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Edgardo Castro-Pérez | Mithalesh Singh | Shreyans Sadangi | Carmen Mela-Sánchez | Vijayasaradhi Setaluri

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REVIEW

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Connecting the dots: Melanoma cell of origin, tumor cell plasticity, trans-differentiation, and drug resistance

Edgardo Castro-Pérez[1,2] | Mithalesh Singh[3] | Shreyans Sadangi[3] | Carmen Mela-Sánchez[2] | Vijayasaradhi Setaluri[3,4]

[1]Center for Cellular and Molecular Biology of Diseases, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT-AIP), City of Knowledge, Panama City, Panama

[2]Department of Genetics and Molecular Biology, University of Panama, Panama City, Panama

[3]Department of Dermatology, University of Wisconsin-Madison School of Medicine and Public Health, Madison, Wisconsin, USA

[4]William S. Middleton VA Hospital, Madison, Wisconsin, USA

Correspondence
Vijayasaradhi Setaluri, Department of Dermatology, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI 53706, USA.
Email: vsetaluri@dermatology.wisc.edu

Edgardo Castro-Pérez, INDICASAT-AIP, City of Knowledge, Building 208, Pullen Street, Clayton, Ancon, Panama City, Panama.
Email: ecastro@indicasat.org.pa

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Abstract

Melanoma, a lethal malignancy that arises from melanocytes, exhibits a multiplicity of clinico-pathologically distinct subtypes in sun-exposed and non-sun-exposed areas. Melanocytes are derived from multipotent neural crest cells and are present in diverse anatomical locations, including skin, eyes, and various mucosal membranes. Tissue-resident melanocyte stem cells and melanocyte precursors contribute to melanocyte renewal. Elegant studies using mouse genetic models have shown that melanoma can arise from either melanocyte stem cells or differentiated pigment-producing melanocytes depending on a combination of tissue and anatomical site of origin and activation of oncogenic mutations (or overexpression) and/or the repression in expression or inactivating mutations in tumor suppressors. This variation raises the possibility that different subtypes of human melanomas (even subsets within each subtype) may also be a manifestation of malignancies of distinct cells of origin. Melanoma is known to exhibit phenotypic plasticity and trans-differentiation (defined as a tendency to differentiate into cell lineages other than the original lineage from which the tumor arose) along vascular and neural lineages. Additionally, stem cell-like properties such as pseudo-epithelial-to-mesenchymal (EMT-like) transition and expression of stem cell-related genes have also been associated with the development of melanoma drug resistance. Recent studies that employed reprogramming melanoma cells to induced pluripotent stem cells have uncovered potential relationships between melanoma plasticity, trans-differentiation, and drug resistance and implications for cell or origin of human cutaneous melanoma. This review provides a comprehensive summary of the current state of knowledge on melanoma cell of origin and the relationship between tumor cell plasticity and drug resistance.

KEYWORDS
BRAF/MEK inhibitors, drug resistance, melanoma cell of origin, melanoma plasticity and trans-differentiation, melanoma stem cells, melanoma-derived iPSC

1 | INTRODUCTION

Melanoma is the leading cause of death from diseases of the skin, the largest organ in humans, with the highest incidence in the United States and other Western countries. Melanoma incidence increased 270% from 1973 to 2002 and continues to increase (Rastrelli et al., 2014). Melanoma is caused by the malignant transformation of melanocytes, the skin-resident pigment-producing cells that accumulate mutations leading to abnormal proliferation (Vultur & Herlyn, 2013).

Cutaneous melanoma is most commonly diagnosed in areas of sun-exposed skin, and it is broadly classified into two main categories based on the anatomical location of its occurrence: chronically sun-damaged (CSD) and non-CSD (Shain & Bastian, 2016). Accordingly, CSD melanomas usually arise on areas of the skin that are naturally prone to long-term exposure to UV radiation throughout life, in particular head and neck regions, mainly in individuals 55 years and older. CSD melanomas typically exhibit a lentiginous (slow) growth pattern, and these lesions are called lentigo malignant melanoma. Acral (palms, soles of feet, or under finger or toenails) and mucosal (respiratory, gastrointestinal, or anogenitourinary tracts) melanomas also have a lentiginous manifestation; however, they do not exhibit sun damage and are classified by different criteria (see below). In contrast to CSD melanocytic lesions, non-CSD melanomas occur in younger individuals in body areas that typically have less frequent or low sun exposure, such as proximal extremities and the trunk. Non-CSD melanomas are classified as superficial spreading melanoma (Shain & Bastian, 2016).

Genomic analyses demonstrated a highly specific distribution of distinct driver mutations among different melanoma subtypes. For example, >60% of all cutaneous melanomas harbor oncogenic mutation T1796A in exon 15 of v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), leading to a substitution of valine by glutamic acid at position 600. This change produces a mutant protein designated as BRAFV600E that is constitutively active and results in hyperactivation of mitogen-activated protein kinase (MAPK) signaling, a critical component for cell survival and proliferation. In contrast, CSD melanomas often harbor mutations in Neurofibromin 1 (*NF1*), Neuroblastoma RAS viral oncogene homolog (*NRAS*), nonV600E *BRAF*, or mast/stem cell growth factor receptor (*KIT*) genes (Bastian, 2014; Flaherty et al., 2012; Robert et al., 2015). The reasons for the particular pattern of these mutations are unknown. It is also unknown whether these clinical and pathological melanoma subtypes represent different mechanisms of tumor initiation or malignancies that reflect different cells of origin. Patients with BRAF mutant melanoma do not respond to traditional chemotherapy such as dacarbazine (DTIC) and show a poor prognosis (Houben et al., 2004; Serrone et al., 2000; Vultur & Herlyn, 2013). However, therapies specifically directed to inhibit oncogenic BRAFV600E (with inhibitors Vemurafenib, Dabrafenib, and Encorafenib; termed collectively hereafter as BRAFi) are highly effective in the short term. Unfortunately, melanoma tumors in some patients show intrinsic resistance to these inhibitors, and most patients sensitive to the initial treatment with these drugs eventually acquire resistance to BRAFi and even to combined therapies of BRAFi with inhibitors of downstream MAPK pathway targeting MEK 1/2 (Trametinib, Cobimetinib, and Binimetinib; termed collectively hereafter as MEKi). Resistance of melanoma to BRAFi/MEKi and aggressiveness of recurrent metastatic tumors are known to be associated with activation of stem cell pathways and the presence of cancer stem cells (CSC), also known as melanoma stem cells (MSC) (Roesch et al., 2010). Therefore, targeting MSC was proposed as an effective strategy for treating melanoma (Schatton & Frank, 2008). Furthermore, self-renewal, cellular plasticity, pluripotency, and trans-differentiation, which are features associated with stem cells, have been implicated in melanoma tumor progression and drug resistance (Kemper et al., 2014; Roesch et al., 2016; Tsoi et al., 2018). However, it is not known whether differences in melanoma plasticity and variable expression of CSC/MSC markers are also related to melanoma cells of origin. Recently, reprogramming of melanoma cells in vitro to induced pluripotent stem-like cells and re-differentiation to a melanocytic lineage has been shown to have the potential to uncover the relationship between melanoma cell plasticity and drug resistance and melanoma cell of origin (Castro-Pérez et al., 2019). In this review, we discuss cellular and molecular pathology-based subtypes of melanoma, and the relationship between the specific patterns of oncogene mutations, developmental precursors of melanocytes, and melanoma cell of origin. We suggest that reprogramming melanoma cells to iPSC might shed some light on the relationship between melanoma cell of origin and the diversity of oncogenic driver mutations in melanoma subtypes.

2 | DEVELOPMENTAL ORIGIN AND ANATOMICAL DISTRIBUTION OF MELANOCYTES

Melanoma arises from melanocytes, specialized cells that produce melanin pigment. Melanocytes in the skin are found predominantly in two distinct locations: the epidermis and hair follicles, where they produce melanin to be exported to epidermal keratinocytes and hair shafts, respectively (Haass et al., 2005; Slominski et al., 2004). Melanocytes are also found in other anatomical locations, including the eyes, inner ear, oral and sinonasal mucosa, anogenital tracts, and the central nervous system (Aoki et al., 2009; Mort et al., 2015). During the neurulation process, melanoblasts, the embryonic melanocyte precursors, develop from the neural crest (NC), a transitory embryonic structure that arises from the neuroectoderm under the influence of the notochord (Dupin et al., 2003; O'Rahilly & Muller, 2007; Young et al., 2009). NC cells are multipotent stem cells with highly migratory and proliferative features, and their developmental fate is determined by their antero-posterior anatomic location (Figure 1). Accordingly, NC cells that migrate along the dorsal pathway in the trunk give rise to peripheral neurons, glial cells, and cutaneous melanocytes in the epidermis and the hair follicles, a source of melanocyte stem cells

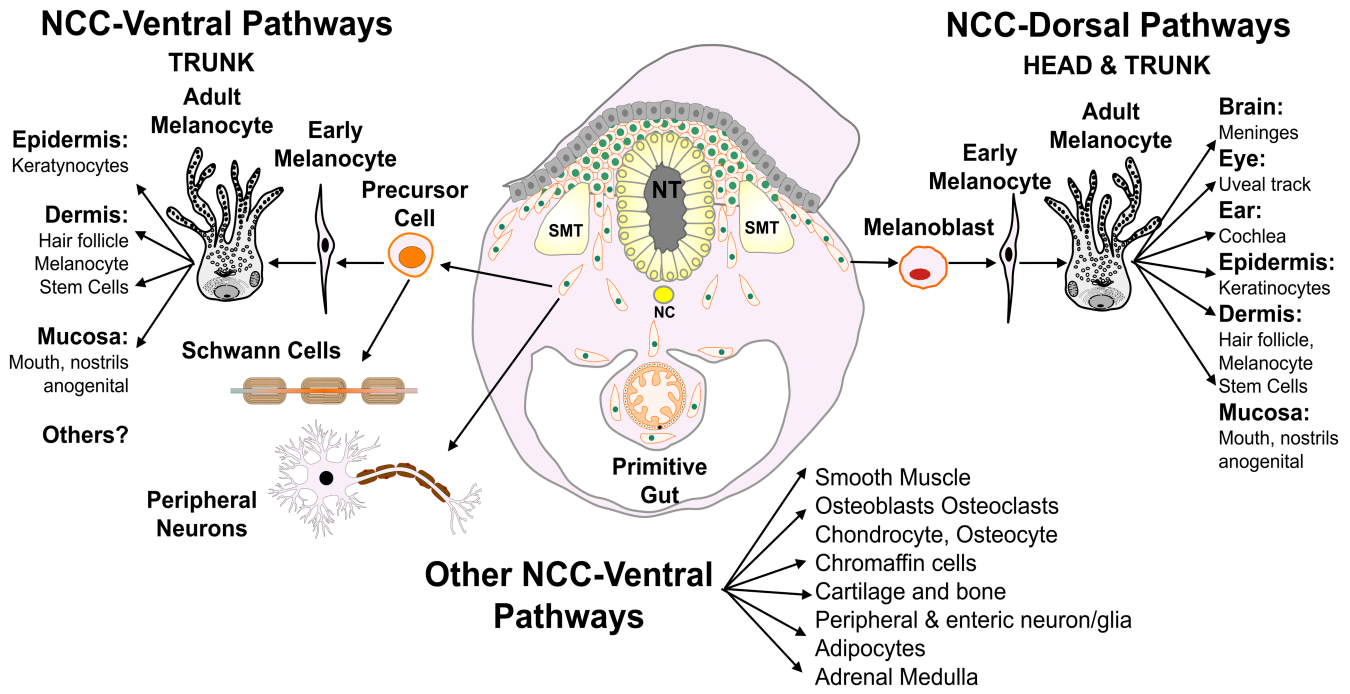

FIGURE 1 Embryonic origin of melanocytes. Melanocyte development starts from multipotent neural crest cells (NCC) and gives rise to several cell types and tissues. Migrating NCC precursors of melanocytes include melanoblasts and a common precursor of Schwann cells that populate the epidermis, dermis, brain, eye, ear mucosa, and other tissues/organs proposed by dorsal and ventral pathway (NC, notochord; NT, neural tube; SMT, somites).

and melanocytic precursors (Sauka-Spengler & Bronner, 2010; Theveneau & Mayor, 2012). Interestingly, although epidermal melanocytes are recognized as molecularly distinct from dermal melanocytes, it is not yet clear if they emerge from distinct precursors (Aoki et al., 2009; Cichorek et al., 2013). A remarkable variety of cells emerge from NC cells that migrate along the ventral pathway, including Schwann cell precursors (SCP), neurons, endocrine glands, smooth muscles, and some cutaneous melanocytes (Adameyko et al., 2009; Harris & Erickson, 2007; Theveneau & Mayor, 2012). In the head region, the diversity of cells that emerge from cranial NC cells and migrate along the dorsal path is even more remarkable, contributing to the formation of bone, cartilage, smooth muscle, connective tissue, glandular tissues, neurons, glia, and melanocytes in the inner ear, the oral cavity, and the scalp skin (Cordero et al., 2011; Snider & Mishina, 2014; Young et al., 2009). Interestingly, cranial melanocytes also emerge from cells that are distinct SCP (Adameyko et al., 2012). Additionally, retinal pigment epithelial cells are a distinct melanocytic lineage that emerges directly from cranial NC-derived SCP and from the neural tube (NT). NC-derived melanocytes also populate some internal anatomical sites, such as the meninges (Cramer & Fesyuk, 2012; Dupin et al., 2003). In addition, based on studies on humans with pigmentary mosaic disorders, it was proposed that a population of melanocyte precursors might emerge in earlier developmental lineages, presumably from mesodermal lineage primitive streak at the time of gastrulation (Kinsler & Larue, 2018). However, more studies in human and animal genetic models are needed to confirm this mesodermal origin of melanocytes.

Given the multiplicity of cell precursors that can give rise to cells of the melanocyte lineages, their plasticity, and migratory patterns, it is tempting to argue that the diversity of oncogenic drivers in melanoma and the subtypes they cause is a manifestation of the diverse origins of melanocytes. Additionally, the various epigenetic modifications acquired during embryonic development and/or microenvironment interactions could also determine the activation of specific driver oncogenes. Consistent with this notion of a diverse cell of origin of melanoma subtypes, expression of markers of stem cells, NC and neural lineages, and different epigenetic patterns have been reported to be associated with the plasticity of melanocytes, melanoma, and drug resistance (Caramel et al., 2013; Johannessen et al., 2013; Larribère et al., 2018; Larribere & Utikal, 2019; Yaar & Park, 2012).

3 | DIVERSITY OF MELANOMA SUBTYPES

The original classification of cutaneous melanoma proposed by Clark et al. (1986) was based on morphologic aspects of the early growth phase and the body site of primary melanocytic lesions and distinguished four main types: superficial spreading melanoma (SSM), lentigo malignant melanoma (LMM), nodular melanoma (NM), and acral-lentiginous melanoma (ALM). The current histopathological classification of melanocytic neoplasms is based on the evidence of chronic sun damage exposure and the anatomical location of the lesions. The 2018 World Health Organization classification of cutaneous, mucosal, and uveal melanoma identifies nine distinct

subtypes distinguishing them by their epidemiology, clinical and histologic morphology, and genomic characteristics (Elder et al., 2020). However, recent evidence suggests that certain tumors might not unequivocally fit into these broad categories based on the origin and distribution of melanocytes and their characteristics (Figure 2). It is not well understood whether these histologically distinct lesions all arise from melanocytes at the same stage of differentiation (even melanocytes residing in different anatomic substructures within the skin like follicular and interfollicular or scale and interscale regions as in mouse tail and may exhibit different susceptibilities to transformation) or represent malignancies of a different cell of origin. For example, cells from skin-resident melanocyte precursors, melanocyte stem cells, or terminally differentiated melanocytes (Grichnik et al., 2014; Kulesa et al., 2006; Yu et al., 2010). As described above, cutaneous melanomas are most frequently diagnosed on hair-bearing skin. Melanoma can also arise in non-hair bearing glabrous skin such as palms, soles of feet, or under fingers, finger/toe-nails, and mucosal surfaces such as respiratory gastrointestinal or genitourinary tracts, ocular sites such as choroid, the ciliary body, and the iris (Bastian, 2014; Shain & Bastian, 2016). Therefore, a better understanding of the relationship between the cellular origin of different melanoma subtypes could improve melanoma classification, diagnosis, and prognosis.

Different clinical and histopathological subsets of melanocytic lesions harbor highly unique patterns of oncogenic driver mutations (Bastian, 2014). For example, common acquired nevi have the highest frequency of BRAF mutation, while lesions associated with sun-induced damage and acral lesions exhibit mostly KIT mutations (Beadling et al., 2008; Curtin et al., 2006; Torres-Cabala et al., 2009), suggesting that biologically distinct types of melanocytic neoplasms arise due to different oncogenic mutations susceptibilities to transformation. However, it is unclear whether these distinct melanocytic lesions all arise from mature, differentiated melanocytes or originate from different precursors along the melanocytic lineage (Grichnik et al., 2014). A more detailed understanding of the biology of the developmentally diverse melanocytes and the clinico-pathological and molecular diversity of melanomas that arise at different anatomical locations may answer these questions.

4 | DIVERSITY AND PREVALENCE OF DRIVER MUTATIONS IN MELANOMA SUBTYPES

An impressive array of concerted international collaborations aimed at creating a comprehensive catalog of genes responsible for melanomagenesis and progression of various human cancers has resulted in whole exome and whole genome sequences of matched sets of tumor-normal samples. Analyzing more than 3080 tumor-normal genomic data from 27 cancer types, it was discovered that extraordinary variation exists in the frequency and spectrum of mutations across the genome among cancer types (Lawrence et al., 2013).

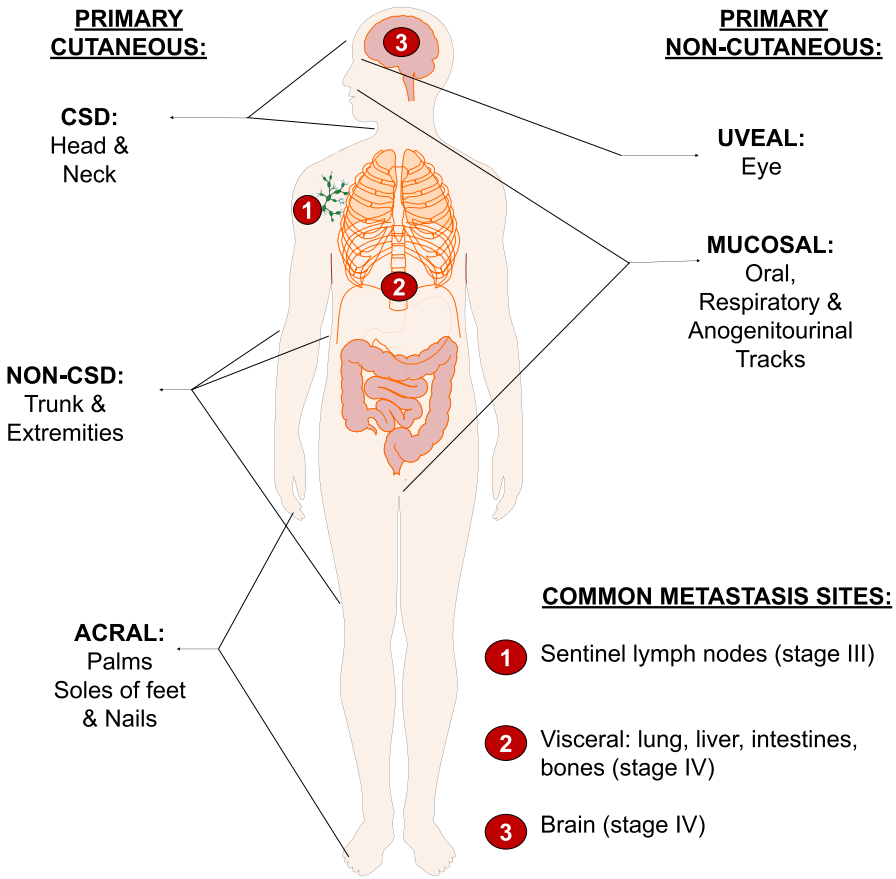

FIGURE 2 Multiple anatomic locations and types of melanomas. The most common type of melanoma (cutaneous, non-acral) arises from the skin, and it is classified as CSD and non-CSD. Other cutaneous melanomas include acral melanoma. Melanoma is also diagnosed in the eye (uveal) mucosa (mucous membrane). Each melanoma subtype exhibits specific patterns of oncogene driver mutations.

For example, as expected, pediatric cancers, as this group is least exposed yet to mutagens compared to older patients, showed the lowest frequencies of mutation rates (~0.1/Mb). In contrast, at the opposite end of the spectrum, melanoma showed the highest rate of mutations exceeding 100/Mb, followed by lung cancers with the second highest mutational rate. It is important to note that the mutation frequencies vary more than 1000-fold between the lowest (pediatric) and the highest (melanoma). The higher mutation rate frequency in lung cancers might be attributable to chronic exposure to the well-known carcinogens in tobacco smoke, whereas the highest rate of mutations in melanoma is attributed to exposure to ultraviolet radiation. However, the remarkable variation in melanoma mutations might be attributed to other biological factors, such as melanomas subtypes, including those that arise in areas not associated with ultraviolet skin exposure. A total of 121 different melanomas subtypes were included in the analyses. Based on these observations, it is appealing to hypothesize that these high rates and diversity of mutations could also reflect the multiplicity of cell lineages derived from N.C. and perhaps other developmental origins.

More recent studies included those led by The Cancer Genome Atlas (TCGA), including whole exomes of 33 cancer types and 1100 tumor samples (Hutter & Zenklusen, 2018) and the Pan-Cancer Analysis of Whole genomes (PCAWG) (a combined effort from TCGA and the International Cancer Genome Consortium ICGC) database contains 2658 whole genomes from 38 types of primary cancers and their matched normal tissues (Campbell et al., 2020). In melanoma, these studies confirmed previously known driver mutations representing over 40 mutated genes (Shaughnessy et al., 2018).

These studies have classified driver mutations in cutaneous melanoma into four major subclasses (percent melanomas): (1) BRAF (>60%) associated with non-CSD, (2) NRAS (28%), and (3) NF1 (14%), both associated with C.S.D., and (4) triple-wild type (15%) (lack BRAF, NRAS, or NF1 mutations), found more frequently in C.S.D. melanomas (Akbani et al., 2015; Cirenajwis et al., 2017; Hayward et al., 2017; Lawrence et al., 2013; Shaughnessy et al., 2018). However, triple wild-type (of the above three genes) melanomas harbor mutations more commonly in *GNA11*, *GNAQ*, *SF3B1*, or *KIT* genes, which are, interestingly, also frequent drivers of uveal melanoma (Akbani et al., 2015; Cirenajwis et al., 2017; Hayward et al., 2017) (Table 1).

The signaling cascades affected by these oncogenic mutations in different melanoma subtypes have been extensively investigated. The BRAF protein is a serine/threonine protein kinase that downstream activates the MAPK/ERK pathway. The V600E mutation represents 80%–90% of all BRAF mutations in melanoma (Cheng et al., 2018; Greaves et al., 2013). NRAS is a member of the family of G-regulatory proteins. NRAS mutations Q61K and Q61R cause constitutive activation leading to BRAF stimulation and consequently causing hyperactivation of the MAPK pathway (Ellerhorst et al., 2011; Polakis & McCormick, 1993). BRAF and NRAS mutations rarely co-occur in the same tumor (Raaijmakers et al., 2016). NF1 is a tumor suppressor gene that encodes the protein neurofibromin 1, and it negatively regulates RAS by converting activated RAS-GTP to inactive RAS-GDP. Inactivating mutations of NF1 result in constitutive activation of both MAPK and phosphoinositide 3-kinase (PI3K) pathways, thus causing a similar effect as BRAF and NRAS driving mutations (Ars et al., 2003; Brastianos et al., 2015; Davies, 2012; Reddy et al., 2017; Shaughnessy et al., 2018; Wee et al., 2009). Other genes, such as telomerase reverse transcriptase (TERT), essential for chromosomal stability, exhibit amplifications or activating mutations in 29% of melanomas (Vinagre et al., 2013). Mutations in cyclin-dependent kinases (CDKs) like CDK inhibitor 2A (CDKN2A) and cyclin-dependent kinase 4 (CDK4), both implicated in susceptibility to familial melanoma, represent about 2% of mutations (Harland et al., 2014; Hayward et al., 2017). Other common genetic alterations in cutaneous melanoma include the loss of the phosphatase and tensin homolog (PTEN), a tumor suppressor and a key regulator of the PI3K pathway, found in about 14% of melanomas (Birck et al., 2000; Shain et al., 2015). PTEN mutations frequently co-occur with BRAF mutations but not with NRAS (Reddy et al., 2017). Additionally, amplifications of the microphthalmia-associated transcription factor (MITF), the melanocyte master regulator required for melanocyte development, are found in 10% of melanomas (Garraway et al., 2005; Wellbrock & Arozarena, 2015). Tumor protein 53 (TP53) inactivating mutations are present in ~19% of melanomas (Hodis et al., 2012).

Mucosal and acral melanomas representing 1.3% and 2% of all melanomas, respectively, are genetically more similar to each other than they are to other melanoma subtypes. Activating mutations in *KIT* (39% of mucosal and 36% of acral melanomas) are the most frequent drivers in these melanomas, with the L576P present in 70% of *KIT* mutated tumors. KIT activating mutations cause dimerization of the receptor activating MAPK/ERK and PI3K/AKT/mTOR. Other genes that exhibit alterations in these melanomas include the platelet-derived growth factor receptor a polypeptide (*PDGFRA*) and cyclin D1 (*CCND1*) (Dominiak et al., 2016; Reddy et al., 2017; Shaughnessy et al., 2018; Vazquez Vde et al., 2016). Some cases also exhibit mutations in *GNAQ/GNA11* genes (Kim et al., 2014; Sheng et al., 2016).

KIT mutations are mutually exclusive with BRAF and NRAS mutations but cause similar effects on MAPK and PI3K pathways (Carvajal et al., 2011; Natali et al., 1992; Shaughnessy et al., 2018). Genetic alterations in the *PDGFRA* gene are predominantly amplifications and are present in 7% of acral and 4% of mucosal melanomas (Dai et al., 2013). Mutations in the *PDGFRA* gene are mutually exclusive with KIT mutations and are associated with non-CSD lesions (Dai et al., 2013; Merkel & Gerami, 2017). Mutations in the *CCND1* gene (Cyclin D1) are mostly amplifications and are present in ~45% of acral melanomas (Sauter et al., 2002; Smalley et al., 2008).

Melanomas may also arise from melanocytes in two anatomical locations of the eye: the uvea/uveal tract and the conjunctiva. Human eye consists of three distinct concentric layers: (1) the outer layer known as the sclera, which comprises the transparent cornea and the opaque white connective tissue; (2) the middle layer composed of the uvea/uveal tract, comprising the iris anteriorly (composed of an anterior layer called the stroma containing melanocytes and a posterior pigmented epithelial layer), an intermediate structure

TABLE 1 Frequency of major melanoma mutations in different melanoma subtypes.

Subtype	Inc.	Mutation	Freq.	Common variants	Manifest.	Exclusive mutations
Cutaneous (non-acral)	91.2	BRAF	60	V600E (80–90%)	Non-CSD	NRAS/NF1
		NRAS	28	Q61K, Q61R	CSD	BRAF/PTEN
		NF1	14	Del/inactivation	CSD	BRAF
		Triple WT	15	GNAQ/GNA11/KIT	CSD	BRAF/NRAS/NF1
		KIT	28	L576P (70%)	CSD	BRAF/NRAS
		PTEN	14	Del/inactivation	Unk/ND	Unk/ND
		MITF	10	Amplif/Activation	Unk/ND	Unk/ND
		CDKN2A	2	Del/inactivation	Familial	Unk/ND
		TERT	29 (all)	Amplif/Activation	Unk/ND	Unk/ND
		TP53	20 (all)	Del/inactivation	Unk/ND	Unk/ND
Mucosal	1.3	KIT	39	L576P, K642E	Non-CSD	BRAF/NRAS/PDGFRA
		PDGFRA	4	Amplif/Activation	Non-CSD	KIT
		GNAQ	4.6	Q209L (92%)	Non-CSD	GNA11
		GNA11	4.9	Q209L (92%)	Non-CSD	GNAQ
		SF3B1	20–35	R625H/S/C	Non-CSD	Unk/ND
Acral	2.3	CCND1	45	Amplification	Non-CSD	Unk/ND
		KIT	36	L576P, activation	Non-CSD	BRAF/NRAS
		PDGFRA	7	Amplif/activation	Non-CSD	KIT
Uveal	5.2	GNAQ	33	Q209L (90%)	CSD	BRAF/NRAS/GNA11
		GNA11	39	Q209L (90%)	CSD	BRAF/NRAS/GNAQ
		BAP1	45	Del/inactivation	CSD	EIF1AX/SF3B1
		EIF1AX	14–20	A11T, N4S	CSD	SF3B1/BAP1
		SF3B1	22	R625C/H/L	CSD	EIF1AX/BAP1

Note: Except for TERT and TP53, representing all melanomas; Manifest, manifestation; Exclusive mutations, Mutually exclusive mutations with mutations/genes in column 3.

Abbreviations: Amplif, amplification; CSD, chronic sun damage; Del, deletion; Freq., Frequency (%); Inc., incidence (%); ND, not determined; TERT, telomerase reverse transcriptase; Unk, unknown.

called the ciliary body (surrounding the iris and located behind the sclera), and the choroid located posteriorly surrounding the middle layer of the eyeball, which supports and nourishes the retina, and (3) the inner layer composed of the retina, which consists of the retinal pigment epithelium (RPE) and the neural retina (Hu, 2005; Hu et al., 2002; Istrate et al., 2020). The RPE is a monolayer of pigment cells that lies between the uveal tract and the neural retina, and although they are in close proximity (uveal melanocytes in the middle and RPE in the inner layer), they have different developmental origins. Melanocytes are derived from the neural crest, while retinal pigment cells share their embryonic origin with the neural retina, arising from the anterior neural plate (Cechmanek & McFarlane, 2017; Esteve & Bovalenta, 2006; Martinez-Morales et al., 2004; Zuber et al., 2003).

In uveal melanomas, the most commonly mutated genes are *GNAQ*, *GNA11*, *BAP1*, *EIF1AX*, and *SF3B1* (Shaughnessy et al., 2018). Mutations in *GNAQ* and *GNA11* genes are present in 33% and 39% of uveal melanomas, respectively, and most commonly in position Q209L (Moore et al., 2018; Reddy et al., 2017; Shoushtari & Carvajal, 2014). Mutations in GNAQ and GNA11 converge on the MAPK/ERK pathway, thus upregulating the M.A.P. kinase pathway, similarly to BRAF and NRAS mutations (Van Raamsdonk et al., 2009, 2010). Mutations in GNAQ/GNA11 are also associated with chronic solar damage (CSD) (de Lange et al., 2015). Mutations in *GNAQ* and *GNA11* genes are mutually exclusive and rarely found in the same tumor (Shaughnessy et al., 2018). Inactivating mutations or loss of the tumor suppressor BRCA1-associated protein 1 (*BAP1*) occur in 45% of uveal melanomas due to the monosomy in chromosome 3 (Ransohoff et al., 2016; Scheuermann et al., 2010; Shaughnessy et al., 2018). Uveal melanomas also exhibit mutations in eukaryotic translation initiation factor 1A X-linked (*EIF1AX*) and six splicing factor 3b subunit 1 (*SF3B1*) at 14% and 22% frequency, respectively (Doherty et al., 2018; Field et al., 2018; Hintzsche et al., 2017; Kong et al., 2014; Quek et al., 2019; Rose et al., 2018; Shaughnessy et al., 2018; Yavuzyigitoglu et al., 2016).

There are also melanocytes located in the *conjunctiva*, the thin mucous membrane that lines the inside of the eyelids and covers the surface of the sclera (the white of the *eye*), and they may give rise to conjunctival melanoma. Conjunctival melanocytes are derived from

the neural crest as uveal and cutaneous melanocytes, although they migrate in different waves (Hu et al., 2007; Iwamoto et al., 2002). Although more population studies are needed, current evidence suggests that the most common driver mutations in conjunctival melanoma are in the BRAF (~30%–70%), NF1 (~20%–30%), NRAS (~17%), and c-KIT (~8%) genes that lead to constitutive activation of the MAPK pathway as well as mutations that result in activation of the PI3K/AKT/mTOR signaling pathway (Gkiala & Palioura, 2020).

Melanoma is the most highly mutated malignancy, and given the remarkable diversity and highly specific patterns of oncogene mutations among subtypes arising from diverse anatomical locations, we argue that this phenomenon may have a relationship with specific competence requirements like transcriptional, epigenetic programs, and tissue microenvironment of different cells of origin for malignant transformation.

5 | SIGNIFICANCE OF DIVERSE PATTERNS OF MELANOMA MUTATIONS

Despite the detailed knowledge gained on the landscape of genetic alterations in melanoma, a relatively small number of clinically relevant and effective drugs are available to treat melanoma. The etiology and interactions of mutations that control melanoma onset and their significance to cell of origin, progression, and therapeutic vulnerabilities are not yet fully understood (Akbani et al., 2015; Shaughnessy et al., 2018). Understanding the complexity of mutations in melanoma continues to be a challenge with implications for deciphering the cell or origin and improving patient care (Davis et al., 2018). Interestingly, melanoma subtypes not only exhibit highly unique driver mutations, but these mutations are often exclusive among them, that is, do not co-exist in the same tumor (Table 1). Moreover, some oncogenic mutations that produce a melanoma subtype in a specific tissue do not cause melanoma in other tissue (Huang et al., 2015; Weiss et al., 2022). Melanoma clinical and molecular subtypes—cutaneous-non-CSD, cutaneous-CSD, mucosal/acral, and uveal melanoma—exhibit distinct biological properties, including metastatic properties and disease progression, and have different prognoses.

The skin is a complex organ harboring a rich array of different cell types and distinct populations of stem cells. For example, populations of skin stem cells that behave spatio-temporally in different manners under healthy and pathological conditions suggest stem cell population heterogeneity with different properties. These skin stem cells produce cell lineages that depend on signals from their local environment but can give rise to all epidermal lineages in response to appropriate stimuli (Li et al., 2004; Mannik et al., 2010). It is known, for instance, that certain human and rodent skin stem cells have the ability to differentiate towards fat, bone, muscle, cartilage, hematopoietic cells, and neural lineages, including NC cells (Waters et al., 2007). Additionally, developmental lineage plasticity and tissue microenvironment appear to influence the susceptibility to specific oncogenic mutations and malignant transformation. Consistent with this notion, it is interesting to note that in a mouse genetic model, activation of oncogenic Gnaq (frequently found in uveal melanomas) was reported to induce proliferation of melanocytes in the eye and meninges but caused an opposite effect in skin melanocytes, which exhibited decreased proliferation and survival with 39% fewer cells than normal (Huang et al., 2015).

Recent studies also showed that the ability to induce malignancy by oncogenic depends on cellular context (Fowler et al., 2021; Weiss et al., 2022). In these studies, DNA sequencing demonstrated that BRAF mutations are enriched in cutaneous melanomas while CRKL amplifications are enriched in acral melanomas. Transgenic zebrafish models supported these findings. It was shown that CRKL-driven tumors are predominantly formed in the fins, the evolutionary anatomic equivalent of limbs and acral location. These data indicate that melanocytes in these acral locations are uniquely susceptible to CRKL mutations. Moreover, RNA-seq data revealed that a transcriptional program controlled by HOXB13 positional identity genes synergized with CRKL to amplify cancer-related signaling pathways (IGF1R–PI3K), making these melanocytes susceptible to malignant transformation to only certain oncogenic mutations (Weiss et al., 2022). The authors also discussed that these results could alternatively be explained by the lineage-specific chromatin state of acral melanocytes that impede their oncogenic competence from responding to activation of MAPK signaling (Baggiolini et al., 2021). Differences in the skin microenvironment were also considered to contribute to the oncogenic mutation specificity in melanomagenesis. Consistent with this view, recent studies also showed a role for keratinocytes in promoting the escape of oncogene-induced senescence in melanocytes (Sadangi et al., 2022). These observations not only suggest differential predisposition of melanocytes to malignant transformation by specific oncogenic mutations but also that differences in the tissue microenvironment, cell of origin, and plasticity could influence melanoma tumor initiation.

Furthermore, published reports suggest that the predisposition of different melanomas to metastasize could be related to the oncogenic driver and/or cell of origin. In particular, it is known that cutaneous BRAF-mutant tumors have a higher tendency to metastasize to the brain than BRAF WT tumors (Ribas & Flaherty, 2011). Conversely, uveal melanoma exhibiting GNAQ or GNA11 mutations predominantly metastasizes through the hematogenous spread, and the most common sites of involvement are the liver, lung, and bone (Harbour, 2012; Reddy et al., 2017). These differences between cutaneous and uveal melanomas in their mode and sites of metastasis and the oncogenic driver mutations they harbor are intriguing because, based on their anatomical proximity to the site of the primary tumor, the brain would be expected to be a more frequent or natural site for metastasis of uveal melanomas than for the spread of BRAF mutant cutaneous melanomas. Similarly, inactivating mutations or deletion of the tumor suppressor *PTEN* co-existing with *BRAF* mutation have been reported in humans and mouse models to correlate with a shorter time to brain metastasis (Bucheit et al., 2014; Krepler et al., 2017). Furthermore, activating mutations in PI3K, which is regulated by PTEN, have also been implicated in brain metastasis,

causing a similar effect as PTEN inactivating mutations (Brastianos et al., 2015). These observations suggested not only that the patterns of metastasis may be related to melanoma driver mutation but also to the melanoma subtype, which in turn is related to the cell of origin. Consistent with this notion, mouse subcutaneous xenograft models of metastasis showed that after inoculation of human melanoma cells, BRAF mutant melanoma cells exhibit, compared to triple-negative melanoma, a significantly higher predisposition to invade the mouse tissue. Several studies have suggested that the differences in the predisposition to metastasize to specific tissues and organs might not just be an indicator of the specificity of driver oncogene but also be an intrinsic biological property of different lineages of melanocytes from which melanoma arises. For example, histological observations showed that melanoma precursor lesions, such as melanocytic nevi, are already capable of dissemination and exhibit some limited colonization capacity. In particular, small deposits of melanocytes are routinely found as a "benign metastasis" in lymph nodes and are known as nodal nevi, which are removed from patients without any history of melanoma (Bautista et al., 1994; Carson et al., 1996). These nodal nevi typically only exhibit BRAFV600E mutation and are especially common in the sentinel lymph node of patients with melanoma that originate from or are associated with nevi (Holt et al., 2004), suggesting that nevus cells could disseminate even before the primary tumor has formed (Taube et al., 2009). These observations also suggest that the differential capacity to disseminate might be related to the cell of origin of melanoma subtypes and not entirely an acquired feature during tumor progression.

6 | MELANOMA CELL OF ORIGIN: HUMAN STUDIES

One limitation in understanding melanocyte development and melanoma cell of origin in humans is that all studies are conducted in animal models (Le Douarin and Kalcheim, 1999; Le Douarin et al., 2004). Although there is very little information on cell of origin derived directly from humans, the concept that human melanomas may have distinct cells of origin was proposed by Houghton et al. (1982) using a panel of surface antigen markers in melanoma cells and melanocytes in different stages of development. In this study, the authors characterized in a comparative analysis the expression of cell surface antigens (termed M1–M10) recognized by a panel of antibodies on metastatic melanoma cells and fetal, neonatal and adult melanocytes. They grouped the antigens into 4 categories: (1) Antigens not expressed on newborn and adult melanocytes and expressed by subsets of melanoma: HLA-DR and melanoma antigens M-I, M-2, and M-3. (2) Antigens expressed on newborn melanocytes but not adult melanocytes: early melanocyte antigens M-4 to M-8. (3) Antigens expressed on adult melanocytes and only weakly (or not at all) on newborn melanocytes: mature melanocyte antigens M-9 and M-10. (4) And antigens detected equally on both newborn and adult melanocytes: melanocytic lineage markers M-11 to M-34. Based on this diversity of expression patterns, it was proposed that antigens present only on melanomas but not on early, intermediate, or adult melanocytes could represent markers of melanocytic precursors, such as presumptive melanoblasts, and a subset of melanomas that express these antigens may arise from such melanocyte precursors or immature melanocytes. However, the expression of antigens, such as HLA-DR, that do not correspond to the neural crest and melanocyte differentiation markers may result from aberrant gene expression during malignant transformation and/or tumor progression. For nearly three decades, not much progress was made in this area until the recent availability of genetic mouse models and elegant methods for in vivo lineage tracing of melanocytes.

7 | LINEAGE TRACING OF MELANOMA CELL OF ORIGIN

The first indication that the diversity in mechanisms of melanoma tumor initiation could be related to differences in the cell of origin came from genetic models of mouse melanoma. These models were first developed using a Tyr-SV40E oncogene expression in ocular and cutaneous mouse melanomas (Bradl et al., 1991; Mintz & Silvers, 1993). More recently, employing $Braf^{V600E}/Pten^{-/-}$ mouse melanoma model, it was demonstrated that melanocytic tumors develop from melanoma-competent melanocyte stem cells (MCSC) upon stimulation by UVB, which induces MCSC activation and translocation from the hair follicle to the epidermis by an inflammation-dependent process (Moon et al., 2017). These observations suggested that hair follicle MCSC, but not differentiated adult epidermal melanocyte, are the cell of origin of cutaneous melanoma that develops in mouse skin upon UV exposure.

Conversely, by employing mouse genetic models with elegant fluorescent reporters to study cell of origin of tumors in $Braf^{V600E}/Pten^{-/-}$ mice, Kohler et al. (2017) showed that MCSCs in the bulge were not susceptible to oncogenic transformation by $Braf^{V600E}$ (Kohler et al., 2017). A possible explanation for this observation is that the Cre recombinase, which is driven by a *Tyr* promoter, is not activated in MCSC that do not express Tyr. Consistent with this notion, a population of differentiated pigmented melanocytic cells in the hair follicle matrix were found to be the targets of malignant transformation when activated by depilation producing multiclonal hyperproliferative lesions. Moreover, these authors studied tail melanocyte cells, which are distributed in two distinct locations—the scale areas and the interscale regions. Importantly, the scale (parakeratotic) and the interscale (orthokeratotic) represent interfollicular regions of the mouse tail epidermis compartments with two cell lineages maintained by distinct stem cell populations (Gomez et al., 2013). The scale melanocytes are pigmented and show dendritic morphology, whereas interscale melanocytes are composed of non-pigmented and pigmented cells. Interestingly, although *Braf* oncogene activation and *Pten* loss could be induced in both populations of melanocytes, hyperproliferative lesions developed only from scale melanocytes and exhibited radial growth. These data showed that melanocytes in very close proximity and existing in

similar tissue microenvironments but originating from different stem cell lineages exhibit differences in susceptibility for melanoma initiation even under the same oncogene driver. These studies highlight the need for detailed analyses of gene expression profiles of melanocytes at diverse anatomical locations and changes in gene expression in response to intrinsic and extrinsic stimuli in the context of their lineage and microenvironment (Soengas & Patton, 2017).

8 | REPROGRAMMING CANCER CELLS INTO IPSC: A MODEL FOR ASSESSING MELANOMA CELL OF ORIGIN, PLASTICITY, AND DRUG RESISTANCE

The concept of cell of origin in cancer refers to normal cells that first acquire the mutations required for malignant transformation and tumor initiation (Visvader, 2011). In order to identify the cells of origin of cancers, it is critical to understand the normal lineage progression that occurs during the development and adulthood and the specific stage at which cells are prone/susceptible to malignant transformation. For instance, previous reports on the cell of origin of chronic myeloid leukemia (CML) indicated that BCR/ABL translocation occurred in hematopoietic stem cells; however, if this translocation occurred in committed progenitor cells, it gave rise to acute lymphoid leukemia (ALL) instead (Li et al., 1999; Tough et al., 1963). Similarly, different types of brain tumors are formed when a specific mutation occurs in neural stem cells, neural progenitor cells, oligodendrocyte progenitors, or neuroepithelial stem cells (Alcantara-Llaguno et al., 2009, 2015; Huang et al., 2019). In several other cases (mentioned below), such as colon cancer, mutations in the tumor suppressor gene of Apc result in tumor formation only in the intestine but not in other cell types (Hashimoto et al., 2017).

In the past decade, the ability to generate patient-derived induced pluripotent cells (iPSC), which exhibit self-renewal, unlimited proliferation, and the capacity to differentiate into nearly all cell types and to generate functional organoids in vitro and in vivo, has revolutionized disease modeling to understand mechanisms of disease pathogenesis and rapid drug screening (Liu et al., 2018; Siller et al., 2013). iPSCs have been used to model cancer cell heterogeneity, plasticity, tumor progression, drug resistance, and for drug screening (Campbell et al., 2020; Czerwińska et al., 2018; Kim, 2015; Sharkis et al., 2012). Also, iPSC reprogramming as a strategy to study cancer cell of origin has been successfully employed in several cancers with different strategies (Friedmann-Morvinski & Verma, 2014; Czerwińska et al., 2018; Kim & Schaniel, 2018; Marin-Navarro et al., 2018). One strategy to study cell of origin in cancer with iPSC is by direct reprogramming of tumor cells. Reports indicate that the cell of origin affects the efficiency of reprogramming and pluripotency of iPSC as a result of retention of some epigenetic marks generating iPSCs that are more amenable to express markers related to the cell of origin and have more tendency to differentiate toward the original lineage (Bar-Nur et al., 2011; Chlebanowska et al., 2020; Hargus et al., 2014; Hu et al., 2016; Kim et al., 2010; Nukaya et al., 2015; Polo et al., 2010; Ruiz et al., 2012; Wang et al., 2018). In the context of melanoma, this strategy could elucidate potential differences in cell of origin, tumor plasticity, and heterogeneity, as suggested by some studies (Bernhardt et al., 2017; Castro-Pérez et al., 2019).

Another strategy successfully applied to investigate cell of origin in cancer with iPSC is to study the effect of oncogenic mutations in different cell types/lineages as candidates of cell of origin. It is known that the spectrum of oncogenic driver mutations differs among cancers in different organs, suggesting the requirement for specific competent cells of origin for their malignant transformation (Friedmann-Morvinski & Verma, 2014; Hashimoto et al., 2017), and we have revised here this possibility among different melanoma subtypes (see above). This effect of an oncogenic mutation in different cell types has been investigated using iPSCs in several cancers, including iPSC-derived from non-small cell lung cancer (NSCLC) and sarcoma cells, that although retaining the oncogenic mutations; they also required differentiation to the cellular context of the cell of origin (e.g., transcriptional, epigenetic programs, and tissue microenvironment) for acquisition and progression of malignant behavior (Mahalingam et al., 2012; Zhang et al., 2013). Another work generated iPSCs from patients with Acute myeloid leukemia (AML) carrying MLL-gene rearrangements; however, only AML-iPS cells that were differentiated into hematopoietic cells gave rise to leukemia in mice, whereas differentiation into other cellular lineages did not (Chao et al., 2017). Furthermore, other reports demonstrated that AML-derived iPSC could be used to map in detail the recapitulation of cell stage-specific progression by introducing or correcting mutations (Kotini et al., 2017). Similarly, glioblastoma- and pancreatic ductal adenocarcinoma (PDAC)-derived iPSCs retain malignancy after differentiation into the context of their cell of origin (Kim et al., 2013; Stricker et al., 2013). In another study, iPSC generated to model cell of origin in colon cancer showed that disruption of tumor suppressor Apc in reprogrammed cells resulted in neoplastic growth that was exclusive to the intestine but not to other cell types. Similarly, iPSC-derived neural precursor cells (NPCs) led to malignant transformation when introduced relevant mutations limited to NPCs and did not have such an effect on any other neural or non-neural cell type (Funato et al., 2014).

Cancer cell-derived c-iPSC and re-differentiation of c-iPSC to normal counterparts have also been employed to study tumorigenicity, plasticity, and mechanisms of drug resistance (Gong et al., 2019). For instance, iPSC derived from sarcoma cells showed terminal differentiation and loss of tumorigenicity (Zhang et al., 2013); pancreatic cancer cell-derived iPSCs showed stable differentiation and loss of tumorigenicity (Miyoshi et al., 2010). B-cell lymphoma and leukemia-derived iPSC exhibited loss of tumorigenicity (Rapino et al., 2013), hepatocellular carcinoma-derived iPSC showed recovery of normal functions and the capacity for liver regeneration (Cheng et al., 2019), and breast cancer-derived iPSC showed adipocyte functions and loss of metastatic potential (Ishay-Ronen et al., 2019). Furthermore, iPSC-derived from colorectal carcinoma cells showed differentiation to benign phenotype and reduction of tumorigenic potential (Zhou et al., 2017); melanoma iPSC exhibited tumorigenicity loss and

increased drug resistance (Bernhardt et al., 2017; Utikal et al., 2009). However, reprogramming cancer cells to iPSC have some limitations, such as intrinsic heterogeneity of the parental tumor cell population, low reprogramming efficiency, and restricted differentiation of iPSC. Barriers to reprogramming present in normal somatic cells can often be more pronounced in cancer cells (Liu et al., 2018). For example, oncogene activation, oncogene-induced cellular senescence, cancer cell-selective signaling pathways such as TGF-β signaling, repression of transcription factors such as c-Jun, and certain microRNAs can act as roadblocks to reprogramming. Moreover, epigenetic factors such as DNA methylation and histone modifications can also impede the generation of iPSCs (Banito et al., 2009; Haridhasapavalan et al., 2020; Liu et al., 2015; Mosteiro et al., 2016).

Leukemia-derived iPSCs strategy was employed to study cancer plasticity in relation to the cell of origin and differential response to imatinib in chronic myeloid leukemia (CML) (Chao et al., 2017; Suknuntha et al., 2015). Reprogramming of CML cells to iPSC followed by differentiation into hematopoietic lineages generated primitive hematopoietic cells with leukemia stem cells features similar to cancer stem cells and exhibited resistance to tyrosine kinase inhibitor imatinib, which is used to treat CML. These studies led to the discovery of the survival factor olfactomedin 4 (OLFM4) as a new and potential therapeutic target to overcome resistance to imatinib in CML (Suknuntha et al., 2015). Overall, these studies showed that it is possible to successfully reprogram cancer cells into iPSC, differentiate them into lineages of the original cell, and generate non-tumorigenic normal cells.

9 | REPROGRAMMING MELANOMA CELLS INTO IPSC: A MODEL FOR UNDERSTANDING MELANOMA CELL OF ORIGIN, PLASTICITY, AND TRANS-DIFFERENTIATION

As mentioned above, several strategies can be used to study cell of origin and plasticity in cancer and melanoma. As some epigenetic marks related to the cell of origin might be retained by melanoma-iPSC, making them more prompt to express and differentiate into lineages of the cell of origin. Therefore, direct reprogramming of melanoma cells could elucidate potential differences in cell of origin, tumor plasticity, and heterogeneity, as suggested by some studies (Bernhardt et al., 2017; Castro-Pérez et al., 2019).

Another reprogramming strategy used in cancer to study cell of origin is by evaluating the role of driver mutations in reprogramming, differentiation/plasticity, and tumorigenesis. For instance, studies by Castro-Pérez et al. (2019) demonstrated that BRAFV600E expression blocks iPSC reprogramming in melanocytes, while the addition of BRAF inhibitor vemurafenib facilitates the reprogramming of BRAFV600E mutant melanoma cell lines. These findings open the possibility of investigating the role of specific mutations in reprogramming, differentiation, and cell of origin in melanoma, as reported in other cancer-derived iPSC studies (Hashimoto et al., 2017; Chao et al., 2017). For example, mutations of specific melanoma subtypes could be induced or corrected in cells with CRISPR/Cas9 before or after reprogramming and evaluate their role as cell of origin after differentiation into different iPSC-differentiated cells such as melanoblasts, melanocytes, keratinocytes, fibroblasts, or other tissue/lineages. Additionally, the role of these melanoma mutant-iPSCs derived cells could be modeled for cell of origin in melanomagenesis in vivo in specific tissues with xenograft mice and in vitro with co-culture models using, for instance, microfluidics platforms and 3D organoids (Bourland et al., 2018; Hill et al., 2015; Nissan et al., 2011; Sadangi et al., 2022). These strategies have the advantage of testing the oncogenic competence of normal cells that might not be easily obtained from live patients (like eye melanocytes, NCCs or melanoblasts during developmental stages) and harboring specific mutations rather than tumor cells containing multiple unknown mutations or even hESCs which have more limited accessibility and different mutations.

As mentioned above, plasticity of cancers has also been evaluated using the strategy of reprogramming. The relevance of phenotypic plasticity and trans-differentiation have recently been highlighted as new functionally distinct hallmarks of cancer pathogenesis (Hanahan, 2022). These are capabilities normally restricted by lineage-specific developmental programs. However, they may be unlocked in cancer by, for example, dedifferentiation back to progenitor/stem-like cell states or changing their morphology to become recognizable as elements of another tissue (Yuan et al., 2019). In order to address plasticity, trans-differentiation properties, and drug resistance in melanoma (Castro-Pérez et al., 2019) employed the iPSC reprogramming strategy. In these studies, skin-derived fibroblast and melanocytes and primary and metastatic human patient-derived melanoma cell lines were reprogrammed into iPSC, and both fibroblast-derived iPSC and melanocyte-derived iPSC were found to readily re-differentiate to pigment-producing melanocytes. However, only a subset of melanoma cell lines was able to generate iPSC, albeit less efficiently than skin fibroblasts and melanocytes. Primary melanoma cells were more efficiently reprogrammed into iPSC than metastatic melanoma cells. In a set of genetically matched primary and metastatic melanoma cells isolated from the same patient, primary melanoma cells efficiently generated iPSC, whereas metastatic cells did not. Melanoma-derived iPSC exhibited pluripotency to differentiate into the primordial germ cells of ectoderm, mesoderm, and endoderm in vitro. Therefore, we asked whether melanoma-iPSC could differentiate back into melanocytes since several studies protocols demonstrating that both ESC and iPSC can be differentiated into melanocytes have been reported (Fang et al., 2006; Ohta et al., 2011; Yang et al., 2011). We found that upon directed differentiation to melanocytes (in vitro), melanoma-iPSC showed neural-like plastic differentiation (Figure 3). These melanoma-iPSC-differentiated cells co-expressed neuronal and melanocyte markers, including MITF, MAP2, neuronal tubulin (TUBB3), and even glial marker GFAP. The expression of these markers varied among different melanoma lines. For example, some melanoma-iPSC-differentiated cells exhibited deficient expression

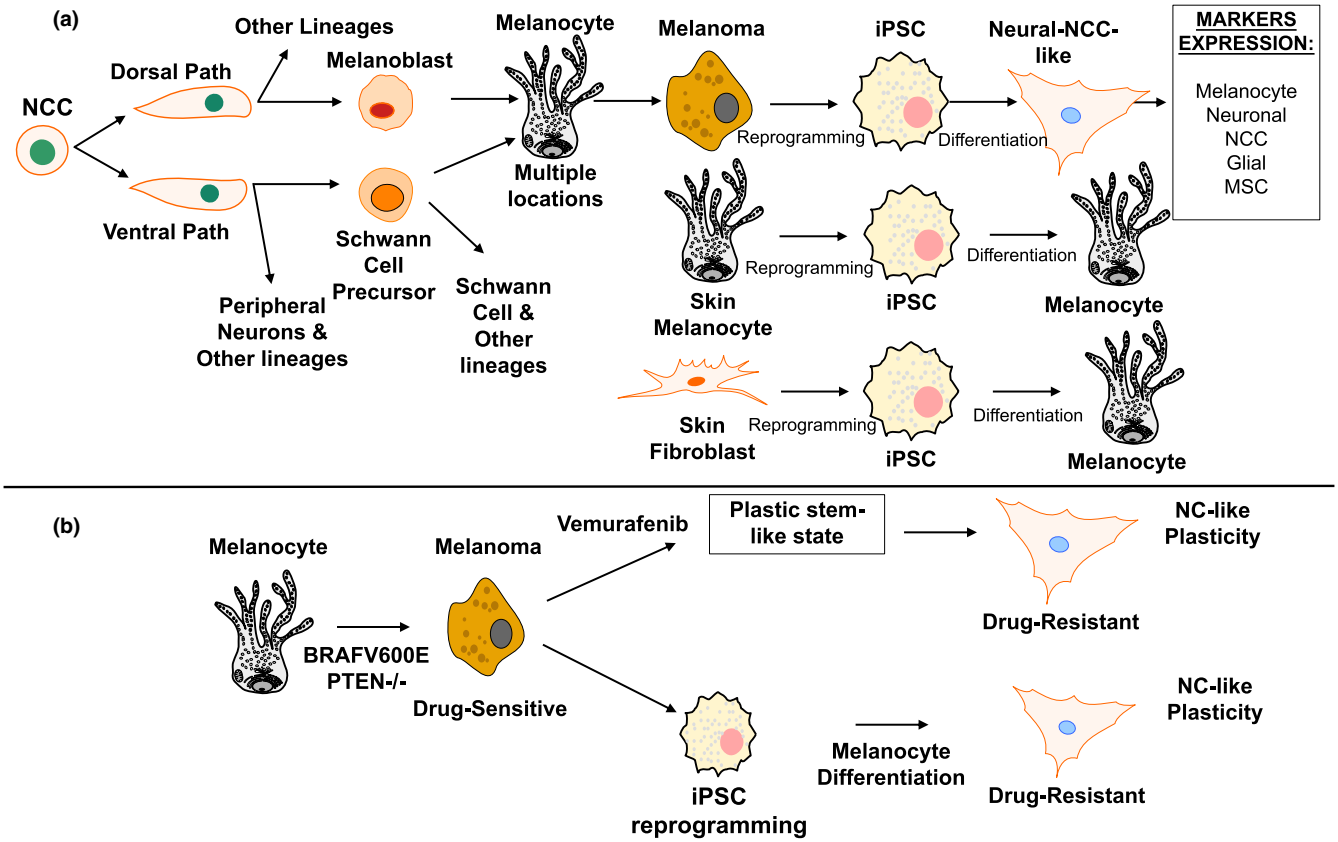

FIGURE 3 Melanoma-derived iPSC to model cell of origin and drug resistance. (a) Melanocytes populate multiple locations as their diverse developmental origins from NCC Melanomas emerge from multiple locations of melanocytes. Reprogramming skin-derived fibroblasts and melanocytes into iPSC and differentiation into melanocytes results in efficient iPSC reprogramming and melanocyte differentiation. However, melanoma cells reprogrammed to iPSC and directed to differentiate back to melanocytes do not exhibit melanocyte differentiation but neural/NCC-like mixed phenotype. Melanoma iPSC-differentiated cells show increased expression of neural/NCC and melanoma stem cell-related markers and decreased expression of melanocytic lineage markers. (b) During malignant transformation, melanocytes acquire BRAFV600E oncogenic mutation and other mutations such as PTEN loss. These cells are initially sensitive to vemurafenib, but after treatment with the inhibitor, the drug induces changes in expression related to cellular plastic changes generating a stem cell-like transitional state. After this transition, many cells survive, have made plastic changes with stem-like characteristics, and exhibit acquired resistance to the drug (top). Reprogramming of BRAFV600E melanoma cells induces the formation of iPSC colonies. iPSC generated do not differentiate into melanocytes but exhibit neural/NCC-like plasticity and show acquired resistance (bottom).

of melanocyte lineage markers, whereas, in others, melanocyte marker expression was barely detectable, but the expression of markers of terminal neuronal differentiation MAP2 and TUBB3 was prominent. It is known that melanocytes derived from human iPSCs transplanted into athymic mouse skin can produce pigmented melanocytic aggregates and express melanocyte markers (Kawakami et al., 2018), suggesting skin microenvironment influences the elaboration of the melanocyte differentiation program. When xenotransplanted into mouse skin, melanoma iPSC-differentiated cells, which show limited melanocyte differentiation in vitro, formed amelanotic tumors that exhibit weak expression of MITF but not key melanocyte differentiation markers TYR, TYRP1, TYRP2, and MART1. In contrast, these tumors exhibit strong expression of neural markers PAX3, TUBB3, MAP2, and GFAP and melanoma stem cell markers SOX9, CD271, and ALDH1, suggesting plasticity to differentiate along multiple lineages. Culture of melanoma-iPSC in media that promote neuronal differentiation facilitated elaboration of neural markers of terminal neural differentiation as evident by the expression of MAP2, TUBB3, SYN1 (Synapsin 1), and GFAP. Melanoma-iPSC generated from different melanoma cell lines exhibited varying patterns of differentiation in melanocyte or neuronal differentiation medium in vitro, in the mouse xenograft skin microenvironment (in vivo). These varying patterns might be a possible indication of different cells of origin among the cell lines analyzed. We argue that cellular plasticity and capacity to trans-differentiate may provide a window into the potential differences in the cell of origin of melanoma subtypes.

10 | TUMOR CELL PLASTICITY AND DRUG RESISTANCE

The cellular plasticity of cancer cells is often associated with phenotypic heterogeneity and drug resistance. However, the specific

mechanisms associated with phenotypic plasticity that promote the survival and proliferation of drug-resistant melanoma cells have not yet been elucidated (Granados et al., 2020). In a certain respect, this plasticity to switch from the proliferative potential of metastatic and drug-resistant melanoma cells is similar to the switch, in reverse, that is, differentiation of embryonic stem cells (ESC)/tissue stem cells (TSC)/induced pluripotent stem cells (iPSC) with self-renewal and pluripotency. Factors and mechanisms that control pluripotency and unlimited self-renewal of ESC/iPSC have been extensively investigated. For example, it was reported that signaling networks involving crosstalk between several pathways culminate in a finely balanced molecular switch that determines the fate of pluripotent cells (Dalton, 2013). In melanoma cells, genes that promote undifferentiated and cancer stem cell-like phenotypes have been reported to be associated with targeted therapy resistance in melanoma-derived induced pluripotent cancer cells (Hüser et al., 2018). Using a strategy of reprogramming human melanoma cells to iPSC showed that culturing melanoma-derived iPSCs in melanocyte differentiation medium produces neural-like lineages that become intrinsically resistant to MAPK inhibitors (Castro-Pérez et al., 2019), suggesting crosstalk between signal pathways involved in trans-differentiation and mechanisms that render melanoma cells resistant to these drugs. These observations suggest that targeting such pathways can block or reverse the emergence of new cell lineage states and make melanoma cells retain sensitivity to these cancer treatments. Trans-differentiation to neuroendocrine phenotype has been reported to act as a treatment resistance mechanism in prostate and lung cancers (Davies et al., 2021; Quintanal-Villalonga et al., 2020; Sequist et al., 2011; Zou et al., 2017). It is not known whether melanoma trans-differentiation also acts as a treatment resistance mechanism.

11 | CONCLUSIONS/PERSPECTIVES

Induced pluripotent stem cell technology has revolutionized regenerative medicine and disease modeling by reprogramming normal and patient-derived cells to iPSC. In particular, the cancer cell-derived iPSC strategy has recently been employed to understand the mechanism of plasticity, drug resistance, and drug screening. Melanocytic neoplasms present a diverse clinical, pathological, and molecular subtype of malignant melanomas that exhibit different patterns of oncogene mutations in a highly specific manner, raising the question of whether these subtypes have a different cell of origin. Reprogramming melanoma-derived iPSC and characterizing their plasticity by directed differentiation is an attractive strategy for understanding the cell of origin in melanoma. A better understanding of the cell of origin could help refine the diagnosis, prognosis, and mechanisms of drug resistance in melanoma. Melanoma-derived iPSC could lead to the identification of genes, proteins, and cellular pathways that control senescence, pluripotency, and oncogenic potential and allow the development and design of novel therapeutics that can overcome drug resistance in malignant melanoma, the primary clinical challenge in this disease.

AUTHOR CONTRIBUTIONS

EC-P and VS conceived, designed, and organized the manuscript. EC-P and VS wrote and edited the manuscript. EC-P, SS, MS, and CM-S wrote different sections of the manuscript. EC-P and CM-S made the figures with input from VS.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

not applicable, because it is a review manuscript with no data

ORCID

Edgardo Castro-Pérez https://orcid.org/0000-0003-4884-9479
Mithalesh Singh https://orcid.org/0000-0002-1812-9860
Shreyans Sadangi https://orcid.org/0000-0002-0160-8806
Carmen Mela-Sánchez https://orcid.org/0000-0001-7178-8702
Vijayasaradhi Setaluri https://orcid.org/0000-0001-6468-7663

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