4 Use of Immunohistochemical and Molecular Studies in the Evaluation of the Sebaceous Neoplasms

Doina Ivan, Victor G. Prieto, and Phyu Aung

Introduction

Sebaceous glands are widely distributed on the body, with the exception of hands and feet. They vary in size and density depending on the anatomic areas and there are more numerous on the head and neck, especially on nose, forehead, and scalp, but also on the midline of the back, external auditory canal, and anogenital area. With few exceptions, sebaceous glands are associated with follicular structures and are connected to the follicular infundibulum. There are also "free sebaceous glands" or "ectopic" which lack the association with the follicular structures and involve the vermillion border of lips, areola, glans penis or labia minora, and even less common, the esophagus and tongue [1–5].

A mature sebaceous gland is composed of sebaceous lobules connected to the follicular infundibulum with a sebaceous duct. At the periphery of the sebaceous lobules there is an outer layer of germinative, immature sebocytes with a basaloid appearance and scant cytoplasm and centrally located are maturing or mature sebocytes cells with abundant multivacuolated cytoplasm and centrally placed, scalloped nuclei. The sebaceous duct is lined by keratinizing stratified squamous epithelium and a compact cornified layer [1, 6].

Sebaceous neoplasms are composed of variable proportion of germinative cells and sebocytes, more or less mature. Usually, the sebaceous nature of a cutaneous adnexal neoplasm is established on the basis of the presence of cells resembling mature sebocytes (i.e., cells with multivacuolated cytoplasm and centrally placed, scalloped nuclei) (Fig. 4.1). However, in some sebaceous tumors (such as poorly differentiated sebaceous carcinomas or some sebaceomas) the predominant cells are those recapitulating the germinative sebaceous cells and their origin is very difficult or impossible to recognize. The less mature sebocytes have rather few intracytoplasmic vacuoles and the cytoplasm is either eosinophilic or finely granular while the nucleus is either displaced to periphery or it is round and centrally placed [1, 6]. The identification of sebaceous duct differentiation and its distinction from other types of ductal differentiation may be difficult, but the presence of unequivocal mature

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sebocytes in vicinity is very helpful in the diagnosis.

Considering all these challenges, histochemical and immunohistochemical studies may be used to aid the diagnosis and confirm the sebaceous differentiation of an adnexal neoplasm. Immunohistochemical markers for sebaceous differentiation are further discussed in this chapter along with other immunohistochemical markers that have been reported to have prognostic significance or therapeutic implications.

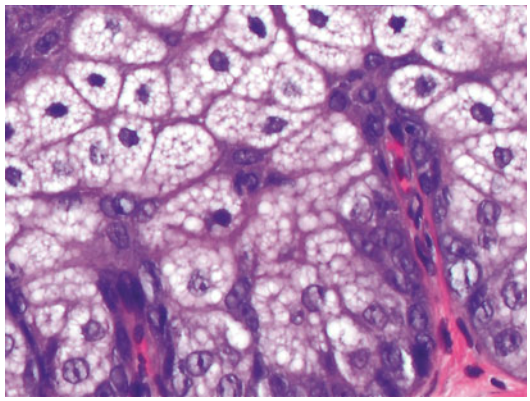

Fig. 4.1 The presence of mature sebocytes in cutaneous adnexal neoplasm is the hallmark for sebaceous differentiation. The cells have multivacuolated cytoplasm and centrally placed, scalloped nuclei

The ectopic and some benign sebaceous lesions, such as sebaceous hyperplasia, are almost exclusively sporadic and there is no currently described their association with systemic syndromes. However, other types of sebaceous neoplasms (especially adenomas, sebaceomas, and less often sebaceous carcinomas) may represent the cutaneous manifestations of systemic syndromes and are best known for their association with Muir–Torre syndrome (MTS), which is an autosomal dominantly inherited phenotypic variant of hereditary nonpolyposis colorectal cancer syndrome (HNPCC) or Lynch Syndrome [7–9]. The potential association of sebaceous tumors with internal malignancies and Muir–Torre syndrome was increasingly recognized in the recent years and their distinction is of utmost clinical significance. Well-characterized genetic alterations have been described and implicated in Muir–Torre syndrome pathogenesis and a detailed description of these along with current recommendations for patient's genetic testing and potential strategies for targeted therapies are provided in this chapter (see Fig. 4.2).

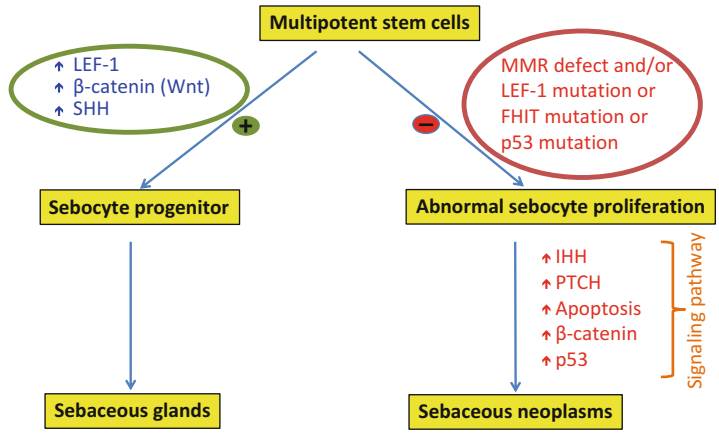

Fig. 4.2 Schematic presentation of the molecular mechanisms involved in the formation of sebaceous neoplasms. The left side of the figure represents the development of the normal sebaceous glands from the multipotent stem cells by normal regulation of β-catenin (Wnt), through Lef-1 transcription factor and sonic hedgehog signaling pathway. In contrast, the right side of the figure demonstrates abnormal proliferation of sebaceous glands (tumorigenesis) by loss of MMR proteins/MSI and/or mutations in *LEF1*, *FHIT* and/or *p53*, leading to activation of the Indian hedgehog and patched pathways, as well as inhibition of p53 and β-catenin (Wnt) signaling pathways.

Clinical Presentation and Histologic Features of Sebaceous Neoplasms

There is a wide spectrum of sebaceous neoplasms ranging from hamartomatous to benign to malignant entities that derive from the sebaceous glands. The classification and the nomenclature of sebaceous neoplasms are relatively confusing and often considered controversial. Sebaceous differentiation in other tumors, other than sebaceous tumors per se, can be encountered in cutaneous adnexal neoplasms with multilineage differentiation as well as in other epithelial lesions, such as squamous cell or basal cell carcinoma, verruca vulgaris or seborrheic keratoses, especially occurring on the face and head and neck area.

Some of the most commonly encountered sebaceous lesions are:

Ectopic Sebaceous Lesions

Sebaceous glands are usually part of the "pilosebaceous unit" and are found in association with hair follicle [1, 2]. However, especially at the mucosal sites, they lack this association and present as small yellow papules, commonly known as Fordyce spots [2, 10]. Their incidence increases with age and since their prevalence is high in general population are considered a normal physiologic variant. They are frequently noted at the vermillion border of the lip, buccal mucosa, medial aspect of labia majora, labia minora, penis (also called Tyson's glands), and rarely in the uterine cervix, vagina, esophagus, or gastroesophageal junction [2–5, 10–13]. They may be also found on the breast areolae, where they are known as Montgomery's tubercules [2, 14, 15]. Histologically the ectopic glands are characterized by sebaceous lobules or small clusters of sebocytes that open directly onto the epithelial surface and lack association with a follicular structure (Fig. 4.3).

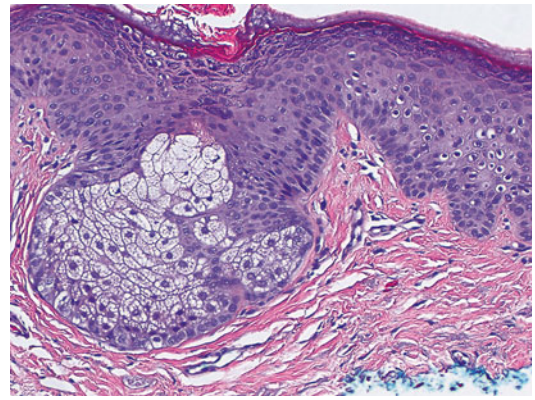

Fig. 4.3 Fordyce spots are ectopic sebaceous glands characterized by lobules or small clusters of sebocytes that open directly onto the epithelial surface and lack association with a follicular structure

Hamartomatous Sebaceous Lesions

Hamartomatous lesions, such nevus sebaceus of Jadassohn, described originally in 1895, represent complex hamartomas involving not only sebaceous glands but also epidermis and dermis. Nevus sebaceus of Jadassohn is also referred to as "organoid nevus" is commonly seen on head and neck area, especially on the scalp, but also forehead, face, postauricular and less commonly the trunk, extremities, oral or perianal region [16–18]. It usually presents since birth as an area of alopecia with a cerebriform appearance and yellow discoloration and it usually enlarges during adolescence under the influence of pubescent hormonal stimulation. In the adults it may also change due to the development of mostly benign and rarely malignant tumors of variable differentiation [17]. It is estimated that about 10–20 % of nevus sebaceus of Jadassohn are complicated by additional proliferations, often multiple and syringocystadenoma papilliferum is the most common association [19–21]. Sebaceous carcinoma arising in nevus sebaceus of Jadassohn is a rare and late occurrence, mostly encountered in the 6th or 7th decade of life [22–24].

Histologically, nevus sebaceus of Jadassohn is characterized by epidermal acanthosis, papillomatosis with an abnormal hair papillae-like proliferations and connections of the sebaceous lobules directly onto the surface epidermis or to the infundibulum of vellus hair follicles. Nevus sebaceus of Jadassohn has a variable number of sebaceous glands, ranging from hyperplastic, increased in number to diminished in number or

even absent and characteristically there is a variation in the morphology and distribution of sebaceous glands. A common finding is the absence or significant reduction in the number of mature hair follicles. Induction of hair follicles also occurs, especially on the scalp lesions, as numerous follicular germinative cells (basaloid cells) proliferate and form basaloid hyperplasia or incipient forms of trichoblastoma. Glandular changes, especially increased number and size of apocrine glands were described in approximately 80% of cases of nevus sebaceus of Jadassohn [16, 17].

In majority of the cases nevus sebaceus of Jadassohn occurs in a sporadic fashion. However, familial cases have been described and sometimes nevus sebaceus of Jadassohn, especially when has a linear appearance, may be part of epidermal nevus syndromes family. and associated with other abnormalities, particularly neurological syndromes, such as mental retardation and seizures, but also with ocular and musculoskeletal deficiencies. The epidermal nevus syndromes that comprises nevus sebaceus of Jadassohn are Schimmelpenning–Feuerstein–Mims syndrome phakomatosis pigmentokeratotica and SCALP (sebaceous nevus, central nervous system malformations, aplasia cutis congenital, limbal dermoid, and pigmented nevus) [25–29].

The pathogenesis of nevus sebaceus of Jadassohn is thought to be caused by genetic mosaicism, but a specific gene responsible for its clinical manifestations is unknown. Loss of heterozygosity of the *PTCH1*(*Drosophila* patched) gene has been described in nevus sebaceus of Jadassohn by Xin et al. but this finding was not supported by subsequent studies [30, 31]. Based on the whole exome sequencing, sebaceous nevi are associated with activating HRAS p.Gly13Arg and KRAS p.Gly12Asp mutations [32].

Steatocystoma Multiplex

Steatocystoma multiplex is characterized by multiple small, yellowish, dome-shaped papules or cysts, usually found in the axillae, chest but also on face, scalp, trunk, extremities, etc. [33, 34]

On histology, there are cysts with undulating, stratified, thin squamous epithelium without a granular layer and with characteristic presence of flattened sebaceous lobules either within or adjacent to the cystic wall [33, 34].

When single (steatocystoma simplex), the lesion occurs in a sporadic fashion. Familial cases of steatocystoma multiplex with autosomal dominant inheritance are well described. Mutations in *KRT17* on chromosome 17 have been documented in families with steatocystoma multiplex and are similar with those seen in pachyonychia congenita type 2 (Jackson–Lawer syndrome) [35, 36]. Rarely multiple steatocystomas have been reported in association with familial syringoma, trichoblastomas, keratoacanthomas, hypertrophic lichen planus, and also hypohidrosis and hypotrichosis [37].

Benign Sebaceous Lesions

Sebaceous Hyperplasia

The most common sebaceous neoplasm is represented by sebaceous hyperplasia which occurs as asymptomatic, solitary or multiple, umbilicated yellow papules on the forehead and face of older individuals and occasionally in younger individuals [1, 38]. Familial cases with early onset have been described. There is a significant increased incidence of sebaceous hyperplasia following renal transplantation, and it was described as being related to therapy with cyclosporine A [39, 40]. The histologic examination reveals an enlarged sebaceous gland with numerous lobules grouped around a centrally located duct.

It is known that the sebaceous gland development is affected by androgens. Sebaceous hyperplasia may also occur after trauma and it is thought that this is due to upregulation of the EGF-EGFR (epidermal growth factor receptor) and the Hedgehog-PTCH signaling pathway [41–43]. There are also studies that show that sebaceous hyperplasia in transgenic mice may be induced by the overexpression of a member of tumor necrosis factor (TNF) ligand family [43].

Sebaceous Adenoma

Sebaceous adenomas are benign tumors derived from sebaceous glands that occur commonly on the head and neck region of older individuals as slowly growing, tan-yellow or pink, small (less than 5 mm) papules [1, 38, 44]. Histologically, they have a lobular, organoid growth pattern, are well-circumscribed and often connected to epithelial surface. Sebaceous adenomas have an increased number of undifferentiated basaloid cells at the periphery of the lobules and more mature sebocytes centrally located. The proportion between these two cell types is variable but sebaceous adenoma is comprised by at least 50 % mature sebocytes [1, 38, 44] (Fig. 4.4).

The association between the presence of usually multiple sebaceous adenomas, often outside of a head and neck location and possible cystic appearance, and Muir–Torre syndrome has been extensively described and is further presented.

It has been recently described that a subset of sebaceous adenomas may harbor inactivating mutations in LEF1, the gene encoding a transcription factor in the Wnt/β-catenin pathway [45]. It was also reported that the Hedgehog and c-Myc pathways may also be involved in the tumorigenesis of sebaceous adenomas [43, 46].

Sebaceoma

The nomenclature of this entity is often confusing and controversial. The term of "sebaceous epithelioma" has been used interchangeably but this mostly refers to cases of basal cell carcinoma with sebaceous differentiation [47, 48]. Sebaceoma appears to be the established nomenclature of choice for this distinctive sebaceous neoplasm which has an increased number of basaloid cells that outnumbers the mature sebaceous component.

Clinically, sebaceomas are usually larger (ranging from 5 to 30 mm), fleshy-yellow, slow-growing, circumscribed nodules or plaques. They often occur in the head and neck region, mostly on the face or scalp, but they were also described on the ear canal or eyelid. There is a female predominance and mostly affect older individuals [47, 48].

On histologic examination, sebaceomas have a lobular growth pattern, similar to sebaceous adenomas, but differ from them by the increased number of basaloid, germinative cells (representing more than 50 % of the lesion) (Fig. 4.5). Often sebaceomas involve dermis but sometimes, connection with epidermal surface is noted. Sebaceomas exhibit numerous histologic patterns, including reticulated, cribriform, cystic, rippled patterns, etc. [47–49] Importantly, sebaceomas are relatively well-circumscribed and

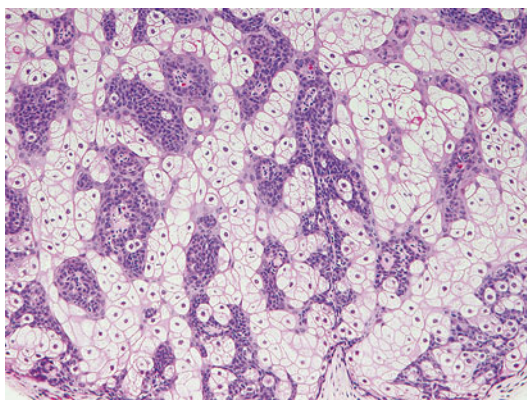

Fig. 4.4 Sebaceous adenomas have a lobular growth pattern and are composed of mature sebocytes (at least 50 % of cells) and an increased number of undifferentiated basaloid cells at the periphery of the lobules

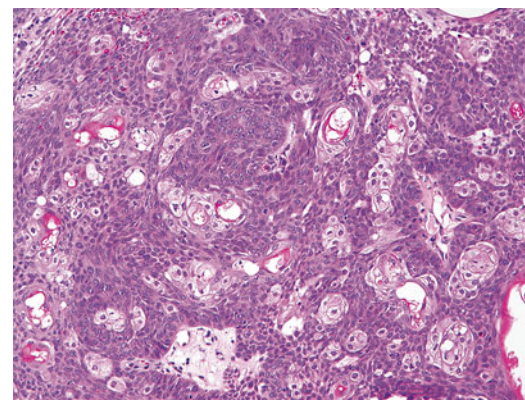

Fig. 4.5 Sebaceomas have a lobular growth pattern, similar to sebaceous adenomas, but differ from them by the increased number of basaloid, germinative cells (representing more than 50 % of the lesion)

lack significant cytologic atypia or an increased number of mitotic figures [50]. However, due to its higher proportion of basaloid, germinative cells and sometimes less obvious presence of mature sebocytes, sebaceomas may be difficult to distinguish from other lesions, such as basal cell carcinomas. The lack of peripheral retraction artifact or associated myxoid stoma is helpful in the differential diagnosis.

Association of multiple sebaceomas with Muir–Torre syndrome has been extensively described and is further discussed.

Malignant Sebaceous Lesions—Sebaceous Carcinoma

Sebaceous carcinomas are relatively uncommon tumors and they may potentially develop from any sebaceous gland and occur at ocular or extraocular sites. Approximately 75 % of cases occur on the eyelids (most common on the upper eyelid), arising mainly from meibomian glands of the tarsal plate and less commonly from the glands of Zeis [51–56]. Extraocular sebaceous carcinomas are predominantly seen in the head and neck area, but can also arise on the trunk, extremities, vulva, penis, etc. [53, 55–57] It was originally believed that the tumors with extraocular location were less aggressive, but it has been shown that these cases have similar metastatic and fatality rates with their ocular counterparts [57, 58]. A recent retrospective review of a large series of cases from the Surveillance, Epidemiology and End-Results (SEER) database of the National Cancer Institute showed no difference in the overall survival between patients with ocular and non-ocular sebaceous carcinoma [58]. Interestingly, the ocular sebaceous carcinomas have a lower likelihood of association with Muir–Torre syndrome than their extraocular counterpart. [57]

Although rare, sebaceous carcinoma is a malignancy with potentially aggressive behavior. Local recurrence complicates 6–29 % of periocular sebaceous cell carcinoma cases [52–56]. Regional or distal metastases affect 14–25 % of patients with a 5-year mortality ranging from 30 % (7–8) to 50–67 % according to different reports [52–58]. The metastatic and mortality rates can be significantly lowered with early detection and treatment but the clinical presentation is notoriously varied and clinical diagnosis is often delayed [59].

Histologically, sebaceous carcinomas are characterized by lobules or sheets of cells with an infiltrative growth pattern in dermis and subcutaneous tissue, or even the underlying skeletal muscle (mostly in the cases involving the eyelid). Sebaceous cell carcinoma in situ often demonstrates pagetoid upward migration of tumor cells, extension into the adnexal structures and may be difficult to distinguish from squamous cell carcinoma. (Fig. 4.6a) The tumor cells of sebaceous carcinoma have often marked cytologic atypia and conspicuous mitotic figures, occasionally atypical (Fig. 4.6b). The degree of sebaceous differentiation varies greatly; in well-differentiated tumors the sebaceous differentiation is usually easily recognized, but in the poorly differentiated forms, mature sebocytes are not conspicuous and the diagnosis is often challenging (Fig. 4.6c, d) Pagetoid intraepithelial spread of neoplastic cells is a common feature and tumor necrosis (sometimes with comedo-like appearance) is often noted [52–58]. Reports of histologically discordant sebaceous neoplasms with architectural features of a benign lesion but significant cytologic atypia have been described in the literature. The authors suggest that these lesions are best fully excised and followed over time, as their behavior is uncertain [56].

Poor prognostic indicators include multicentricity, size greater than 1 cm in diameter, extensive infiltrative growth pattern, and lymphovascular invasion [58].

Sebaceous tumor may occur de novo, but their potential association with internal malignancies and Muir–Torre syndrome has been increasingly recognized in the recent years and this distinction is of utmost clinical significance and is further discussed.

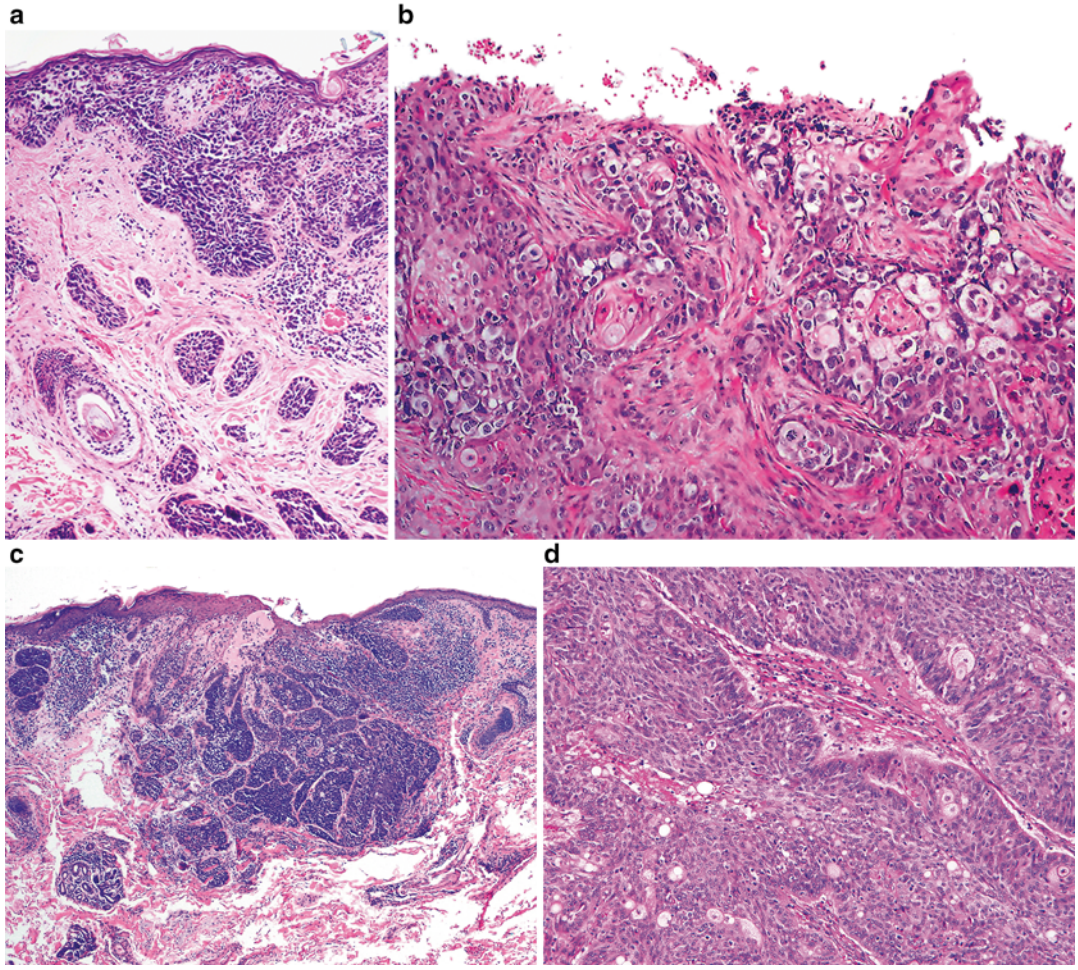

Fig. 4.6 (**a**) Sebaceous carcinomas have an infiltrative growth pattern, extension into adnexal structures, (**b**) demonstrates pagetoid upward migration of tumor cells, and have marked cytologic atypia, conspicuous mitotic figures, and sometimes necrosis. In the poorly differentiated sebaceous carcinoma the mature sebocytes are not conspicuous and the diagnosis is often challenging: (**c**) low power view (magnification 4×) and (**d**) high power view (magnification 4×)

Use of Immunohistochemical Studies in Evaluation of Sebaceous Neoplasms

Immunohistochemical Studies that Support the Sebaceous Differentiation

The presence of mature sebocytes is the hallmark of sebaceous neoplasms. Histologically, the mature sebocytes are recognized by their characteristically centrally placed, indented nuclei and numerous intracytoplasmic lipid droplets (scalloped morphology). However, this distinction may not be so obvious and they may confused with other cells with clear cell histology, such as the clear cells derived from the follicular outer root sheath cells that contain a large amount of glycogen that usually pushes the nucleus to the periphery [2]. These clear cells may be encountered in squamous cell or basal cell carcinomas and their distinction from poorly differentiated sebaceous carcinomas can be

difficult. However, this differential diagnosis has a paramount importance in view of the distinct prognostic features of these lesions.

Traditionally, Oil-Red O and Sudan Black IV have been used to identify intracytoplasmic lipid droplets in the sebocytes of the sebaceous lesions. However, these stains require fresh, frozen tissue for analysis and have a relatively low sensitivity, of approximately 40 % [60].

There is a large number of reports in the literature that describe the use of immunohistochemical studies performed on formalin-fixed paraffin-embedded sections to distinguish sebaceous differentiation, with widely variable results. Single markers or panels of antibodies including CK7, AR, CAM 5.2, EMA and Ber-EP4 were used, but numerous limitations were noted and sometimes contradictory results were obtained.

The androgen receptors (AR) are nuclear proteins that frequently are expressed in normal skin and the sebaceous glands, and their greater prominence in the pilosebaceous units of men than in that of women has led to investigations of their influence on the development of male pattern baldness [61]. Among skin tumors, AR have been found in both benign and malignant sebaceous tumors and it was suggested that the presence of AR by immunohistochemical studies is a reliable and highly sensitive marker of sebaceous differentiation [62]. However, further studies reveal that AR may also be present in up to 60 % of basal cell carcinomas [63, 64].

The recommendation to use CK7 to differentiate ocular sebaceous carcinoma from both squamous and basal cell carcinoma has been reported [60, 65, 66]. However, later studies showed that all these tumors can be positive for CK7, in variable proportions, and there is no definite diagnostic utility of this marker in the differential diagnostic of these tumors, especially when trying to distinguish sebaceous carcinoma from basal cell carcinoma [65, 66].

EMA (epithelial membrane antigen) is a cell membrane-associated glycoprotein that is positive in a number of glandular or secretory tumors, including sebaceous lesions. Several studies have been published describing the usefulness of EMA antibodies in diagnosing sebaceous carcinoma, as most of the sebaceous tumors are positive for EMA. Currently, it appears that the immunohistochemical detection of EMA is useful in differentiating sebaceous carcinoma from basal cell carcinoma (which labels less often for EMA), but not from squamous cell carcinoma. One study showed that all cases of squamous cell carcinoma and 80 % of sebaceous carcinomas were positive for EMA, while the marker was negative in all basal cell carcinoma cases. However, in poorly) differentiated cases of sebaceous carcinoma, EMA can be negative or only focally positive [60, 66].

Ber-EP4 expression has been reported in up to 80 % of sebaceous carcinomas. Fan et al. reported that the use of an immunohistochemical panel using EMA and Ber-EP4 may be especially useful [65]. While sebaceous carcinomas are EMA-positive and Ber-EP4-positive, an immunophenotype with EMA-negative and Ber-EP4-positive supports the diagnosis of basal cell carcinoma. The authors also noted that EMA-positive and Ber-EP4-negative labeling favors squamous cell carcinoma [66, 67].

Undifferentiated basaloid cells situated at the periphery of the sebaceous lobules or part of the sebaceous neoplasms express CK15, a stem cell marker, and also D2-40 and p63 [68–70]. Germinative sebaceous cells are positive for androgen receptors and variably positive for CK8/18, CK19 and also stain for SOX9, while the mature sebocytes are usually negative for the marker [71, 72].

Recently, the use of antibodies against the lipid droplet-associated proteins, including adipophilin and perilipin, has gained interest and has been proven to have a significant role in the identification of sebaceous differentiation and the differential diagnosis of these tumors.

Adipophilin is present in milk fat globule membranes and on the surface of lipid droplets in various normal cell types, including the cells of lactating mammary epithelium, adrenal cortex, steatotic hepatocytes in alcoholic cirrhosis, renal cell carcinoma, hepatocellular carcinomas, pancreatic carcinomas, prostatic carcinomas, and liposarcomas, thus suggesting that lipid droplet accumulation is not an uncommon feature of neoplastic cells. The perilipins are a family of

phosphoproteins found on the surface of intracellular lipid droplets and in the adrenal gland, Leydig cells, and both brown and white fat [73, 74].

A monoclonal antibody against adipophilin can be used on paraffin-embedded tissue assisting the identification of intracytoplasmic lipids, and it has been proven to be very helpful in identifying sebaceous differentiation, including in poorly differentiated neoplasms. Muthusamy et al. showed that adipophilin and perilipin were positive in 88 % (23/26) and respectively 38 % (10/26) of sebaceous carcinomas [73]. Osler et al. studied the expression of adipophilin in 117 sebaceous lesions and other cutaneous tumors with clear cell histology which may mimic sebaceous tumors and noted that adipophilin was positive in 92 % of sebaceous carcinomas and all cases of sebaceous adenoma, xanthelasmas, and 65 % of metastatic renal cell carcinomas [75]. Subsequent studies similarly have shown remarkable sensitivity of adipophilin in detection of sebaceous carcinoma (97–100 %), but a lower specificity (ranging from 35 to 77 %) than the one originally noted. This discrepancy stems in part from differences in the interpretation of what constitutes true positive staining [76–78]. Ostler et al. noted the membranous and vesicular ("mulberry") staining of intracytoplasmic lipid vacuoles in sebocytes (Fig. 4.7) and also a "granular, non-specific" labeling in the background stroma or other cells. This was originally attributed to possible cross-reactivity with keratohyalin granules and Odland bodies (lamellar bodies, composed of phospholipids associated with lysosomal membranes) [75]. However, Boussahmain and colleagues proposed that "granular" staining is not nonspecific, but rather reflects reactivity with small intracytoplasmic lipid droplets, which show clustering and localization to the outer nuclear membrane in a reproducible pattern. It was also noted the presence of lipid droplets in a wide variety of normal metabolically active cells and in cells altered by neoplastic processes [76]. Straub and colleagues found that adipophilin is nearly ubiquitously expressed in normal human tissues, including in the basal keratinocytes of epidermis with a "dot-like" or "granular" pattern

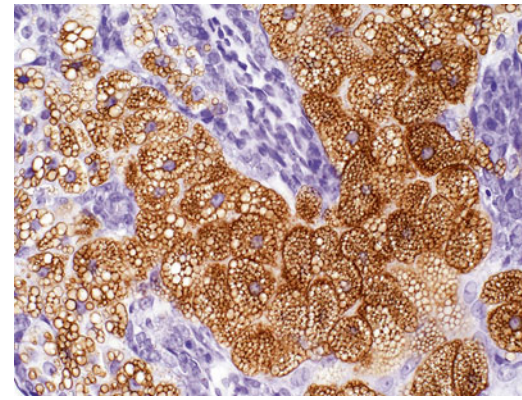

Fig. 4.7 Adipophilin—membranous and vesicular staining of intracytoplasmic lipid vacuoles in sebocytes

and further demonstrated in normal sebocytes and sebaceous neoplasms the "vacuolar" adipophilin labeling of the lipid droplets, by far exceeding the extent expected by light microscopy [73] (Fig. 4.8a, b). Recently, Milman et al. supports the observation on frequent expression of lipid droplet-associated proteins in neoplastic cells due to steatogenesis, but concurs with Ostler et al. that the pattern of adipophilin expression can be useful in distinguishing sebaceous carcinoma from other masquerading periocular neoplasms. [78] They found that the presence of greater than 5 % vacuoles and less than 95 % granules to be 100 % sensitive and 100 % specific in distinguishing sebaceous carcinoma from other periocular and ocular carcinomas [78]. In a recent study of Plaza et al. it has been noted adipophilin expression in all cases of sebaceous carcinoma with a membranous labeling of intracytoplasmic lipid globules and granular uptake in the cytoplasm of 76 % of basal cell carcinoma and 50 % of squamous cell carcinoma (none of those cases showed membranous labeling of intracytoplasmic lipid globules) [66].

In conclusion, when attempting to differentiate tumors with clear cell histology in periocular area, especially sebaceous cell carcinoma from squamous cell or basal cell carcinoma with clear cell features, adipophilin is a very useful immunohistochemical marker, with particular attention being given to the pattern of staining: intracytoplasmic membranous and vesicular type.

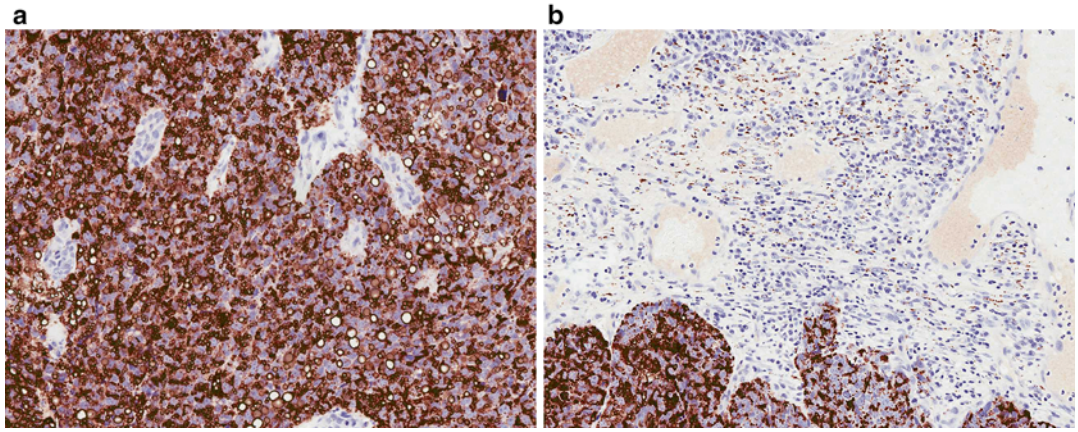

Fig. 4.8 (**a**) Adipohilin expression in poorly differentiated sebaceous carcinoma with membranous and vesicular labeling of intracytoplasmic lipid vacuoles and (**b**) adipophilin background, granular, "nonspecific" labeling

Recent work suggests the differential diagnostic value in sebaceous neoplasms of immunohistochemical studies for proteins involved in lipid synthesis and/or processing, namely alpha/beta hydrolase domain-containing protein 5 (ABHD5), progesterone receptor membrane component-1 (PGRMC1) and squalene synthase (SQS) [73, 79]. Perilipin regulates lipolysis by physically binding to the co-lipase ABHD5, thus reducing the interaction of ABHD5 with adipose triglyceride lipase. Mutations in *ABHD5* results in decreased lipid degradation. PGRMC1 is part of a multiprotein complex that binds to progesterone and other steroids to link extracellular signals to P450 activation. It plays an important role in regulating cholesterol and hormone synthesis and turnover. SQS, also known as farnesyl-diphosphate farnesyltransferase 1, catalyzes the biosynthesis of squalene and cholesterol, the major lipid components of sebum [73].

Plaza et al. found that these markers are very specific, but not very sensitive for sebaceous carcinoma, since PGRMC1 was expressed in 81.4 %, SQS in 51.8 %, and ABHD5 in 70.3 % of cases. None of basal cell or squamous cell carcinoma included in the study expressed any of these markers [66].

In conclusion, it is recommended that an immunohistochemical panel consisting of adipophilin, EMA and possibly AR to be used for the highest sensitivity and specificity for the differential diagnosis of periocular sebaceous carcinoma from basal or squamous cell carcinoma with clear cell differentiation.

Immunohistochemical Studies with Prognostic Significance or Potential Therapeutic Implications in Sebaceous Neoplasms

P53 is a transcription factor protein encoded by the p53 tumor suppressor gene that induces apoptosis or cell cycle arrest in cells with damaged DNA. Mutation of p53 in non-melanoma skin cancers and previous reports noting p53 staining in sebaceous carcinomas are well documented [80]. Aberrant or absent p53 signaling was identified as potential mediator of an alternative mechanism of malignant sebaceous tumorigenesis (distinct from the microsatellite instability pathway) [57, 80]. Cabral et al. examined 27 benign and malignant sebaceous lesions and found statistically significant increased percentages of p53-positive cells in carcinomas compared with adenomas and a trend for the intensity of p53 staining to be greater in carcinomas compared with benign lesions [81]. Shalin et al. showed that nearly one-quarter of examined carcinomas showed p53 staining, whereas all adenomas were negative and only 1 sebaceoma was positive [57]. Moreover, the same study

demonstrated a strong association between p53 dysregulation and periocular tumor location. Interestingly, when p53 staining was compared with expression of DNA mismatch repair proteins in sebaceous lesions, in cases with p53 overexpression, mismatch repair proteins were intact, confirming microsatellite stability, suggesting a divergent signaling mechanisms can contribute to sebaceous neoplasia [57].

A recent study by Kiyosaki et al. found a high percentage of p53 mutations in a small group of eyelid sebaceous carcinomas, but the mutations were not the typical tandem mutations induced by UV damage, raising the possibility that p53 dysregulation as a mechanism of sebaceous tumorigenesis may occur independent of UV damage [82]. In the same study, there was no significant correlation between p53 expression and clinical-pathologic findings, but p21, an inhibitor of cyclin-dependent kinases, induced by p53-dependent and independent pathways, has been shown that inversely correlates with disease stage and lymph node metastases of sebaceous carcinoma. Therefore, it was suggested that p21 immunoreactivity may be used as a tool for prediction of nodal metastasis in sebaceous carcinoma of the eyelid [82].

Dysregulation of cell cycle progression is strongly associated with the development of cancer and tumor progression. Kim et al. showed that high expression of p21, p27, cyclin E, and p16 was found in the majority of cells of sebaceous carcinoma, whereas these proteins were rarely expressed in the normal sebaceous glands [83]. Notably, it was reported that decreased p27 expression correlate with poor prognosis and increased metastatic potential [83]. Loss of $p21^{WAF1}$ compartmentalization in sebaceous carcinoma has been described being helpful for the differential diagnosis from sebaceous adenomas when used as a part of the panel including p53, Ki67, bcl-2, and p21 [84].

Proliferative markers, including PCNA and Ki-67 (MIB-1), are typically elevated in sebaceous carcinomas, and Hasebe et al. showed that carcinomas with a PCNA index greater than 20 % had a worse prognosis [85]. Cabral et al. found that carcinomas had statistically significantly increased levels of p53 in comparison with sebaceous adenomas (50 % versus 11 %, respectively) and Ki-67 (30 % versus 10 %). The carcinomas also had significantly reduced levels of bcl-2 (7 % versus 56 %) and p21 (16 % versus 34 %) compared to the adenomas [81].

Survivin is a member of the inhibitor of apoptosis family of proteins implicated in the inhibition of apoptosis and cell cycle control, both crucial in the progression to malignancy. A study of Calder et al. shows that survivin is expressed more often in sebaceous carcinoma in comparison with sebaceous adenoma and hyperplasia but the study has limitations due the relatively small number of cases studied [86].

Epithelial–mesenchymal transition (EMT) plays an important role in tumor invasion and metastasis in various malignancies and ZEB2/SIP1 is an important EMT regulator and downregulates E-cadherin expression [87]. A recent study reported cytoplasmic overexpression of ZEB2 and membranous loss of E-cadherin were seen in 68 % and respectively 66 % of 65 cases of eyelid sebaceous carcinomas ($P=0.002$) and correlated with high-risk features such as advanced tumor stages and large tumor size. Overexpression of ZEB2 also showed significant association with lymph node metastasis ($P=0.046$), orbital invasion ($P=0.049$) and poor survival [88].

Epidermal growth factor receptor (EGFR), a tyrosine kinase growth factor receptor is normally expressed in periocular surface epithelium, in the conjunctival goblet cells and sebocytes. Ivan et al. showed that EGFR expression is greater in extraocular than periocular sebaceous carcinomas in terms of both distribution and intensity, suggesting a different pathogenic mechanism. Interestingly, the sebaceous carcinomas associated with Muir–Torre syndrome showed a trend towards lower expression of EGFR since they also tend to behave in less aggressive fashion than their microsatellite-stable counterpart. *EGFR* gene mutations were not identified in the study [89].

Human epidermal growth factor receptor 2 protein (HER2) is a transmembrane receptor protein with tyrosine kinase activity that once is activated could potentially inhibit apoptosis, promote

cellular proliferation, stimulate tumor-induced neovascularization, and activate invasion and metastasis . A recent study showed that increased copies of the HER2 gene were identified in 5 of 42 ocular sebaceous carcinoma samples (11.9 %), including two with amplification. The study also demonstrated EGFR amplification. HER2 protein overexpression and *HER2* amplification in 2 of 33 (6.1 %) cases of sebaceous carcinoma [90]. This study is of particular importance, since potential targeted therapies against HER2 and EGFR might be beneficial for a subset of patients with sebaceous carcinomas.

Molecular Aspects of Sebaceous Neoplasms

Muir–Torre Syndrome

Muir Torre Syndrome (MTS) is a rare autosomal dominant genodermatosis with a high degree of penetrance and variable expressivity, which was originally reported by Muir and Torre in 1967 and 1968, respectively [7, 91, 92]. MTS is recognized as a phenotypic variant of hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome [8]. It is characterized by the occurrence of sebaceous neoplasms such as adenoma, sebaceoma, sebaceous carcinoma, and/or keratoacanthomas with visceral malignancies, including gastrointestinal and genitourinary cancers [8]. In almost 40 % of MTS patients a sebaceous neoplasm was the first clinical manifestation of the syndrome and it was reported that as many as 63 % of the MTS patients presenting with a sebaceous neoplasm have a concurrent internal malignancy or develop an additional one [57]. Therefore, early diagnosis of MTS is crucial not only for the patient, but also may prompt familial genetic testing. Colorectal carcinoma and uterine carcinomas are the most commonly associated internal malignancies when associated with MTS and occur in younger patients (typically <50 years old) in comparison with sporadic cases. Tumors of the renal pelvis and breast have also been recognized as being part of the syndrome. One of the cutaneous manifestation of MTS is keratoacanthoma, that occurs in up to 20 % of MTS patients with or without a concurrent sebaceous neoplasm [9]. Hybrid lesions (keratoacanthoma and sebaceous adenoma), called "seboacanthoma" are rare, but considered to be highly suggestive of MTS [7, 8, 93].

Genetic Aspects of Muir–Torre Syndrome

The deoxyribonucleic acid (DNA) mismatch repair (MMR) genes are essential for the maintenance of genomic integrity. These genes eliminate mismatches in base pairing occurring during DNA replication [7, 94]. Microsatellites are repeated sequences of DNA of 1–6 base pairs in length that are normally constant for a given individual. Certain tumors, as is the case in MTS, will show variation in the size of the microsatellite repeats when compared to normal cells from the same individual. The abnormal length of microsatellites occurs as a result of microsatellite instability (MSI) due to defects in the DNA repair process [7, 95, 96]. A germline mutation in one or more of MMR genes, combined with a second somatic mutational "hit" of the remaining functional allele usually causes genetically unstable tumors by the accumulation of replication errors in microsatellite sequences in patients with MTS [97]. The mismatch repair system is composed of human mutL homolog 1 (hMLH1), human mutS homolog 2 (hMSH2), human mutS homolog 6 (hMSH6), human mutS homolog 3 (hMSH3), human post meiotic segregation increased 2 (hPMS2) proteins, among others. Initially a complex of hMSH2 and hMSH6 binds to erroneous DNA segment and then recruits hMLH1 and hPMS2 leading to excision of DNA segment. Many of the patients with HNPCC demonstrate germ-line mutations in genes encoding DNA MMR proteins MLH1 and/or MSH2 and, less commonly, MSH6, MSH3, MLH3, PMS1, and PMS2 [98, 99]. In MTS mutation of *MHS2* locus are more commonly seen (90 %) that *MLH1* gene mutations [57]. The lack of expression of MSH6 in sebaceous lesions of MTS patients suggests that a *MSH6* gene mutation is also common and considering that MSH6 forms a heterodimer with MSH2, it is conceivable that mutations of

MSH2 lead to MSH6 loss and is not necessary representing a germline mutation in *MSH6* [57]. Isolated mutations in *MSH6* are exceptionally noted. MTS is not definitely yet linked to isolated loss of MSH3 or PMS. [57]

Sebaceous lesions that may be potentially associated with MTS include both benign lesions, such as adenomas and sebaceomas as well as malignant (sebaceous carcinoma). Given the frequent occurrence of sebaceous hyperplasia in the general population and its rare association with MTS (0–10 %) reported by some studies, the association of sebaceous hyperplasia with MTS is still clinically insignificant [7, 8, 100–102]. In contrast, the remaining other sebaceous neoplasms are reported to play an important role as markers of MTS. Among them, sebaceous adenoma seems to be the most common tumor found in association with MTS, with a reported frequency of 25–60 % [7, 100, 101]. On the other hand, association of MTS with sebaceoma and carcinoma ranges from 31 to 86 % and from 66 to 100 %, respectively [101].

Several reports have documented that loss of MMR proteins in sebaceous tumors occurring outside of the head and neck region, in patients less than 50 years old, multiple sebaceous neoplasms, with keratoacanthoma-like and cystic changes, and/or increased intratumoral lymphocytes may be a strong indicator for MTS [93, 102–104]. However, there are different opinions in this matter; for instance one of the reports did not find cystic change to be statistically associated with sebaceous tumors demonstrating loss of mismatch repair protein expression [93].

Molecular and Immunohistochemical Testing for Muir–Torre Syndrome

The test of choice for identifying genetic instability of tumors in MTS, caused by defects in MMR genes, is the detection of microsatellite instability (MSI) by polymerase chain reaction (PCR). Most studies assess MSI in MTS using the five Bethesda markers, which were recommended by the National Cancer Institute as the standard screen for assessing MSI in tumors from patients with HNPCC [105]. The markers include three dinucleotide repeats (D2S123, D5S346, and D17S250) and two mononucleotide tracts (BAT25, BAT26). If MSI is detected in any two of the five markers, it is considered a positive result and indicative of a high probability of MSI [7, 96, 106].

However, in the daily pathology practice this PCR testing is costly and not always available. Immunohistochemical studies have become the preferred initial screen test, as studies have shown them to be excellent surrogates of underlying MMR gene function although it is not well established as in the case of colonic adenocarcinomas [107]. This technique, which is the initial screening test, uses antibodies directed against the MMR proteins, such as MLH1, MSH2, MSH6, and PMS1, and it is relatively easy to perform and interpret because of their nuclear staining pattern. Loss of MMR protein expression is indicated by complete absence of nuclear staining in the lesional tissue [7, 95, 106] (Fig. 4.9a–d).

In studies on unselected sebaceous neoplasms, the positive predictive value of lack of expression of each of the MMR proteins for MTS varies from 33 to 88 % for MLH1, 55 to 66 % for MSH2, and is approximately 67 % for MSH6 [57, 100]. The positive predictive value for MTS increases when the markers are used in combination: is 55 % for MTS tumors with combined loss of MSH2 and MSH6, and 100 % for neoplasms with either dual loss of MLH1 and MSH6, or loss of all three (MLH1, MSH2, and MSH6) markers [105]. In their functional state, the MMR proteins form heterodimers. MSH2 dimerizes with MSH6, forming the functional complex, MutSα; and MLH1 dimerizes with PMS2, forming MutLα. It has been shown that the MSH2 and MLH1 proteins are the obligatory partners of their respective heterodimer. Due to this heterodimeric nature of the MMR proteins, loss of expression of a particular protein may in fact be due to the loss of expression of its paired obligatory partner protein. For example, loss of PMS2 alone indicates a defect in PMS2, whereas, when expression of both MLH1 and PMS2 are lost, this is likely due to loss of MLH1 and results in unstable PMS2. The same is true for MSH6 and MSH2, respectively [50]. Therefore, some authors say that MSH6 and PMS2 may be enough and there

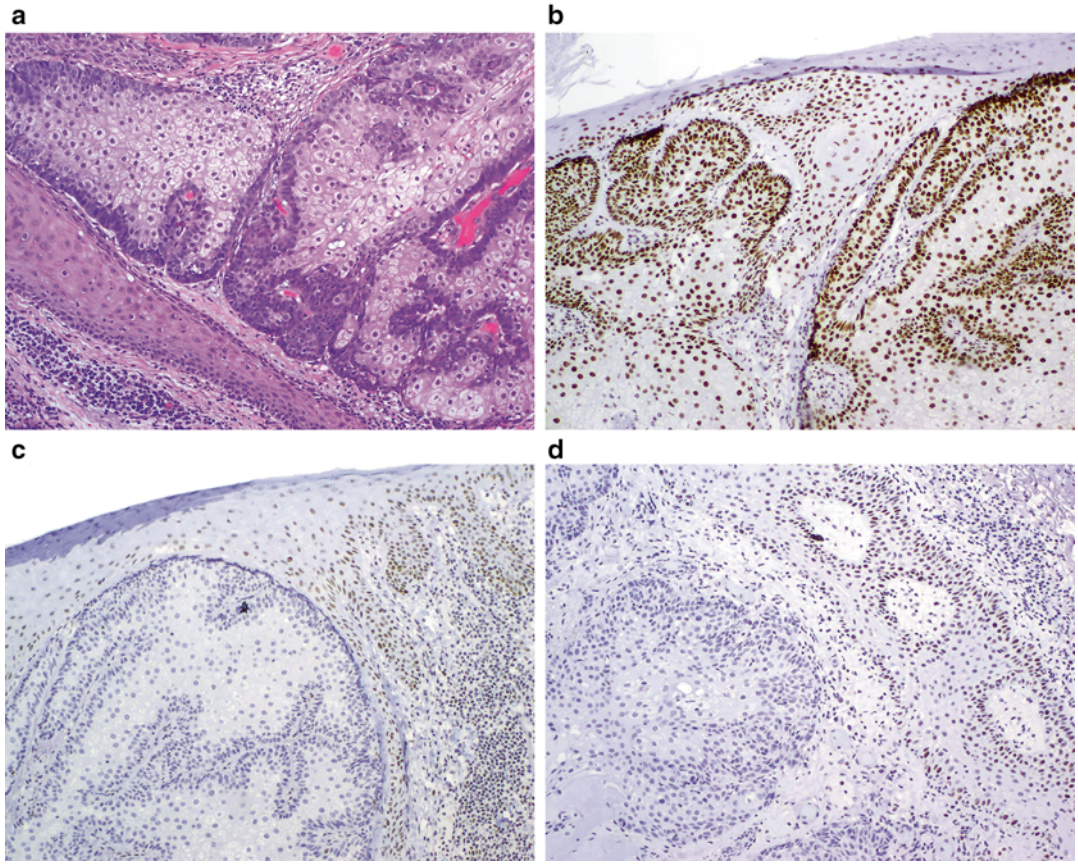

Fig. 4.9 Immunohistochemical studies are used as an initial screening test to determine the loss of MMR protein expression in patients with Muir–Torre syndrome and show: (**a**) Histologic image of sebaceous adenoma (hematoxylin and eosin) with preservation of nuclear labeling of (**b**) MLH1 and loss of nuclear expression for (**c**) MSH2 and (**d**) MSH6

is no need for testing for MSH2 and MLH1 respectively.

Loss of nuclear staining of MLH1 and MSH2 in tumor cells are more commonly found in Lynch syndrome and MTS, although sporadic, non-germline mutated tumors may also show loss of staining. To distinguish sporadic vs. germline MMR loss, hypermethylation of the upstream MLH1 promoter (and subsequent silencing of the MLH1 gene) or BRAF (V600E) mutation testing can be performed on a tumor sample. Presence of either BRAF mutation or hypermethylation is strongly suggestive of sporadic loss of MMR. [108]

In addition to MSI, there must be other mechanisms involved in the pathogenesis of MTS because not all patients with sebaceous neoplasia and/or a characteristic internal malignancy demonstrate MSI. One study reported that 80 % of the internal malignancy-associated sebaceous neoplasms showed loss of expression of MSH-2 or MLH-1 by immunohistochemical method [109]. For instance, it has been reported that the presence of sebaceous neoplasms in patients with MYH (mutY Homolog) mutation-associated gastrointestinal polyposis syndrome did not exhibit MSI [108]. MYH is a protein involved in DNA base excision repair following DNA oxidative damage. The mechanisms by which the genomic instability due to loss of MMR proteins promotes sebaceous tumorigenesis are not well understood.

Studies in mouse models have shown that Wnt/β-catenin, Indian hedgehog, and p53 signaling

pathways along with mutations in numerous tumor suppressor genes such as FHIT (Fragile Histidine Triad), DNA mismatch repair genes, and P53 may contribute to sebaceous tumor formation. After translocation of β-catenin to the nucleus by activation of Wnt signaling, it binds to proteins such as lymphocyte enhancing factor 1 (Lef-1) for gene transcription. A defective β-catenin binding site in the Lef-1 protein in a transgenic mouse causes sebaceous skin tumors due to defective transcriptional activity [110]. Upregulation of Indian hedgehog protein expression occurs in Lef-1 transgenic mice, which increases proliferation of sebaceous precursor cells [43]. This study also suggests that aberrations in β-catenin and hedgehog signaling pathways may promote various cutaneous tumors [108]. Takeda and colleagues found double-nucleotide substitutions in the same *LEF1* allele, irrespective of DNA mismatch repair status, in one-third of human sebaceous adenomas and sebaceomas, resulting in impaired β-catenin binding and decreased transcriptional activity [45].

In addition, the FHIT gene, a tumor suppressor, is a member of the histidine triad proteins and it has been shown that gastrointestinal malignancies and sebaceous lesions develop in transgenic heterozygous Fhit mice, when exposed to carcinogens [111]. Interestingly, no MSI was detected in these tumors [112]. FHIT mutations caused defective programmed cell death, and inhibit β-catenin transcriptional activity [45, 113]. FHIT mutations have), been found in human periocular sebaceous carcinomas regardless of MSI status [114, 115].

Mutations in the DNA-binding regions of p53 are common in skin cancers associated with ultraviolet irradiation [116]. Some sebaceous neoplasms showed increased nuclear immunoreactivity for p53 due to mutations and/or dysregulation of p53 signaling [117]. In contrast, sebaceous tumors developed in transgenic Lef-1 mutated mice showed no expression of p53 protein because of downregulation of its binding partner ARF, a tumor suppressor protein [36]. It has been hypothesized that p53 signaling alterations may represent an early, primary event in a subset of sebaceous malignancies with p53 expression. On the other hand, *LEF1* (a downstream gene) mutations may indicate the secondary effect within the other sebaceous neoplasms with LEF1 mutations [57].

Genetic Testing for Muir–Torre Syndrome

It has been recommended by some authors that the diagnosis of a sebaceous neoplasm located outside the head and neck area in a young patient (<50 years of age) should undergo additional testing for MSI [93]. Immunohistochemical analysis is used as a first line to detect the expression of MMR proteins (especially the more common ones such as MSH2, MLH1 and MSH6). Lack of expression of any one of these proteins should be followed by MSI analyses. If MSI is detected, it should be followed by germline mutation analysis. In a patient with positive results in all the tests mentioned above, cancer surveillance will be required in both the patient and family members. However, not all tumors with loss of these proteins are associated with MTS, and the positive predictive value of a loss of one of these markers is poor unless the clinical setting is taken into consideration. When patients with a positive family history of colon cancer in at least one relative are chosen for testing, the positive predictive value of loss of MMR proteins increased from 22 % to 92 % [105]. If there is a high clinical suspicion of MTS, germline mutation analysis should be done even after a normal finding in the first line immunohistochemical test for MMR proteins and/or the second line MSI PCR analysis [103, 118, 119]. However, if MSI is not detected in a patient with a negative family history, additional genetic tests are not required.

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