Key Signaling Pathways in Normal and Neoplastic Melanocytes

4

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Contents

Abstract

Signal transduction pathways regulate the proliferation, differentiation, migration, and survival of melanocytes. These signaling pathways are dysregulated during the transformation of melanocytes, often due to somatic mutation of genes within the pathway. One major signaling pathway that highlights this paradigm is the mitogen-activated protein (MAP) kinase pathway. Growth factor signaling via the MAP kinase pathway is required for melanocyte proliferation and survival. MAP kinase signaling is activated in the majority of melanomas through somatic mutations in NRAS, BRAF, and MEK1/2. Regulation of proliferation and survival is also controlled by phosphatidyl-inositol 3′-kinase (PI3K) signaling. PI3K is a major regulator of melanocyte biology and is commonly activated through the mutation/loss of expression of negative not

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pathway regulators such as PTEN. Alterations in cyclin-dependent kinase signaling are also frequent in melanoma and promote aberrant cell cycle progression. Other pathways such as Gαq, Wnt (canonical and noncanonical), Hippo, Notch, and signaling downstream of Rho family GTPases also play important roles in the aforementioned biological processes, and in some cases are altered in selective subsets of melanoma. The high mutation burden within genes in signaling pathways, the important role of these pathways in melanocytic neoplasms, and the knowledge that melanomas adapt their signaling mechanisms in response to targeted inhibitors make it essential to have a thorough understanding of the key signaling pathways in melanocytes and melanomas.

Keywords
RAS · BRAF · ERK1/2 · PI3K · PTEN · AKT · GNAQ · GNA11 · Rac · Rho · PREX · CDK4/6 · Wnt · β-catenin · YAP · TAZ · Notch

Introduction

Regulation of melanocyte proliferation and differentiation is modulated by a diverse set of cues that cells receive from their extracellular milieu. Stimuli range from soluble peptide growth factors to interaction via cell-cell adhesion molecules and generate a range of signal transduction pathways that tightly control processes in normal melanocytes and are aberrantly regulated during melanoma initiation and progression. Pathways often de-regulated in melanoma range from protein kinase and lipid kinase transduction events to heterotrimeric and monomeric GTPase signaling. In this chapter, we review the key signaling pathways that regulate melanocyte proliferation, growth, and migration/invasion. Furthermore, we illustrate how these pathways become deregulated during the transformation of melanocytes to melanoma.

Mitogen-Activated Protein (MAP) Kinase Pathway

The mitogen-activated protein (MAP) kinase cascade is one of the ubiquitously important signaling cascades in different cell types. In normal melanocytes, as in other normal cells, MAP kinase cascade activation is triggered by soluble peptide growth factors binding to their cognate receptor tyrosine kinases (RTKs), which are expressed on the cell surface. Growth factor binding leads to dimerization and autophosphorylation of receptors. Subsequent transphosphorylation of additional residues within the intracellular C-termini of RTKs which serve as binding sites to recruit Src homology 2 (SH2)-domain and phosphotyrosine binding (PTB) domain-containing adaptor proteins. These adaptor proteins then link through to signaling networks. One example of an adaptor protein is Grb2, which binds to phosphorylated tyrosine residues of RTKs and localizes SOS, a guanine nucleotide exchange factor, to the plasma membrane. In turn, SOS catalyzes GDP to GTP nucleotide exchange in not of RAS GTPases.

A key step between RTKs and the MAP kinase pathway is the activation of RAS. There are three main forms of RAS: N-RAS, H-RAS, and K-RAS, which act as molecular switches being active in their GTP-bound state. RAS binds multiple effectors but relevant to the MAP kinases ERK1 and 2 are the RAF (*R*apidly *A*ccelerated *F*ibrosarcoma) serine-threonine kinases ARAF, BRAF, and CRAF. RAS recruits RAFs to the plasma membrane where they are activated and initiate a phospho-relay cascade. RAFs phosphorylate and activate the dual specificity kinases MEKs at key serine residues, S218 and S222. RAFs act as dimers, with BRAF-CRAF heterodimers eliciting higher activity than homo-dimers (Rushworth et al. 2006). C-terminal 14-3-3 binding sites in RAF (serine 621 in CRAF; serine 729 in BRAF) also play an important role in RAF dimerization (Weber et al. 2001). Activated MEKs phosphorylate and activate ERK1/2. ERK1/2 phosphorylate a variety of cytoplasmic

targets (e.g., focal adhesion kinase, p90RSK, Bim) as well as translocate to the nucleus to control transcriptional events (Balmanno and Cook 2009).

The ERK1/2 pathway is fine-tuned through multiple adaptor complexes, scaffolding molecules, and feedback pathways. As noted above, 14-3-3 proteins are highly conserved adapters that bind phosphorylated residues in RAFs creating an inhibitory intra-molecular bridge, which is relieved by activated RAS. Kinase suppressor of RAS (KSR) is a pseudokinase that translocates to the plasma membrane and provides a scaffold function by binding and co-localizing RAFs, MEKs, and ERK1/2 (McKay et al. 2011). Negative control of the pathway is mediated by negative feedback loops including direct phosphorylation of SOS and C-terminal sites on BRAFs by ERK1/2 (Brummer et al. 2003), upregulation of Sprouty proteins that inhibit RAF activity (Pratilas et al. 2009), and regulation of dual-specificity phosphatases (DUSP) 4 and 6 that de-phosphorylate the activation sites on ERK1/2 (Lito et al. 2012).

Regulation of signaling through the ERK1/2 pathway is critical for melanocyte proliferation. Basic FGF that is secreted from keratinocytes promotes proliferation and activates ERK1/2 signaling in primary human melanocytes. Additional growth factors such as stem cell factor and endothelin 1 also activate the pathway. ERK1/2 signaling is key to G1-S cell cycle progression in normal human melanocytes through control of cyclin D1 levels as well as downregulation of the cyclin-dependent kinase (CDK) inhibitor, p27Kip1 (Bhatt et al. 2005). ERK1/2 signaling also impacts survival mechanisms by controlling the levels of the pro-apoptotic proteins, Bim EL and Bmf. In addition to RTK stimulation, MAP kinase signaling is also triggered downstream of G-protein-coupled receptors (GPCRs). For example, melanocortin 1 receptor (MC1R), expressed in melanocytes, is required for melanocyte proliferation and is a major determinant of skin pigmentation. Binding of the ligand, alpha melanocyte stimulating hormone, to MC1R activates adenylate cyclase-mediated production of cyclic adenosine monophosphate (cAMP) via the Gs alpha subunit, as well as ERK1/2 signaling (Busca et al. 2000). The role of cAMP in ERK1/2 activation may differ between model systems.

Cutaneous melanomas have a high burden of somatic mutations. The advent of next generation sequencing technologies has led to the identification of multiple recurrent mutations within components of the RAS-RAF-MEK-ERK1/2 pathway (Fig. 1). The first oncogene identified as being mutated in melanoma was NRAS. Most NRAS mutations occur at the Q61 locus and less frequently at the G12 or G13 loci. Other forms of RAS are mutated infrequently in melanoma; mutation rates in HRAS and KRAS are both ~1%. NRAS mutations at the Q61 locus impair the GTP hydrolysis activity resulting in a GTP-bound state for NRAS and constitutive activation of downstream effector pathways.

Approximately 50% of cutaneous melanomas harbor an activating mutation within the BRAF gene (Davies et al. 2002; TCGA 2015). The most frequent BRAF mutation is a thymine (T) to adenine (A) transversion that results in a valine to glutamic acid substitution at codon 600. This V600E alteration introduces a phosphomimetic change within the activation loop of BRAF and leads to constitutive, RAS-independent activation of BRAF and downstream MEK-ERK1/2 signaling. Furthermore, BRAF V600E mainly exists as a monomer and is refractory to negative feedback mechanisms mediated by Sprouty proteins (Brady et al. 2009). Additional non-V600 mutations have been identified; most are activating but a subset of mutations actually inactivate BRAF kinase activity. Nevertheless, these mutations still activate MEK, as the kinase-defective BRAF protomer can dimerize with CRAF and lead to an activated heterodimer (Wan et al. 2004). Other forms of RAF are mutated at very low frequencies; <2% for ARAF and 1.1–3.6% for CRAF according to the dataset in cBioPortal for Cancer Genomics. More recently, BRAF fusions have been identified, in which the intact kinase domain of BRAF is fused to a broad variety of different 5′ partner

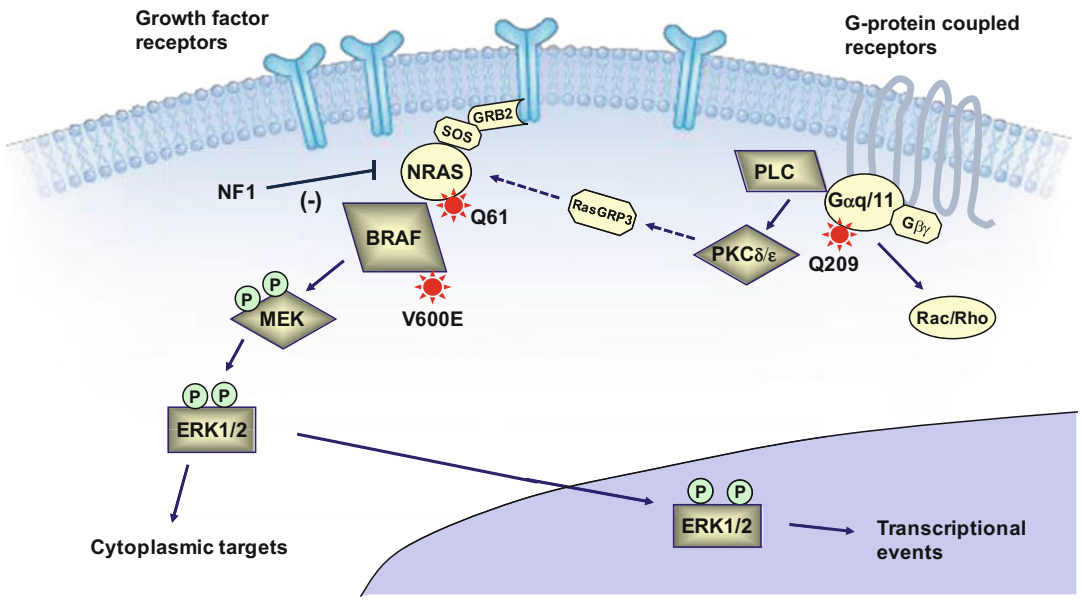

Fig. 1 Mutations in the RAF-MEK-ERK1/2 signaling pathway associated with cutaneous and uveal melanoma. The MAP kinase signaling is activated in the majority of cutaneous melanomas through somatic mutations in NRAS, BRAF, and MEK1/2. This pathway is activated in uveal melanoma by mutations in GNAQ and GNA11 and downstream signaling via PKC and RasGRP3

genes (Hutchinson et al. 2013; Botton et al. 2013). The 5′ partners often promote dimerization of fusion kinases, resulting in constitutive activation.

A third frequent alteration in the MAP kinase pathway affects the *NF1* gene. NF1 encodes a GTPase activating protein (GAP) that upregulates the GTPase activity of RAS proteins. NF1 is mutated frequently in melanomas on chronically sun-damaged skin and desmoplastic melanoma (Krauthammer et al. 2015; Shain et al. 2015). Although all the mutations have not been functionally analyzed, many are damaging alterations in both alleles and therefore likely result in loss of NF1 function. NF1 mutations can co-occur with NRAS mutations but are anticorrelated with BRAF V600 mutations, which arise in a specific melanoma subtype. Less frequent mutations have also been detected within MEK1 and -2 (Nikolaev et al. 2012).

As noted above, the ERK1/2 pathway is regulated by scaffold proteins including kinase suppressor of RAS (KSR), IQGAP, and MEK partner 1 (MP1). However, as exemplified by studies in KSR, while a role for scaffold proteins in modulating the ERK1/2 pathway has been shown in developmental biology models and mouse embryo fibroblasts, there have been few studies in melanocytes and melanoma cells.

BRAF V600E signaling via the MEK-ERK pathway contributes to several malignant traits in melanoma. BRAF V600E signaling promotes cell cycle progression via upregulation of cyclin D1 and downregulation of p27Kip1 (Bhatt et al. 2007). Resistance to apoptotic cell death is mediated by pathway signaling leading to downregulation of two BH3-only proteins, Bim-EL (BCL2-like 11) and Bmf (BCL2-modifying factor) (Brocklin et al. 2009; Boisvert-Adamo and Aplin 2008; Cartlidge et al. 2008; Shao and Aplin 2012). Additionally, phosphorylation of dynamin-related protein 1, a protein involved in mitochondrial division, may also contribute to transformation mediated by MEK-ERK signaling. Migratory and invasive properties of melanoma cells are enhanced by BRAF V600E-MEK-ERK upregulation of the Rho family GTPase, Rnd3 (Klein et al. 2008), and the EMT transcription factor Twist1 (Weiss et al. 2012) and downregulation of the cGMP-selective

phosphodiesterase, PDE5A (Arozarena et al. 2011a). MAP kinase signaling also cross-talks to metabolic pathways via ERK1/2 and RSK phosphorylating LKB1, which suppresses LKB1 function and promotes BRAF V600E-driven tumorigenesis (Zheng et al. 2009). By contrast, expression of BRAF V600E in normal melanocytes leads to senescence (Michaloglou et al. 2005; Gray-Schopfer et al. 2006; Dankort et al. 2009) and the formation of nevus-like structures in mice (Dankort et al. 2009). These data are consistent with the notion of oncogene-induced senescence and the presence of BRAF mutations in the majority of human nevi (moles) (Pollock et al. 2003).

The MAP kinase cascade is also activated via mutations in NRAS, which occur in a mutually exclusive manner from BRAF mutations and are more frequent in older patients (Jakob et al. 2012). Mutations in NRAS at Q61, G12, and G13 lead to a persistent GTP-bound state of NRAS and constitutive activation of downstream effector pathways. Furthermore, inactivation of NF1 occurs frequently and is associated with a high level of active RAS, although additional mechanisms of RAS activity regulation appear to be important in some NF1-null tumors (Krauthammer et al. 2015). In contrast to their BRAF-mutant counterpart, NRAS mutant melanomas signal predominantly via CRAF, which is associated with a disruption in cAMP metabolism (Dumaz et al. 2006). Other major effectors downstream of activated RAS are phosphatidyl-inositol 3′-kinase (PI3K) and Ral GEF. PI3K signaling will be discussed in section "Phosphatidyl-Inositol 3′-Kinase (PI3K) Pathway."

Suppression of NRAS expression in human melanoma cell lines harboring NRAS mutations can induce apoptosis (Eskandarpour et al. 2005); however, the effects are likely variable across a larger panel of lines. More compelling data for a driver role for mutant NRAS comes from mouse models of melanoma. Expression of NRas Q61K in the melanocytic lineage (under the control of tyrosinase-regulatory sequences) leads to skin hyperpigmentation and increased phosphorylation of ERK1/2 (Ackermann et al. 2005). Tyr: NRas Q61K mice crossed onto a p16Ink4a null background develop primary melanoma with high penetrance and short latency, a subset of which metastasize to the liver and lung (Ackermann et al. 2005). Expression of a mutant form of CDK4 that is resistant to inactivation by p16INK4a in combination with NRAS Q61K in melanocytes leads to dysplastic nevus-like lesions and metastatic melanoma, although the time to onset and multiplicity is dependent on the mouse strain utilized (Ferguson et al. 2015). By contrast, tyrosinase-driven HRas G12V expression in p16INK4a null mice gives rise to amelanotic melanomas but few/no metastases (Chin et al. 1997).

Due to the varied mechanisms of activation and multiple opportunities for therapeutic targeting, considerable efforts have been focused on targeting the ERK1/2 pathway in melanoma. However, the dependency of melanoma cell lines on the ERK1/2 pathway varies with BRAF V600E/K mutant cell lines being highly sensitive to MEK inhibitors, inhibiting G1-S progression and proliferation in vitro and in vivo (Solit et al. 2006). BRAF dependency of BRAF V600E/K lines has also been demonstrated with selective inhibition by vemurafenib/PLX4032 (Joseph et al. 2010). These studies have formed the initial basis for therapeutic blockade of the BRAF-MEK-ERK1/2 signaling pathway in BRAF V600 melanomas. Other factors also influence the pathway and provide therapeutic strategies. For example, copper influx binding to MEK1 enhances MEK1 phosphorylation of ERK1/2, and disrupting this binding inhibits BRAF V600E signaling through MEK-ERK1/2 and tumorigenesis (Eskandarpour et al. 2005). This study forms the basis of copper chelation strategies to enhance the effects of BRAF-MEK-ERK1/2 pathway inhibitors in mutant BRAF melanoma patients.

Phosphatidyl-Inositol 3′-Kinase (PI3K) Pathway

A second pathway strongly implicated in melanoma initiation and metastasis is the phosphatidyl-inositol 3′-kinase (PI3K) signaling cascade. Class I PI3Ks consist of a regulatory p85 subunit and a catalytic p110 subunit. Typically, growth

factor signaling leads to PI3K activation via the phosphorylation of cytoplasmic adaptor proteins containing Y-x-x-M motifs that serve as strong docking sites for the p85 subunit. However, direct binding to a receptor may also occur. For example, neuregulin binding to ERBB3 leads to receptor heterodimerization with a co-receptor (typically EGFR, ERBB2 or EBB4) and phosphorylation of Y-x-x-M motifs in the cytoplasmic domain of ERBB3. As with adaptor proteins, phosphorylation of Y-x-x-M motifs leads to PI3K binding and activation. The p110 catalytic subunit phosphorylates phospholipid phosphatidylinositol -4,5-bisphosphate (PIP2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP3) to trigger downstream signaling. PIP3 binds to pleckstrin-homology domains within several proteins to transmit signals. These targets include phosphoinositide-dependent protein kinase-1 (PDK1), AKT1-3, and serum/glucocorticoid-regulated kinase (SGK) 1-3. The PI3K signaling pathway is dampened by the action of phosphatases. Phosphatase and tensin homologue (PTEN) dephosphorylates PIP3 at the 3′ position and inositol polyphosphate 4-phosphatase type II (INPP4B) dephosphorylates PI(3,4)P2 to PI(3)P. In normal melanocytes, PI3K is activated in vitro by growth factors such as stem cell factor and provides a pro-survival signal (Larribere et al. 2004). Furthermore, PTEN deletion in melanocytes in mice leads to increased numbers of melanocytes in the dermis and retention of coat color by resisting hair greying (Inoue-Narita et al. 2008). Inpp4b-/- mice are viable (Li Chew et al. 2015), but the effect of loss of Inpp4b on melanocytes in vivo is yet to be described. INPP4B loss has been reported in 21% of melanoma (Gewinner et al. 2009); however, genetic alterations are less frequent in more recent next-generation sequencing analyses of melanoma. Further studies are warranted to understand the influence of INPP4B in melanoma initiation and progression.

PI3K signaling plays a key role in growth, survival, and metabolism; thus, it is unsurprising that PI3K signaling has been linked to melanoma progression. Importantly, use of patient samples has linked PI3K signaling to melanoma brain metastasis (Anastas et al. 2014), providing a strategy to limit a severe complication of melanoma that is currently an unmet clinical need. Activating mutations are frequent in the catalytic PIK3CA subunit are prevalent in breast cancer, colorectal cancer, and uterine corpus endometrial carcinoma. By contrast, PIK3CA mutations are they are infrequent (~3%) and in cutaneous melanoma and their contribution to the malignant phenotype is poorly characterized. A frequent alteration leading to activation of PI3K signaling in melanoma is the loss of PTEN (Fig. 1). Alterations in PTEN occur through frameshift mutations leading to premature truncations, intragenic microdeletions and epigenetic silencing (Xing et al. 2012). Loss of PTEN has been strongly linked to progression of melanoma, often occurring in concert with BRAF mutations and in wild-type BRAF/NRAS tumors. PTEN loss is rare in mutant NRAS melanomas. Mice with the loss of Pten in melanocytes do not develop spontaneous melanomas unless induced with a carcinogen (Inoue-Narita et al. 2008). Additionally, while expression of BRAF V600E leads to the formation of nevus-like structures in mice, concurrent loss of PTEN leads to the formation of invasive tumors with 100% penetrance and metastasis to the lymph and lungs (Dankort et al. 2009). In this model, melanoma growth was prevented by inhibitors of mTorc1 (Dankort et al. 2009). There is also growing evidence of PTEN modulating the response to targeted therapy and immune checkpoint inhibitors (Paraiso et al. 2011). Overall, these data strongly implicate PTEN loss in the malignant progression of melanomas.

In BRAF V600E/PTEN null tumors, combinations of BRAF inhibitors and PI3K inhibitors elicit more potent effects in vivo than either alone (Marsh Durban et al. 2013; Deuker et al. 2015); thus, it is important to understand the signaling downstream of PI3K. PDK1 plays an active role since its depletion or pharmacological inhibition impairs tumor growth in BRAFV600E CDKN2A -/- mice (Scortegagna et al. 2014). PDK1 phosphorylates AKT at threonine 308 within the activation loop but is not frequently altered in melanomas. E17K alterations in the pleckstrin homology domains in AKT isoforms 1 and 3 have been identified at low

frequencies in melanoma tumors and cell lines (Davies et al. 2008). However, AKT3 is frequently (20–40%) deregulated in melanomas through copy number increases and loss of PTEN expression (Stahl et al. 2004). AKT signaling contributes to malignant properties of melanomas through control of mTOR signaling, phosphorylation of PRAS40 (proline-rich Akt substrate of 40 kDa) (Madhunapantula et al. 2007), and regulation of Notch1 (Bedogni et al. 2008). Importantly, in mouse models, expression of a constitutively active (myristoylated) form of Akt1 promotes melanoma formation and metastasis to the lung and brain in the setting of BRaf V600E and silencing of p16INK4A and p19ARF. PI3K and PDK1-dependent and AKT-independent signaling may also contribute to malignant traits. AKT inhibitors demonstrate only modest effects on growth in BRAF V600E/PTEN null tumors (Marsh Durban et al. 2013), and SGK3 has been implicated as a major target downstream of PDK1 that contributes to the growth of mutant BRAF melanoma (Scortegagna et al. 2015). PTEN may also signal independent of its role in the PI3K-AKT pathway, for example, utilizing protein phosphatase activity, to contribute to malignant behavior. PTEN represses β-catenin nuclear localization and transcriptional activity via a caveolin-1 regulated mechanism (Conde-Perez et al. 2015). PTEN inhibits the activity of the Rac-selective GTP exchange factor PREX2 (Mense et al. 2015). PREX2 mutations have been found in ~14% of melanomas (see below), and expression of mutant PREX may promote the transformation of TERT-immortalized mutant NRAS human melanocytes (Berger et al. 2012). However, the exact role for PREX2 requires further examination (Horrigan et al. 2017).

Gαq Signaling

In contrast to the high frequency of *BRAF* and *NRAS* mutations found in cutaneous melanoma, alterations in are absent in nevi, or melanocytomas of the central nervous system. Rather, activating mutations in GNAQ and GNA11 genes (typically Q209 in exon 5 but also R183 in exon 4) are found in 80–90% of uveal melanomas. GNAQ mutations have also been identified at a high frequency in blue nevi (Van Raamsdonk et al. 2009, 2010) and melanocytomas (Koelsche et al. 2015). Alterations in GNAQ and GNA11 occur early in disease progression; however, additional mutations are required, often including inactivating BAP1 mutations on chromosome 3. BAP1 encodes a deubiquitylating enzyme and loss of function mutations in this gene are found in 32–50% of primary uveal melanomas and are associated with aggressive disease and higher likelihood of metastasis (Harbour et al. 2010).

GNAQ and GNA11 encode Gqα and G11α, the alpha subunits of heterotrimeric G-proteins signaling downstream of G-protein coupled receptors (GPCR). Normally, Gα is in a complex with Gβ and Gγ subunits, but following receptor stimulation the α subunit switches from a GDP-bound form to a GTP-bound form and dissociates from the β and γ subunits. Of note, Gqα has been shown to be downstream of the endothelin receptor, which is required for melanoblast migration during neural crest development (Shin et al. 1999). A major effector of Gqα and G11α is phospholipase Cβ, which when activated hydrolyzes membrane PIP2 to release diacylglyerol (DAG) and inositol triphosphate (IP3). These second messengers ultimately lead to activation of members of the protein kinase C (PKC) family and the RAS GEF, RasGRP3 (Chen et al. 2014, 2017). The Q209 residue in Gqα and G11α lies within the RAS-like domain and its mutation disrupts GTP-hydrolysis, leading to constitutive activation of the aforementioned pathways and transformation of immortalized melanocytes (Van Raamsdonk et al. 2009). Downstream of PKC and RasGRP3 signaling, the MEK-ERK1/2 pathway is activated in mutant GNAQ/11 uveal cell lines (Fig. 1). These findings have contributed to the basis for phase II and phase III clinical trials to target MEK in uveal melanoma patients. To date, these trials have met with limited success (Carvajal et al. 2014).

Gqα and G11α may also signal independently of phospholipase Cβ. Several guanine nucleotide

exchange factors (GEFs) for Rho family GTPases are also effectors of Gqα and G11α. Specifically, LARG, p115 RhoGEF, PDZ Rho GEF, lbc Rho-GEF, and p63 Rho-GEF have all been reported to interact with Gqα and/or G11α. A distinct Rho/Rac GEF, Trio, was identified through a genome-wide RNA interference screen to signal downstream of Gqα and is required for the growth of mutant GNAQ uveal melanoma cells. Trio promotes Rho and Rac GTPases activity, actin stress fiber formation, and YAP1 (Yes associated protein 1) nuclear translocation and YAP-dependent transcription. YAP is a transcriptional co-activator within the Hippo signaling pathway. Phosphorylation at serine 127 leads to the cytoplasmic sequestration of YAP and inhibition of its transcriptional activity. The Notch ligand, Jag-1, and the Notch target gene, Hes-1, are possible downstream targets of YAP signaling in uveal melanoma cells (Liu et al. 2015). Uveal melanoma display high levels of nuclear YAP and depletion of YAP impairs the growth of uveal melanoma lines in vitro and in vivo. Thus, selectively targeting YAP represents a therapeutic strategy for advanced-stage uveal melanoma patients. A recent advance toward testing such strategies is the generation of a transgenic mouse model of uveal melanoma. Mice with MITF-Cre regulated expression of GNAQ Q209L develop melanocytic neoplasms with a 100% penetrance after 3 months with evidence of invasive lesions and lung metastasis (Huang et al. 2015).

The Rho/Rac Signaling Pathway

The Ras superfamily is comprised of multiple members. Of these, the Rho/Rac subfamily is, in itself, comprised of 26 members, in varying isoforms of Rho, Rac, Cdc42, Rnd, and the atypical RhoBTB and RhoT/Miro GTPases. In this chapter, we will focus on Rho/Rac GTPases specifically. Rho/Rac GTPases control motility, and other processes critical to melanocyte function, and melanoma progression. Rho/Rac GTPases, when bound to guanosine di-phosphate (GDP), are inactive. When GDP are exchanged for guanosine tri-phosphate (GTP), with the help of guanosine exchange factors (GEFs), Rho/Rac proteins are activated. Specificity of Rho/Rac activities is determined by (1) lipid modifications, which determine their subcellular localization, (2) the GTPase effector proteins present in those locales, and (3) phosphorylation by molecules such as PKC and PKA.

Rho and Rac signaling have both collaborative and divergent functions in cytoskeletal remodeling. Rho proteins are involved in the formation of stress fibers, and Rac proteins dictate the formation of lamellipodia and dendrite extension. In response to UV irradiation, melanocytes form dendrites, which allow them to pass melanosomes to neighboring keratinocytes, to protect them. This process of dendrite formation is dictated by Rho/Rac signaling and is very similar to that observed in the formation of dendrites from neurons, perhaps reflecting the neural crest origins of melanocytes. After exposure to UV, the hormone alpha-MSH is increased, which results in stimulation of cAMP. cAMP signals to activate PKA, which in turn signals to deactivate Rho, and increase Rac signaling (Scott et al. 1997). Activation of Rac, therefore, is a major contributor to cell motility.

In melanoma cells, Rac can be activated in multiple ways. One mechanism is via the mutation of the NRAS oncogene. When NRAS is mutated, Rac is activated (Li et al. 2012). While this does not affect the motility of melanoblasts, it does promote their survival. Inhibiting Rac1 suppresses tumor growth and progression. In addition to upstream mutations, it has recently been discovered that Rac1 itself is recurrently mutated in melanoma. Unlike BRAF V600E, NRAS, or GNAQ/11, Rac1 P29S is not thought to be an initiating mutation, as it is not present in nevi. The mutation is likely environmentally induced as is formed through a C to T transition, at a dipyrimidine, as typical for UV-induced mutations. This mutation renders Rac1 constitutively active, allowing it to maintain binding with molecules such as PAK1, driving transformation and a metastatic program (Watson et al. 2014). Data showing that inhibiting Rac in Rac1 mutant melanocytes inhibits malignant transformation suggest that this might make Rac1 P29S a good target for therapy.

Finally, mutations in one additional family of Rac exchange factors have been identified in melanoma. The PIP3-dependent Rac GEFs PREX1 and -2 have recently been shown to play a role in melanoma (Welch 2015). These proteins are large >180 kDa proteins that encompass pleckstrin homology domains, a Dbl homology domain, DEP domains, and PDZ domains among others. The most important interaction in melanoma is likely the DH-PH domains, as this both acts as a Rac GEF and allows for the binding of PREX to PTEN. This inhibits PTEN activity, resulting in the activation of AKT and therefore cell survival. Knockout of PREX1 in mice causes the development of white bellies, indicative of a melanoblast migration defect, and crossing these mice to melanoma-prone mice results in suppression of tumor growth and metastasis (Lindsay et al. 2011, 2015).

PREX2 binds and inactivates PTEN. In turn, loss of PTEN can increase PREX2 activity (Mense et al. 2015). PTEN is commonly deleted in melanoma, and this has been shown to have multiple implications for both melanoma progression and therapy resistance (Dankort et al. 2009). Intriguingly, melanoma cells that develop resistance to BRAF inhibitors are largely PTEN deficient. In addition to this, it has been shown that the metabolism of these cells is altered as well (Baenke et al. 2016) and vemurafenib resistant cells tend to rely more on oxidative phosphorylation and less on glucose (Zhang et al. 2016). Since PREX2 knockout mice have been shown to be insulin resistant due to defects in the metabolism and uptake of glucose, it may be that PREX2 can also contribute to therapy resistance in melanoma. Overall, melanoma cells appear to find multiple ways to maintain and promote Rac1 activity, resulting in increases in both tumor progression and therapy resistance.

CDK4/6-RB1 Pathway

Abnormal cell cycle progression is one of the hallmark features of cancer. Notably the progression through G1 phase, through the restriction point, and into the DNA synthesis (S) phase is a tightly regulated process. The molecular machinery controlling the G1-S progression comprises a series of cyclins and cyclin-dependent kinases (CDKs). Within the early phase of G1, D-type cyclins (D1, D2 and D3) form a complex with two closely related CDKs, CDK4 and CDK6. Later in G1, cyclins E and A bind to CDK2 and CDK1 to promote G1-S and M phase progression. In general, cyclin levels fluctuate across the cell cycle, whereas CDK levels are more constant. A major function for cyclin-CDK complexes is to phosphorylate and inactivate the pocket protein, retinoblastoma (RB), and its related proteins, p107 and p130. Hyper-phosphorylation of RB releases E2F transcription factors that can activate transcription of genes involved in further cell cycle progression and division. Opposing the kinase activities of cyclin-CDK complexes are two families of cyclin-dependent kinase inhibitors (CDKI). The Cip/Kip family of CDKIs includes p21Cip1, p27Kip1, p57Kip2, which primarily inhibit cyclin E and cyclin A-bound CDK complexes by forming ternary complexes. By contrast, the INK4 family, which includes p16 INK4A, p15 INK4B, p18 INK4C, and p19 INK4D/ARF, more selectively inhibits D-type binding CDK4 and CDK6 complexes through an allosteric mechanism.

Multiple mechanisms drive aberrant G1-S cell cycle progression in melanoma and lead to aberrant proliferation. D-type cyclins act as mitogenic sensors within G1. The RAF-MEK-ERK1/2 pathway, which is constitutively activated by mutations in NRAS (20–30% frequency) and BRAF (~50% of melanomas), transcriptionally upregulates cyclin D1 and cyclin D3, as well as downregulates p21Cip1 and p27Kip1 (Bhatt et al. 2005). Cyclin D1 is also regulated by ubiquitin-proteasomal systems. In melanoma, cyclin D1 may be posttranscriptionally upregulated through mutation of the E3 ubiquitin ligase, FBXO4, and thus impairs ubiquitylation and degradation of cyclin D1 (Lee et al. 2013). However, the frequency of these mutations in the melanoma TGCA dataset seems very low at approximately 1%. Cyclin D1 is recurrently amplified, in particular in melanomas on acral and mucosal sites (Sauter et al. 2002). Inactivation of RB1 may

also occur through mutations in CDK4 and loss of CDKI expression and, to a lesser degree, loss of RB1 itself. Somatic and germline R24C/H mutations in CDK4 are detected in cutaneous melanoma (Wolfel et al. 1995), and CDK4 amplifications occur in a mutually exclusive pattern with CDKN2A deletions (Curtin et al. 2005). The CDK4 mutations occur within the p16INK4a domain and render CDK4 less susceptible to inhibition by INK family proteins. CDK4 R24C knock-in mice are susceptible to melanoma induced by chemical carcinogenesis (Sotillo et al. 2001). Loss of the Ink4a/Arf tumor suppressor locus is a more frequent mechanism leading to RB-deregulation in melanoma. In mouse models, deletion of p16Inka in combination with expression of mutant RAS promotes aggressive melanoma formation (Monahan et al. 2010), and loss of p19Arf in combination with tyrosinase-driven mutant HRAS promotes melanomagenesis, an effect that is exacerbated following UV irradiation (Kannan et al. 2003). Deficiency in both Ink4a and Arf promotes UV-induced melanomagenesis in a HGF transgenic mouse model (Ha et al. 2007). Thirdly, the RB1 gene is mutated albeit infrequently (3–5% in cBioportal databases) in melanoma. RB1 loss may occur either through focal intragenic homozygous deletion or through truncation mutations (Xing et al. 2012).

CDKs are targetable with small molecule inhibitors; however, early generation CDK inhibitors were relatively nonselective and elicited limited therapeutic effects in melanoma patients. Parke-Davies developed PD0332991/palbociclib/IBRNACE, an orally available, highly selective inhibitor of CDK4/6. A very similar compound LEE011/ribociclib from Novartis and a CDK4/6/9 inhibitor, LY2835219/abemaciclib from Eli Lilly have also been developed. The FDA-approval of palbociclib in postmenopausal estrogen receptor (ER)-positive/HER2-negative breast cancer has re-ignited interest in targeting cell cycle progression in cancer. In melanoma, palbociclib inhibits the hyper-phosphorylation of RB and blocks the proliferation of RB-proficient melanoma cell lines. Also, the combination of palbociclib with inhibition of MEK leads to apoptosis in vitro and tumor shrinkage in vivo (Kwong et al. 2012). These preclinical findings have translated into a clinical trial of ribociclib and the MEK inhibitor, MEK162/binimetinib, in late-stage, mutant NRAS melanoma patients. In summary, the CDK4/6 pathway is activated across most, if not all melanoma subtypes, and provides an attractive option for therapeutic intervention in combination with other targeted agents.

The Wnt Signaling Pathway

The Wnt signaling pathway involves 19 known Wnt ligands, which signal via any one of 10 frizzled (fzd) receptors to activate G-protein signaling downstream of ligand-binding. Wnt signaling can be divided into canonical and non-canonical signaling (Fig. 2), where canonical Wnt signaling involves the activation of the DIX-domain protein, Disheveled (Dsh), ultimately activating β-catenin (Webster et al. 2015a). β-Catenin plays important roles both at the cell surface, where it regulates adhesion, and in the nucleus where it activates the transcription of genes involved in cell proliferation. β-Catenin activity is regulated by the GSK3β/APC/Axin destruction complex. In this complex, Axin acts as a docking protein, as it has a GSK3β docking motif as well as a β-catenin docking motif, bringing together the two proteins in close proximity. This is further enhanced by APC, a protein with multiple β-catenin docking sites. When β-catenin is recruited to this complex, which is what occurs in the absence of Wnt ligand, it is phosphorylated and targeted for ubiquitination and degradation. Once Wnt ligand binds its cognate receptor, Dsh is activated. Dsh inhibits the ability of GSK3β to phosphorylate β-catenin to target it for destruction, increasing the levels and activity of β-catenin and enhancing cellular proliferation. Canonical Wnt ligands mostly include Wnt1, 3A, 7, 8, and 8b, and the canonical Fzd receptors that most commonly mediate this signaling include Fzd 1 and 7 (Webster et al. 2015a).

In noncanonical Wnt signaling, β-catenin is not a key component and in fact, as discussed below, may even be targeted for degradation in a GSK3-β-independent manner. There are two key non-canonical pathways, the Wnt/Ca^{2+} pathway, and the planar cell polarity (PCP) pathway (Fig. 2).

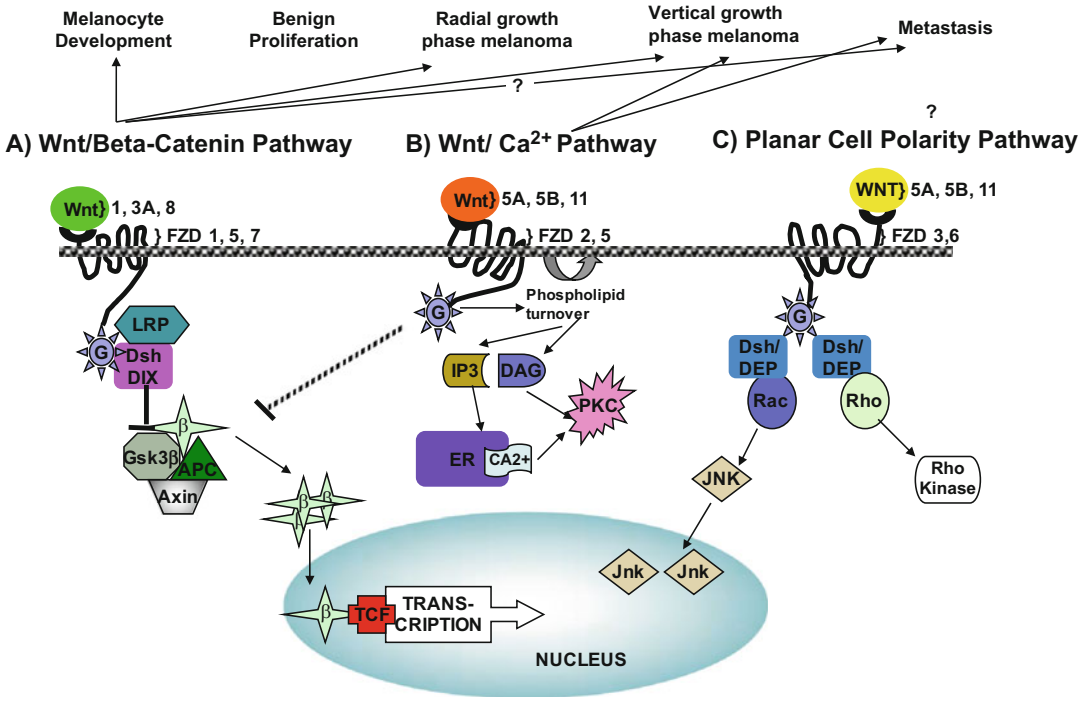

Fig. 2 The Wnt signaling pathway. The three main Wnt signaling pathways (Wnt/β-catenin; Wnt/Ca^{2+} and planar cell polarity) and their probable roles in melanocyte development, and melanoma progression

The planar cell polarity pathway results in the activation of Rho kinases. First, Wnt binds to its receptor, activating Dsh, which signals to activate the disheveled associated activator of morphogenesis (Daam1). Daam1 activates Rho signaling via the activation of GEFs. In the PCP pathway, Dsh can also complex with Rac, activating Jun kinases (JNK). Both the activation of Rho and Rac can result in cytoskeletal remodeling, thereby promoting migration. In the Wnt/Ca^{2+} pathway, Wnt binding to its receptor triggers the direct activation of Gαq. This results in the activation of PLCγ, lipid turnover in the membrane, and the generation of the second messengers, diacylglycerol (DAG) and inositol triphosphate (IP3). DAG can signal to activate PKC, and IP3 translocates to the endoplasmic reticulum where it releases calcium from its intracellular stores. PKC has multiple downstream signaling effects, and calcium release results in the activation of CAMKII and calcineurin, which also signal to activate and inhibit multiple different pathways. Noncanonical Wnt signaling is most commonly mediated by Wnts 4, 5, 5B and 11, and Fzd 2 and 5 (recently reviewed in (Webster et al. 2015a)).

In addition to the Fzd receptors, Wnt signals require co-receptors for transduction. These co-receptors help to determine through which pathway the signals are transduced, as the Wnt/Fzd combination can otherwise be rather promiscuous. Low-density lipoprotein receptors (LRPs), specifically LRP5 and 6, are single transmembrane receptors that complex with Fzd to mediate canonical Wnt signaling. Orphan receptor tyrosine kinases ROR1 and ROR2 are also single transmembrane receptors, but have intracellular tyrosine and serine-threonine kinase domains, as well as extracellular immunoglobulin-like, cysteine-rich domains. These receptors seem to play antagonizing roles to each other in melanoma, and most commonly mediate noncanonical Wnt signaling (O'Connell et al. 2013). In addition, there are other co-receptors that are not well described in humans, including Ryk/Derailed and FRL1/Crypto. Given the enormous amount of possible ligand/receptor/

co-receptor interactions, and the multiple downstream amplification steps in this cascade, Wnt signaling plays multiple, varied roles in the development and pathogenesis of cancer.

In melanoma, canonical and noncanonical Wnt signaling (specifically, the Wnt/Ca^{2+} pathway) play important and opposing roles. First, Wnt signaling directs the migration of melanocytes from the neural crest. Then, during the early stages of melanomagenesis, β-catenin is critical for the immortalization of melanocytes and the bypassing of melanocyte senescence (Delmas et al. 2007; Larue et al. 2009). Activation of the POU-domain transcription factor, Brn2, by β-catenin activates the melanocyte transcription factor MITF, leading to increased proliferation of melanocytes. As melanoma cells become more aggressive, they switch from a canonical Wnt signaling state, where they are very proliferative, to a noncanonical Wnt signaling state, where they now become invasive (O'Connell and Weeraratna 2009). This delineation of proliferation versus invasion in melanoma is known as phenotype switching. When melanoma cells switch to an invasive phenotype, they express higher levels of Wnt5A. Wnt5A has been shown to play multiple roles in melanoma metastasis from modulating the cytoskeleton, to promoting an EMT-like switch, to activating a series of metalloproteinases and tumor homing antigens such as CD44 (Weeraratna et al. 2002; Dissanayake et al. 2008). Many of these effects require the presence and activity of ROR2, but not ROR1, which is not present in metastatic melanoma.

The role of β-catenin in melanoma metastasis is controversial. While some studies have shown that β-catenin is inhibitory to melanoma invasion (Arozarena et al. 2011b), others have shown that β-catenin promotes invasion (Damsky et al. 2011). Studies on human samples have shown a loss of β-catenin during the progression from nevi to metastatic melanoma, and high levels of β-catenin correlate with better prognosis for melanoma patients in multiple studies (Hoek et al. 2006; Chien et al. 2009; Bachmann et al. 2005). Recently, a new study also showed that an inhibitor of β-catenin signaling, sFRP2, is increased in the aged microenvironment (specifically fibroblasts) and shuts off β-catenin signaling in melanoma cells, contributing to age-related increases in melanoma metastasis in both human samples, and in vivo in mice (Kaur et al. 2016). Additionally, in mice, overexpression of the canonical Wnt ligand, Wnt3A, inhibited tumorigenesis. However, as mentioned, other mouse models show that stabilizing β-catenin promotes metastasis (Damsky et al. 2011). The difference between these studies could be due to whether or not β-catenin is mutated (constitutively stabilized). Therefore, studies that look at genetically altered β-catenin tend to show associations with invasion, but studies that look at endogenous β-catenin may show the opposite.

The canonical WNT pathway is constitutively activated in a subset of melanocytic neoplasms called deep penetrating nevi (DPN) (Yeh et al. 2017). DPN are deeply invasive, highly pigmented lesions that are considered benign, but which can occasionally metastasize. DPNs are characterized genetically by a combination of a MAPK pathway activating mutation at the level of BRAF or MEK1 and an activating mutation of β-catenin. It has been shown that WNT signaling in benign nevi with only a BRAF V600E mutation decreases gradually with increasing distance of nevus cells from epithelial structures such as the epidermis and hair follicles and coincides in a decrease in cell size and pigmentation. The latter phenomenon is called "maturation" by pathologists and considered as an important criterion for benignancy. DPN do not show maturation and show constitutive activation of the WNT pathway, as the mutations activating β-catenin override the dependency on secreted WNTs, allowing the tumor cells to invade deeply.

As well as effects on metastasis, the dichotomy between β-catenin and Wnt5A also dictates therapy resistance in melanoma. Activation of β-catenin in cells with a loss of Axin1 has been shown to sensitize melanoma cells to vemurafenib-induced apoptosis, and overexpression of Wnt3A does the same (Biechele et al. 2012). Inhibiting β-catenin induced resistance to PLX4720, and this was confirmed in a later study, where Wnt5A was shown to increase resistance to PLX4720, in part via the hypoxia/

Siah2-induced degradation of β-catenin (O'Connell et al. 2013) and in part via the induction of a pseudo-senescent state (Webster et al. 2015b). Additionally, in aged mice, β-catenin loss correlated to increased resistance to BRAF inhibitor. MEK inhibitor (AZD6244) treatment of NRAS mutant tumors showed a similar dependence on β-catenin for effective apoptosis, suggesting that this might be a universal response to inhibition of the RAS/RAF/MEK/ERK pathway (Yeh et al. 2017). Intriguingly, however, continued, long-term treatment of melanoma patients with vemurafenib did not show the same association between nuclear β-catenin and response, suggesting that this pathway may get reactivated after long-term BRAF inhibitor treatment (Chien et al. 2014).

In addition to targeted therapy, immune therapy plays a large role in the current clinical landscape of melanoma. Wnt5A plays critical roles in hematopoietic stem cell self-renewal, suppressing β-catenin to maintain stem cell quiescence (Nemeth et al. 2007). In melanoma, we have shown that Wnt5A signals to decrease MITF and shuts off melanocytic antigens such as GP100 and MART1, thereby rendering melanoma cells less recognizable to the immune microenvironment (Dissanayake et al. 2008). β-catenin, especially mutant β-catenin, has been considered a melanoma antigen for some time and indeed was used to design some of the earlier tumor-infiltrating lymphocyte therapies (Robbins et al. 1996). A new study suggests that mutant β-catenin may actually do the opposite and decrease immune infiltration, but this has yet to be confirmed in other studies (Spranger et al. 2015). Overall, Wnt signaling plays critical roles in the development and pathogenesis of melanoma. The incredible complexity of this signaling pathway leads to its roles in multiple aspects of melanoma, from metastasis to therapy resistance.

Hippo Signaling

The Hippo signaling pathway (known as MST1/2 in humans) is important in mediating the interplay between contact inhibition and mitogenic signaling. Thus, it is the key pathway that regulates organ size during development and as such is highly conserved. MST1/2 are serine/threonine kinases that phosphorylate LATS1/2, which is activated and signals to phosphorylate and inhibit the activities of the Yes-associated protein (YAP) and the transcriptional coactivator with PDZ-binding motif (TAZ). Once YAP/TAZ signaling is inhibited they are sequestered in the cytoplasm via binding to 14-3-3 proteins. By contrast, unphosphorylated YAP is able to translocate to the nucleus and activate the transcription of a number of genes involved in mitosis and proliferation, such as cyclin A and cyclin E, as well as the TEA family of transcription factors. In addition to MST1/2 (Hippo), this signaling cascade can also be initiated by other MAP kinases, including MAP4K4/6/7 and MAP4K1/2/3/5 (Sanchez and Aplin 2014).

In cancer, the aberrant activation of YAP/TAZ leads to a loss of contact inhibition and subsequently, uncontrolled proliferation of tumor cells. This can be regulated in part by molecules involved in cell-cell adhesion, such as E-cadherin. As cells transition towards a more metastatic phenotype, they lose E-cadherin, allowing for increased activation of YAP/TAZ. YAP/TAZ are expressed in both melanocytes and melanoma cells, but are increased in metastatic disease. In one study, staining of YAP/TAZ in nevi and early stage melanoma revealed a mixed cytoplasmic/nuclear pattern with increased TAZ levels in more invasive lesions. Knockdown of YAP/TAZ led to a loss of tumorigenic and metastatic properties, while overexpressing YAP in melanoma cells increased their ability to metastasize to distant sites (Nallet-Staub et al. 2014).

The observed increase in invasion in YAP-overexpressing cells was attributed to the activation of TEA domain transcription factors, which recognize TEA/ATTS elements as their DNA binding domains (Lamar et al. 2012). YAP activation of TEADs was shown to increase transcription of the connective tissue growth factor, which is also overexpressed in metastatic melanoma, and thought to contribute to melanoma invasion. Transcriptome analysis of melanoma indicated that TEAD was a key regulator of the

invasive phenotype in melanoma (Lamar et al. 2012). In keeping with this, TEAD knockdown has been shown to decrease invasion and increase sensitivity of melanoma cells to targeted therapy. This suggests then that inhibition of the Hippo/YAP/TEAD pathway might be of interest for melanoma therapy. Indeed, the small molecule verteporfin, which inhibits the YAP-TEAD interaction, has been shown to decrease metastatic outgrowth of YAP overexpressing melanoma cells. Importantly, this drug may be effective not only in cutaneous melanoma but also in uveal melanoma, as YAP/TAZ signaling can be activated by mutant GNAQ/11, the predominant mutation in uveal melanoma. Verteporfin has been shown to inhibit the growth/invasion of uveal melanomas bearing GNAQ/11 mutations (Feng et al. 2014). This pathway holds great potential for the discovery of therapies that might affect multiple subtypes of melanoma.

The Notch Signaling Pathway

Like the Wnt pathway, the Notch pathway has been extensively studied in melanoma and can be divided into canonical and noncanonical pathways. Canonical Notch signaling is made up of the four transmembrane Notch receptors (Notch 1-4) and the membrane-bound ligands Jag 1 and 2 and delta-like (dll) 1-3. Signals transduced from these ligands, with the exception of dll3, are very similar, resulting in cleavage of notch by γ-secretase, thereby releasing the Notch intracellular domain (NICD). NICD interacts with MAML and other transcription factors in the nucleus, as part of a DNA binding complex known as the CSL (CBF1/Suppressor of Hairless/LAG-1) complex. One intriguing feature of this pathway is that there are no amplification steps in the signaling cascade, i.e., no phosphorylation of a number of different proteins. This, together with the fact that the receptor is cleaved in order to be activated such that one NICD is generated per activated receptor, means that any genetic alterations that change gene dosage can have dramatic effects (Ranganathan et al. 2011).

Unlike canonical Notch signaling, noncanonical Notch signaling occurs independently of CSL. The mediators of noncanonical Notch signaling are largely unknown, but the output is the activation of canonical Wnt signaling (β-catenin). This may have to do with the fact that Dsh, a key component of the Wnt signaling pathway, can bind to the NICD. NICD can also bind to components of the β-catenin destruction complex such as Axin and GSK3β, thereby affecting the stability of β-catenin. In turn, Wnt signaling can also regulate Notch signaling by upregulating ligands such as jagged1 and Dll4, as well as Notch itself (Borggrefe et al. 2016). Given the importance of Wnt signaling in melanoma, investigators explored the relevance of Notch in this disease. In melanocytes, deletion of Notch results in defects in melanoblast formation and the elimination of melanocyte stem cells. In addition to this, the localization of melanoblasts and melanocyte stem cells is also controlled by Notch signaling. Despite this, Notch1 is not expressed at high levels in melanocytes or benign melanocytic lesions, and forced overexpression of active Notch (Notch-NIC) can transform melanocytes both in the skin and in the retinoid pigmented epithelium (Bedogni 2014).

Notch signaling may also play roles in the metastatic progression of melanoma (Bedogni 2014). Notch 3 and Notch 4 have been implicated in angiogenesis and vasculogenic mimicry. Notch 1 was demonstrated to activate NFκB in pancreatic cancer. In melanoma, it has also been shown that NFκB can increase Notch expression via the PI3K/AKT pathway. Depletion of Notch in pancreatic cancer cells led not only to decreases in NFκB, but also in MMP-9, VEGF, survivin, and COX2. Since all of these molecules have also been implicated in metastatic dissemination of melanoma, it is reasonable to assume that a similar signaling cascade exists in melanoma. Indeed, treating melanoma cells with a γ-secretase inhibitor overcomes survivin expression and leads to apoptosis. In addition, forced activation of Notch in primary melanoma cells led to their progression to a metastatic state via the Wnt pathway, which as described earlier can also signal to increase Notch. Finally, Notch signaling can be activated in

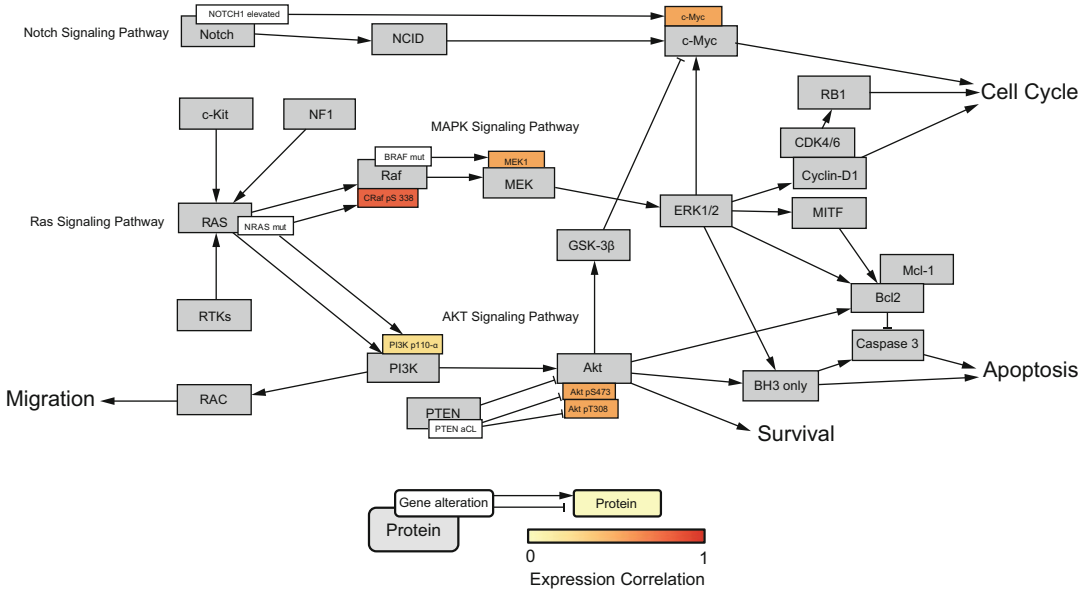

Fig. 3 A network map showing interactions between proteins in cutaneous melanoma signaling pathways. Individual proteins and phosphoproteins are colored according to the spearman correlation results between either mRNA or protein levels and the downstream protein levels for TCGA's cutaneous melanoma samples harboring a gene with a mutation (mut), copy loss (CL), or elevated expression level (elevated). The mutation profile, RNA Sequencing and protein data were generated by the TCGA Research Network: http://cancergenome.nih.gov/. Calculations were performed using Matlab® (v2015b). We acknowledge the help from Tim Purwin (Thomas Jefferson University) for the generation of this figure

situations known to promote metastatic progression such as hypoxia. HIF-1α can regulate Notch1, increasing its expression. This allows for the AKT-dependent transformation of melanocytes to a malignant state, and in the absence of Notch1 this transformation cannot occur (Bedogni et al. 2008).

In addition to hypoxia, other microenvironmental factors can regulate Notch expression in melanoma. It has been shown that fibroblasts transfected with active Notch1 increase their levels of the Wnt-inhibitory secreted protein WISP1. When this happens, Wnt signaling in adjacent melanocytes is inhibited, and their transformation to a malignant state does not occur (Shao et al. 2011). Finally, Notch is also an important player in the immune system as it is critical for hematopoietic stem cell self-renewal and lineage decisions (Bigas et al. 2013). It is not yet known how Notch in infiltrating immune cells may affect the survival, growth, and metastatic progression of melanoma. Understanding the interplay between Notch in the microenvironment and Notch in the tumor cell will be critical before therapies targeting this pathway can be conceived.

Conclusion

The heterogeneity and plasticity of melanoma is reflected in the vast complexity of the signaling pathways described herein (Fig. 3) and indeed in many other pathways considered of minor relevance that were not covered in this review. Studies to understand these signaling pathways mechanistically have provided vital insights into the acquisition of the malignant traits of the melanoma cell. Additionally, they have highlighted strategies to target oncogene addiction, which have translated into the development of novel therapies. The role of these signaling pathways is currently being interrogated for alterations in immune cell infiltration/activation in the melanoma tumor microenvironment.

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