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Wnt signaling in the phenotype and function of tumor-associated macrophages

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Introduction

Activation of the Wnt/β-catenin pathway is associated with poor prognosis in a variety of cancers. Perhaps the most notable example is colorectal carcinoma, where mutations of the tumor suppressor gene *APC* are present in over 80% of cases (1). With the recent renaissance in tumor immunology and checkpoint inhibitor therapy, there has been increased interest in elucidating drivers of tumor immune evasion, in particular the roles of Wnt signaling. Transcriptional analyses of melanoma patients treated with anti-PD1 immunotherapy shows that non-responders have higher Wnt ligand expression and activation of both β-catenin-dependent and β-catenin-independent Wnt pathways compared to responders (2). Wnt/β-catenin signaling is known to be important for the development, maturation, and differentiation of many immune cells including cytotoxic NK cells and undifferentiated and memory $CD8^+$ T cells (3,4). Wnt signaling is downregulated in effector $CD8^+$ T cells, which are important drivers of anti-tumor immunity (4). Wnt driven immunotherapy resistance may also involve exclusion of cytotoxic $CD8^+$ T cells from the tumor microenvironment all together (5,6). Indeed, dendritic cells and tumor-associated macrophages (TAMs) may be critical components of the immune microenvironment orchestrating this T cell exclusion (2,6-8). While the role of Wnt signaling in dendritic cell-driven anti-tumor immunity was recently reviewed (9), the roles of Wnt signaling

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in TAM-mediated tumor immunity have not been thoroughly explored. In addition to suppressing anti-tumor immune responses, TAMs can directly promote tumor progression and metastasis (8,10). Meta-analyses in several different cancers have found a correlation between a high-density of TAMs and poor patient prognosis (11-14). Understanding the signaling pathways that support TAM enrichment in the tumor microenvironment can offer strategies for future therapeutic intervention (8,15,16). This review will focus on the ways in which Wnt signaling affects the establishment of TAMs in the tumor microenvironment, TAM polarization, and TAM promotion of cancer progression.

Macrophages in the tumor microenvironment

Origin of macrophages

Macrophages are phagocytic antigen-presenting cells of the innate immune system that are found in tissues throughout the body. Tissue-resident macrophages are tissue-specific and exhibit specialized and heterogenous functions. Examples include liver Kupffer cells, alveolar macrophages in the lung, and microglia of the brain. Generally, these macrophages are thought to be embryonically-derived and can self-renew within a given tissue under normal conditions (17). However, there is also evidence that when depleted or in states of inflammation, tissue-resident macrophages can be replenished by circulating monocytes derived from hematopoietic stem cells (HSCs) (18,19).

HSC-derived monocytes originate from the bone marrow and circulate in the blood (20). Monocytes can respond to inflammatory signals by extravasating through the endothelium and differentiating into macrophages or dendritic cells within the inflamed tissue (21-23). Colony stimulating factor 1 (CSF1; also known as M-CSF) has been identified as an essential factor for monocyte differentiation into macrophages, as well as for establishment of tissue-resident macrophages (23,24). Murine bone marrow derived monocytes or human monocytes isolated from peripheral blood can be stimulated with M-CSF *in vitro* to produce macrophages (25). Both circulating monocytes and tissue-resident macrophages are believed to be important sources of TAMs (reviewed in (26)). In order to target TAMs, it is important to define the signals that attract monocytes into tumors and support macrophage renewal and survival within a tissue.

Macrophage polarization

Upon tissue recruitment and differentiation, macrophages play an important role in tissue homeostasis and the immune response. Although there is still debate concerning nomenclature, macrophages are traditionally classified based on 1) phenotypic expression of particular protein markers and 2) cytokines or other factors that induce their distinct phenotypes *in vitro* (reviewed in (27)). Using this system, macrophages can be placed on a spectrum of *functional polarization*, with M1-type macrophages representing an immune-promoting phenotype and M2-type macrophages being considered anti-inflammatory and cancer-promoting (Figure 1A). Pro-inflammatory macrophages are stimulated *in vitro* by IFN-γ and/or LPS, secrete cytokines and chemokines such as IL-12, TNF, and CXCL10, and recruit other immune cells to promote inflammation (27-29). Pro-inflammatory macrophages can be further distinguished by markers including MHCII, iNOS, and CD80,

among others (Figure 1A) (28,29). On the other end of the spectrum are M2-type, anti-inflammatory macrophages, which can be generated via stimulation with IL-4/IL-10 *in vitro* (27). Notable anti-inflammatory markers include CD163, CD206, CCL17, and TGFβ, among others (Figure 1A) (27,29). M2 polarized macrophages are thought to be important in wound healing and tissue remodeling (30). Macrophages that are generated from monocytes *in vitro* and have not been stimulated with polarizing cytokines can be labeled as unpolarized or M0-like macrophages and can be used as a baseline for comparison of gene or protein expression profiles (27).

The significant plasticity of macrophages, which comprise a wide spectrum of activation states *in vivo*, is not fully reflected in the current model of inflammatory versus anti-inflammatory polarization (27,29,31). Additionally, TAMs likely have a phenotype that is distinct from tissue-resident macrophages or traditionally polarized macrophages altogether (32-35). Still, much of the literature continues to distinguish TAMs as either M1-like or M2-like based on their expression of established markers (36). While depicting TAMs in this binary system may over-simplify their diverse functionality, there is strong evidence that TAMs expressing M1 markers are pro-inflammatory and anti-tumorigenic, whereas M2-like TAMs support tumor growth and progression (14,37-40) (Figure 1B).

TAMs can influence tumor progression through a number of mechanisms including growth factor secretion, promotion of angiogenesis, cytokine secretion to regulate immune cell recruitment and tolerization, and support of cancer cell invasion and metastasis (11,13) (reviewed in (41)). In numerous cancers, including pancreatic, breast, and colorectal cancer among others, a higher density of M2-like TAMs in tissue correlates with an aggressive tumor phenotype and worse prognosis (14,37,38) (reviewed in (42)). In contrast, M1-like TAM infiltration has been correlated with a favorable prognosis in non-small cell lung cancer, hepatocellular, ovarian, and gastric cancer (42,43). However, the role of TAMs in oncology may not always be dependent on M1 vs. M2 polarization, as numerous inflammatory cytokines, such as IL-6 and IL-1β, are known to promote tumor development, and diverse TAMs can be associated with increased T cell activation and tumor clearance (43,44). Nonetheless, efforts to develop therapies aimed at eliminating TAMs, enhancing M1 and limiting M2 macrophage polarization, and inhibiting macrophage recruitment into tumors are noteworthy (reviewed in (16,45)). Further elucidation of the role of Wnt signaling in modulating pro-tumor TAM phenotypes is essential for assessing whether Wnt represents a potential therapeutic target.

Wnt signaling

The wingless (*wg*) gene was first discovered in the 1970s and was shown to be critical for proper development in *Drosophila melanogaster* (46). In the ensuing decades, 19 distinct vertebrate Wnt proteins have been identified. Wnts are cysteine-rich, lipid-modified, secreted proteins that generally have a short-range of action due to their limited solubility. Wnts are morphogens and act as both paracrine and autocrine signals in a variety of human tissues. Wnt signaling is critical for normal development, maintenance of stem cells in adult tissues, and cancer initiation and progression. Wnt ligands can bind with varying affinities to their receptors and co-receptors (47). The receptors/co-receptors for Wnts include Frizzled

(FZD) proteins (10 in humans), which are seven-transmembrane receptors, the single-pass low-density lipoprotein receptor-related proteins (LRP5 and LRP6), as well as the ROR family of receptor tyrosine kinase-like orphan receptors (ROR1 and ROR2) (48,49).

Wnt/β-catenin signaling

Historically, Wnt signaling is classified as either canonical or non-canonical. Canonical signaling (i.e., Wnt/β-catenin) encompasses a well-described conserved signaling cascade that is β-catenin-dependent. In the absence of Wnt, a complex consisting of glycogen synthase kinase 3 (GSK3), adenomatosis polyposis coli (APC), the scaffold protein axin, and casein kinase Iα (CKIα) comprise the "destruction complex", which targets β-catenin for phosphorylation and subsequent ubiquitin-mediated proteasomal degradation (50) (Figure 2A). Binding of a Wnt ligand to FZD recruits Dishevelled to the receptor and, in a process that is still somewhat ill-defined, mediates oligomerization, receptor/co-receptor activation, and inhibition of the destruction complex (51). This allows stabilization and accumulation of cytoplasmic β-catenin. Cytosolic β-catenin then enters the nucleus, where it associates with family members of the transcriptional factors T cell factor (TCF) and lymphoid enhancer-binding factor (LEF1) to form a transcriptional complex and recruits transcriptional co-activators, such as cyclic AMP response element-binding protein (CBP) or its homolog p300 (47,52,53). This complex activates *Myc, Jun*, and a host of other Wnt target genes involved in cell differentiation and proliferation (47). Nuclear localization of β-catenin is often used as a surrogate marker of Wnt/β-catenin pathway activation (Figure 2A).

β-catenin-independent Wnt signaling

Non-canonical Wnt signaling encompasses numerous β-catenin-independent pathways that are not fully understood at the molecular level (reviewed in (54)). Wnt mediated inhibition of GSK3 and the destruction complex has even been proposed to affect the degradation of several proteins besides β-catenin (55). Other β-catenin-independent pathways rely on different receptors and/or co-receptors. There is evidence that some of these pathways may overlap with each other or with the β-catenin pathway. Some of the most studied non-canonical pathways are the planar cell polarity, Wnt-Ca^{2+}, and Wnt/ROR signaling pathways. However, tools needed to examine these pathways are still not well developed.

Much of the diversity pertaining to how Wnt ligands affect downstream signaling cascades and thus cellular phenotypes is determined by receptor and co-receptor availability (reviewed in (56,57)). Although several Wnt ligands may preferentially activate β-catenin-dependent or -independent pathways, it is now understood that receptor/co-receptor combination is critical in determining which downstream signaling pathway will be activated (56-59). Because the expression of specific Wnt receptors is both cell type and context dependent, it cannot be assumed that a particular Wnt ligand will activate the same signaling cascade in different contexts. Wnt5a, Wnt7a, and Wnt11, specifically, are ligands that are traditionally considered non-canonical activators, but are able to trigger or inhibit Wnt/β-catenin signaling or activate any of the β-catenin-independent pathways, depending on the cellular context (49,60-70). Recognizing these nuances in Wnt signaling is important to consider when designing and interpreting experiments that imply activation of a certain

signaling cascade based only on which Wnt ligands are used. Here we review roles that various Wnt ligands and their potential downstream signaling pathways play in the biology of TAMs.

Wnt signaling in the establishment of TAMs

TAM formation from tissue-resident macrophages

There are two proposed origins for TAMs: tissue-resident macrophages and circulating monocytes (26). One recent study suggests that Wnt/β-catenin signaling may be important for the formation of TAMs from tissue-resident macrophages. To study TAMs derived from tissue-resident macrophages (Kupffer cells) in the liver, Ye and colleagues (2019) blocked TAM differentiation from monocytes, which increased the Kupffer cell-like TAM ($F4/80^{hi}Cd11b^{lo}Ly6G^{-}Ly6C^{lo}$) population in Hepa 1-6 liver tumors (71). Wnt/β-catenin signaling was activated in these Kupffer cell-like TAMs as measured by elevated total and nuclear β-catenin, as well as elevated levels of β-catenin target genes *Axin2, c-Myc*, and *cyclin D1*. ICG-001 is a small molecule that disrupts the transcription of Wnt target genes through inhibiting CBP, a scaffold protein that potentiates β-catenin-mediated transcription (72). Through this inhibition of Wnt signaling, and potentially though additional off-target effects, ICG-001 reduced the number and proliferation index of Kupffer cell-like TAMs and suppressed tumor growth (71). This study suggests that in orthotopic liver tumors, Wnt signaling is important for the proliferation of pro-tumorigenic TAMs derived from tissue-resident Kupffer cells. Given the diversity of tissue-resident macrophages in the body, more research is needed to determine if this relationship with β-catenin signaling exists in other organ systems.

Monocyte recruitment

Circulating monocytes, likely the primary source of TAMs in most tumors, migrate from the blood into a tissue before differentiating into macrophages (26,73). It is unclear whether Wnt signaling is involved in monocyte recruitment and trafficking (22). Few, if any, published papers have looked at the direct effects of Wnt ligands and downstream Wnt signaling on monocyte migration into tumors. Nonetheless, there is evidence that Wnt signaling can promote monocyte/macrophage motility and migration. In a murine orthotopic model of HCC, knockout of *Wntless*, a transmembrane protein necessary for secretion of all Wnt ligands, led to recruitment of fewer $F4/80^{+}CD11b^{+}$ $Ly6G^{-}$ TAMs in resected tumors and decreased proliferation of Hepa1-6 cells (74). The authors could find no difference in TAM proliferation, as measured by Ki67 staining of resected tumors, suggesting that disruption of Wnt ligand secretion in this setting directly impeded monocyte recruitment, though the effect was modest (74). This study also did not determine which downstream Wnt signaling pathways were affected. Other *in vitro* studies demonstrated that upstream or downstream activation of the Wnt/β-catenin pathway enhanced macrophage migration (75,76). Neither of these studies directly examined Wnt ligand mediated effects. Additionally, both *in vitro* studies addressed macrophage migration rather than the migratory properties of undifferentiated monocytes, which are the population that would need to be recruited from the blood and travel through the endothelium into tissues to become TAMs.

Therefore, further work is needed to resolve the roles that Wnt signaling plays in monocyte recruitment *in vivo*.

Wnt Signaling in TAM differentiation and polarization

Once monocytes have infiltrated into a tumor, Wnt signaling may play a critical role in promoting TAM differentiation and polarization. RNA sequencing of patient samples has validated that $CD206^+$ TAMs in lung cancer exhibit increased Wnt/β-catenin signaling compared to matched normal macrophages (33). $CD68^+MR^+/ARG1^+$ M2-like TAMs in HCC have Wnt/β-catenin pathway activation as measured by β-catenin nuclear localization (74). General $CD68^+$ TAMs in colorectal carcinoma (CRC) and M2-like, $CD163^+$ TAMs in breast cancer express the Wnt ligand, Wnt5a, which can promote Wnt signaling through a variety of pathways in both cancer and stromal cells (54,77,78). Additionally, it has been shown that a high $Wnt5a^+CD68^+/Wnt5a^-CD68^+$ TAM ratio is associated with poor recurrence-free and overall survival in CRC patients (77). This underlies the importance of Wnt pathway activation in and by TAMs.

Activation of Wnt/β-catenin signaling promotes an M2 phenotype in macrophages

Higher infiltration of M2-like TAMs has been correlated with poor prognosis in a variety of cancers (14,37-39). In infectious or other inflammatory settings, significant experimental evidence supports a role for Wnt/β-catenin pathway activation in promoting anti-inflammatory macrophage polarization. *In vitro*, recombinant Wnt3a can promote macrophage polarization to an M2-like phenotype despite co-administration of M1 inducers, such as LPS + IFN-γ (74). Similarly, Wnt3a conditioned media (CM) decreased the inflammatory (M1-type) response in macrophages infected with *M. tuberculosis* or treated with IFN-γ, as measured by TNF levels (79). β-catenin-dependent signaling may also be important in models of lung fibrosis, which are thought to rely on M2 macrophage function (80). Wnt2b overexpression in macrophages derived from the human leukemic THP-1 cell line, which has been used extensively to study macrophage function, led to an increase in mRNA levels of M2 markers and a decrease in M1 markers (81,82). Wnt1 increased expression of M2 markers in THP-1 cells and promoted the expression of CD36, which is upregulated in M2 macrophages, during macrophage differentiation from monocytes (83-85). Likewise, blockade of Wnt/β-catenin signaling with ICG-001 or β-catenin deletion in macrophages reduced the expression of M2 markers following stimulation with IL-4, an inducer of M2 polarization (74,86). Wnt/β-catenin signaling enhances, and may be necessary for, M2 polarization of macrophages in these inflammatory, non-cancerous settings.

Activation of Wnt signaling promotes an M2-like phenotype in TAMs

While TAMs are a heterogeneous population and exhibit phenotypes distinct from traditionally polarized macrophages, there is strong evidence that Wnt signaling also supports an immunosuppressive phenotype in TAMs. *In vitro*, TAMs are often generated by treatment with tumor-conditioned media (TCM) or co-culturing with tumor cells, which generally promotes an immunosuppressive phenotype that more closely resembles *in vivo* TAMs compared to IL-4/IL-10 stimulated macrophages (32,33,81). Co-culturing THP-1

macrophages with thyroid cancer cells upregulated Wnt1 and Wnt3a expression (87). TCM from HCC cell lines also upregulated the expression of Wnt2b and other Wnt ligands by undifferentiated macrophages and promoted nuclear localization of β-catenin (74,81). Further, silencing *Wnt2b* or *CTNNB1* (the gene for β-catenin), knockdown of *Wntless*, or inhibiting the Wnt/β-catenin pathway with ICG-001 reversed the upregulation of M2 markers following treatment with TCM (74,81). Similarly, when *ex vivo* lung-tumor TAMs ($CD68^+$) and M2-like co-culture-generated TAMs were transfected with *CTNNB1* shRNA, M1 markers were upregulated and M2 markers were downregulated (33). While there is some limited evidence that Wnt activation can promote an M1-like phenotype in tissue resident microglia of the brain, others demonstrated that microglia treated with glioblastoma TCM+Wnt3a indeed had increased markers of M2 polarization (88-90). These studies suggest both an increase in Wnt ligand production by TAMs and a reliance of the M2-like phenotype on Wnt/β-catenin signaling within TAMs in nearly all tumor contexts.

Although Wnt/β-catenin signaling has been shown to be important for anti-inflammatory polarization in macrophages and TAMs, the effects of β-catenin-independent Wnt signaling on macrophage polarization are less clear. TAMs in both human breast and CRC tumors express Wnt5a, which is known to act through both β-catenin-dependent and β-catenin-independent pathways (54,77,78). There is evidence to suggest that signaling mediated by Wnt5a is necessary for the secretion of pro-inflammatory cytokines by M1 macrophages (91,92). However, there is also substantial evidence in both cancerous and non-cancerous settings that Wnt5a induces a tolerogenic, M2-like phenotype of macrophages (77,78,93,94). Distinct β-catenin-independent pathways are triggered by Wnt5a in these divergent contexts (77,78,92-94). A recent review of Wnt5a in the tumor microenvironment suggests that activation of different signaling pathways at different times permits Wnt5a to exhibit both pro-inflammatory and immunosuppressive effects (95). Additionally, there is evidence that Wnt7a, which can also activate multiple β-catenin-independent Wnt pathways, decreases the production of both pro- and anti-inflammatory cytokines by macrophages under different stimulation conditions (96). This study did not assess the downstream pathways affected by Wnt7a in these macrophages. These works highlight the need to carefully examine which downstream signaling pathways are being activated by unique Wnt ligands in different contexts in order to fully appreciate the role that Wnt signaling plays in macrophage polarization and to develop successful therapeutic strategies targeting these pathways.

Wnt signaling supports TAMs as drivers of tumor growth

TAM-intrinsic Wnt signaling supports the pro-tumorigenic functions of TAMs

Wnt signaling increases M2 markers on TAMs generated *in vitro*, and M2-like TAMs exhibit tumor-promoting roles *in vitro* and *in vivo* (14,37-40). Multiple studies have validated the importance of Wnt signaling on the tumor-supporting functions of TAMs. Specifically, culturing tumor cells with macrophages (or CM from macrophages) with downregulated Wnt/β-catenin signaling reduced colony formation, migration, and invasion (33,34,74,81,87). *In vivo* experiments using small molecule inhibitors of the Wnt/β-catenin pathway support these *in vitro* models. Treating orthotopic hepatocellular tumors with ICG-001 suppressed tumor growth and reduced $F4/80^{hi}CD11b^{lo}$ TAM proliferation and

IL-10 production (71). In three different lung cancer models in mice, treatment with XAV939 (a tankyrase inhibitor that promotes β-catenin degradation by stabilizing axin within the destruction complex) significantly reduced the growth of tumors and shifted $F4/80^+$ TAMs to a tumor-inhibiting M1-like phenotype (33,97,98). Wnt/β-catenin signaling within M2-like TAMs clearly plays a role in supporting their tumor-promoting functions.

TAM-derived Wnt ligands support tumorigenesis

Aside from TAM-intrinsic Wnt signaling, production of Wnt ligands by TAMs may also influence tumor growth through TAM-tumor crosstalk. CM of *Wnt2b* overexpressing macrophages promoted epithelial to mesenchymal transition (EMT) of HCC cells, supporting a malignant phenotype (81). *Wnt5b* knockdown in M2 polarized macrophages decreased self-renewal of ovarian cancer stem cells (CSC), increased their sensitivity to carboplatin, and decreased migration and growth of CSC/M2 hetero-spheroids *in vitro* and *in vivo* (99). Knockdown of Wnt1 or Wnt3a in THP-1 macrophages co-cultured with thyroid cancer cells led to decreased tumor growth and decreased expression of genes associated with EMT in an *in vivo* model (87). Additionally, in xenograft models of colorectal carcinoma, Wnt5a production by TAMs supported tumor growth, likely through a β-catenin-independent pathway (77).

In breast and other major cancer types, TAMs have been implicated in facilitating cancer cell invasion and metastasis (10,100). Further studies of TAMs in breast cancer found macrophage derived Wnt ligands to play an important role in this process. In whole human breast cancer samples, increased expression of Wnt7b was associated with positive lymph node metastasis (101). Ojalvo and colleagues (2010) found that expression of *Wnt5b* and *Wnt7b* were upregulated in invasive TAMs, which were associated with breast cancer cell invasion and metastasis, as compared to general TAMs isolated from mouse mammary tumors or splenic macrophages (101). Other studies demonstrated that *Wnt5a* expression was upregulated in macrophages cultured with the MCF-7 breast cancer cell line, and Wnt5a-containing microvesicles isolated from these TAMs enhanced the invasiveness of MCF-7 cells *in vitro* (102,103). These studies suggest that TAM-derived Wnt ligands support cancer growth, invasion, and disease progression. Inhibition of Wnt signaling could therefore impact tumor progression in two distinct ways: by limiting the pro-tumorigenic effects of M2-like TAMs and directly affecting cancer cell proliferation, invasion, and metastasis that may be in part driven by TAM-secreted Wnt ligands.

Additionally, it is known that Wnt signaling in T cells decreases the propensity of $CD8^+$ T cells to become cytotoxic effector T cells and inhibits $CD4^+$ T cell differentiation into type 1 and type 17 helper T cells (4). TAMs are known to inhibit these inflammatory phenotypes of T cells as well, while promoting an immunosuppressive environment. *In vivo*, inhibition of Wnt signaling improves CD8+ T cell infiltration into tumors (104). Whether TAM-derived Wnt ligands are the primary mediators of these anti-inflammatory T cell phenotypes has not been well studied. One group did demonstrate *in vivo* that TAM-intrinsic Wnt/B-catenin signaling was important for TAM inhibition of CD8+ T cell proliferation (74). Further work is needed to determine how Wnt facilitates interactions between macrophages and T cells in the tumor microenvironment.

Conclusion

There are 19 total Wnt ligands and various Wnt signaling pathways that could have a role in regulating TAM polarization and pro-tumorigenic TAM function. While Wnt ligand production by tumors likely stimulates monocyte recruitment and supports TAM development from tissue-resident macrophages, research on this topic is limited. On the other hand, there is ample evidence establishing a role for various Wnt ligands and Wnt/β-catenin signaling in the polarization and pro-tumorigenic functions of M2-like TAMs (Figure 2B). Wnt ligands and their signaling pathways are highly complex, posing a challenge for studying their effect on TAMs. Additionally, mapping which Wnt ligands and receptors are uniquely expressed in different tumor types is a technically challenging endeavor that could be important for determining how Wnt signaling may be facilitating tumor-stroma crosstalk and tumor growth. Moreover, in the field of TAM biology, there is still debate about whether defining TAMs as M1-like and M2-like adequately describes the complex spectrum of *in vivo* TAM functionality (27).

The complex interactions between tumors and their stromal and immune environments are a major topic of ongoing research. Targeting these interactions to impede tumor growth is an enticing and sometimes very effective therapeutic strategy. Because TAMs have been shown to impede infiltration of CD8 T cells into tumors and may play a role in resistance to immunotherapies, they are being considered as a novel therapeutic target in combination with immune checkpoint inhibitors (8,16). Clinical trials with a variety of TAM-targeting therapies are currently underway (reviewed in (16)). Many of these therapies aim to reprogram TAMs towards an M1-like phenotype, rather than eliminate them, in order to potentially benefit from the pro-inflammatory properties of M1 macrophages. CSF1 receptor inhibitors are just one class of inhibitors developed with this goal; unfortunately, early clinical trials of these drugs used in combination with checkpoint inhibitors do not show promising results (105). A major current limitation of developing TAM-targeted therapies is the lack of reliable and specific markers of pro-tumorigenic TAMs. Combining inhibitors of Wnt signaling and immune checkpoint blockade may be another promising approach to reprogram TAMs and combat T cell exclusion, with several ongoing Phase I and II clinical trials currently testing this drug combination (2,106). Wnt inhibitors, however, do have dose-limiting toxicities affecting organ systems that rely on Wnt signaling for normal functioning, such as the gastrointestinal tract and the skeletal system. Finding ways to target Wnt inhibitors to a tumor or the tumor microenvironment represent a significant challenge in the field.

Understanding how Wnt signaling mediates an immunosuppressive environment through recruitment and polarization of M2-like TAMs could allow for more expansive yet targeted use of these novel combinatorial therapies. For instance, Wnt inhibitors could be considered as preferential therapy in patients without known aberrations in Wnt signaling but with high TAM infiltration. In numerous tumors mentioned in this review, including hepatocellular, breast, thyroid, lung, and pancreas, Wnt signaling promotes TAM polarization toward an M2-like phenotype. Given the poor prognosis associated with TAM infiltration, patients with highly macrophage-ridden tumors may stand to benefit from these clinical trials immensely.

Indeed, targeting TAM recruitment and polarization through inhibitors of Wnt signaling may prove beneficial to patients with a wide range of cancer types.

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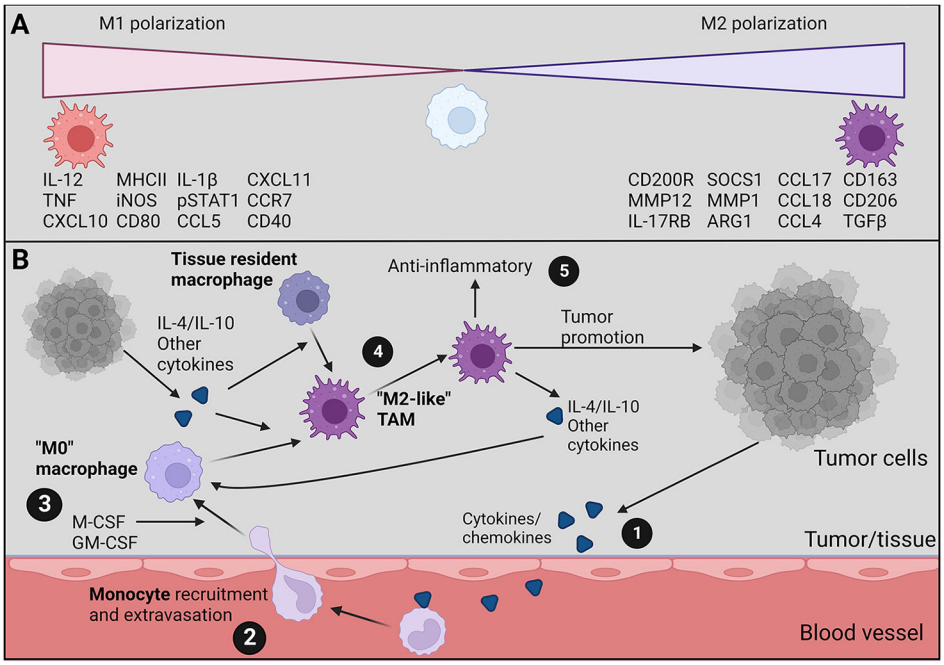

Figure 1. A: Macrophage polarization spectrum.

A simplified illustration of macrophage polarization and associated phenotypic markers (27). **1B: Steps in the establishment of immunosuppressive, M2-like tumor-associated macrophages (TAMs) and the potential role of Wnt signaling**. 1-2: Tumor cells or other cells within the tumor stroma secrete molecules that recruit monocytes from nearby vasculature, which then extravasate through the endothelium into the tissue. 3: Monocytes differentiate into macrophages within the tissue. 4: Monocyte-derived macrophages and tissue-resident macrophages are polarized to become anti-inflammatory, M2-like TAMs by cytokines, chemokines, and other molecules secreted into the tumor microenvironment. 5: M2-like TAMs promote tumor growth and progression and support an anti-inflammatory environment. Figure created with BioRender.com.

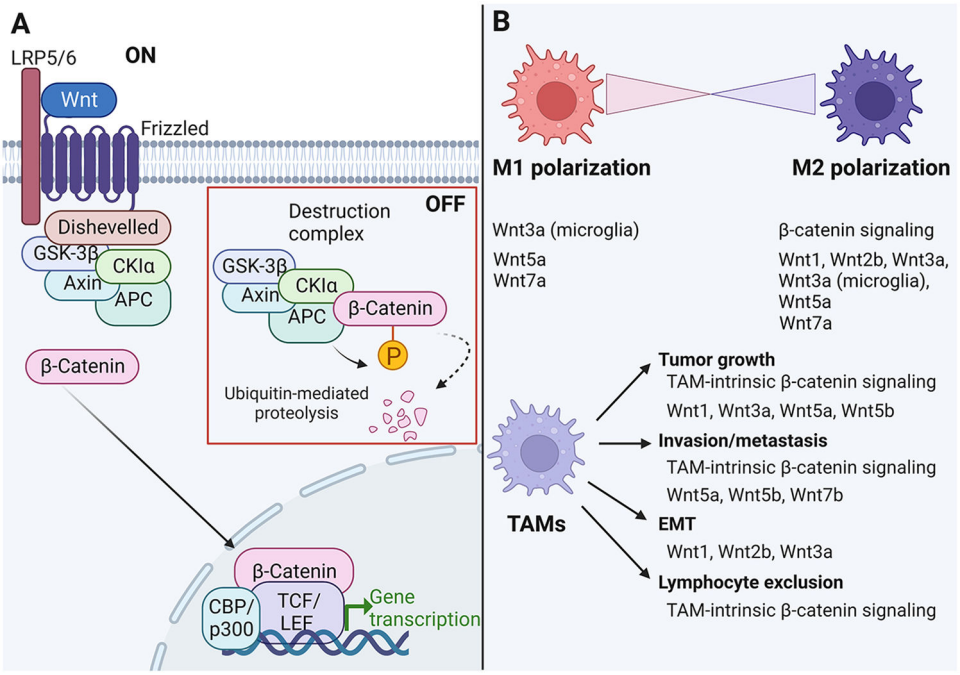

Figure 2. A. Wnt/β-catenin Signaling.

In the absence of Wnt, a complex consisting of glycogen synthase kinase 3 (GSK3), adenomatosis polyposis coli (APC), Axin, and casein kinase Iα (CKIα) comprise the "destruction complex", which targets β-catenin for phosphorylation and subsequent ubiquitin-mediated proteasomal degradation. When Wnt ligand binds to FZD and recruit Dishevelled and LRP5/6 to the membrane, the destruction complex is inhibited and β-catenin degradation is blocked. Consequently, β-catenin enters the nucleus and associates with transcription factors T cell factor (TCF) and lymphoid enhancer-binding factor (LEF1), and the transcriptional co-activators, cyclic AMP response element-binding protein (CBP) or p300, to activate transcription of Wnt target genes, including *c-Myc, c-Jun*, and other genes involved in cell differentiation and proliferation. **2B. The roles of Wnt signaling and specific Wnt ligands in TAM polarization and tumor promotion.** Figure created with BioRender.com.