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REVIEW OPEN ACCESS

The Potential of Phytochemicals to Overcome Multidrug Resistance in Metastatic Melanoma

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Received: 25 August 2025 | **Revised:** 24 October 2025 | **Accepted:** 28 October 2025

Keywords: melanoma stem cells | metastatic melanoma | multidrug resistance | phytochemicals

ABSTRACT

Metastatic melanoma is the most lethal form of skin cancer, accounting for most skin cancer-related deaths. Immunotherapies and targeted therapies have improved overall and progression-free survival rates in metastatic melanoma patients. The effectiveness of these therapies decreases due to multidrug resistance (MDR). In contrast to previous reviews, this review extensively highlights the hallmarks of MDR and strategies for reversal of MDR. The review also critically evaluates the challenges in clinical translation of

Abbreviations: A375, human malignant melanoma; ABC, ATP-binding cassette; ABCA9, ATP-binding cassette subfamily A member 9; ABCB1, ATP-binding cassette subfamily B member 1; ABCB5, ATP-binding cassette subfamily B member 5; ABCB8, ATP-binding cassette subfamily B member 8; ABCC1, ATP-binding cassette subfamily C member 1; ABCC2, ATP-binding cassette subfamily C member 2; ABCD1, ATP-binding cassette subfamily D member 1; ABCG2, ATP-binding cassette subfamily G member 2; ACSL4, acyl-CoA synthetase long chain family member 4; ADAM, A disintegrin and metalloproteinase; AKT, protein kinase B; ALDH, aldehyde dehydrogenase; AP-1, activator protein 1; ARNT, aryl hydrocarbon receptor nuclear translocator; AXL, Axl receptor tyrosine kinase;; B16F10, murine melanoma cells; Bax, B-cell lymphoma 2 associated X protein; Bcl2, B-cell lymphoma 2; BCRP, breast cancer resistance protein; BRAF, Braf proto-oncogene; BRAFV600E, B-raf proto-oncogene serine/threonine kinase with V600E mutation (substitution of valine by glutamic acid); C8161, human malignant melanoma; CBF1, C-promoter binding factor 1; CD133, prominin-1; CD147, cluster of differentiation 147; CD20, cluster of differentiation 20; CD271, nerve growth factor receptor; CD44, cluster of differentiation 44; c-fos, cellular proto-oncogene; c-Jun, cellular proto-oncogene Jun; CK1, casein kinase 1; CLS, capillary-like structures; c-myc, cellular myelocytomatosis; CpG, cytosine-phosphate-guanine dinucleotide; CREB, cAMP response element binding protein; CSL, CBF1/RBP-Jk/Su(H)/LAG-1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CYP3A4, cytochrome P450 monooxygenase 3A4; DHh, desert hedgehog; DLL1/DLL3/DLL4, delta-like protein family; DMT1, divalent metal transporter; DPPH, α,α-diphenyl-β-picrylhydrazyl; ECG, epigallocatechin gallate; ECGC, epigallocatechin gallate catechin; EF24, diphenyl difluoroketone; EMA, European Medicines Agency; EMT, epithelial–mesenchymal transition; EphA2, epithelial cell kinase; ER, endoplasmic reticulum; ERK, extracellular regulated kinase; ESRP1, epithelial splicing regulatory protein 1; FAS-L, Fas ligand; FDA, Food and Drug Administration; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; G361, human malignant melanoma; GEF, guanine nucleotide exchange factors; GliA, glioblastoma associated oncogene homolog A; Glu-Cys, glutamate-cysteine; GPX4, glutathione peroxidase 4; GR, glutathione reductase; Grb2, growth factor receptor bound protein 2 adapter protein; GSH, glutathione; GSK3B, glycogen synthase kinase 3 β; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; H460, nonsmall cell lung cancer; HDAC, histone deacetylases; $HER2^-$, human epidermal growth factor receptor-2 negative breast cancer; HES-1, hairy and enhancer of split-1; HEY-1, hairy/enhancer-of-split related with YPRW motif protein-1; Hh, hedgehog signaling pathway; HIF-1-α/β, hypoxia-inducible growth factor-alpha/beta; HMM, human malignant melanoma; HPLC-DAD, high-performance liquid chromatography diode array detector; HR^+, hormone receptor positive breast cancer; HRE, hypoxia response element; Hs-294T, human melanoma cell line; IHh, Indian hedgehog; IRS1, insulin receptor substrate 1; JAG1/2, jagged protein family; JARID1B, H3K4me3 demethylase; K1735, murine melanoma cells; Keap1/NRF2/HO-1, Kelch-like ECH-associated protein 1/Nuclear factor erythroid-2-related factor 2/Heme oxygenase 1; LAG-1, LAG-1 family transcription factor; LOH, lipid alcohols; LOOH, lipid hydroperoxides; LOX/5, lipooxygenase/5; LPCAT3, lysophosphatidylcholine acyltransferase 3; LPS, lipopolysaccharide; Lu1205, human melanoma cell line; MAM, mastermind; MAPK, mitogen-activated protein kinase; Me3, trimethylation; MEK, mitogen-activated protein (MAP) kinase extracellular signal regulated kinase 1 and 2; MELII, human melanoma cells; MIB, mindbomb; miR-200, microRNA-200; miR216-a, microRNA 216-a; miR-9, microRNA-9; MITF, microphthalmia-associated transcription factor; MMP, matrix metalloproteinase; mRNA, messenger RNA; MRP1, multidrug resistance associated protein 1; mTOR, mechanistic target of rapamycin; MV3, human melanoma cells; MX20, multidrug resistant ovarian cancer cell line; MYC, MYC proto-oncogene; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; N-cadherin, neural cadherin; NCI, National Cancer Institute; NECD, Notch extracellular domain; NF-κB, nuclear factor kappa-light chain-enhancer of activated B cells; NICD, notch intracellular domain; NOTCH-1, neurogenic locus notch homolog protein 1; NRAS, neuroblastoma RAS viral oncogene homolog; OH, hydroxyl radical; p300, histone acetyltransferase; p65, protein 65; p70S6K, ribosomal protein S6 kinase β-1; PARP, poly(ADP-ribose) polymerase; PCBP1/2, poly(rC)-binding protein 1; PD-L1, programmed death ligand 1; PCR, polymerase chain reaction; PDK1/2, 3-phosphoinositide-dependent protein kinase 1/2; P-gp, permeation glycoprotein; PI3K, phosphatidylinositol-3-kinase; p-p38, phosphorylated p38 mitogen-activated protein kinase; PRC2, polycomb repressive complex 2; PROTACs, proteolysis targeting chimeras; Ptch, patched; PTEN, phosphatase tensin homolog; PUFA-PE, polyunsaturated fatty acid-phosphatidylethanolamine; PUFAs, polyunsaturated fatty acids; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RAF, rapidly accelerated fibrosarcoma; RAS-GDP, rat sarcoma virus bound to guanosine diphosphate; RAS-GTP, rat sarcoma virus bound to guanosine triphosphate; RBP-Jk, recombining binding protein suppressor of hairless; RNAPII, RNA polymerase II; ROS, reactive oxygen species; RSK, ribosomal S6 kinase; S6, ribosomal protein S6; SAM, *S*-adenosyl methionine; SEER, Surveillance, Epidemiology and End Results; SEL-10, suppressor of lin-12 like protein 10; SH2, Src homology domain 2; SH3, Src homology domain 3; SHh, sonic hedgehog; ShRNA, short hairpin RNA; SI-MI-80, human melanoma cell line; SiRNA, small-interfering RNA; SKMEL-14, SKMEL-19, SKMEL-28, SKMEL-103, SKMEL-173, SKMEL-192, SKMEL-239, human malignant melanoma cell lines; Slug, snail family transcription repressor 1; Smo, smoothened; Snail, snail family transcription repressor 2; SOS/-1, son of sevenless/-1; SOX2/10, sex determining region Y (SRY) box transcription factor 2; STAT3, signal transducer and activator of transcription 3; STEAP3, six transmembrane epithelial antigens of the prostate 3; Su(H), suppressor of hairless; Sufu, suppressor of fusion; TF, transferrin; TFR1, transferrin receptor 1; TFs, transcription factors; TWIST, twist related protein 1; UHPLC-MS, ultra performance liquid chromatography mass spectrometry; VEGF, vascular endothelial growth factor; VM, vasculogenic mimicry; WM115, WM793, WM983, WM4235, human malignant melanoma cell lines; XIAP, X-linked inhibitor of apoptosis; Zeb1, zinc finger E-box binding homeobox; γ-secretase, gamma-secretase.

phytochemicals for metastatic melanoma and strategies to overcome these challenges. This review also highlights the various gaps that exist in metastatic melanoma (e.g., vascularized organ-on-a-chip model has not been developed for melanoma which is largely influenced by angiogenesis). These gaps offer novel avenues that could be explored for clinical translation of phytochemicals.

1 | Introduction

Melanoma is the most lethal form of skin cancer and accounts for most skin cancer related deaths. The 5-year survival rate, based on the Surveillance, Epidemiology, and End results (SEER) database (maintained by the National Cancer Institute [NCI], Bethesda, USA) reported that 35% of people diagnosed with melanoma had a distant SEER stage (describing melanoma that has metastasized to distant sites in the body such as the liver, lungs, or brain) highlighting the aggressiveness of metastatic melanoma [1]. Conventional treatments such as surgery, chemotherapy, and radiotherapy have been utilized but are often ineffective [2], which led to the development of targeted therapies and immunotherapies [3, 4]. Targeted therapies and immunotherapies display limited clinical translation because of the shortcomings related to pharmacokinetics and pharmacodynamics [5, 6]. The development of multidrug resistance due to the activation of various hallmarks of multidrug resistance: signaling cascades (mitogen-activated protein kinase [MAPK], phosphatidylinositol-3-kinase [PI3K]/protein kinase B [Akt]/mechanistic target of rapamycin [mTOR], sonic hedgehog [SHh] and notch signaling pathway), overexpression of ATP-binding cassette (ABC) transporters that efflux drugs from the cytoplasm to the extracellular matrix, hypoxia, epigenetic modifications, antiapoptotic proteins, vasculogenic mimicry (VM) and epithelial–mesenchymal transition (EMT) [7–9]. Cancer stem cells were initially identified in leukemia cancer cells but in recent times, cancer stem cells also became apparent in solid cancers (breast, colon, pancreatic, brain and melanoma) [10, 11]. Cancer stem cells are characterized as cells with the unique capability of regeneration, differentiation and EMT, enabling the acquisition of metastatic and invasive properties [12]. The development of multidrug resistance in human malignant melanoma was recently linked to melanoma stem cells as these display an overexpression of various hallmarks of multidrug resistance and remain after treatment with targeted or immunotherapies [13, 14]. The deregulation of the hallmarks of multidrug resistance accounts for the maintenance of melanoma stem cells and inhibitors of these hallmarks have displayed reduced proliferation, differentiation and EMT of melanoma stem cells [13, 14]. Various inhibitors of the hallmarks of multidrug resistance have displayed efficacy in eradicating melanoma stem cells, however these therapies have not been clinically translated and display various side effects [15], therefore phytochemicals derived from natural products came to the fore for the reversal of multidrug resistance in metastatic melanoma [16]. Various phytochemicals such as curcumin, genistein, and thymoquinone have reversed multidrug resistance in melanoma through the inhibition of key effectors of multidrug resistance (ABC transporters, antiapoptotic proteins, EMT, signaling cascades, hypoxia, epigenetic modifications and VM) [17–19]. The aim of this review is to elucidate the efficacy of phytochemicals for the reversal of multidrug resistance in melanoma and melanoma stem cells as well as highlighting the conceptual advance for the integration of phytochemicals into the treatment regime for HMM, thereby enhancing treatment efficacy and diminishing dose-limiting toxicities, which is critically evaluated in the discussion. Furthermore, this review elevates the existing knowledge on phytochemicals as a novel approach for eradicating existing treatment challenges.

2 | Methodology

The literature search was conducted from 2022 to 2025 using search engines such as Google Scholar, PubMed and Scopus. The search terms included “phytochemicals” AND “multi-drug resistance”, “phytochemicals” AND “melanoma stem cells”, “phytochemicals” AND “melanoma” and “collaterally sensitive phytochemicals” where research articles published from 2018 to 2025 were reviewed. The search identified 190 articles, and the abstracts were reviewed for relevance to the search terms to establish the foundation for the review article.

3 | Melanoma Stem Cells

The concept of cancer stem cells came to the fore as an explanation for the development of multidrug resistance in various cancers [20]. In melanoma, targeted therapies and immunotherapies are modestly effective but within a year, acquired resistance occurs [21]. The acquired resistance could be linked to melanoma stem cells that drive metastasis and invasion in malignant melanoma [21]. The subpopulation of melanoma stem cells are identified through the expression of certain biomarkers such as ATP-binding cassette subfamily B member 5 (ABCB5) [22, 23], cluster of differentiation (CD20) [23, 24], nerve growth factor receptor (CD271) [23, 25], aldehyde dehydrogenase (ALDH) [23, 26], and prominin-1 (CD133) [23, 27] (Table 1).

3.1 | Multidrug Resistance in Melanoma

Despite the substantial improvement in the treatment of melanoma, metastatic melanoma is still regarded to be the most lethal, heterogeneous, and substantially mutated cancer compared to other cancers [40, 41]. Chemotherapies such as dacarbazine and fotemustine were historically used for the treatment of advanced metastatic melanoma but only displayed 5-year overall survival (OS) rates of approximately 6%–10% in patients with Stage IV metastatic melanoma [41, 42]. Targeted therapies and immunotherapies have displayed superior progression-free and OS rates compared to chemotherapies [41]. Combined B-raf proto-oncogene (BRAF) inhibitors (BRAFi) and mitogen-activated protein (MAP) kinase extracellular signal regulated kinase 1 and 2 (MEK) inhibitors (MEKi) displayed a 5-year OS of 34% and patients harboring a BRAF mutation, treated with

TABLE 1 | The role of stemness molecular markers.

Stemness molecular marker	Role	Reference
ATP-binding cassette subfamily B member 5 (ABCB5)	ABCB5 maintains membrane hyperpolarization in progenitor cells and serves as an efflux pump that mediates resistance to doxorubicin in melanoma. A subpopulation of ABCB5$^+$ cells in melanoma have been identified as melanoma stem cells that possess the capabilities to self-renew like embryonic stem cells. Furthermore, ABCB5 may be an imperative factor for the promotion of melanoma metastasis through maintenance of the nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) pathway and enhancement of protein 65 (p65) protein stability through the inhibition of p65 ubiquitination	[28, 29]
ATP-binding cassette subfamily G member 2 (ABCG2)	ABCG2 is expressed in normal stem cells, sustains the stem cell phenotype, and regulates stem cell proliferation. Furthermore, CD133$^+$ and ABCG2$^+$ were identified as markers of melanoma stem cells in melanoma biopsies and a highly tumorigenic melanoma cell line (WM115). The subpopulation of melanoma cells expressing ABCG2$^+$ display enhanced efflux potential and multidrug resistance	[30, 31]
Cluster of differentiation 20 (CD20)	CD20 has been identified as a phosphoprotein predominantly expressed on the surface of B cells (normal and malignant). CD20 has also been identified as a melanoma stem cell marker and rituximab (monoclonal antibody) for CD20 also displayed a therapeutic effect in Stage 4 metastatic melanoma. Furthermore, doxorubicin-loaded exosomes with anti-CD20 aptamers selectively suppressed CD20$^+$ melanoma stem cells. This highlights the potential that complete eradication of metastatic melanoma may be achieved through the eradication of melanoma stem cells expressing CD20	[24, 32–34]
Nerve growth factor receptor (CD271)	CD271 regulates apoptosis and survival of neurons but in melanoma, it regulates self-renewal, phenotype switching and tumorigenicity. Thus, CD271 has been identified as a molecular marker of melanoma stem cells and in A375 cells. The overexpression of CD271 resulted in enhanced migration and metastasis of melanoma	[25, 35]
Aldehyde dehydrogenase (ALDH)	ALDH has been validated as a marker of melanoma stem cells. ALDH$^+$ cells in melanoma were associated with phenotype switching, melanomagenesis, drug resistance, and metastasis	[26, 36, 37]
Prominin-1 (CD133)	CD133 is a glycoprotein that regulates survival, proliferation, and DNA repair in normal stem cells and retinal development in humans. The subpopulation of melanoma cells expressing CD133 mediate drug resistance in melanoma and CD133 has also been linked to the ABC transporter, ABCG2. Enhanced sensitivity to dabrafenib and trametinib occurred through small-interfering RNA (siRNA) knockdown of CD133 but microarray analysis revealed that ABCG2 mediates this response as siRNA knockdown of CD133 resulted in decreased ABCG2 and knockdown of ABCG2 re-sensitized CD133 cells to therapeutics	[38, 39]

a combination of ipilimumab and nivolumab, displayed a 5-year OS of 60% [41, 43, 44]. The lower 5-year OS of combined BRAFi/MEKi therapy could be due to the presence of melanoma stem cells. Metastatic melanoma cells (A375) treated with delta (δ)-tocotrienol lacked stem cell forming capabilities, whereas A375 cells treated with vemurafenib were capable of forming stem cells suggesting that efficacy of targeted therapies, such as vemurafenib, are lowered due to development of melanoma stem cells [44, 45]. The variants of multidrug resistance in melanoma and melanoma stem cells discussed below highlight the various molecular targets and signaling pathways that lead to multidrug resistance of monotherapies and combination therapies.

4 | Variants of Multidrug Resistance in Melanoma and Melanoma Stem Cells

4.1 | ABC Transporters

Increased drug efflux of chemotherapeutics or targeted therapeutics results in a low intracellular concentration (below the therapeutic dose), thereby resulting in chemotherapy/targeted therapy resistance [46]. ABC transporters are integral membrane proteins that comprise of two nucleotide binding domains and two transmembrane domains that can be classified into seven subfamilies ranging from ABCA to ABCG, based on the organization and sequence of the nucleotide binding domain

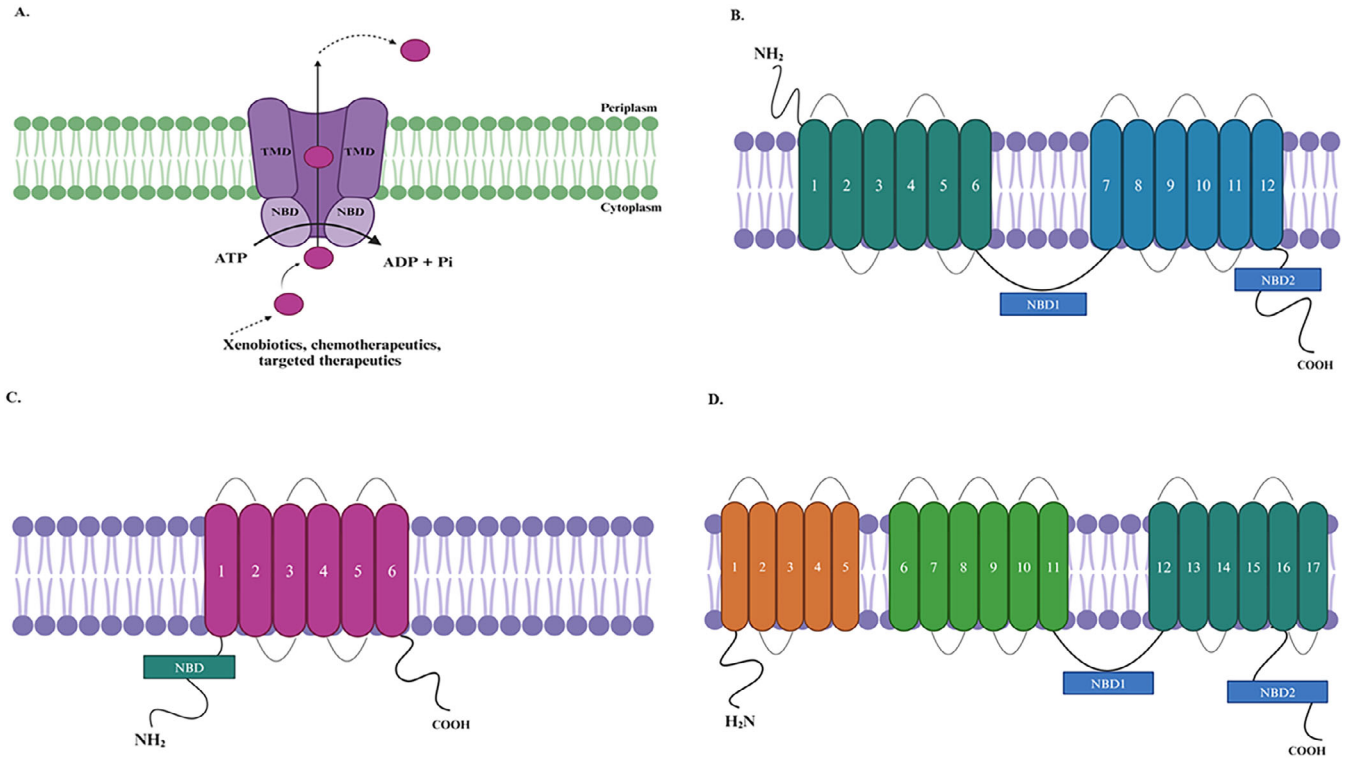

FIGURE 1 | (A) Basic structure of ABC transporters. (B) Permeation glycoprotein (P-glycoprotein), also referred to as ABCB1 and cluster of differentiation (CD243), has two homologous halves with six transmembrane domains. The transmembrane domains facilitate the transport of substrates outside the cell. The nucleotide binding domains, which contain ATP, supply energy for the transport of substrates out the cell through the hydrolyzation of ATP. (C) ATP-binding cassette subfamily G-member 2 (ABCG2), also referred to as breast cancer resistance protein (BCRP), is a half transporter with one transmembrane domain and nucleotide binding domain. ABCG2 also facilitates the transport of substances such as chemotherapeutics and xenobiotic substances through the transmembrane domain using energy supplied by the hydrolyzation of ATP in the nucleotide binding domain. (B and C): ATP-binding cassette sub-family B member 5 (ABCB5), which is highly expressed in metastatic melanoma cells, is both a full transporter (two transmembrane domains and two nucleotide binding domains) and a half transporter (one transmembrane domain and nucleotide binding domain). ABCB5 is functionally related to P-glycoprotein and serves as a marker for stemness in metastatic melanoma cells. (D) Multidrug resistance associated protein 1 (MRP1), also referred to as ATP-binding cassette subfamily C member 1 (ABCC1), consists of three membrane spanning domains and two nucleotide binding domains. This transporter also facilitates the transport of chemotherapeutics and xenobiotic substances outside the cell.

[47, 48] (Figure 1A). These transporters actively transport lipophilic compounds out of the cell membrane [49, 50]. Twelve ABC transporters contribute to multidrug resistance whereas the three main contributors are: Permeation glycoprotein (P-glycoprotein [P-gp])/ATP-binding cassette subfamily B member 1 (ABCB1) (Figure 1B), multidrug resistance protein 1 (MRP1)/ATP-binding cassette subfamily C member 1 (ABCC1) (Figure 1D) and breast cancer resistance protein/ATP-binding cassette subfamily G member 2 (BCRP/ABCG2) (Figure 1C) [51, 52]. In addition to the ABC transporters mentioned previously, overexpression of ABCB5 in malignant melanoma and stem cell populations has also been linked to the development of multidrug resistance (Figure 1B,C) [47, 52]. Various ABC transporters (ATP-binding cassette subfamily A member 9 (ABCA9), ABCB1, ABCB5, ATP-binding cassette subfamily B member 8 (ABCB8), ABCC1, ATP-binding cassette subfamily C member 2 (ABCC2) and ATP-binding cassette subfamily D member 1 (ABCD1) are expressed in melanoma cell lines and may be linked to the resistance of melanoma cells to targeted therapies [53]. The ABC transporters that also serve as stemness markers in melanoma (ABCB5 and ABCG2) have been linked to the enhanced tumorigenicity and treatment resistance displayed by melanoma stem cells [54].

4.2 | Signaling Cascades That Drive the Proliferation of Melanoma and Melanoma Stem Cells

4.2.1 | MAPK Pathway

The MAPK pathway is a major driver of melanoma development and the development of resistance to targeted therapies [55]. Deregulation of the MAPK pathway is largely driven by mutations in the BRAF gene (occur in 40%–68% of metastatic melanomas) and neuroblastoma RAS viral oncogene homolog (NRAS) (occurs in 15%–20% of melanomas) [55–58]. The binding of a growth factor (e.g., epidermal growth factor [EGF]) to a receptor (epidermal growth factor receptor [EGFR]) results in the EGFR molecules joining and activating through phosphorylation [59–61]. The growth factor receptor bound protein 2 (Grb2) adaptor protein together with the Src homology (SH2) domain binds to the site on the protein where phosphate is added. This promotes the binding of the son of sevenless (SOS) adapter protein to the Src homology domain 3 (SH3) domain of Grb2 [59, 62]. The binding of SOS results in the recruitment of guanine nucleotide exchange factors (GEFs), which converts rat sarcoma virus bound to

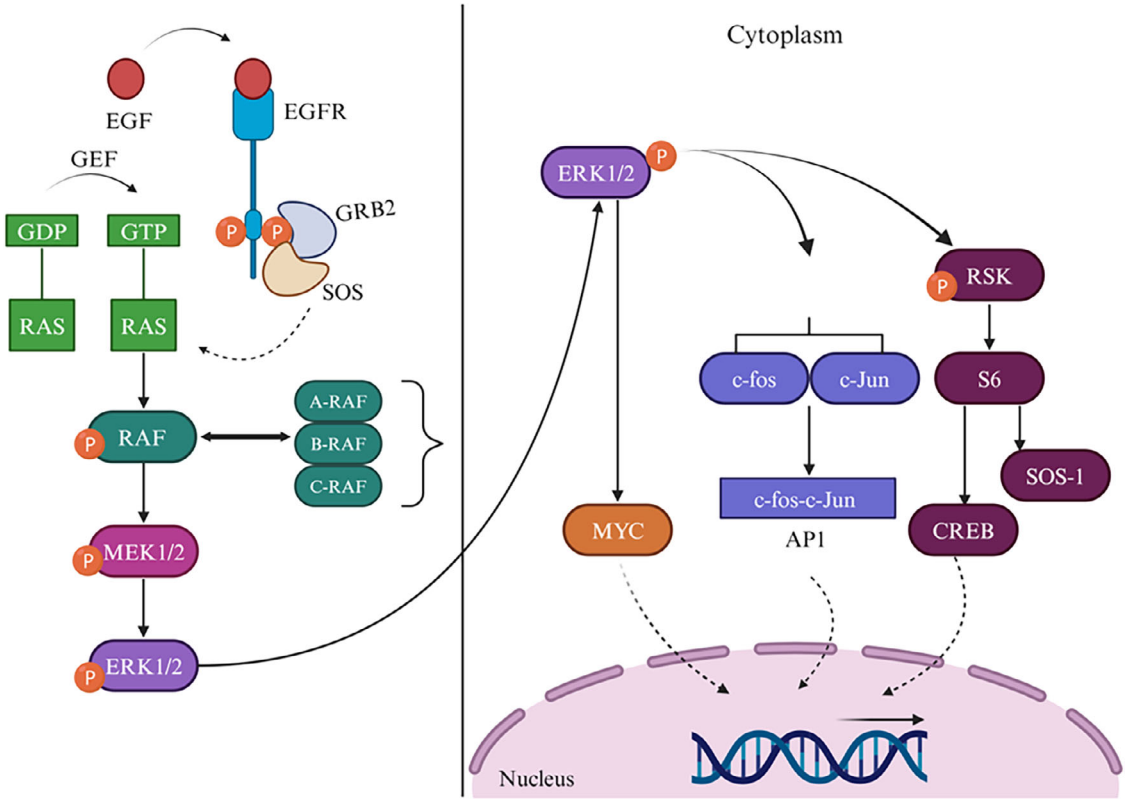

FIGURE 2 | The depiction of the MAPK pathway where the binding of EGF to EGFR triggers dimerization and autophosphorylation of EGFR. Grb2 adapter protein together with the SH2 domain binds to the phosphotyrosine residue which facilitates the binding of SOS adapter protein to the SH3 domain of Grb2. The binding of SOS leads to the recruitment of GEFs which convert RAS-GDP to RAS-GTP. RAS-GTP activates RAF (A-RAF, B-RAF, and C-RAF), MEK1/2 and ERK1/2. ERK1/2 dimerizes c-fos and c-Jun in the cytoplasm forming activator protein-1 which enters the nucleus, binds to mRNA, and initiates transcription. AP-1, activator protein-1; c-fos, cellular proto-oncogene fos; c-Jun, cellular proto-oncogene Jun; CREB, cAMP response element binding protein; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal regulated kinase; GEF, guanine nucleotide exchange factors; Grb2, growth factor receptor bound protein 2 adapter protein; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MYC, MYC proto-oncogene; RAF, rapidly accelerated fibrosarcoma; RAS-GDP, rat sarcoma virus bound to guanosine diphosphate; RAS-GTP, rat sarcoma virus bound to guanosine triphosphate; RSK, ribosomal S6 kinase; S6, ribosomal protein S6; SH2, Src homology domain 2; SH3, Src homology domain 3; Sos, son of sevenless; SOS-1, son of sevenless homolog 1.

guanosine diphosphate (RAS-GDP) to Rat sarcoma virus bound to guanosine triphosphate (RAS-GTP) [59, 62]. The activated RAS-GTP activates rapidly accelerated fibrosarcoma (RAF) (A-RAF, B-RAF, and C-RAF) [63], MAP kinase extracellular signal regulated kinase 1 and 2 (MEK1/2), and extracellular regulated kinase (ERK1/2) [63]. ERK1/2 joins cellular proto-oncogene (c-fos) and cellular proto-oncogene Jun (c-Jun) in the cytoplasm forming c-fos–c-Jun (activator protein-1 [AP-1]). AP-1 enters the nucleus, binds to messenger RNA (mRNA) and triggers transcription (Figure 2). The initiation of transcription results in increased angiogenesis through increased vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) [59, 64]. The MAPK signaling pathway has been identified as a major driver of melanoma oncogenesis, but it has not been identified as a direct driver of oncogenesis in melanoma stem cells [21, 65]. The MAPK and Akt pathways have been identified as drivers in colon cancer stem cells that express prominin-1 ($CD133^+$) [66]. In the $CD133^+$ subpopulation of colon cancer stem cells, inhibitors of the Akt and MAPK pathways resulted in decreased colony formations and proliferation. This decrease indicated the significance of the pathways in maintaining tumor development in $CD133^+$ colon cancer stem cells. [66]. In melanoma stem cells, the neurogenic locus notch homolog protein 1 (Notch-1)/MAPK signaling axis-maintained tumor development of $CD133^+$ melanoma stem cells [21, 65]. The MAPK signaling pathway is linked to the tumor development of $CD133^+$ melanoma stem cells. The markers of the MAPK signaling pathway (phosphorylated p38 mitogen-activated protein kinase [p-p38]), c-fos, and c-jun) were upregulated in $CD133^+$ melanoma stem cells, confirming the association [21, 65]. To confirm maintenance of tumor development in $CD133^+$ melanoma stem cells is not exclusively mediated through MAPK, the stem cells were treated with the p38 MAPK inhibitor (SB203580). SB203580 had no effect on CD133 but altered p-p38, c-fos, and c-jun [21, 65]. Melanoma stem cells were further treated with a notch signaling pathway inhibitor, GSI-IX, and expression of notch intraceullar domain 1 (NICD1), p-p38, c-fos, and c-jun was analyzed through Western blotting [21, 65]. GSI-IX, inhibited NICD1, p-p38, c-fos, c-jun, and downregulated CD133. Inhibition by GSI-IX confirmed that both (MAPK and NOTCH) pathways are required to regulate tumor development in $CD133^+$ melanoma stem cells [21, 65]. In colon cancer, the pathways (MAPK and AKT) were both required to regulate tumor development by $CD133^+$ colon cancer stem cells. This demonstrated how different signaling pathways regulate the

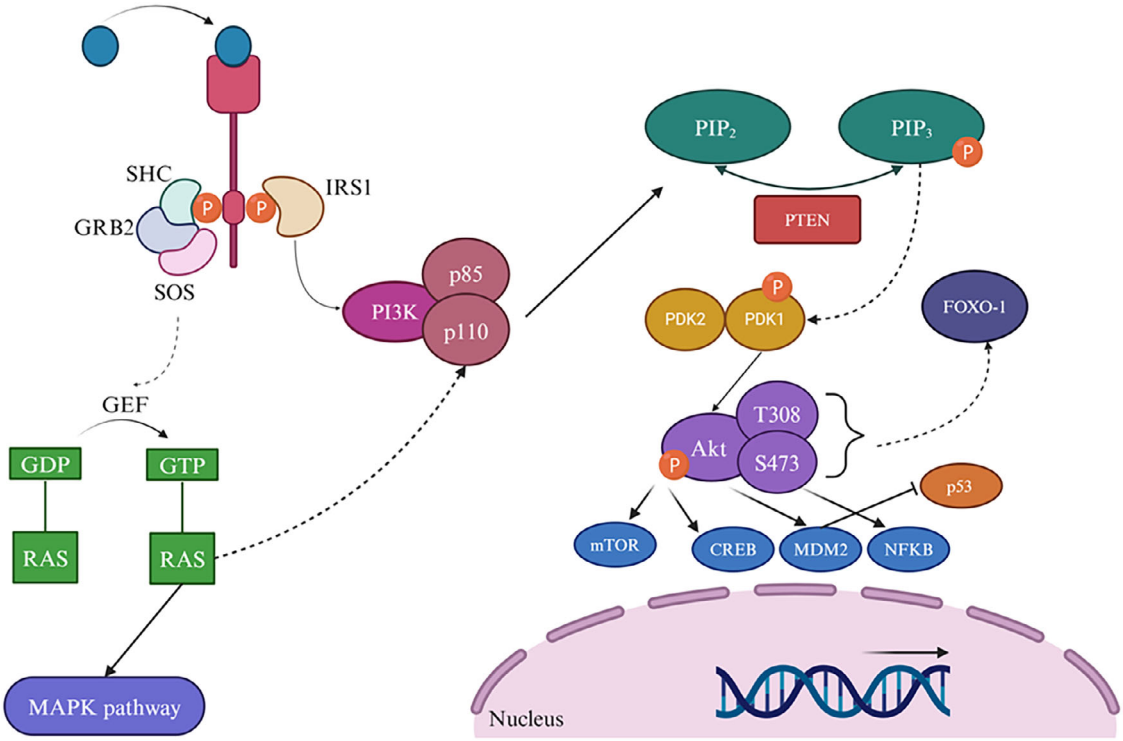

FIGURE 3 | Depiction of the PI3K pathway where the binding of IGF/EGF to EGFR/IGFR triggers autophosphorylation. The IRS1 adapter protein binds to the phosphotyrosine residue thereby recruiting PI3K. PI3K comprises of a p85 regulatory subunit and p110 catalytic subunit. The recruitment of the SHC-Grb2-SOS domain to the phosphotyrosine residue facilitates the recruitment of GEFs that convert RAS-GDP to RAS-GTP. RAS-GTP activates p110 which activates PDK1 and PDK2. PDK1 activates Akt (comprising of a threonine (T308) and serine (S473) subunit which regulates various processes such as apoptosis through FOXO-1. The conversion of PIP_2 to PIP_3 is regulated by the tumor suppressor, PTEN. Akt, protein kinase B; CREB, cAMP response element binding protein; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FOXO-1: forkhead box 1; GEF, guanine nucleotide exchange factors; Grb2, growth factor receptor bound protein 2; IGF, insulin growth factor; IGFR, insulin growth factor receptor; IRS1, insulin receptor substrate 1; MDM2, mouse double minute 2 homolog; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa B; p53, tumor suppressor protein p53; PDK1, 3-phosphoinsositide-dependent protein kinase 1; PI3K, phosphatidylinositol-3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-triphosphate; PTEN, phosphate tensin homolog; RAS-GDP, rat sarcoma virus bound to guanosine diphosphate; RAS-GTP, rat sarcoma virus bound to guanosine triphosphate; SHC, Src homology domain; SOS, son of sevenless.

same target. Highlighting the differing mechanisms of action for different cancers, thus, personalized therapeutic development for a specific cancer is important.

4.2.2 | PI3K/Akt/mTOR Pathway

The PI3K pathway is a significant oncogenic pathway in melanoma. Similarly, to the MAPK pathway, a growth factor (insulin or EGF) binds to the receptor tyrosine kinase and activates through phosphorylation [67–69]. The insulin receptor substrate 1 (IRS1) adapter protein binds to the site on the protein where phosphate is added, thereby recruiting PI3K [67, 68]. PI3K consists of a p85 regulatory subunit and a p110 catalytic subunit [70]. The recruitment of the Src homology domain-growth factor receptor bound protein 2 adapter protein-son of sevenless (SHC-Grb2-SOS) domain to the site on the protein where phosphate is added promotes GEFs that convert RAS-GDP to RAS-GTP [71]. RAS-GTP activates p110 which activates 3-phosphoinositide-dependent protein kinase 1/2 (PDK1 and PDK2) [72, 73]. PDK1 activates Akt which regulates various cellular processes [72, 73]. The PI3K pathway is also regulated by the lipid phosphatase activity of the tumor suppressor (phosphatase tensin homolog [PTEN]) [74, 75] (Figure 3). PTEN is deactivated in 10%–30% of cutaneous melanomas thereby driving the constant activation of Akt [74]. The PI3K/Akt/mTOR has also been identified as a major driver of melanoma development in melanoma stem cells [76, 77]. The PI3K pathway maintains migration, differentiation, VM and cellular growth in melanoma stem cells [76, 77]. Jamal et al. isolated $CD133^+$ cells (molecular marker for melanoma stem cells) from a metastatic melanoma cell line [78]. The subpopulation of $CD133^+$ cells were evaluated for antiapoptotic activity using fotemustine (antiapoptotic agent) and the activity was reduced compared to melanoma cells [78]. Thus, the acquired resistance was linked to the activation of the PI3K signaling pathway in melanoma stem cells. The interaction of CD133 and the p85 regulatory subunit of PI3K led to the inhibition of apoptosis and reduced efficacy of fotemustine [78, 79], highlighting the role of signaling pathways in resistance.

4.2.3 | Hedgehog Signaling Pathway (Hh Pathway)

In addition to the MAPK and PI3K pathway, the hedgehog (Hh) pathway also plays a role in melanoma development [80, 81]. The Hh signaling pathway is important during embryological

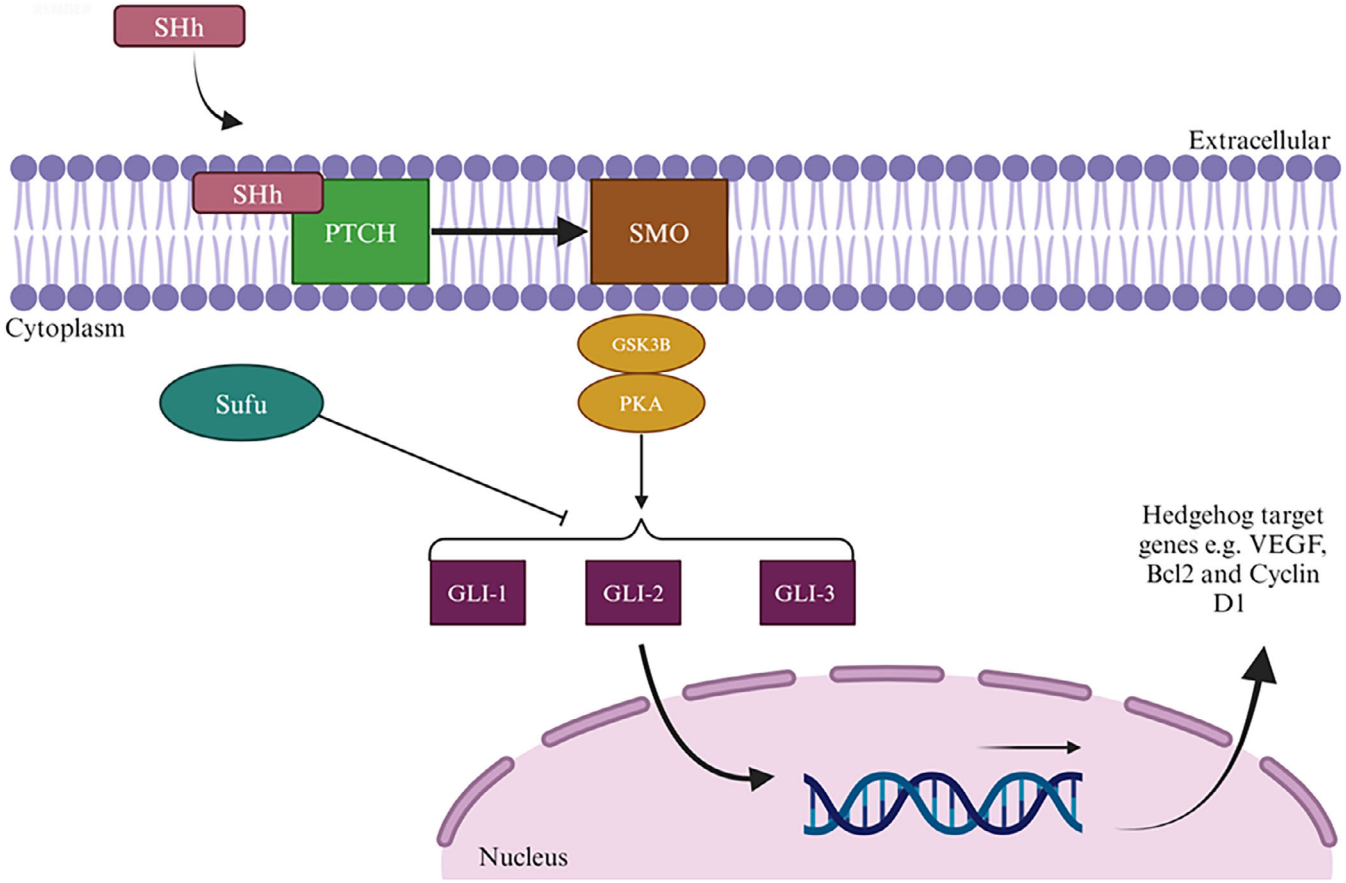

FIGURE 4 | Depiction of the Hh pathway where activated and secreted SHh binds to a transmembrane protein, Ptch, which enables the activation of transmembrane protein, Smo. The binding of SHh to Ptch alleviates the repression of Smo and Smo is subsequently phosphorylated by PKA and CK1 which initiates a phosphorylation cascade enabling the recruitment of transcriptional activator, GliA. The Sufu serves the role of a negative regulator by binding Gli transcription factors (Gli1, Gli2, and Gli3) preventing the association of Smo and Gli in the absence of SHh binding to Ptch. Once Smo is activated and Ptch degraded, the Sufu-Gli complex is dissociated and GliA translocates to the nucleus and initiates transcription of target genes associated with angiogenesis (VEGF), apoptosis (Bcl2), proliferation (cyclin D1), and stemness (SOX2). BCL2, B-cell lymphoma 2; CK1, casein kinase 1; cyclin D1, cyclin D1; GliA, glioblastoma associated oncogene homolog A; Hh, hedgehog signaling pathway; PKA, protein kinase A; Ptch: patched; SHh, sonic hedgehog; Smo, smoothened; SOX2, SRY-box transcription factor 2; Sufu, suppressor of fusion; VEGF, vascular endothelial growth factor.

development, but abnormal activation may drive tumorigenesis, metastasis, and multidrug resistance [82, 83]. The Hh signaling pathway consists of homologous ligands: SHh, Indian hedgehog (IHh), and desert hedgehog (DHh) but SHh is the most studied one [84]. Upon activation and secretion, SHh binds to a transmembrane protein, patched (Ptch), which enables the activation of transmembrane protein, smoothened (Smo) [85, 86]. The binding of SHh to Ptch alleviates the repression of Smo and Smo is subsequently phosphorylated by protein kinase A (PKA) and casein kinase 1 (CK1), thereby initiating a phosphorylation cascade enabling the recruitment of the transcriptional activator, GliA [87, 88]. The suppressor of fusion (Sufu) plays an important role as a negative regulator through binding Gli transcription factors (Gli 1, Gli 2, and Gli 3) and preventing association of Smo and Gli in the absence of SHh binding to Ptch [86, 89]. Upon Smo activation and Ptch degradation, the Sufu-Gli complex is dissociated, and GliA translocates to the nucleus. GliA initiates transcription of target genes associated with angiogenesis (VEGF), apoptosis (B-cell lymphoma 2 [BCL2]), proliferation (cyclin D1), and stemness (SOX2) (Figure 4) [86, 89, 90].

The Hh signaling pathway was also upregulated in melanoma stem cells. Santini et al. investigated the capability of metastatic melanomas harbouing cancer stem cells [91]. Amongst other melanoma cell lines, A375 cells were grown in human embryonic or neural stem cell media to enrich for cancer stem cells. This resulted in the formation of melanoma spheres highlighting the existence of stem-like cells in melanoma. ALDH activity also serves as a marker for cancer stem cells [91, 92]. The A375 cells displayed a high percentage of $ALDH^+$ cells, and these cells displayed a higher percentage of (Gli), through Western blotting. This study demonstrated the upregulated Hh signaling in melanoma stem cells [91, 93]. This highlighted the increase in Hh signaling in melanoma stem cells and the importance of targeting this pathway in stem cells.

4.2.4 | Neurogenic Locus Notch Homolog Protein 1 (Notch) Signaling Pathway

The Notch signaling pathway has also been identified as a major driver of melanoma tumorigenesis [94]. The Notch signaling

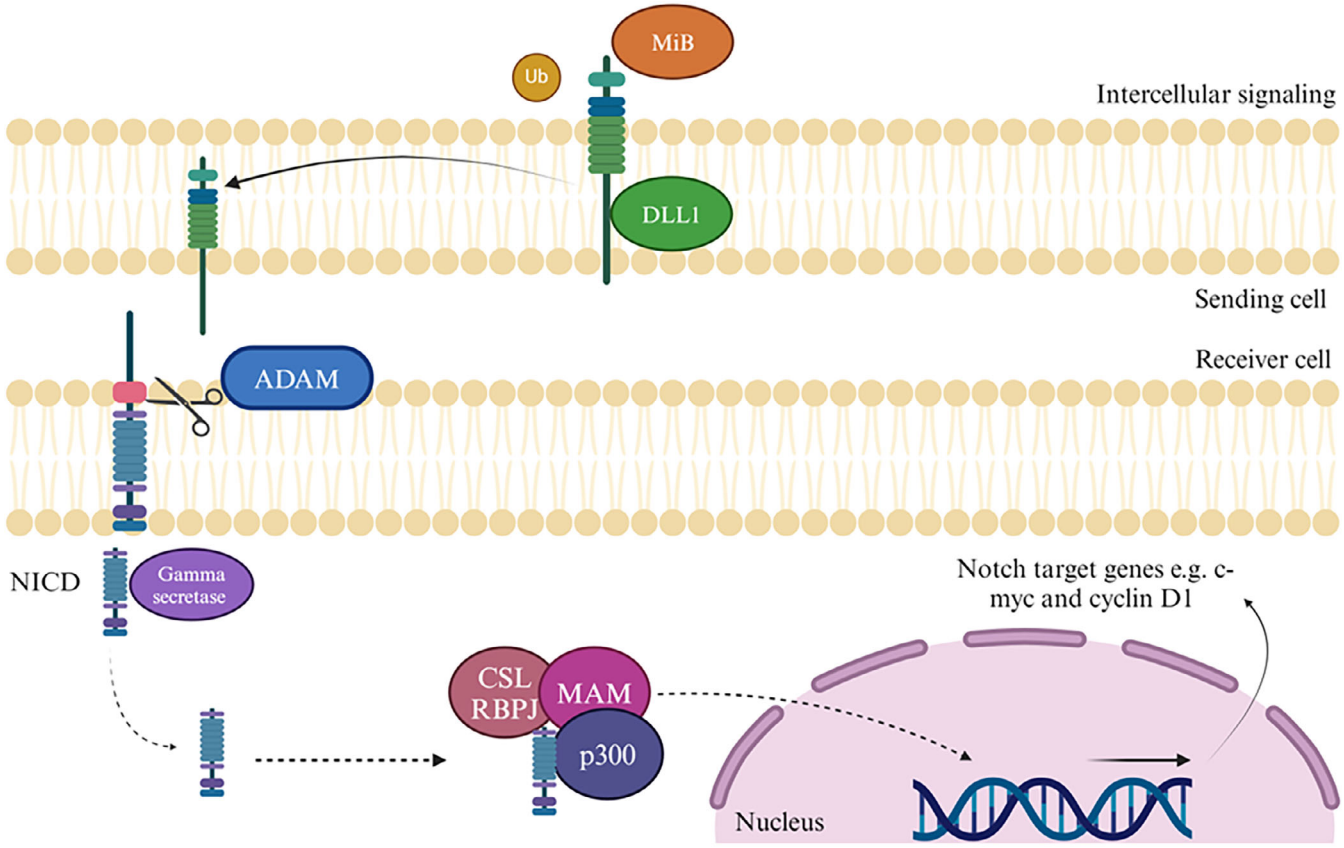

FIGURE 5 | Depiction of the notch intercellular signaling pathway where activated DLL1 binds to NECD resulting in the cleavage of the extracellular domain by ADAM (S2 cleavage). Followed by the cleavage of NICD by γ-secretase (S3 cleavage). NICD is free to bind to a complex in the cytosol comprising of CSL (CBF1, Su(H), Lag-1), MAM, and p^{300}. After binding, the complex is translocated into the nucleus and facilitates the transcription of Notch target genes (c-myc and cyclin D1). Signaling stops through the ubiquitination of NICD by SEL-10 resulting in proteasomal degradation of NICD. ADAM, A disintegrin and metalloproteinase; CBF1, c-promoter binding factor 1; c-Myc, cellular myelocytomatosis; CSL, CBF1/RBP-Jk/Su(H)/LAG-1; DLL1, delta like protein 1; LAG-1, LAG-1 family transcription factor; MAM, mastermind; MiB, mindbomb; NECD, notch extracellular domain; NICD, notch intracellular domain; p^{300}, histone acetyltransferase; RBP-Jk, recombining binding protein suppressor of hairless; SEL-10, suppressor of lin-12-like protein 10; Su(H), suppressor of hairless; γ-secretase, gamma secretase.

pathway consists of four notch receptors (Notch 1–4), ligands such as the jagged protein family (JAG1 and JAG2) and delta-like protein family (DLL1, DLL3, and DLL4) [95]. This pathway is an intercellular signaling pathway and once the ligand, DLL1 is activated by mindbomb (MIB, E3 ubiquitin ligase) through ubiquitination, DLL1 binds to the extracellular domain (NECD). A disintegrin and metalloproteinase (ADAM) catalyzes the cleavage of NECD extracellular domain (S2 cleavage) [96, 97]. This is followed by the cleavage of the intracellular domain, NICD, by a protein called gamma (γ)-secretase (S3 cleavage) [95, 98]. The NICD is free to bind to a complex in the cytosol comprising of CSL (C-promoter binding factor 1 [CBF1], suppressor of hairless [Su(H)], LAG-1 family transcription factor [Lag-1]), mastermind (MAM, co-activator) and histone acetyltransferase (p^{300}). After binding, the complex is translocated into the nucleus and promotes the transcription of notch target genes (cellular myelocytomatosis, c-myc, and cyclin D1) (Figure 5) [99, 100]. The pathway is inhibited through the ubiquitination of NICD by suppressor of lin-12 like protein 10 (SEL-10) resulting in proteasomal degradation of NICD [101].

The development of multidrug resistance in melanoma has also been linked to the protumorigenic properties of melanoma stem cells [54]. The overexpression of Notch 1, correlated with the overexpression of CD133 in melanoma stem cells and the Notch1/CD133 axis, activates the MAPK pathway [102]. Activation of the MAPK pathway upregulates genes involved in angiogenesis (VEGF)/VM (MMPs) as well as metastasis snail family transcription factor repressor 1 (Snail)/Snail family transcription repressor 2 (Slug) [54, 65, 103]. To highlight the significance of the Notch pathway in melanoma stem cells, the effect of honokiol on Notch-2 was evaluated [104]. Honokiol decreased the expression of Notch-2, which was shown through Western blotting [104]. To validate the role of Notch-2 in melanoma stem cells, NICD1 and NICD2 were overexpressed [104]. The overexpression of NICD-2 led to increased expression of hairy and enhancer of split 1 (HES-1), cyclin D1, and melanosphere formation [104]. HES-1, cyclin D1, and melanosphere formation are characteristic of melanoma stem cells suggesting that Notch signaling is increased in melanoma stem cells.

4.3 | Hypoxia-Mediated Multidrug Resistance

Hypoxia is induced in the tumor microenvironment when oxygen levels are between 5 and 10 mmHg. The decreased oxygen

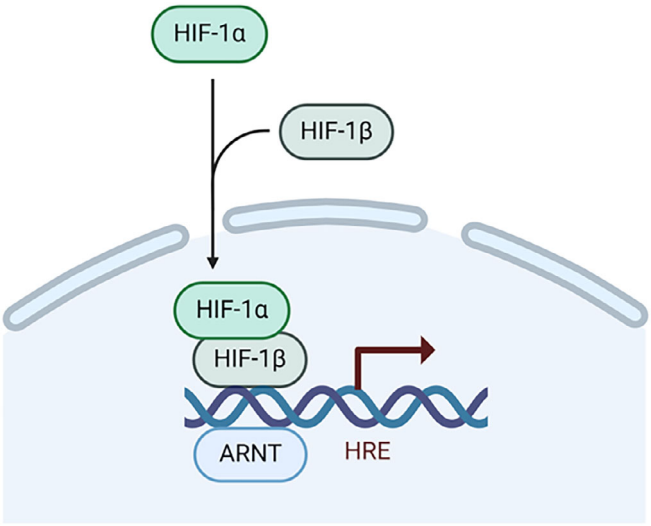

FIGURE 6 | Under hypoxic conditions, HIF-1-α, is stabilized and forms a complex with HIF-1-β through joining together. The complex binds to hypoxia response elements in the promoter of target genes, resulting in the transcription of these genes. ARNT, aryl hydrocarbon receptor nuclear translocator; HIF-1-α, hypoxia-inducible growth factor alpha; HIF-1-β, hypoxia-inducible growth factor β; HRE, hypoxia response element.

pressure occurs due to an imbalance between consumption and supply of oxygen due to the rapid growth of the tumor (Figure 6) [105]. Hypoxia triggers angiogenesis, invasion, migration, anaerobic glycolysis, and multidrug resistance in tumor cells [105]. In genes that encode MRP1, ABCC1, and ABCG2, the deletion of the hypoxia response element enhanced drug efflux through hypoxia dependent activation. In several cancers, hypoxia has led to the inhibition of pro-apoptotic proteins such as B-cell lymphoma 2 associated X protein (BAX) and enhanced expression of antiapoptotic proteins such as BCL-2 [106]. Enhanced levels of reactive oxygen species (ROS) (superoxide anion [O_2^-], hydrogen peroxide [H_2O_2], and hydroxyl radicals [OH^-]) due to external factors (xenobiotics, ionizing radiation, ultraviolet [UV] light, chemotherapeutics, alcohol, tobacco, bacterial, and viral infections) lead to the activation and stabilization of transcription factors (TFs) such as hypoxia-inducible growth factor-alpha (HIF-1-α). The generation of ROS could also be due to the lack of oxygen which is required in the mitochondrial respiratory chain. The lack of oxygen in the mitochondrial respiratory chain results in the depletion of existing oxygen molecules and generation of radicals. Enhanced ROS generation due to hypoxia is observed in several secondary cell lines and the stabilization of HIF-1-α leads to the expression of VEGF-A which induces resistance to chemotherapeutics (etoposide and doxorubicin). In melanoma stem cells, Li et al. explored the role of nodal expression due to enhanced hypoxia in the maintenance of stemness in A375 melanoma stem cells [107]. Upon stimulation of hypoxia, nodal expression was enhanced leading to resistance to dacarbazine, enhanced self-renewal capabilities and invasion capabilities [107]. Knockdown of Nodal and treatment with small molecule inhibitor, SB431542, sensitized A375 melanoma stem cells to dacarbazine [107]. Furthermore, $BRAF^{V600E}$ mutations enhance HIF-1-α signaling, illustrating the link between signaling pathways and hypoxia. This also highlights the link between the different hallmarks of cancer [108].

4.4 | Epigenetic Modifications That Sustain Multidrug Resistance

Epigenetic modifications consist of histone modifications, DNA methylation, chromatin remodeling, and noncoding RNA (Figure 7) [109, 110]. Irregular histone methylation patterns, due to deregulated histone demethylases, results in enhanced melanoma tumorigenesis [111]. The H3K4me3 demethylase, JARID1B, was characterized as a biomarker for a subpopulation of slow cycling melanoma cells with enhanced self-renewal in vitro [111]. The subpopulation of melanoma cells expressing high levels of JARID1B also displayed the expression of mitochondrial bioenergetic enzymes. Through inhibition of the mitochondrial respiratory chain, intrinsic multidrug resistance in melanoma was reduced [112]. In addition to histone modifications, DNA methylation served as an epigenetic hallmark in melanoma as it plays a role in the initiation of melanoma [109]. A total of 120 of 200 (60%) samples obtained from patients with cutaneous melanoma, displayed methylation of the PTEN promoter through methylation specific PCR [113]. Melanoma stem cells may be oncogenic derivatives of normal tissue and progenitor stem cells [114]. Latexin, a negative regulator of hematopoietic stem cell populations, reduced the transition of stem cells into cancer stem cells [114, 115]. Latexin was downregulated in 50% of melanomas and the cytosine-phosphate-guanine dinucleotide (CpG) island promoter of the latexin gene was hypermethylated in other cancers and melanoma [114, 116]. This highlights the role of epigenetic modifications in the maintenance of melanoma stem cell subpopulations. Noncoding RNAs also play an important role in the epigenome [117]. Notably, microRNAs (miRNAs) (a subset of noncoding RNAs that negatively regulate gene expression), are the most frequently studied noncoding RNAs [118]. The miR-200 family regulated EMT and also regulated stemness through the regulation of TFs associated with EMT (transcriptional repressor of E-cadherin), zinc finger E box binding HOX 1/2 (Zeb 1/2) [119]. In metastatic melanoma, miR-9 was downregulated, and this correlated with enhanced E-cadherin expression and downregulation of the NF-κB–Snail1 pathway [120].

4.5 | Antiapoptotic Proteins Implicated in Multidrug Resistance

Apoptosis is a process necessary for normal tissue development and homeostasis, but cancer cells evade apoptosis, leading to the development of resistance to various therapeutics (Figure 8). In melanoma, a key tumor suppressor, PTEN, is mutated in approximately 30% of melanomas and the deregulation of PTEN results in the maintenance of antiapoptotic protein, survivin [121, 122]. The deregulation of PTEN also leads to the overactivation of Akt, thereby leading to the expression of X-linked apoptosis protein (XIAP). Apoptosis is also evaded by melanoma stem cells. The combination of a BCL-2 inhibitor with a retinoid derivative (tenretinide) was a promising treatment for melanoma cells, melanoma cells harboring the $BRAF^{V600E}$ mutation, non-melanoma stem cells and melanoma stem cells [123]. The combination decreased the number of $ALDH^{high}$ cells, inhibited

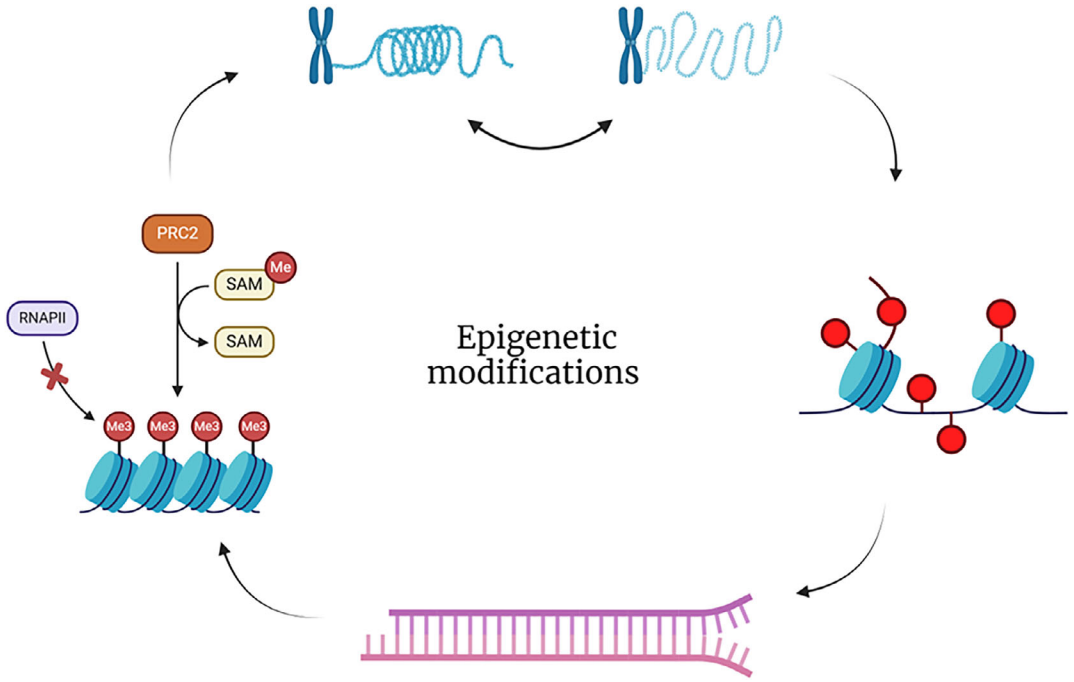

FIGURE 7 | Depiction of various epigenetic modifications such as DNA methylation, chromatin remodelling, and histone modification. Me3, trimethylation; PRC2, polycomb repressive complex 2; RNAPII, RNA polymerase II; SAM, *S*-adenosyl methionine.

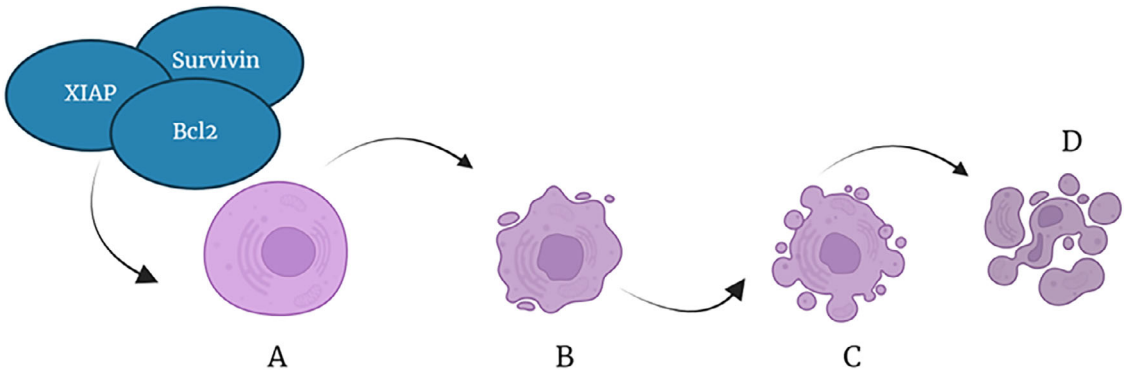

FIGURE 8 | Depiction of antiapoptotic proteins (XIAP, Bcl2, and survivin) suppressing apoptosis. The cell morphological changes during apoptosis. (A) Normal cell, (B) cell shrinkage and chromatin condensation, (C) membrane blebbing, and (D) formation of apoptotic bodies. BCL2, B-cell lymphoma 2; XIAP, X-linked inhibitor of apoptosis.

the ability of melanoma stem cells to self-renew, and inhibited the growth of melanoma cells in vitro and in vivo [123].

4.6 | EMT

The EMT is important for the embryonic process, which enables the acquisition of motility properties [124]. Abnormal expression of EMT TFs drives invasive and metastatic characteristics in cancer [125–127]. In melanoma, zinc finger E-box binding homeobox 1 (ZEB1, EMT TF) has been linked to the acquisition of stemness properties in melanoma, which has further been linked to the development of resistance to MAPK inhibitors (MAPKi) [126, 128]. This was validated through tumor cell lines and biopsies derived from patient tumors. The cell lines, biopsies and patient tumors displayed increased ZEB1 and resistance to MAPKi [126, 128]. EMT has also been identified as a driver in melanoma stem cells. The expression of EMT TFs (Snail family transcription repressor 2 [Slug], Snail, Zeb, Twist-related protein 1 [Twist], sex determining region Y box transcription factor [SOX], microphthalmia-associated transcription factor [MITF], and epithelial-splicing regulatory protein 1 [ESRP1]) have been linked to stemness traits in melanoma (Figure 9). The expression of Zeb1 has been linked to increased expression of molecular melanoma stem cell markers (CD133 and CD44) in murine melanoma (B16F10) and the knockdown of Zeb1 resulted in decreased metastatic potential of melanoma stem cells.

4.7 | VM

Maniotis et al. were the first to report the phenomenon of VM which was first identified in cutaneous and uveal melanoma [129, 130]. In VM, typically triggered during hypoxia, tumor

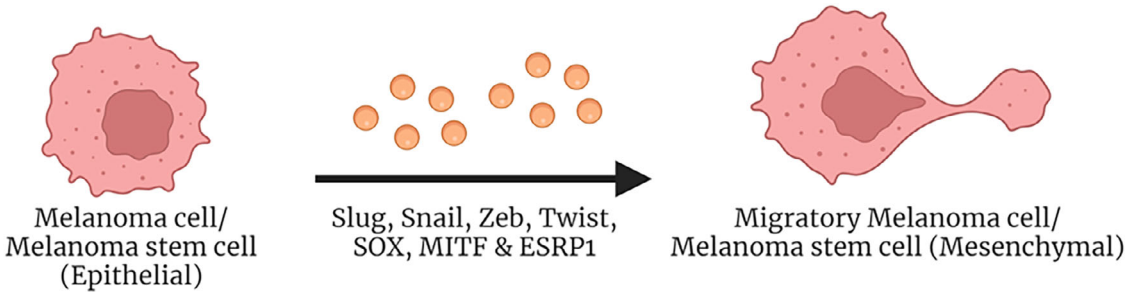

FIGURE 9 | Depiction of the acquisition of stemness factors (Slug, Snail, Zeb, Twist, SOX, MITF, and ESRP1) triggering the shift from an epithelial to a mesenchymal cell state. ESRP1, epithelial splicing regulatory protein 1; MITF, microphthalmia-associated transcription factor; Slug, Snail family transcription repressor 2; Snail, Snail family transcriptional repressor 1; SOX, SRY (sex determining region Y) box transcription factor; Twist, Twist-related protein 1; ZEB, zinc finger E-box-binding homeobox.

cells display the same characteristics as endothelial cells through the formation of capillaries and the provision of blood supply to tumors [8, 129]. The transition of melanoma cells from the radial growth phase to the vertical growth phase requires angiogenesis for the supply of nutrients and oxygens [131]. Due to the significance of angiogenesis in melanoma, various inhibitors of an angiogenic target, namely, VEGF, were developed but these inhibitors often displayed the acquisition of inherent or acquired resistance due to VEGF-independent mechanisms of angiogenesis such as VM [8, 132]. The acquisition of resistance to angiogenic inhibitors was further displayed by the monoclonal antibody, bevacizumab, enhancing VM [133]. Melanoma stem cells have also been implicated in VM [77, 134]. Melanoma cells undergoing VM display stemness and EMT which are traits linked to melanoma stem cells (Figure 9) [135]. The TFs expressed by melanoma cells undergoing VM are like those for EMT, and melanoma stem cells are found to actively participate in VM [77, 134].

4.8 | Ferroptosis

In addition to the various hallmarks of multidrug resistance in melanoma, ferroptosis, has also been identified as a novel driver of multidrug resistance in melanoma [136]. Ferroptosis, which was discovered in 2012, is an iron dependent form of programmed cell death driven by oxidative damage to cell membranes [137]. Melanoma cells preferentially metastasize through the lymphatic system, avoiding ferroptosis [136]. Higher levels of glutathione (GSH), oleic acid and lower levels of iron in the lymph provides an environment that protects melanoma cells from ferroptosis [136]. Various chemotherapy drugs induce ferroptosis but the evasion of ferroptosis in melanoma suggests that this could be one of the factors leading to multidrug resistance in melanoma [136]. There are three main pathways that regulate ferroptosis that are deregulated in melanoma: the canonical glutathione peroxidase 4 (GPX4) regulated pathway, iron metabolism pathway and lipid metabolism pathway [138]. The GPX4 regulated pathway is present in various cells as cells have antioxidant defense systems to protect against ferroptosis and oxidative stress. One of the key antioxidants, GSH, prevents oxidative damage to cell membranes and neutralizes ROS. In cells with high oxidative stress, GSH is depleted making the cell more vulnerable to ferroptosis (Figure 11). GPX4 can repair oxidative damage and scavenge ROS. The iron metabolism pathway is important for various cellular functions and plays an essential role in ferroptosis. Excessive amounts of iron in the iron metabolism pathway may lead to production of ROS through the Fenton reaction. Excessive amounts of ROS lead to lipid peroxidation (cell membrane damage) and cell death (Figure 10). Another key pathway in ferroptosis is the lipid metabolism pathway. Lipids are major components of cell membranes, specifically polyunsaturated fatty acids (PUFAs). Under high levels of oxidative stress (during ferroptosis) the PUFAs are affected by lipid hydroperoxides which alter membrane integrity and function. The alteration in membrane integrity and function results in cell death (Figure 12).

5 | Strategies to Overcome Multidrug Resistance in Melanoma and Melanoma Stem Cells

For the treatment of melanoma, chemotherapies, targeted therapies, and immunotherapies have been used but develop resistance. Reversing multidrug resistance in melanoma and melanoma stem cells may enhance the effectiveness of melanoma treatments. ABC transporters (ABCB5 and ABCG2) have been implicated in melanoma and melanoma stem cells.

5.1 | Inhibitors of ABC Transporters in Melanoma (ABCG2 and ABCB5)

The first ABCG2 inhibitor was a mycotoxin derived from *Aspergillus fumigatus*, FTC, which displayed promising in vitro activity but displayed neurotoxicity in vivo. Thus, derivatives of FTC, which displayed lower neurotoxic activity in vivo, were explored, such as Ko143 [139–141]. In addition to Ko143, elacridar (GF120918) and tariquidar (XR9576) were also frequently used as ABCG2 inhibitors [142, 143]. Considering that there is no Food and Drug Administration (FDA) or European Medicines Agency (EMA) approved ABCG2 inhibitor, febuxostat (clinically approved for hyperuricemia) was investigated and found to strongly inhibit ABCG2 in vitro and in vivo [143, 144]. Furthermore, febuxostat was effective at clinically relevant doses. It was identified as a potential ABCG2 inhibitor that could be used in humans [143, 144]. Furthermore, δ-tocotrienol, reduced the expression of ABCG2 in A375 melanospheres [145]. ABCB5 serves a dual role as an ABC transporter and as a stemness marker [146]. In melanoma cells treated with chemotherapeutics (dacarbazine and temozolomide), the selection pressure (treatment triggers

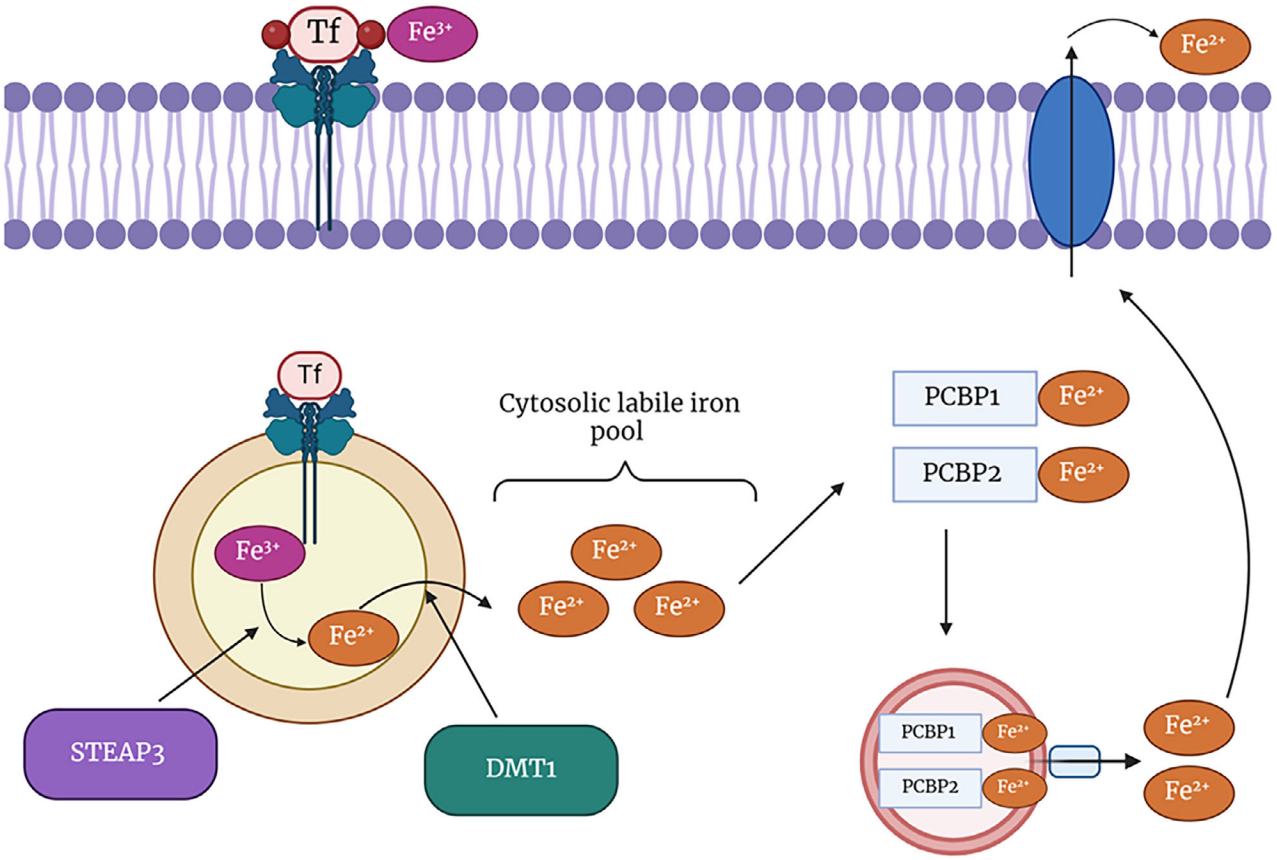

FIGURE 10 | Depiction of the iron metabolism pathway where TF binds Fe^{3+} and the iron loaded TF binds to TFR1. The TFR1 bound with TF is endocytosed and STEAP3 facilitates the conversion of Fe^{3+} to Fe^{2+} in the endosome. Fe^{2+} is transported into the cytosol by DMT1 forming a cytosolic labile iron pool. Fe^{2+} forms a complex with PCBP1/PCBP2 and is then transported to ferritin where Fe^{2+} is converted back to Fe^{3+} for storage. Ferritin can be degraded in the lysosome to form Fe^{2+} which is released in the cytosol and can be used for other cellular processes and transported out of the cell by ferroportin. DMT1, divalent metal transporter; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; PCBP1/PCBP2, poly(rC)-binding protein 1; STEAP3, six transmembrane epithelial antigens of the prostate 3; TF, transferrin; TFR1, transferrin receptor 1.

favor for resistant cells) for $ABCB5^{+}$ was increased leading to the development of resistance [147]. The inhibition of ABCB5 by a monoclonal antibody or short hairpin RNA (shRNA) sensitized glioblastoma cells to temozolomide resulting in apoptosis in vitro [148].

5.2 | Inhibitors of Major Signaling Pathways That Drive Multidrug Resistance in Melanoma

Several signaling pathways, such as the MAPK, PI3K, Hh, and notch signaling pathway are implicated in melanoma. The deregulation of the signaling pathways are linked to multidrug resistance in melanoma and melanoma stem cells. The table below displays various inhibitors of signaling pathways implicated in multidrug resistance in melanoma (Table 2).

5.3 | Inhibitors of Hypoxia

Various strategies have been developed in melanoma for the inhibition of hypoxia, such as reoxygenation through nano drugs that deliver oxygen to target organs [149, 150], inhibitors of HIF-1-α (semaxanib and thalidomide) [149, 151, 152] and hypoxia activated prodrugs [149, 153]. These strategies result in the inhibition of downstream target genes, such as VEGF, which regulates tumor angiogenesis [149, 154]. The inhibition of hypoxia also enhances the effectiveness of chemotherapeutics such as oxaliplatin due to the reversal of multidrug resistance [149, 155]. This highlights the significance of hypoxia inhibitors in melanoma.

5.4 | Reversal of Epigenetic Modifications

Histone deacetylases (HDACs) silence key tumor suppressor genes in melanoma such as PTEN [156]. Giles et al. investigated the silencing of PTEN by HDACs in seven melanoma cell lines (SKMEL239, WM4235, WM983. SKMEL173, SKMEL103, SKMEL192, and SKMEL14) using the HDAC inhibitor, panobinostat, at 15 nmol/L for 24 h [156]. Panobinostat enhanced PTEN expression in 57% of the melanoma cell lines and this was further confirmed by the decreased expression of p-Akt which is regulated by PTEN in the PI3K/Akt pathway [156].

5.5 | Inducers of Apoptosis

Apoptosis is a major hallmark of melanoma and various factors contribute to apoptosis such as antiapoptotic proteins (survivin and XIAP) [157–159]. Survivin is overexpressed in cell lines

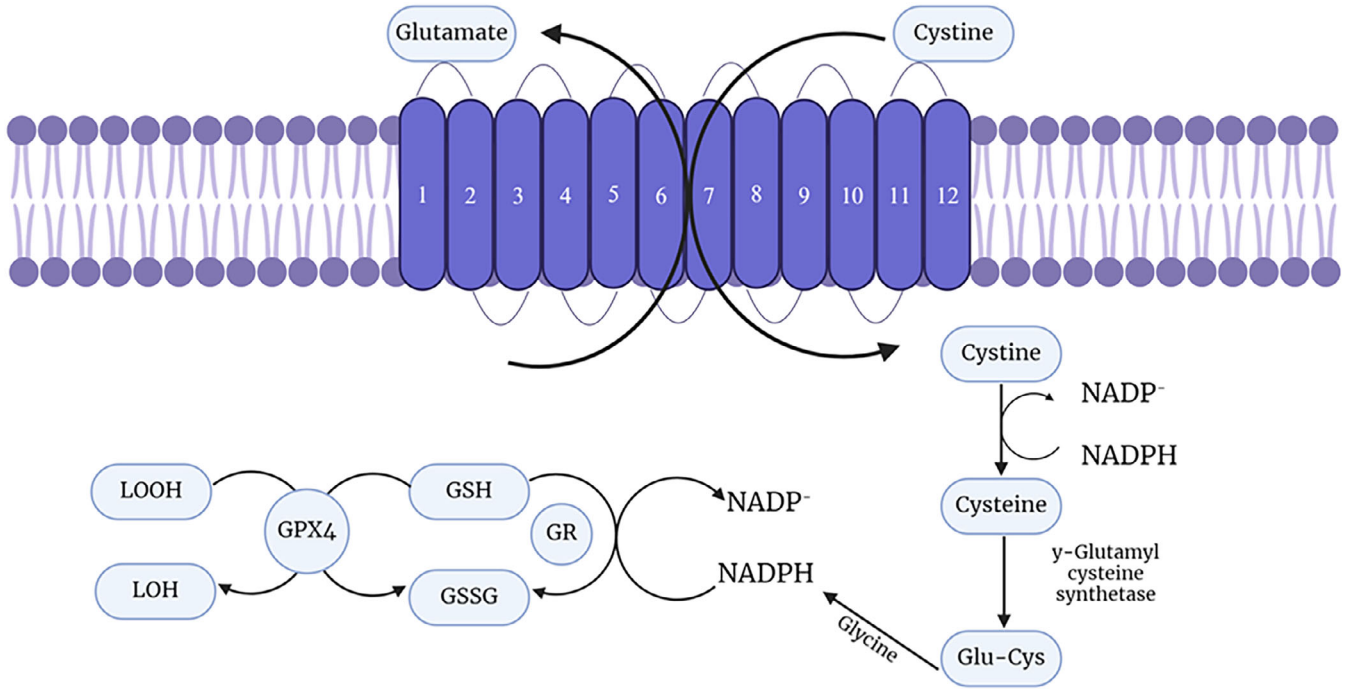

FIGURE 11 | Depiction of the canonical GPX4 pathway where cystine is transported into the cell by the cystine/glutamate antiporter (xCT) system. Cystine is subsequently converted to cysteine, then to Glu-Cys, and then converted to GSH by glycine. GPX4 facilitates the conversion of GSH to GSSG and LOOH to LOH. The accumulation of LOOH triggers ferroptosis and the Fenton reaction also enhances the production of LOOH in the presence of disfunctioning GPX4, thereby enhancing LOOH and triggering ferroptosis. Glu-Cys, glutamate–cysteine; GPX4, glutathione peroxidase 4; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; LOH, lipid alcohols; LOOH, lipid hydroperoxides; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate.

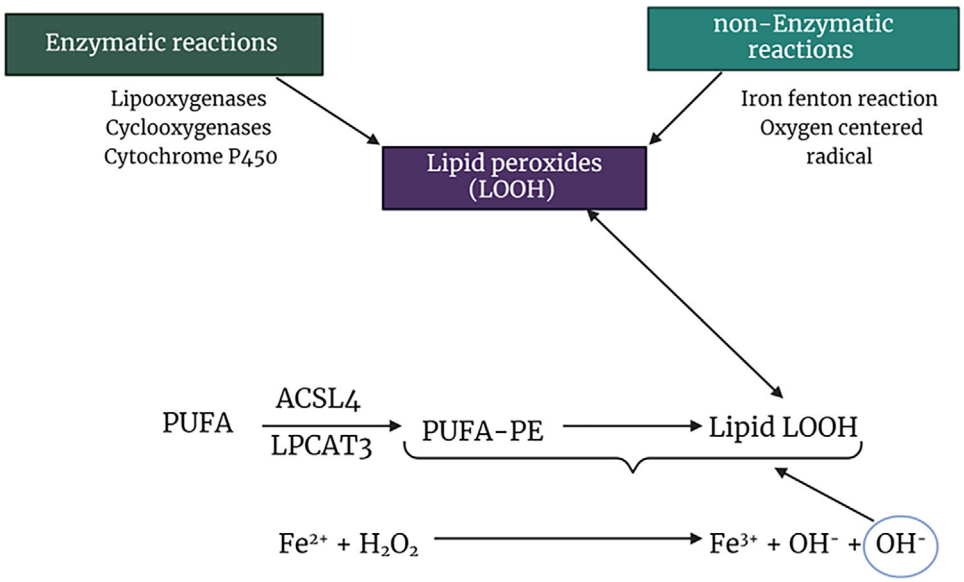

FIGURE 12 | Depiction of the lipid metabolism pathway in ferroptosis which comprises of enzymatic and nonenzymatic reactions. PUFA forms acyl-coA groups through ACSL4 and LPCAT3 forms PE using acyl-coA groups resulting in the formation of a PUFA-PE complex. The Fenton reaction where intracellular Fe^{2+} + H_2O_2 is converted to Fe^{3+} and OH^-. The OH^- trigger the conversion of PUFA-PE to LOOH. The accumulation of LOOH triggers ferroptosis. LOXs such as LOX-5 can also directly react with PUFA forming intermediates that are susceptible to lipid peroxidation. Cytochromes as well as cyclooxygenases contribute to ferroptosis through the generation of ROS that can trigger lipid peroxidation thereby triggering ferroptosis. ACSL4, acyl-CoA synthetase long chain family member 4; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; H_2O_2, hydrogen peroxide; LOOH, lipid hydroperoxides; LOX, lipooxygenase; LOX-5, lipooxygenase-5; LPCAT3, lysophosphatidylcholine acyltransferase; OH^-, hydroxyl radical; PUFA, polyunsaturated fatty acids; PUFA-PE, polyunsaturated fatty acid-phosphatidylethanolamine; ROS, reactive oxygen species.

TABLE 2 | Small molecule inhibitors of signaling pathways implicated in multidrug resistance in melanoma.

Signaling pathway	Target in signaling pathway	Inhibitor	Mechanism of action	Clinical studies	Phase	FDA approved	Multidrug resistance marker	References
MAPK	BRAF	Vemurafenib	Selectively inhibits $BRAF^{V600E}$ mutated melanoma cells, thereby reducing MAPK activation	NCT01271803 and NCT01400451	Phase IB and Phase I	Approved in 2011 for metastatic melanoma specifically with the $BRAF^{V600E}$ mutation		[265–267]
	MEK1/2	Trametinib	The MAPK pathway consists of Ras/Raf/MEK/ERK and BRAF activates MEK, thereby activating downstream targets. Trametinib is a selective MEK1/MEK2 inhibitor, elicits G1 cell cycle arrest and activates apoptosis	NCT01245062	Phase III	Approved in 2013 for BRAF mutant metastatic melanoma. Combination of trametinib and dabrafenib approved in 2014 for BRAF mutant metastatic melanoma	Qiu et al. displayed the inhibition of ABCB1 drug efflux through increased intracellular accumulation of rhodamine 123 and doxorubicin. Trametinib also increased the effect of vincristine on ABCB1-overexpressing xenograft cancer cells in nude mice	[268–271]
	BRAF and CRAF	Dabrafenib	Inhibitor of BRAF and CRAF through ATP competitive binding leading to decreased activation of MEK and ERK and subsequent increase in apoptosis and G1 cell cycle arrest	NCT01227889, NCT01584648, and NCT01597908	Phase III	Approved in 2013 as a monotherapy for BRAF mutant metastatic melanoma. Combination of dabrafenib and trametinib was approved in 2014 for BRAF mutant metastatic melanoma	Dabrafenib inhibited ABCG2 and enhanced the effects of mitoxantrone and docetaxel on their respective resistant cell lines	[269, 272–276]

(Continues)

TABLE 2 | (Continued)

Signaling pathway	Target in signaling pathway	Inhibitor	Mechanism of action	Clinical studies	Phase	FDA approved	Multidrug resistance marker	References
PI3K	All PI3K isoforms	Buparlisib	Pan-class I inhibitor of PI3K thus, it inhibits the four isomers of PI3K (PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ). Buparlisib antagonizes proliferation, angiogenesis, and enhances apoptosis	NCT01297452 NCT01155453	Phase Ib		In breast cancer, multidrug resistance is promoted by the activation of the PI3K pathway which results in reduced or abolished efficacy of cytotoxic drugs and the increase in cells with cancer stem cell traits. The inhibition of the PI3K pathway with a pan-PI3K inhibitor (buparlisib, BKM120) was explored resulting in enhanced apoptosis and decrease in sphere formation by the multidrug resistance (MDR) breast cancer cells	[277–280]
	PI3Kα	Alpelisib	Alpelisib is an isoform specific and oral selective inhibitor of PI3Kα	NCT02273219	Phase Ib	Combination of alpelisib and fulvestrant was approved by the FDA in 2019 for hormone receptor-positive (HR^+) and hormone receptor-negative ($HER2^-$) breast cancers		[281–283]

(Continues)

TABLE 2 | (Continued)

Signaling pathway	Target in signaling pathway	Inhibitor	Mechanism of action	Clinical studies	Phase	FDA approved	Multidrug resistance marker	References
	AKT	MK-2206	Allosteric inhibitor of AKT	NCT01480154	Phase I		MK-2206 enhanced the efficacy of SN-38, mitoxantrone and topotecan (substrates of ABCG2) at 0.3 and 1 μM in H460/MX20 and SI-MI-80 cells. Thus, MK-2206 also enhanced ABCG2 ATPase thus reversing MDR in ABCG2 overexpressing cells and hence enhanced the efficacy of SN-38, mitoxantrone and topotecan	[284, 285]
	AKT	GDC-0068	Selective competitive inhibitor of AKT	NCT03072238	Phase III			[286]
	AKT	AZD5363	Selective inhibitor of AKT in melanoma					[287]
	AKT	Perifosine	Alkylphospholipid, perifosine, allosterically inhibited Akt phosphorylation and activation. Richardson et al. further displayed the mechanism by which perifosine inhibited Akt in melanoma	NCT00053781	Phase II			[288–290]

(Continues)

TABLE 2 | (Continued)

Signaling pathway	Target in signaling pathway	Inhibitor	Mechanism of action	Clinical studies	Phase	FDA approved	Multidrug resistance marker	References
Sonic hedgehog signaling pathway	Gli	Cyclopamine	Downregulation of the sonic hedgehog signaling pathway through the inhibition of Gli				In prostate cancer cells (PC3), cyclopamine (natural steroid alkaloid) inhibits smoothened (SMO) and subsequently downregulated the expression of ABC transporters (ABCB1 and ABCG2)	[291–293]
	Smoothened (SMO)	NVP-LDE225	Inhibition of smoothened signaling mediator in sonic hedgehog signaling pathway			Approved on the July 24, 2015 for locally advanced basal cell carcinoma		[294, 295]
	Gli 1/2	GANT61	Inhibited Gli 1/2 in melanoma				In vemurafenib-resistant melanoma cells, GANT61 decreased invasion of melanoma cells in a 3D skin reconstruct model. Furthermore, treatment with GANT61 resensitized vemurafenib resistant melanoma cells to vemurafenib thus, reversed resistance to vemurafenib	[296]

(Continues)

TABLE 2 | (Continued)

Signaling pathway	Target in signaling pathway	Inhibitor	Mechanism of action	Clinical studies	Phase	FDA approved	Multidrug resistance marker	References
Notch signaling pathway	Gamma (γ)-secretase inhibitors	MK-0752	The downstream effector of the NOTCH pathway, HES1, was downregulated by MK-0752 which was displayed through Western blotting		Phase I			[297–300]
		R04929097	Activation of the NOTCH pathway occurs through the catalytic cleavage of the γ-secretase complex; thus, inhibition of the γ-secretase complex ensures that the NOTCH pathway is inactive. R04929097 inhibits effectors of the NOTCH pathway (HES1 and HEY-1)	NCT01120275	Phase II		R04929097 impairs the formation of melanospheres	[297, 299, 301]

Abbreviations: ABCB1, ATP-binding cassette subfamily B member 1; ABCG2, ATP-binding cassette subfamily G member 2; Akt, protein kinase B; BRAF, B-raf proto-oncogene; BRAF-V600E, B-raf proto-oncogene serine/threonine kinase with V600E mutation (substitution of valine by glutamic acid at position 600); ERK, extracellular regulated kinase; FDA, Food and Drug Administration; Gli, glioblastoma-associated oncogene; H460, nonsmall cell lung cancer cell line; $HER2^{-}$, human epidermal growth factor receptor-2 negative breast cancer; HES-1, hairy and enhancer of split-1; HEY-1, hairy/enhancer-of-split related with YRPW motif protein 1; HR^{+}, hormone receptor positive breast cancer; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein (MAP) kinase extracellular signal regulated kinase 1/2; MX20, multidrug-resistant ovarian cancer cell line; PI3K, phosphatidylinositol-3-kinase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma virus; SI-MI-80, human melanoma cell line.

derived from melanoma patients thus inhibitors of survivin have been explored [160]. Prodigiosin (isolated from marine bacteria) displayed cytostatic effects after 24 h on SKMEL19 and SKMEL28 cells [160]. Prodigiosin was found to induce apoptosis through enhanced cleavage of caspase-3, DNA damage, and downregulation of survivin [160].

5.6 | Inhibitors of EMT in Melanoma

Several factors drive EMT in melanoma, such as ZEB1(128). The inhibition of ZEB1 results in the sensitization of melanoma cells to BRAF inhibitors as ZEB1 largely accounts for phenotype switching, which leads to the formation of melanoma stem cells that are resistant to BRAF inhibitors [128]. Thus, the inhibition of ZEB1 is a potential strategy for the reversal of multidrug resistance in melanoma [161]. Small noncoding RNA molecules (miRNA) can silence gene expression of a specific mRNA by binding to the 3′-untranslated region of the mRNA. MicroRNA-3662 specifically targeted ZEB1 and inhibited EMT, metastasis and invasion in melanoma [161].

5.7 | Inhibitors of VM in Melanoma

Angiogenesis, the formation of blood vessels from pre-existing blood vessels, is required for physiological processes such as wound healing, menstrual cycle, and embryonic development [162]. Tumor cells use angiogenesis for nutrients and oxygen to maintain the metastasis of tumor cells, tumor growth and development [162]. Tumor cells also form vascular structures that resemble blood vessels for nutrients and oxygen independently of angiogenesis termed VM [163, 164]. Various strategies have been employed for the inhibition of VM [164]. The formation of tubular structures by melanoma cells was investigated on a 3D gel matrix for VM [164, 165]. The melanoma cells (C8161 and WM793) formed tubular structures indicative of VM after 24 h [164, 165]. The cells were treated with tivantinib (1 μM), which inhibited VM formation in both C8161 and WM793 cells [164, 165]. Thus, tivantinib could be explored for the inhibition of VM in melanoma [164, 165].

5.8 | Inducers of Ferroptosis

Ferroptosis is a novel driver of multidrug resistance in melanoma. The pharmacological and genetic regulation of the three main pathways governing ferroptosis (canonical GPX4 regulated pathway, iron metabolism pathway, and lipid metabolism pathway) reversed multidrug resistance in melanoma [137]. The MAPK pathway is a major oncogenic signaling pathway in melanoma and several inhibitors, for example, vemurafenib, have been developed to target BRAF (predominant driver of MAPK pathway) [55, 56]. These inhibitors are often ineffective due to the development of multidrug resistance. The co-administration of BRAF inhibitors and ferroptosis inducing agents in melanoma have displayed promising results [137, 166]. The BRAF inhibitor (vemurafenib), co-administered with the Axl receptor tyrosine kinase (AXL) inhibitor (BGB324), induced ferroptosis and sensitized melanoma cells (A375) to vemurafenib [137, 166]. Another small molecule inhibitor, sorafenib, also enhanced the sensitivity of metastatic melanoma cells to vemurafenib through ferroptosis [137, 167].

6 | Reversal of Multidrug Resistance Using Phytochemicals

Various therapeutics for the treatment of melanoma have been derived from medicinal plants with superior efficacy such as curcumin, resveratrol, quercetin, epigallocatechin gallate (ECG), genistein, and berberine. The conventional treatment modalities for melanoma are surgery, chemotherapy, and radiotherapy. These conventional treatment modalities often show modest treatment success followed by relapse and the development of multidrug resistance. Thus, targeted therapies and immunotherapies came to the fore but the treatment failures with these therapeutics is largely due to the development of multidrug resistance (various mechanisms of multidrug resistance discussed previously) as well as the presence of subpopulations of melanoma stem cells that are resistant to the therapeutics discussed previously due to the enhanced expression of multidrug resistance drivers particularly in melanoma stem cells.

The vitamin E derivatives (δ- and γ-tocotrienol) (Figure 13A,B) displayed inhibition of multidrug resistance in melanoma [45, 168]. Particularly, δ-tocotrienol, specifically targets the ABCG2 positive cancer stem cells subpopulation in the melanoma cell line, A375, preventing the formation of melanospheres and inducing disaggregation [45, 168]. The inhibition of apoptosis is viewed as a means of inhibition of multidrug resistance and δ-tocotrienol exerts significant apoptotic activity in cutaneous melanoma cells by triggering the endoplasmic reticulum (ER) stress pro death pathway [45, 168]. γ-Tocotrienol elicited apoptosis in G361 melanoma cells after treatment with γ-tocotrienol at 60 μM [169]. The activation of apoptosis was evident through an enhanced sub-G1 population and activation of procaspase-3, -7, and -9 and poly(ADP-ribose) polymerase (PARP) [169]. γ-Tocotrienol (at 10 and 30 μM) inhibited cell invasion which was evidenced as determined by the Matrigel cell invasion assay [169]. Furthermore, γ-tocotrienol (at 40 and 60 μM) upregulated E-cadherin, β-catenin, γ-catenin (epithelial markers), and repressed Snail (repressor of E-cadherin) as measured by Western blotting [169].

Another phytochemical displaying reversal of multidrug resistance is curcumin (Figure 13C) [170]. Curcumin (active component of turmeric) is derived from the rhizome of *Curcuma longa* L. [170]. Curcumin (at 25 and 15 μM) suppressed the expression of proteins downstream of PI3K (p-Akt, p-mTOR, and ribosomal protein S6 kinase β-1 [p70S6K]) [170]. Treatment with curcumin at the same concentrations incubated for 72 h significantly reduced the invasive potential of A375 and C1861 melanoma cells compared to untreated cells in the Matrigel model of the basement membrane [170]. VM is also regarded to be a major contributor of multidrug resistance in melanoma and in one study, the choroidal murine melanoma model was used to assess VM [171]. The mice were treated with 100 mg/kg curcumin for 18 days which commenced on Day 3 of the experiment [171]. After treatment with curcumin, VM was reduced, through immunohistochemical analysis, epithelial cell kinase (EphA2), PI3K and MMP-2 and -9 were decreased compared to the control

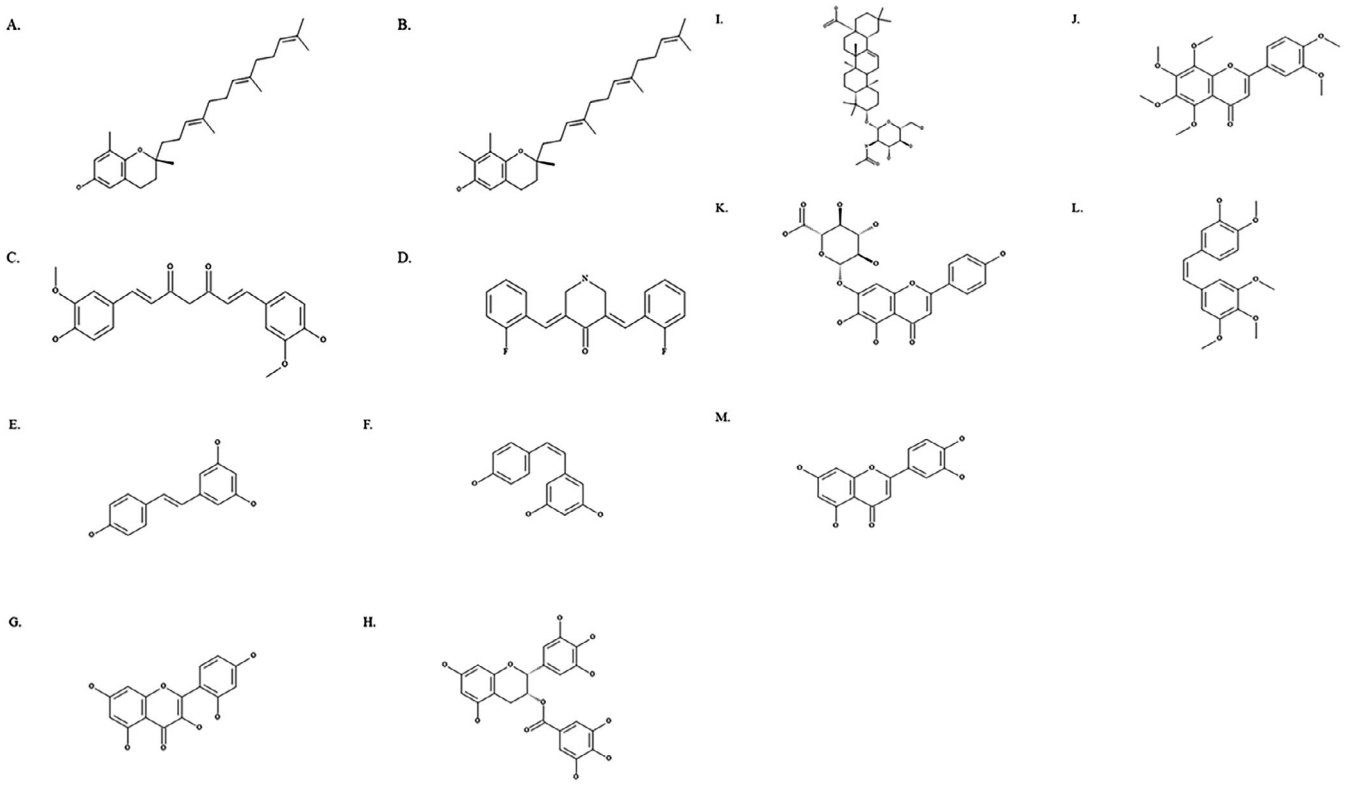

FIGURE 13 | Chemical structures of compounds that reversed the hallmarks of multidrug resistance in metastatic melanoma. (A and B) Vitamin E derivatives (gamma and delta tocotrienol), (C) curcumin, (D) diphenyl-difluoroketone (EF24, analog of curcumin), (E) resveratrol, (F) morin, (G) epigallocatechin gallate catechin (EGCG), (H) aridanin, (I) nobiletin, (J) gambogic acid, (K) scutellarin, (L) combrestatin A4, and (M) luteolin.

group (100 mg/kg poloxamer-F68) [171]. Through real time PCR, the mRNA levels of EphA2, PI3K, MMP-2, and MMP-9 were reduced [171]. In another study, an analog of curcumin, diphenyl difluoroketone (EF24) displayed enhanced bioavailability and tolerance compared to free curcumin (Figure 13D) [172]. Diphenyl difluoroketone (EF24) also abrogated the metastatic behavior and EMT in melanoma cells (Lu1205 and A375) through induction of E-cadherin, dephosphorylation of STAT3, and downregulation of vimentin and neural-cadherin (N-cadherin) [172]. The treatment of human melanoma cells with curcumin has also resulted in the inhibition of the expression of notch-1 (NOTCH signaling pathway) and sex-determining region Y box transcription factor 10 (SOX10) (nuclear TF that serves as a marker for metastatic melanoma) [173, 174].

Resveratrol is another phytochemical with noteworthy multidrug resistance reversal potential. Resveratrol was initially isolated from the roots of *Polygonum cuspidatum* Siebold & Zucc. but is also found in peanuts, grapes and berries [175]. Resveratrol occurs as two geometric isomers: cis and trans-resveratrol, whereby the biological activity exhibited by resveratrol is often associated with *trans*-resveratrol (Figure 13E,F) [175]. In a mouse melanoma model, lipopolysaccharide (LPS)-induced EMT and markers of EMT were significantly inhibited by resveratrol thereby prolonging animal survival and reducing tumor size [176]. Through immunoblot assays, resveratrol inhibited N-cadherin and snail at 3.2 and 16 μg/mL, respectively, in murine melanoma cells (K1735) [176]. Vartanian et al. postulated that VM evidenced by the formation of capillary-like structures (CLS) was related to the ROS level. Thus, antioxidants such as resveratrol (10 μmol/L) reduced the formation of CLS in human melanoma (Mel II) cells [177, 178]. Resveratrol in a mouse melanoma model intraperitoneally administered in vivo at 2.5 and 10 mg/kg of bodyweight ip body weight inhibited vascular channel formation [178]. Hypoxia is regarded to be a major mediator of multidrug resistance in melanoma and resveratrol (50 μM) significantly inhibited HIF-1-α after 48 h in A375 cells [179].

In terms of the inhibition of mediators of multidrug resistance in melanoma by phytochemicals, melanoma stem cells also play a pivotal role in the mediation of multidrug resistance as discussed previously, thus the subpopulation of melanoma (MV3) cells that express $CD133^+$ were treated with the polyphenol, morin, which at 50 μM displayed a fivefold increase in microRNA-216a (miR216-a) through qRT-PCR (Figure 13G) [180]. The enhanced expression of miR216-a resulted in decreased cell proliferation, reduced sphere formation and reduced stemness markers (CD20, CD133, and CD44) [180, 181].

Epigallocatechin gallate catechin (EGCG), a major polyphenol found in green tea (*Camellia sinensis* (L.) Kuntze) (Figure 13H), suppressed the formation of CLS in Mel II cells at a concentration of 50 μM after 24 h, highlighting the inhibition of VM in melanoma [178, 182]. As mentioned previously, the deregulation of apoptosis mediates multidrug resistance in melanoma. EGCG at 10 μg/mL triggered apoptosis through the significant upregulation of Fas ligand (FAS-L) after 48 h in melanoma (Hs-294T cells), which was evaluated through flow cytometry

[183]. A novel EGCG analog (4-(*S*)-(2,4,6-trimethylthiobenzyl)-ECG induced apoptosis in B16F10 melanoma cells by triggering the activation of autophagy and ROS [184]. A nanocomplex comprising of EGCG and lanthanide metal ions (Sm^{3+}), Sm^{III}-EGCG, inhibited melanoma cell migration in vitro. Furthermore, SM^{III}-EGCG inhibited the migration of metastatic lung melanoma in a mouse melanoma tumor model [185].

The *N*-acetylglucoside of oleanolic acid, aridanin (triterpenoid saponin widely isolated from *Tetrapleura tetraptera* (Schumach. & Thonn.) Taub. (Figure 13I), induced ferroptosis in melanoma cells through the deactivation of antioxidant systems and induction of ROS, thereby inducing ferroptosis [186, 187]. Nobiletin (polymethoxyflavone) (Figure 13J) induced ferroptosis in SK-MEL-28 melanoma cells through overexpression of glycogen synthase kinase 3β (GSK3β), which led to the inhibition of the pathway regulating the antioxidant system Kelch-like-ECH-associated protein 1/nuclear factor erythoid-2-related factor 2/heme oxygenase 1 (Keap1/NRF2/HO-1) [186, 188].

Gambogic acid (polyprenylated xanthone) extracted from the resin of *Garciania hanburyi* Hook.f. mitigated multidrug resistance in metastatic melanoma through the inhibition of migration, invasion, and angiogenesis [173, 189]. The inhibition of migration, invasion, and angiogenesis by gambogic acid in melanoma was mediated through the inhibition of the PI3K and MAPK signaling pathways which lead to the suppression of angiogenesis and EMT [173, 189]. Ipobscurine, an indole alkaloid from *Ipomoea obscura* (L.) Ker Gawl. inhibited migration and invasion in murine melanoma cell models and exerted the anti-angiogenic activity in vivo [173, 190, 191]. Scutellarin, a flavone isolated from *Erigeron breviscapus* (Vaniot) Hand.-Mazz. (Figure 13K), inhibited migration, invasion, and angiogenesis through the inhibition of EMT and angiogenesis via the inhibition of the PI3K/Akt/mTOR pathway in melanoma cells [192].

DIM-D, bis(triethylammonium)tris[1,1-bis(indol-3-yl)-1-(3,4-catecholate)-methane]vandate(IV) complex enhanced the formation of ROS mediated through the loss of mitochondrial membrane potential and subsequently mitochondrial damage leading to G2/M cell cycle arrest in 518A2 melanoma cells [193, 194]. Combrestatin A4 (cis-stilbene), isolated from the South African *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (Figure 13L), was used to derive two *N*-heterocyclic gold complexes [10, 11, 195]. These complexes reorganized the actin cytoskeleton in 518A2 melanoma cells resulting in the formation of stress fibers, displayed through immunofluorescence [195]. The effect on the cell cycle was evaluated and complex 10 and 11 induced G1 cell cycle arrest, whereas combrestatin A4 had elicited G2/M cell cycle arrest in line with complete destruction of the microtubule cytoskeleton [195]. Complex 10 and 11 inhibited cell migration which was displayed through a wound healing assay and was comparable to combrestatin A4 in 518A2 melanoma cells [195]. Luteolin (flavonoid) (Figure 13M) inhibited EMT in melanoma cells (A375 and B16F10) cells which was displayed through the upregulation of E-cadherin, downregulation of N-cadherin and vimentin at the mRNA and protein levels [196, 197]. The inhibition of EMT in melanoma cells subsequently led to the inhibition of the HIF-1α/VEGF cascade in melanoma cells [196, 197].

7 | Strategies for Improved Clinical Translation of Phytochemicals

7.1 | Effect of Plant Part, Extraction Method, and Solvent

The limited clinical translation of phytochemicals is due to differing biological activity across batches. To ensure consistency, standardize the plant part, extraction method and solvent for the target phytochemical or biological activity. Dziki et al. reported on the extraction of the leaves and roots of *Ajuga repens* L. through ultrasound-assisted extraction (UAE) using 70% ethanol. The phytochemical profiles of the root and leaf extracts were evaluated through high performance-liquid chromatography diode array detector (HPLC-DAD). The leaves contained flavonoids (apigenin and quercetin), phenethyl glycosides (verbacoside and isoverbacoside) and phenolic acids (chlorogenic acid, gallic acid and neochlorogenic acid), whereas in the roots, flavonoids and phenolic acids were not detected. The antioxidant and anti-collagenase activity was evaluated, where the leaf extract showed significant antioxidant potential (47.76%) compared to the roots (16.47%) in the DPPH assay, whereas the roots significantly inhibited collagenase (66.96%) compared to the leaves (49.37%) [198]. This study showed how different plant parts have different phytochemical profiles and biological activities. In another study, the ethanolic and aqueous extracts of *Madhuca longifolia* (J. Koenig ex L.) J.F. Macbr. (MLE and MLA) showed different phytochemical profiles by UHPLC-MS. The MLE contained phenolic acids, procyanidins and triterpenoids but triterpenoids were absent in MLA. MLE showed the most significant activity against human melanoma cell lines (1205-Lu and Me45) with 50% minimum inhibitory concentration (IC_{50}) values of 2.57 ± 0.36 and 15.13 ± 1.02 μg/mL, respectively, whereas MLA had IC_{50} values > 200 μg/mL against the same cell lines [199]. This study showed that extracts made with different solvents have different biological activities. Triterpenoids in MLE have been linked to chemopreventive and anticancer effects in melanoma, implying that different solvents with different polarities yield different compounds. Brown algae (*Sargassum polycystum* C.Agardh) was extracted using cold maceration (CM) and UAE. The total flavonoid and phenolic content (TFC and TPC) of brown algae in CM and UAE were compared. UAE extracts had significantly higher TPC (55 mg GAE/g) than CM extracts (21 mg GAE/g), however. UAE and CM extracts had the same TFC. Furthermore, the UAE yield was higher (6.5%) than CM (2.5%) as UAE breaks cell walls and membranes enabling the release of more phenolic acids. The effect of *S. polycystum* C.Agardh (UAE and CM) was evaluated on a murine melanoma (B16F10) cell line. The IC_{50} values obtained for UAE and CM were 70.89 ± 1.85 and 259.5 ± 2.41 μg/mL, showing a statistically significantly ($p < 0.01$) difference in activity [200]. This study further showed how an extraction method changed the phytochemical profile and biological activity.

The studies above collectively showed how different solvents, extraction methods and plant parts yield different biological activity. Thus, it is important that the most effective solvent, extraction method or plant part is selected to ensure batch-to-batch consistency. Dziki et al. illustrated the different biological activities of the leaves and roots which could be due to the

different physiological roles of the plant parts. Leaves produce flavonoids and phenolic acids under sunlight, while roots produce terpenoids and alkaloids in response to fungal endophytes [201, 202]. Leaves are better for antioxidants, while roots are better for anti-collagenase activity. Thus, if the wrong plant part is used, the potency of the biological activity may be reduced resulting in the upregulation of multidrug resistance markers, limiting clinical translation of phytochemicals.

7.2 | Improved Pharmacokinetic Profile

The absorption, distribution, breakdown, and removal of phytochemicals from the body can be described as pharmacokinetics. Phytochemicals, such as resveratrol, can display low aqueous solubility, stability and bioavailability [203]. For the improvement of the poor pharmacokinetic properties of resveratrol, derivatives of the compound have been synthesized. Natural or synthetic dimethyl derivatives displayed increased lipophilicity, metabolic stability and fast maximal absorption in the bloodstream [204]. Basri et al. showed that pterostilbene, dimethyl derivative of resveratrol, was more active at lower concentrations compared to resveratrol [205]. In ultraviolet radiation B (UVB) irradiated B164A5 mouse melanoma cells treated with pterostilbene (10 μM) and resveratrol (100 μM), the melanin content was reduced to 27.34 ± 0.98 and 25.54 ± 3.04 μg/mL, respectively [205]. This highlighted that at a lower concentration of 10 μM, pterostilbene displayed comparable activity to resveratrol at 100 μM. The improved pharmacokinetic profile of pterostilbene displays improved or comparable biological activity. Pterostilbene significantly inhibited amelanotic (C32) and melanotic (A2058) melanoma cell proliferation. The IC_{50} values against C32 were 21.45 μM, while against A2058, they were 42.70 μM. Pterostilbene (40 μM) increased the expression of apoptosis related proteins (BAX, caspase-3 and -9) at the transcriptional level [206]. Curcumin and its analogs were evaluated against human melanoma (A375) and normal human fibroblasts (NHF) through the (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetraziol]-1,3-benzene disulfonate) (WST-1 assay) [206]. Curcumin had an $IC_{50} > 20$ μM against A375, while monocarbonyl analogs (Compounds A and I) had IC_{50} values of 1 and 2 μM. Compounds A and I decreased cell viability at 2.5 μM on NHF, while curcumin showed no decrease in cell viability [207]. In a Phase I study, curcumin taken orally at the highest dose of 3.6 g was detected in plasma together with metabolites (curcumin sulfates and curcumin glucoronides). The only toxicity observed for doses as high as 3.6 g was mild diarrhea. Curcumin and associated metabolites were detected in urine and feces due to low systemic bioavailability [208]. In a Phase I dose-escalation study for advanced or metastatic cancer (melanoma), liposomal curcumin delivered intravenously at 300 mg/m^2 for 6 and 8 h displayed higher plasma concentrations of 1428 and 1641 ng/mL, respectively, during infusion [209]. The plasma concentrations dropped immediately after infusion with only one patient displaying a drop to 251 ng/mL, 45 min post-infusion [209]. This illustrates how liposomes can improve the pharmacokinetic profile but also highlights the need for alternative formulations to further improve the maintenance of plasma concentrations post infusion. In another Phase I clinical trial evaluating the effect of an oleoresin based turmeric formulation (CURCUGEN) compared to a standardized curcuminoid extract (C-95) [210]. CURCUGEN displayed a higher C_{max} (peak concentration in the blood) and AUC (area under the curve, exposure time) was higher than the standard curcuminoid extract (C-95), showing that CURCUGEN, delivers more curcumin and active metabolites to the bloodstream than C-95 [210]. Highlighting how different formulations can alter the pharmacokinetic profile and how additional research is required as improved pharmacokinetics corresponds with higher biological activity. Improving the pharmacokinetic profiles of phytochemicals could escalate their clinical translation.

The studies illustrated the importance of formulation and pharmacokinetic profile for biological activity. Pterostilbene (10 μM) showed comparable activity to resveratrol (100 μM). Resveratrol metabolizes rapidly, while Pterostilbene metabolizes slower due to its improved profile. The biological activity is linked to the parent compound and longer circulation enhances biological activity. With increased metabolism of resveratrol, metabolites are formed, and the concentration of the biologically active compound decreases. This leads to subpopulations of resistant cells with acquired mutations that drive multidrug resistance, reducing treatment efficacy. Further validating the use of derivatives and other formulations to improve the pharmacokinetic profile.

7.3 | Improved Preclinical Methods

Preclinical models aid with the identification and evaluation of potential lead compounds for subsequent clinical trials. The most common model used for screening the pharmacodynamic potential of phytochemicals against cancer are 2D cell culture models. 2D cell cultures display different results due to genetic variability and do not mimic the same microenvironment that cancer cells are found within in the human body. Therefore, positive results obtained from 2D cell cultures do not translate clinically. Factors such as improved cellular differentiation, mechanical properties, physiologically relevant cell morphology, drug metabolism and secretion profiles make 3D cell cultures more suitable for drug screening [211]. Chinembiri et al. evaluated the in vitro efficacy and selectivity of *Withania somnifera* (L.) Dunal using 2D and 3D human melanoma (A375) models [212]. The IC_{50} of the 80% ethanolic extract of *W. somnifera* (L.) Dunal against A375 (2D) and A375 (3D) was 0.51 ± 0.02 and 21.88 ± 0.17 μg/mL, respectively, whereas the IC_{50} values against human keratinocytes (HaCat) were 0.52 ± 0.02 and 12.23 ± 0.05 μg/mL, respectively. The selectivity index (SI) values calculated by dividing the IC_{50} (HaCat, nontumorigenic) by the IC_{50} (A375, tumorigenic) were higher for 2D (1.02) compared to 3D (0.56). This highlights that through a model that closely mimics tumor tissue and the extracellular matrix, the values differ substantially, however, the compound (Withaferin A) displayed comparable IC_{50} values of 26.25 ± 1.16 (A375, 2D) and 29.14 ± 1.16 (A375, 3D). A higher IC_{50} was observed for the *W. somnifera* 80% ethanolic extract on the 3D model compared to the 2D model [212]. This suggests the phytochemical is less active and ineffective for clinical translation on a physiologically relevant model. The SI was lower on the 3D model, indicating increased toxicity and making the phytochemical unsuitable. 2D and 3D models may display similar results. 3D models are more accurate but develop cellular heterogeneity, reducing accuracy. 2D models are effective for initial high-throughput screening but should not

be used for clinical translation. 3D cell models mimic tissue and tumor complexity, providing an accurate representation of cell interactions and tissue architecture, making them more suitable for therapeutics due to their physiological relevance [213].

As highlighted by Angeli et al. 2D models are not accurate for the effect of phytochemicals on MDR markers, while 3D models are more accurate as they mimic tumor complexity and cell interactions. 3D models are also more heterogenous and carry mutational profiles like tumors, making them a better representation for clinical translation. Evaluating the effect of phytochemicals on MDR markers would increase clinical translation, as MDR remains a major drawback for clinical translation.

Spheroids, clusters of single or different cells, represent limited drug absorption and decreased drug effectiveness due to hypoxia [213]. Organoids are more beneficial when developed using patient biopsies to maintain tumor heterogeneity and mutational landscape, providing an accurate representation of phytochemical activity in humans with tumor heterogeneity [214], therefore screening on organoids is more relevant than 2D and 3D spheroid models. Patient-derived organoids generated using dissociated melanoma brain metastasis from patients, which were seeded on ultra-low attachment plates in melanoma brain metastasis patient-derived organoid culture media. The mutational landscape of the organoids was determined through next-generation sequencing (NGS). Five out of seven of the organoids were $BRAF^{V600E}$ and two were BRAF wild-type but had NRAS and KIT mutations, respectively. The organoids harboring $BRAF^{V600E}$ were sensitive to BRAF and MEK inhibitors, whereas the two with NRAS and KIT mutations were resistant to BRAF and MEK inhibitors [215]. There is limited data on the generation of patient-derived organoids from cutaneous melanoma. Ou et al. highlighted the generation of patient-derived organoids from patients with diverse mutation profiles. The screening of small molecule inhibitors: PI3K inhibitor (AZD8186, copanlisib), Bcl-xl inhibitor (navitoclax), HDAC inhibitor (entinostat) yielded varying results through the CCK-8 assay [216]. The varying results underscored tumor heterogeneity, so response aligns with the mutational landscape. This highlights how personalized medicine aids in patient-specific treatments.

The development of more complex models that closely mimic the tumor microenvironment and display the extensive vascular network would further aid in the accurate assessment of biological activity in a physiologically relevant model. Quintard et al. developed an organ-on-a-chip model that consists of mesenchymal and pancreatic islet spheroids. These spheroids are connected to blood vessel organoids generated from stem cells that perfuse oxygen and nutrients to the spheroids through the microfluidic chip [217]. This vascularized organ-on-a-chip model offers a physiologically relevant model. Fisetin inhibited growth of colorectal cancer patient-derived organoids dose-dependently. In a colorectal cancer patient-derived organoid xenograft (PDOX) model, A-kinase anchoring protein 12 (AKAP12) was increased by Fisetin. The upregulation of AKAP12 inhibited VEGF and epithelial cell adhesion molecule (EpCAM) [218]. Thus, for improved preclinical evaluation of phytochemicals for melanoma, patient-derived organoids or vascularized patient-derived organ-on-a-chip-model would increase clinical translation.

7.4 | Improved Pharmacodynamic Profile

Biomarkers detected in blood (liquid biopsies) are preferred over more invasive procedures, such as tissue biopsies for improved detection and monitoring of melanoma [219]. Exosomes are extracellular vesicles that are secreted by most cells and facilitate intercellular communication. Exosomes carry molecular markers such as noncoding RNAs (miRNAs, lncRNAs, and circRNAs), DNA and RNA. The level of drug resistance and immune evasion can be determined by the exosomal miRNA. Exosomes also serve as more efficient biomarkers due to the secretion of metastasis markers, therefore, metastasis can be detected earlier increasing treatment effectiveness [220]. Immunotherapies have revolutionized the treatment of metastatic melanoma. In some instances, the effectiveness of immunotherapies decreases and biomarkers such as ZEB1 can be monitored through liquid biopsies. Exosomes may also contain ZEB1 mRNA or ZEB1 protein which can be detected and linked to efficacy of immunotherapies. The ZEB1 microRNAs (miR200 or miR205) serve as more reliable biomarkers in liquid biopsies as they are expressed more abundantly [221]. Circulating tumor cells (CTCs) and circulating tumor DNA (CtDNA) in liquid biopsies can serve as biomarkers for metastatic melanoma. CTCs correlate with OS, disease progression- and relapse-free survival (RFS). In a clinical trial, CTCs above 1 (>1) predicted relapse. CtDNA in plasma correlates with decreased OS and adjuvant therapy effectiveness. In 32 BRAF mutant melanoma patients, CtDNA detection correlated with decreased survival. Of 11 of 32 patients with CtDNA, the OS rate was 54.6% compared to 95% in CtDNA negative patients [222–224]. This highlights how optimization of dosage of phytochemicals may decrease CTCs or CtDNA for enhanced efficacy. Monitoring these markers after treatment with phytochemicals will help elucidate the efficacy of phytochemicals in patients with varying CTCs or CtDNA levels. These strategies aid clinical translation of phytochemicals across patient cohorts.

8 | Discussion

Various treatment modalities have been developed for the treatment of metastatic melanoma such as surgery, radiation therapy, and chemotherapy [225]. These conventional treatment modalities are often ineffective for metastatic melanoma [225]. Targeted therapies and immunotherapies have largely displayed enhanced progression-free survival and OS rates in clinical studies [2]. These enhanced effects are often obscured by the development of multidrug resistance [2]. Small molecule drugs that have already been approved for the treatment of solid cancers (e.g., metastatic melanoma) have been further repurposed as potential inhibitors of the hallmarks of multidrug resistance [226] (Table 2). These small molecule drugs, although efficacious, often display dose-limiting toxicities and thus, phytochemicals have also been explored as inhibitors of hallmarks of multidrug resistance [227]. Most small molecule drugs approved from 1981 to 2014 originate from natural products and around 50% of anticancer drugs approved from 1940 to 2014 were derived from natural products highlighting the relevance of phytochemicals as potential multidrug resistance inhibitors [227–229].

Phytochemicals from various classes (flavonoids, phenolics, terpenoids, alkaloids, carotenoids, stilbenoids, lignans, polyketides,

nitrogen-containing compounds, and curcuminoids) have multifactorial effects such as inhibiting more than one of the various hallmarks of multidrug resistance [228, 230]. The development of multidrug resistance in cancer was largely attributed to the overexpression of ABC transporters such as P-gp, ABCB5, and ABCG2 [52]. Three generations of inhibitors were developed to enhance the efficacy of chemotherapeutics. The first-generation ABC transporter inhibitors (verapamil, quinidine, and cylosporin A) were ineffective and toxic at therapeutic doses [231, 232]. Second generation inhibitors such as valspodar (PSC-833) were more effective but elicited pharmacokinetic interactions with cytochrome P450 3A4 (CYP3A4), thereby reducing drug metabolism and clearance [231, 233]. Reduced drug metabolism and clearance resulted in increased chemotherapeutic concentrations and therefore, increased adverse effects linked to the chemotherapeutics [231, 233]. To circumvent this, the doses of chemotherapeutics were reduced in trials assessing valspodar but due to the variable expression of CYP3A4 in patients, some patients' doses were too low or too high [231, 233]. Third generation inhibitors (tariquidar [XR9576], zosuquidar [LY-335979], laniquidar [R101933], and CBT-1 [CP100356]) were more potent, minimal pharmacokinetic interactions and nontoxic [231, 234]. Tariquidar was found to reduce the efflux of the P-gp substrate (rhodamine 123) for 48 h after a single dose and in a Phase I study, tariquidar in combination with chemotherapeutics (vinorelbine, paclitaxel, or doxorubicin) showed no pharmacokinetic interactions or significant side effects [231, 235]. However, two Phase II clinical studies were suspended due to the toxicities displayed after the combination of tariquidar with chemotherapeutics for patients with nonsmall cell lung cancer (NSCLC) [231, 236, 237].

Results from clinical trials investigating whether the inhibition of ABC transporters may lead to enhanced accumulation of chemotherapeutics, targeted therapeutics, and immunotherapies may be misleading as a standardized methodology for assessing the effect of the inhibition of ABC transporters has not been established [231]. Preclinical studies are often based on highly drug-resistant murine models and a moderate increase in P-gp expression resulted in doxorubicin resistance in a mouse model for hereditary breast cancer [231, 238]. The moderate increase in P-gp expression was below the level of expression of P-gp in normal tissues (e.g., gut, liver, and kidneys) [231, 238]. The determination of the level of P-gp expression for patient selection would result in more accurate and reliable clinical trial data [231, 238].

Phytochemicals have also been identified as "fourth generation" ABC transporter modulators and offer a plethora of potential modulators as the chemical structures of phytochemicals can be altered thereby enhancing their efficacy as ABC transporter modulators [16, 239]. Phytochemicals also overcame multidrug resistance in synergy with existing anticancer drugs which is favorable as the first line treatments for cancer are still surgery (benign cancers) and chemotherapy (metastatic cancers) [16, 240]. Although the efficacy of phytochemicals for the reversal of multidrug resistance has not been confirmed clinically, phytochemicals have displayed the reversal of multidrug resistance in several preclinical studies [16]. In addition to ABC transporters, there are other hallmarks of multidrug resistance in melanoma such as the deregulation of signaling cascades (MAPK, PI3K, Hh, and Notch signaling pathways), enhanced hypoxia, epigenetic modifications, antiapoptotic proteins, EMT, VM, and ferroptosis. Various phytochemicals such as curcumin and EGCG altered various hallmarks of multidrug resistance in melanoma, thereby making phytochemicals a promising reservoir for new targeted therapeutics and adjuvants for melanoma.

In addition to the previously discussed hallmarks of multidrug resistance, the gut microbiome could also be considered in the response to treatment modalities for melanoma [241]. The two main forms of therapies for metastatic melanoma are targeted therapies and immunotherapies [242]. Immunotherapy has displayed favorable results for the treatment of melanoma and checkpoint inhibitors such as ipilimumab which blocks cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and atezolizumab (which blocks programmed death ligand 1, PD-L1) enhances thymus-derived cell (T-cell) activation and proliferation thereby enhancing the immune response to melanoma [243]. The response to anti-PD-L1 treatment in mice was enhanced if the level of *Bifidobacterium* spp. was also enhanced in the microbiome of mice [244]. This highlighted the role of the gut microbiome in the response to immune checkpoint inhibitors. The immune-related adverse effects associated with anti-CTLA-4 treatment (ipilimumab) were also lowered with enhanced *Bacteroides fragilis* which suppressed the inflammatory response that typically occurs with anti-CTLA-4 treatment thereby reducing the occurrence of colitis (inflammation of the colon) [245]. Fecal microbiota transplantation (FMT), which is the transplantation of fecal matter from a donor to a patient to alter the gut microbiome, has been studied in metastatic melanoma models to enhance the response to immunotherapy [246, 247]. In a study conducted by Gopalakrishnan et al., mice with FMT from anti-PD-1 responders, abundant in fecalibacterium, displayed enhanced cluster of differentiation 8 positive T cells ($CD8^+$ T cells) and innate immune cells which correlated with decreased tumor growth [246], whereas mice with FMT from non-responders had enhanced T-helper 17 (Th17) cells and regulatory T cells (Tregs) resulting in an immunosuppressive response [246, 247]. Furthermore, in a phase I trial (NCT03353402), 10 patients with anti-PD-1 resistant metastatic melanoma with FMT from donors who attained a complete response for over a year after receiving nivolumab monotherapy and evaluated the safety and feasibility of nivolumab reinduction. Three recipients obtained progression-free survival for six months, among them two partial responses (PR) and one complete response (CR) [248, 249]. The cross talk between the immune system and the gut microbiome could be through phytochemicals [250, 251]. Phytochemicals correct the gut microbiome imbalance in patients treated with immune checkpoint inhibitor therapy (ICT), thereby enhancing the response to ICT [250, 251]. In a study, 39 melanoma patients treated with ICT who responded to treatment had high levels of anacardic acid [250, 251]. Anacardic acid is a derivative of salicyclic acid and is mainly found in the nutshell of cashews [252]. Anacardic acid aids in immune response as it stimulates neutrophils and enhances T-cell recruitment [252]. The gut microbiome may also serve as a biomarker for the prevention of multidrug resistance in metastatic melanoma.

Proteolysis targeting chimeras (PROTACs) have come to the fore in drug discovery and development as PROTACs can selectively degrade proteins within cells [253]. The conjugation of phytochemicals to PROTACs may also offer another avenue of

inhibiting multidrug resistance in metastatic melanoma [254]. Pseudolaric acid B (isolated from golden larch bark) has been conjugated with thalidomide derivatives for the generation of PROTACs [254, 255]. These PROTACs were designed to target the transmembrane glycoprotein, cluster of differentiation 147 (CD147), in metastatic melanoma [254, 255]. CD147 plays an imperative role in the metastasis and progression of metastatic melanoma through the regulation of MMP-9 which degrades the extracellular matrix enabling cell invasion and metastasis [256]. Furthermore, CD147, activated the MAPK and PI3K pathway, thereby facilitating the proliferation of metastatic melanoma cells [257, 258], promoting tumor angiogenesis through enhanced expression of VEGF [259], as well as enhancing expression of ABC transporters and stemness markers [260, 261]. The degradation of CD147 was favorable for the treatment and inhibition of multidrug resistance in metastatic melanoma. One of the PROTACs generated after the conjugation of pseudolaric acid B to a thalidomide derivative reduced the proliferation of metastatic melanoma cells (SK-MEL-28) and induced degradation of CD147 [254, 255]. In an in vivo study using BALB/c female nude mice, the PROTAC reduced CD147 levels, decreased the volume and weight of tumors [254, 255].

Although phytochemicals display favorable alteration of key hallmarks of multidrug resistance in metastatic melanoma, there are various hurdles that limit clinical translation of phytochemicals. These hurdles include short plasma half-life whereby the phytochemical fails to bind to plasma proteins leading to rapid elimination and clearance, thereby limiting circulation in the body and efficacy [262]. Phytochemicals also display poor absorption in the gastrointestinal tract thereby lowering systemic circulation and, thus, efficacy [263]. The efficacy of berbamine (BBM), a bisbenzylisoquinoline alkaloid extracted from *Berbaris amurensis* Rupr., has been studied for the treatment of metastasis in melanoma in vivo [264]. BBM displayed a short plasma half-life, thereby limiting clinical translation [264]. Thus, BBM was encapsulated in lipid nanoparticles which enhanced the efficacy of BBM in vivo at 30 mg/kg body weight against metastasis C57BL6 mice melanoma model injected with B16F10 cells into the tail vein in comparison to the untreated and unencapsulated BBM [264]. This example highlighted, how nanotechnology can bridge the gap between phytochemicals and clinical translation.

Author Contributions

Jacqueline Maphutha: Conceptualization, writing – original draft, writing – review and editing. **Danielle Twilley, Mona Dawood, Thomas Efferth, and Namrita Lall**: Writing – review and editing.

Acknowledgments

The figures were created with Biorender.com.

Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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