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Common Skin Diseases: Skin Cancer

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5.1 Introduction

Skin cancers – basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma – are very common cancers with high environmental risk factors that lead to the highest mutation rates among all cancers [1–3]. Although strong niche-dependent mechanisms help resist oncogenic transformation in the skin despite the presence of oncogenic drivers [4, 5], the staggering amount of accumulated mutations lead to aberrant overgrowths that can destroy local tissues, invade into the underlying dermis, and eventually metastasize to distant sites. Surgical excision of these cancers displays high cure and low mortality rates partly because the identification of precancerous and early-stage lesions is far easier than a tumor growing internally. Metastatic tumors, although rare when compared with the overall incidence rates, require systemic approaches and range from general chemotherapies to targeted precision therapies that typically have high resistance rates. Defining the oncogenic drivers and using combination therapies may present the best method to treat advanced and metastatic tumors. In this chapter, we will discuss the common skin cancers and focus on their risk factors, origins, signaling pathways that drive tumor growth, and common treatments.

5.2 Basal Cell Carcinoma

BCCs are locally invasive epithelial cancers driven by activating mutations in the Hedgehog (HH) pathway [6]. They are the most common skin cancers and make up approximately 60–80% of all skin cancer cases [7], affecting approximately 4.88 million patients in the United States [8], 1.28 million in Europe [9], less than 1 million in Asia [10], and approximately 450 000 in Australia [11] based

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on current population counts. Accurate numbers are difficult to pin down due to a lack of uniform reporting guidelines for non-melanoma skin cancer and a lack of universal application of a staging system by dermatologists. Although BCCs are typically treated by surgical excision, five-year recurrence rates of 3.3% indicate that a large number of patients are not cured by this method [12]. While alternative therapies such as SMO inhibitors (SMOinh: i.e. vismodegib and sonidegib) are FDA-approved to treat advanced BCCs by targeting and suppressing the HH pathway [6], nearly 60% of advanced tumors display inherent SMOinh resistance and 20% of tumors that initially respond acquire resistance every year [13]. This is a highly relevant issue as advanced BCC cases, which are defined as surgically non-resectable, are estimated at 1–10% of total BCC cases [14]. Inappropriate HH pathway activation also drives growth of a variety of cancers including blood, bone, brain, breast, lung, pancreas, prostate, and stomach cancer, accounting for up to 25% of all human cancer deaths [15]. These statistics point to a need to understand how oncogenic HH signaling is controlled and how we can target this pathway to suppress HH-driven cancers.

5.2.1 Risk Factors

The likelihood of developing BCC derives from a combination of genetic and environmental factors. On the far end of the genetic spectrum, basal cell nevus syndrome (BCNS) patients harbor germline alterations in the *PTCH1* gene and can develop hundreds of BCCs throughout their lifetimes [16], presumably driven by persistently high activation levels of the HH pathway. On the other end of the environmental spectrum, ultraviolet radiation from indoor tanning significantly increases the risk of BCC by approximately 4% alone as determined by a systematic review and meta-analysis of appropriate research articles [17]. A combination of these two risk factors significantly increases the number of tumors developed by BCNS patients [18]. Interestingly, genetics seems to play a role in preventing too many more tumors from forming in BCNS patient population as these patients display a lower mutational load, lower proportion of UV mutagenesis, and increased genomic stability in comparison to patients with sporadic BCC tumors that often develop after a lifetime of UV exposure. Light-skinned individuals have far higher incidence than darker-skinned individuals, as seen in the statistics at the beginning of the chapter and reviewed by Porcia Bradford [19]. Photosensitizing drugs that induce either a phototoxic or photoallergic reaction upon UV exposure have been shown to enhance the risk of developing BCC in a population-based case-control study [20]. Other types of radiation, such as those exposed to the atomic bomb, have also been shown to have higher rates of BCC formation, especially when the exposure occurred earlier in life [21]. Of final note on the risk factors for BCC, immunosuppression is a frequently reported significant risk factor that increases over time, especially in organ transplant recipients [22]. The risk of subsequent BCC tumors after the first tumor appearance is high in transplant recipients, where patients who develop BCCs typically continue to develop BCCs [23]. Frequent BCC formation is also a clinical marker for inherited cancer risk of other malignancies, presumably due in part to an increase in pathogenic mutations in DNA repair genes [24].

5.2.2 Classification

BCCs are broadly classified into nodular, superficial, infiltrative, and micronodular subtypes, ordered here with respect to their relative frequencies [25]. BCCs are typically classified by their histological presentation as it is difficult to determine the subtype upon visual inspection of the patient [26]. Nodular BCCs are the most common clinical subtype and typically appear with small telangiectasia (spider veins) and rolled borders. Histologically, they are characterized by large basophilic cell nodules with the surrounding stroma seemingly retracting from the tumors. Micronodular BCCs describe tumors with many microscopic nodules smaller than 15 μm . Superficial BCCs often appear as reddened patches and histologically present as buds of malignant cells extending down from the basal layer of the epidermis. Infiltrative (or morpheaform) BCCs are an aggressive subtype that typically appears as an ivory lesion that may have telangiectasia and may resemble a scar. Histologically, they are characterized as strands of tumor cells invading deeply into the underlying dermis.

The staging of BCC is similar to SCC and have been defined succinctly by the Cancer Treatment Centers of America and summarized in Table 5.1. The staging ranges from small tumors present only locally in the epidermis to larger tumors that have metastasized to distant parts of the body. Although rare, metastatic BCCs have been reported at rates as low as 0.0028% from a questionnaire sent to members of the Australasian College of Dermatologists asking them to estimate the average numbers of BCCs they see each month and extrapolating the total number from the working life of all members [27], to high rates of approximately 0.1% from case series of 9050 cases from the United States [28] and 3634 cases in Europe [29].

5.2.3 Cell of Origin

Multiple stem cell populations are found in the basal layer of the epidermis and hair follicles in both mice and humans. At least in mice, BCC can arise from nearly all of the stem cell populations in the basal layer depending on the strength of the oncogenic driver. Loss of PTCH1 induces tumor formation in $\sim 70\%$ of

Table 5.1 Staging of BCC as defined by the Cancer Treatment Centers of America.

Stage	Stage description
0	Also called carcinoma <i>in situ</i> , the cancer is only present in the epidermis and has not spread deeper into the dermis
I	The cancer is less than 2 cm across, has not spread to nearby lymph nodes or organs, and has one or fewer high-risk features
II	The cancer is larger than 2 cm across, has not spread to nearby organs or lymph nodes, or has two or more high-risk features
III	The cancer has spread locally into facial bones or a nearby lymph node, but not to distant organs
IV	The cancer can be any size and may have spread to the lymph nodes and other distant sites in the body

human BCCs, whereas overexpression of constitutively active SMO is found in ~20% of tumors and constitutively active GLI2 in ~8% of tumors [2]. In mice, loss of *Ptch1* can induce BCCs in the hair follicle bulge, secondary hair germ, and epidermis [30, 31]. Interestingly, when *Ptch1* is lost using specific drivers within the skin and hair follicle compartments, the isthmus and infundibulum also show tumor induction, but the epidermis is largely resistant to tumor growth except at the touch dome epithelia where nerve innervation promotes HH pathway activity [32]. Overexpression of constitutively active SMO, a relatively weak oncogenic driver, can induce BCCs from the upper infundibulum and epidermis [33, 34]. Using a strong oncogenic driver like constitutively active GLI2 can drive nodular BCC formation when expressed in the lower bulge, secondary hair germ, infundibulum, and sebaceous gland compartments of the hair follicle, whereas such expression in the epidermis resulted in superficial BCCs [35]. In humans, BCCs are observed to arise from the epidermis and hair follicle, but the exact cells of origin are not known. If one were to assume superficial BCCs arise from the epidermis and nodular BCCs arise from the hair follicle, ~16% of human BCCs are superficial and 57–78% of human BCCs are nodular [36, 37]. One intriguing observation is that Merkel cells, which are associated with the mouse touch dome, are seen in more superficial human tumors, suggesting at least one common origin at mechanosensory niches [32].

5.2.4 Signaling Pathways

Inappropriate activation of the HH pathway and its target genes are thought to be the sole drivers of BCC with enrichment of GLI protein or RNA signal used as a clinical marker that is present in all BCCs so far observed [38]. HH signaling is essential for the development of all vertebrates and drives proliferation, migration, and differentiation of progenitor cells to pattern organs [39]. Vertebrate HH signals through a microtubule-based sensory organelle called the primary cilium, which largely resides in progenitor cells of the skin and hair follicle [40]. HH signaling components are recruited to this membrane-enclosed structure to sense and respond to extracellular cues. HH ligand binds and inactivates the receptor PTCH1, removing it from the primary cilium and allowing the G protein-coupled receptor SMO to traffic into the cilium to activate the GLI transcription factors. Loss of the primary cilium suppresses tumor growth when the driving mutation is at the receptor level; however, tumor growth can be accelerated in the absence of cilia when the driving mutation is at the level of GLI [41].

Stem cell compartments that are competent to receive HH signal and activate GLI and its target genes drive tumor growth [42–44]. Additionally, pathways that enhance HH signaling are often intimately entwined with tumor growth. For instance, atypical protein kinase C ι/λ (aPKC), a HH target gene and GLI1 kinase, phosphorylates and activates GLI1 to maintain high HH pathway activation levels in both normal and tumor contexts [45]. Inappropriate aPKC activity correlates with poor patient clinical outcome and mortality [46] and loss of aPKC results in severe reduction in GLI activity and tumor growth [45]. aPKC-specific phosphorylation of GLI1 recruits HDAC1, promoting GLI1 deacetylation and activity, and that pharmacological suppression of HDAC1

also suppresses tumor growth [47]. Actin cytoskeletal dynamics driven by RHO can activate SRF and MLK1, which serve as GLI1 coactivators on target genes to drive tumor growth [48]. These are but a few examples of pathways that also activate HH signaling to drive tumor growth.

Recent genomic analysis of all types and stages of BCC reinforces the idea of the HH pathway as a driver of tumor growth [2]. However, the data also point to single nucleotide variants (SNVs) and somatic copy number aberrations (SCNAs) that exist in other pathways thought to be outside of the HH pathway axis. For instance, the Hippo-YAP pathway showed a high frequency of SNVs and SCNAs in PPP6C, LATS1/2, and PTPN14. Subsequent work by others have shown that driving tumor formation with constitutively active SMO activates the Hippo-YAP pathway and that overexpression of Hippo-YAP pathway can drive BCC growth in part by activating the HH pathway [49, 50]. The RAS-PI3K pathway shows lower levels of SNVs and SCNAs in ERBB2, RAS, PIK3CA, and RAC1 [2]. Yet, these pathways are also intimately tied to HH signaling, with the PI3K pathway essential for HH signaling [51] and the RAS pathway serving more as a secondary driver that is suppressed when the HH pathway is active and driving a fate switch to SCC when the HH pathway is suppressed [52, 53]. Finally, the NOTCH pathway is highly altered in BCCs with *Notch1*-deficient mice showing susceptibility to forming both BCCs and SCCs [2, 54]. However, Notch does not seem to be a driver of tumor growth as Notch-inhibited keratinocytes do not form tumors in mice despite spreading rapidly throughout the skin [55]. Notch does seem to help make the tumor susceptible to SMO inhibition [56], suggesting a more nuanced role bridging HH signaling and apoptosis.

5.2.5 Common Treatments

Surgical excision is the most common therapy to treat BCC, although management is often guided by anatomical location and histological features [57]. Common surgical methods include cryosurgery, curettage, electrodissection, Mohs surgery, and standard surgical excision. These methods are typically used for superficial and nodular BCC but are inappropriate for tumors in cosmetically sensitive areas or tumors that are overly large or metastatic. Mohs surgery does appear to be the most effective way to prevent recurrence in primary BCCs and recurrent BCCs compared with standard surgical excision [58].

Nonsurgical methods to treat BCC include radiotherapy, photodynamic therapy, and chemotherapy. Radiotherapy is used for patients who are not good candidates for surgery because of the tumor location or metastasis and is typically avoided in patients younger than 60 years of age. Photodynamic therapy is an alternative way to treat BCC, which uses visible light to activate a photosensitizing drug to generate reactive oxygen species and kill tumor cells [59]. This type of therapy is more effective for superficial BCC than the nodular subtype, and for smaller tumors, where response rate increases with more sessions [60]. For larger tumors, photodynamic therapy leads to higher recurrence rates compared with surgical excision [61], so a trade-off between a better cosmetic outcome with photodynamic therapy compared with surgical excision must be weighed when using this therapy.

The major types of topical chemotherapy treatments include imiquimod, 5-fluorouracil (5-FU), and SMOinh (i.e. vismodegib and sonidegib). Imiquimod is a Toll-like receptor 7 agonist that induces local skin inflammation through interferon-alpha, TNF- α , and other cytokines and can clear up to 75% of superficial BCCs [62]. Serendipitously, imiquimod also serves to suppress HH pathway activation by reducing the active form of GLI [63]. 5-FU is a well-tolerated treatment for BCC with little to no pain or scarring and works by interfering with DNA synthesis by blocking methylation of deoxyuridylic acid and inhibiting thymidylate synthetase [64]. In a five-year randomized controlled trial comparing the effectiveness of photodynamic therapy of imiquimod and 5-FU in 601 patients with superficial BCC, imiquimod resulted in an 80.5% tumor-free survival compared with 70% for 5-FU and 62.7% for photodynamic therapy [65].

Vismodegib was the first SMOinh to be FDA-approved to treat locally advanced and metastatic BCC and is quite effective at suppressing both late-stage tumor growth and Gorlin's syndrome patients [66–68]. However, many tumors regrow after cessation of drug therapy with nearly 60% of advanced tumors displaying innate resistance and 20% of tumors that do respond to drug acquire resistance every year [13], suggesting use of SMOinh are more appropriate for neoadjuvant therapy in combination with a surgical technique. Side effects of this therapy are more pronounced than the other topical chemotherapy treatments and include alopecia, decreased appetite, diarrhea, fatigue, muscle spasms, nausea, and taste and weight loss. Acquired resistance to SMOinh are mainly SMO mutations that prevent drug interaction or promote constitutive activity even in the presence of drug [38]. As such, alternative therapies to treat SMOinh-resistant BCC should target genes that promote HH pathway activity downstream of SMO, such as the SRF-MKL1 [48], aPKC [45], HDAC1 [47], and other pathways described in the “Signaling Pathways” section. Interestingly, recent data point to hair follicle-derived BCCs as more sensitive to SMOinh than ones with an epidermal-derived origin [69, 70]. BCCs are plastic and can switch from a hair-follicle signature to an epidermal signature to evade SMO inhibition and persist in the presence of drug. However, targeting with a WNT inhibitor in combination with SMOinh leads to the eradication of resistant tumor cells and prevents tumor relapse.

5.3 Squamous Cell Carcinoma

Cutaneous squamous cell carcinoma (cSCC) is the second most common of the skin cancers and arise from suprabasal keratinocytes. As such, they have a high degree of cellular heterogeneity where keratinocytes at various stages of post-mitotic differentiation can become proliferative, resulting in difficulty in treating this disease using targeted therapeutics [71]. Although BCCs arise *de novo*, cSCCs are thought to arise from precursor lesions like actinic keratoses (AKs) or Bowen disease (SCC *in situ*) [72]. cSCCs are commonly thought to make up approximately 20% of all skin cancer cases. However, rates of cSCC are estimated to be as high as BCC in the aging US population by administrative data

from the Medicare and Medicaid databases from over 2 million documented fee-for-service procedures [73] and as high as 50% of skin cancer cases in the Singaporean Asian population [10]. At the least, these statistics would indicate cSCCs affect over one million patients in the United States, over 600 000 in Europe, and approximately 500 000 in Asia each year. As with BCC, surgical excision is the most common treatment method where five-year recurrence rates of 8.1% indicate a poorer prognosis compared with BCC. No widespread targeted therapeutics are available to treat cSCCs, pointing to a need to define appropriate therapeutic targets.

5.3.1 Risk Factors

cSCC has similar risk factors as BCC, where genetics and environment play important roles. For instance, indoor tanning more than doubles the risk of cSCC compared with BCC at approximately 8% [17]. Photosensitizing drugs in light-skinned individuals and other types of radiation also increase the risk of cSCC [20, 21]. Immunosuppression also plays a commonly strong role in developing cSCC where the risk factor increases over time and the risk of subsequent cSCC tumors after the first tumor appearance is high [22, 23]. The genetic landscape is far more varied. Instead of one pathway controlling tumor growth, genes involved in cell cycle control, cell survival, activation of mitogens, and gene expression are all implicated in cSCC [74]. Determining which gene is a driver for cSCC is more difficult, as many putative oncogenic mutations are also found in phenotypically normal skin [5].

Where cSCC risk factors diverge from BCC are the role infectious agents play in oncogenesis, human papillomavirus (HPV) produces oncoproteins upon integration into the genome of host keratinocytes and is found in nearly all cervical SCC [75]. Epidermodysplasia verruciformis (EV) patients, a rare autosomal recessive skin disorder, are especially susceptible to HPV-induced cSCC [76]. HPV has been detected in skin and tumor samples, especially those affecting the genital areas or the skin surrounding the fingernails, whereas HPV infection that results in warts on the hands and feet are not associated with cancer [77, 78].

5.3.2 Classification

cSCCs are broadly classified by their histological appearance where they may appear as single cells, small groups of cells, or a single mass [26]. Their diagnosis is always made by skin biopsy. Precursor AK lesions have a dry adherent scale with ill-defined borders, and Bowen disease have sharp borders surrounding scaling plaques. Once the precursor lesion evolves into cSCC, they commonly look like firm, flesh-colored papules or plaques. cSCCs are subdivided into adenoid, tubular pattern with acantholysis; clear cell, keratinocytes appear clear from cytoplasmic swelling and accumulation of lipid vacuoles; spindle cell, spindle-shaped atypical cells; and signet-ring cell, concentric rings of keratin and dilated endoplasmic reticulum. Low-grade tumors resemble mature keratinocytes with intracellular bridges and high amount of keratin, whereas high-grade tumors show atypical features, with loss of intracellular bridges and minimal keratin protein.

The staging of cSCC is similar to BCC and is defined in Table 5.1. The staging ranges from precursor lesions to larger tumors that have metastasized to distant parts of the body. Poorly differentiated disease has a 33% risk of metastasis with over 90% recurrent disease after three years [79], far higher than BCC and much more dangerous.

5.3.3 Cell of Origin

Stratified epithelial cells are the cell of origin for cSCC. However, these cells also line the cavities of the head and neck region, digestive and respiratory tracts, and hollow organs and tumors can arise in these locations as well. In mice, overexpression of constitutively active Ras in the hair follicle bulge, hair germ, outer root sheath, and interfollicular epidermis lead to benign precursor lesions that do not typically progress to invasive cSCC [80, 81]. Constitutively active Ras overexpression in combination with loss of p53 was able to induce invasive cSCC, suggesting a more stepwise evolution of tumorigenesis. These data make sense as cSCCs are thought to arise from precursor lesions like AKs or Bowen disease [72]. AKs are essentially benign intraepithelial cSCCs originating from abnormal keratinocyte proliferation in response to prolonged UV exposure. They are also one of the strongest predictors of subsequent invasive cSCC development [82]. Of note, a systemic analysis of AK literature reveals a very low 0.075% rate an AK lesion will develop into cSCC each year, although this number increases to 0.53% if patients with a prior history of non-melanoma skin cancer [83].

5.3.4 Signaling Pathways

A combination of genetic alterations is thought to drive cSCC development in a stepwise fashion as evident from the cSCC cell of origin studies [80, 81]. This idea makes sense as cSCC is the second most mutated cancer in humans at 45.2 mutations/Mb [3], behind only BCC at 65 mutations/Mb [2], and that a large fraction of putative oncogenic mutations are found in phenotypically normal skin [5]. cSCC tumors are also quite genetically heterogeneous, with TP53 (mutated in ~54% of cSCC), NOTCH1 (~47%), NOTCH2 (~42%), CDKN2A (~37%), HRAS (~13%), and KRAS (~10%) mutations that are highly enriched and can be found in different parts of the same tumor [84, 85], suggesting that some of these mutations act as codrivers of tumor growth and others as passengers. Adding to the list of commonly mutated genes includes KNSTRN (~19%), a kinetochore gene whose disruption increases aneuploidy and enhances cSCC tumor growth [84], mitogens like AJUBA (~18%) and BRAF (~18%), cadherens like FAT1 (~44%), chromatin modifiers like KMT2D (~69%) and KMT2C (~39%), and cell survival genes like CASP8 (~23%) [74]. These highly mutated genes begin to illuminate how cSCC progression can occur where keratinocytes need to initiate cell proliferation (RAS and RAF), lose connections and communication between neighboring cells (NOTCH and FAT1), reduce apoptosis (CASP8), and stimulate a mutator phenotype (TP53, CDKN2A, KNSTRN, KMT2) to accumulate genetic alterations that facilitates tumor growth and invasion. One begins to see why monotherapies may not be effective measures to treat cSCC.

5.3.5 Common Treatments

As with BCCs, surgical excision remains one of the most common ways to treat cSCC. The use of different surgical methods such as electrodesiccation and curettage, complete excision, or cryosurgery has been shown to eliminate up to 96% of tumors with a low risk of recurrence [86]. Although cryotherapy and electrodesiccation are common and inexpensive, the more costly surgical excision offers lower rates for recurrence. For electrodesiccation and curettage, the tumor and surrounding tissues are cauterized and scraped with a curet, resulting in a 96% cure rate for small tumors [79]. Surgical excision allows for verification of tumor margins and ensuring complete tumor removal; however, there is greater risk of surgical complications and cure rates that do not differ much from electrodesiccation. Cryotherapy is often used for small tumors, where liquid nitrogen is used to freeze the tumor to promote necroptosis and have high cure rates [87]. Mohs surgery once again seems to be the most effective way to prevent tumor recurrence, similar to BCC and has been shown to have a five-year disease-free rate of ~97% in all sites other than the lips [79].

Nonsurgical methods to treat cSCC include radiotherapy, which may be used in combination with other therapies for aggressive or recurrent tumors. Radiotherapy does have a high cost, needs multiple visits to the clinic, and recurrent tumors tend to be highly aggressive [86]. Topical chemotherapy like 5-FU and imiquimod (may not be appropriate for invasive cSCC due to poor penetration into the dermis) or systemic chemotherapies like cisplatin, bleomycin, and doxorubicin show varying levels of effectiveness [88]. Epidermal growth factor receptor (EGFR) inhibitors are effective in several forms of cancer by shutting down the growth pathway of tumor cells [89]. Cetuximab, a humanized monoclonal antibody that binds and inhibits EGFR, and Gefitinib and Erlotinib, both of which prevent ATP binding to EGFR, seem to be effective therapeutic options for cSCC [88].

Recently, the FDA approved the use of cemiplimab for patients with locally advanced or metastatic cSCC. This is the first therapeutic to be approved by the FDA specifically for cSCC. Cemiplimab belongs to a family of immune checkpoint inhibitors, which work to strengthen the immune response against tumors. Forty-eight percent of patients in a phase 1 and phase 2 trial showed a positive response to cemiplimab [90]. Fifty-seven percent of the patients who had a response continued to respond after six months, and 82% of those patients continued to respond past 14 months. Furthermore, only 7% of patients discontinued treatment because of an adverse event.

HPV vaccines may also be a way to effectively treat inoperable cSCCs. An elderly woman with multiple, inoperable cSCCs was treated with a combination of systemic and intratumoral injection of the 9-valent HPV vaccine for roughly a year [91]. The 9-valent HPV vaccine is FDA approved to prevent genital warts and anogenital cancer caused by HPV infection. A reduction in tumor size and number was observed eight weeks after initial treatment, no detectable tumors nine months later, and no recurrence was observed 24 months after initial treatment. Although this represents one case, no systemic adverse effects were reported besides mild pain at the site of injection on the day of the procedure, suggesting that this may be a strong alternative to treat cSCCs.

5.4 Melanoma

Melanoma is the deadliest and most aggressive form of skin cancer and originates from melanocytes residing in the basal epidermis. In rare cases, melanomas may also arise in the eyes, nails, inner ears, or other neural crest-derived tissues. According to the latest American Cancer Society report, melanoma of the skin is estimated to inflict 91 270 Americans and result in 9320 deaths in 2018 [92]. Melanoma rates in Asia are ~10% of BCC rates [10]. In 2012, WHO reported more than 230 000 of new cases and 55 000 deaths globally [93]. Melanomas are caused by cumulative mutations acquired through sun exposure and/or genetic susceptibility. These tumors display high mutation rates in NRAS and BRAE, or other tumor suppressor genes that lead to uncontrolled growth of melanocytes. With early detection, benign melanomas can largely be removed by surgeries, yet advanced and metastatic tumors require more targeted approaches that can confer resistance. Recently, immunotherapy and vaccines that stimulate T-lymphocytes are being developed to treat melanomas, while combinatorial therapies continue to improve treatment and prolong patients' life.

5.4.1 Risk Factors

As with BCC and cSCC, UV exposure increases the risk of melanoma. On a basic level, UV exposure from tanning beds show a positive association with melanoma risk that increases if the exposure occurred as a young adult according to a systematic review and meta-analysis of the literature [94]. Intermittent sun exposure but not heavy occupational exposure significantly increases the risk of melanoma at all ages with an especially pronounced risk in childhood [95, 96]. Tumors typically arise in sun-exposed skin areas with men particularly susceptible in the trunk and upper back, whereas women see more tumors in the lower legs [97]. Immunosuppression can also increase the risk of melanoma from two- to five-fold compared with the general population, although the risk is less than BCC (10-fold) and cSCC (65-fold to 250-fold) [98].

Genetics play a vital role during melanoma development with both physical markers and genetic ones. For instance, risk for melanoma is strongly related to the number of nevi (moles). Patients with atypical nevi have at least a two-fold increase risk of melanoma that increases with more nevi [99]. Adults with more than 100 small nevi or children with more than 50 small nevi are also at a greater risk. Although melanoma do arise from nevi, with ~26% of melanomas histologically associated with nevi, most melanoma arise *de novo* [100]. Although there is an increased risk of melanoma with large congenital nevi greater than 20 cm, there is no increased risk of melanoma for isolated small-to-medium nevi [101].

No complete cure exists for advanced and metastatic melanomas. Yet, early detection often ensures that surgical excisions are efficiently curative. Therefore, it is important to observe and note the irregular signs on the skin, especially the appearance of a new or changes in an existing nevus using the ABCDE rule: A (asymmetry), B (border irregularity), C (color variation), D (diameter >6 mm), and E (evolving over time).

5.4.2 Classification

Melanomas can be categorized based on histopathological type, vertical growth, and level of metastasis. The most common subtype of melanoma is the superficial spreading melanoma (SSM), which accounts for ~70% of all cases [26]. SSM have irregular borders and pigmentation that spread slowly over months to years. Nodular melanoma occurs in 15–30% of cases and appears darker with a dome shape. These lesions progress quickly and often arise *de novo*. Lentigo maligna is a freckle-like tan-brown macule with irregular shape and slow progression that can develop into lentigo maligna melanoma (LMM) overtime in older patients. LMM comprises 10–15% of melanoma cases. Acral lentiginous melanoma (ALM) comprises 2–8% of cases and appears dark brown to black and more frequently affects darker-pigmented individuals, with ~60–72% of ALM cases found in individuals of African descent.

The staging of melanoma by the American Joint Committee on Cancer is summarized in Table 5.2 [102]. It ranges from *in situ* lesions like lentigo maligna to larger tumors that have metastasized to distant parts of the body.

Table 5.2 Staging of melanoma as defined by the American Joint Committee on Cancer.

<u>Stage</u>	<u>Stage description</u>
0	<i>In situ</i> , confined to the epidermis
I	>2 mm thick, optional ulceration, and no lymph node or distant sites
II	1.01–4.0 mm, optional ulceration, and no lymph node or distant sites
IIIA	<2.0 mm thick, optional ulceration, to three or less lymph nodes, no distant sites
IIIB	No sign of the primary cancer, to one lymph node or to nearby skin but no distant sites
	OR
	<4.0 mm thick, optional ulceration, to ≥ 1 lymph nodes or to nearby skin but no distant sites
IIIC	No sign of primary cancer, to only one or clumped lymph nodes or to nearby skin but no distant sites
	OR
	<4.0 mm thick, optional ulceration, to ≥ 1 or clumped lymph nodes or to nearby skin but no distant sites
	OR
	2.1–4.0 mm or more thick, optional ulceration, to ≥ 1 or clumped lymph nodes or to nearby skin but no distant sites
	OR
	<4.0 mm thick, ulcerated, to ≤ 3 lymph nodes or to nearby skin but no distant sites
IIID	<4.0 mm thick, ulcerated, to 4+ or clumped lymph nodes or to nearby skin but no distant sites

5.4.3 Cell of Origin

Melanoblasts, the precursors of melanocytes, originate from the neural crest and migrate to colonize the skin, hair follicles, uveal tract of the eyes, inner ear, anogenital tract, adrenal gland, and meninges during embryonic development [103]. In human skin and the mouse tail, melanocytes reside in the basal layer of the epidermis. Melanocytes also colonize the hair follicles in human skin and mouse dorsal skin. Recent *in vivo* lineage tracing experiments examined at what point melanocytes are competent to initiate melanoma as many melanomas arise from skin without a precursor lesion [104, 105]. $Braf^{V600E}$ overexpression coupled with *Pten* loss under the Tyr-CreERT2 promoter induced dermal melanomas in dorsal skin, but UV irradiation induced a switch to epidermal melanoma growth that did not seem to be dependent on dedifferentiation of the melanocyte. In the tail, only pigmented melanocytes and not their non-pigmented counterparts in the scale areas could be induced to proliferate. Interestingly, the pigmented melanocytes in the scale region lost their pigmentation as they became invasive. Mature, pigmented melanocytes in the hair follicle matrix could also be driven to transform under certain conditions. The hair follicle bulge was resistant to melanoma formation. How this work translates to human melanomas is still an open question.

5.4.4 Signaling Pathways

Many signaling pathways are significantly mutated in melanomas and include drivers of cell cycle progression, cell death, differentiation, proliferation and survival, chromatin modifications, and telomere maintenance [106, 107]. The most commonly found mutational variants for cutaneous melanoma lie in the MAPK pathway, where BRAF (~55% of tumors contain a SNV) and NRAS (~27%) mutations dominate. The PI3K pathway, operating downstream of MAPK, was also significantly altered, where PTEN mutations (~8%) or deletions (31%) dominate. TP53 mutations (~18%) or deletions (~36%) and CDKN2A/B mutations (~17%) and deletions (~46%) are commonly seen and affect the cell death and progression pathways. Interestingly, CDKN2A mutations account for nearly 70% of hereditary melanoma cases in Europe, United States, and Australia combined [108]. BRAF, NRAS, and NF1 mutations dominate acral melanoma, whereas SF3B1 mutations dominate mucosal melanoma. Cutaneous melanomas were most often mutated at a rate of ~49 mutations/Mb. Acral and mucosal melanoma had a nearly 20-fold decrease in mutation rate at ~2.6 mutations/Mb but had more unstable genomes where copy number variations were more prominent than in cutaneous melanoma.

5.4.5 Common Treatments

Surgical excision remains the most common treatment for localized melanomas [109]. Typically, an excisional biopsy with narrow margins is performed. Such margins depend on the depth and size of the histologically verified tumors. Partial or complete amputation of the digits may be done if a melanoma on a toe or finger

has grown too deep. Lymph node dissection can be done if the sentinel lymph nodes are notably enlarged or hardened and its biopsy shows presence of cancer cells. However, complete lymph node dissection may be appropriate to prevent the spread of melanoma. Once melanomas have metastasized to the lungs, liver, or brain, surgical efforts are meant to control the cancer or relieve the symptoms instead of curing it.

Nonsurgical methods to treat melanoma include chemotherapy, where individual or combinatory treatments of alkylating agents dacarbazine and temozolomide are modestly effective but have high rates of resistance [110]. Electrochemotherapy, where cytotoxic drugs like bleomycin and cisplatin are administered with high-intensity electric pulses to help facilitate drug delivery, can be quite effective with an overall response rate of 85% and a complete response rate of ~74% regardless of tumor type and nodule size [111]. Immunotherapies are becoming increasingly popular treatments for metastatic melanomas. Interferon α -2b was the first adjuvant therapy approved by the FDA for the treatment of resected stage IIb/III melanoma and can significantly reduce the risk of tumor recurrence and improves patient survival by activating a variety of immune cell types [112]. Other FDA-approved therapies include Interleukin-2, a cytokine that activates T-cells and shows an overall response rate of ~20% [113]; ipilimumab, an anti-CTLA-4 antibody that promotes T-cell activation and suppresses immune tolerance and shows greater overall survival than standard systemic therapies for unresectable stage III/IV melanoma [114]; anti-PD-1 monoclonal antibodies activate T-cells and may be more suitable therapies than ipilimumab [115, 116]; and T-VEC, a genetically modified herpes simplex virus type 1 that is injected directly into a tumor and specifically replicates in tumor cells, promotes tumor cell lysis, release of tumor-specific antigens, and leads to T-cell activation [117]. Many other immunotherapies are in clinical trials to treat metastatic melanoma [118].

Targeted therapies like BRAF inhibitors are FDA approved for the treatment of unresectable or metastatic melanomas with BRAF^{V600E} mutations and show high rate of overall survival approaching 85% at six months [119]. Unfortunately, rapid development of resistance occurs where acquired resistance is often associated with the reactivation of the MAPK and/or PI3K-AKT pathways [120, 121]. BRAF copy number gain and alternative splicing have, respectively, been found in 20% and 32% of BRAF inhibitor-treated melanomas. Notably, expression of splicing isoforms of BRAF^{V600E} that dimerize in a RAS-independent manner promotes resistance to BRAF inhibitors [120]. Considering the complex mutational patterns of melanoma driven by their high mutational rates, combinatory treatments would likely increase the progression-free survival rate.

5.5 Concluding Remarks

High mutation rates from UV irradiation commonly underlie skin cancers. In fact, frequent skin cancer development is a clinical marker for general inherited cancer susceptibility where individuals have an increased prevalence of germline

mutations and oncogenic malignancy [24]. As mutations accumulate, resistance to targeted therapeutics can develop, especially if the tumor is under selective pressure [38, 122]. Defining the signaling pathways that are preferentially hit will lead to a better understanding of skin cancer growth and the development of next-generation therapies. However, enlisting the immune system to help eradicate tumor growth holds much promise as a general therapeutic strategy. Combining these efforts may prove vital in our collective path to eliminate skin cancer.

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