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Cell Senescence and the Genetics of Melanoma Development

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ABSTRACT

Cutaneous malignant melanoma is an aggressive skin cancer with an approximate lifetime risk of 1 in 38 in the UK. While exposure to ultraviolet radiation is a key environmental risk factor for melanoma, up to ~10% of patients report a family history of melanoma, and ~1% have a strong family history. The understanding of causal mutations in melanoma has been critical to the development of novel targeted therapies that have contributed to improved outcomes for late-stage patients. Here, we review current knowledge of the genes affected by familial melanoma mutations and their partial overlap with driver genes commonly mutated in sporadic melanoma development. One theme linking a set of susceptibility loci/genes is the regulation of skin pigmentation and suntanning. The largest functional set of susceptibility variants, typically with high penetrance, includes *CDKN2A*, *RB1*, and telomerase reverse transcriptase (*TERT*) mutations, associated with attenuation of cell senescence. We discuss the mechanisms of action of these gene sets in the biology and progression of nevi and melanoma.

1 | Introduction

1.1 | Sporadic and Familial Melanoma

Cutaneous malignant melanoma (hereafter referred to as melanoma) is a highly aggressive human skin cancer that arises from an epidermal melanocyte (pigment cell) via genetic mutations, mostly mediated by solar ultraviolet light (UV) [1–4]. The incidence of melanoma has been increasing for decades [2, 4, 5], with an approximate lifetime risk now of ~1 in 38 in the UK (born in 1961) [5]. The incidence varies between countries since the causes of melanoma are both heterogeneous and complex. There is a major environmental risk factor, exposure to ultraviolet (UV) radiation, while genetically influenced phenotypic characteristics such as numerous benign and atypical melanocytic nevi (moles), Caucasian skin types, red hair, and a family history of melanoma are also important [2–4, 6, 7].

Clinically, four subtypes of melanoma can be distinguished: superficial spreading (beginning as thin and flat); nodular (growing vertically into the dermis forming a nodule, with no flat precursor phase); lentigo maligna melanoma (slow-growing, common on sun-exposed skin) and acral lentiginous melanoma (typically on the sole or palm, or under a nail). Superficial spreading melanomas are the commonest of these, comprising 70% of all melanomas [1, 7]. Rarer subtypes such as uveal (in the eye), desmoplastic, spitzoid, mucosal, and intracranial melanomas account for only around 5% of melanomas, although frequently associated with a poor prognosis [1, 8]. Treatment for melanoma depends on anatomical location, stage, and grade of tumor as well as treatment intent. Options include surgical excision, lymph-node dissection, adjuvant radiotherapy, topical/systemic chemotherapy, and immunotherapy [9, 10]. In the last decade, a multitude of genomic-mutation-targeted small molecules and novel immunotherapies have been licensed, which have dramatically improved outcomes for metastatic melanoma [10].

Most melanomas are sporadic, arising only via somatic mutations, but between 5% and 15% of patients report one or more relatives with melanoma, with some geographical variation

[4, 11, 12]. Although family members often share similar environmental UV exposures, twin studies indicate relatively high heritability of melanoma susceptibility, for example, 58% in a large Nordic study [13]. A recent large meta-analysis of genome-wide and transcriptome-wide association studies identified no less than 85 melanoma susceptibility loci [6], with likely influences on both sporadic and familial melanoma development. Familial melanoma is suspected when patients present with a history of melanoma in multiple relatives and/or with multiple melanomas (Box 1); such a "strong" family history applies to only around 1% of patients [11]. Inheritance in these families generally follows an autosomal dominant pattern with variable degrees of penetrance, in part by latitude of residence [3, 6, 11]. National UK guidelines recommend germline screening for such individuals at high risk of melanoma, to improve early detection and outcomes (NICE NG14) [9].

Well-known loci for melanoma susceptibility are summarized in Table 1. The Genomics England tool PanelApp for melanoma includes an overlapping list: the genes *CDKN2A*, *CDK4*, *BAP1*, and *POT1* within the diagnostic-grade "Green" virtual panel, and *TERF2IP*, telomerase reverse transcriptase (*TERT*), *BRCA2*, and *ACD* within the "Amber" (borderline) panel [33]. However, *BRCA2* has recently been reported as making minimal, if any, contribution to familial melanoma [34]. Remarkably, all the other PanelApp genes are related to a common cellular regulatory mechanism, namely cell senescence,

BOX 1 | UK testing criteria for familial melanoma: Excerpted from the UK National Genome Testing Directory [14]. Note that this testing is for familial melanoma alone, though some gene variants increase the risk for other cancers too; see Table 1.

R254 Familial Melanoma

Testing Criteria

Testing of the phenotypically affected individual (proband) where the individual +/− family history meets ONE of the following criteria. The proband has:

a. ≥ 2 melanomas and/or melanomas in situ age < 30 years, OR

b. ≥ 3 melanoma and/or melanomas in situ at any age, OR

c. Melanoma and/or melanoma in situ AND ≥ 2 relatives (first/second/third-degree relatives) with melanoma and/or melanoma in situ, OR

d. Melanoma and/or melanoma in situ AND ≥ 1 first-degree relative with melanoma and/or melanoma in situ; one individual has multiple melanomas and/or melanomas in situ, OR

e. ≥ 1 Melanoma and/or melanoma in situ OR melanoma and/or melanoma in situ and atypical moles AND ≥ 1 first-degree relative with pancreatic cancer aged < 60, OR

f. Atypical moles AND ≥ 2 relatives (first/second degree relatives) with melanoma and/or melanoma in situ, OR

g. Deceased affected individual (proband) where (i) the individual +/− family history meets one of the above criteria, (ii) appropriate tissue is available (tumor or normal), and (iii) no living affected individual is available for genetic testing.

TABLE 1 | Main genes associated with familial melanoma.

Gene penetrance	Gene	Normal function	Biological effect of mutation	References
High	*CDKN2A* p16	Senescence effector, tumor suppressor	Impaired cell senescence	[17–19]
	CDKN2A ARF	Tumor suppressor, activator of p53	Impaired cell senescence	[11, 20]
	CDK4	Cell-cycle regulator, senescence	Impaired cell senescence	[21–23]
	TERT	Telomere maintenance and repair	Overexpression. Delay or absence of cell senescence	[12, 24, 25]
	POT1	Telomere protection	Delayed cell senescence	[22, 26–28]
Medium	*BAP1*	Nuclear deubiquitinase and tumor suppressor	> 1 pathway proposed	[29, 30]
	MITF	Melanocyte development and differentiation	Melanoma risk possibly through impaired cell senescence	[31–33]
Low (but common)	*MC1R*	Suntanning response (melanin synthesis); melanocyte differentiation	Reduced UV protection, higher risk of mutation	[31, 34]

Note: Summary of their normal functions and biological effects of mutations. The two protein products of CDKN2A are separated. The table is adapted and updated from Rossi et al. [3]. See text for further discussion of these and more rarely reported melanoma-associated loci: *TERF2IP*, *TINF2*, and *ACD*. Note that variants in *BAP1*, *POT1*, and *TERT* also appear to increase the risk of other cancers besides cutaneous melanoma (more below), so that their overall penetrance for cancer is higher than for melanoma alone, though currently poorly defined because of their rarity.

together with its predominant trigger, DNA damage signaling. We therefore now introduce this mechanism. Section 2 will explain how specific melanoma susceptibility genes relate to cell senescence, or another common theme among susceptibility loci, the regulation of skin pigmentation. We will then consider related somatic driver mutations in sporadic melanoma (Section 3), leading to an overview and interpretation of the genetic causes of melanoma.

1.2 | Cell Senescence

Cell senescence is a programmed arrest of cell proliferation, permanent in the absence of genetic change, following specific triggers, and accompanied by specific phenotypic changes [35–39]. Cells arrest with both G1 and G2 (2n and 4n) DNA content [40]. Senescent cells in culture or in tissues can be distinguished from terminally differentiated or quiescent (reversibly arrested) cells by a range of morphological differences (large, flattened [in culture] cells with large nuclei and prominent nucleoli) and molecular markers [36, 39]. (See Section 3.2 for a list relevant to melanocytes and nevi.) The molecular characterization of senescent cells has been further refined by the identification of transcriptomic gene signatures [41, 42]. Although senescence is now commonly viewed as a hallmark of aging [43], senescent cells also positively regulate tissue remodeling and repair in normal development [44] and adult life [45, 46]. Importantly, cell senescence is now appreciated to be key in limiting tumorigenesis, by restricting the replication of premalignant cells in response to oncogenic mutations [39, 47–50]. In humans there are two main intracellular molecular pathways effecting cell senescence by arresting the cell cycle, respectively regulated by proteins p53 and p16 [35, 38] and summarized in Figure 1. p53 and p16 are encoded by the two genes most commonly sporadically altered in human metastatic cancers, and RB1 (downstream of p16) by the fifth commonest [51], underlining the importance of senescence in cancer suppression.

Various subtypes of senescence have been described, according to the external trigger, including stresses (radiation, cytotoxic drugs, and oxidative stress), oncogene overexpression, and extensive cell division [37, 47, 52, 53]. However, it is now appreciated that these nearly all work through a common internal trigger, namely the cellular DNA-damage response (DDR) [35, 36, 47] (Figure 1).

The best-known subtype is replicative senescence, arrest following extended cell division and associated in humans with telomere shortening [36, 54, 55]. Telomeres are DNA-protein complexes that protect the ends of chromosomes from degradation or aberrant ligation to other telomeres by DNA repair [11, 36]. In the germline and early embryos, telomeres are maintained by the telomere-synthesizing enzyme telomerase, but

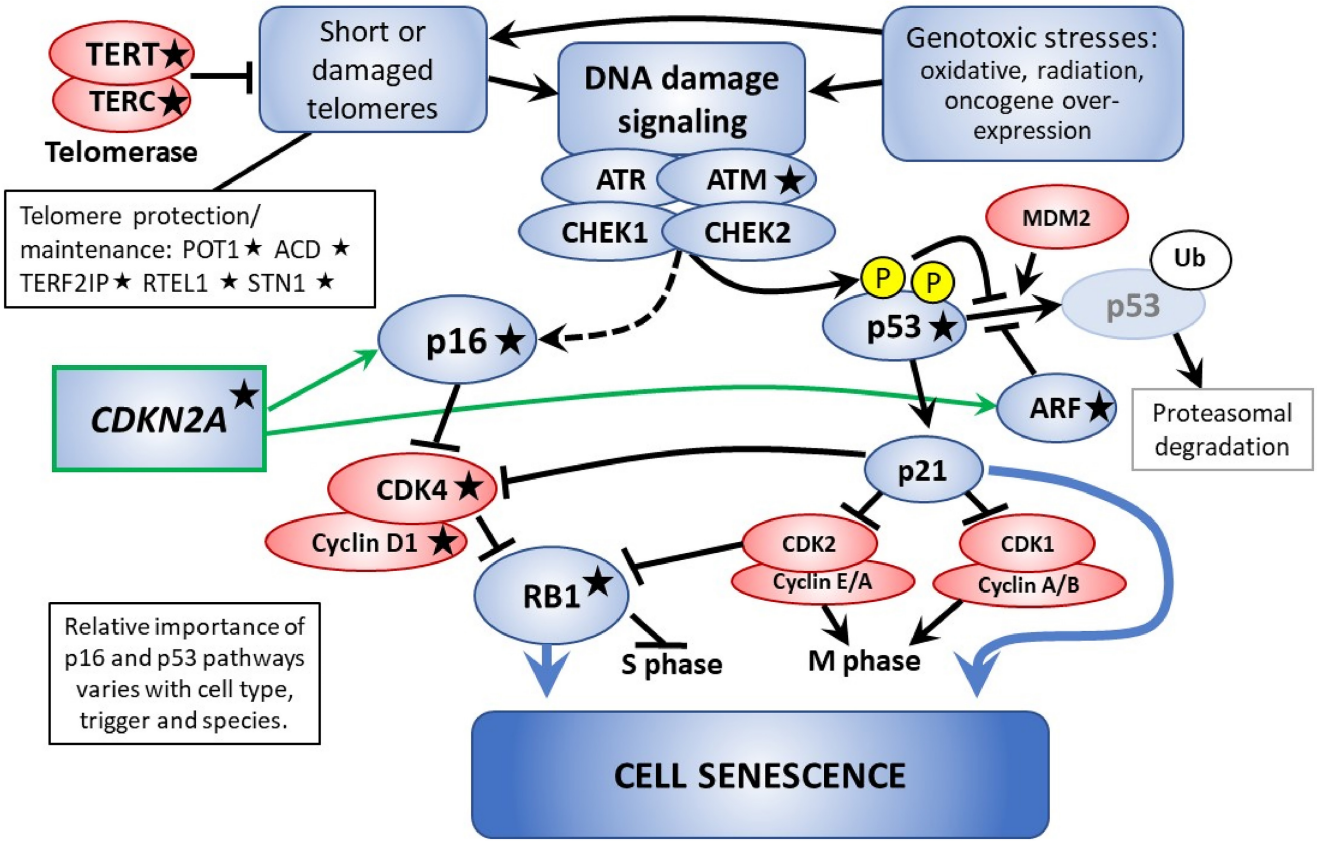

FIGURE 1 | Main human cell senescence pathways, via p16 and p53. Adapted from Bennett [35, 39]; see Reference [35] for further details of pathways. Showing links with DNA-damage signaling and telomere dysfunction. Blue: a pro-senescence component. Red: pro-proliferation, antisenescence component. Arrows: stimulation. T-bars: inhibition. P: phosphorylation, stabilizing p53. (★) Component with known germline mutations or significant GWAS locus [6] linked to melanoma susceptibility.

after birth, telomerase is inactivated in most human somatic cells, by their no longer expressing the catalytic subunit TERT [55–57]. Telomeres now gradually shorten over many divisions, until at a critical length, protection is lost and the DNA end is recognized as a break. This triggers a persistent DDR, resulting in cell senescence [36, 55, 57] (Figure 1). The activation of p53 by dysfunctional telomeres and DDRs is well understood [36, 47, 55], whereas that of p16 is not (dashed arrow, Figure 1). However, upregulation of human p16 by telomere dysfunction [58] and DNA damage (ultraviolet irradiation) [59] have been demonstrated, and p16 is well established as a marker of aging and replicative senescence (telomere dysfunction) in humans [60, 61].

Another much-studied form is oncogene-induced senescence (OIS), an arrest triggered rapidly in cultured cells by engineered overexpression of an oncoprotein such as an activated RAS or RAF [47, 49, 50, 53, 62, 63]. This is reported to create DNA damage through DNA hyperreplication and/or reactive oxygen species generation, triggering senescence [47, 64]. However, the relevance of this mechanism to human tumor development is unclear, since cells acquiring such an oncogene by mutation alone, in vivo or in culture, do not overexpress it nor arrest it immediately; they can proliferate extensively before becoming senescent [39, 65].

Senescent cells signal to nearby cells by secreting numerous factors known collectively as the 'senescence-associated secretory phenotype' (SASP), including inflammatory cytokines, growth factors, and proteases [61, 66, 67]. SASP factors can often promote the immune clearance of senescent cells [68]. However, chronic stress or immune dysfunction can lead to the accumulation of these cells within tissues [52], now believed to be a fundamental pathogenic mechanism for age-related diseases [36, 43, 67, 69]. SASP secretion by stromal cells of tumors is also now implicated positively in tumorigenesis [36, 38, 70]. In short, the senescence of precancerous cells appears primarily protective, but conversely, the senescence of neighboring normal cells may promote tumor growth.

2 | Familial Melanoma

Over the last 30 years, many germline variants have been significantly associated with familial melanoma risk [3, 6, 11, 15, 71]. Nonetheless, despite the increasing identification of culprit familial melanoma genes using high-throughput sequencing techniques, only ~20% of familial melanoma pedigrees were explained by high-risk mutations in one large study [22] (Figure 2). The main known high-penetrance familial melanoma genes include *CDKN2A*, *CDK4*, *POT1*, *ACD*, *TERF2IP*, *TINF2*, and *TERT* alleles, with medium- or low-penetrance genes including *BAP1*, *MC1R*, and *MITF* alleles [3, 12, 72] (Table 1). These genes are discussed below. Importantly, many of them interact, displaying epistasis and altering mutation pathogenicity accordingly.

2.1 | High-Penetrance Genes

2.1.1 | CDKN2A

Early seminal linkage and positional cloning studies identified cyclin-dependent kinase inhibitor 2A (*CDKN2A*, 9p21.3) as the first high-risk gene to be associated with familial melanoma [17, 71]. Around 20% to over 50% of melanoma pedigrees harbor *CDKN2A* mutations, varying greatly by geographical location, and with the chosen definition of a pedigree (number of cases) [7, 11, 18]. The small *CDKN2A* gene contains four exons (1α, 1β, 2, and 3) (Figure 3) [73] encoding two unrelated proteins: $p16^{INK4A}$ ([Inhibitor of kinase 4]A), commonly called p16, and $p14^{ARF}$ (Alternative Reading Frame), often called ARF [4, 11, 73]. p16 is encoded by exons 1α, 2, and part of 3, while ARF is encoded by exon 1β and part of 2 in a different reading frame from p16 [11, 73].

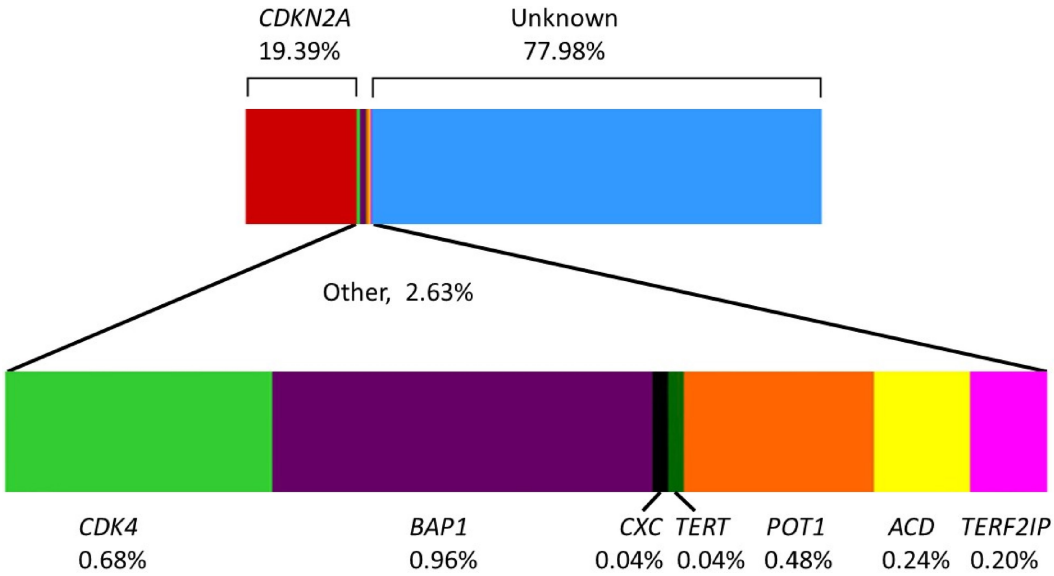

FIGURE 2 | Prevalence of high-risk gene mutations responsible for familial melanoma among >2500 melanoma pedigrees. Figure reformatted from Potrony et al. [22]. *CXC*: Duplication of a set of CXC cytokine genes in one family. Although high-risk mutations in further genes (*TINF2*, *MITF*) have since been identified, they are estimated to account for only a further 2% of melanoma families. Most culprit genes remain as yet unidentified [26].

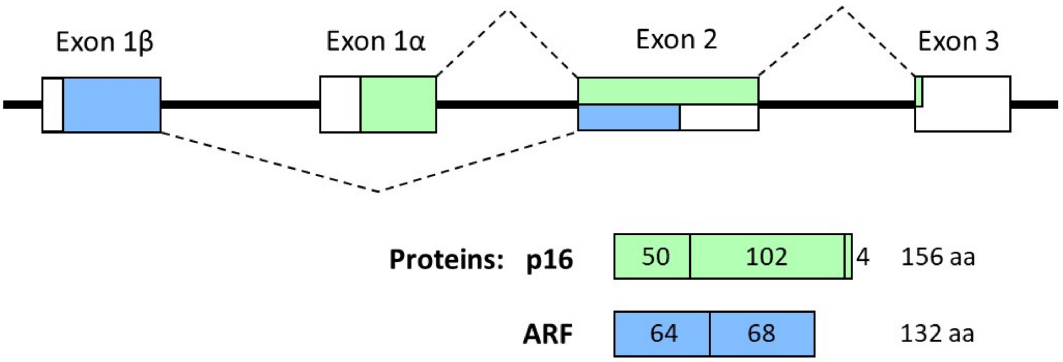

FIGURE 3 | Organization of the human *CDKN2A* locus and its two products. p16 and ARF are encoded by separate first exons (1α, 1β). They share sequences in exon 2 but in different reading frames, resulting in unrelated protein sequences. Dashed lines indicate splicing. Figure adapted from James and Peters [73].

More than 190 pathogenic germline and sporadic mutations in human *CDKN2A* are included without conflicts in the ClinVar database to date [74], associated with melanoma as well as other cancers and other diseases. Numerous heterozygous germline *CDKN2A* variants have been described in melanoma families, which result in loss or defective function of p16 and sometimes ARF [7, 11, 65]. Most *CDKN2A* mutations occur in exons 1α and 2, affecting p16 protein alone or both p16 and ARF. Those affecting only ARF are rare and are reported to be associated with neural system tumors and melanoma [11].

These families display further phenotypic heterogeneity. Besides malignant melanoma, *CDKN2A* variants can cosegregate with familial atypical multiple mole-melanoma (FAMMM, OMIM #155601, Box 2A) and/or FAMMM-pancreatic cancer syndromes (OMIM #606719, Box 2B), as well as other tumors [3, 4].

Melanocytic nevi appear to result from cell senescence following oncogene-induced proliferation of a melanocyte (see Section 3.2). It is not clear why some *CDKN2A* mutations lead to larger than normal nevi (atypical or dysplastic nevi) and increased numbers of nevi while others do not; the site of mutation and interacting effects from other risk loci may affect nevus development [6].

p16 and ARF both function in cell senescence and tumor suppression, within two key pathways (Figure 1) [35, 43]. In mouse models, deletion of p16 and ARF together [76] or either separately [20] has been found to disrupt the replicative senescence of melanocytes [20, 76], although mouse $p19^{ARF}$ differs from human $p14^{ARF}$ and is thought to be more dominant in cell senescence [39].

p16 binds cyclin-dependent kinases CDK4 and CDK6, preventing their phosphorylation of retinoblastoma protein (RB1), although CDK4 (rather than CDK6) appears the main p16 target relevant to human melanoma [7, 11, 35, 73] (Figure 1). There is no clear evidence for any molecular role for p16 besides mediating senescence; indeed it is often used as a marker of senescence, being absent from normal, nonsenescent cells [35, 36, 61]. Thus, although p16 is sometimes called a cell-cycle regulator, it does not have that role in nonsenescent cells. Hypophosphorylated RB1 binds the transcription-factor protein family E2F in the cell nucleus, blocking E2F interaction with S-phase genes and thus blocking the cell-cycle transition from G1 to S phase. In the presence of p16, RB1 thus induces and maintains cell-cycle arrest and senescence [4, 77]. Pathogenic mutation or deletion in p16 leads to defective inhibition of CDK4, usually by impaired binding, and thus to defective senescence [11, 19, 35, 76, 77].

BOX 2 | Criteria and guidelines related to familial melanoma. (A) Diagnostic criteria for FAMMM, adapted from Rossi et al. [3]. (B) UK NICE guidelines for surveillance for pancreatic cancer in patients carrying *CDKN2A* variants with or without a diagnosis of FAMMM [75].

(A) Diagnostic criteria for FAMMM

1. Cutaneous malignant melanoma in ≥first-or second-degree relatives
2. Total body nevus count > 50 and multiple atypical nevi
3. Atypical nevi features on histology

(B) National Institute for Health and Care Excellence (NICE) guidelines for pancreatic cancer surveillance [NG85] given *CDKN2A* variant

1. Pathogenic variant in *CDKN2A* gene plus
2. Pancreatic cancer in one or more first-degree relatives

ARF acts to stabilize tumor suppressor p53 by inhibiting the ubiquitin-protein ligase MDM2 (mouse double minute two homolog), thereby promoting senescence [20, 35] (Figure 1). Oncogenic mutations or deletion of *ARF* thus enhance the ubiquitination and proteasomal degradation of p53 [78]. Sometimes called the "Guardian of the genome," p53 is a transcription factor that regulates the expression of thousands of target genes [79]. p53 is activated when it is phosphorylated and stabilized by DNA-damage signaling (Figure 1) [47]. This can trigger apoptosis or—with lesser DNA damage and varying with other inputs—cell senescence [79, 80]. Among p53 effectors, the cyclin-dependent kinase inhibitor p21 (*CDKN1A*) is vital in mediating cell senescence in relevant cell types [20, 80] (Figure 1), and also promotes cell viability by inhibiting pro-apoptotic effectors [81]. Disruption of p53 allows evasion of cell senescence, thus division in the presence of DNA damage and accumulation of potential tumorigenic mutations

[3, 35, 82]. It is interesting though that p21 and its downstream components have not emerged as linked to melanoma risk, unlike those downstream of p16 [6] (Figure 1, note stars).

Crosstalk or cooperation between the p16/RB1 and ARF/p53/p21 pathways is important in the induction and maintenance of cell senescence in some cells and tissues [80]. In some cell types, p16 is insufficient and p53 is required for senescence; in others, p16 is sufficient [19, 67, 80]. In cultured human melanocytes, p16 is necessary for normal replicative senescence; p16-deficient melanocytes senesce only after many more divisions than normal, when they do upregulate p53, providing a "backup" [19]. Further interactions between the p53/p21 and p16/RB1 pathways are also known [35].

2.1.2 | *CDK4* and Related Genes

Further evidence connecting cell senescence and familial melanoma comes from the identification of rare germline mutations in *CDK4* (cyclin-dependent kinase 4, 12q14) [3, 11, 22]. CDK4 is a serine/threonine kinase activated by forming a heterodimer with D-type cyclins such as cyclin D1 (*CCND1*, 11q13.3). CDK4 is important in initiating S-phase of the cell cycle, by the phosphorylation of RB1. This is inhibited by p16-binding (Figure 1) [22, 36, 83]. CDK4 also activates the G2/M-phase master transcription factor FOXM1, so its inhibition by p16 may arrest cells in both G1 and G2 [35]. All known *CDK4* pathogenic/likely pathogenic germline variants in familial melanoma affect the p16-binding site at residue 24 [3, 21]. Impaired p16 action thus results in defective p16/RB1-mediated senescence [19, 35, 83]. Carriers of pathogenic *CDK4* variants are phenotypically similar to *CDKN2A* variant carriers [20, 23]. These patients were reported to develop a narrower range of nonmelanoma tumors [4], although few families were available for study.

Among other pathway components (Figure 1), *CCND1* (cyclin D1) is at or near a germline locus for melanoma risk [6], and is often somatically amplified in some melanoma types (Section 3). *RB1* is the gene mutated in familial retinoblastoma, and melanoma risk is greatly increased (~17-fold) as a second malignancy in familial retinoblastoma patients [84]. All these mutations are expected to impair or delay melanocyte senescence.

2.1.3 | Telomerase Genes

The telomerase complex contains two main subunits: the highly conserved TERT (encoded by *TERT*, 5p15.33), and the telomerase RNA component TERC [55, 56]. Somatic *TERT* gene reactivation has been associated with over 90% of all human cancers [12], allowing repair and re-lengthening of dysfunctional telomeres, thus terminating the DDR that sustains cell senescence [39, 55, 56]. Both germline and somatic activating *TERT* mutations are associated with melanoma, being almost exclusively located within the promoter [12]. Horn et al. (2013) identified a novel T > G mutation (at −57) within the *TERT* promoter in a 4-generation family susceptible to melanoma [24]. This was found to act as a new binding site for ETS-family transcription factors. Authors also observed higher telomerase transcription levels in luciferase reporter gene assays [27], and constitutively long telomeres [64]. This specific mutation has been described in only one additional familial melanoma pedigree [25], and other germline variants have not yet been found; germline *TERT* mutations are therefore estimated to account for only a tiny proportion (0.04%) of familial melanoma cases [22, 25]. It is intriguing that these families were prone especially to melanoma since somatic *TERT* promoter mutations are common in many cancer types [12].

Other loci encoding components in telomere elongation have been identified by meta-analysis of GWAS (genome-wide association studies), as having risk alleles for familial melanoma [6]. These include *TERC*, also *RTEL1* (Regulator of telomere elongation helicase (1)), and *STN1/OBFC1* (Homolog of S. pombe *Stn1*), involved in telomeric 5′ strand fill-in after telomerase elongation of the 3′ strand.

2.1.4 | Shelterin Genes: *POT1*

Mutations in shelterin genes have been implicated in ~1% of familial melanoma cases (Figure 2) [11, 22, 85, 86]. Shelterin is a 6-protein complex (Figure 4), many copies of which coat telomeric DNA, stabilizing and protecting chromosomal ends from DDRs and from "repair" by nonhomologous end-joining to other telomeres [57]. Shelterin has three core subunits encoded by *TERF1* (telomere repeat factor 1, also known as *TRF1*), *TERF2* (telomere repeat factor 2/*TRF2*), and *POT1* (protection of

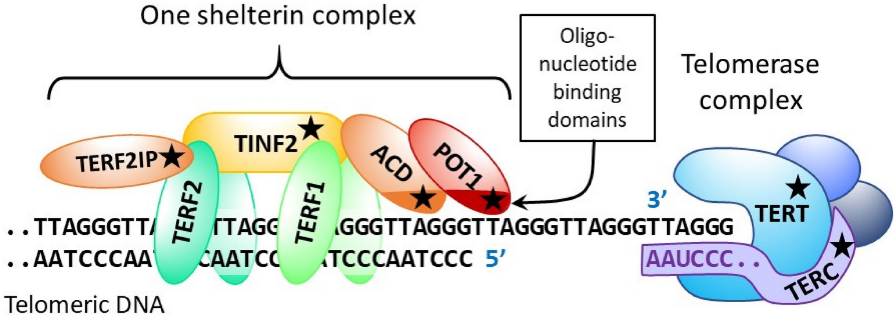

FIGURE 4 | Diagram of the shelterin and telomerase complexes on a telomere. The DNA 3′ overhang is normally arranged within the T-loop (not shown). (★): Component with germline mutations or GWAS locus (*TERC*) linked to melanoma susceptibility. Figure adapted and updated from Aoude et al. [11].

telomeres 1; 7q31.33). Interconnecting proteins are encoded by *TINF2* (TERF1-interacting nuclear factor 2; 14q12, also known as *TIN2*), *ACD* (adrenocortical dysplasia associated; 16q22.1, also known as *TPP1*) and *TERF2IP* (TERF2-interacting protein, 16q23.1, also known as RAP1) (Figure 4) [11].

POT1 is a crucial, highly conserved shelterin gene [11]. Together with ACD, POT1 binds to the overhanging 3′ single-stranded telomeric DNA (ss-DNA) at the end of chromosomes, through two oligonucleotide-/oligosaccharide-binding (OB) domains [11, 12, 56] (Figure 4). This facilitates the binding of shelterin to DNA as well as the binding of ss-DNA into a "t-loop," preventing free telomere ends from being recognized by DNA repair machinery or extended by telomerase [11, 12, 56, 72].

Both missense and loss-of-function variants in *POT1* were identified in a small proportion (~4%) of *CDKN2A*-negative melanoma families [28, 85]. Mutated POT1 was unable to complex with DNA, and carriers of *POT1* mutations had longer telomeres [85]. A recent systematic annotation of all known *POT1* variants found that mutations associated with familial melanoma clustered within the OB domains (Figure 4) [26]. It is thought that reduced *POT1* binding reduces the protection of telomeric ends, which can then be accessed by telomerase [11], potentially delaying cell senescence and promoting melanoma development.

Interestingly, invasive melanoma samples from patients with *POT1* mutations more often displayed a spitzoid morphology than samples from *CDKN2A* and *CDK4* variant carriers, which were histologically similar to sporadic melanomas [87], suggesting a role for telomeric dysfunction in spitzoid differentiation [12].

Additional germline *POT1* variants have been described in other hereditary cancers, but not associated with familial melanoma, suggesting that individual variants may give rise to specific tumor phenotypes [12], although ascertainment bias is another possible explanation. Although somatic *POT1* mutations have not robustly been associated with sporadic melanoma [85], variants affecting the OB domains are found in aggressive subtypes of acquired chronic lymphocytic leukemia [11].

2.1.5 | Shelterin Genes: *ACD*, *TERF2IP*, and *TINF2*

Additional shelterin-complex gene mutations have been associated with familial melanoma development [12]. Novel nonsense mutations within *ACD* and *TERF2IP* were identified by Aoude et al. [86] among non-*CDKN2A* melanoma families. Loss of *ACD* in mice and cell cultures results in low levels of telomeric POT1, reduced telomerase function, DDR activation, and cellular arrest [87]. ACD also facilitates interaction between shelterin and TERT [86], with germline *ACD* mutations reported to synergize with somatic *TERT* mutations to immortalize melanoma cells [88].

TERF2IP binds TERF2 (Figure 4). Protein-truncating TERF2IP variants identified in familial melanoma disrupt the TERF2 binding site, predicted in silico to impair TERF2IP-shelterin binding [86]. TERF2IP is reported to repress homology-directed double-stranded-DNA repair [86], and to regulate other processes including metabolism, inflammation, and DDR [89]. Other germline *ACD* and *TERF2IP* mutations are associated with solid and hematological malignancies [12], but not robustly with familial melanomas [86].

Germline *TINF2* mutations have been implicated in familial melanoma [72] and multiple primary melanoma [90]. In a three-generation family with high cancer rates, He and colleagues reported a novel truncating *TINF2* mutation that cosegregated with papillary thyroid carcinoma and melanoma, and was associated with longer telomeres and impaired TERF2 binding [91].

No germline mutations in *TERF1* or *TERF2* have yet been associated with familial melanoma.

2.2 | Medium- and Low-Penetrance Genes

2.2.1 | BAP1

BAP1 (BRCA1-associated protein 1, 3p21.1) encodes a nuclear deubiquitinase and tumor suppressor [92], which associates with the DNA repair component BRCA1, and also with ASXL1/2 in the polycomb group repressive deubiquitinase complex [92, 93]. BAP1 overexpression can trigger cell senescence [92]; other normal functions are reported in melanocyte differentiation and DDRs [3, 11]. Heterozygous loss-of-function germline variants in *BAP1* are associated with a high risk of early-onset tumors including mesothelioma, renal cell carcinoma, cutaneous melanoma, and uveal melanoma (tumor predisposition syndrome 1, TPDS1) [94]. Under 1% of familial cutaneous melanoma patients carry *BAP1* mutations [11, 22, 29], and only 18% of *BAP1*-TPDS1 patients develop cutaneous melanoma, a medium risk [30]. However, 28% of *BAP1*-TPDS1 patients develop uveal melanoma, with a high risk of metastasis [93] and very poor prognosis [30]. Thus, while medium-penetrance for melanoma specifically, *BAP1* mutations rate as high-penetrance for cancer in general.

2.2.2 | TP53

TP53 (tumor protein p53, 17p13.1) encodes the tumor suppressor and senescence mediator p53 (see Section 1.2 and Figure 1). *TP53* is not a locus for familial melanoma specifically, but for Li-Fraumeni Syndrome, a susceptibility to multiple cancers including breast cancers, sarcomas, leukemias, and others. However, cutaneous melanoma does show significantly increased incidence in carriers of pathogenic *TP53* mutations; a US NCI study found an increased lifetime melanoma risk of sevenfold compared with the general population, or 12.6% of patients by age 70 [95].

2.2.3 | Pigmentation-Related Genes: *MC1R*, *MITF* and Others

Melanin pigment locates over basal keratinocyte nuclei in the human epidermis, and protects these proliferative cells and the underlying melanocytes from UV in sunlight [1]. Thus it is not surprising that variants of genes regulating melanin synthesis can contribute to melanoma risk.

Germline *MC1R* (melanocortin-1 receptor, 16q24.3) and *MITF* (microphthalmia-associated transcription factor, 3p13) mutations increase the risk of melanoma by ~1.6–2.7-fold [31]. Nonsynonymous germline variants in *MITF* are associated with melanoma development [11], with a functional variant $MITF^{E318K}$ being found in up to 2.8% of familial melanoma patients [31].

MITF is the master gene regulator for melanocyte differentiation; it transcriptionally upregulates many components, including MC1R and proteins for building melanosomes and synthesizing melanin [32, 96, 97]. *MITF* is also reported to regulate cell senescence through p16 and p21, and potentially also via DNA-damage repair and oxidative stress pathways, as well as via SASP signaling [98]. Additionally, it is implicated in epigenetic phenotypic switching in advanced melanomas [32]. Evidence has been presented that the specific germline $MITF^{E318K}$ mutation impairs cell senescence. Cultured $MITF^{E318K}$-variant human melanocytes showed some resistance to OIS, while mice coexpressing $MITF^{E318K}$ and $BRAF^{V600E}$ had higher nevus counts, perhaps indicating extended melanocyte proliferation before senescence [33].

MC1R is the receptor for α-melanocyte-stimulating hormone (MSH), which promotes melanocyte differentiation, melanin synthesis, and the switch from pheomelanin (red/yellow) to eumelanin (black/brown) synthesis [96]. Human *MC1R* exhibits numerous polymorphisms associated with varying red or fair hair and pale skin phenotypes [3, 99]. *MC1R* is also part of the suntanning response to UV radiation, through DNA damage/DDRs triggering MSH secretion by keratinocytes [100]. April and Barsh, studying gene expression in *Mc1r*-mutant mouse skin, found an overrepresentation of proliferation-associated gene products [34]. Coinheritance of *MC1R* polymorphisms further increases the risk of melanoma in *CDKN2A* families [3].

The *MC1R* locus notably yields the highest significance in the genome for melanoma risk, by meta-analysis of GWAS ($p < 10^{-51}$) [6], attributable at least partly to the high frequency and diversity of variants in pale-skinned human populations [97]. The same approach yields peaks of high significance close to other pigmentary loci, including *ASIP* (Agouti signal protein), *TYR* (tyrosinase), *OCA2* (oculocutaneous albinism 2), *TYRP1* (tyrosinase-related protein 1), and *SLC45A2* (Solute carrier family 45, member 2) [6]. ASIP is an antagonist at the MC1R, promoting red-yellow melanin synthesis. *TYR*, *OCA2*, *TYRP1*, and *SLC45A2* encode components of the melanosome (pigment granule) and are major loci for oculocutaneous albinism (OCA)—also known respectively as *OCA1*, *OCA2*, *OCA3*, and OCA4 [96, 97]. They are polymorphic among human ethnic populations and associated with skin color, thus contributing to a molecular basis for the well-known protective effect of dark skin color against skin cancer [97].

3 | Somatic Mutations and Steps in Melanoma Development

Cutaneous malignant melanoma from sun-exposed body sites has the highest rate of somatic mutations of all solid tumors. Much is known about its mutational landscape, though doubtless not all. Many driver mutations have been identified for human melanoma [35, 101–104], the therapeutic targeting of which has transformed the treatment options available [10]. Table 2 summarizes the best-established and commoner driver genes. Here, we review these somatic alterations, including deletions and epigenetic changes.

For context, we use a framework that aims to explain how familial and/or somatic mutations synergize in metastatic melanoma development. This model for the minimal required genetic (or epigenetic) changes in melanoma progression has been developed and refined over two decades [35, 39, 105]. An update is shown in Figure 5, influenced by the important work of Shain et al. [106, 107] and Suram et al. [108]. The figure shows the commonest order in which four required changes are observed, although other orders are also found [35]. Typically advanced melanomas will also have many other driver and nondriver mutations, but these four appear to be the functional minimum [109]. They also provide a framework to understand familial melanoma genes. Common somatic driver mutations are now reviewed in the context of these four steps (Figure 5). They are also summarized in Table 2. For brevity, we discuss only the best-known/commonest mutated loci for each step.

3.1 | Initiation: Mitogenic Somatic Mutations

To form any tumor, cell proliferation has to be dysregulated such that cells can proliferate to an abnormally high local density. Driver mutations specifically promoting proliferation are called mitogenic; they almost always activate the MAPK signaling pathway that normally links growth-factor receptors, through the RAS, RAF, MAP2K, and MAPK protein families, to the cell-cycle rate-regulators CDK4/CCND1 and MYC [35, 102, 103, 106, 107] (full pathway diagram in ref. [35]). Mitogenesis is most commonly the first mutation in somatic oncogenesis, as the proliferation generates a clone of hundreds to millions (or more) of mutated cells, proportionately increasing the probability of second and further subclonal mutations. In melanoma, the commonest mitogenic mutations are in the genes *BRAF* and *NRAS* [35, 102, 103, 107].

3.1.1 | BRAF

Around 45%–50% of melanomas carry activating point mutations in *BRAF* (v-Raf murine sarcoma viral oncogene homolog B, 7q34) [35, 102], usually $BRAF^{V600E103}$. $BRAF^{V600E}$ mutations lead to sustained activation of the MAPK pathway, promoting cell proliferation [103]. The continued growth of cancer depends on its mitogenic drivers, so these make favored therapeutic targets. The genomic testing of patient melanoma tissue samples for mutant *BRAF* is now routine in stage II to IV melanomas. Specific mutant *BRAF* inhibitors, such as dabrafenib, encorafenib, and vemurafenib, are in use in late-stage $BRAF^{V600E}$ melanomas, though tending to be superseded now by immunotherapies [9, 128].

Of note, $Braf^{V600E}$ germline mutation is embryonic lethal in a mouse model [129], a potential explanation for why this does not feature among inherited melanoma syndromes.

TABLE 2 | Common somatic driver mutations, epimutations, and copy-number variations found in cutaneous melanoma.

Gene	Change	Cellular pathway	Main effect(s) of mutation	References
Mitogenic drivers				
BRAF	Activating mutation	MAPK	Mitogenic driver	[101–103]
NRAS	Activating mutation	MAPK+PI3K	Mitogenic driver; Suppression of apoptosis	[102, 110]
MYC	Amplification	Core mitogenic regulator	Mitogenic driver; TERT upregulation	[35, 111]
NF1	Mutation	MAPK+PI3K	RAS activation: mitogenic driver Suppression of apoptosis	[102, 103, 112, 113]
PTPRD	Mutation, deletion	RTK to MAPK+PI3K	Mitogenic driver; Suppression of apoptosis	[114]
ERBB4	Activating mutation	RTK to MAPK+PI3K	Mitogenic driver; Suppression of apoptosis	[115]
MITF	Amplification	Master regulator, cell differentiation	[?]Mitogenesis; [?]others	[32, 35]
PPP6C	Mutation	MAPK	Mitogenic driver	[102, 116]
Antisenescence				
TERT	Promoter mutation	Telomere extension	Suppression of senescence	[24, 117, 118]
CDKN2A *CDKN2B*	Deletion, methylation, mutation	CDKN-CDK4-RB1; ARF-p53	Evasion of senescence	[35, 49, 119, 120]
TBX2	Amplification, mutation	p53[?]	Evasion of senescence	[121]
APC	Methylation	β-catenin-AP1 and MITF	[?]Evasion of cell senescence [?]Mitogenesis	[122]
ARID2	Mutation	Chromatin remodeling	[?]Evasion of senescence Immunoresistance	[101, 102, 123]
Antiapoptotic				
PTEN	Deletion, mutation	PI3K	Suppression of apoptosis	[102, 124, 125]
TP53	Mutation	p53	Suppression of apoptosis; Evasion of senescence	[102, 126]
PREX2	Activating mutation	PI3K	Suppression of apoptosis [?]Mitogenic driver	[127]
APAF1	Deletion, methylation	Caspase activation	Suppression of apoptosis	[35]

Note: Adapted and updated from Bennett (2016) [35]. (Red): genes activated in melanoma. (Blue): genes defective/deleted in melanoma. Mutations form three functional groups as indicated, and are listed in approximate order of prevalence within groups (this varies between studies). Not all are discussed in the text. Pathway acronyms in table order (see also text): (MAPK) mitogen-activated protein kinase mitogenic pathway. (PI3K) phosphoinositide 3-kinase antiapoptotic pathway. (RTK) receptor tyrosine kinase. (GPCR) G-protein coupled receptor. (PRKC) protein kinase C. (AP1) activator protein 1 transcription-factor complex: activates proliferation through cyclin D and MYC.

[?]Denotes possible effect but not certain.

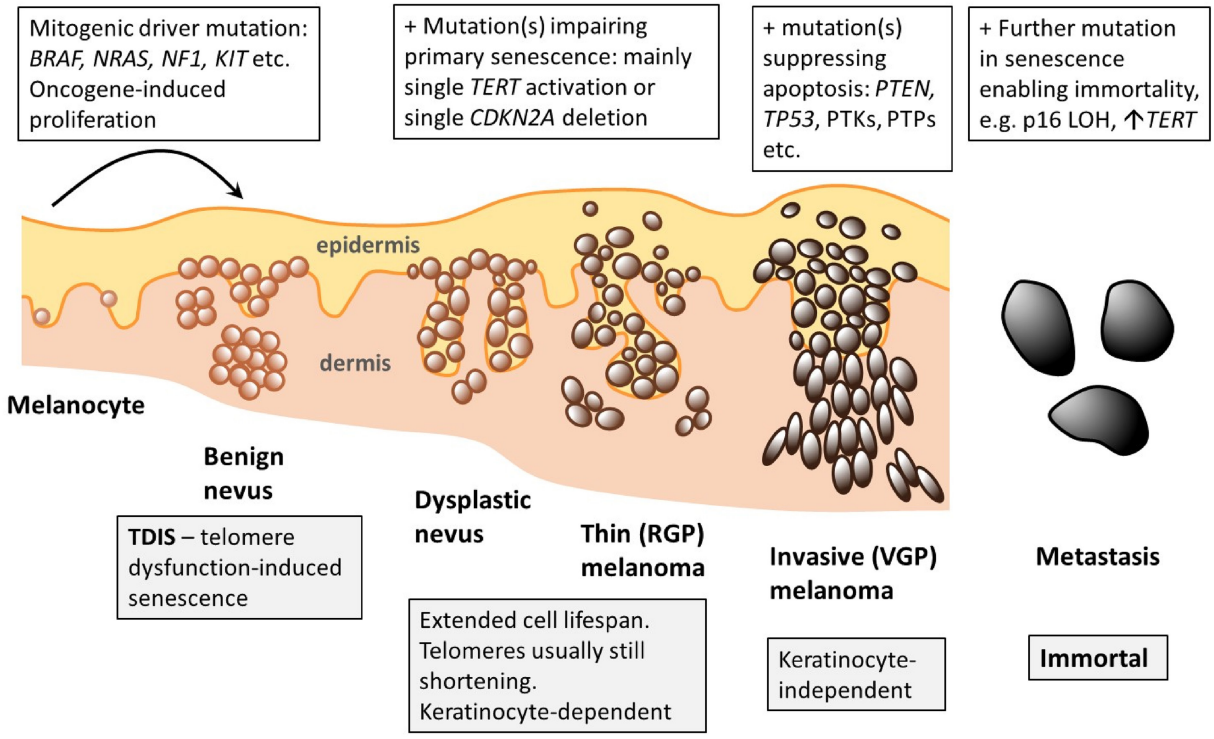

FIGURE 5 | Updated minimal genetic model for melanoma progression. Adapted from Bennett [35, 39] and Shain and Bastian [106]. RGP: radial growth phase. VGP: vertical growth phase (invasion to the deeper dermis). This shows that benign nevi often proliferate extensively in the dermis before senescing, while dysplastic nevi and thin melanomas are typically largely confined to the epidermis, although rete ridges can become crowded with tumor cells and expanded. See text for further discussion.

3.1.2 | NRAS

NRAS (Neuroblastoma RAS Viral Oncogene Homolog, 1p13.2) encodes NRAS, a member of the RAS family of small G-regulatory proteins. Activating mutations in *NRAS* at codons 12, 13, and especially 61 are found in about 25% of melanoma tissue samples [102]. *NRAS* (like other *RAS*) driver mutations activates the MAPK pathway [35, 103] and also the PI3K-AKT cell survival pathway [35], thus achieving both proliferation and reduced apoptosis (Section 3.4) at once [110]. Not surprisingly, *NRAS* mutations in melanoma are associated with aggressive disease [103].

3.1.3 | NF1

NF1 (neurofibromin 1 or neurofibromatosis 1, 17q11.2) encodes NF1, a negative regulator of RAS proteins. Inactivating *NF1* mutations thus increase RAS activity, and constitutive activity of both the MAPK and PI3K-AKT pathways [103]. Somatic *NF1* mutations are found in around 11%–14% of melanomas, 90% of which cause loss of function in silico [119, 133]. *NF1*-mutant melanomas are also associated with metastasis [113]. Hodis et al. [101] and The Cancer Genome Atlas (TCGA) Network [102] reported that *BRAF*, *NRAS*, and *NF1* driver mutations in melanoma are almost always mutually exclusive, and classified melanomas into these three groups and the remainder, called triple wild-type. Krauthammer et al. noted that *NF1* mutations in human melanoma samples were frequently accompanied by co-mutations including in IDH1, a metabolic enzyme, and RASA2, another inhibitor of NRAS mitogenic activity [112, 130]. It is possible that co-mutations are required because *NF1* inactivation does not activate RAS signaling as much as RAS mutations themselves. Mutant *IDH1* is also found in conjunction with oncogenic mutations in *BRAF* and *NRAS* among others [102, 131, 132]. Its oncogenic role is unclear.

3.1.4 | Other Mitogenic Drivers

It seems likely that all the "triple-wild-type" melanomas have some mitogenic driver since they do proliferate to high cell densities. Rarer reported MAPK-activating oncogenes in melanoma include mutated versions of receptor tyrosine kinase genes like *ERBB4* and *KIT*, a glutamate receptor *GRM3*, other RAS genes *KRAS* and *HRAS*, and *MAP2K1* (MEK) [35, 102, 112]. PPP6C is the catalytic (C) subunit of protein phosphatase 6. which negatively regulates MAPK by dephosphorylation of MAP2K1 [133]. Nonsynonymous mutations in *PPP6C* were found in ~9% of sporadic melanomas [102], although almost exclusively in conjunction with mutant *BRAF* or *NRAS*, implying that it is not a complete driver [102]. Maskin et al. recently reported that inactivating *PPP6C* mutations in melanoma cell lines also increase *MITF* activity (see Section 2.2.3) and reduce sensitivity to therapeutic *BRAF* inhibitors [116].

MITF is somatically upregulated in ~11% of melanomas by focal or broader gene amplification [98], and it upregulates growth-factor receptors among other targets; so its somatic

mutations are tentatively included here under mitogenic drivers, although its roles in melanoma appear complex and even contradictory (see also Section 2.2.3). The melanoma-associated germline *MITF*^{E318K} mutation appears to impair cell senescence [33]. Higher *MITF* in human melanoma samples was reported to correlate either with improved patient outcomes [134] or with metastasis [135]. *MITF* evidently has multifaceted and incompletely understood roles in melanoma genesis.

One type of mitogenic genetic change does not affect MAPK signaling, namely, those that act downstream of MAPK to upregulate its targets CCND1 (cyclin D1) and MYC. Somatic ***CCND1*** gene amplification was reported in ~7% of melanomas [102], and amplification of the 8q24 region containing ***MYC*** in over 30% (reviewed [35]). *CCND1* amplification also doubles as antagonistic to the p16 senescence pathway (Figure 1).

3.2 | Cell Senescence and Nevus Formation

Much evidence has accumulated that activation of a mitogenic oncogene alone is insufficient to generate melanoma (among other cancers) in humans. Specifically, the common benign melanocytic nevus arises through oncogene-induced proliferation, followed by cell senescence and arrest (Section 1.2, Figure 5) [39]. 75%–80% of benign acquired melanocyte nevi carry a *BRAF*^{V600E} mutation [35, 50, 64, 103], and ~8% carry an *NRAS*^{Q61K} mutation [35], showing that these are each insufficient to generate melanoma. Similarly, activating *NRAS* mutations are reported in ~70% of benign congenital melanocytic nevi [136] and mutant *HRAS* in 30% of benign Spitz nevi [135]. Nevus senescence is often described as oncogene-induced senescence, but this raises difficulties because "classical" OIS in cell culture differs in immediacy and phenotype [39], and human nevus senescence is influenced by telomeres (more below). Thus, findings about OIS in culture cannot necessarily be assumed to apply to nevi.

The evidence that human benign nevi are composed predominantly of senescent cells was reviewed by Bennett [39], including notably that very few nevus cells can proliferate when explanted, unlike normal melanocytes [105]. Nevi express many molecular markers of senescence, in ~50% to > 90% of the cells, including p16, p15 (CDKN2B), β-galactosidase, PML bodies, γH2AX, H2AFY (macroH2A), H4K40Me3, and DDR markers phospho-CHEK2 and 53BP1 [39, 49, 50, 108, 136]. There is strong evidence that, as with cultured human melanocytes [19], nevus senescence is primarily p16-mediated: carriers of melanoma-associated p16 variants commonly have more large nevi and abnormal nevi than others (delayed senescence) (Section 2.1.1, FAMMM syndrome) and benign nevi express little p53 or p21 [138]. Telomere shortening is also genetically implicated: longer telomere length among humans is associated with more numerous and large nevi [139]. Moreover, congenital nevi—initiated in embryonic life when telomeres are longer—are typically larger than acquired nevi [136].

It was previously reported that telomeres do not appear critically short in nevi [50]; but more data may be needed on this, as the shortest telomeres can be missed by the assay used [140]. Moreover, DNA damage within telomeres is poorly repaired and can also lead to cell senescence [108]. Importantly, Suram et al. reported that cells in nevi (among other benign human lesions) display telomere-associated DNA damage, a marker of telomere dysfunction-associated senescence (TDIS), senescence mediated by critical telomere shortening or unrepaired DNA damage within telomeres [108]. Ongoing UV exposure could very plausibly cause such damage in developing nevi.

Thus TDIS with p16-mediated arrest now seems the best-supported model mechanism for nevus senescence (Figure 5). This can explain why so many melanoma susceptibility genes are linked to either the p16 pathway or to telomere maintenance and protection. It also clarifies the pattern of the first additional mutations seen in lesions progressing from nevus toward melanoma, as now discussed.

3.3 | Progression 1: Mutations Impairing Primary Cell Senescence

Around 1/3 of melanomas appear to arise from preexisting nevi [106, 141], or 89% of superficial spreading melanomas in one study [141]. Shain et al. and others have conducted revealing studies of additional mutations found in "intermediate" (atypical, dysplastic, or early malignant) lesions compared with the nevi from which they arose [107, 142].

3.3.1 | *TERT* Promoter Mutations (TPMs)

The commonest second pathogenic change found in addition to a mitogenic driver was an activating TPM, in no less than 77% of intermediate lesions and in-situ melanomas [106, 107]. This resembles the overall frequency of 70%–80% for TPMs in all melanomas at nonacral sites [117]. These are UV-signature C > T mutations at −124 and−146 from the transcription start site, either of which creates a new binding site for ETS-family transcription factors [24, 117], as seen in familial melanoma. ETS-related factor GABP is a reported candidate for activation of TERT transcription via such mutated sites in cancer generally [143]; while ETS1 is substantially upregulated in nevi and melanomas versus normal epidermal melanocytes (it is downstream of the MAPK pathway), providing another candidate in these lesions [144].

Frequent TPMs early in progression were initially surprising since short telomeres (exerting selection pressure for TERT expression) had not been observed in nevi; moreover, telomerase expression is associated with cell immortality (ability to proliferate indefinitely), whereas early melanomas rarely display immortality [105]. However, Chiba et al. found that a single TPM was insufficient to prevent the shortening of longer telomeres, but promoted the repair of telomeric DNA damage and very short telomeres [145]. This repair could therefore also mute the DDR signal in nevus cells (Section 3.2) and downregulate senescence signaling, an attractive explanation for these mutations.

These early TPMs were rarely found in lesions with *BRAF* mutations, mainly with *NRAS* mutations and in older patients [107, 142].

3.3.2 | CDKN2A

CDKN2A heterozygous deletions were the next commonest second pathological mutations found in intermediate lesions in the above studies, especially in lesions with *BRAF* mutations [107, 142]. This implies that two intact copies of *CDKN2A* are required for BRAF-associated nevus senescence. p16 is typically expressed in a patchwork fashion within benign nevi (at least those excised as suspicious) [49, 50, 138], suggesting subclonal inactivation already at this stage. Biallelic *CDKN2A* loss was seen only later in progression (Section 3.5).

3.3.3 | *ARID* Family

Somatic loss-of-function mutations in *ARID2* (AT-rich interaction domain-containing protein 2, 12q12) have been identified overall in ~10%–30% of melanomas [35, 101, 102]. Loss of this or other ARID family members *ARID1A* and *ARID1B* were also identified among early progression mutations [107]. They encode subunits of the SWI/SNF chromatin-remodeling complex which regulates gene transcription. These early defects suggest a function in senescence, but this is unclear. Their role in melanoma progression may be complicated, as inactivation may also be associated with increased tumor immunogenicity and susceptibility to immunotherapies [123].

3.4 | Progression 2: Apoptosis Reduction

Our model (Figure 5) proposes that a mutation reducing apoptotic tendency is required for the development of invasiveness in melanoma, based on the phenotype of p16-deficient (senescence-impaired) human melanocytes in culture. These show enhanced apoptosis compared to normal melanocytes, which are suppressed in the presence of keratinocytes or keratinocyte-derived growth factors [19]. The mechanism remains unknown, but this keratinocyte-dependence after p16 loss can potentially explain the thin, radially growing phenotype commonly seen in early melanomas, even though benign nevi often grow in the deeper dermis. Various less-common mutations seen in advanced melanomas can also reduce apoptosis, such as activations of receptor tyrosine kinases, defects of tyrosine phosphatases, and mutations of *PREX2* and *APAF1* (Table 2) [35], but here we will discuss only the best-studied examples.

3.4.1 | TP53

Although mutations of the p53 gene (*TP53*, 17p13.1) are frequent overall in human cancer, they are seen in only around 5%–20% of sporadic melanomas [35, 102, 126]. This may reflect the unusual predominance of p16 in normal human melanocyte (and nevus) senescence, with apparent uncoupling of p53 upregulation from the DDR [138]. p53 is however upregulated and can mediate delayed senescence after extensive proliferation in p16-null, ARF-competent melanocytes [19], giving a later selection pressure for its loss or antagonism. p53 can also mediate apoptosis as well as senescence, so its loss may reduce keratinocyte-dependence [35]. Interestingly MDM4 (mouse double minute 4 homolog), which binds p53 and inhibits its activity, is frequently upregulated in sporadic melanomas, which may be the predominant route to impaired p53 function in melanoma [146]. *CDKN2A* also encodes ARF, a stabilizer of p53 (Figure 1), so *CDKN2A* deletion is likely to reduce p53 activity. Early cell culture studies identified a selective deletion of the ARF-specific exon 1β in two metastatic melanoma cell lines [120], so ARF deletion is not always a by-product of p16 deletion.

3.4.2 | PTEN

The PI3K-AKT pathway is upregulated in the development of many cancers, including melanoma [102, 106]. PI3K-AKT signaling both suppresses apoptosis and promotes proliferation [124, 147], although upregulation is not found without other mitogenic driver mutations in melanoma [107], suggesting that apoptosis reduction may be key. Upregulation is often through the deletion of *PTEN* (phosphatase and tensin homolog deleted on chromosome 10, 10q23.31). *PTEN* is a tumor suppressor gene that negatively regulates the PI3K-AKT pathway. Deleterious *PTEN* mutation or deletion has been found in 20%–35% of sporadic melanomas [35, 124], but only later in progression [107].

Loss of *PTEN* is found especially in conjunction with *BRAF* (not *NRAS*) mutations [125]. The PI3K-AKT pathway is already upregulated by mutant *NRAS* (Section 3.1.2), so perhaps there is no selection there for *PTEN* loss. Sustained hyperactivation of the PI3K-AKT pathway in nontumoral cells has been found to result in an OIS-like state [147], reminiscent of OIS induced by artificial overexpression of mitogenic drivers. The physiological relevance is unclear.

3.5 | Immortalization

As mentioned, a single (heterozygous) TERT activation and/or p16 defect appears not to immortalize cells; telomere shortening with proliferation is still typically occurring in dysplasias and early melanomas [138, 145]. In the absence of normal senescence, this leads to telomeric crisis, with end-to-end chromosome fusions leading to mitotic abnormalities like anaphase bridging, multipolarity, and chromosome rearrangements, as seen in cultured p16-deficient melanocytes [19] or in uncultured early melanomas [138]. In further progression, especially metastasis [35], changes leading to full immortalization are seen.

3.5.1 | p16 Pathway, Further Inactivation

Biallelic *CDKN2A* inactivation is found only in advanced, invasive melanoma [107]. Up to ~88% of human advanced melanomas harbor *CDKN2A* loss-of-function alterations, comprising ~50% deletions, ~19% promoter hypermethylation [148], and ~19% mutations [35, 119]. Percentages of lesional cells expressing nuclear p16 decrease with progression to ~10% in metastatic melanoma [49, 138, 149].

Alternative defects in the p16 pathway (Figure 1) are also found at later stages. Somatic mutations or amplifications of *CDK4* are

mainly associated with higher risk and rarer clinical subtypes of melanoma including acral and mucosal melanoma [149]. TCGA Network estimated that (including these subtypes), 8% of melanomas overall showed *CDK4* amplification and 7% amplification of *CCND1*, while *RB1* was also significantly mutated, notably in *NF1*-driven melanomas (10%) [102]. Numbers seem consistent with the possibility that p16 pathway disruption is required as well as telomere maintenance in advanced melanoma and for immortalization, as seen for the immortalization of cultured human melanocytes [49].

3.5.2 | *TERT*, Further Upregulation

Activating *TERT* PMs were observed in 85% of metastatic melanomas [24] and, as already mentioned, are already common in dysplasias and early melanomas [107]. Immortality is believed essential to generate enough cells for cancer metastasis [35, 145], but Chiba et al. [145] reported that, in melanoma cells carrying single TPMs, telomeres could continue to shorten leading to telomeric crisis, as also seen in early melanomas in vivo [138]. They also observed however that such cultured melanoma cells with critically short telomeres then tended to upregulate the expression of TERT further, which made a second step in immortalization allowing stable telomere maintenance [145]. The mechanism of upregulation was not determined, though additional mechanisms of TERT upregulation have been found in many cancer types, including frequent hypermethylation of a repressive promoter region [150]. Mitogenic transcription factor MYC has several activating binding sites in the TERT promoter [151], so the known common *MYC* amplifications in advanced melanoma (Section 3.1.4) give another potential route for further TERT upregulation.

4 | Conclusions

In summary, the genes disrupted in familial melanoma fall into two groups. One, not surprisingly, is related to skin pigmentation, reflecting the established role of melanin in protection against DNA damage and mutation. The other group, including all major high-penetrance melanoma genes, relates strikingly to cell senescence, especially telomere regulation and the p16/CDK4/RB1 senescence pathway. This second group reappears among somatic mutations found commonly in sporadic melanoma. Cell senescence evidently plays a vital role in suppressing the development of both familial and sporadic melanoma, through the formation of nevi and TDIS (Section 3.2).

Uncovering the roles of these genes has directly impacted patient diagnosis, prognosis, and treatment, and illustrates the power of the genetic and genomic tools now available to us for understanding cancer. As diagnostic whole-genome sequencing becomes embedded into healthcare pathways, it is likely that novel pathogenic germline variants will be discovered in melanoma-prone families, with implications for individual patient prognosis, family planning, and preventative sun-protection strategies. Such future studies are likely to help identify new therapeutic avenues and continue to improve outcomes for patients with melanoma.

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Data Availability Statement

No unpublished original data are associated with this review.

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