internal calcification. Lesions with little or no calcification have a regular structure and low to medium reflectivity, whereas highly calcified lesions are irregularly structured with mainly medium to high reflectivity with marked acoustic shadowing. Due to the lack of intrinsic vessel formation within retinoblastoma lesions, acoustic vascularity is absent.

21.6.2 Persistent Fetal Vasculature (PFV)

PFV, formally known as persistent hyperplastic primary vitreous (PHPV), is a unilateral condition that is usually associated with microphthalmos or shallow anterior chamber. On B-scan a taut, nonmobile vitreous band, persistent hyaloid remnant, is detected connecting the optic nerve to the posterior lens capsule (Fig. 21.16). Often, the vitreous band is very thin, and it cannot be imaged in entirety. In some cases, the band is very thick and resembles a closed total retinal detachment. For these reasons, it is necessary to examine the eye at both a high gain to delineate the thin membranes and a low gain to rule out retinal detachment. On diagnostic A-scan, the band will exhibit an initial spike that is not 100 % of the tissue sensitivity, further excluding retinal detachment.

21.6.3 Retinopathy or Prematurity (ROP)

ROP occurs in a setting of low birth weight and exposure to supplemental oxygen in the neonatal period due to premature birth. In contrast to eyes with retinoblastoma, which are usually of normal axial eye length and unilateral or bilateral, eyes with ROP have shorter axial length than normal and presentation is typically bilateral. On B-scan, ROP appears as a highly reflective, nonmobile, thick, closed funnel-shaped retinal detachment inserting into the disc (Fig. 21.17). Diagnostic A-scan shows an initial spike that is 100 % of the tissue sensitivity confirming retinal detachment.

21.6.4 Coats' Disease

Coats' disease is a retinal vascular disorder that is usually unilateral and most commonly diagnosed in young males between 4 and 10 years of age [24]. On B-scan, Coats' disease usually appears as a poorly defined fundus lesion with focal areas of high reflectivity (Fig. 21.18). In advanced cases, total retinal detachment is also observed. Diagnostic A-scan evaluation most commonly shows a solid mass with regular to slightly irregular structure, mainly high reflectivity, and absent internal vascularity. Ultrasonography is often most helpful in the differentiation of Coats' disease from retinoblastoma. On B-scan

examination at a low gain, highly reflective calcium deposits (retinoblastoma) display shadowing, whereas medium to highly reflective cholesterol deposits (Coats' disease) do not exhibit acoustic shadowing.

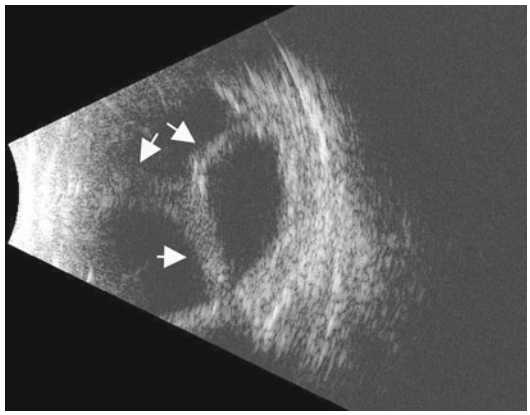

Fig. 21.17 ROP (retinopathy of prematurity). Retinopathy of prematurity. Longitudinal B-scan demonstrates a highly reflective, closed funnel-shaped retinal detachment (*arrows*) inserting into the disc (Reproduced with permission from Fu et al. [37])

21.7 Differentiation of Choroidal Tumors

21.7.1 Choroidal Nevus

Choroidal nevi are usually less than 1.0 mm in height and have a greatest basal dimension of less than 5.0 mm [25]. Often, choroidal nevi are too flat to be well evaluated with ultrasonography. On B-scan, they appear only as focal elevations of the choroid with sloping basal margins that are difficult to delineate. Most lesions under 0.8 mm cannot be evaluated with diagnostic A-scan. Lesions with sufficient apical height appear as regularly structured, highly reflective, and avascular (Fig. 21.19).

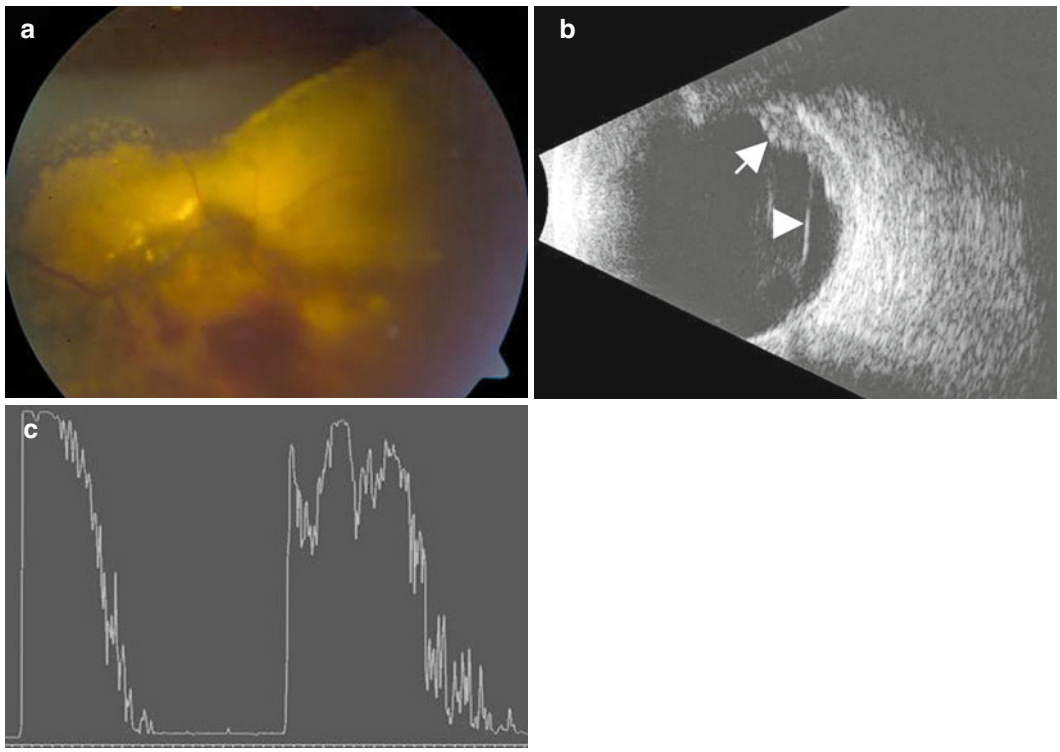

Fig. 21.18 Coats's disease. Clinical photograph showing lipid exudation (**a**). B-scan demonstrating exudative retinal detachment (**b**, *arrow*) and vitreous band (**b**, *arrowhead*) and A-scan with high internal reflectivity (**c**) (Reproduced with permission from Turell et al. [36])

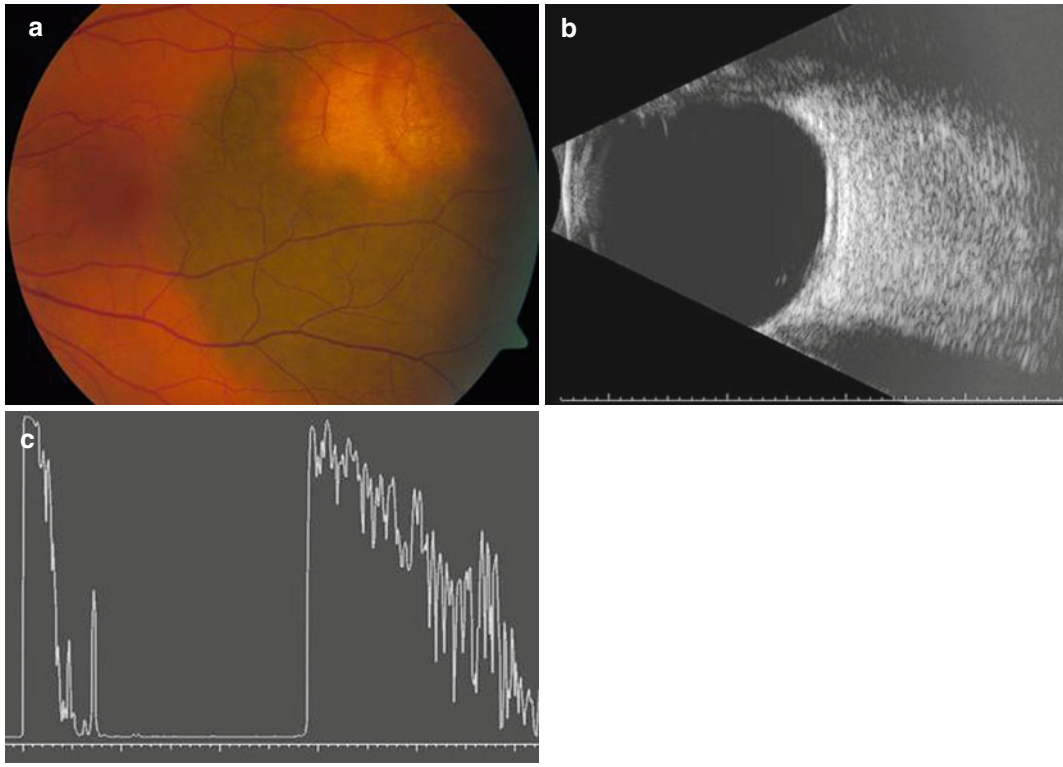

Fig. 21.19 Choroidal nevus. Clinical photograph showing partially amelanotic nevus with overlying drusen. Note absence of orange pigmentation or subretinal fluid (**a**). B-scan demonstrating that nevus is less than 1 mm height (**b**). A-scan with high internal reflectivity (**c**) (Reproduced with permission from Turell et al. [36])

21.7.2 Choroidal Melanoma

21.7.2.1 Topographic

Choroidal melanomas are solid and most commonly collar button, dome, or lobulated in shape. Although unusual, choroidal melanomas can present in a diffuse pattern or as a ring lesion. They have no predilection for lateral or radial locations in the eye and can be detected at all clock hours both anterior and posterior to the equator. Determination of the apical height of the tumor is essential in the diagnosis and treatment of choroidal melanoma [14, 15]. In most cases, diagnostic A-scan is the preferred, most accurate method to obtain apical height. As long as the sound beam is directed perpendicular to the most elevated portion of the lesion, the inner scleral spike is easily identified. Obtaining the measurement with diagnostic A-scan is particularly helpful in the measurement of treated choroidal melanoma where the lesion becomes highly reflective and the inner sclera is difficult to delineate on B-scan. Diagnostic A-scan can also identify thickening of the retina and subretinal fluid at the apex of the lesion, which appear as high double peaks at the tumor surface. In most cases, both the choroidal melanoma and its overlying shallow retinal irregularity should be included in the apical measurement. In cases where there is a large exudative retinal detachment, the measurement is up to the tumor surface. B-scan ultrasonography, utilizing both transverse and longitudinal scans, is the preferred method to obtain apical height for small lesions (<1.5 mm) and is necessary to obtain basal dimensions. In some cases, the clear delineation of basal boundaries is difficult to identify on B-scan, and in such cases, clinical correlation is essential.

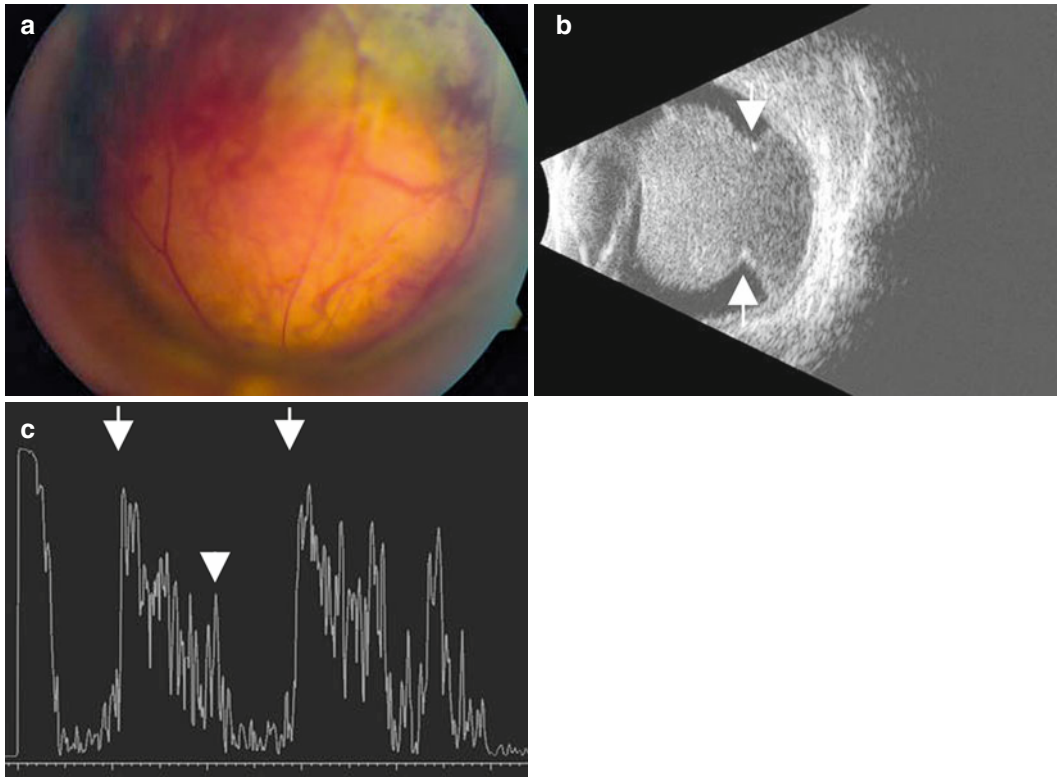

Fig. 21.20 Choroidal melanoma. Clinical photograph showing large, partially amelanotic dome-shaped choroidal mass (**a**). B-scan reveals a large mushroom-shaped choroidal mass that has broken through Bruch's membrane (*arrows*) touching the posterior surface of the lens (**b**). A-scan demonstrates internal reflectivity (*arrows*) with medium to high reflectivity in the anterior portion and low reflectivity beneath Bruch's membrane (*arrowhead*) in the posterior portion of the lesion (**c**) (Reproduced with permission from Turell et al. [36])

21.7.2.2 Quantitative and Kinetic

Due to homogeneous cell structure, choroidal melanoma is usually regularly structured, low reflective, and moderate to markedly vascular lesion [18, 26]. However, unlike dome or lobulated shape lesions, collar button-shaped melanoma is the result of the tumor breaking through Bruch's membrane; therefore, they can differ somewhat in quantitative features. The anterior portion often has higher reflectivity and varying degrees of vascularity (Fig. 21.20), while at the tumor base, reflectivity is low and in some cases echolucent, known as acoustic hollowing [27].

21.7.2.3 Special Cases

Associated Hemorrhage

Vitreous hemorrhage, subretinal hemorrhage, and intratumoral hemorrhage can occur in choroidal melanoma making standardized echography challenging. Dense vitreous hemorrhage and subretinal hemorrhage can mask an underlying choroidal melanoma. In order to correctly image the tumor surface and borders, it is essential to lower B-scan gain because choroidal melanoma is almost always more dense than layered vitreous hemorrhage and subretinal hemorrhage. Intratumoral hemorrhage can change the quantitative properties of choroidal melanoma by disrupting the typical homogenous structure. Instead of appearing regular in structure, low reflective, and vascular, these tumors are frequently irregular in structure with reflectivity ranging from low to high, and intrinsic pulsations indicative of vascularity may be absent.

Extraocular Extension

Extraocular extensions of choroidal melanoma appear as echolucent nodule adjacent to the

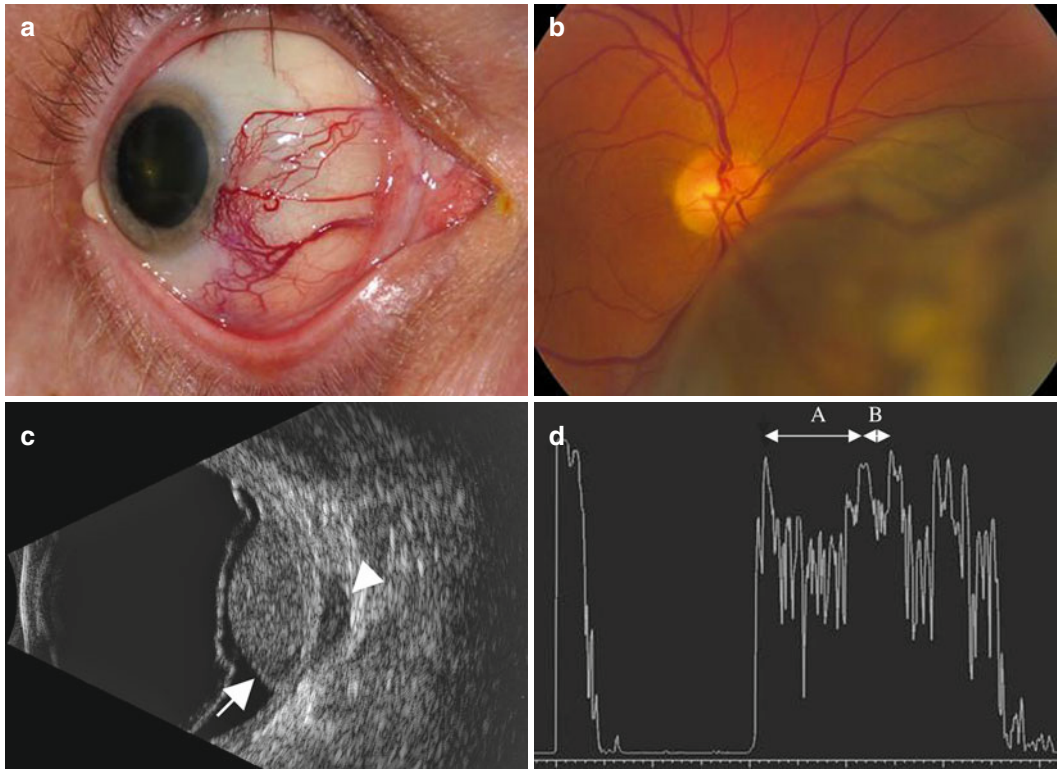

Fig. 21.21 Choroidal melanoma with extrascleral extension. Slit lamp photograph with sentinel vessels (**a**). Fundus photograph showing a large choroidal mass (**b**). B-scan demonstrating dome-shaped choroidal mass (*arrow*) with extrascleral extension (*arrowhead*) (**c**). A-scan shows medium to high internal reflectivity of the choroidal lesion (*arrows-A*) and high internal reflectivity of the extraocular extension (*arrows-B*) (**d**) (Reproduced with permission from Turell et al. [36])

sclera at the tumor base (Fig. 21.21). The low reflectivity of the extraocular component compared to intraocular component is due to sound attenuation from the sclera. High-resolution examination of the posterior segment frequently reveals slight excavation of the inner sclera at the tumor base or the suggestion of a tiny break in the sclera representing a transocular vessel.

Plaque Radiation

Intraoperatively, ultrasonography can aid in the precise radioactive plaque placement. The iodine-125 plaque, used in the Collaborative Ocular Melanoma Study (COMS), produces distinct hyper- and hyporeflective patterns on B-scan clearly delineating the plaques edges and causing shadowing of orbital structures behind the lesion (Fig. 21.22) [28, 29].

Postoperatively, serial examination with standardized echography following radiation plaque treatment of choroidal melanoma is essential for the precise measurement of decreasing tumor apical height. In addition to topographic changes, marked changes in quantitative and kinetic properties of the treated lesions also occur. The internal structure often becomes irregular, the reflectivity higher, and the vascularity absent.

21.7.3 Choroidal Hemangioma

21.7.3.1 Topographic

Circumscribed hemangiomas are dome shaped with smooth sloping edges and usually measure less than 6.0 mm in apical height [30]. On B-scan, dimensions can be difficult to delineate due to hyperreflectivity of the lesion and sloping base

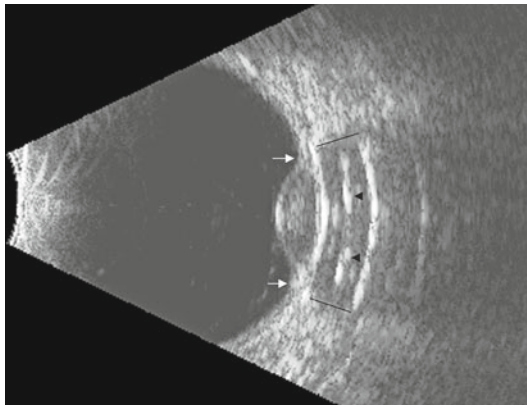

Fig. 21.22 Placement of iodine-125 radiation plaque. Transverse B-scan demonstrates a dome-shaped intraocular lesion with a concave radiation plaque behind the lesion and adjacent to the sclera. The highly reflective linear points within the plaque correspond to the I-125 seeds (*arrow heads*). Note that the margins of the tumor (*arrows*) are well within the margins of the plaque (*lines*) (Reproduced with permission from Fu et al. [37])

(Fig. 21.23). Diagnostic A-scan is recommended to determine the maximal apical height and ophthalmoscopy to estimate the basal dimensions. Diffuse choroidal hemangioma (Sturge-Weber syndrome) appears as diffuse thickening in all quadrants of the choroid, posterior to the equator with maximal elevation usually in the macula.

21.7.3.2 Quantitative and Kinetic

Choroidal hemangioma is regularly structured and highly reflective on standardized echography. Although these lesions are composed of a network of fine lacy vessels, on ultrasonographic examination, vascularity is not detected perhaps due to non pulsatile blood flow within them. These quantitative and kinetic features are markedly different from the low reflective and vascular properties of choroidal melanoma (Table 21.2).

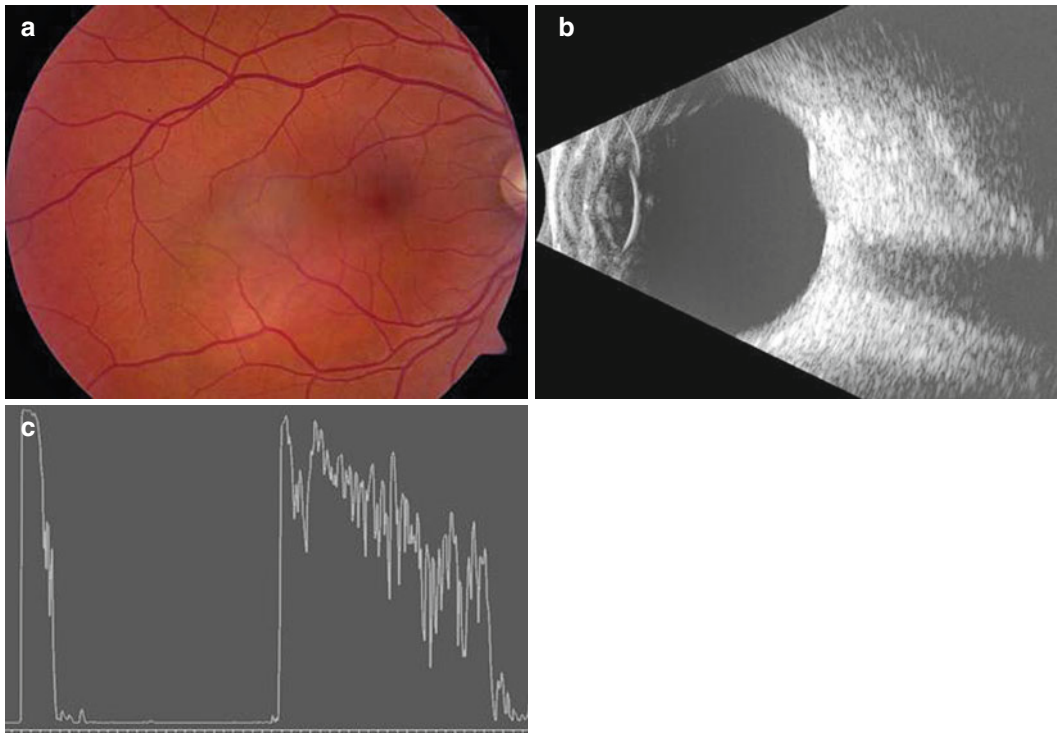

Fig. 21.23 Circumscribed choroidal hemangioma. Clinical photograph with elevated choroidal mass with indistinct borders (**a**), Axial B-scan showing dome-shaped mass (**b**), A-scan with high internal reflectivity (**c**) (Reproduced with permission from Turell et al. [36])

Table 21.2 Differential diagnosis of choroidal melanoma

Condition	Shape	Reflectivity	Attenuation	Vascularity	Specific features
Choroidal melanoma	Collar button/dome/lobulated	Low–medium	High	High	Regular internal structure Acoustic hollowness Choroidal excavation
Choroidal nevus	Flat/dome	Medium–high	High	No	Height less than 2 mm
Choroidal hemangioma	Dome	High	No	No	Regular internal structure
Choroidal metastasis	Placoid/irregular/multiple	Medium–high	Low	No	Multiple lesions
ARMD with hemorrhage	Dome/irregular	High	No	No	Irregular internal structure

Adapted with permission from Turell et al. [36]
ARMD age-related macular degeneration

21.7.4 Choroidal Metastasis

21.7.4.1 Topographic

Choroidal metastasis is usually flat or dome shaped with smooth or irregular surface and most frequently present in the posterior pole (Fig. 21.24). They can be focal, multifocal, or diffuse lesions, occurring unilaterally or bilaterally. Extensive associated retinal detachment is frequently detected. If diffuse infiltrative metastasis to the choroid is suspected, an axial B-scan examination is recommended to delineate peripapillary involvement and orbital extension.

21.7.4.2 Quantitative and Kinetic

On diagnostic A-scan, the internal structure is usually slightly irregular and most commonly medium to highly reflective. However, irregular metastatic lesions of the choroid can present with reflectivity ranging from low to high. Vascularity is usually absent or very minimal within the lesion, yet the surrounding choroid appears to have a marked increase in vascularity.

21.7.5 Hemorrhagic Disciform Lesion

Subretinal hemorrhage or disciform lesion from age-related macular degeneration (AMD) can resemble choroidal melanoma. Solidified

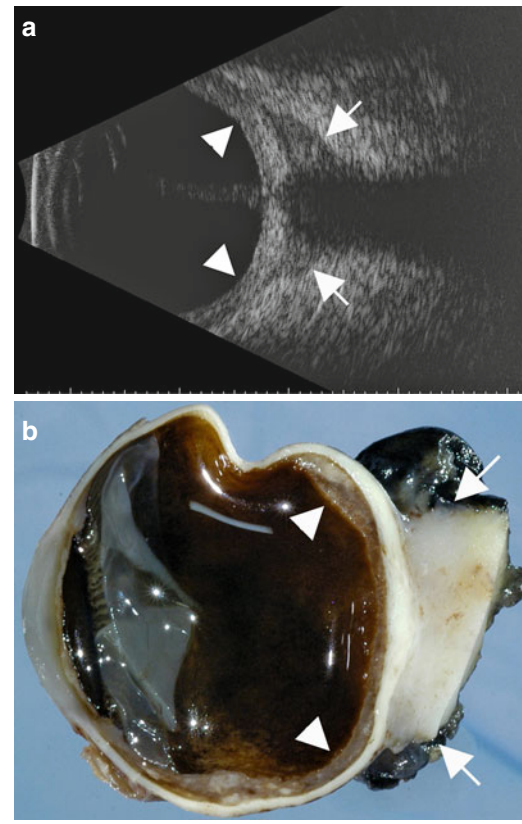

Fig. 21.24 Choroidal metastasis. B-scan demonstrating a total retinal detachment, diffuse choroidal thickening (*arrowheads*), and extraocular extension (*arrows*) near the retrobulbar optic nerve (**a**). Gross photograph of opened globe showing chalky white tumor deposits involving most of choroid (*arrowheads*) and tumor extending outside of globe (*arrows*-**b**, 2× magnification. Hematoxylin and eosin stain) (Reproduced with permission from Turell et al. [36])

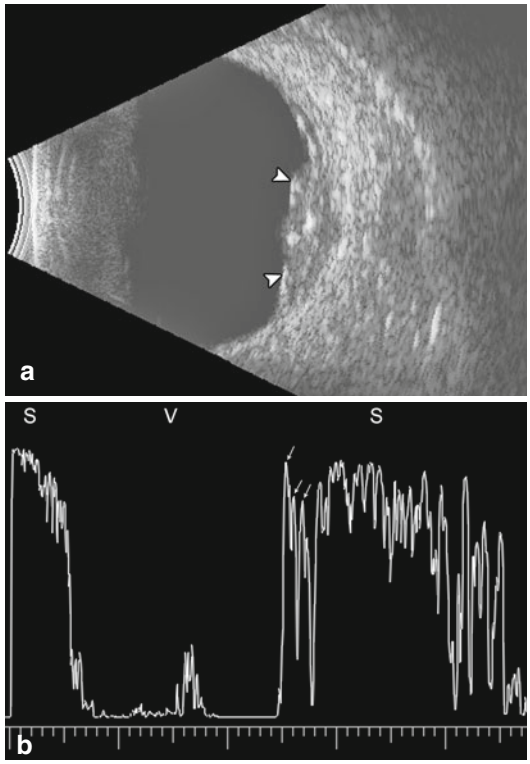

Fig. 21.25 Disciform lesion. B-scan shows mildly elevated lesion in the macular region (*arrowheads*) (**a**). A-scan shows multiple highly reflective peaks (*arrows*) corresponding to the lesion (**b**, *S* sclera, *V* vitreous) (Reproduced with permission from Sharma et al. [38])

subretinal and intrachoroidal hemorrhage is usually irregularly shaped with poorly defined margins but can appear smooth and dome shaped (Fig. 21.25). Due to the heterogeneous internal structure, most disciform lesions are irregularly structured and are medium to highly reflective, thus easily differentiated from choroidal melanoma. However, over time, subretinal hemorrhage can become organized with choroidal excavation becoming regularly structured and low to medium reflectivity, mimicking choroidal melanoma. Complete absence of internal vascularity is the distinguishing kinetic feature of AMD lesions, differentiating them from highly vascular choroidal melanoma.

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