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The Wnts of Change: How Wnts Regulate Phenotype Switching in Melanoma

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Abstract

The outgrowth of metastatic and therapy-resistant subpopulations in cancer remains a critical barrier for the successful treatment of this disease. In melanoma, invasion and proliferation are uncoupled, such that highly proliferative melanoma cells are less likely to be invasive, and vice versa. The transition between each state is likely a dynamic rather than a static, permanent change. This is referred to as “phenotype switching”. Wnt signaling pathways drive phenotypic changes and promote therapy resistance in melanoma, as well as play roles in the modulation of the immune microenvironment. Three Wnt signaling pathways play a role in melanoma progression, canonical (β-catenin dependent), polar cell polarity (PCP), and the Wnt/Ca^{2+} pathway. Here we summarize phenotype plasticity, and its role in therapy resistance and immune evasion. Targeting the Wnt signaling pathways may be an effective way to overcome tumor plasticity in melanoma.

Keywords

phenotype switching; melanoma; Wnt signaling; therapy resistance; immune microenvironment

Introduction

1. Phenotype switching in melanoma

Phenotype switching between proliferative and invasive phenotypes promotes metastasis, progression, and therapy resistance in melanoma (1). Markers of proliferative and invasive phenotypes following dynamic switching are similar to markers observed in proliferative and invasive states during epithelial to mesenchymal transitions (EMT). Recent studies have also observed that invasive cells have characteristics similar to cancer stem cells, which can switch between proliferative and invasive phenotypes (1–5), potentially promoting survival and therapy resistance (6, 7).

Proliferative subpopulations of melanoma can be uniquely identified by differential expression of markers associated with melanocyte differentiation, such as MITF and

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MART1. Cells that produce these markers are typically epithelial in morphology, highly proliferative, but less invasive (8). When melanoma cells lose these differentiation antigens, they become more mesenchymal in appearance and highly invasive. Clinically, this also makes them harder to detect, as melanocytic antigens are used to detect melanoma metastases in lymph nodes and other organs. We have previously shown that non-canonical Wnt signaling down-regulates MART1 expression (9), and is a key mediator of the switch from a proliferative and less invasive state to a mesenchymal and highly invasive one (10). On the other hand, it is known that canonical Wnt signaling, via β-catenin, plays a key role in the genesis of melanoma (11, 12). Although its role in melanoma metastasis is unclear, most data suggests loss of β-catenin activity contributes to metastasis in melanoma (13–17). This is consistent with the finding that in some cell systems, Wnt5A can inhibit β-catenin activity by increasing its degradation (18, 19). In this review, we will assess what is known about the interplay between canonical and non-canonical Wnt signaling in melanoma, and the implication of phenotype switching not only for metastatic progression of melanoma, but also for therapy resistance and immune surveillance.

2. Wnt signaling guides phenotype plasticity in melanoma

The Wnt signaling pathways promote dichotomous phenotypes in melanoma through the canonical and non-canonical pathways mediated by the Wnt family of proteins. The Wnt family of proteins can signal via three distinct pathways involving three key mediators: β-catenin (the canonical Wnt signaling pathway), Jnk (the planar cell polarity pathway) and PKC/ Ca2+ (non-canonical Wnt signaling pathway) (20). Each of these three pathways has distinct, but not always separate functions. Our lab has shown that when melanoma cells switch to a more invasive phenotype, the non-canonical Wnt5A signaling pathway is activated. Activation of the non-canonical pathway results in the activation of PKC and the release of intracellular Ca^{2+}, which in turn activates downstream signaling intermediates such as CAMKII (10). This results in a decrease in canonical Wnt signaling and melanocytic antigen expression (9), and a switch to a mesenchymal morphology (10). Current data suggest that melanoma cells utilize canonical Wnt signaling for growth and transformation (21, 22) and non-canonical Wnt signaling for metastasis (4, 23–26). (Figure 1)

2.1 Wnt ligands, receptors and co-receptors—Canonical and non-canonical Wnt signaling pathways are mediated through a combination of Wnt ligands, receptors, and co-receptors which initiate specific downstream signaling pathways. Canonical Wnt signaling is activated by Wnt ligands Wnt1, 3A, 7, 8, and 8B, whereas non-canonical Wnt signaling is activated by Wnt4, 5A, 5B, and 11 (reviewed in Weeraratna, 2005). In melanocytes, the Wnt ligands Wnt1 and 3A activate the canonical pathway, whereas in melanoma, Wnt5A activates the non-canonical pathway. Wnt proteins interact with the Frizzled (Fzd) family of receptors, which consist of 10 known members. They are characterized by an extracellular N-terminal cysteine-rich domain that interacts with both Wnts and Wnt co-receptors (27, 28). The Fzd receptors are seven-pass transmembrane G-protein coupled receptors, which upon Wnt binding, require heterotrimeric G-proteins for downstream signaling (29). The activation of specific G-protein subunits depends on the Wnt ligand subtype and the Fzd receptor to which it binds (30, 31).

Co-receptors also play an important role in Wnt signaling. Low-density lipoprotein receptor-related proteins (LRPs), and receptor tyrosine kinase-like orphan receptors (RORs) are known co-receptors important in Wnt signaling in melanoma. LRP co-receptors, LRP-5 and LRP-6, are single pass transmembrane proteins that form a co-receptor complex with the Fzd receptor and activate downstream signaling pathways (32). The ROR co-receptors, ROR1 and ROR2, are single pass-transmembrane proteins with extracellular immunoglobulin-like, cysteine-rich and kringle domains (33–35). The intracellular domains of these receptors contain both tyrosine and serine-threonine kinase domains (36). ROR1 and ROR2 are inversely expressed in proliferative and invasive melanoma cells. ROR1 expression promotes survival and proliferation whereas ROR2 expression promotes invasion. Other co-receptors, less well-described in humans include Ryk, Derailed (drosophila) and FRL1/Crypto (xenopus).

2.2 Canonical Wnt Signaling in Melanoma—The canonical Wnt pathway is mediated through β-catenin. Canonical Wnt signaling is activated by the binding of canonical Wnts, Wnt1/ Wnt3A, to their receptors, FRZD1/7, and also co-receptors, LRP5/6 and ROR1. In the absence of Wnt signaling, β-catenin is targeted for degradation via ubiquitination by GSK3-β. GSK3-β phosphorylates β-catenin thereby priming it for ubiquitination. Upon initiation of canonical Wnt signaling, the downstream mediator disheveled becomes activated and prevents β-catenin degradation by inhibiting GSK3-β. Stabilization of β-catenin, allows β-catenin to enter the nucleus and associate with transcription factors such as LEF (lymphoid enhanced transcription factor) and TCF (T cell transcription factor). In melanoma, canonical Wnts, Wnt1 and Wnt3A, are critical for melanocyte transformation and bypassing melanocyte senescence (21, 22, 37). While it is clear that β-catenin is critical in the early stages of melanocyte transformation, and melanomagenesis, conflicting studies on the role of β-catenin in melanoma metastasis have been published. Paradoxically, melanoma patients with higher levels of β-catenin have a better prognosis (13, 16), implying that the mechanisms that drive melanomagenesis are distinct from those that dictate outcome. However, in defined genetic contexts such as in an animal models of melanoma where BRAF is mutated, PTEN is deleted, and β-catenin is stabilized, metastasis is increased (38). Further, the receptor and co-receptor context at different stages of melanoma progression may also define the path of Wnt signaling (39).

Canonical Wnt signaling co-operates with MAP kinase signaling to regulate the expression and activity of the master transcription regulator microphthalmia-associated transcription factor (MITF) (8, 40, 41). In proliferative melanoma cells, LEF1/β-catenin signaling activates MITF which in turn activates melanocyte specific target genes including melan-A (Mart-1), Dct, and Tyr. MITF also activates cell cycle regulators p16, p21 and CdK2 (1, 41–44). The regulation of melanocyte specific target genes and cell cycle regulators suggests a role for Wnt signaling in immune surveillance and therapy resistance as discussed below. The effects of Wnt/β-catenin and MITF signaling may be context-dependent, with activating mutations and intracellular localization β-catenin playing a role (45–48). Intracellular localization of β-catenin also correlates with cellular phenotype, with membrane-associated β-catenin found in proliferative cells and cytosolic β- catenin in invasive cells (23). Nuclear localization of β-catenin does not appear to correlate with cellular phenotype, but expression

of co-factors LEF1 and TCF4 correlates with proliferative and invasive phenotypes, respectively. This suggests that cofactors play a role in regulating β-catenin signaling. Prevention of nuclear β-catenin by Wnt5A activity leads to suppression of LEF1 (23), upregulation of cofactor TCF4, and a switch to a more invasive phenotype. The interplay of Wnt signaling pathways controls the conversion of melanoma from a primary tumor, to a more deadly metastatic disease.

2.3 Non-canonical Wnt Signaling in melanoma—Down-regulation of canonical Wnt signaling and up-regulation of non-canonical Wnt signaling is thought to promote the phenotype switch of melanoma cells from a proliferative state, to a more motile, invasive state. Non-canonical Wnt5A signaling initiates this switch by inhibiting canonical Wnt signaling in a Siah2 and APC dependent manner (49). Specifically, Wnt5A signaling increases Siah2 expression, leading to ubiquitination of β-catenin, proteasome-dependent degradation, and down-regulation of β-catenin dependent Wnt signaling. Wnt5A has also been shown to inhibit canonical Wnt signaling by binding to Fzd receptors. Wnt5A competes with Wnt3A for binding to Fzd2, preventing Wnt3A-dependent phosphorylation of LRP6 and accumulation of β-catenin. (50) Prevention of nuclear β-catenin by Wnt5A activity leads to suppression of LEF1, upregulation of cofactor TCF4, and a switch to a more invasive phenotype (23).

The non-canonical Wnt/Ca^{2+} signaling pathway, mediated by Wnt5A, is associated with increasing metastatic potential of melanoma cells and tumor grade (4, 24–26, 51, 52). Wnt5A promotes invasion via PKC/Ca^{2+} signaling in part through remodeling of intracellular actin and filamin to promote membrane contractility. Wnt5A mediates Ca^{2+} dependent signaling through activation of G proteins, resulting in the increase of intracellular inositol 1,4,5-triphosphate (IP3), 1,2 diacylglycerol (DAG), and Ca^{2+}. The increase in intracellular Ca^{2+} activates calpain-1 and calcineurin (Cn). Calpain-dependent cleavage of filaminA mediates the remodeling of the cytoskeleton and promotes motility (4). Release of Ca^{2+} also leads to the activation of calcium-dependent signaling proteins such as calmodulin-dependent protein kinase II (CAMKII) and protein kinase C (PKC). Wnt5A signaling via PKC has been shown to mediate the motility of melanoma cells by inhibiting metastasis repressors such as KISS-1 and ECAD, and up-regulating metastasis-associated molecules such as CD44 and SNAIL (53–56). PKC also activates STAT3 leading to inhibition of MITF and consequently down-regulation of melanocytic antigens (4).

3. Microenvironmental Regulation of Wnt-mediated phenotype signaling

Several types of stress can promote environmental changes that induce phenotypic changes in melanocytes and melanoma cells. UV light induces keratinocytes to secrete α-MSH, which increases expression of PGC-1α and PGC-1β (PGC-1s) in melanocytes, leading to an increase in MITF signaling and melanin synthesis (57). Melanin synthesis is an energy demanding and oxidative process that is thought to generate high levels of reactive oxygen species (ROS) (58). PGC-1α, a co-activator of Peroxisome proliferator activated receptor (PPAR) gamma, controls ROS levels in many cell types including melanocytes (59). In melanoma cells, MITF drives PGC-1α expression, increasing the ability of these cells to

detoxify ROS, and promoting survival under high oxidative stress conditions (60). This makes these cells more sensitive to targeted therapies in combination with ROS inhibitors.

Interestingly, Wnt signaling downstream of β-catenin is also known to modulate responses to ROS by increasing the redox effector APE-1via MITF (61). APE-1, (apurinic/apyrimidinic endonuclease) is a redox sensor and DNA endonuclease critical for repair of oxidative DNA damage. APE-1 creates a nick in the phosphodiester backbone of DNA to promote the removal of mismatched or damaged DNA via the DNA base excision repair pathway. Loss of MITF blocks the induction of APE-1 by ROS and reduces the ability of melanoma cells to survive damage due to ROS. Loss of MITF expression during melanoma progression and in therapy resistance may increase carcinogenesis through an increase in ROS induced DNA damage. Increased ROS due to oxidative stress results in regulation of genetic and epigenetic cascades underlying changes in gene expression during cancer progression (62). Epigenetic changes during tumor progression may block the expression of multiple tumor suppressor genes and allow cells to exert a survival advantage (63–67).

In addition, microenvironmental pressure can exert changes in the expression of Wnt receptors which promotes phenotype plasticity in melanoma. The orphan tyrosine kinase receptors ROR1 and ROR2 promote a more proliferative or a more invasive phenotype respectively. ROR1 and ROR2 are also inversely expressed in melanoma, and negatively regulate each other. Microenvironmental factors such as an increase in hypoxia, can drive the expression of ROR2, leading to an increase in Wnt5A signaling, and an increase in invasion (68). Wnt signaling via Wnt5A and ROR2 leads to down-regulation of canonical Wnt signaling and up-regulation of pathways involved in cell motility. Wnt5A signaling is reinforced through activation of PKC, which leads to stabilization of Wnt5A mRNA, an increase in Wnt5A secretion, and reinforcement of non-canonical Wnt signaling pathways. Wnt5A signaling can also be increased following extracellular signaling through TGFβ. TGFβ, which is a marker of an invasive phenotype in melanoma, increases Wnt5A signaling through the Smad complex and promotes the expression and secretion of proteins that regulate the microenvironment (1, 69). The microenvironment can play a large role in tumor heterogeneity and phenotype switching in melanoma.

4. Phenotype Switching in Therapy Resistance

The ability of cells to switch phenotypes has implications not only for melanoma metastasis but also for therapy resistance. 50% of melanomas harbor a mutation in the BRAF gene, resulting in a valine to glutamic acid substitution at residue 600 and leading to constitutive activation of the MAP kinase pathway. Mutant BRAF inhibitors have had great initial success in the clinic, however resistance to these therapies quickly arises. Understanding why this occurs and how to stop this is a key area of interest in melanoma biology. In addition, intrinsic resistance also poses a problem for some patients. While the majority of mutant BRAF patients have some degree of response to mutant BRAF inhibitors, about 10–20% of these patients do not. Konieczkowski et al found that patients who are sensitive and resistant to BRAF targeted therapy have distinct transcriptional profiles (70). Patients who are sensitive have high MITF, whereas patients who are non-responsive have low MITF expression and higher NF-kB and AXL expression. Patients who express high NF-kB and

AXL are resistant to single and combinatorial inhibition of RAF and MEK kinases. NF-kB signaling antagonizes MITF and increases Wnt5A signaling (68, 69, 71) (Figure 2).

4.1. Wnt signaling in therapy resistance

The increase in Wnt5A is consistent with the observation that intrinsically resistant and acquired resistant subpopulations, which emerge following MAPK pathway targeted therapy, express increased markers of an invasive phenotype (68, 70–75). Wnt5A signaling, which is increased in invasive melanoma cells, drastically decreases sensitivity to $BRAF^{V600E}$ targeted therapy. Our lab has shown that microenvironmental stress causes phenotype switching in BRAF-mutant melanoma cells. This switch decreases sensitivity to BRAF inhibitors and induces an adaptive stress response following $BRAF^{V600E}$ targeted therapy and DNA damage (68, 71, 76). Tumors which have a high level of Wnt5A expression prior to therapy are intrinsically resistant to $BRAF^{V600E}$ targeted therapy. Intriguingly, tumors which express low Wnt5A, but ultimately recur, also begin to express Wnt5A, suggesting Wnt5A plays a role in acquired resistance as well. This occurs, in part, via the Wnt5A mediated degradation of b-catenin. Resistance to $BRAF^{V600E}$ targeted therapy has been connected to a loss of β-catenin signaling due to an increase in AXIN1, a negative regulator of β-catenin (77). Interestingly, our data suggests that Wnt5A may further contribute to β-catenin loss, and perhaps suggests a co-operative interaction between Wnt5A and Axin. Finally, oncogenic BRAF has also been shown to decrease MITF and PGC1α expression (78). This suggests that oncogenic BRAF itself may contribute to beta-catenin loss, though this has not yet been explored.

4.2. Cell fate and therapy resistance

One more contributing factor to phenotype switching- induced therapy resistance is the generation of cells with stem cell like characteristics (6, 7). Invasive cells from multiple types of cancer including breast, prostate, and epithelial exhibit stem-like characteristics (6, 79, 80). In melanoma, slow cycling cells, which express JARID1B, are required for tumor maintenance and growth (81). Slow cycling cells are also increased in response to BRAF targeted therapy (82), suggesting they play a role in therapy resistance. Therapy resistant melanoma cell populations show an increase in Wnt5A. We have shown that Wnt5A promotes a p21 dependent senescent like state, which we call “pseudosenescence”, that results in the ability of these cells to evade targeted therapy, yet also promotes their metastasis. Together these data suggest that a switch from canonical to non-canonical Wnt signaling may guide a more aggressive tumor state encompassing both metastasis and therapy resistance.

5. Immune regulation and Wnt signaling

Melanoma is a highly immunogenic disease, but it has been shown that immune checkpoints play a significant role in suppressing the immunogenicity of melanoma. Regulatory checkpoints such as CTLA4 and PD-1 signal to control T-cell proliferation and T-cell activity respectively. By targeting these immune suppressive receptors, drugs such as ipilimumab (anti-CTLA4) and nivolimumab (anti-PD1) result in response rates of about 10–40% in patients (83, 84). More recent advancements in immune based therapies have led to

combination treatments with these two drugs capable of reaching response rates of 50–70%, however this is accompanied by an increase in toxicities (84). Although these response rates are generally much lower than the mutant BRAF inhibitors mentioned earlier, patients who respond to immune based therapies tend to have much longer overall and progression free survival (83, 84). As a result, an emphasis has been placed on understanding why certain patients do not respond to immune based therapies, and evidence exists to suggest Wnt/β-catenin signaling in melanomas may be playing a role.

5.1 Non-canonical Wnt signaling in immune therapy resistance

Non-canonical Wnt signaling appears to contribute to the immune suppressive environment in melanoma via paracrine and autocrine mechanisms. In melanoma cells, Wnt5A plays a role in suppressing the expression of melanoma antigens, specifically MART1. MART1 is a downstream target of MITF, and has been used in vaccine based therapies in melanoma patients as early as 1999. Phase I data from a study using a vaccine to promote immune recognition of the melanoma antigen recognized by T cells 1 (MART1) showed a correlation between regression free responses (RFS) and cytokine release in patients who had a response to the vaccine. All patients who had a high cytokine response remained relapse free, whereas patients who relapsed had either no or weak cytokine responses (85). Interestingly, Wnt5A expression inversely correlates with MART1 expression in melanoma cells.

In melanoma patient metastases, where Wnt5A is highly expressed, there is a loss of MART1 expression viewed by IHC analysis compared to nevus samples where Wnt5A is low (4). A review of the literature suggests a potential regulation of MART1 by PKC, STAT3, and MITF through non-canonical Wnt5A signaling (86–88). Consistent with this, treatment of Wnt5A low expressing melanoma cell lines with recombinant Wnt5A results in an increase in PKC and STAT3 activation, and a decrease in MART-1 expression (4). Additionally, PKC inhibition by GO6983 or STAT3 inhibition by a STAT3 inhibitor peptide (PpYLKTKAAVLLPVLLAAP) in Wnt5A low expressing cells prevents loss of MART1 in the presence of recombinant Wnt5A. The loss of MART1 in Wnt5A expressing cells also reduced T cell recognition of melanoma cell lines. Co-culturing of Wnt5A low, MART1 high melanoma cell lines with T cells primed against MART1 induced IFN-γ release *in vitro*, whereas Wnt5A high, MART1 low cell lines did not induce IFN- γ release (4). Importantly, recombinant Wnt5A alone has no effect on T cells, suggesting the IFN- γ release is not the result of Wnt5A signaling in T cells. Similar results were observed in the Wnt5A low expressing B16 murine melanoma cell line where treatment with recombinant Wnt5A led to loss of the melanoma antigen gp100 and failure to activate gp100 primed T cells *in vitro*. This response was recapitulated *in vivo* by treating mice bearing B16 murine melanomas with either vehicle or recombinant Wnt5A by tail vein injection. All mice treated with recombinant Wnt5A developed lung metastases whereas only 3 of 10 mice in the vehicle group had detectable lung metastases (4). These data indicate a role of Wnt5A in immune evasion through loss of melanoma antigens.

Wnt5A also plays a paracrine role in suppressing the immune microenvironment. Cytokines present in the immune microenvironment which regulate T cell exhaustion and immune

suppression are key for melanoma to avoid immune detection and can promote resistance to immune based therapies (89). WNT/β-catenin signaling in dendritic cells has been shown to lead to an upregulation of FOXP3+ regulatory T cells (Tregs), and a loss of immune stimulating cytokine production by the dendritic cells (90). Recently, a link between tumor derived Wnt5A and increased Indoleamine 2,3-dioxygenase (IDO) expression by dendritic cells (DCs) has been described in melanoma (91). IDO is a tryptophan degrading enzyme expressed by antigen presenting cells (APCs) which contributes to immune suppression in a variety of ways. IDO directly inhibits T cells by causing cell cycle arrest through loss of tryptophan, indirectly inhibits them by altering antigen presentation functions of APCs, and may also play a role in Treg generation and proliferation (92).

Holtzhausen et al. has shown that gene expression levels of *WNT5A* from the Oncomine Cancer Profiling Database strongly correlate with the Treg marker, *FOXP3*, in melanoma. Consistent with this observation, there is an increase in *FOXP3*+ Tregs in the tumor draining lymph nodes (TDLNs) of mice harboring Wnt5A expressing $BRAF^{V600E}PTEN^{-/-}$ murine melanomas compared to mice harboring $BRAF^{V600E}PTEN^{-/-}$-$WNT5A^{KD}$ melanomas (91). The effect seen in the lymph nodes of mice suggests Wnt5A secreted by the tumors causes the observed increase in Tregs in the lymph nodes. Indeed, purified bone marrow derived dendritic cells (BMDCs) co-cultured with spleen derived CD4+ T cells induced *FOXP3*+ Treg expansion when incubated with Wnt5A compared to other DC stimuli *in vitro* (91). A TCF/LEF1-luciferase reporter system and qRT-PCR of common β-catenin target genes identified induction of the β-catenin pathway in dendritic cells incubated with WNT5A. qRT-PCR also identified an increase in *Ido1* transcripts in tumor infiltrating dendritic cells (TIDCs) and TDLN-derived DCs compared to non-tumor bearing mice. Although these data indicate Wnt5A may be signaling in a canonical manner in DCs, contrary to non-canonical signaling more often seen in melanoma, this could be explained by the receptors present on dendritic cells (93). A corresponding increase in dendritic cell IDO expression was also observed in $BRAF^{V600E}PTEN^{-/-}$-NTC compared to dendritic cells isolated from $WNT5A^{KD}$ tumor bearing mice. Together these data provide a role for melanoma secreted Wnt5A in promoting a tumor suppressive phenotype *in vivo*, both via paracrine and autocrine mechanisms.

5.2. Canonical Wnt signaling in immune therapy resistance

A role for canonical Wnt/β-catenin signaling in resistance to immune checkpoint therapies has also been described in melanoma (94). Spranger et al. has described an inverse correlation between active β-catenin signaling and T cell infiltration in patient tumors. This was also observed in the $BRAF^{V600E}PTEN^{-/-}$ genetically engineered mouse (GEM) model, where tumors expressing mutant β-catenin, which accumulates in the nucleus, failed to accumulate CD3+ T cells (94). The loss of T cell infiltration in the β-catenin expressing GEM tumors was shown to be the result of a failure of T cell priming due to the inability of β-catenin expressing tumors to recruit and activate CD103+ dendritic cells (94). Loss of dendritic cell recruitment and activation in β-catenin expressing GEM tumors correlated with a reduction in CCL4, a chemokine part of the macrophage inflammatory protein family known to recruit various immune effector cells (95).

It has previously been shown that the transcription factor ATF3 is regulated downstream of the Wnt/β-catenin pathway, and is a negative regulator of *Ccl4* expression in murine macrophages (68). This regulation was also found in β-catenin expressing GEM cells, where siRNA mediated knockdown of either *Atf3* or *Ctnnb1* restored *Ccl4* expression *in vitro* (94). Importantly, only a partial restoration of *Ccl4* was observed with *Atf3 or Ctnnb1* knock down. Additionally, there is evidence to suggest that CCL4 in the tumor microenvironment may also attract CD4+ regulatory T cells (71). In this case, a loss of CCL4 as a result of β-catenin signaling in the tumor cells would actually have an immune stimulating effect, as opposed to the suppressive effect described here. Nonetheless, β-catenin expressing GEM tumors were resistant to anti- CTLA4 and anti-PD1 treatment *in vivo*, and intratumoral injection of activated dendritic cells was able to resensitize the tumors to treatment (94). Together these data provide a role of canonical Wnt/β-catenin signaling in immune suppression and resistance to checkpoint inhibitor therapy. However, it should be noted that although *WNT7B* and *FZD3* were shown in the genetic analysis of patient data, Wnt ligands were not tested in the animal models described here.

As our understanding of immune based therapies in melanoma advances, it is becoming clear that communication between signaling pathways in the cancer cells and the immune microenvironment is essential for cancers to evade immune detection and resist immune based therapies. More research is needed on Wnt signaling and its effects on the immune response to cancer.

6. Controversies of Wnt Signaling in Phenotype Switching

While Wnt5A plays pro-metastatic roles in some cancers, such as melanoma, prostate, gastric and renal cell cancers, it appears to play a tumor suppressive role in other cancers such as ovarian, colon and breast. It may be that these hormone dependent cancers (breast/ovarian) activate different pathways downstream of Wnt signaling that have yet to be uncovered (96). Another possibility is that Wnt5A effects may be dependent on the maintenance of the wild type form of some genes, such as p53, APC, or β-catenin, which remain wild type in melanoma, but are commonly mutated in ovarian, colon, and breast cancers (97–99). This would also account for some of the discrepancy we observe in data surrounding the role of beta-catenin in melanoma metastasis. While the majority of data point to an anti-metastatic and therapy sensitive phenotype for β-catenin, there are some studies that implicate increased β-catenin in melanoma metastasis (45, 100). However, the majority of these studies are performed in cells in which β-catenin has been artificially stabilized, via mutations that maintain constitutive expression of β-catenin in the nucleus. When this is the case, nuclear β-catenin appears to drive invasion, and make cells more aggressive, and less responsive to immune therapies, as discussed above. It may also be that other intermediates, such as ARF6, promote nuclear translocation of β-catenin, in Wnt5A high cells (101). It is of note that we have rarely observed nuclear beta-catenin in human melanoma samples. Further, despite an early study claiming a preponderance (23%) of mutations in β-catenin (102), subsequent data, including data from the TCGA, indicate that these mutations are not common (2–4%) in melanoma (103). Teasing apart the contributions and relevance of nuclear vs cytoplasmic/ membrane β-catenin to phenotype switching and melanoma progression in general should be an area of investigation.

7. Targeting Wnt signaling to improve cancer therapy

The various roles the Wnt signaling pathway can play in immune suppression and resistance suggests a potential target to help improve immune based therapies in melanoma. Recent studies have explored activating Wnt/ β-catenin signaling in order to increase patient survival and response to therapy. Wnt/ β-catenin signaling increases markers associated with a better prognosis including MITF and MART1 (104–106). Activation of the Wnt/ β-catenin pathway via inhibitors specific to GSK3β and GSK3α increased β-catenin levels. Stabilization of β-catenin led to an increase in Axin2, a downstream target of Wnt signaling (107). Whether or not this strategy will yield a long-term response is yet unknown, since it appears that tumors readily switch between canonical and non-canonical Wnt signaling to evade death and promote tumor maintenance. Further, increasing tumor growth, which would be a function of increasing b-catenin via Wnt5A inhibition, even in the context of inhibiting metastasis is not a preferred option for many patients. Therefore, drugs such as LGK974 which inhibit both canonical and non-canonical Wnt signaling through inhibition of the Wnt specific acetyltransferase Porcupine (76), may be highly effective in combination with current treatments in melanoma patients, as they will inhibit both tumor growth, and tumor metastasis.

References

1. O'Connell MP, et al. Hypoxia Induces Phenotypic Plasticity and Therapy Resistance in Melanoma via the Tyrosine Kinase Receptors ROR1 and ROR2. Cancer Discovery. 2013; 3(12):1378–1393. [PubMed: 24104062]
2. Biddle A, et al. Cancer Stem Cells in Squamous Cell Carcinoma Switch between Two Distinct Phenotypes That Are Preferentially Migratory or Proliferative. Cancer Research. 2011; 71(15): 5317–5326. [PubMed: 21685475]
3. Dang H, Ding W, Emerson D, Rountree CB. Snail1 induces epithelial-to-mesenchymal transition and tumor initiating stem cell characteristics. BMC Cancer. 2011; 11(1):396. [PubMed: 21929801]
4. Dissanayake SK, et al. Wnt5A Regulates Expression of Tumor-Associated Antigens in Melanoma via Changes in Signal Transducers and Activators of Transcription 3 Phosphorylation. Cancer Research. 2008; 68(24):10205–10214. [PubMed: 19074888]
5. Witze ES, Litman ES, Argast GM, Moon RT, Ahn NG. Wnt5a Control of Cell Polarity and Directional Movement by Polarized Redistribution of Adhesion Receptors. Science. 2008; 320(5874):365–369. [PubMed: 18420933]
6. Mani S, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell. 2008; 133:704–715. [PubMed: 18485877]
7. Morel A, et al. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS ONE. 2008; 3:e2888. [PubMed: 18682804]
8. Hoek KS, et al. In vivo Switching of Human Melanoma Cells between Proliferative and Invasive States. Cancer Research. 2008; 68(3):650–656. [PubMed: 18245463]
9. Dissanayake SK, et al. Wnt5A regulates expression of tumor-associated antigens in melanoma via changes in signal transducers and activators of transcription 3 phosphorylation. Cancer Res. 2008; 68(24):10205–10214. [PubMed: 19074888]
10. Dissanayake SK, et al. The Wnt5A/protein kinase C pathway mediates motility in melanoma cells via the inhibition of metastasis suppressors and initiation of an epithelial to mesenchymal transition. J Biol Chem. 2007; 282(23):17259–17271. [PubMed: 17426020]
11. Curley DP, Bosenberg MW. A new mechanism of release from senescence: suppression of p16INK4a by beta-catenin. Pigment Cell Melanoma Res. 2008; 21(1):5–6. [PubMed: 18353136]

12. Delmas V, et al. Beta-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. Genes Dev. 2007; 21(22):2923–2935. [PubMed: 18006687]
13. Bachmann IM, Straume O, Puntervoll HE, Kalvenes MB, Akslen LA. Importance of P-cadherin, betacatenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. Clin Cancer Res. 2005; 11(24 Pt 1):8606–8614. [PubMed: 16361544]
14. Biechele TL, et al. Chemical-genetic screen identifies riluzole as an enhancer of Wnt/beta-catenin signaling in melanoma. Chem Biol. 2010; 17(11):1177–1182. [PubMed: 21095567]
15. Biechele TL, Chien AJ. Re: Specific targeting of Wnt/beta-catenin signaling in human melanoma cells by a dietary triterpene lupeol. Carcinogenesis. 2011; 32(1):120. author reply 121. [PubMed: 20980347]
16. Chien AJ, et al. Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. Proc Natl Acad Sci U S A. 2009; 106(4):1193–1198. [PubMed: 19144919]
17. Demunter A, Libbrecht L, Degreef H, De Wolf-Peeters C, van den Oord JJ. Loss of membranous expression of beta-catenin is associated with tumor progression in cutaneous melanoma and rarely caused by exon 3 mutations. Mod Pathol. 2002; 15(4):454–461. [PubMed: 11950921]
18. Nemeth MJ, Topol L, Anderson SM, Yang Y, Bodine DM. Wnt5a inhibits canonical Wnt signaling in hematopoietic stem cells and enhances repopulation. Proc Natl Acad Sci U S A. 2007; 104(39): 15436–15441. [PubMed: 17881570]
19. Topol L, et al. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. J Cell Biol. 2003; 162(5):899–908. [PubMed: 12952940]
20. O'Connell MP, Weeraratna AT. Hear the Wnt Ror: how melanoma cells adjust to changes in Wnt. Pigment Cell Melanoma Res. 2009
21. Cheng M, et al. The p21Cip1 and p27Kip1 CDK ‘inhibitors’ are essential activators of cyclin D-dependent kinases in murine fibroblasts. The EMBO Journal. 1999; 18(6):1571–1583. [PubMed: 10075928]
22. Goodall J, et al. Brn-2 Expression Controls Melanoma Proliferation and Is Directly Regulated by β-Catenin. Molecular and Cellular Biology. 2004; 24(7):2915–2922. [PubMed: 15024079]
23. Ghosh MC, et al. Activation of Wnt5A signaling is required for CXC chemokine ligand 12–mediated T-cell migration. Blood. 2009; 114(7):1366–1373. [PubMed: 19520808]
24. Bergenfelz C, et al. Wnt5a Induces a Tolerogenic Phenotype of Macrophages in Sepsis and Breast Cancer Patients. The Journal of Immunology. 2012; 188(11):5448–5458. [PubMed: 22547701]
25. Pereira C, Schaer DJ, Bachli EB, Kurrer MO, Schoedon G. Wnt5A/CaMKII Signaling Contributes to the Inflammatory Response of Macrophages and Is a Target for the Antiinflammatory Action of Activated Protein C and Interleukin-10. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008; 28(3):504–510.
26. Ramirez AG, Wages NA, Hu Y, Smolkin ME, Slingluff CL Jr. Defining the effects of age and gender on immune response and outcomes to melanoma vaccination: a retrospective analysis of a single-institution clinical trials' experience. Cancer immunology, immunotherapy : CII. 2015
27. Nusse R, Varmus HE. Wnt genes. Cell. 1992; 69(7):1073–1087. [PubMed: 1617723]
28. Miller J. The Wnts. Genome Biology. 2001; 3(1) reviews3001.3001 - reviews3001.3015.
29. Slusarski DC, Corces VG, Moon RT. Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. Nature. 1997; 390(6658):410–413. [PubMed: 9389482]
30. Liu X, et al. Activation of a frizzled-2/beta-adrenergic receptor chimera promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via Galphao and Galphat2. Proc Natl Acad Sci USA. 1999; 96:14383–14388. [PubMed: 10588714]
31. Malbon CC, Wang H-y, Moon RT. Wnt Signaling and Heterotrimeric G-Proteins: Strange Bedfellows or a Classic Romance? Biochemical and Biophysical Research Communications. 2001; 287(3):589–593. [PubMed: 11563835]
32. Li Y, Bu G. LRP5/6 in Wnt signaling and tumorigenesis. Future Oncology. 2005; 1(5):673–681. [PubMed: 16556044]

33. Masiakowski P, Yancopoulos GD. The Wnt receptor CRD domain is also found in MuSK and related orphan receptor tyrosine kinases. Curr. Biol. 1998; 8(12):R407. [PubMed: 9637909]
34. Rehn M, Pihlajaniemi T, Hofmann K, Bucher P. The frizzled motif: in how many different protein families does it occur? Trends in Biochemical Sciences. 1998; 23(11):415–417. [PubMed: 9852758]
35. Katoh MKM. Comparative genomics on ROR1 and ROR2 orthologs. Oncology Reports. 2005; 14(5):1381–1384. [PubMed: 16211313]
36. Masiakowski P, Carroll RD. A novel family of cell surface receptors with tyrosine kinase-like domain. J. Biol. Chem. 1992; 267(36):26181–26190. [PubMed: 1334494]
37. Delmas V, et al. β-Catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. Genes Dev. 2007; 21(22):2923–2935. [PubMed: 18006687]
38. Damsky WE, et al. beta-catenin signaling controls metastasis in Braf-activated Pten-deficient melanomas. Cancer Cell. 2011; 20(6):741–754. [PubMed: 22172720]
39. Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. PLoS Biol. 2006; 4(4):e115. [PubMed: 16602827]
40. Wellbrock C, Arozarena I. Microphthalmia-associated transcription factor in melanoma development and MAP-kinase pathway targeted therapy. Pigment Cell Melanoma Res. 2015; 28(4):390–406. [PubMed: 25818589]
41. Widlund HR, et al. β-Catenin–induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. The Journal of Cell Biology. 2002; 158(6):1079–1087. [PubMed: 12235125]
42. Novak ADS. Signaling through beta-catenin and lef/Tcf. Cell Mol Life Sci. 1999; 56:523–537. [PubMed: 11212302]
43. Carreira S, et al. Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression. Nature. 2005; 433(7027):764–769. [PubMed: 15716956]
44. Loercher AE, Tank EMH, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. The Journal of Cell Biology. 2005; 168(1):35–40. [PubMed: 15623583]
45. Damsky William E, et al. β-Catenin Signaling Controls Metastasis in Braf-Activated Pten-Deficient Melanomas. Cancer Cell. 2011; 20(6):741–754. [PubMed: 22172720]
46. Damsky WE, Theodosakis N, Bosenberg M. Melanoma metastasis: new concepts and evolving paradigms. Oncogene. 2014; 33(19):2413–2422. [PubMed: 23728340]
47. Park J-W, Jang M-A, Lee YH, Passaniti A, Kwon TK. p53-Independent Elevation of p21 Expression by PMA Results from PKC-Mediated mRNA Stabilization. Biochemical and Biophysical Research Communications. 2001; 280(1):244–248. [PubMed: 11162506]
48. Yaguchi T, et al. Immune Suppression and Resistance Mediated by Constitutive Activation of Wnt/β-Catenin Signaling in Human Melanoma Cells. The Journal of Immunology. 2012; 189(5):2110–2117. [PubMed: 22815287]
49. Topol L, et al. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3–independent β-catenin degradation. The Journal of Cell Biology. 2003; 162(5):899–908. [PubMed: 12952940]
50. Sato A, Yamamoto H, Sakane H, Koyama H, Kikuchi A. Wnt5a regulates distinct signalling pathways by binding to Frizzled2. The EMBO Journal. 2010; 29(1):41–54. [PubMed: 19910923]
51. Da Forno PD, et al. WNT5A Expression Increases during Melanoma Progression and Correlates with Outcome. Clinical Cancer Research. 2008; 14(18):5825–5832. [PubMed: 18794093]
52. Bittner M, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature. 2000; 406(6795):536–540. [PubMed: 10952317]
53. Cano A, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol. 2000; 2:76–83. [PubMed: 10655586]
54. Dissanayake SK. The Wnt5A/protein kinase C pathway mediates motility in melanoma cells via the inhibition of metastasis suppressors and initiation of an epithelial to mesenchymal transition. J. Biol. Chem. 2007; 282:17259–17271. [PubMed: 17426020]

55. Hao L, Ha JR, Kuzel P, Garcia E, Persad S. Cadherin switch from E- to N-cadherin in melanoma progression is regulated by the PI3K/PTEN pathway through Twist and Snail. Br. J. Dermatol. 2012; 166(6):1184–1197. [PubMed: 22332917]
56. Lee J-H, et al. KiSS-1, a Novel Human Malignant Melanoma Metastasis-Suppressor Gene. Journal of the National Cancer Institute. 1996; 88(23):1731–1737. [PubMed: 8944003]
57. Shoag J, et al. PGC-1 Coactivators Regulate MITF and the Tanning Response. Mol. Cell. 2013; 49(1):145–157. [PubMed: 23201126]
58. Wittgen HGM, van Kempen LCLT. Reactive oxygen species in melanoma and its therapeutic implications. Melanoma Research. 2007; 17(6):400–409. [PubMed: 17992124]
59. St-Pierre J, et al. Suppression of Reactive Oxygen Species and Neurodegeneration by the PGC-1 Transcriptional Coactivators. Cell. 2006; 127(2):397–408. [PubMed: 17055439]
60. Vazquez F, et al. PGC1α Expression Defines a Subset of Human Melanoma Tumors with Increased Mitochondrial Capacity and Resistance to Oxidative Stress. Cancer Cell. 2013; 23(3): 287–301. [PubMed: 23416000]
61. Liu F, Fu Y, Meyskens FL Jr. MiTF Regulates Cellular Response to Reactive Oxygen Species through Transcriptional Regulation of APE-1/Ref-1. J Invest Dermatol. 2008; 129(2):422–431. [PubMed: 18971960]
62. Ziech D, Franco R, Pappa A, Panayiotidis MI. Reactive Oxygen Species (ROS)—Induced genetic and epigenetic alterations in human carcinogenesis. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2011; 711(1–2):167–173. [PubMed: 21419141]
63. De Donatis GM, et al. NF-kB2 induces senescence bypass in melanoma via a direct transcriptional activation of EZH2. Oncogene. 2015
64. Patino WDSJ. Epigenetics of cutaneous melanoma. Adv Dermatol. 2008; 24:59–70. [PubMed: 19256305]
65. Venza M, et al. Epigenetic regulation of p14ARF and p16INK4A expression in cutaneous and uveal melanoma. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms. 2015; 1849(3):247–256. [PubMed: 25497382]
66. Lauss M, et al. Genome-Wide DNA Methylation Analysis in Melanoma Reveals the Importance of CpG Methylation in MITF Regulation. J Invest Dermatol. 2015; 135(7):1820–1828. [PubMed: 25705847]
67. Kostaki M, et al. High-frequency p16INK4A promoter methylation is associated with histone methyltransferase SETDB1 expression in sporadic cutaneous melanoma. Experimental Dermatology. 2014; 23(5):332–338. [PubMed: 24673285]
68. Khuu CH, Barrozo RM, Hai T, Weinstein SL. Activating transcription factor 3 (ATF3) represses the expression of CCL4 in murine macrophages. Molecular Immunology. 2007; 44(7):1598–1605. [PubMed: 16982098]
69. Katoh MKM. Transcriptional mechanisms of WNT5A based on NF-κB, Hedgehog, TGFβ, and Notch signaling cascades. International Journal of Molecular Medicine. 2009; 23:763–769. [PubMed: 19424602]
70. Konieczkowski DJ, et al. A Melanoma Cell State Distinction Influences Sensitivity to MAPK Pathway Inhibitors. Cancer Discovery. 2014; 4(7):816–827. [PubMed: 24771846]
71. Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APCs recruit regulatory T cells via CCL4. Nat Immunol. 2001; 2(12):1126–1132. [PubMed: 11702067]
72. Tap WD, et al. Pharmacodynamic Characterization of the Efficacy Signals Due to Selective BRAF Inhibition with PLX4032 in Malignant Melanoma. Neoplasia (New York, N.Y.). 2010; 12(8):637–649.
73. Zipser MC, et al. A proliferative melanoma cell phenotype is responsive to RAF/MEK inhibition independent of BRAF mutation status. Pigment Cell & Melanoma Research. 2011; 24(2):326–333. [PubMed: 21176117]
74. Larkin J, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. New England Journal of Medicine. 2015; 373(1):23–34. [PubMed: 26027431]
75. Byers LA, et al. An Epithelial–Mesenchymal Transition Gene Signature Predicts Resistance to EGFR and PI3K Inhibitors and Identifies Axl as a Therapeutic Target for Overcoming EGFR Inhibitor Resistance. Clinical Cancer Research. 2013; 19(1):279–290. [PubMed: 23091115]

76. Liu J, et al. Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110(50): 20224–20229. [PubMed: 24277854]
77. Biechele TL, et al. Wnt/β-Catenin Signaling and AXIN1 Regulate Apoptosis Triggered by Inhibition of the Mutant Kinase BRAFV600E in Human Melanoma. Sci. Signal. 2012; 5(206):ra3–ra3. [PubMed: 22234612]
78. Haq R, et al. Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF. Cancer Cell. 2013; 23(3):302–315. [PubMed: 23477830]
79. Klarmann G, et al. Invasive prostate cancer cells are tumor initiating cells that have a stem cell-like genomic signature. Clin Exp Metastasis. 2009; 26(5):433–446. [PubMed: 19221883]
80. Sabbah M, et al. Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers. Drug Resistance Updates. 2008; 11(4–5):123–151. [PubMed: 18718806]
81. Roesch A, et al. A Temporarily Distinct Subpopulation of Slow-Cycling Melanoma Cells Is Required for Continuous Tumor Growth. Cell. 2010; 141(4):583–594. [PubMed: 20478252]
82. Roesch A, et al. Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B high cells. Cancer Cell. 2013; 23(6): 811–825. [PubMed: 23764003]
83. Robert C, et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. N. Engl. J.Med. 2011; 364(26):2517–2526. [PubMed: 21639810]
84. Adams PD. Remodeling of chromatin structure in senescent cells and its potential impact on tumor suppression and aging. Gene. 2007; 397(1–2):84–93. [PubMed: 17544228]
85. Wang F, et al. Phase I Trial of a MART-1 Peptide Vaccine with Incomplete Freund's Adjuvant for Resected High-Risk Melanoma. Clin. Cancer Res. 1999; 5(10):2756–2765. [PubMed: 10537339]
86. Murisier FF. Genetics of pigment cells: lessons from the tyrosinase gene family. Histology and histopathology. 21(5):567–578. [PubMed: 16493586]
87. Gartsbein M, et al. The role of protein kinase C δ activation and STAT3 Ser727 phosphorylation in insulin-induced keratinocyte proliferation. J. Cell Sci. 2006; 119(3):470–481. [PubMed: 16418226]
88. Sheldahl LC, Park M, Malbon CC, Moon RT. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in aG-protein-dependent manner. Curr. Biol. 1999; 9(13):S695–S691.
89. Aird KM, Zhang R. Detection of senescence-associated heterochromatin foci (SAHF). Methods Mol Biol. 2013; 965:185–196. [PubMed: 23296659]
90. Niculescu AB, et al. Effects of p21Cip1/Waf1 at Both the G1/S and the G2/M Cell Cycle Transitions: pRb Is a Critical Determinant in Blocking DNA Replication and in Preventing Endoreduplication. Molecular and Cellular Biology. 1998; 18(1):629–643. [PubMed: 9418909]
91. Holtzhausen A, et al. Melanoma-Derived Wnt5a Promotes Local Dendritic-Cell Expression of IDO and Immunotolerance: Opportunities for Pharmacologic Enhancement of Immunotherapy. Cancer Immuno. Res. 2015; 3(9):1082–1095.
92. Mellor AL, Munn DH. Ido expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol. 2004; 4(10):762–774. [PubMed: 15459668]
93. Mikels AJ, Nusse R. Purified Wnt5a Protein Activates or Inhibits β-Catenin–TCF Signaling Depending on Receptor Context. PLoS Biol. 2006; 4(4):e115. [PubMed: 16602827]
94. Rene H, Medema RK, Veronique AJ Smits, Gert Rijksen. $p21^{waf1}$ can block cells at two points in the cell cycle, but does not interfere with processive DNA-replication or stress-activated kinases. Oncogene. 1998; 16(4):431–441. [PubMed: 9484832]
95. Ye X, et al. Downregulation of Wnt Signaling Is a Trigger for Formation of Facultative Heterochromatin and Onset of Cell Senescence in Primary Human Cells. Molecular Cell. 2007; 27(2):183–196. [PubMed: 17643369]
96. Geyer FC, et al. [beta]-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol. 2011; 24(2):209–231. [PubMed: 21076461]
97. Fearnhead NS, Britton MP, Bodmer WF. The ABC of APC. Human Molecular Genetics. 2001; 10(7):721–733. [PubMed: 11257105]

98. Morin PJ, et al. Activation of β-Catenin-Tcf Signaling in Colon Cancer by Mutations in β-Catenin or APC. Science. 1997; 275(5307):1787–1790. [PubMed: 9065402]

99. Palacios J, Gamallo C. Mutations in the β-Catenin Gene (CTNNB1) in Endometrioid Ovarian Carcinomas. Cancer Res. 1998; 58(7):1344–1347. [PubMed: 9537226]

100. Conde-Perez A, et al. A caveolin-dependent and PI3K/AKT-independent role of PTEN in [beta]-catenin transcriptional activity. Nat Commun. 2015; 6

101. Grossmann AH, et al. The Small GTPase ARF6 Stimulates β-Catenin Transcriptional Activity During WNT5A-Mediated Melanoma Invasion and Metastasis. Sci. Signal. 2013; 6(265):ra14–ra14. [PubMed: 23462101]

102. Rubinfeld B, et al. Stabilization of β-Catenin by Genetic Defects in Melanoma Cell Lines. Science. 1997; 275(5307):1790–1792. [PubMed: 9065403]

103. Pollock PMHN. Mutations in exon 3 of the beta-catenin gene are rare in melanoma cell lines. Melanoma Res. 2002; 12(2):183–186. [PubMed: 11930117]

104. Salti GI, et al. Micropthalmia Transcription Factor: A New Prognostic Marker in Intermediate-thickness Cutaneous Malignant Melanoma. Cancer Res. 2000; 60(18):5012–5016. [PubMed: 11016620]

105. Selzer E, et al. The Melanocyte-specific Isoform of the Microphthalmia Transcription Factor Affects the Phenotype of Human Melanoma. Cancer Res. 2002; 62(7):2098–2103. [PubMed: 11929831]

106. Hofbauer GFKJ, Geertsen R, Boni R, Dummer R. Melan A/MART-1 immunoreactivity in formalin-fixed paraffin-embedded primary and metastatic melanoma: frequency and distribution. Melanoma Res. 1998; 8(4):337–343. [PubMed: 9764809]

107. Jho, E-h, et al. Wnt/β-Catenin/Tcf Signaling Induces the Transcription of Axin2, a Negative Regulator of the Signaling Pathway. Molecular and Cellular Biology. 2002; 22(4):1172–1183. [PubMed: 11809808]

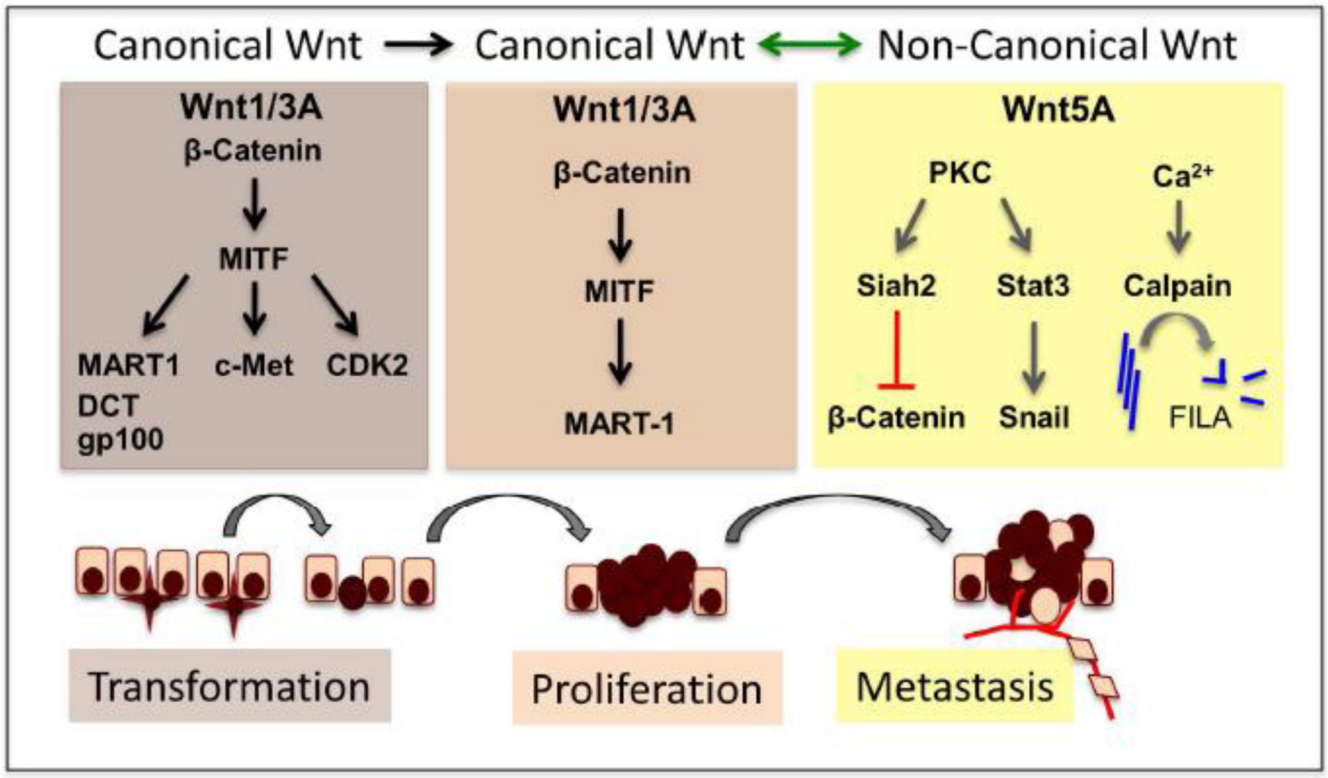

Figure 1. Overview of Wnt signaling in melanoma

Canonical Wnt signaling via β-catenin and MITF allows melanocytes to bypass senescence, transform and proliferate. Non-canonical Wnt signaling via Wnt5A antagonizes canonical Wnt signaling and promotes invasion and metastasis of melanoma cells.

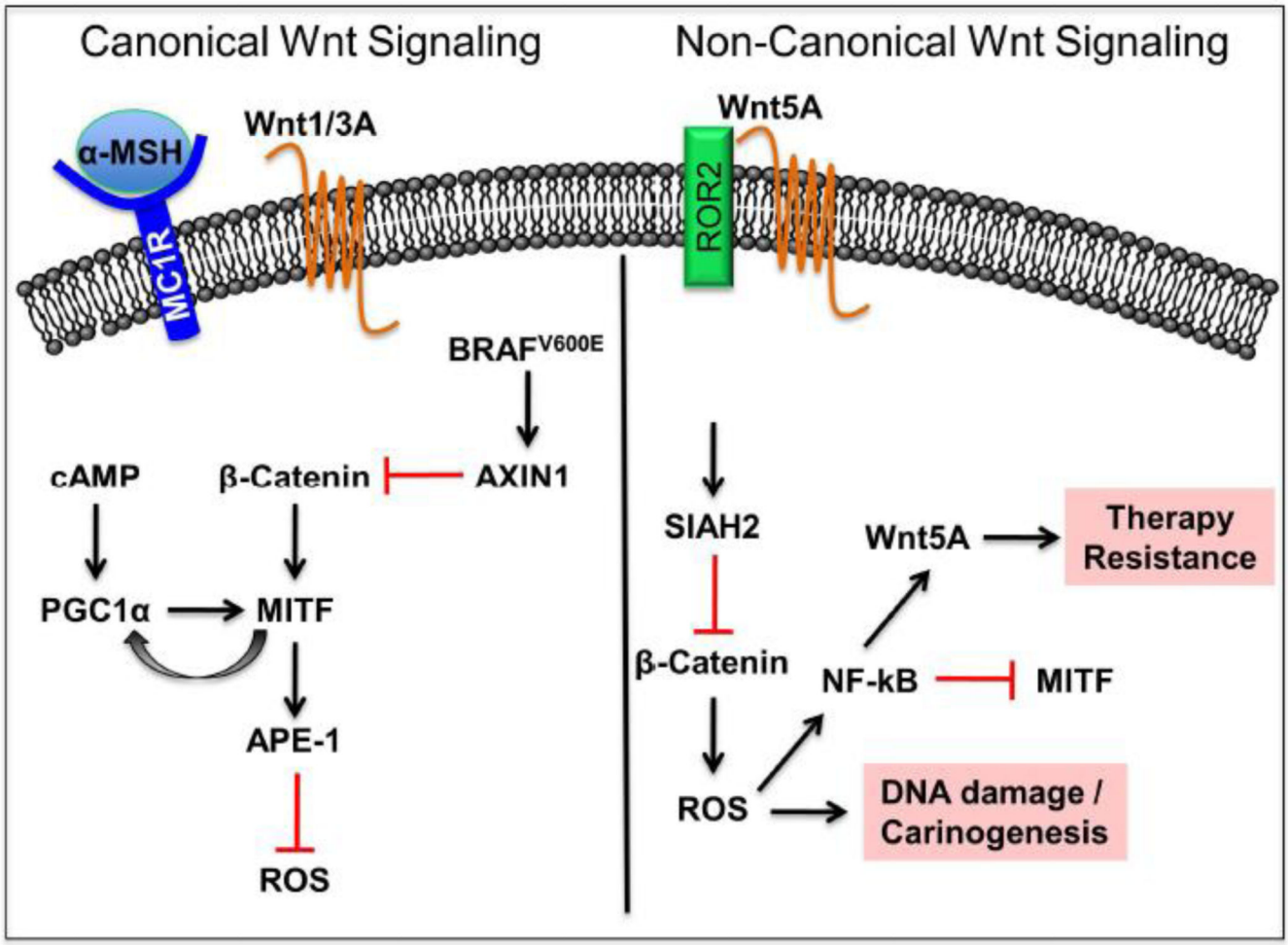

Figure 2. Loss of β-catenin promotes therapy resistance

Canonical Wnt signaling promotes the expression of MITF and APE-1, which protect melanoma cells from cellular damage due to reactive oxygen species (ROS). Non-Canonical Wnt signaling, decreases MITF and APE-1 expression, leading to an increase in ROS, increased carcinogenesis, and therapy resistance.

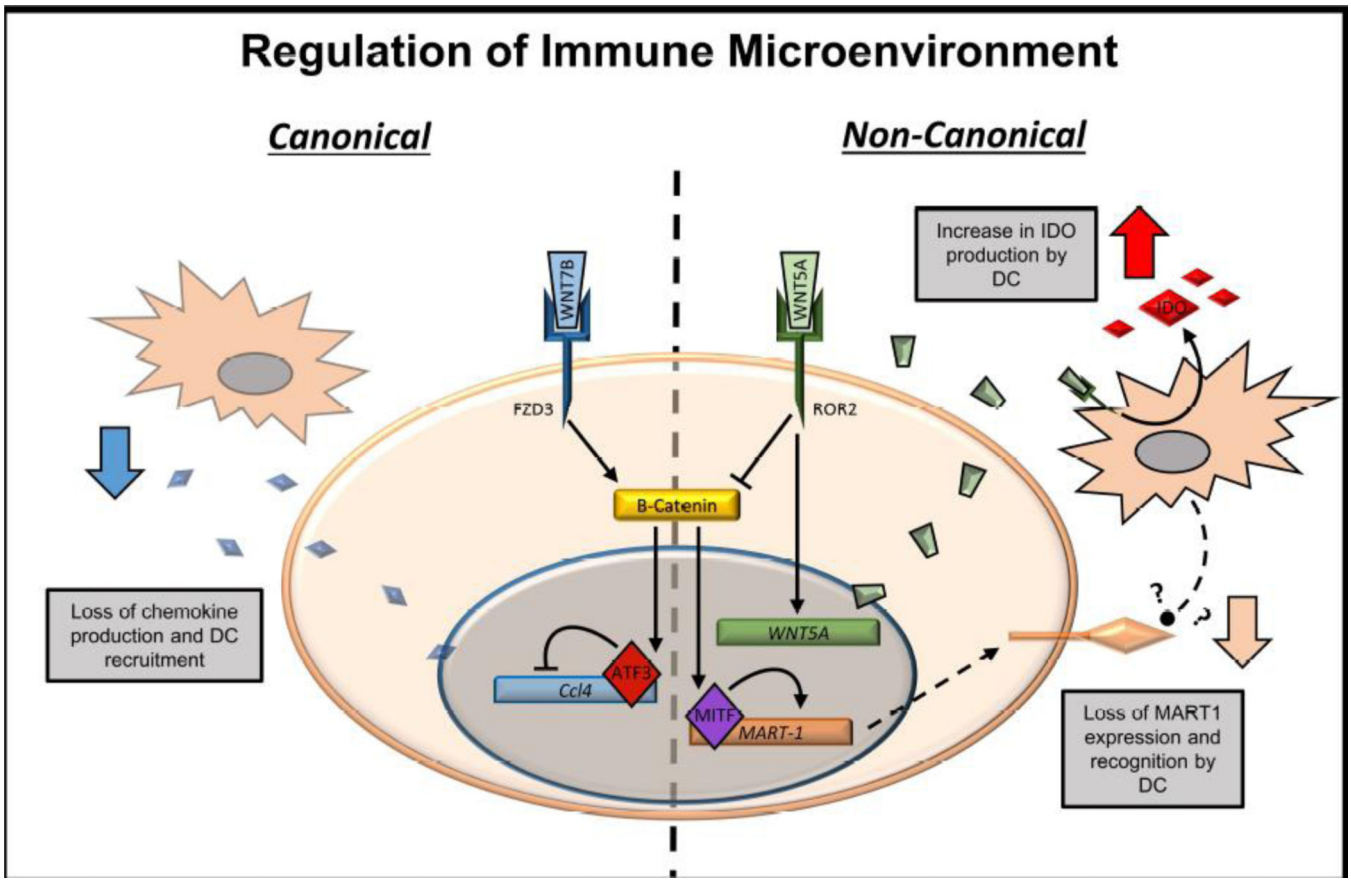

Figure 3. Wnt signaling and regulation of immune microenvironment

Canonical Wnt signaling leads to the upregulation of the transcriptional repressor ATF3 which inhibits production of CCL4 and reduces the recruitment of dendritic cells helping to evade detection by the immune system. Non-Canonical signaling also helps to evade immune detection by down regulating the melanoma antigen MART1 expression through loss of MITF. Non-canonical signaling also increases secreted WNT5A, which can signal in a canonical fashion in dendritic cells leading to the secretion of the immune suppressor molecule IDO.