Review

Beyond the Surface: Cell Cycle Targeting in Skin Cancer Treatment

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Beyond the Surface: Cell Cycle Targeting in Skin Cancer Treatment

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Abstract: Skin cancers, including melanoma, and non-melanoma- cutaneous basal cell carcinoma and cutaneous squamous cell carcinoma, are a diverse group of malignancies characterized by variations in their molecular and cellular features. Melanoma is a highly heterogenous tumor characterized by the dysregulation of a myriad of cell-cycle associated signaling pathways. In the non-melanoma skin cancers, such as cutaneous basal and cutaneous squamous cell carcinoma, the role of cyclin-dependent kinases and cyclins remains poorly understood with relatively limited investigation. Although revolutionary therapies against diverse molecular targets have been introduced over the last decade leading to significant prognostic benefit, advanced stages of melanoma remain exceptionally difficult to treat. The three cancers are united by evidence of stem-like, cancer initiating cell populations which dictate highly dynamic microenvironments and rely on disrupted cell cycle signaling to support treatment resistance and cancer progression. Potential of cell cycle targeting in skin cancer in underexplored in terms of identification of specific mechanisms supporting the maintenance of skin cancer stem cell pools and could offer potential targets to advance the therapy in these malignancies. In this review, we comprise the existing data on major cell of cell cycle regulators in skin cancer, the regulation of cancer stem cells and most recent developments and limitations surrounding cell cycle-targeted therapies, with a focus on the application of CDK inhibitors.

Keywords: skin cancer; cell cycle; CDK inhibitors; skin cancer stem cells

1. Skin Cancer - Overview of Pathophysiology and Epidemiology

1.1. Melanoma

Melanoma is currently the fifth most common malignancy in both men and women [1]. Although the 5-year overall survival of early-stages of melanoma (94%) has significantly improved thanks to advances in therapeutical strategies, the prognosis of metastatic melanoma remains dire, with the 5-year survival hovering near 30% [2,3].

Melanoma is classified into subtypes by distinguishable histopathological characteristics, with variable genetic profiles depending on the primary tumor site (cutaneous, mucosal and uveal types). Clinically recognized histopathological subtypes of cutaneous melanoma (CM) include the most common superficial spreading (41%), nodular melanoma (16%), lentigo maligna (2.7-14%) and acral lentiginous melanoma (1-5%), in addition to less frequently observed subtypes such as desmoplastic and amelanotic melanoma (Table 1) [4,5].

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The incidence of melanoma continues to rise globally, and is currently reported at 21.2 per 100,000 adults in the USA [6]. Fair-skinned (Fitzpatrick phenotype I and II) adults in developed countries represent the predominant demographic of melanoma. Ultraviolet radiation exposure remains the dominant risk factor of CM, with other contributory risk factors being indoor tanning, presence of melanocytic or dysplastic naevi, chronic immunosuppression, and a personal or familial history of melanoma [5,7]. Interestingly, some subtypes of melanoma are independent of UV-exposure, such as acral lentiginous, mucosal and uveal melanomas [5].

Embryologically, melanocytes originate from a neural crest precursor, beginning as pluripotent melanoblasts that migrate to the basal epidermis and hair follicles to ultimately mature into pigment-producing skin cells [8]. The photoprotective pigment, melanin, is packaged into small granular organelles known as melanosomes, that are transported into neighbouring keratinocytes, to absorb UV radiation. In addition to skin, melanin is synthesized in the retina, meninges, gastrointestinal tract, among other tissues [9]. Epidermal melanocytes transform into cancerous cells primarily through the accumulation of somatic oncogenic driver mutations. CM houses a high mutation burden, which is more than mucosal or uveal melanomas. Oncogenic signaling in human CM , results from a variety of somatic mutations, the most frequent include*CDKN2A* (germline mutation), the serine/threonine kinase *BRAF* (driving ERK/MAPK signaling), *NRAS*, *NF-1*, *TERT* promoter, *PTEN* (driving PI3K signaling), *TP53*, *APC* (driving Wnt/ β-catenin signaling) [10]. These driver mutations are used to classify CM into subtypes: the most common are mutant *BRAF* (classically, the V600E mutation), mutant *RAS* (*NRAS, KRAS, HRAS*) and mutant NF1 [11].

With early diagnosis, surgical resection of localized melanoma is associated with a good prognosis [6]. However, melanoma can spread quickly and aggressively, and advanced melanoma is associated with poor patient prognosis. Analyses of the transcriptomes and mutations of melanoma subtypes has carved a path for the development of targeted therapies. Accordingly, recent prognosis of early and metastatic melanoma has improved relative to previous treatment with non-specific chemotherapies (e.g. dacarbazine, fludarabine, Bcl-2 anti-sense) [12]. The introduction of targeted therapy such as small molecule inhibition of mutant BRAF (vemurafenib) and immunotherapy (e.g. PD-1 checkpoint inhibitor antibodies such as ipilimumab, nivolumab, pembrolizumab) has provided significant prognostic benefit [13,14]. For example, patients suffering from advanced melanoma have seen their median prognosis rise from 6 months to 6 years. However, a prominent issue that remains is the frequent therapy resistance exhibited by this highly mutated and genomically unstable malignancy [15].

1.2. Cutaneous Basal Cell Carcinoma (cBCC)

Cutaneous basal cell carcinoma is the most common malignant neoplasm worldwide, primarily arising from sun-exposed regions of the skin. Although it bears a highly mutated genome, it has a limited tendency for metastasis and is defined by its slow rate of growth and local invasion allowing for earlier detection and treatment while accounting for the low mortality rate [16]. cBCC, which bears a high de novo mutation burden, arises in a cancerized field of resulting in frequent recurrence post initial treatment [17,18]. Classification of BCC is broad, with numerous subtypes mentioned in literature, though the more frequently discussed histopathological subtypes include the lower-risk nodular (most common), superficial, pigmented BCCs, and the higher-risk sclerosing/morpheaform, infiltrating and micronodular types (Table 1) [16,19].

In North America, the incidence of BCC in all age groups has increased at an approximate annual rate of 2%, and as high as 5% in Europe, with millions of new cases each year in the USA alone [20–22]. It is grouped with cutaneous squamous cell carcinoma as a non-melanoma skin cancer (NMSC), with BCC alone comprising nearly 80% of such tumors [23]. Most commonly, BCC occurs in high UV geographic regions, as UVB-induced injury is the leading cause of BCC formation, primarily on the head and neck. Like melanoma, BCC is most prevalent in fair-skinned adults. Additional risk factors include personal history of BCC (10-fold more likely recurrence), family history of skin malignancy, and heritable mutations (basal cell nevus syndrome, xeroderma pigmentosum) [24].

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Identifying the cellular lineage of BCC has remained challenging, but it is presently thought that the malignancy arises from UV-induced mutation of multipotent stem cells found the bulge of hair follicles [25]. The initiation and progression of sporadic BCC is primarily mediated via mutational activation of of Sonic Hedgehog (SHH signalling, with 85% of tumors possessing SHH-related gene mutations. The majority of these occur in the cBCC suppressor gene, *PTCH1* (Patched1), and to a lesser extent in SMO (Smoothened) [26]. Inactivation of *PTCH1* results in constitutive activation of *SMO* (SmoM2), normally suppressed by *PTCH1*, that drives oncogenic transformation [27,28]. Additional mutations have been implicated in cBCC, including the tumour suppressor *TP53* (found in over half of tumors and required for sustained cBCC transformation in experimental models), as well as to a lesser extent in *GLI1, SUFU, RAS, ERBB2, PIK3CA*. Aberrant signaling through Hippo-Yap and APC pathways may also contribute to cBCC [29,30].

Although rarely fatal, delayed management can lead to local disfiguration and damage, depending on proximity to surrounding tissue. BCC is highly curable in the early stage, with treatment primarily consisting of surgical resection. If excision is not sufficient, alternative techniques such as electrodesiccation and curettage, cryosurgery, and Mohs micrographic surgery can be applied with high success rates (95%) [7]. The recurrence rates of cBCC are as follows: Mohs, 1.0%; surgical excision, 10.1%; electrodesiccation and curettage, 7.7%; radiation therapy, 8.7%; and cryosurgery, 7.5% [31]. Within the last decade, targeted therapy against the SHH pathway, primarily SMO inhibitors such as vismodegib and sonidegib, results in initial tumor control but recurrence was noted in 20-30% of cases treated with vismodegib, and high-rates of development of resistance within the first year of therapy [32,33].

1.3. Cutaneous Squamous Cell Carcinoma (cSCC)

Cutaneous squamous cell carcinoma (cSCC) is a keratocyte carcinoma and is the second most common human malignancy, trailing only behind BCC.In contrast to cBCC,, cSCC can progress to an advanced stage, exhibiting higher rates of metastasis (5%) and mortality [34]. Like cBCC, Precursor lesions, including actinic keratosis, spontaneously involuting keratoacanthomas and cSCC in situ (e.g. Bowen's disease) arise in a cancerized field of UV-induced somatic mutations While the primary risk factor for actinic keratosis and cSCC is chronic UV exposure, additional factors include chronic infection and immunosuppression, primarily in fair-skin individuals, as well as genetic predisposition [35,36]. Like melanoma and cBCC, the incidence of cSCC continues to rise, with rates similar to that of BCC [20,21]. There is debate over the annual rate of progression of actinic keratosis to cSCC, though it is evident that a stepwise progression exists, emphasizing that early diagnosis is imperative [37,38].

Generally there is a good prognosis for most cSCC patients, though prognosis is difficult due to heterogeneous presentation [34]. Attempts to risk-stratify have been made but objective measures such as tumor size and depth of invasion provide more accurate prognostic information [39].

Histologically, cSCC is the abnormal proliferation of keratinocytes in the spinous or outer layer of the epidermis. The variants can be classified based on differentiation status into the more invasive acantholytic type, or the more differentiated spindle cell and verrucous types. As seen in CM and in BCC, UV exposure results in genetic damage of squamous cells, promoting cell cycle dysregulation [40]. The majority of cSCC possess a UV-signature mutation (cytosine to thymine) in *TP53*, which implicates aberrant p53 functions with NMSC tumor initiation [41]. Additional cSCC driver mutations include inactivating mutations in *NOTCH1/2* and *CDKN2A*, and less frequently in *PTEN*, as well as activating mutations in *RAS, ERBB4,* and *PIK3CA* (Table 1). Although mutations in EGFR (40-80% of cases) have also been reported, a phase II clinical trials using EGFR inhibitors for metastatic cSCC yielded poor response rates [42].

Topical imiquimod and 5-fluorouracil containing agents are successfully used on AK to prevent progression to tumorigenesis. Surgical excision of tumors is the preferred tumor management, including Moh's micrographic surgery for higher-risk SCC to ensure tumor margin assessment [43]. Alternative but less frequent treatments include local radiation therapy, cryosurgery and laser treatment.

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2. Cell Cycle Deregulation in Skin Cancer

Control of the cell cycle hinges on the tight regulation of cyclin-dependent kinases (CDKs) and their dynamic interactions with oscillating cyclins, which together orchestrate cellular division (Table 2). Targeting these various functional complexes at different checkpoints restricts aberrant proliferation. When this intricate signaling network is compromised, CDK-cyclin complexes are detached from protective cellular mechanisms to drive uncontrolled growth. Cutaneous malignancies, particularly melanoma, demonstrate substantial dysregulation of the cell cycle network, which contributes to tumor initiation and progression.

2.1. CDKs and p16^{INK4}

The cyclin-dependent kinase-2A gene (*CDKN2A*) is clustered in the region of chromosome 9p21.3. This tumor suppressor gene encodes p16^{INK4a}, a pivotal regulator of the G1-S checkpoint. The loss of regulation at this checkpoint is critical in the progression of cancer. p16^{INK4a} prevents proliferation by binding to CDK4 and CDK6, inhibiting their interaction with cyclin D and thus preventing the formation of an activated complex.

Undoubtedly, progression of melanoma is closely linked to direct cell cycle regulators. Walker et al., found that 43 of 45 melanoma cell lines exhibited genetic aberrations in *CDK2NA*, with deletions being more prevalent than point mutations or methylations *[44]*. Over 50% of melanomas were found to have deletions in this locus, implicating *CDKN2A* as one of the most common alterations in cutaneous melanoma. The inactivation of this protective gene is a key factor in melanoma susceptibility, particularly in familial cases due to heterozygous germline mutations, and to a lesser extent, in sporadic melanoma. Despite the pivotal role of p16^{INK4a} in melanoma progression, its expression alone has not proven to be a reliable indicator of tumor recurrence or patient survival [45]. It is more common to see multiple mutations rather than an isolated p16^{INK4a} aberration. An *in vivo* murine model mimicking human somatic loss of p16^{INK4a} and activation of *RAS* in human melanoma demonstrated rapid growth and development of unpigmented melanomas from adult melanocytes [46]. This highlighted the potential oncogenicity throughout cell maturity and the synergy of different genetic alterations used by melanocytes to exploit cell cycle regulation.

As noted, binding partners CDK4/6 and cyclin D1 are crucial propellors of the G1-S transition point, via sequential inactivating phosphorylation of the stage-specific tumor suppressor, Retinoblastoma (Rb), ultimately leading to expression downstream transcription factors [47]. Acting as independent oncogenes, CDK4 and CCND1 amplifications are most common in acral melanomas, where median survival of patients with CDK4 amplification and p16^{INK4a} loss is significantly decreased [48]. Activation of CDK4 can result from various mutations, such as loss of CDK4 sensitivity to p16^{INK4A} or the germline CDK4^{R2C} mutation that disrupts binding of CDK4-p16^{INK4a}, both preventing negative regulation of the G1-S transition [49]. In studies, homozygous mouse knock-in of CDK4^{R2C} mutation led to widespread formation of tumors within 8 to 10 months, including skin tissue. The dependency of melanoma progression on CDK4 is further emphasized in the work of Zou et al., where CDK4 and cyclin D1 null mice experienced significant reduction in tumorigenic foci relative to wildtype CDK4, and similarly did not lead to tumor production *in vivo* [50]. The team of Sauter et al. demonstrated the oncogenic potential of cyclin D1, using anti-sense therapy to target cyclin D1 in melanoma lines overexpressing this protein. Importantly, targeting of cyclin D1 induced apoptosis *in vitro* with significantly decreased tumor burden in mice models observed selectively in the mutated melanocytes [51,52]. With upwards of 90% of melanomas demonstrating mutation in various segments of the CDK4/6 pathway, the deep investigation of cyclin-dependent kinase inhibitors (CKIs) in targeted therapy is of no surprise [53]. Notably, both the independent and concurrent amplification of these cell cycle regulators has been implicated in enhancing therapy resistance in variously mutated melanomas [54,55].

Though not as classically defined, deregulation of the G1-S cell cycle transition also contributes to the progression of the non-melanoma cutaneous malignancies. Screening of the *CDKN2A* locus for genetic aberrancy in 15 cases of freshly-frozen BCC tissue by Kanellou et al. revealed a previously described G442 (Ala148Thr) polymorphism in three cases that did not hamper the regulatory role of

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p16^{INK4a} on CDK4/6-cyclin D1 [56]. In this same study, a decrease in p16 transcript levels was observed in 14 of 15 samples, implying potential inactivation of the tumor suppressor similar to what is seen in melanoma. In contrast, the team of Eshkoor et al. found that ten samples of paraffin-embedded skin BCCs had significantly increased p16 protein in the nucleus and cytoplasm, with a corresponding significant increase in gene expression [57]. Additionally, when assessing the role of HPV in BCC, Paolini et al. found that dysregulated keratinocytes overexpressing p16^{INK4A} in 94% of samples (35 of 37), with 8 samples exhibiting elevated protein levels in >30% of the immunostained cells [58]. Notably, this variable expression between different research teams may have been a result of differing sample processing and experimental methods.

The mechanism behind the upregulation of p16^{INK4a} remains undetermined. An alternate study by Eshkoor et al. of ten BCC tissue samples displayed significantly increased protein and mRNA expression of CDK6 and cyclin D1 [59]. Considering this, BCC appears to be influenced by the p16^{INK4a}-cyclin D/CDK-pRb signaling pathway to an extent. Accordingly, one might postulate an elevation in p16^{INK4a} levels as the expected response to increased proliferation within the tumor cells. Upon investigating for regional variability in p16 levels among different subtypes of BCC, Svensson et al. note a correlation of expression with invasiveness, as protein levels were highest in cells along the infiltrating tumor periphery. Persistence of p16^{INK4a} functionality in this aggressive tumor edge was associated with downregulation of the proliferative marker Ki-67, indicating an inverse relationship between proliferation and infiltration that may be influenced by p16^{INK4a} [60].

The risk of BCC, like melanoma, which is augmented by UV radiation-induced DNA damage [61]. results in augmented p16 expression in comparison to non-sun-exposed skin. Hence, upregulation of p16^{INK4a} could indicate a broad cellular stress response. An increased expression of p16^{INK4a} was observed in recurrent BCC lesions relative to non-recurrent, suggesting an association with therapy resistance and/or tumor recurrence [62]. Most research on BCC has focused upon the role of chronic activation of SHH signaling although the common development of resistance to SMO inhibitors predicts the involvement of other factors such as deregulation of the cell cycle. This possibility remains understudied. An instructive role for aberrant cell cycle regulation in cSCC is also not well investigated although an association of dysregulated cell cycle is broad range of dysplastic cells (10-80%) isolated from samples of actinic keratoses (pre-cancerous lesions), Bowen's carcinoma (squamous cell carcinoma *in* situ) and cutaneous squamous cell carcinoma cells displayed overexpression of p16^{INK4a} relative to normal tissue [63]. As well, the involvement of p16^{INK4a}, cyclin D1 and Rb has been demonstrated in other SCC [64]. Importantly, *in vitro* targeting of cyclin D1 in SCC lines (head and neck, facial and vulvar tissue) and *in vivo* immunodeficient mice revealed significant reduction in tumor growth [52].

Both cBCC and cSCC often bear allelic loss at the *CDKN2a* locus [65]. although the consequence to tumor initiation or progression is not yet clear. Immunohistochemical staining by Zheng et al. suggested a variability in p16^{INK4a} expression between BCC and SCC. Here, 15% of BCC (47 cases) revealed low levels of positive staining (1+), relative to 80% of cSCC (44 cases) while 20% of cSCC exhibit significantly protein expression [66]. These results suggest cSCC progression is associated with deregulation of cell cycle mediators but establishing cause and effect requires further study.

2.2. p14^{ARF} and p53

The *CDKN2a* locus is alternatively known as ARF-INK4a, due to the alternatively spliced product being p14^{ARF}, a tumor suppressor with identified inactivating mutations demonstrated in various cancers. Importantly, p14^{ARF} functions to inhibit the p53-degrading protein MDM2, thereby stabilizing p53 and stabilizing its activity as a crucial cell-cycle and apoptotic regulator [67]. Moreover, p14^{ARF} is recognized as a connector of Rb and p53 [68,69]. Upon phosphorylation of Rb, associated E2F transcription factors are untethered, which then induce expression of p14^{ARF}. This increase in p14^{ARF} activates the p53/p21 pathway, providing an additional layer of control to inhibit cellular proliferation, underscoring the interplay between these regulators in maintaining cellular homeostasis [70,71].

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There is strong evidence highlighting an inverse relationship of $p14^{ARF}$ with melanoma progression. In general, exon mutations impacting $p16^{INK4A}$ function are more frequent than in $p14^{ARF}$, although a downregulation of $p14^{ARF}$ expression can contribute to melanoma oncogenesis [72,73]. However, some studies of metastatic melanoma have reported increased mutation of ARF relative to $p16^{INK4a}$, which is perhaps sample-dependent [74]. Regardless, mutations in both genes are significant, and their concurrent inactivation is reported in upwards of 40% of melanoma. [75] Dobrowolski et al. compared $p14^{ARF}$ protein levels in increasingly aggressive human melanomas, revealing 11 of 14 benign nevi, 3 of 12 melanomas and 0 of 6 metastatic melanomas showing positive staining for $p14^{ARF}$, supporting a role for this protein in melanoma progression [76]. Moreover, the introduction of FLAG-tagged truncated $p14^{ARF}$ constructs into the NM39 melanoma cell line resulted in blunted G1 arrest relative to wild-type $p14^{ARF}$ [75].

Upon comparison of select tumor suppressor genes in AK and cSCC, Kanellou et al. found downregulation of both $p14^{ARF}$ and p53 in SCC relative to the pre-cancerous AK lesions [77] Likewise, investigation of the *CDKN2A* locus in 40 human cSCC samples revealed alterations in 76% of samples, with variable point mutations and promoter methylation representing the most common causes of inactivation. [78] Similarly, Pacifico et al. show a similar inactivation in BCC, with only 1 of 16 sun-exposed patient tumors staining positively for protein expression of $p14^{ARF}$ [79]. These studies highlight the potential role of inactivation of $p14^{ARF}$ in the tumourigenic process for both melanoma and NMSCs.

Nevertheless, mutations in p16 or its loss of expression is likely insufficient for cutaneous tumorigenicity. For example, individuals homozygous for the *CDKN2A* germline mutation can remain disease-free. Thus, inactivation of this tumor suppressor in humans is presumed to cooperate with additional driving or spontaneous mutations to orchestrate tumor initiation and progression [80]. However, experimentally, genomic loss of ARF in mice (murine $p19^{ARF}$ is equivalent to human p14) led to increased cSCC tumor formation at multiple cutaneous sites when treated with the carcinogen DMBA [81]. Loss of ARF was found to be independent of p53 signalling, which remained functional via alternate activation pathways, as demonstrated by the continued induction of p21 expression in the context of non-functional $p14^{ARF}$ [75,82].

TP53 is one of the most frequently inactivated regulators in cancer, yet an association of *TP53* function loss in melanoma is controversial. Early studies reported variable *TP53* mutations in melanoma (0-20%). More recent and higher resolution whole exome sequencing analysis have detected inactivating p53 mutation in 15-20% of melanoma samples [83,84]. In contrast to NMSCs, which exhibit early mutations of *TP53*, these mutations are more often detected as late events associated with advanced melanoma [85]. The current consensus is that wildtype *TP53* predominates in over 80% of melanomas [86]. Moreover, elevated p53 expression is detected with increasing tumor progression [87,88]. These results imply that wildtype *TP53* function is aberrant in melanoma. This possibility is supported by one study that demonstrated failure of p53 to induce apoptosis. Other studies implicate p53 as a driver of therapy resistance and aggressiveness through expression of shorter isoforms [86,89,90]. Additional mechanisms include disrupted $p14^{ARF}$ signaling, which causes persistent MDM2-mediated inactivation of p53 [91]. However, as mentioned earlier, alternative studies have demonstrated p53 can retain its normal function in the presence of inactivated $p14^{ARF}$ [81].

In skin cancer, deletions of *CDKN2A* are more common than point mutations, the latter more commonly associated with UV exposure [92]. UV-signature mutations (C to T and CC to TT dipyrimidine sequences) are present in NMSCs and pre-cancerous lesions, likely serving as an early step in carcinogenesis [79]. Comparison of mutant p53 in aggressive and non-aggressive BCCs revealed detection in 38% and 66% of human samples, respectively [93]. Moreover, increased tumorigenicity of NMSC was exhibited in heterozygous p53 mutant mice compared to wildtype [94]. In cSCC, mutant p53 has been reported to be found in greater than 50% of human tumor samples analyzed [93,95]. This early involvement implicates p53 in tumor initiation, as noted by the detection of mutations in actinic keratosis, Bowen's disease and cSCC in situ [96,97]. Progression is influenced by additional tumor suppressor mutations at the *CDKN2A* locus, especially in the presence of loss-

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of-function p53 mutants resulting in elevated proliferation, metastatic potential and drug-resistance [92,98,99]. In experimental models of cBCC loss-of-function mutations in *Trp53* is required for sustained tumor transformation. Unlike NMSCs, mutant p53 is less significantly implicated in melanoma.

Collectively these reports suggest that both the initiation and progression of human melanoma and NMSCs may be impacted by dysregulated cell cycle protein functions. Additional studies focusing on specific growth pathways and stemness regulation further implicate the discussed proteins, though are out of the scope of this review.

3. Skin Cancer Stem Cells (sCSCs) and Therapy Resistance

3.1. CSC and sCSCs

The intricate processes of tissue regeneration and homeostasis require the presence and activity of resident adult stem cell (ASC) populations. Cellular turnover in tissues balances rates of cellular renewal and death, a process that is heavily modulated by crosstalk between the ASCs and the niche microenvironment they occupy. [100,101] This warrants that for tissues to be replenished, resident ASCs must have the inherent plasticity to reconstitute tissue-specific cells following damage while maintaining persistent, equipotent stem cells and/or oligopotent transit-amplifying (TA) progenitor populations. [102] Tissue regeneration by ASCs can be represented as a hierarchical organization, a model initially developed by Till and McCullough in 1961 to explain hematopoietic stem cells [103]. At the apex of the hierarchy lies a quiescent multipotent ASC capable of establishing progenies through asymmetrical or symmetric division [102,104]. Asymmetrical division describes the formation of an equipotent stem-cell and a more differentiated progenitor of a certain lineage. On the other hand, symmetric division can see the formation of either two equipotent stem cells from a parent or two less-plastic progenitors [105]. While many tissue types in the body have some capability to regenerate; the epithelium is one of the organs with the highest regenerative capacity [104]. This is in large part because there is a constant need to replenish lost cells that following continuous stressor insults from the environment, such as ultraviolet radiation on the epidermal or microbial stressors faced by intestinal epithelia [106,107].

Spatial organization of distinct stem cell populations within a tissue is evident in epithelial structures. In skin, both the interfollicular epidermis and sebaceous glands have a high turnover to regenerate the stratified skin barrier and sebaceous glands. The interfollicular epidermis (IFE) regenerates from a stem cell pool in the basal layer while sebaceous gland regenerate from hair follicle stem cells [108,109]. On the other hand, the hair follicle (HF) is unique in this organization as it follows a cyclic process of hair growth (anagen) and death that does not warrant the same frequency of resident SC recruitment as seen in the IFE or SG[110]. The hair follicle stem cells (HFSCs) are resident at the base of the follicle in a permanent structure known as the *hair bulge* in addition to the distally placed hair germ (HG)[111]. While SCs in both the bulge and HG are multipotent and express stemness markers including Lgr5 and Sox9 their plasticity vary given their unique target niche [112,113]. This is clearly demonstrated by bulge SCs which are capable of migrating from their primary microenvironment at the base of the follicle to other areas within the structure during the hair growth cycle as well as into the epidermis during cutaneous wound healing. [104,114,115] This highlights that not all stem cells permanently reside in their primary niche and are capable of homing to new areas. Hence, the fate and potency of a SC is greatly influenced by the niche it currently resides in. Remarkably, Morris et al. demonstrated that engraftment of bulge SCs into transgenic mice exhibited increased potency within the non-resident niche, with the capability of giving rise to all cell lineages found in the cutaneous structure, including those in the IFE and SG [116]. Contrary to the classical model that explains a unidirectional differentiation pattern of stem cells, recent data has supported the idea lineage dedifferentiation or switching, as observed in the epithelia of the lung and skin [117,118]. For instance, melanocyte stem cells (McSC) demonstrate a high level of plasticity, capable of switching between a TA and multipotent phenotype depending on the SC niche the McSC resides [118]. Provided specific signalling programmes within a microenvironment, the plastic potential of a SC is highly dynamic.

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Skin cancers remarkably demonstrate similar hierarchical architectures and mechanisms like those used in the regeneration of the epidermis [102]. However, it is clear that cancerous masses do not respond the same way to the processes that control tissue homeostasis. Research has shown that within various cancers exists a high degree of intratumoural heterogeneity and plasticity[110]. This is in large part due to populations of tumor-initiating or cancer stem cells that have the ability to self-renew and give rise to various committed lineages within the skin lesion. Along the tumoral CSC hierarchy lies a spectrum of differing plastic and differentiated cellular states, providing multiple integration points by which an oncogenic event can drive neoplastic growth with differing levels of malignancy [102]. The accumulation of oncogenic triggers in highly plastic cells leads to a significant proportion of cancer stem cells (CSCs) forming malignancies, primarily arising from the bulge area of the hair follicle (HF) [119]. In contrast, an increased mutational burden in the interfollicular epidermis (IFE) has been implicated in the development of benign papillomas [120]. Understanding the fundamental molecular mechanisms behind the transformation of CSC in tumor initiation and progression will shed greater insight into phenomena like chemoprotection and relapse.

3.2. Molecular Mechanisms of Stemness in sCSC

3.2.1. Melanoma Cancer Stem Cells

Research has been increasingly focused on the role of CSCs and their potential to initiate and recapitulate a heterogenous tumor post-treatment. Initial work with melanoma cell cultures lead to the discovery of the presence of such CSC subpopulations, which exhibit a multipotent and self-renewal plasticity that is similar to their lineage progenitor, neural crest. Work by Fang *et. al* clearly demonstrated the existence of multipotent melanoma cell subsets from clinical tumor samples. Spheroids, which are non-adherent subpopulations, of these multipotent melanoma cells exhibited persistence after serial cloning *in vitro* and demonstrated self-renewal both *in vitro* and *in vivo* [121]. Several subpopulations in human melanomas are capable of self-renewal, differentiation, tumorigenicity and/or drug resistance. One subset is enriched for the B-cell lineage marker, CD20, that has been implicated in B-cell lymphomas and that is linked to melanoma metastasis [122,123]. In comparison to differentiated adherent melanoma cells which were CD20 deficient, *in vivo* engraftment of CD20+ cells had increased tumorigenic potential and ability to initiate and maintain tumors with sustained plastic potential, which is characteristic of CSC. The role of CD20 in the maintenance of cancer stem cell subpopulations is not well understood, but is associated with poor prognosis and increased aggressiveness of melanoma tumors [124]. These findings not only underscore the tumor-initiating potential of certain cells within the tumor mass, but it also establishes CD20 as a non-canonical melanoma CSC marker which can be targeted by combination therapies such as nanoparticle and immunotherapy technologies [9,126].

Following chemotherapy, a multitude of melanoma CSC biomarkers play significant roles in renewal, metastasis and therapeutic resistance. Aldehyde dehydrogenase (ALDH) isoenzymes mediate oxidation of intracellular aldehyde pools which has been linked to increased metabolism of cytostatic agents, increased retinoic acid synthesis, and adaptation of core metabolic pathways [127]. ALDH overexpression within CSC subpopulations serve as stemness biomarkers and has been implicated in increased tumorigenic potential, with ALDH1A1 being one of the most well documented isoforms in cancers not limited to melanoma [128–131]. Certain isoforms, such as ALDH1L2, have been shown to mediate melanoma metastasis when adapting to oxidative stress [132]. Lu et al. recently demonstrated using a zebrafish melanoma model that tumor cell subpopulations exhibited ALDH1A3^{high} phenotype following BRAF targeted therapy, in addition to increased stemness markers like SOX2, SOX10 and TFAP2B. Consequently, the ALDH^{high} tumors became vulnerable to ALDH inhibition using nifuroxazide, highlighting the importance of identifying changes in CSC subpopulations as a tumor management approach [133]. Melanoma CSCs upregulate expression of other multidrug resistance associated proteins, such as drug efflux ABC transporters like ABCB5, which confer increased tumorigenic and plastic potential [134,135,136]. ABCB5 is a reliable marker for isolating CSCs as it identifies quiescent, slow-cycling melanoma subsets and correlates with the expression of stemness markers such has nestin, CD144, CD20, and

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PECAM1[137]. Recent studies have delineated a unique role for ABCB5 in melanoma where its activity leads to upregulated interleukin-8 (IL-8) in a Wnt-dependent fashion, maintaining quiescent melanoma subpopulations [135]. IL-8 induces inflammation in the niche environment which has been implicated in increased malignancy potential, angiogenesis, immune evasion and metastatic potential in late melanoma stages [135,138,139]. Melanoma CSC confer the heterogenous tumor mass with protective mechanisms that in the end provide mechanisms of chemoresistance and immune evasion to occur, leading to poorer prognostic outcomes.

3.2.2. Basal Cell Carcinoma Stem Cells

Experimental models suggest that CSC initiating cBCC occur in both the IFE basal layer and also HF stem cell niches such as the bulge [102,140]. A BCC origin in human cBCC has not yet been conclusively demonstrated. CBCC is a highly mutated human cancer that initiates from oncogenic driver mutations in sonic Hedgehog (SHH)/GLI signalling. As noted, the majority of human cBCC is driven by loss-of-function mutations of Patched1 (*PTCH1*) and/or gain-of-function Smoothened (*SMO*) mutations [24,99,141]. Although *Ptch1* mutations are sufficient to initiate cBCC in experimental models exposed to UVB, the acquisition of secondary mutations in *Gli1* and *Gli2*, *Notch1*, *Trp53* and elevated MYCN expression are required for stable tumor progression by hyperactivating SHH signaling, and promoting tumor persistence, genomic instability and proliferation respectively [142]. **The SHH targets, *Gli1* and *Gli2*** are members of the zinc finger family transcriptional factors and their elevated expression in mouse models is sufficient to induce BCC-like lesions UV exposure [141].

As previously discussed, cancers tend to mirror the hierarchical SC organization observed during tissue homeostasis. CBCC and cSCC clearly reflect this phenomenon. Mounting evidence suggests that in addition to a cancerized field of mutations established by UV exposure, chronic and acute wounding of the epidermis primes the injured area for the formation of keratinocyte tumors bearing driver mutations in the SHH pathway [143]. Lineage tracing techniques have established that Lgr5+ bulge SCs regenerate the IFE following a cutaneous wound while IFE stem cells do not. Interestingly, using an inducible Cre-flox Ptch1 mouse model driven by an epithelial-specific promoter, Ptch1-deficient Lrg5+ cells demonstrated the ability to develop cBCC. These included the expression of the bulge stemness marker Sox9 and the HG-specific marker P-cadherin [143]. KRT15+ bulge stem cells also contribute to repair of IFE wounds and can initiate cBCC in the context of a wound microenvironment. Hence, tumor-initiating cells are capable of migrating from the bulge niche into the IFE microenvironment, contributing to the formation of neoplastic lesions [143,144]. Several studies suggest a contextual dependency of the Lrg5+ bulge SC this tumorigenesis. For instance, work by Wong and Reiter show that activating mutations of *SmoM2* driven by a KRT15 promoter was not sufficient to induce cBCC in mice while mice expressing *SmoM2* driven by KRT14 promoter formed tumors. However, following wounding and migration of mutant bulge CSCs into the IFE area, *SmoM2* driven by a KRT15 promoter was capable of initiating cBCC [119]. Given the absence of Gli1 expression in the HF niche, it has been postulated that the varying tumorigenic potential of *SmoM2* may result from differential Gli1 expression between the HF and IFE niches [141,145,146]. This emphasizes the nuanced complexity and relevance of considering the niche microenvironment when studying BCC tumor initiation and the contribution of specific stem cells as tumor initiators.

3.2.3. Cutaneous Squamous Cell Carcinoma Stem Cells

Research over the past decade has begun to delineate the role of multipotent tumor-initiating cells in cSCC neoplasia using transgenic mouse models and shown that stem cell compartments in HF and IEF can give rise to cSCC in a mutation and niche-specific manner. Unlike BCC which is driven by CSCs harbouring *Ptch1*/*Smo* mutations in SHH signaling pathway cSCC requires two critical mutations for oncogenic transformation. For example, in mouse models, KRT15+ stem cells from the HF bulge bearing a $KRas^{G12D}$ mutation form benign papillomas while an additional and p53 loss develop invasive cSCC[147]. KRT14 driven KrasG12D or Lrg5 driven Kras12G12D combined

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with Trp53 loss result in cSCC initiation from the IEF and outer root sheath of the HF respectively. Thus, both IEF and HF stem cells are competent to initiate cSCC when these two critical mutations occur. In synergy with mutant *RAS*, ectopic expression of *NFKBIA* inactivates *NFKB1* signalling, which is an inhibitor of epidermal growth, thereby producing cSCC lesions [148].

The maintenance of CSC subpopulations in cSCC is dependent on aberrant Wnt/*β*-catenin signalling. [99,102] Malanchi et al. demonstrated that murine HF stem cells that expressed the surface marker, CD34, define a subset of cells within the HF bulge niche capable of initiating SCC following tumor initiation and promotion by DMBA (7,12-dimethylbenz[a]anthracene) and TPA (12-*O*-tetradecanoylphorbol-13-acetate) treatment, respectively [149]. Interestingly, *β*-catenin loss sensitized CD34+ CSC pools to depletion, causing loss of their tumorigenic potential to produce secondary lesions [149]. It has been postulated that constitutively active β-catenin in cSCC lesions acts through the *β*-catenin/LEF/TCF transcription factor axis, directly upregulating Wnt-responsive targets and aiding tumor progression [102,149–151].

Other CSC-associated genes are reliable prognostic markers and targets to isolate cSCC-initiating subpopulations, including CD133, CD44 and CD29. CD133 is a putative stemness and tumor burden marker extensively studied in a variety of cancers [152–154]. In cSCC, CD133+ tumor-initiating cells, which demonstrate stemness by limiting dilution cloning, have tumorigenic potential [155]. Work by Geng and colleagues explored the role of CD29 and CD44 in promoting epithelial to mesenchymal transition (EMT) of cSCC tumor-initiating cells. $CD29^{high}/CD44^{high}$ CSCs demonstrated a mesenchymal phenotype and transcriptome, (e.g., reduced E-cadherin and increased vimentin, N-cadherin, fibronectin and nuclear *β*-catenin) [156]. These CSCs exhibit increased Wnt/*β*-catenin signalling and became largely localized at the tumor-stromal interface [156]. While the mechanisms of EMT and how crosstalk with the microenvironment niche triggers this phenotype is poorly understood, a significant area of research has become devoted to understanding these underlying processes [157].

Although most SCC mouse models use CD34 as a biomarker for identifying hair follicle CSC, there is currently no evidence in the literature for CD34 expression in human hair follicle or IEF CSC subpopulations, complicating translation of experimental models to humans and prompting studies that broaden the array of human CSC biomarkers [102,158]. Siegle and colleagues demonstrated that the stemness marker, SOX2, is essential for initiating invasive human and mouse cSCC [159]. By inducing angiogenic mimicry through the Nrp1/VEGF pathway, SOX2 facilitates the expansion of tumor-initiating cell populations along the tumoral boundary [159]. Given that SOX2 is not expressed in murine and human epidermal homeostasis it has emerged as an important marker of tumor-initiating cells in cSCC neoplasms [160].

The concept of cancer stem cells has become a central paradigm in probing the hierarchical organization of tumors. While major strides have been made to understand the roles of CSCs in the etiologies of melanoma, BCC, and cSCC, major questions remain about the complex interactions between CSCs and their niche microenvironments, their role immune evasion, metastasis, and relapse, as well as defining the events modulating lineage commitment of CSC compartments. Identification of stemness biomarkers will help in isolating and studying sCSC biology but also serve as potential targets for theragnostic approaches to treatment in skin cancers.

3.3. Mechanisms of Therapy Resistance in sCSCs

Distinct features of sCSCs are pivotal in therapy resistance of all three major forms of skin cancer. Despite numerous therapy treatments designed to specifically target the molecular mechanisms behind unregulated cell cycle progression, these unpredictable cells have taken advantage of various alternative pathways, leading to a quickly developed resistance to common primary therapies. In fact, it is suggested that cases of metastatic or recurring melanoma show an expression of CD133 that is two-fold higher compared to non-recurring instances [155].

Melanoma therapy treatments often involve the use of BRAF inhibitors (e.g. vemurafenib), blocking RAS-RAF signaling, a frequently overexpressed pathway in CM that serves to augment cell proliferation and survival [161]. Although this treatment has demonstrated promising efficacy, use

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of these therapies eventually leads to tumor recurrence through secondary signaling pathways [162,163]. Stem cells utilize CD133 in activating PI3K pathways, which are known to inhibit multiple MAPKs (p38 and JNK) and influence other tumor suppressor genes such as p53. Other pathways related to tumor recurrence directly reactivate the RAS-RAF pathway blocked by the BRAF inhibitors. RHOB, a gene found to be overexpressed in melanoma cells treated with BRAF-inhibitors, desensitizes the effects of BRAF inhibitors, leading to a reversal of expression that was previously suppressed by therapy treatments, or a lack of proper execution to begin [152,164]. Evidently, melanoma cells, specifically those that exhibit recapitulation when treated with current therapies, exploit the heterogeneity of tumors to override anti-proliferative mechanisms and increase survival.

Basal cell carcinoma demonstrates similar resistance to current therapies. Targeted primary therapies such as vismodegib exhibit a response rate of 43% in cBCC patients but resistance to this treatment is common [165]. Functionally, SMO-inhibitors effectively deactivate mutant Hedgehog/Gli-1 signaling but resistant tumor cells promote signaling through transcription factors such as AP-1 to reactivate expression of SMO target genes initially blocked by therapy [166]. In cBCC, a key player in promoting stemness in the sCSC subpopulation is the transcription factor SOX2. Direct targeting of SOX2 has led to reduced tumor invasion, migration, and survival. Thus, factors in stem cells contribute greatly the resistance and regrowth of tumors initially targeted through primary therapies, as they demonstrate a unique subpopulation that monotherapies struggle to effectively treat. Not sure what this means.

sCSCs in SCC develop unique mechanisms to evade primary therapies and promote tumor recurrence. TGF-β is a stem-cell regulator that can drive tumorigenesis when expression levels are low, and promote therapy resistance by increasing expression levels and stemness characteristics [167,168]. Furthermore, stem cells contain properties that enhance tumor survival. Many resistant forms of cSCC utilize the NF-κB pathway mediated by NOTCH, a key stem-cell factor [169]. Upon activation of NF-κB by NOTCH, tumors have been shown to demonstrate increased angiogenesis and metastasis, and thus overall strength and resistance to therapies [170]. Clearly, sCSCs are crucial in avoiding complete tumor-elimination and promoting tumor recurrence following therapy.

In melanoma and NMSCs, therapy resistance is promoted by stem-like subpopulations with a capacity to overcome targeted therapies through exploitation of alternative mechanisms known to reverse or directly combat primary treatment. Although each driving force is unique, sCSCs all demonstrate the ability to resist anti-tumorigenic effects. Currently, research behind these mechanisms is limited, especially in NMSCs, and a better understanding is essential in determining the measures to combat these heterogenous systems.

4. Cell Cycle Targeting Approaches

4.1. Concept of Cell Cycle Targeting in Skin Cancer

Cell cycle targeting is a promising strategy for skin cancer therapy, focusing on the regulation of CDKs and other key cell cycle regulators (Table 4) [171,172]. A key focus is on CDKs such as CDK4/6 and CDK2, which are crucial for the regulated progression through different phases of the cell cycle [53,171,172]. CDK4/6 inhibitors, including palbociclib (PD0332991), ribociclib (LEE011), and abemaciclib (LY2835219), have shown efficacy in preclinical and clinical studies, particularly in melanoma [53,171,173]. These inhibitors work by preventing the phosphorylation of the retinoblastoma (Rb) protein, thereby halting cell cycle progression from the G1 to the S phase [53]. Additionally, CDK2 inhibitors like dinaciclib have shown promise in preclinical models by inducing apoptosis in cancer cells [174]. CDK2 plays a significant role in the transition from the G1 to the S phase, and its inhibition can lead to cell cycle arrest and subsequent cell death [174].

Another strategy involves targeting G2-M checkpoint kinases such as CHK1 and CHK2, which are integral to the DNA damage response and repair mechanisms [172,175]. Inhibitors like prexasertib (LY2606368) and AZD7762 aim to restore normal cell cycle checkpoints that are often bypassed in cancer cells, leading to increased tumor cell death [176–178]. Studies have shown that these inhibitors can enhance the efficacy of other treatments, such as chemotherapy and radiation, by preventing cancer cells from repairing DNA damage [176–178].

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Reactivating tumor suppressor proteins, particularly p53, is another crucial approach in cell cycle targeting. MDM2 inhibitors, such as nutlin-3 and RG7388, work by blocking the interaction between MDM2 and p53, thereby restoring p53's ability to induce cell cycle arrest and apoptosis [172,179,180]. This strategy is particularly relevant in cancers where p53 is inactivated due to MDM2 overexpression. Clinical trials have demonstrated that these inhibitors can reactivate p53 function, leading to significant antitumor effects in various cancer types [179]. Each of these cell cycle targeting strategies holds potential for improving the efficacy of existing treatments and achieving better therapeutic outcomes for skin cancer patients.

4.2. Synthetic CKIs

The development of synthetic cyclin-dependent kinase inhibitors (CKIs) has evolved from broad-spectrum pan-CKIs to highly specific agents targeting individual CDKs [171,172,181]. Early examples include roscovitine (seliciclib), which inhibits CDK1/2/5/7/9 but showed limited efficacy and high toxicity in phase I clinical studies. Flavopridol (alvocidib), a pan-CKI targeting CDK1/2/4/7/9, showed greater promise in preclinical studies but did not achieve the desired response in phase II trials [172]. The failure of multiple broad-spectrum CKIs in clinical trials has led to a shift towards more selective CDK-targeting approaches [173].

Current research is focused on several specific CKIs for melanoma therapy. CDK4/6 is of particular interest due to its role in mediating cell progression through the G1 phase of the cell cycle. The most developed CDK4/6 inhibitors for melanoma include palbociclib, abemaciclib, and ribociclib [53,172]. These orally available selective inhibitors bind to the ATP-binding domain of CDK4/6, leading to cell cycle arrest at the G1/S checkpoint. Notably, abemaciclib and ribociclib exhibit greater selectivity for CDK4 over CDK6, while palbociclib inhibits both CDK4 and CDK6 with similar potency [182].

In preclinical studies, palbociclib reduces Rb phosphorylation and the Ki67 proliferation marker, as well as downregulate E2F target genes at nanomolar concentrations, indicating effective inhibition of CDK4/6 activity [171]. In addition to cell cycle arrest, palbociclib can induce senescence and increase cell death in melanoma cell lines [53]. Abemaciclib achieves CDK4/6 inhibition at nanomolar concentrations with higher potency and has been shown to cross the blood-brain barrier, which is significant for potentially treating brain metastases, a common issue in melanoma [182,183]. Moreover, abemaciclib has broader activity against other cyclin-dependent kinases such as CDK1, CDK2, CDK7, and CDK9, which may contribute to more comprehensive tumor cell proliferation inhibition and enhanced efficacy in combination therapies. Ribociclib exhibits inhibitory activity against CDK4/cyclin D1 and CDK6 complexes at sub-micromolar concentrations and has demonstrated in vivo antitumor activity in melanoma, although it requires functional Rb protein [184,185]. Phase I studies have established recommended dosing levels: 125 mg daily for 3-4 weeks or 200 mg daily for 2-3 weeks for Palbociclib; 200 mg twice daily continuously for Abemaciclib; and 600 mg daily for 3-4 weeks for Ribociclib [171,172]. Phase II clinical trials for all three CDK4/6 inhibitors are ongoing.

Genetic alterations, particularly in the MAPK pathway, are critical in the development of metastatic melanomas. The $BRAF^{V600E}$ mutation occurs in approximately 66% of melanoma cases, making it a key therapeutic target.[186] Vemurafenib, an FDA-approved $BRAF^{V600E}$ inhibitor, is effective but often leads to tumor relapse due to acquired resistance [187,188]. Research by Yoshida et al. has shown that vemurafenib-resistant tumors remain sensitive to palbociclib, suggesting that sequential treatment with vemurafenib followed by palbociclib could potentially overcome resistance [189]. Similarly, a study by Yadav et al. found that abemaciclib could also overcome vemurafenib resistance in V600E-mutant melanoma cell lines, indicating the potential of combining CDK4/6 inhibitors with MAPK pathway inhibitors [190].

Activating *NRAS* mutations, which occur in approximately 15% to 20% of melanomas, drive tumor progression through the MAPK pathway [191]. Combining CKIs with mitogen-activated protein kinase (MEK) inhibitors has shown promise in treating this subtype [172]. A preclinical study by Kwong et al. found that while MEK inhibition induces apoptosis in murine models of *NRAS*-

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mutant melanoma, it fails to effectively arrest the cell cycle [192]. The combination of *MEK* and CDK4 inhibitors, however, demonstrated significant *in vivo* synergy, addressing the limitations of *MEK* inhibition alone by targeting both apoptotic pathways and cell cycle progression. In clinical studies, the combination of ribociclib and MEK inhibitor binimetinib in *NRAS*-mutant melanoma showed favorable efficacy and manageable toxicity, with a 35% partial response rate and a median progression-free survival of 6.7 months [193,194]. Additionally, the triple combination of encorafenib (BRAFi) and binimetinib (MEKi) with ribociclib in $BRAF^{V600E}$-mutant melanoma achieved an objective response rate of 52% [195]. These combination therapies provide promising approaches for effectively treating specific melanoma subtypes.

Although CDK2 inhibition is less studied in melanoma compared to CDK4/6 inhibition, it could offer significant benefits for controlling melanoma cell proliferation. Dinaciclib, a selective CKI targeting CDK2 while also affecting CDK1/5/9, has shown significant anti-tumor activity in preclinical melanoma models [173]. Desai et al. found that while CDK2 is dispensable for most tumors, it is essential for melanoma cell proliferation, regulated by MITF [174]. Dinaciclib inhibited melanoma cell growth, induced G2/M cell arrest and apoptosis, and caused tumor regression in mouse xenografts by reducing Rb phosphorylation and Bcl-2 expression. The drug's pro-apoptotic effects require p53 activation, as knocking down p53 completely abolished apoptosis. Newly synthesized quinazolinone-based CDK2 inhibitors are also being investigated for their potential anti-cancer effects through selective CDK2 inhibition [196].

Of importance to therapeutical regulation of CDK activity are non-canonical CDK binding partners such as Spy1. Spy1 directly binds and activates both CDK1 and CDK2 independent of post-translational modification known to regulate access to the CDK active site [197,198,199,200] and regulates expansion of CSC populations in different types of cancer [201,202]. Although this unique activation of CDKs may render Spy1-CDK complex insensitive to inhibition with synthetic CKIs, designed to target canonical cyclin-CDK complexes, it offers a new and attractive therapeutical target.

While most research on CKIs has focused on melanoma, BCC and cSCC are less studied despite sharing disrupted pathways involving cell cycle regulation. Given the similarities in the molecular mechanisms driving these skin cancers, CDK4/6 and CDK2 inhibitors hold potential as effective treatments. Further research is essential to evaluate their efficacy and safety specifically in BCC and cSCC, ensuring a comprehensive understanding of their therapeutic potential in these more common forms of skin cancer.

4.3. Other Cell Cycle Targeting Drugs

As discussed, an important target in melanoma therapy is the inhibition of the MAPK signal transduction pathway, of which the ubiquitously known kinase, BRAF, is a member of. Additional pathway members include *RAS*, *RAF*, *MEK* and *ERK*, all of which have been previously targeted using small molecular inhibition, antisense therapy and antibodies [203]. Examples described in literature include both mono- and combination therapy with vemurafenib ($BRAF^{V600E}$ mutation), anti-angiogenics such as sorafenib and bevacizumab, RAF-265 inhibitor, as well as trametinib and binimetinib (MEKi) [188,204–206]. Using a fluorescent cell cycle reporter (FUCCI), Haass et al. found that melanoma cells arrested in G1 through MAPKi exhibited resistance to G2-M phase drugs (bortezomib, temozolomide), highlighting cell cycle phase-specific drug sensitivity in melanoma [207]. Moreover, some studies have taken advantage of cell cycle dysregulation in melanoma. Tumor cells with an abrogated G1 checkpoint rely on G2-M for DNA repair, presenting the opportunity of G2-M specific targeting. Barnaba et al. showed that these checkpoint-deficient melanoma cells were more successfully eliminated with inhibition of the G2-M kinase, CHK1 [208]. These studies emphasize the use of sequential and/or combination therapy to counter-act therapy resistance.

The focus toward additional cell cycle targeting in NMSCs is relatively inadequate. Existing therapy of BCC includes targeting of the Hedgehog pathway with inhibitors such as vismodegib and sonidegib [209]. Some studies have suggested exploring the potential of combination regimens through simultaneous inhibition of other signaling pathways indirectly related to Hh, such as PI3K/Akt/mTOR, EGFR and the MAPK pathways [210,211]. Darido et al. demonstrated that the

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inhibition of PI3K/mTOR signalling with a dual inhibitor (NVP-BEZ235) successfully prevented tumor initiation in carcinogen exposed mice, as well as delaying progression of induced papillomas. However, when applied to mice with existing cSCC, this oncogenic inhibitor displayed minimal effect [212]. Zou et al. revealed similar anti-proliferative success with administration of the PI3K/Akt/mTOR inhibitor LY3023414, blocking tumor initiation in immunodeficient mice [213].

Overall, there is some promise in preliminary studies that validate a need for further exploration of cell cycle associated intricacies, although quantity and replicability of existing studies does remain limited.

5. Active Clinical Trials and Potential of Cell Cycle Trageting

Emerging advancements in the treatment of skin cancer are notable in immunotherapy and target-specific treatments. The effects of short-term fasting in combination with PD-1/PD-L1 inhibitors like pembrolizumab, nivolumab, and others were studied in patients with advanced or metastatic skin cancer. The impact of those therapies on cell cycle or potential combination with cell cycle targeting approaches are understudied. Here we present the most current clinical trials using novel therapy approaches and include potential therapy approaches using treatment combined with cell cycle targeting reported in other types of cancer (Table 5).

Short-term fasting induced a state of metabolic stress that affected cancer cells more significantly than normal cells. The study assessed the impact on tumor response, quality of life, and key biomarkers related to oxidative stress, insulin, and immune signaling pathways. Additionally, this novel approach aimed to decrease side effects of immunotherapy and study the cancer-fighting capability [214]. Fasting mimicking diet (FMD) was shown before to increase treatment efficacy of triple negative breast cancer and block cancer stem cell escape by potentiating the effects of CDK4/6 inhibitor, palbociclib [215]. In melanoma, palbociclib showed promising synergistic results *in vitro* and *in vivo* with other therapeutics [216]. Hence, a combination of FMD with palbociclib is a potential approach in skin cancer treatment awaiting assessment. Another, phase I/II clinical trial, is looking at the efficacy of gene-modified FH-MCVA2TCR T-cells for the treatment of metastatic or unresectable Merkel cell carcinoma. This trial is designed to explore the safety and effectiveness of these modified immune cells, which are engineered to target specific antigens on tumour cells. Initially, patients receive interferon gamma-1b to support immune function, followed by intravenous administration of the FH-MCVA2TCR T-cells. Subsequent treatment with either avelumab or pembrolizumab, based on patient response, aims to enhance therapeutic outcomes. This study also includes long-term follow-up to monitor treatment efficacy and safety [217]. Inhibitors of CDK4/6 can significantly enhance activation of T cells [218]. Introducing of CDK4/6 inhibitors upon administration of FH-MCVA2TCR cells could potentially contribute to increased activation of those cells against types of skin cancer.

Avelumab, a human IgG1 monoclonal antibody immune checkpoint inhibitor, with and without cetuximab, are tested in a phase II clinical trial that is currently underway in patients with metastatic squamous cell carcinoma of the skin. Avelumab inhibits PD-L1 therefore enhancing the immune system's ability to attack cancer cells, while cetuximab inhibits the EGFR. The primary objective of the trial is to determine if combining avelumab with cetuximab prolongs progression-free survival compared to avelumab alone. Secondary objectives include evaluating the confirmed objective response rate, clinical benefit rate, and overall survival for each treatment arm, as well as assessing toxicity. Combining these therapies may enhance immune response and disrupt cancer cell proliferation by targeting both immune evasion and growth signaling pathways, potentially leading to better clinical outcomes [219]. Efficacy of EGFR inhibition was significantly enhanced in combination with palbociclib which blocked the emergence of EGFR resistance *in vitro* in oesophageal squamous cell carcinoma [220]. CDK4/6 activity was also shown to attenuate the effects of EGFR inhibitor treatment of non-small cell lung cancer [221]. Combining treatment with avelumab with cetuximab plus CDK4/6 inhibitor, could potentially improve the efficacy of the avelumab-cetuximab approach. Similarly, a phase II clinical trial is evaluating the efficacy of adjuvant nivolumab, with or without cabozantinib, in preventing the recurrence of resected mucosal melanoma. Nivolumab is an

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immune checkpoint inhibitor that targets PD-1, assisting the immune system's ability to attack cancer cells. Cabozantinib is a tyrosine kinase inhibitor that blocks multiple pathways involved in tumor growth and angiogenesis. The rationale for combining these drugs is to potentially enhance treatment efficacy. Nivolumab aims to boost immune response against remaining cancer cells, while cabozantinib targets additional cancer growth mechanisms. This combination may provide a more comprehensive approach to reducing the risk of melanoma recurrence after surgery [222].

Combination of nivolumab with abemaciclib, a CDK4/6 inhibitor was tested in phase II of clinical trial in HR+/HER2 breast cancer patients and despite observed antitumour effect it significant adverse immune effects [223]. However, cabozantinib synergized before with dasatinib an inhibitor of CDK1,2,4, and 6 to induce tumour regression in renal cell carcinoma, suggesting that targeting of CDKs could potentiate the effects of cabozantinib allowing for testing of lower doses of the combination towards reduced side effects. [224,225] A phase II randomized trial is investigating the combination of fecal microbiota transplantation (FMT) with immune checkpoint blockade compared to immune checkpoint blockade alone in patients with advanced melanoma. The rationale behind this approach is based on emerging evidence that gut microbiota can influence the immune system and potentially increase responses to immunotherapy. By improving the gut microbiome, FMT may help boost the efficacy of immune checkpoint inhibitors, leading to better patient outcomes. The trial aims to determine if this combination provides impressive clinical benefits compared to immune checkpoint blockade alone [226]. It is hypothesized that FMT can improve response to CDK4/6 inhibitors [227]. With reported priming activity of CDK inhibition for anti PD-L1 treatment, combination of the three therapeutic approaches can lead to potentiated anti-tumour effects in skin cancer [228].

The effectiveness of cemiplimab administered prior to surgery is tested in patients with high-risk skin cancer that is either localized, locally recurrent, or regionally advanced but still resectable [229]. Cemiplimab is another monoclonal antibody that targets PD-1, a protein that helps cancer cells evade the immune system. By blocking PD-1, cemiplimab aims to enhance the immune system's ability to recognize and attack cancer cells. The trial aims to determine whether pre-surgical treatment with cemiplimab can hopefully improve outcomes by shrinking tumors or enhancing the immune response, potentially leading to better surgical results and reducing the risk of cancer recurrence. Although there is no significant data available describeing effects of cemiplimab combined with CDK inhibition, an ongoing clinical trial is studying the effects of cemiplimab in combination with palbociclib in patients with liposarcoma [230]. Another study compares two treatment approaches for resectable stage III melanoma. One group receives neoadjuvant therapy with BCD-217 (a combination of Nurulimab and Prolgolimab, monoclonal antibodies targeting cytotoxic T-lymphocyte associated protein (CTLA-4) and a PD-1 inhibitor, respectively) before surgery, while the other receives standard adjuvant therapy with pembrolizumab after surgery. Treatment continues for up to 12 months or until disease progression or unacceptable toxicity occurs. The objective of this study is to assess whether BCD-217 is more effective or safer than pembrolizumab alone in managing the disease, which again highlights the importance of targeting cell cycle check points in treating skin cancer [231]. Pembrolizumab was demonstrated previously to synergize with CDK4/6 inhibitor, abemaciclib, however combination of CDK inhibitors with BCD 217 has not been tested to date [232].

6. Summary

Cutaneous melanoma is a highly dynamic and heterogeneous malignancy. Despite the revolution of targeted therapy, melanoma poses recurrent difficulty in treatment while maintaining its notoriously poor prognosis in advanced stages. Improved characterization of the landscape of driving mutations has been valuable in furthering our understanding of cell cycle dysregulation in the malignancy, while simultaneous attempt at better deciphering the role of sCSCs in tumorigenesis, progression and therapy resistance is required. Likewise, our understanding of basal cell and cutaneous squamous cell carcinoma has continued to expand, yet the impact of a disrupted cell cycle does not appear to be as profound in tumorigenesis and prognosis relative to melanoma, perhaps secondary to the clear under-exploration of this conserved cell function. Regarding therapy of

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melanoma, there is apparent promise in checkpoint inhibition with the combined use of CKIs, particularly noted in the partnership of CDK4/6 and MEK inhibition. There has been a shift from pan-CKI use to phase-specific inhibition, with discussion of the overall success still pending on-going clinical trials. Contrastingly, such application has remained limited in NMSCs, with low-volume evidence suggesting promise in anti-proliferative targeting. Despite acknowledgement of a potential role, there has been minimal investigation in cBCC and cSCC. There is an evident role of the cell cycle in skin cancer, with promising pre-clinical and clinical data in melanoma. The venue for applied therapy remains open to further investigation.

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17-48. doi:10.3322/caac.21763
2. Melanoma of the Skin - Cancer Stat Facts. SEER. Accessed August 4, 2024. https://seer-cancer-gov.proxy.bib.uottawa.ca/statfacts/html/melan.html
3. Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of Melanoma. *Med Sci Basel Switz*. 2021;9(4):63. doi:10.3390/medsci9040063
4. El Sharouni MA, van Diest PJ, Witkamp AJ, Sigurdsson V, van Gils CH. Subtyping Cutaneous Melanoma Matters. *JNCI Cancer Spectr*. 2020;4(6):pkaa097. doi:10.1093/jncics/pkaa097
5. Rabbie R, Ferguson P, Molina-Aguilar C, Adams DJ, Robles-Espinoza CD. Melanoma subtypes: genomic profiles, prognostic molecular markers and therapeutic possibilities. *J Pathol*. 2019;247(5):539-551. doi:10.1002/path.5213
6. Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. *Cancer Biol Ther*. 2019;20(11):1366-1379. doi:10.1080/15384047.2019.1640032
7. Wunderlich K, Suppa M, Gandini S, Lipski J, White JM, Del Marmol V. Risk Factors and Innovations in Risk Assessment for Melanoma, Basal Cell Carcinoma, and Squamous Cell Carcinoma. *Cancers*. 2024;16(5):1016. doi:10.3390/cancers16051016
8. Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. *Nature*. 2007;445(7130):843-850. doi:10.1038/nature05660
9. Hu DN, Savage HE, Roberts JE. Uveal melanocytes, ocular pigment epithelium, and Müller cells in culture: in vitro toxicology. *Int J Toxicol*. 2002;21(6):465-472. doi:10.1080/10915810290169891
10. Goel VK, Lazar AJF, Warneke CL, Redston MS, Haluska FG. Examination of mutations in BRAF, NRAS, and PTEN in primary cutaneous melanoma. *J Invest Dermatol*. 2006;126(1):154-160. doi:10.1038/sj.jid.5700026
11. Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. *Cell*. 2015;161(7):1681-1696. doi:10.1016/j.cell.2015.05.044
12. Bedikian AY, Millward M, Pehamberger H, et al. Bcl-2 antisense (oblimersen sodium) plus dacarbazine in patients with advanced melanoma: the Oblimersen Melanoma Study Group. *J Clin Oncol Off J Am Soc Clin Oncol*. 2006;24(29):4738-4745. doi:10.1200/JCO.2006.06.0483
13. Knight A, Karapetyan L, Kirkwood JM. Immunotherapy in Melanoma: Recent Advances and Future Directions. *Cancers*. 2023;15(4):1106. doi:10.3390/cancers15041106
14. Proietti I, Skroza N, Michelini S, et al. BRAF Inhibitors: Molecular Targeting and Immunomodulatory Actions. *Cancers*. 2020;12(7):1823. doi:10.3390/cancers12071823
15. Patel M, Eckburg A, Gantiwala S, et al. Resistance to Molecularly Targeted Therapies in Melanoma. *Cancers*. 2021;13(5):1115. doi:10.3390/cancers13051115
16. Cameron MC, Lee E, Hibler BP, et al. Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and disease associations. *J Am Acad Dermatol*. 2019;80(2):303-317. doi:10.1016/j.jaad.2018.03.060
17. Riew TR, Kim YS. Mutational Landscapes of Normal Skin and Their Potential Implications in the Development of Skin Cancer: A Comprehensive Narrative Review. *J Clin Med*. 2024;13(16):4815. doi:10.3390/jcm13164815
18. Martincorena I, Roshan A, Gerstung M, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science*. 2015;348(6237):880-886. doi:10.1126/science.aaa6806
19. Scrivener Y, Grosshans E, Cribier B. Variations of basal cell carcinomas according to gender, age, location and histopathological subtype. *Br J Dermatol*. 2002;147(1):41-47. doi:10.1046/j.1365-2133.2002.04804.x
20. Rogers HW, Weinstock MA, Feldman SR, Coldiron BM. Incidence Estimate of Nonmelanoma Skin Cancer (Keratinocyte Carcinomas) in the US Population, 2012. *JAMA Dermatol*. 2015;151(10):1081-1086. doi:10.1001/jamadermatol.2015.1187

21. Muzic JG, Schmitt AR, Wright AC, et al. Incidence and Trends of Basal Cell Carcinoma and Cutaneous Squamous Cell Carcinoma: A Population-Based Study in Olmsted County, Minnesota, 2000 to 2010. *Mayo Clin Proc*. 2017;92(6):890-898. doi:10.1016/j.mayocp.2017.02.015
22. Epidemiology of basal cell carcinoma: scholarly review | British Journal of Dermatology | Oxford Academic. Accessed August 1, 2024. https://academic.oup.com/bjd/article-abstract/177/2/359/6668659?redirectedFrom=fulltext&login=false
23. Eisemann N, Waldmann A, Geller AC, et al. Non-melanoma skin cancer incidence and impact of skin cancer screening on incidence. *J Invest Dermatol*. 2014;134(1):43-50. doi:10.1038/jid.2013.304
24. Johnson RL, Rothman AL, Xie J, et al. Human Homolog of patched, a Candidate Gene for the Basal Cell Nevus Syndrome. *Science*. 1996;272(5268):1668-1671. doi:10.1126/science.272.5268.1668
25. Youssef KK, Van Keymeulen A, Lapouge G, et al. Identification of the cell lineage at the origin of basal cell carcinoma. *Nat Cell Biol*. 2010;12(3):299-305. doi:10.1038/ncb2031
26. Dika E, Scarfì F, Ferracin M, et al. Basal Cell Carcinoma: A Comprehensive Review. *Int J Mol Sci*. 2020;21(15):5572. doi:10.3390/ijms21155572
27. Athar M, Tang X, Lee JL, Kopelovich L, Kim AL. Hedgehog signalling in skin development and cancer. *Exp Dermatol*. 2006;15(9):667-677. doi:10.1111/j.1600-0625.2006.00473.x
28. Epstein EH. Basal cell carcinomas: attack of the hedgehog. *Nat Rev Cancer*. 2008;8(10):743-754. doi:10.1038/nrc2503
29. Bonilla X, Parmentier L, King B, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nat Genet*. 2016;48(4):398-406. doi:10.1038/ng.3525
30. Di Nardo L, Pellegrini C, Di Stefani A, et al. Molecular alterations in basal cell carcinoma subtypes. *Sci Rep*. 2021;11(1):13206. doi:10.1038/s41598-021-92592-3
31. McDaniel B, Badri T, Steele RB. Basal Cell Carcinoma. In: *StatPearls*. StatPearls Publishing; 2024. Accessed September 14, 2024. http://www.ncbi.nlm.nih.gov/books/NBK482439/
32. LoRusso PM, Rudin CM, Reddy JC, et al. Phase I Trial of Hedgehog Pathway Inhibitor Vismodegib (GDC-0449) in Patients with Refractory, Locally Advanced or Metastatic Solid Tumors. *Clin Cancer Res*. 2011;17(8):2502-2511. doi:10.1158/1078-0432.CCR-10-2745
33. Chang ALS, Oro AE. Initial Assessment of Tumor Regrowth After Vismodegib in Advanced Basal Cell Carcinoma. *Arch Dermatol*. 2012;148(11):1324-1325. doi:10.1001/archdermatol.2012.2354
34. Cassarino DS, Derienzo DP, Barr RJ. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. Part one. *J Cutan Pathol*. 2006;33(3):191-206. doi:10.1111/j.0303-6987.2006.00516_1.x
35. Clinicopathologic variants of cutaneous squamous cell carcinoma - PubMed. Accessed August 4, 2024. https://pubmed-ncbi-nlm-nih-gov.proxy.bib.uottawa.ca/11152092/
36. Winge MCG, Kellman LN, Guo K, et al. Advances in cutaneous squamous cell carcinoma. *Nat Rev Cancer*. 2023;23(7):430-449. doi:10.1038/s41568-023-00583-5
37. Lober BA, Lober CW, Accola J. Actinic keratosis is squamous cell carcinoma. *J Am Acad Dermatol*. 2000;43(5):881. doi:10.1067/mjd.2000.108373
38. From actinic keratosis to squamous cell carcinoma | British Journal of Dermatology | Oxford Academic. Accessed August 2, 2024. https://academic.oup.com/bjd/article-abstract/146/s61/20/6634287?login=false
39. Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA. Factors Predictive of Recurrence and Death From Cutaneous Squamous Cell Carcinoma: A 10-Year, Single-Institution Cohort Study. *JAMA Dermatol*. 2013;149(5):541-547. doi:10.1001/jamadermatol.2013.2139
40. Chow RY, Jeon US, Levee TM, et al. PI3K Promotes Basal Cell Carcinoma Growth Through Kinase-Induced p21 Degradation. *Front Oncol*. 2021;11:668247. doi:10.3389/fonc.2021.668247
41. Rünger TM, Kappes UP. Mechanisms of mutation formation with long-wave ultraviolet light (UVA). *Photodermatol Photoimmunol Photomed*. 2008;24(1):2-10. doi:10.1111/j.1600-0781.2008.00319.x
42. Gold KA, Kies MS, William WN, Johnson FM, Lee JJ, Glisson BS. Erlotinib in the Treatment of Recurrent or Metastatic Cutaneous Squamous Cell Carcinoma: A Single Arm Phase II Clinical Trial. *Cancer*. 2018;124(10):2169-2173. doi:10.1002/cncr.31346
43. Guidelines of care for the management of cutaneous squamous cell carcinoma - PMC. Accessed August 2, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6652228/
44. Walker GJ, Flores JF, Glendening JM, Lin A (Hsui T, Markl IDC, Fountain JW. Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. *Genes Chromosomes Cancer*. 1998;22(2):157-163. doi:10.1002/(SICI)1098-2264(199806)22:2<157::AID-GCC11>3.0.CO;2-N
45. Sanki A, Li W, Colman M, Karim RZ, Thompson JF, Scolyer RA. Reduced expression of p16 and p27 is correlated with tumour progression in cutaneous melanoma. *Pathology (Phila)*. 2007;39(6):551-557. doi:10.1080/00313020701684409
46. Somatic p16INK4a loss accelerates melanomagenesis - PMC. Accessed August 4, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3007178/

47. Narasimha AM, Kaulich M, Shapiro GS, Choi YJ, Sicinski P, Dowdy SF. Cyclin D activates the Rb tumor suppressor by mono-phosphorylation. *eLife*. 2014;3:e02872. doi:10.7554/eLife.02872
48. Kong Y, Sheng X, Wu X, et al. Frequent Genetic Aberrations in the CDK4 Pathway in Acral Melanoma Indicate the Potential for CDK4/6 Inhibitors in Targeted Therapy. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2017;23(22):6946-6957. doi:10.1158/1078-0432.CCR-17-0070
49. Rane SG, Cosenza SC, Mettus RV, Reddy EP. Germ line transmission of the Cdk4(R24C) mutation facilitates tumorigenesis and escape from cellular senescence. *Mol Cell Biol*. 2002;22(2):644-656. doi:10.1128/MCB.22.2.644-656.2002
50. Zou X, Ray D, Aziyu A, et al. Cdk4 disruption renders primary mouse cells resistant to oncogenic transformation, leading to Arf/p53-independent senescence. *Genes Dev*. 2002;16(22):2923-2934. doi:10.1101/gad.1033002
51. Sauter ER, Yeo UC, von Stemm A, et al. Cyclin D1 is a candidate oncogene in cutaneous melanoma. *Cancer Res*. 2002;62(11):3200-3206.
52. Sauter ER, Nesbit M, Litwin S, Klein-Szanto AJP, Cheffetz S, Herlyn M. Antisense Cyclin D1 Induces Apoptosis and Tumor Shrinkage in Human Squamous Carcinomas1. *Cancer Res*. 1999;59(19):4876-4881.
53. Sheppard KE, McArthur GA. The Cell-Cycle Regulator CDK4: An Emerging Therapeutic Target in Melanoma. *Clin Cancer Res*. 2013;19(19):5320-5328. doi:10.1158/1078-0432.CCR-13-0259
54. Smalley KSM, Lioni M, Palma MD, et al. Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E–mutated melanomas. *Mol Cancer Ther*. 2008;7(9):2876-2883. doi:10.1158/1535-7163.MCT-08-0431
55. Young RJ, Waldeck K, Martin C, et al. Loss of CDKN2A expression is a frequent event in primary invasive melanoma and correlates with sensitivity to the CDK4/6 inhibitor PD0332991 in melanoma cell lines. *Pigment Cell Melanoma Res*. 2014;27(4):590-600. doi:10.1111/pcmr.12228
56. Deregulation of the tumour suppressor genes p14ARF, p15INK4b, p16INK4a and p53 in basal cell carcinoma - Kanellou - 2009 - British Journal of Dermatology - Wiley Online Library. Accessed July 4, 2024. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2133.2009.09079.x
57. Eshkoor SA, Ismail P, Rahman SA, Oshkour SA. p16 Gene Expression in Basal Cell Carcinoma. *Arch Med Res*. 2008;39(7):668-673. doi:10.1016/j.arcmed.2008.06.003
58. Paolini F, Carbone A, Benevolo M, et al. Human Papillomaviruses, p16INK4a and Akt expression in basal cell carcinoma. *J Exp Clin Cancer Res*. 2011;30(1):108. doi:10.1186/1756-9966-30-108
59. Eshkoor S, Rahman S. Gene Expression of CDK 6 and CCND 1 Genes in Basal Cell Carcinoma. In: ; 2008. Accessed August 4, 2024. https://www.semanticscholar.org/paper/Gene-Expression-of-CDK-6-and-CCND-1-Genes-in-Basal-Eshkoor-Rahman/099697740b7d77f89b0053441ee527bda6b0adcf
60. Svensson S, Nilsson K, Ringberg A, Landberg G. Invade or proliferate? Two contrasting events in malignant behavior governed by p16(INK4a) and an intact Rb pathway illustrated by a model system of basal cell carcinoma. *Cancer Res*. 2003;63(8):1737-1742.
61. Teng Y, Yu Y, Li S, et al. Ultraviolet Radiation and Basal Cell Carcinoma: An Environmental Perspective. *Front Public Health*. 2021;9. doi:10.3389/fpubh.2021.666528
62. Hasan A, Kandil AM, Al-Ghamdi HS, et al. Sun-Exposed versus Sun-Protected Cutaneous Basal Cell Carcinoma: Clinico-Pathological Profile and p16 Immunostaining. *Diagn Basel Switz*. 2023;13(7):1271. doi:10.3390/diagnostics13071271
63. Nindl I, Meyer T, Schmook T, et al. Human papillomavirus and overexpression of P16INK4a in nonmelanoma skin cancer. *Dermatol Surg Off Publ Am Soc Dermatol Surg Al*. 2004;30(3):409-414. doi:10.1111/j.1524-4725.2004.30111.x
64. Park HW, Song SY, Lee TJ, Jeong D, Lee TY. Abrogation of the p16-retinoblastoma-cyclin D1 pathway in head and neck squamous cell carcinomas. *Oncol Rep*. 2007;18(1):267-272.
65. Quinn AG, Campbell C, Healy E, Rees JL. Chromosome 9 Allele Loss Occurs in both Basal and Squamous Cell Carcinomas of the Skin. *J Invest Dermatol*. 1994;102(3):300-303. doi:10.1111/1523-1747.ep12371786
66. Zheng Z, Kye Y, Zhang X, Kim A, Kim I. Expression of p63, bcl-2, bcl-6 and p16 in Basal Cell Carcinoma and Squamous Cell Carcinoma of the Skin.
67. Brady CA, Attardi LD. p53 at a glance. *J Cell Sci*. 2010;123(15):2527-2532. doi:10.1242/jcs.064501
68. Shen L, Kondo Y, Hamilton SR, Rashid A, Issa JJ. p14 methylation in human colon cancer is associated with microsatellite instability and wild-type p53. *Gastroenterology*. 2003;124(3):626-633. doi:10.1053/gast.2003.50102
69. Bates S, Phillips AC, Clark PA, et al. p14ARF links the tumour suppressors RB and p53. *Nature*. 1998;395(6698):124-125. doi:10.1038/25867
70. Stott FJ, Bates S, James MC, et al. The alternative product from the human CDKN2A locus, p14ARF, participates in a regulatory feedback loop with p53 and MDM2. *EMBO J*. 1998;17(17):5001-5014. doi:10.1093/emboj/17.17.5001
71. Engeland K. Cell cycle regulation: p53-p21-RB signaling. *Cell Death Differ*. 2022;29(5):946-960. doi:10.1038/s41418-022-00988-z

72. Fargnoli MC, Chimenti S, Peris K, et al. CDKN2a/p16INK4a Mutations and Lack of p19ARF Involvement in Familial Melanoma Kindreds. *J Invest Dermatol*. 1998;111(6):1202-1206. doi:10.1046/j.1523-1747.1998.00412.x
73. Bachmann IM, Straume O, Akslen LA. Altered expression of cell cycle regulators Cyclin D1, p14, p16, CDK4 and Rb in nodular melanomas. *Int J Oncol*. 2004;25(6):1559-1565. doi:10.3892/ijo.25.6.1559
74. Freedberg DE, Rigas SH, Russak J, et al. Frequent p16-Independent Inactivation of p14ARF in Human Melanoma. *JNCI J Natl Cancer Inst*. 2008;100(11):784-795. doi:10.1093/jnci/djn157
75. Rizos H, Darmanian AP, Holland EA, Mann GJ, Kefford RF. Mutations in the INK4a/ARF Melanoma Susceptibility Locus Functionally Impair p14ARF*. *J Biol Chem*. 2001;276(44):41424-41434. doi:10.1074/jbc.M105299200
76. Dobrowolski R, Hein R, Buettner R, Bosserhoff A. Loss of p14ARF expression in melanoma. *Arch Dermatol Res*. 2002;293(11):545-551. doi:10.1007/s00403-001-0274-y
77. Kanellou P, Zaravinos A, Zioga M, et al. Genomic instability, mutations and expression analysis of the tumour suppressor genes p14ARF, p15INK4b, p16INK4a and p53 in actinic keratosis. *Cancer Lett*. 2008;264(1):145-161. doi:10.1016/j.canlet.2008.01.042
78. Brown VL, Harwood CA, Crook T, Cronin JG, Kelsell DP, Proby CM. p16INK4a and p14ARF Tumor Suppressor Genes Are Commonly Inactivated in Cutaneous Squamous Cell Carcinoma. *J Invest Dermatol*. 2004;122(5):1284-1292. doi:10.1111/j.0022-202X.2004.22501.x
79. Pacifico A, Goldberg LH, Peris K, Chimenti S, Leone G, Ananthaswamy HN. Loss of CDKN2A and p14ARF expression occurs frequently in human nonmelanoma skin cancers. *Br J Dermatol*. 2008;158(2):291-297. doi:10.1111/j.1365-2133.2007.08360.x
80. Sharpless NE. *INK4a/ARF*: A multifunctional tumor suppressor locus. *Mutat Res Mol Mech Mutagen*. 2005;576(1):22-38. doi:10.1016/j.mrfmmm.2004.08.021
81. Kamijo T, Zindy F, Roussel MF, et al. Tumor Suppression at the Mouse *INK4a* Locus Mediated by the Alternative Reading Frame Product p19 *ARF*. *Cell*. 1997;91(5):649-659. doi:10.1016/S0092-8674(00)80452-3
82. Russell JL, Weaks RL, Berton TR, Johnson DG. E2F1 suppresses skin carcinogenesis via the ARF-p53 pathway. *Oncogene*. 2006;25(6):867-876. doi:10.1038/sj.onc.1209120
83. Zhang T, Dutton-Regester K, Brown KM, Hayward NK. The genomic landscape of cutaneous melanoma. *Pigment Cell Melanoma Res*. 2016;29(3):266-283. doi:10.1111/pcmr.12459
84. Hodis E, Watson IR, Kryukov GV, et al. A Landscape of Driver Mutations in Melanoma. *Cell*. 2012;150(2):251-263. doi:10.1016/j.cell.2012.06.024
85. Shain AH, Yeh I, Kovalyshyn I, et al. The Genetic Evolution of Melanoma from Precursor Lesions. *N Engl J Med*. 2015;373(20):1926-1936. doi:10.1056/NEJMoa1502583
86. Webster MR, Fane ME, Alicea GM, et al. Paradoxical Role for Wild-Type p53 in Driving Therapy Resistance in Melanoma. *Mol Cell*. 2020;77(3):633-644.e5. doi:10.1016/j.molcel.2019.11.009
87. Terzian T, Torchia EC, Dai D, et al. p53 prevents progression of nevi to melanoma predominantly through cell cycle regulation. *Pigment Cell Melanoma Res*. 2010;23(6):781-794. doi:10.1111/j.1755-148X.2010.00773.x
88. Houben R, Hesbacher S, Schmid CP, et al. High-Level Expression of Wild-Type p53 in Melanoma Cells is Frequently Associated with Inactivity in p53 Reporter Gene Assays. *PLOS ONE*. 2011;6(7):e22096. doi:10.1371/journal.pone.0022096
89. Li W, Sanki A, Karim RZ, et al. The role of cell cycle regulatory proteins in the pathogenesis of melanoma. *Pathology (Phila)*. 2006;38(4):287-301. doi:10.1080/00313020600817951
90. Avery-Kiejda KA, Bowden NA, Croft AJ, et al. P53 in human melanoma fails to regulate target genes associated with apoptosis and the cell cycle and may contribute to proliferation. *BMC Cancer*. 2011;11(1):203. doi:10.1186/1471-2407-11-203
91. Loureiro JB, Abrantes M, Oliveira PA, Saraiva L. P53 in skin cancer: From a master player to a privileged target for prevention and therapy. *Biochim Biophys Acta BBA - Rev Cancer*. 2020;1874(2):188438. doi:10.1016/j.bbcan.2020.188438
92. JCI - An inducible mouse model for skin cancer reveals distinct roles for gain- and loss-of-function p53 mutations. Accessed July 22, 2024. https://www.jci.org/articles/view/31721
93. Bolshakov S, Walker CM, Strom SS, et al. p53 Mutations in Human Aggressive and Nonaggressive Basal and Squamous Cell Carcinomas1. *Clin Cancer Res*. 2003;9(1):228-234.
94. Rigby CM, Roy S, Deep G, et al. Role of p53 in silibinin-mediated inhibition of ultraviolet B radiation-induced DNA damage, inflammation and skin carcinogenesis. *Carcinogenesis*. 2017;38(1):40-50. doi:10.1093/carcin/bgw106
95. Ananthaswamy HN, Loughlin SM, Ullrich SE, Kripke ML. Inhibition of UV-induced p53 mutations by sunscreens: implications for skin cancer prevention. *J Investig Dermatol Symp Proc*. 1998;3(1):52-56.
96. Campbell C, Quinn AG, Ro YS, Angus B, Rees JL. p53 mutations are common and early events that precede tumor invasion in squamous cell neoplasia of the skin. *J Invest Dermatol*. 1993;100(6):746-748. doi:10.1111/1523-1747.ep12475717

97. Yilmaz AS, Ozer HG, Gillespie JL, et al. Differential mutation frequencies in metastatic cutaneous squamous cell carcinomas versus primary tumors. *Cancer*. 2017;123(7):1184-1193. doi:10.1002/cncr.30459
98. Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal | Science Signaling. Accessed July 22, 2024. https://www.science.org/doi/abs/10.1126/scisignal.2004088
99. Boeckmann L, Martens MC, Emmert S. Molecular Biology of Basal and Squamous Cell Carcinomas. In: Reichrath J, ed. *Sunlight, Vitamin D and Skin Cancer*. Springer International Publishing; 2020:171-191. doi:10.1007/978-3-030-46227-7_9
100. Jin Y, Li S, Yu Q, Chen T, Liu D. Application of stem cells in regeneration medicine. *MedComm*. 2023;4(4):e291. doi:10.1002/mco2.291
101. Mannino G, Russo C, Maugeri G, et al. Adult stem cell niches for tissue homeostasis. *J Cell Physiol*. 2022;237(1):239-257. doi:10.1002/jcp.30562
102. Perez-Losada J, Balmain A. Stem-cell hierarchy in skin cancer. *Nat Rev Cancer*. 2003;3(6):434-443. doi:10.1038/nrc1095
103. Till JE, McCULLOCH EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res*. 1961;14:213-222.
104. Fuchs E. Skin stem cells: rising to the surface. *J Cell Biol*. 2008;180:273-284. doi:10.1083/jcb.200708185
105. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther*. 2019;10(1):68. doi:10.1186/s13287-019-1165-5
106. Burns EM, Ahmed H, Isedeh PN, et al. Ultraviolet radiation, both UVA and UVB, influences the composition of the skin microbiome. *Exp Dermatol*. 2019;28(2):136-141. doi:10.1111/exd.13854
107. Liu Y, Chen YG. Intestinal epithelial plasticity and regeneration via cell dedifferentiation. *Cell Regen*. 2020;9:14. doi:10.1186/s13619-020-00053-5
108. Banjac I, Maimets M, Jensen KB. Maintenance of high-turnover tissues during and beyond homeostasis. *Cell Stem Cell*. 2023;30(4):348-361. doi:10.1016/j.stem.2023.03.008
109. Distinct mechanisms for sebaceous gland self-renewal and regeneration provide durability in response to injury - PubMed. Accessed September 14, 2024. https://pubmed-ncbi-nlm-nih-gov.proxy.bib.uottawa.ca/37205445/
110. Donati G, Watt FM. Stem Cell Heterogeneity and Plasticity in Epithelia. *Cell Stem Cell*. 2015;16(5):465-476. doi:10.1016/j.stem.2015.04.014
111. Blanpain C, Fuchs E. Plasticity of epithelial stem cells in tissue regeneration. *Science*. 2014;344(6189):1242281. doi:10.1126/science.1242281
112. Jaks V, Barker N, Kasper M, et al. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet*. 2008;40(11):1291-1299. doi:10.1038/ng.239
113. Nowak JA, Polak L, Pasolli HA, Fuchs E. Hair Follicle Stem Cells Are Specified and Function in Early Skin Morphogenesis. *Cell Stem Cell*. 2008;3(1):33-43. doi:10.1016/j.stem.2008.05.009
114. Ito M, Liu Y, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med*. 2005;11(12):1351-1354. doi:10.1038/nm1328
115. Zhang YV, Cheong J, Ciapurin N, McDermitt DJ, Tumbar T. Distinct Self-renewal and Differentiation Phases in the Niche of Infrequently Dividing Hair Follicle Stem Cells. *Cell Stem Cell*. 2009;5(3):267-278. doi:10.1016/j.stem.2009.06.004
116. Morris RJ, Liu Y, Marles L, et al. Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol*. 2004;22(4):411-417. doi:10.1038/nbt950
117. Kotton DN, Morrisey EE. Lung regeneration: mechanisms, applications and emerging stem cell populations. *Nat Med*. 2014;20(8):822-832. doi:10.1038/nm.3642
118. Belote RL, Le D, Maynard A, et al. Human melanocyte development and melanoma dedifferentiation at single-cell resolution. *Nat Cell Biol*. 2021;23(9):1035-1047. doi:10.1038/s41556-021-00740-8
119. Wong SY, Reiter JF. Wounding mobilizes hair follicle stem cells to form tumors. *Proc Natl Acad Sci*. 2011;108(10):4093-4098. doi:10.1073/pnas.1013098108
120. Yuan S, Stewart KS, Yang Y, et al. Ras drives malignancy through stem cell crosstalk with the microenvironment. *Nature*. 2022;612(7940):555-563. doi:10.1038/s41586-022-05475-6
121. Fang D, Nguyen TK, Leishear K, et al. A Tumorigenic Subpopulation with Stem Cell Properties in Melanomas. *Cancer Res*. 2005;65(20):9328-9337. doi:10.1158/0008-5472.CAN-05-1343
122. Prevodnik VK, Lavrenčak J, Horvat M, Novakovič BJ. The predictive significance of CD20 expression in B-cell lymphomas. *Diagn Pathol*. 2011;6:33. doi:10.1186/1746-1596-6-33
123. Pinc A, Somasundaram R, Wagner C, et al. Targeting CD20 in melanoma patients at high risk of disease recurrence. *Mol Ther J Am Soc Gene Ther*. 2012;20(5):1056-1062. doi:10.1038/mt.2012.27
124. Maurer M, Somasundaram R, Herlyn M, Wagner SN. Immunotargeting of tumor subpopulations in melanoma patients. *Oncoimmunology*. 2012;1(8):1454-1456. doi:10.4161/onci.21357
125. Zeng Y bin, Yu Z chong, He Y ni, et al. Salinomycin-loaded lipid-polymer nanoparticles with anti-CD20 aptamers selectively suppress human CD20+ melanoma stem cells. *Acta Pharmacol Sin*. 2018;39(2):261-274. doi:10.1038/aps.2017.166

126. Mukhtar AB, Morgan HJ, Gibbs A, Davies GE, Lovatt C, Patel GK. Targeting CD20-expressing malignant melanoma cells augments BRAF inhibitor killing. *Br J Dermatol*. 2024;190(5):729-739. doi:10.1093/bjd/ljad502
127. Kang JH, Lee SH, Hong D, et al. Aldehyde dehydrogenase is used by cancer cells for energy metabolism. *Exp Mol Med*. 2016;48(11):e272. doi:10.1038/emm.2016.103
128. Yang L, Ren Y, Yu X, et al. ALDH1A1 defines invasive cancer stem-like cells and predicts poor prognosis in patients with esophageal squamous cell carcinoma. *Mod Pathol*. 2014;27(5):775-783. doi:10.1038/modpathol.2013.189
129. Panigoro SS, Kurnia D, Kurnia A, Haryono SJ, Albar ZA. ALDH1 Cancer Stem Cell Marker as a Prognostic Factor in Triple-Negative Breast Cancer. *Int J Surg Oncol*. 2020;2020:7863243. doi:10.1155/2020/7863243
130. Liu H, Qiu W, Sun T, et al. Therapeutic strategies of glioblastoma (GBM): The current advances in the molecular targets and bioactive small molecule compounds. *Acta Pharm Sin B*. 2022;12(4):1781-1804. doi:10.1016/j.apsb.2021.12.019
131. Püschel J, Dubrovska A, Gorodetska I. The Multifaceted Role of Aldehyde Dehydrogenases in Prostate Cancer Stem Cells. *Cancers*. 2021;13(18):4703. doi:10.3390/cancers13184703
132. Piskounova E, Agathocleous M, Murphy MM, et al. Oxidative stress inhibits distant metastasis by human melanoma cells. *Nature*. 2015;527(7577):186-191. doi:10.1038/nature15726
133. Lu Y, Travnickova J, Badonyi M, et al. ALDH1A3-acetaldehyde metabolism potentiates transcriptional heterogeneity in melanoma. *Cell Rep*. 2024;43(7). doi:10.1016/j.celrep.2024.114406
134. Guo Q, Grimmig T, Gonzalez G, et al. ATP-binding cassette member B5 (ABCB5) promotes tumor cell invasiveness in human colorectal cancer. *J Biol Chem*. 2018;293(28):11166-11178. doi:10.1074/jbc.RA118.003187
135. Wilson BJ, Saab KR, Ma J, et al. ABCB5 maintains melanoma-initiating cells through a pro-inflammatory cytokine signaling circuit. *Cancer Res*. 2014;74(15):4196-4207. doi:10.1158/0008-5472.CAN-14-0582
136. Niebergall-Roth E, Frank NY, Ganss C, Frank MH, Kluth MA. Skin-Derived ABCB5+ Mesenchymal Stem Cells for High-Medical-Need Inflammatory Diseases: From Discovery to Entering Clinical Routine. *Int J Mol Sci*. 2023;24(1):66. doi:10.3390/ijms24010066
137. Schatton T, Murphy GF, Frank NY, et al. Identification of cells initiating human melanomas. *Nature*. 2008;451(7176):345-349. doi:10.1038/nature06489
138. Waugh DJJ, Wilson C. The Interleukin-8 Pathway in Cancer. *Clin Cancer Res*. 2008;14(21):6735-6741. doi:10.1158/1078-0432.CCR-07-4843
139. Li A, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 Directly Enhanced Endothelial Cell Survival, Proliferation, and Matrix Metalloproteinases Production and Regulated Angiogenesis1. *J Immunol*. 2003;170(6):3369-3376. doi:10.4049/jimmunol.170.6.3369
140. Tan ST, Ghaznawie M, Heenan PJ, Dosan R. Basal Cell Carcinoma Arises from Interfollicular Layer of Epidermis. *J Oncol*. 2018;2018:3098940. doi:10.1155/2018/3098940
141. Nilsson M, Undèn AB, Krause D, et al. Induction of basal cell carcinomas and trichoepitheliomas in mice overexpressing GLI-1. *Proc Natl Acad Sci*. 2000;97(7):3438-3443. doi:10.1073/pnas.97.7.3438
142. Barresi G, Inferrera C, Gemelli M, De Luca F. Morphological aspects of the endocrine pancreas in a prednisolone-treated infant. *Histopathology*. 1977;1(3):201-208. doi:10.1111/j.1365-2559.1977.tb01659.x
143. Kasper M, Jaks V, Hohl D, Toftgård R. Basal cell carcinoma — molecular biology and potential new therapies. *J Clin Invest*. 2012;122(2):455-463. doi:10.1172/JCI58779
144. Blanpain C, Fuchs E. Plasticity of epithelial stem cells in tissue regeneration. *Science*. 2014;344(6189):1242281. doi:10.1126/science.1242281
145. Zhang B, Chen T. Local and systemic mechanisms that control the hair follicle stem cell niche. *Nat Rev Mol Cell Biol*. 2024;25(2):87-100. doi:10.1038/s41580-023-00662-3
146. Dahmane N, Lee J, Robins P, Heller P, Altaba AR i. Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. *Nature*. 1997;389(6653):876-881. doi:10.1038/39918
147. Lapouge G, Youssef KK, Vokaer B, et al. Identifying the cellular origin of squamous skin tumors. *Proc Natl Acad Sci U S A*. 2011;108(18):7431-7436. doi:10.1073/pnas.1012720108
148. Mulero MC, Ferres-Marco D, Islam A, et al. Chromatin-Bound IκBα Regulates a Subset of Polycomb Target Genes in Differentiation and Cancer. *Cancer Cell*. 2013;24(2):151-166. doi:10.1016/j.ccr.2013.06.003
149. Malanchi I, Peinado H, Kassen D, et al. Cutaneous cancer stem cell maintenance is dependent on β-catenin signalling. *Nature*. 2008;452(7187):650-653. doi:10.1038/nature06835
150. Celso CL, Prowse D, Watt F. Transient activation of β-catenin signalling in adult mouse epidermis is sufficient to induce new hair follicles but continuous activation is required to maintain hair follicle tumours. Published online 2004. doi:10.1242/dev.01052
151. Danek P, Kardosova M, Janeckova L, et al. β-Catenin–TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. *Blood*. 2020;136(22):2574-2587. doi:10.1182/blood.2019004664

152. Jamal SME, Alamodi A, Wahl RU, et al. Melanoma stem cell maintenance and chemo-resistance are mediated by CD133 signal to PI3K-dependent pathways. *Oncogene*. 2020;39(32):5468-5478. doi:10.1038/s41388-020-1373-6
153. Ahmed SI, Javed G, Laghari AA, et al. CD133 Expression in Glioblastoma Multiforme: A Literature Review. *Cureus*. 10(10):e3439. doi:10.7759/cureus.3439
154. Tume L, Paco K, Ubidia-Incio R, Moya J. CD133 in breast cancer cells and in breast cancer stem cells as another target for immunotherapy. *Gac Mex Oncol*. 2015;15(1):22-30. doi:10.1016/j.gamo.2016.01.003
155. Sharma BK, Manglik V, O'Connell M, et al. Clonal dominance of CD133+ subset population as risk factor in tumor progression and disease recurrence of human cutaneous melanoma. *Int J Oncol*. 2012;41(5):1570-1576. doi:10.3892/ijo.2012.1590
156. Geng S, Guo Y, Wang Q, Li L, Wang J. Cancer stem-like cells enriched with CD29 and CD44 markers exhibit molecular characteristics with epithelial-mesenchymal transition in squamous cell carcinoma. *Arch Dermatol Res*. 2013;305(1):35-47. doi:10.1007/s00403-012-1260-2
157. Liang L, Kaufmann AM. The Significance of Cancer Stem Cells and Epithelial–Mesenchymal Transition in Metastasis and Anti-Cancer Therapy. *Int J Mol Sci*. 2023;24(3):2555. doi:10.3390/ijms24032555
158. Sireesha K, Samundeshwari EL, Surekha K, Chandrasekhar C, Sarma PVGK. In vitro generation of epidermal keratinocytes from human CD34-positive hematopoietic stem cells. *Vitro Cell Dev Biol - Anim*. 2024;60(3):236-248. doi:10.1007/s11626-024-00862-x
159. Siegle JM, Basin A, Sastre-Perona A, et al. SOX2 is a cancer-specific regulator of tumour initiating potential in cutaneous squamous cell carcinoma. *Nat Commun*. 2014;5:4511. doi:10.1038/ncomms5511
160. Boumahdi S, Driessens G, Lapouge G, et al. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature*. 2014;511(7508):246-250. doi:10.1038/nature13305
161. Shi C, Ju H, Wu Y, Ma X, Zhang Z, Ren G. Potential role of cyclin-dependent kinase 4/6 inhibitors in the treatment of mucosal melanoma. *Holist Integr Oncol*. 2024;3(1):24. doi:10.1007/s44178-024-00090-z
162. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of Mutated, Activated BRAF in Metastatic Melanoma. *N Engl J Med*. 2010;363(9):809-819. doi:10.1056/NEJMoa1002011
163. Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600–Mutant Advanced Melanoma Treated with Vemurafenib. *N Engl J Med*. 2012;366(8):707-714. doi:10.1056/NEJMoa1112302
164. Delmas A, Cherier J, Pohorecka M, et al. The c-Jun/RHOB/AKT pathway confers resistance of BRAF-mutant melanoma cells to MAPK inhibitors. *Oncotarget*. 2015;6(17):15250-15264.
165. Sekulic A, Migden MR, Oro AE, et al. Efficacy and Safety of Vismodegib in Advanced Basal-Cell Carcinoma. *N Engl J Med*. 2012;366(23):2171-2179. doi:10.1056/NEJMoa1113713
166. Yao CD, Haensel D, Gaddam S, et al. AP-1 and TGFß cooperativity drives non-canonical Hedgehog signaling in resistant basal cell carcinoma. *Nat Commun*. 2020;11(1):5079. doi:10.1038/s41467-020-18762-5
167. Cammareri P, Rose AM, Vincent DF, et al. Inactivation of TGFβ receptors in stem cells drives cutaneous squamous cell carcinoma. *Nat Commun*. 2016;7(1):12493. doi:10.1038/ncomms12493
168. Brown JA, Yonekubo Y, Hanson N, et al. TGF-β-Induced Quiescence Mediates Chemoresistance of Tumor-Propagating Cells in Squamous Cell Carcinoma. *Cell Stem Cell*. 2017;21(5):650-664.e8. doi:10.1016/j.stem.2017.10.001
169. Quan XX, Hawk NV, Chen W, et al. Targeting Notch1 and IKKα Enhanced NF-κB Activation in CD133+ Skin Cancer Stem Cells. *Mol Cancer Ther*. 2018;17(9):2034-2048. doi:10.1158/1535-7163.MCT-17-0421
170. Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-κB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. *Oncogene*. 2001;20(31):4188-4197. doi:10.1038/sj.onc.1204535
171. Lee B, Sandhu S, McArthur G. Cell cycle control as a promising target in melanoma. *Curr Opin Oncol*. 2015;27(2):141-150. doi:10.1097/CCO.0000000000000159
172. Xu W, McArthur G. Cell Cycle Regulation and Melanoma. *Curr Oncol Rep*. 2016;18(6):34. doi:10.1007/s11912-016-0524-y
173. Julve M, Clark JJ, Lythgoe MP. Advances in cyclin-dependent kinase inhibitors for the treatment of melanoma. *Expert Opin Pharmacother*. 2021;22(3):351-361. doi:10.1080/14656566.2020.1828348
174. Desai BM, Villanueva J, Nguyen TTK, et al. The Anti-Melanoma Activity of Dinaciclib, a Cyclin-Dependent Kinase Inhibitor, Is Dependent on p53 Signaling. *PLOS ONE*. 2013;8(3):e59588. doi:10.1371/journal.pone.0059588
175. Hwang BJ, Adhikary G, Eckert RL, Lu AL. Chk1 inhibition as a novel therapeutic strategy in melanoma. *Oncotarget*. 2018;9(54):30450-30464. doi:10.18632/oncotarget.25765
176. Lowery CD, Dowless M, Renschler M, et al. Broad spectrum activity of the checkpoint kinase 1 inhibitor prexasertib as a single agent or chemopotentiator across a range of preclinical pediatric tumor models. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2019;25(7):2278-2289. doi:10.1158/1078-0432.CCR-18-2728
177. Ditano JP, Eastman A. Comparative Activity and Off-Target Effects in Cells of the CHK1 Inhibitors MK-8776, SRA737, and LY2606368. *ACS Pharmacol Transl Sci*. 2021;4(2):730-743. doi:10.1021/acsptsci.0c00201

178. Zabludoff SD, Deng C, Grondine MR, et al. AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. *Mol Cancer Ther*. 2008;7(9):2955-2966. doi:10.1158/1535-7163.MCT-08-0492
179. Gupta A, Shah K, Oza MJ, Behl T. Reactivation of p53 gene by MDM2 inhibitors: A novel therapy for cancer treatment. *Biomed Pharmacother*. 2019;109:484-492. doi:10.1016/j.biopha.2018.10.155
180. Discovery of RG7388, a Potent and Selective p53–MDM2 Inhibitor in Clinical Development | Journal of Medicinal Chemistry. Accessed August 2, 2024. https://pubs.acs.org/doi/10.1021/jm400487c
181. Shapiro GI. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol Off J Am Soc Clin Oncol*. 2006;24(11):1770-1783. doi:10.1200/JCO.2005.03.7689
182. Garutti M, Targato G, Buriolla S, Palmero L, Minisini AM, Puglisi F. CDK4/6 Inhibitors in Melanoma: A Comprehensive Review. *Cells*. 2021;10(6):1334. doi:10.3390/cells10061334
183. Sanchez-Martinez C, Raub T, Dios A, et al. Abemaciclib (LY2835219) is an Oral Inhibitor of the Cyclin-Dependent Kinases 4/6 that Crosses the Blood-Brain Barrier and Demonstrates In Vivo Activity Against Intracranial Human Brain Tumor Xenografts. *Drug Metab Dispos*. 2015;43. doi:10.1124/dmd.114.062745
184. Zhang YX, Sicinska E, Czaplinski JT, et al. Antiproliferative effects of CDK4/6 inhibition in CDK4-amplified human liposarcoma in vitro and in vivo. *Mol Cancer Ther*. 2014;13(9):2184-2193. doi:10.1158/1535-7163.MCT-14-0387
185. Kim S, Loo A, Chopra R, et al. Abstract PR02: LEE011: An orally bioavailable, selective small molecule inhibitor of CDK4/6– Reactivating Rb in cancer. *Mol Cancer Ther*. 2013;12(11_Supplement):PR02. doi:10.1158/1535-7163.TARG-13-PR02
186. Brose MS, Volpe P, Feldman M, et al. BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res*. 2002;62(23):6997-7000.
187. Kim G, McKee AE, Ning YM, et al. FDA approval summary: vemurafenib for treatment of unresectable or metastatic melanoma with the BRAFV600E mutation. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2014;20(19):4994-5000. doi:10.1158/1078-0432.CCR-14-0776
188. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011;364(26):2507-2516. doi:10.1056/NEJMoa1103782
189. Yoshida A, Lee EK, Diehl JA. Induction of Therapeutic Senescence in Vemurafenib-Resistant Melanoma by Extended Inhibition of CDK4/6. *Cancer Res*. 2016;76(10):2990-3002. doi:10.1158/0008-5472.CAN-15-2931
190. The CDK4/6 Inhibitor LY2835219 Overcomes Vemurafenib Resistance Resulting from MAPK Reactivation and Cyclin D1 Upregulation | Molecular Cancer Therapeutics | American Association for Cancer Research. Accessed July 3, 2024. https://aacrjournals.org/mct/article/13/10/2253/129494/The-CDK4-6-Inhibitor-LY2835219-Overcomes
191. Muñoz-Couselo E, Adelantado EZ, Ortiz C, García JS, Perez-Garcia J. NRAS-mutant melanoma: current challenges and future prospect. *OncoTargets Ther*. 2017;10:3941-3947. doi:10.2147/OTT.S117121
192. Kwong LN, Costello JC, Liu H, et al. Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. *Nat Med*. 2012;18(10):1503-1510. doi:10.1038/nm.2941
193. Sosman JA, Kittaneh M, Lolkema MPJK, et al. A phase 1b/2 study of LEE011 in combination with binimetinib (MEK162) in patients with NRAS-mutant melanoma: Early encouraging clinical activity. *J Clin Oncol*. 2014;32(15_suppl):9009-9009. doi:10.1200/jco.2014.32.15_suppl.9009
194. Schuler MH, Ascierto PA, De Vos FYFL, et al. Phase 1b/2 trial of ribociclib+binimetinib in metastatic NRAS-mutant melanoma: Safety, efficacy, and recommended phase 2 dose (RP2D). *J Clin Oncol*. 2017;35(15_suppl):9519-9519. doi:10.1200/JCO.2017.35.15_suppl.9519
195. Ascierto PA, Bechter O, Wolter P, et al. A phase Ib/II dose-escalation study evaluating triple combination therapy with a BRAF (encorafenib), MEK (binimetinib), and CDK 4/6 (ribociclib) inhibitor in patients (Pts) with BRAF V600-mutant solid tumors and melanoma. *J Clin Oncol*. 2017;35(15_suppl):9518-9518. doi:10.1200/JCO.2017.35.15_suppl.9518
196. Mohammed ER, Elmasry GF. Development of newly synthesised quinazolinone-based CDK2 inhibitors with potent efficacy against melanoma. *J Enzyme Inhib Med Chem*. 2022;37(1):686-700. doi:10.1080/14756366.2022.2036985
197. Porter LA, Dellinger RW, Tynan JA, et al. Human Speedy: a novel cell cycle regulator that enhances proliferation through activation of Cdk2. *J Cell Biol*. 2002;157(3):357-366. doi:10.1083/jcb.200109045
198. Lenormand JL, Dellinger RW, Knudsen KE, Subramani S, Donoghue DJ. Speedy: a novel cell cycle regulator of the G2/M transition. *EMBO J*. 1999;18(7):1869-1877. doi:10.1093/emboj/18.7.1869
199. Structural basis of divergent cyclin-dependent kinase activation by Spy1/RINGO proteins | The EMBO Journal. Accessed September 19, 2024. https://www.embopress.org/doi/full/10.15252/embj.201796905
200. Biochemical characterization of Cdk2-Speedy/Ringo A2 - PMC. Accessed September 19, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1262692/
201. Lubanska D, Market-Velker BA, deCarvalho AC, Mikkelsen T, Fidalgo da Silva E, Porter LA. The Cyclin-like Protein Spy1 Regulates Growth and Division Characteristics of the CD133+ Population in Human Glioma. *Cancer Cell*. 2014;25(1):64-76. doi:10.1016/j.ccr.2013.12.006

202. Lubanska D, Porter LA. The atypical cell cycle regulator Spy1 suppresses differentiation of the neuroblastoma stem cell population. *Oncoscience*. 2014;1(5):336-348. doi:10.18632/oncoscience.36
203. Fecher LA, Amaravadi RK, Flaherty KT. The MAPK pathway in melanoma. *Curr Opin Oncol*. 2008;20(2):183. doi:10.1097/CCO.0b013e3282f5271c
204. Al Mahi A, Ablain J. RAS pathway regulation in melanoma. *Dis Model Mech*. 2022;15(2):dmm049229. doi:10.1242/dmm.049229
205. Amaravadi R, Schuchter LM, McDermott DF, et al. Updated results of a randomized phase II study comparing two schedules of temozolomide in combination with sorafenib in patients with advanced melanoma. *J Clin Oncol*. 2007;25(18_suppl):8527-8527. doi:10.1200/jco.2007.25.18_suppl.8527
206. Grimaldi AM, Simeone E, Festino L, Vanella V, Strudel M, Ascierto PA. MEK Inhibitors in the Treatment of Metastatic Melanoma and Solid Tumors. *Am J Clin Dermatol*. 2017;18(6):745-754. doi:10.1007/s40257-017-0292-y
207. Haass NK, Gabrielli B. Cell cycle-tailored targeting of metastatic melanoma: Challenges and opportunities. *Exp Dermatol*. 2017;26(7):649-655. doi:10.1111/exd.13303
208. Barnaba N, LaRocque JR. Targeting cell cycle regulation via the G2-M checkpoint for synthetic lethality in melanoma. *Cell Cycle*. 2021;20(11):1041-1051. doi:10.1080/15384101.2021.1922806
209. Dummer R, Ascierto P a., Basset-Seguin N, et al. Sonidegib and vismodegib in the treatment of patients with locally advanced basal cell carcinoma: a joint expert opinion. *J Eur Acad Dermatol Venereol*. 2020;34(9):1944-1956. doi:10.1111/jdv.16230
210. Dreier J, Dummer R, Felderer L, Nägeli M, Gobbi S, Kunstfeld R. Emerging drugs and combination strategies for basal cell carcinoma. *Expert Opin Emerg Drugs*. 2014;19(3):353-365. doi:10.1517/14728214.2014.914171
211. Piérard-Franchimont C, Hermanns-Lê T, Paquet P, Herfs M, Delvenne P, Piérard GE. Hedgehog- and mTOR-Targeted Therapies for Advanced Basal Cell Carcinomas. *Future Oncol*. 2015;11(22):2997-3002. doi:10.2217/fon.15.181
212. Darido C, Georgy SR, Cullinane C, et al. Stage-dependent therapeutic efficacy in PI3K/mTOR-driven squamous cell carcinoma of the skin. *Cell Death Differ*. 2018;25(6):1146-1159. doi:10.1038/s41418-017-0032-0
213. Zou Y, Ge M, Wang X. Targeting PI3K-AKT-mTOR by LY3023414 inhibits human skin squamous cell carcinoma cell growth *in vitro* and *in vivo*. *Biochem Biophys Res Commun*. 2017;490(2):385-392. doi:10.1016/j.bbrc.2017.06.052
214. Short-term Fasting prior to Standard of Care PD-1/PD-L1 Inhibitor Therapy for the Treatment of Advanced or Metastatic Skin Malignancy - NCI. June 23, 2016. Accessed September 14, 2024. https://www.cancer.gov/about-cancer/treatment/clinical-trials/search/v?id=NCI-2020-01590
215. Salvadori G, Zanardi F, Iannelli F, Lobefaro R, Vernieri C, Longo VD. Fasting-mimicking diet blocks triple-negative breast cancer and cancer stem cell escape. *Cell Metab*. 2021;33(11):2247. doi:10.1016/j.cmet.2021.10.008
216. Zhu L, Yang Q, Hu R, et al. Novel therapeutic strategy for melanoma based on albendazole and the CDK4/6 inhibitor palbociclib. *Sci Rep*. 2022;12(1):5706. doi:10.1038/s41598-022-09592-0
217. Gene-Modified Immune Cells (FH-MCVA2TCR) in Treating Patients with Metastatic or Unresectable Merkel Cell Cancer - NCI. June 23, 2016. Accessed September 18, 2024. https://www.cancer.gov/about-cancer/treatment/clinical-trials/search/v?id=NCI-2018-02483
218. Deng J, Wang ES, Jenkins RW, et al. CDK4/6 Inhibition Augments Anti-Tumor Immunity by Enhancing T Cell Activation. *Cancer Discov*. 2018;8(2):216-233. doi:10.1158/2159-8290.CD-17-0915
219. Study Details | Avelumab With or Without Cetuximab in Treating Patients With Advanced Skin Squamous Cell Cancer | ClinicalTrials.gov. Accessed September 18, 2024. https://clinicaltrials.gov/study/NCT03944941?intr=NCT03944941&rank=1
220. Zhou J, Wu Z, Wong G, et al. CDK4/6 or MAPK blockade enhances efficacy of EGFR inhibition in oesophageal squamous cell carcinoma. *Nat Commun*. 2017;8(1):13897. doi:10.1038/ncomms13897
221. Hara N, Ichihara E, Kano H, et al. CDK4/6 signaling attenuates the effect of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer. *Transl Lung Cancer Res*. 2023;12(10):2098-2112. doi:10.21037/tlcr-23-99
222. Study Details | Using Nivolumab Alone or With Cabozantinib to Prevent Mucosal Melanoma Return After Surgery | ClinicalTrials.gov. Accessed September 18, 2024. https://clinicaltrials.gov/study/NCT05111574
223. Masuda J, Sakai H, Tsurutani J, et al. Efficacy, safety, and biomarker analysis of nivolumab in combination with abemaciclib plus endocrine therapy in patients with HR-positive HER2-negative metastatic breast cancer: a phase II study (WJOG11418B NEWFLAME trial). *J Immunother Cancer*. 2023;11(9):e007126. doi:10.1136/jitc-2023-007126
224. Williams ESCP, Szaniawski MA, Martins LJ, et al. Dasatinib: effects on the macrophage phospho proteome with a focus on SAMHD1 and HIV-1 infection. *Clin Res HIVAIDS*. 2022;8(1):1053.
225. Cabozantinib and dasatinib synergize to induce tumor regression in non-clear cell renal cell carcinoma - PMC. Accessed September 18, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8149375/

226. Study Details | Fecal Microbial Transplantation in Combination With Immunotherapy in Melanoma Patients (MIMic) | ClinicalTrials.gov. Accessed September 18, 2024. https://clinicaltrials.gov/study/NCT03772899
227. Schettini F, Fontana A, Gattazzo F, et al. Faecal microbiota composition is related to response to CDK4/6-inhibitors in metastatic breast cancer: A prospective cross-sectional exploratory study. *Eur J Cancer*. 2023;191:112948. doi:10.1016/j.ejca.2023.112948
228. CDK Inhibition Primes for Anti-PD-L1 Treatment in Triple-Negative Breast Cancer Models - PMC. Accessed September 18, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9322647/
229. Study Details | A PD-1 Checkpoint Inhibitor (Cemiplimab) for High-Risk Localized, Locally Recurrent, or Regionally Advanced Skin Cancer | ClinicalTrials.gov. Accessed September 18, 2024. https://clinicaltrials.gov/study/NCT04315701?cond=NCT04315701&rank=1
230. Testing the Addition of Cemiplimab to Palbociclib for the Treatment of Advanced Dedifferentiated Liposarcoma - NCI. June 23, 2016. Accessed September 18, 2024. https://www.cancer.gov/about-cancer/treatment/clinical-trials/search/v?id=NCI-2022-08568
231. Study Details | A Study of Neoadjuvant Therapy With BCD-217 (Nurulimab + Prolgolimab) in Patients With Resectable Stage III Skin Melanoma | ClinicalTrials.gov. Accessed September 19, 2024. https://clinicaltrials.gov/study/NCT05751928?intr=NCT05751928&rank=1
232. Rugo HS, Kabos P, Beck JT, et al. Abemaciclib in combination with pembrolizumab for HR+, HER2− metastatic breast cancer: Phase 1b study. *Npj Breast Cancer*. 2022;8(1):1-8. doi:10.1038/s41523-022-00482-2
233. Heistein JB, Acharya U, Mukkamalla SKR. Malignant Melanoma. In: *StatPearls*. StatPearls Publishing; 2024. Accessed September 17, 2024. http://www.ncbi.nlm.nih.gov/books/NBK470409/
234. Conway JR, Dietlein F, Taylor-Weiner A, et al. Integrated molecular drivers coordinate biological and clinical states in melanoma. *Nat Genet*. 2020;52(12):1373-1383. doi:10.1038/s41588-020-00739-1
235. Tímár J, Ladányi A. Molecular Pathology of Skin Melanoma: Epidemiology, Differential Diagnostics, Prognosis and Therapy Prediction. *Int J Mol Sci*. 2022;23(10):5384. doi:10.3390/ijms23105384
236. Cs W, Rc S, Jt L. Basal cell carcinoma. *BMJ*. 2003;327(7418). doi:10.1136/bmj.327.7418.794
237. McDaniel B, Badri T, Steele RB. Basal Cell Carcinoma. In: *StatPearls*. StatPearls Publishing; 2024. Accessed September 17, 2024. http://www.ncbi.nlm.nih.gov/books/NBK482439/
238. Agbai ON, Buster K, Sanchez M, et al. Skin cancer and photoprotection in people of color: a review and recommendations for physicians and the public. *J Am Acad Dermatol*. 2014;70(4):748-762. doi:10.1016/j.jaad.2013.11.038
239. Kailas A, Solomon JA, Mostow EN, Rigel DS, Kittles R, Taylor SC. Gaps in the understanding and treatment of skin cancer in people of color. *J Am Acad Dermatol*. 2016;74(5):1020-1021. doi:10.1016/j.jaad.2015.11.028
240. Incidence ratio of basal cell carcinoma to squamous cell carcinoma equalizes with age - Journal of the American Academy of Dermatology. Accessed September 17, 2024. https://www.jaad.org/article/S0190-9622(16)30638-7/fulltext
241. cancer CCS/ S canadienne du. Types of non-melanoma skin cancer. Canadian Cancer Society. Accessed September 17, 2024. https://cancer.ca/en/cancer-information/cancer-types/skin-non-melanoma/what-is-non-melanoma-skin-cancer/types-of-non-melanoma
242. Dalal AJ, Ingham J, Collard B, Merrick G. Review of outcomes of 500 consecutive cases of non-melanoma skin cancer of the head and neck managed in an oral and maxillofacial surgical unit in a District General Hospital. *Br J Oral Maxillofac Surg*. 2018;56(9):805-809. doi:10.1016/j.bjoms.2018.08.015
243. Higgins S, Nazemi A, Chow M, Wysong A. Review of Nonmelanoma Skin Cancer in African Americans, Hispanics, and Asians. *Dermatol Surg Off Publ Am Soc Dermatol Surg Al*. 2018;44(7):903-910. doi:10.1097/DSS.0000000000001547
244. Skin cancer in African Americans - Halder - 1995 - Cancer - Wiley Online Library. Accessed September 17, 2024. https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/1097-0142(19950115)75:2+%3C667::AID-CNCR2820751409%3E3.0.CO;2-I
245. Dhir A, Orengo I, Bruce S, Kolbusz RV, Alford E, Goldberg L. Basal Cell Carcinoma on the Scalp of an Indian Patient. *Dermatol Surg*. 1995;21(3):247-250. doi:10.1111/j.1524-4725.1995.tb00165.x
246. Sotillo R, García JF, Ortega S, et al. Invasive melanoma in Cdk4-targeted mice. *Proc Natl Acad Sci U S A*. 2001;98(23):13312-13317. doi:10.1073/pnas.241338598
247. Puntervoll HE, Yang XR, Vetti HH, et al. Melanoma prone families with CDK4 germline mutation: phenotypic profile and associations with MC1R variants. *J Med Genet*. 2013;50(4):264-270. doi:10.1136/jmedgenet-2012-101455
248. Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2013;19(19):5320-5328. doi:10.1158/1078-0432.CCR-13-0259
249. Burnworth B, Popp S, Stark HJ, et al. Gain of 11q/cyclin D1 overexpression is an essential early step in skin cancer development and causes abnormal tissue organization and differentiation. *Oncogene*. 2006;25(32):4399-4412. doi:10.1038/sj.onc.1209474

250. Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature*. 2001;413(6851):83-86. doi:10.1038/35092584
251. Kreuger IZM, Slieker RC, van Groningen T, van Doorn R. Therapeutic Strategies for Targeting *CDKN2A* Loss in Melanoma. *J Invest Dermatol*. 2023;143(1):18-25.e1. doi:10.1016/j.jid.2022.07.016
252. Goel VK, Ibrahim N, Jiang G, et al. Melanocytic nevus-like hyperplasia and melanoma in transgenic BRAFV600E mice. *Oncogene*. 2009;28(23):2289-2298. doi:10.1038/onc.2009.95
253. Recio JA, Noonan FP, Takayama H, et al. Ink4a/arf deficiency promotes ultraviolet radiation-induced melanomagenesis. *Cancer Res*. 2002;62(22):6724-6730.
254. You MJ, Castrillon DH, Bastian BC, et al. Genetic analysis of Pten and Ink4a/Arf interactions in the suppression of tumorigenesis in mice. *Proc Natl Acad Sci U S A*. 2002;99(3):1455-1460. doi:10.1073/pnas.022632099
255. Rizos H, Darmanian AP, Holland EA, Mann GJ, Kefford RF. Mutations in the INK4a/ARF Melanoma Susceptibility Locus Functionally Impair p14ARF*. *J Biol Chem*. 2001;276(44):41424-41434. doi:10.1074/jbc.M105299200
256. Ming Z, Lim SY, Rizos H. Genetic Alterations in the INK4a/ARF Locus: Effects on Melanoma Development and Progression. *Biomolecules*. 2020;10(10):1447. doi:10.3390/biom10101447
257. Kreuger IZM, Slieker RC, van Groningen T, van Doorn R. Therapeutic Strategies for Targeting *CDKN2A* Loss in Melanoma. *J Invest Dermatol*. 2023;143(1):18-25.e1. doi:10.1016/j.jid.2022.07.016
258. Kannan K, Sharpless NE, Xu J, O'Hagan RC, Bosenberg M, Chin L. Components of the Rb pathway are critical targets of UV mutagenesis in a murine melanoma model. *Proc Natl Acad Sci U S A*. 2003;100(3):1221-1225. doi:10.1073/pnas.0336397100
259. Bardeesy N, Bastian BC, Hezel A, Pinkel D, DePinho RA, Chin L. Dual inactivation of RB and p53 pathways in RAS-induced melanomas. *Mol Cell Biol*. 2001;21(6):2144-2153. doi:10.1128/MCB.21.6.2144-2153.2001
260. Giglia-Mari G, Sarasin A. TP53 mutations in human skin cancers. *Hum Mutat*. 2003;21(3):217-228. doi:10.1002/humu.10179
261. Soto JL, Cabrera CM, Serrano S, López-Nevot MÁ. Mutation analysis of genes that control the G1/S cell cycle in melanoma: TP53, CDKN1A, CDKN2A, and CDKN2B. *BMC Cancer*. 2005;5(1):36. doi:10.1186/1471-2407-5-36
262. Luo C, Sheng J, Hu MG, et al. Loss of ARF sensitizes transgenic BRAFV600E mice to UV-induced melanoma via suppression of XPC. *Cancer Res*. 2013;73(14):4337-4348. doi:10.1158/0008-5472.CAN-12-4454
263. Fröhlich LM, Makino E, Sinnberg T, Schittek B. Enhanced expression of p21 promotes sensitivity of melanoma cells towards targeted therapies. *Exp Dermatol*. 2022;31(8):1243-1252. doi:10.1111/exd.14585
264. Tubita A, Lombardi Z, Tusa I, et al. Inhibition of ERK5 Elicits Cellular Senescence in Melanoma via the Cyclin-Dependent Kinase Inhibitor p21. *Cancer Res*. 2022;82(3):447-457. doi:10.1158/0008-5472.CAN-21-0993
265. Stoyanova T, Roy N, Bhattacharjee S, et al. p21 Cooperates with DDB2 Protein in Suppression of Ultraviolet Ray-induced Skin Malignancies*. *J Biol Chem*. 2012;287(5):3019-3028. doi:10.1074/jbc.M111.295816
266. Wang X, Gorospe M, Huang Y, Holbrook NJ. p27Kip1 overexpression causes apoptotic death of mammalian cells. *Oncogene*. 1997;15(24):2991-2997. doi:10.1038/sj.onc.1201450
267. Chen G, Cheng Y, Zhang Z, Martinka M, Li G. Prognostic Significance of Cytoplasmic p27 Expression in Human Melanoma. *Cancer Epidemiol Biomarkers Prev*. 2011;20(10):2212-2221. doi:10.1158/1055-9965.EPI-11-0472
268. Al Hmada Y, Brodell RT, Kharouf N, et al. Mechanisms of Melanoma Progression and Treatment Resistance: Role of Cancer Stem-like Cells. *Cancers*. 2024;16(2):470. doi:10.3390/cancers16020470
269. Lionetti MC, Fumagalli MR, La Porta CAM. Cancer stem cells, plasticity, and drug resistance. *Cancer Drug Resist*. 2020;3(2):140-148. doi:10.20517/cdr.2019.112
270. Whitson RJ, Lee A, Urman NM, et al. Noncanonical hedgehog pathway activation through SRF-MKL1 promotes drug resistance in basal cell carcinomas. *Nat Med*. 2018;24(3):271-281. doi:10.1038/nm.4476
271. Atwood SX, Sarin KY, Whitson RJ, et al. Smoothened variants explain the majority of drug resistance in basal cell carcinoma. *Cancer Cell*. 2015;27(3):342-353. doi:10.1016/j.ccell.2015.02.002
272. Lorenzo-Sanz L, Lopez-Cerda M, da Silva-Diz V, et al. Cancer cell plasticity defines response to immunotherapy in cutaneous squamous cell carcinoma. *Nat Commun*. 2024;15(1):5352. doi:10.1038/s41467-024-49718-8
273. Overcoming Resistance to Immunotherapy in Advanced Cutaneous Squamous Cell Carcinoma - PMC. Accessed September 18, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8533861/
274. Hadian Y, Howell JY, Ramsey ML. Cutaneous Squamous Cell Carcinoma. In: *StatPearls*. StatPearls Publishing; 2024. Accessed September 18, 2024. http://www.ncbi.nlm.nih.gov/books/NBK441939/
275. Jost T, Heinzerling L, Fietkau R, Hecht M, Distel LV. Palbociclib Induces Senescence in Melanoma and Breast Cancer Cells and Leads to Additive Growth Arrest in Combination With Irradiation. *Front Oncol*. 2021;11:740002. doi:10.3389/fonc.2021.740002

276. Kwong LN, Costello JC, Liu H, et al. Oncogenic NRAS Signaling Differentially Regulates Survival and Proliferation in Melanoma. *Nat Med*. 2012;18(10):1503-1510. doi:10.1038/nm.2941
277. Aldawsari MF, Kamal MA, Balaha MF, et al. Optimized Ribociclib nanostructured lipid carrier for the amelioration of skin cancer: Inferences from ex-vivo skin permeation and dermatokinetic studies. *Saudi Pharm J SPJ*. 2024;32(3):101984. doi:10.1016/j.jsps.2024.101984
278. The CDK4/6 inhibitor LY2835219 overcomes vemurafenib resistance resulting from MAPK reactivation and cyclin D1 upregulation - PubMed. Accessed September 19, 2024. https://pubmed.ncbi.nlm.nih.gov/25122067/
279. Desai BM, Villanueva J, Nguyen TTK, et al. The anti-melanoma activity of dinaciclib, a cyclin-dependent kinase inhibitor, is dependent on p53 signaling. *PloS One*. 2013;8(3):e59588. doi:10.1371/journal.pone.0059588
280. Short-term Fasting prior to Standard of Care PD-1/PD-L1 Inhibitor Therapy for the Treatment of Advanced or Metastatic Skin Malignancy - NCI. June 23, 2016. Accessed September 18, 2024. https://www.cancer.gov/about-cancer/treatment/clinical-trials/search/v?id=NCI-2020-01590
281. Abemaciclib in Combination With Pembrolizumab for Stage IV KRAS-Mutant or Squamous NSCLC: A Phase 1b Study - PMC. Accessed September 19, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8551846/