Oncology: Pathologist's View

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Abstract

Cancer is a leading cause of death in the present century and despite spectacular advances in diagnosis and treatment and a considerable up-gradation in the survival graph, we are still bothered about it. Risk of developing cancer increases with age and is related to certain risk factors, which vary according to the site of cancer, but the real demon is the disorder of gene expression, and epigenetic factors are mainly responsible for demonizing certain proteins that are related to our normal growth and development. We cannot regulate our genes directly, but their control through epigenetic factors has their stakeholders in environment, food, and lifestyle, which influence carcinogenesis, the process of transformation of a normal cell to a cancerous one. These transformations occur through mutational changes in certain genes, which give the cell some survival advantage over others, which is again an evolutionary process. It is interesting that during this transformation, which is generally a gradual and multistep process, body defense mechanisms play a dubious role of preventing the cancer cell to survive and proliferate on the one hand, while actively helping them to evade the defense surveillance system on the other. Previously, we were of opinion that cancer cells in a particular tumor are monoclonal, i.e., arise from a single transformed cell and gradually progress through obtaining different mutations that give them definite survival advantage over their neighbors and ultimately become autonomous. But recent progress in molecular study tools like genome-wide association studies (GWAS) reveals that cancer cells are heterogeneous and the different subclones exist with different characters from the very beginning of the tumor and only those subclones survive that win a fight against normal cells by their

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advantageous mutations. If mutations in a particular subclone could not confer the survival advantage, that particular subclone will extinct.

Keywords

Neoplasia · Cell cycle regulators · Carcinogenesis · Stem cell · Pathology

10.1 Introduction

The human society has faced death through violence, war, accidents, natural disasters, and fearsome array of infectious diseases over time immemorial. The leading causes of death in the twentieth century were pneumonia, influenza, and tuberculosis as are evident in the world literature. In the present century, the leading causes of death are heart diseases, cerebral strokes, and cancer. Antibiotics and other modern medicines have reduced morbidity from infectious diseases to a large extent; a conscious effort to change the lifestyle has reduced fatal heart ailments and strokes to some extent. But we are still bothered with mortality from cancer. A lot of progress has been achieved in the field of diagnostics and treatment, and consequently a considerable up-gradation in 5-year and 10-year survival graphs, but the "final diagnosis" still rests on the seemingly fragile shoulders of pathologists, the mysterious people sitting always with a microscope and writing reports in a language full of confounding words and dubious logic. Whenever the clinician thinks for a second opinion, the confusions often get exaggerated and the decision making becomes tougher. The irony is that hardly these two persons, that is, the clinician and the pathologist, meet and discuss, which might almost reduce the entire impasse.

This is the most talked-about perspective of a pathologist; they expect to receive a bit more information regarding the illness on the so-called requisition slip and sometimes a little bit of interaction with the clinician to arrive at an unequivocal tissue diagnosis with proper staging and grading that can save valuable time, which in turn may prove life-saving for the patient. The modern medicine as a subject experienced tremendous advancement in the last two to three decades as a consequence of human genome project and its aftermath, particularly in the areas of human genetics, immunology, therapeutics, and field of radiation oncology. Even the supportive fields like terminal care and rehabilitation have progressed a lot. Still, it is the early diagnosis, proper categorization, and prognostic evaluations that are vital for the choice of treatment from the available options.

10.2 Oncology, Neoplasia, and Cancer

Oncology is an integrated discipline of medicine dealing with the prevention, diagnosis, and treatment of cancer. A medical professional who practices oncology is designated as an oncologist, a comparatively new speciality not heard even a few decades ago. Oncology specialists include medical oncologists, surgical oncologists,

radiation oncologists, and oncopathologists in their respective field of activities. But for a pathologist, the term "onco" does not always mean cancer.

Neoplasia literally means "new growth," which obviously encompasses both "benign" or good tumors and "malignant" or bad tumors, that is, cancer. The term "tumor" means any swelling that we often encounter in our daily living, even after a blunt injury or ankle sprain or following an allergic inflammation. So, neither tumor nor neoplasia literally means cancer. Therefore, a pathologist has to write his diagnosis in a proper and acceptable bunch of words that may appear Greek even to a literate common folk but carry meaningful information for the clinicians. Moreover, the "biopsy" report is not an expression of quantity as in the case of reporting blood glucose or lipid profile. The utmost concern of a "histopathologist" (the pathologist trained for and designated with reporting a biopsy sample) is to provide maximum information evident in a sample of tissue for the clinician so that he/she can make a proper choice of therapy for the best treatment outcome of the patient in the given situation.

So, cancer, presumably a malignant tumor or "bad tumor," can be defined as n growth of tissue that is autonomous, has escaped normal checkpoints of cell proliferation, and exhibits various degrees of similarity to their precursors (Strayer and Kluwer 2015). People often consider cancer as a disease of modern age and a curse of civilization, but the fact is that it is a disease of ancient times. Evidence of bone tumors has been found in prehistoric remains, and the disease is mentioned in some forms in the ancient literary works from India, Egypt, Babylonia, and Greece. Hippocrates is reported to have introduced the term *karkinos,* from which the term carcinoma is derived (Strayer and Kluwer 2015).

10.3 Risk Group for Developing Cancer

The incidence of cancer, in general, increases with age. Therefore, with gradually rising life expectancy, an increasing proportion of older population are falling prey to the claws of this horrifying demon. But the demon is actually a disorder in gene expression, may it be structural or functional deviation from so-called normal genome. The claws of the demon were blurred to our vision before the completion of Human Genome Project, but after that these are becoming clearer day by day. Now, we know the major proteins involved in development of carcinoma, its invasion and metastasis, and the genes coding these proteins. We are also exploring the factors that are involved in errant behavior of these proteins, factors that are known as "epigenetic" factors (epi = outside, of genes). These epigenetic factors that are mainly responsible for demonization of these benign proteins (which are mostly involved in normal growth of our cells and smooth running of cell cycles with a watchful policing of the whole complex events) are largely associated with our environment and lifestyle. Those cancers where the passwords (causative factors) are yet to be cracked will fall in line with others with further advance in cancer research, the cancer biologists presume.

The environmental factors that lead to carcinogenesis are a common threat for all of us, and we have to solve this threat ourselves. Regarding lifestyle, we may think of reverting to our younger days when we were less greedy, physically active, and socially more accessible to our friends and neighbor, be satisfied with minor gains, and used to live a simpler and happier life. But it is not possible to reverse the time line. Therefore, we have to innovate the ways of our living, which will be healthier, yet acceptable and feasible to all of us. For this, we should clearly understand the happenings during the development of cancer.

10.4 Carcinogenesis

Every living entity is composed of cell(s), which we can consider as the unit of life. It is basically the structural, functional, and biological unit of all known living organisms. The cell can replicate independently. It consists of cytoplasm enclosed within a membrane, which contains many biomolecules such as proteins and nucleic acids. Humans contain more than 10 trillion (10^{13}) cells. The cell was discovered by Botanist Robert Hooke, who coined the term "cell" for their resemblance to the cells inhabited by the Christian monks in the monastery. Human cells along with those of plants, animals, fungi, and protozoa belong to eukaryotic cells, although prokaryotic cells were the first form of life on earth. In eukaryotic cells, there is cell membrane, cytoplasm, different organelles, and vacuoles, but the most important among these is the cell nucleus that contains the cell's DNA or the genome. Human genome contains roughly 3.2 billion DNA base pairs. Within the genome, there are only around 20,000 protein-encoding genes, comprising less than 1.5% of the genome. The rest 98.5% of the human genome does not encode proteins, but here the DNA lies in long stretches, separating the protein-coding genes, almost like a buffer zone. The recently concluded ENCODE (Encyclopaedia of DNA Elements) shows that 80% of this noncoding DNA is involved in regulating gene expression (Kumar et al. 2014).

Now, the question is how the protein-encoding genes, or the genome, are implicated in cancer? Cancer or in the broad sense, neoplasia (new growth) is defined by British oncologist Willis as "an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change." Therefore, basically a cancer alias tumor alias neoplasia is a disorder of cell growth that is triggered by a series of acquired mutations (i.e., permanent DNA alterations). These mutations affect the normal mechanism of cell growth by setting the growth cycle out of gear, thus attaining freedom from the growth control mechanisms. Even the behavior of a tumor, whether it will be a benign one like lipoma (a tumor consisting of fat cells) or it will transform to a malignant or cancerous tumor-like liposarcoma, also depends on which set or sets of genes are mutated and at which stage of cell growth.

With this background, we may proceed into the mystery of carcinogenesis, that is, the genesis of cancer. The main objective behind the transformation of a normal cell

into cancer cell is the attainment of survival advantage. This means the transformed cell will survive longer than its neighboring nontransformed cells. These mutations (i.e., a change in the primary nucleotide structure of the DNA) that will provide a survival advantage invariably involve those genes that are somewhat related to cell growth and differentiation. Those mutations that provide a survival advantage to the mutated cell will empower the cell to survive longer than its nonmutated neighbors, hence have a fair chance to undergo another mutation, which might not be possible without survival advantage acquired by the first mutation. This second mutation may or may not provide any survival or growth advantage. If it does not provide such advantage to the cells with the 1st mutation, then the matter ends there and this population of cells will face their natural death. But if, by any chance, this 2nd mutation also provides another survival advantage, then the descendants of these cells will survive longer and be prepared for undergoing the 3rd mutation. These survival advantages may be in the form of survival in low oxygenated condition or empowerment to avoid growth-inhibiting proteins or to avoid apoptosis and similar other advantages. After getting a series of such adventitious mutations, the cell population will attain "immortality"; that is, they will be free off normal regulations of cell cycle. We call this mutated population as "transformed" cell population. This transformed cell population forms the nidus for cancer.

10.5 Cell Cycle Regulators and their Role in Cancer

The most important drive for any living organism is its struggle for survival and growth. Dialectic materialism, which was theorized by Karl Marx in nineteenth century and applied in the evolution of human society, also influenced Charles Darwin who developed the great concept of evolutionary drive and survival of the fittest in animal and in plant kingdom. The experience an organism gains during its struggle for existence has to be shared with its progeny for their survival interest. This is being done by exchange of chromosome material during gametogenesis, and the gametes carry this experience in the form of genetic code to future generations. Therefore, cell division is a crucial step and it should be regulated properly so that no error, whether intentional or unintentional, occurs during cell division. This is ensured by a series of checks and counter checks by a gamut of proteins.

All cells do not have the same potential for cell division. Some cells do not divide at all after birth, for example, brain and heart (permanent cells); others have only minimal proliferative activity but are capable of dividing when called for like liver, kidney, pancreas (stable cells), and the rest of the cells are continuously being lost and replaced by proliferation, for example, bone marrow cells, skin, oral mucosa, gastrointestinal tract, and endometrium (labile cells). This compartmentalization is not always rigid; even permanent cells can be pushed from its quiescence state to activity of cell cycle through genetic switching.

Whenever a cell undergoes cell division, it has to pass through certain stages. First, it has to double its DNA blueprint so that the progeny cells get a normal DNA copy that is compatible for its survival and further growth. This is known as S

(synthesis) phase as DNA synthesis occurs in this phase when the whole genome is copied. As the 3.2 billion base pairs are to be copied in a very tight schedule, there are possibilities for occurrence of some errors during copying and errors do occur at an approximate rate of 1 error in every million base pair and some of these errors are important enough to jeopardize the functioning of the progeny cells. Therefore, these errors have to be detected and rectified before the cell enters into its final lap of cell division that is M (mitosis) phase. There are proteins dedicated for detecting (spellchecking) the errors generated during copying and for rectifying (mismatch repairing) those errors. But this process will obviously take some time, therefore necessitating a temporary halt or applying brake to the cell cycle. The errant cell is then isolated from the highway of the cell cycle, and the repair can be accomplished properly and in time in the roadside garage and again place it on the pathway of cell cycle after successful repair. To supervise the whole process of detection of defect, putting a brake on the cell cycle, making necessary repair, and then put them back to cell cycle, strong supervisors are necessary who will do their job meticulously. This supervision is again no individual's job and a set of proteins are there under the able guidance of their team leader, the p53 protein, which has also a nickname in his team—the guardian of the genome. The team has their own standard operating procedure (SOP), which has been updated many times during the evolutionary process and currently the SOP includes thorough on-road checking of all cells that have completed the S phase and then going to the final destination, that is, M phase, so that no cell can enter M phase with a DNA defect. The SOP also includes erecting roadblocks by a separate group of proteins (cyclins, cyclin-dependent kinases, and CDK inhibitors) so that all cells running in the cell cycle have to stop and only be allowed to proceed further if the inspectors and supervisors found it ok and give signal to remove the roadblock. All these checks and counterchecks are intelligently designed so that they can function in an integrated way to ensure a full-proof result.

These are all about normal control of cell cycle. Problem begins if the above-mentioned checks and counterchecks did not function properly. Then, the errors generated in S phase go unnoticed and the cells enter the M phase of cell cycle either unchecked or underchecked or with faulty check. Some of these errors may be due to changes (mutations) in DNA that occurred due to environmental or chemical factors (carcinogens), while others are spontaneous errors of copying. The combination of genetic errors due to mutations and copying may provide the survival advantages that are prerequisites for survival advantages and subsequent malignant transformations.

10.6 Carcinogenesis Model

Our access to molecular analysis of biological specimens is expanding day by day, and the cost is also coming down; cancer genetics are revealing newer and unexpected information. The previous concept of monoclonal origin of cancer cells is being replaced by cancer heterogeneity. Previously, it was conceived that a series of mutations ultimately transform a cell irreversibly to a cancer cell with the

characteristics of autonomy and other features like dysplasia, invasion, and lack of cohesion that imparts the phenotypic characters of a malignant lesion. Hence, it was presumed that all the cells present in a cancerous growth bear the same set of mutations and therefore same phenotypic characters.

Genome-wide sequencing (GWS) studies of cancer cells revealed that as few as ten or so mutations are required for the necessary transformation. The classical prototype is colorectal cancer. This multistep clonal model also revealed that not all mutations have the ability of transforming cells. Those mutations that are vital for transformation are known as "driver" mutations, and the other mutations that are nonvital but help propagating the driver mutations are known as "passenger" mutations. This theory says that tumor progressively accumulates carcinogenic mutations. These mutations occur in stepwise fashion. During the progression of clonal evolution, "driver" lesions that have some selective advantages lead to acquisition of "hallmarks of cancer" (e.g., sustained proliferation, avoiding growth suppression mechanisms and cell death, attaining replicative immortality, angiogenesis, invasion, and metastasis) (Flanagan 2016).

Now the genome-wide association studies (GWAS) are revealing that all cancer cases do not follow this clonal carcinogenesis model. Instead, there are multiple subclones in a tumor mass having different driver mutations. This proves that *cancers are heterogeneous rather than homogenous as all the cells do not carry the same set of mutations* and also they vary in their behavioral phenotype. This intratumor heterogeneity is clearly evident in cancers of kidney, lung, breast, ovary, leukemia, etc. According to the recently hypothesized "*Big Bang*" model of carcinogenesis, subclones do not always expand deterministically within tumors. Chance plays a great role in deciding the spread and success of a clonal growth. Because of the lack of selection, it is the exact timing of mutation that determines clone size, and mutations arising early in the tumor will tend to form larger subclones, whereas late mutations will form clones of restricted size. Further, all sizeable subclones arise early during cancer expansion. Thereafter, these subclones have to compete with the resident cells for survival and eventually their growth rate will slow down. Late arising subclones are extremely unlikely to expand to the size detectable by sampling. In contrast, early-arising subclone mutations have the advantage of sweeping through the population as the population size is small enough for new clones to be established. Hence, the naming of Big Bang model—the clonal composition of a tumor—is determined early on and remains effectively static thereafter. This is analogous to cosmological model wherein perturbations during the initial expansion of the universe still dominate in the present day (Flanagan 2016).

In sequential model, each driver mutation bestows a large increase in fitness to the recipient clone. Phenotypic evolution proceeds in an incremental fashion. In Big Bang model, significant selection occurs at the outset of a cancer growth, and the subsequent evolutionary selection within the expanding population is of negligible magnitude and/or consequence. The Big Bang model is thus an example of punctuated equilibrium (genotype–phenotype differentiation) whereby large phenotypic leaps can suddenly occur in an otherwise phenotypically static population. Most preinvasive and premalignant lesions are in a state of evolutionary stasis, most

will not progress to malignancy. The main driver mutations occur early in the neoplastic process—"born to be bad" (Flanagan 2016).

Do these observations mean the multistage carcinogenesis theory has been totally replaced by the Big Bang theory? The answer is not affirmative as in many cases multistage carcinogenesis is still valid.

10.7 Evolution and Cancer

Animals have evolved some potent tumor suppressor mechanisms to prevent cancer development. These mechanisms at the earlier stage of evolution were important for growth of multicellular organisms and large-body animals. Thus, the development of animals was evolutionarily constrained by the need to limit cancer. Cancer development within an individual is thus an evolutionary process, and it often mimics evolution of different species.

What are the hallmarks of species evolution? Species are evolved by mutation and by the process of selection acting on individuals in a population. Tumor cells also reveal these characteristics of evolving by mutation and selection in a tissue, same as that of evolution of individuals in a population. Cancer evolution leaves "information" in cellular genomes that evolutionary theory can decode. Species evolution also leaves such information in the genome; in cancer, such information is easily verifiable.

From a biological point of view, cancer is an evolutionary disease. In cancer, cells not only evolve morphologically, but also functionally. These new set of functions thus acquired by the cancer cells are beneficial for them, but ultimately lethal to their hosts. What new functions do the cancer cells gain by these evolutions? Apart from growing quickly, it also ignores signals to die (evade apoptosis), evades host immune defenses, grows blood vessels to obtain nutrients (angiogenesis), invades surrounding tissue, survives in bloodstream, and establishes new colonies throughout the body (metastasis), and they may even resist treatment (chemo- and radioresistant) (Swamidass 2017; Casás-Selves and DeGregori 2011).

All these gains in function are related to overcoming the growth control mechanisms in some way or other. Earlier, we have mentioned that the growth-controlling (or tumor suppressor) mechanisms are robust in comparison with the growth-promoting mechanisms, and this is an integral part of evolution of species. But whenever an individual cell has to attain autonomy in growth, which is also an evolutionary drive, it has to overcome the stubborn attitude of an array of tumor suppressor proteins, and this is achieved by a series of sequential mutations of genes encoding these tumor suppressor proteins.

10.8 Cancer Stem Cells

The concept of stem cell is not new, but the evidence indicating the presence of cancer stem cells (CSCs) is a recent development. We know that stem cells are those cells that have *pluripotency* to develop different types of mature cells. Let us give an example. When an embryo has formed after the successful fusion of a sperm and an ovum, it forms a tiny bubble-like aggregate of cells within a few days, which we call *blastocyst*. It undergoes cell division, and the cell number increases by doubling. Now, these cells are capable of creating an entire human embryo and then into a fetus and ultimately a full-formed newborn. To achieve this highly specialized form, all the cells in a blastocyst have the potential to create an entire baby. These are known as pluripotent embryonal stem cells. These are the most potent stem cells that can give rise to any type of mature cells needed for organogenesis. There are other types of stem cells, for example, multipotent stem cells (hematopoietic stem cells that form different blood cells), oligopotent stem cells (neural stem cells that are more restricted to neuron cell formation), and unipotent or monopotent stem cells (restricted for developing single type of cells). All these stem cells have some common characteristics. Stem cells are usually immortal; that is, they have an unlimited replication potential. When a stem cell divides, it is usually asymmetric; i.e., it always gives rise to a stem cell (to replenish stem cell population) and a differentiated cell (that will be a mature and functional cell). Stem cells can be induced in vitro to differentiate into any organ or tissue-specific cells by genetic reprogramming. These induced stem cells are known as induced pluripotent stem cells or iPSC (Nature Outlook: Cancer 2014).

A lot of research work is going on stem cells and its application in targeted therapy in different cancers. One may find frequent news items on some recent controversies related to stem cell research, particularly on ethical issues, religious controversies, federal funding, and other issues. Stem cells have already become an integral part of our survival. In different blood cancers and lymphomas, hematopoietic stem cell transplantation is the only hope for survival. Stem cell transplantation is also coming up for the treatment of different neurological diseases as well.

How did the scientists come to know about the existence of cancer stem cells? Obviously, this is not an accidental finding. As we mentioned earlier, almost all labile tissues in our body (those tissues which have a high cellular turnover, e.g., skin, and gastric and intestinal mucosa) do harbor adult stem cells, which are basically unipotent stem cells destined to maintain a high turnover of adult cell population. With the advancement on stem cell research, attention was focused also on these adult stem cells in cancer tissue. As these stem cells are immortal (in the sense that they always maintain a constant pool of themselves by pursuing asymmetric cell division), they have more chance of acquiring and propagating the mutations that are necessary for transforming them from adult stem cells into cancer stem cells. The evidences in favor of this theory came in the form of immune histochemical (IHC) marker study.

IHC is detection of expression of different cellular proteins by specific antibodies tagged with fluorescent dye. The proteins being antigenic in nature will be identified by these tagged antibodies, and after washing, those tagged fluorescent markers will be detected using a fluorescent microscope. Detection can also be done by nonfluorescent tagging, for example, horseradish peroxidase. These techniques have been utilized for detecting different cancer cells since the 1970s. IHC has been universally accepted as markers of cancer cells as they are definite evidences of the cell of origin, markers of prognostic factors that determine the survival rate, and, the most important one, the target molecules for therapeutic approach. Initially, only a few markers were used for cancer cell study, but with tremendous advancement of molecular biology more and more protein molecules are being identified for depicting the functional heterogeneity of mutated cancer cells. Some of these IHC markers were successfully developed for detection of stem cells also. These stem cell markers were also applicable for detection of stem cell population in different adult tissue to locate their residence (known as *niche*) and study their behavior and interaction with the microenvironment that dictates their activation and hibernation.

The first conclusive evidence for CSCs came in 1997 when Bonnet and Dick were succeeded to isolate a subpopulation of leukemia cells that expressed surface marker CD34, but not CD38. The authors established that the $CD34^{+}/CD38^{-}$cell subpopulation is capable of tumorigenesis in mice that were histologically similar to the donor. The first evidence of a solid tumor containing cancer stem-like cell found in 2002 with the discovery of a clonogenic, sphere-forming cell isolated and characterized from human brain gliomas. More evidence comes from histology. Many tumors are heterogeneous, and they contain multiple cell types that are native to the host organ. Tumor heterogeneity is commonly expressed by tumor metastases. This suggests that the cell that produced them had the potentiality to generate multiple cell types, a classical hallmark of stem cells. IHC marker study also confirmed this property of "stemness." The existence of leukemia stem cells prompted research into other cancers. CSCs have recently been identified in several solid tumors (e.g., brain, breast, colon, ovary, pancreas, and prostate), melanoma, nonmelanoma skin cancers, and multiple myeloma.

10.9 Cancer Stem Cells and Growth of Cancer

Cancer stem cells possess the characteristics of normal stem cells, particularly self-renewal and differentiation into multiple cell types. CSCs are therefore tumorigenic cancer cells that acquire driver mutations, in contrast to other nontumorigenic cancer cells that form the bulk of the tumor by passenger mutations. CSCs persist in a tumor as a separate population and are responsible for cancer relapse and metastasis. Development of specific therapies targeted at CSCs holds hope for the improvement of survival and quality of life of cancer patients, especially for patients with metastatic disease. Therefore, the histopathologists are now more interested in studying CSC by IHC markers and a lot of publications are on the way. But there are some challenges also: Some of these markers are shared by non-CSCs present in

the tumor mass, therefore compromising the specificity of these markers, Not all the cases of a particular tumor express these CSC markers, If we accept this CSC model of carcinogenesis, then the question is how we will explain cancer formation in stable or permanent tissue (where cell turnover is low or absent), which do not harbor adult stem cells that are subsequently converted into CSCs.

However, this CSC model is now widely accepted as a valid model of carcinogenesis in a good number of cancer growths.

10.10 Pathologist in Diagnosis of Cancer

Cancer is suspected by the clinician by carefully listening the complaints of the patient or the relatives, asking him/her a few leading questions, which include the family history, food habit, working conditions, personal habits and hygiene, addictions if any, history of medication, and then a thorough clinical examination. The clinician then advises some investigations to confirm or exclude his clinical suspicion. In this whole process, the most definite part of the diagnosis is rendered by the pathologist in the form of cytopathology or/and histopathology. Other diagnostic methods like radiodiagnosis and biochemistry have also an important ancillary role, but final diagnosis rests on pathologists. But there are some important issues that need to be solved.

Whenever cancer is suspected by the clinician, he/she should act promptly to confirm the diagnosis. Tumor load grows in geometric progression, and therefore, we should not miss the opportunity of offering early treatment to the patient. It can be readily calculated that the originally transformed cell must undergo at least 30 doublings (1 doubling = 1 cell division) to produce 10^9 cells weighing 1 gm., which is the smallest clinically detectable mass. In contrast, only 10 further doubling cycles are required to produce a tumor containing 10^{12} cells weighing approximately 1 kg, which is usually the maximal size compatible for life (Kumar et al. 2014).

For making a faster diagnosis, cytopathology is an important tool. Fine needle aspiration cytology (FNAC) is an approach, which is widely used. The procedure involves aspirating cells with a small-bore needle, followed by cytological examination of the stained smear. This method is used most commonly for assessing readily palpable tumors such as breast, thyroid, and lymph node. Modern imaging techniques like ultrasonography, CT, or MRI permit guided FNAC of deep-seated lesions and nonpalpable ones. It is an outpatient procedure and obviates surgical biopsy and its attendant risks.

Cytological smears provide yet another method for early detection of cancer and are widely used to screen for carcinoma of cervix. Detection of cancer cells can also be made in other body secretions and excretions like bronchial fluid and sputum in lung cancer, urine in bladder and prostate cancer, gastric fluid in stomach cancer, and for identification of cancer cells in abdominal, pleural, cerebrospinal, and joint fluids. The cancer cells that are exfoliated in different body fluids due to their lack of cohesion when they are transformed into malignant cells exhibit a range of

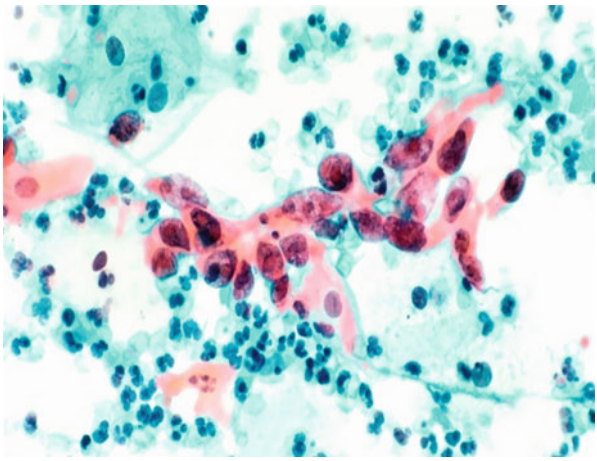

Fig. 10.1 An abnormal cervicovaginal smear showing numerous malignant cells that have pleomorphic, hyperchromatic nuclei

morphological changes encompassed by the term *anaplasia,* which literally means "to form backward," implying a reversal of differentiation to a more primitive level.

Lack of *differentiation* usually means morphological variations in a cell from its normal counterpart and can be detected microscopically by observing some characteristics: (i) variation in size and shape, which are termed as *pleomorphism*, (ii) abnormal nuclear morphology, which include nuclei being disproportionately large for the cell, and the nuclear-to-cytoplasm ratio is increased and may approach 1:1 instead of normal 1:4 or 1:6, (iii) the nuclear membrane is irregular and the chromatin is often coarsely clumped and distributed along the nuclear membrane, giving it a vesicular appearance, (iv) nucleus is more darkly stained than normal, which we call *hyperchromatic* nuclei, and (v) more frequent mitotic figures than normal and atypical mitosis, giving it a bizarre look. But cytological reporting of a cancer requires more experience as judgment must be rendered based on the features of individual cells or, at most, a clump of cells without the supporting evidence of loss of orientation of one cell to another (loss of polarity) and the evidence of invasion, which can be judged only in histology.

Figure 10.1 points to an abnormal cervicovaginal smear showing numerous malignant cells that have pleomorphic, hyperchromatic nuclei. Figure 10.2 shows anticytokeratin immune peroxidase stain (IHC) of a tumor of epithelial origin. Figure 10.3 shows papillary adenoma of colon, which is a benign tumor. Figure 10.4 shows beautiful transition from normal surface squamous epithelium to invasive carcinoma. Figure 10.5 shows well-differentiated squamous cell carcinoma showing prominent "pearl" formation. Figure 10.6 shows well-differentiated adenocarcinoma of colon. Note the cellular distinguishing it from Figure 10.3, *i.e.*, papillary adenoma. Figure 10.7 shows anaplastic large cell carcinoma of lung showing marked cellular pleomorphism. Figure 10.8 shows highly dysplastic epithelium with high N:C ratio, nuclear hyperchromatism, mitotic figures, and loss of polarity.

Histopathology or biopsy is the mainstay in cancer diagnosis, but it needs a very good histology laboratory with modern instruments and facilities but, more

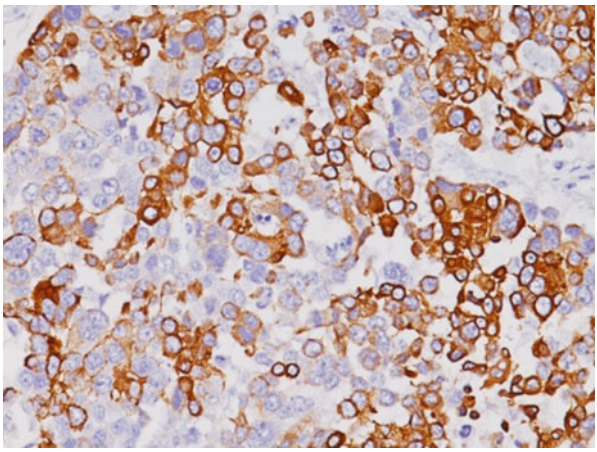

Fig. 10.2 Anticytokeratin immunoperoxidase stain (IHC) of a tumor of epithelial origin

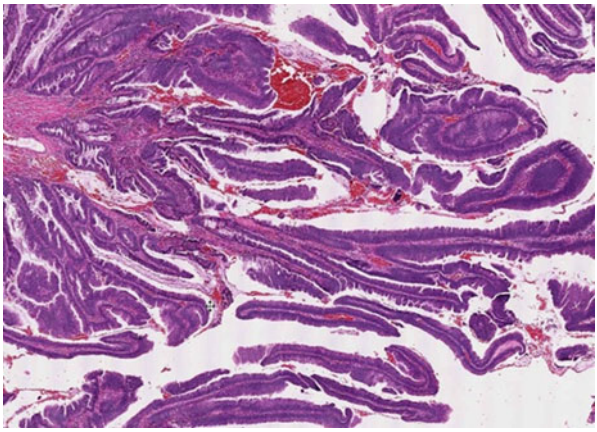

Fig. 10.3 Papillary adenoma of colon showing finger-like projections

importantly, trained manpower, which is often lacking. The pathologist, while reporting a histology slide under a microscope, will require a good quality thin section, appropriately stained, to ascertain the architectural and morphological characteristics and to arrive at a logical conclusion. He/she also needs a morphological description of the specimen from which the tissue section is taken and the relevant clinical information with other diagnostic parameters. Therefore, good coordinating information flow among clinician, pathologist, and laboratory staff is an essential prerequisite for proper diagnosis.

While reporting the pathologist should adhere to standard protocol of reporting, as provided by the nomenclature guidelines of WHO and cancer protocol templates by CAP (College of American Pathologists) or cancer staging manual of AJCC (American Joint Committee on Cancer), reporting based on standard guidelines and protocols carries pertinent information to clinicians to decide for the best choice of treatment, which has an immense value for cure and survival.

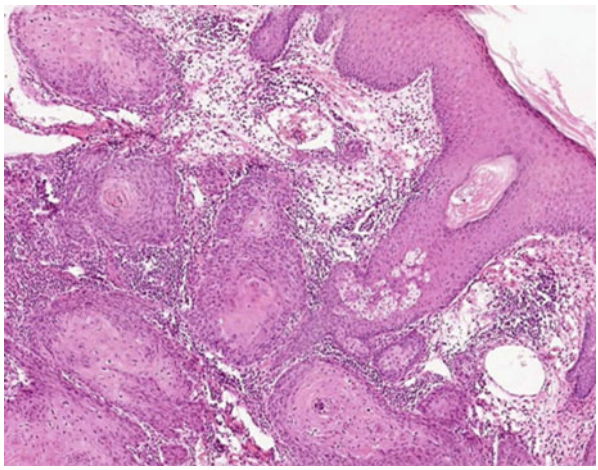

Fig. 10.4 Transition from normal squamous epithelium to invasive carcinoma showing invasion

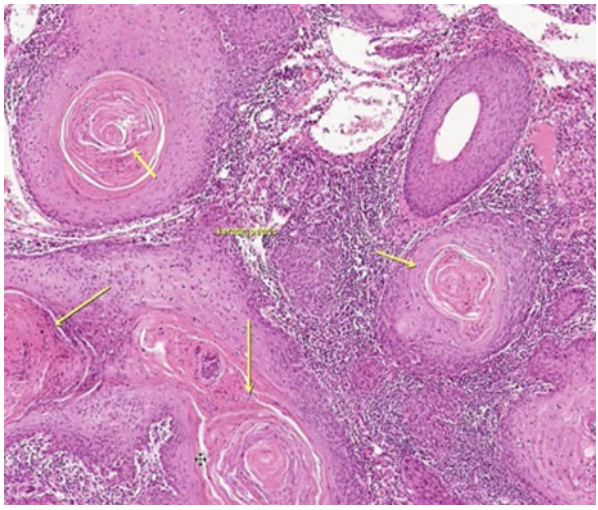

Fig. 10.5 Squamous cell carcinoma, well-differentiated, showing "pearls"

The availability of specific antibodies has greatly facilitated the identification of cell product by immunohistochemistry (IHC). But this facility requires judicious use of IHC markers in a cost-effective manner. Nowadays, most of the cancers are categorized by their protein expression, which is of immense importance for targeted therapy. Therefore, all laboratories reporting cancer must be equipped with IHC facility. This is particularly important for (i) categorization of undifferentiated tumors, that is, when the histology has failed to recognize the tumor properly, (ii) determination of site of origin of metastatic tumor, that is, when we could not ascertain the tissue of origin of a metastatic or far away deposit of a cancer, and (iii) detection of protein molecules expressed by the cancer cells that have prognostic (predicting the outcome) or therapeutic significance.

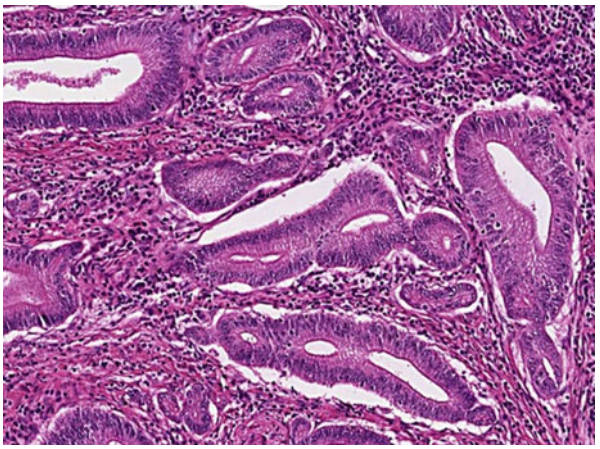

Fig. 10.6 Adenocarcinoma of colon. Glandular pattern is well-recognized

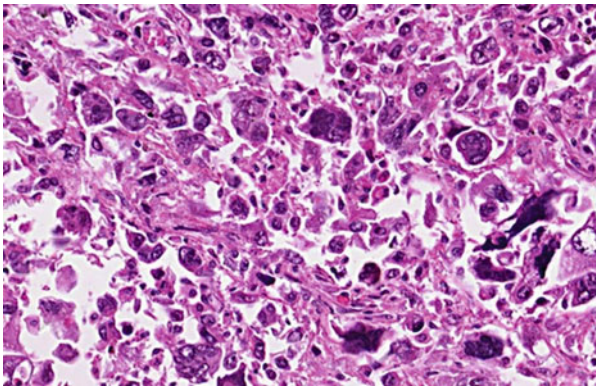

Fig. 10.7 Anaplastic large cell carcinoma of lung showing marked pleomorphism

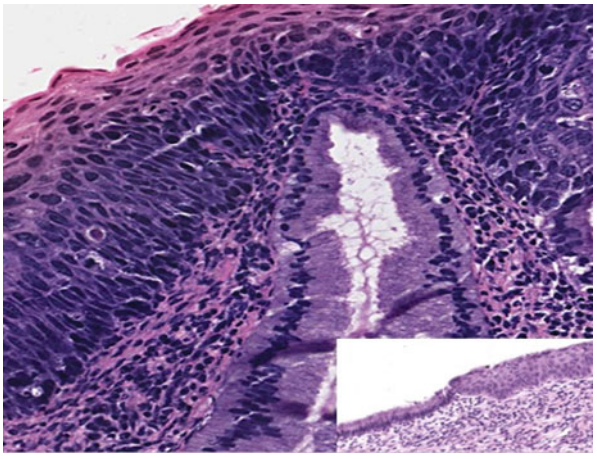

Fig. 10.8 Dysplastic epithelium showing high N:C ratio, nuclear hyperchromatism, mitotic activity, and loss of polarity

10.11 Histopathology Techniques in Biopsy Interpretation

Pathology literally means "study of diseases" and pathologists are a speciality of doctors trained for examining different body samples and providing their opinions, based on which the clinician will diagnose and treat the patient. This broad speciality has subsequently become compartmentalized into several subdisciplines: Histopathologists study morphological alterations in tissues and cells. Microbiologists study the microbes, biochemists study the alterations in biochemical substances, and so on. Cancer is basically an alteration in growth of tissue and therefore has implications on almost all specialities in medical sciences. A cancer patient may come to a clinician of any speciality for certain complaints related to the organ affected. Cancer may be suspected during investigation procedure for any other disease. The process can affect any age-group, although it is much more common in middle-aged or elderly patients, probably due to the time taken for a chain of mutations that need to occur for the malignant transformations. However, whenever a clinician suspects a cancer in his/her patient, the final diagnosis (this will remind us of the famous namesake novel by Arthur Hailey centered on the chief pathologist Joe Pearson) usually rests on the pathologists who have to take all the responsibilities to declare the presence of this dreadful disease.

The clinician usually sends a small tissue sample from the suspected organ which he collects either in an OT setup or in an outpatient procedure room which we called "biopsy." If the biopsy sample is collected by taking out a small bit of tissue from the tumor or growth, we call it incisional biopsy, and if it is collected after taking out the whole tumor, we call it excisional biopsy. Whether it is a small incisional biopsy or the whole specimen, it should be sent to the pathology department in an appropriate container containing formol saline, a fluid mixture of formalin and normal saline. This fluid maintains the tissue architecture and prevents degeneration of cells. This biopsy sample should be accompanied by a requisition slip that contains detailed information regarding the tissue, the clinician's impression about the growth accompanied by relevant history of current illness, relevant operative note, and few other technical information that are absolutely necessary for the pathologists to derive an evidence-based conclusion. Whenever the pathology department receives a biopsy sample, the sequential steps that follow are as follows:

A unique identification number is provided after entering into a register.
The tissue is kept in freshly prepared fixative solution overnight.
On the next day, the tissue piece or organ is taken out from the container, and if the tissue is small, as in the case of incisional biopsy or biopsy collected through endoscopy, the whole specimen is put in a cassette for histological processing. If the tissue is not a small one, its appearance, size, surface, etc., are noted, then it is dissected on a paraffin tray with the help of dissection instruments (forceps, knives, scissors, and others) following an established protocol and the details of description noted on the requisition form. This process is known as *grossing*. This is very much important for diagnosis of cancer as the cancerous tissue has some characteristic features that are revealed during this process and without which the

diagnosis of cancer will be incomplete. These gross findings are an important part of final report.

After grossing is done and small tissue pieces thus obtained are put into the cassettes, labeled with the identification number provided to it earlier during registration, and these cassettes are then put into an automated tissue processor or *histokinet* where these cassettes containing tissue pieces are dipped into different chemical solutions for a particular period of time (set by timer) to drive out the water portion of the tissue and make it suitable for paraffin embedding.

Paraffin embedding is a process by which the tissue pieces contained in the cassettes are immersed in paraffin bath (hot and liquefied paraffin wax) and then cooled down to make a paraffin-embedded block in which the tissue is impregnated inside the 3D paraffin blocks. Now, these tissues are called formalin-fixed paraffin-embedded (FFPE) tissues. This embedding is meant for hardening the tissue so that sections can be made for examination under microscope.

Now, these paraffin blocks, with the identification or accession number inscribed on one side of the block, are cut into thin sections or slices of about 3–5 micron thick in a microtome machine.

These sections are then taken on glass slides, deparaffinized, and stained to make the tissue and the cells in it to view under microscope.

10.12 Final Diagnosis by a Pathologist

The task of a histopathologist is quite difficult because, unlike the clinician, he is not examining the patient directly, and therefore, he has to depend solely on the clinical information provided in the requisition slip by his/her clinician friend. After examining the histology slides under microscope, he/she has to correlate his/her microscopic findings with that of the clinical and operative notes, biochemical reports, and the findings noted during grossing to arrive at a logical conclusion. When he corroborates the positive with the negative findings for a particular diagnosis, his analytic mind follows the dialectic pathway to arrive at the final diagnosis.

Why the process of deduction is dialectic in nature? Because the pathologist has to explore all possibilities with an open mind without any clinical bias on the one hand, while on the other hand trying to integrate the clinical findings with the histological findings so that the final diagnosis is a logical conclusion in that particular case. As biological science as a subject is full of variances, and multiple variants act on the disease process (e.g.. age, sex, geographical location, ethnicity, food habit, lifestyle, education, and culture), the same logic is not applicable in all similar cases, that is, the logic varies from case to case, depending on the variables involved in each case. As this logical conclusion is the highest level of cognitive function, error is not a rare thing and this might sometimes prove costlier for the patient. Several procedures may be followed by the pathologist to minimize errors: using proper and relevant clinical information along with the reports of biochemical and radiological investigations, interviewing the patient and/or the relatives for bridging the gap in information, regressing the specimen to corroborate with

histology findings, recutting the section from the block to get a thinner and deeper section, and using special histochemical stains if needed. Ancillary methods like immunohistochemistry (stains tagged with antibody to detect antigens expressed in the tissue), cytogenetics (alterations in chromosomes that are characteristics of a particular tumor), and molecular genetic study may be used where indicated. Apart from these routine techniques for diagnosis of cancer and predicting the prognosis and guidance to therapy, there are certain ancillary diagnostic tools available at state-of-the-art laboratories to help the final diagnosis. These are as follows:

Flow cytometry can rapidly and quantitatively measure several cellular antigens, particularly in cases of leukemias and lymphomas. It has the advantage over IHC as multiple proteins (antigens) can be assessed simultaneously on individual cells.

Circulating tumor cells can be detected in blood as in cases of many carcinomas and melanomas, and their identity is confirmed by coating them with antibodies.

Molecular and cytogenetic techniques such as antigen receptor gene rearrangement detected by PCR-based evaluation in T- and B-cell neoplasms, detection of genetic translocation by routine cytogenetic analysis, or FISH (fluorescent in situ hybridization) in leukemias and lymphomas, NGS (Next Generation Sequencing) that can cover entire human genome to detect any genetic mutation anywhere in the genome, or SNP (single nucleotide polymorphism) chips which allow high-resolution mapping of nucleotides (either deletions or amplification). Have also profound implications in prognosis of malignant neoplasms, detection of minimal residual disease after treatment, diagnosis of hereditary predisposition to cancer, and guiding therapy with oncoprotein-directed drugs.

10.13 Molecular Profiling of Tumors: The Future of Cancer Diagnostics

Until recently, molecular studies of tumors involve the analysis of individual genes in selected cancer. However, the past decade, particularly after completion of Human Genome Project, has seen the introduction of revolutionary technologies that can rapidly sequence an entire human genome; assess epigenetic or around the gene modifications (the epigenome); quantify all the RNA expressed in a cell population (the transcriptome); measure many proteins simultaneously (the proteome); and take a snapshot of all of the cell's metabolites (the metabolome). DNA sequencing is technically simpler than RNA sequencing, permitting the development of massively parallel sequencing methods (so-called next-generation [NextGen] sequencing). The time taken for NextGen for an individual tumor today is 28 days, and the cost has fallen under $3,000.

These advances have enabled systemic sequencing and cataloging the genomic alterations in various human cancers under a consortium, The Cancer Genome Atlas (TCGA). The complexity of the genetic aberrations identified in these genome-wide studies has inspired biomedical informaticians to display the data in a creative way, known as circos plot, which provides a snapshot of all the genetic alterations present

in a particular tumor. This information is required for a "personalized" approach if targeted therapy is to succeed.

Another molecular method that is moving rapidly in clinical practice is "DNA microarray" to identify changes in DNA copy number, such as amplifications and deletions. Other "omics" such as proteomics and epigenomics are currently being used mainly in the field of clinical research.

These developments in technology have led some scientists to predict that the end of histopathology is in sight. Though it sounds as a premature one, it can well be argued that the histopathologists will remain the key anchor for the show; we are probably in the midst of a paradigm shift in which the most important workup of a cancer specimen is the identification of molecular targets, rather than histopathological diagnosis. For example, the histologically distinct cancers all often harbor the same gain-in-function mutation of BRAF, a serine/threonine kinase, and a component of RAS signaling pathways. All these "BRAFomas" are candidates for treatment with BRAF inhibitors. But again for predicting the response to such therapy, histological subtypes are important, which obviously fall within the domain of histopathologists. Moreover, histopathological inspection of tumor will provide information about other important characteristics such as anaplasia, invasiveness, and tumor heterogeneity. Thus, what lies ahead is not the replacement of one set of techniques by another. On the contrary, the most accurate diagnosis and assessment of prognosis in cancer patients will be arrived by a combination of morphologic and molecular techniques.

Medical science has been immensely enriched by fundamental research in other scientific fields including basic sciences. The knowledge base in genetics and immunology is expanding in an exponential way, and it is impossible for an individual to keep track with these developments. Therefore, the perspectives of a pathologist are gradually drifting from an individual effort to a team effort. Moreover, integration with clinical and allied diagnostic departments is a must for an effective problem-solving approach to the patients. A pathologist will definitely sharpen his macroscopic and microscopic skills and pursue a dialectic approach of problem-solving, but he has to confirm his diagnosis also by judicious use of special stain, genetic markers, immune markers, and other ancillary techniques. Only a good morphological observation is not sufficient nowadays, due to advent of targeted therapy. Therefore, the perspectives are changing and a pathologist should remain updated and relevant in the diagnosis and management in oncology.

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