Published in final edited form as:

J Mammary Gland Biol Neoplasia. 2011 June ; 16(2): 157–167. doi:10.1007/s10911-011-9205-5.

Wnt5a as an Effector of TGF β in Mammary Development and Cancer

Rosa Serra, Stephanie L. Easter, Wen Jiang, and Sarah E. Baxley

Department of Cell Biology, University of Alabama at Birmingham, 1918 University Blvd., 660 MCLM, Birmingham, AL 35294-0005, USA

Rosa Serra: rserra@uab.edu

Abstract

Wnt5a is a member of the Wingless-related/MMTV-integration family of secreted growth factors, which are involved in a wide range of cellular processes. Wnt signaling can be broadly divided into two categories the canonical, β -catenin-dependent pathway and the non-canonical β -catenin-independent pathway. Wnt5a is a non-canonical signaling member of the Wnt family. Loss of Wnt5a is associated with early relapse of invasive breast cancer, increased metastasis, and poor survival in humans. It has been shown that TGF- β directly regulates expression of Wnt5a in mammary gland and that Wnt5a mediates the effects of TGF- β on branching during mammary gland development. Here we review the evidence suggesting Wnt5a acts as an effector of TGF- β actions in breast cancer. It is suggested that the tumor suppressive functions of TGF- β involve Wnt5a-mediated antagonism of Wnt/ β -catenin signaling and limiting the stem cell population. Interactions between TGF- β and Wnt5a in metastasis appear to be more complex, and may depend on specific cues from the microenvironment as well as activation of specific intracellular signaling pathways.

Keywords

Wnt5a; TGF- β ; Mammary gland; Breast cancer; Stem cell; Migration; Metastasis

Introduction

Identification of Wnt5a as a Down-Stream Target of TGF- β

Due to the complex nature of mammary gland development and breast cancer, it is likely that coordination of signaling by many factors is involved. The Transforming Growth Factor-beta (TGF- β) family of polypeptides consists of multifunctional factors involved in the development of many organ systems and in the progression of disease (reviewed in [1, 2]). TGF- β 1, β 2, and β 3 are members of this multi gene family, which also includes inhibins, bone morphogenic proteins and growth and differentiation factors [3, 4]. TGF- β s have long been associated with diverse cellular processes including but not limited to growth, differentiation and cell migration. TGF- β s are differentially regulated during postnatal mammary gland development [5–8]. All three isoforms have been detected in the terminal end buds. Expression of TGF- β 2 and TGF- β 3 are up regulated in response to pregnancy, while TGF- β 1 levels remain constant. The level of all three isoforms of TGF- β is dramatically reduced during lactation. TGF- β 1 expression then rises during involution [9,

10]. TGF- β receptors type I and type II (Tgfr1 and Tgfr2) are expressed throughout mammary gland development in both epithelial and stromal cells [11].

TGF- β has been shown to have several important functions during mammary development including regulation of ductal outgrowth, lateral branching, lactation, and apoptosis (Reviewed in [5, 6]). In addition, it was shown that TGF- β can limit regeneration of the mammary gland during serial transplantation of mammary epithelial cells into mouse cleared fat pads [12–14]. Members of the TGF- β family also have a complex and important role in mediating tumor progression and metastasis (Reviewed in [6, 15]). TGF- β has biphasic effects on tumor progression; acting as a tumor suppressor in early stages of breast cancer but either promoting or inhibiting tumor metastasis later depending on microenvironmental factors. The mechanisms by which TGF- β act during tumor progression are not completely known.

In an effort to understand the role of TGF- β signaling in mammary gland development and cancer in vivo, we previously constructed transgenic mice that express a truncated, dominant-negative mutation of Tgfr2 (DNIR) [11, 16, 17]. Microarray analysis was used to identify genes that were regulated by alterations in TGF- β signaling in the mammary gland. Wingless-related MMTV Integration Site 5A (Wnt5a), a non-canonical signaling Wnt, was identified in this screen. Direct regulation of Wnt5a by TGF- β was verified in primary cells in culture and Smad binding sites were identified in the Wnt5a promoter [18, 19]. Wnt5a is associated with poor prognosis and early relapse of invasive breast cancer in humans suggesting that, like TGF- β , Wnt5a can act as a tumor suppressor [20–22]. Using Northern blot analysis it was shown that, similarly to TGF- β , Wnt5a is expressed at all stages of mammary development except lactation [23, 24]. During embryonic development, Wnt5a mRNA was localized by whole mount in situ hybridization to a broad strip of mesenchyme underlying the area where the mammary placodes form [25]. In adult virgin mice, expression was detected by Northern blot in RNA isolated from cleared fat pads, suggesting that Wnt5a is expressed in the mammary stroma [24]. More recently, it was shown using microarray screens that Wnt5a is preferentially expressed in the terminal end buds (TEB) relative to the mature ducts [26] and we have shown using in situ hybridization that Wnt5a is also expressed in both the ductal epithelium and stroma of adult mice.

Wnt Signaling

The Wnt family of proteins consists of at least 19 members whose functions contribute to the regulation of a wide range of cellular processes including proliferation and differentiation (Reviewed in [27, 28]). Wnts have also been implicated in tumor formation [29, 30]. Wnts were first identified in mammals as the proto-oncogenic integration site (int-1) for the Mouse Mammary Tumor Virus (MMTV). It was subsequently shown that the int-1 protein was homologous to an important patterning protein, wingless, in *Drosophila*. The name Wnt evolved as a combination of the two terms. Wnts activate many signaling cascades that can be broadly divided into two general categories 1) the canonical, β -catenin pathway and [2] the non-canonical β -catenin independent pathways including the planar cell polarity pathway (PCP) and the Wnt/ Ca^{2+} pathway (Fig. 1; [27, 28, 31, 32]).

Canonical Wnts transmit their signals by binding to a subset of members within a family of seven-pass-transmembrane-spanning receptors, termed Frizzled receptors, in addition to co-receptors that belong to the LDL-receptor-related protein family. In the absence of Wnt, Glycogen Synthase Kinase-3 β (GSK-3 β) is active and phosphorylates β -catenin, targeting it for degradation. In the presence of Wnt, the cytoplasmic protein, dishelved (Dsh), acts to inhibit the activity of GSK-3 β , which is in a complex with the Adenomatous Polyposis Coli protein and Axin resulting in stabilization of the β -catenin protein and its subsequent translocation to the nucleus. Nuclear β -catenin associates with the Lymphoid Enhancer

Factor/T-Cell-Specific Transcription Factor (LEF/TCF) family of transcription factors and activates transcription of Wnt target genes (Reviewed in [28]).

The non-canonical signaling pathways are not as well characterized. Wnt5a and Wnt11 are representative examples of non-canonical signaling Wnts both of which are expressed in the mammary gland. The signaling pathways are termed non-canonical in that they do not involve stabilization of β -catenin. Like canonical signaling, the PCP pathway signals through Dsh; however, this pathway results in cytoskeletal changes and cell movement via activation of RhoA, and c-Jun N-terminal kinase (JNK) [29]. The Wnt/ Ca^{2+} pathway leads to the release of intracellular Ca^{2+} , which can have effects on Calmodulin Kinase II and/or Phospho Kinase C (PKC) depending on the cell type. This pathway also involves the activation of a $\text{G}_{i/o}$ class G-protein since it is inhibited by pertussis toxin [32–34]. In addition, it has been shown that some non-canonical signaling Wnts can directly antagonize canonical signaling although the mechanism appears to vary from cell type to cell type [35–37]. Non-canonical Wnts also utilize Frizzled receptors. Frizzled 2, 3, 5, and 6 are representative receptors for non-canonical Wnts. In addition, the phospho-tyrosine kinase receptors, ROR1, ROR2, and PTK7, can act as co-receptors for some of the non-canonical signals [37, 38]. It has also been shown that receptor blocking antibodies specific to Frizzled 5 blocked the effects of Wnt5a on breast cancer motility. Receptor blocking antibodies targeted to Frizzled 2 had no effect suggesting Frizzled 5 is a receptor for Wnt5a in breast cancer [39].

Wnt5a Mediates the Effects of TGF- β on Branching in the Mammary Gland

Similarly to TGF- β , Wnt5a regulates mammary development [18]. Slow release pellets containing Wnt5a inhibited both ductal extension and lateral branching when implanted into mouse mammary glands. Delayed development was associated with a decrease in cell proliferation as measured by BrdU incorporation. Furthermore, Wnt5a inhibited branching in mammary organoids grown in 3-dimensional culture. Loss of Wnt5a resulted in large terminal end bud size and increased branching and proliferation. The effects of losing Wnt5a were observed when Wnt5a null epithelium combined with Wnt5a null stroma was grown under the kidney capsule of host mice as well as when null epithelium alone was placed into the cleared fat pad of a wild type host. This result suggested that even though Wnt5a is expressed and regulated by TGF- β in both epithelial and stromal compartments, Wnt5a from the host stroma is not sufficient to mediate branching. Since Wnt5a has similar effects as TGF- β on mammary development, it was hypothesized that Wnt5a could act as a downstream mediator of TGF- β 's action. When slow release pellets containing TGF- β were placed in glands containing Wnt5a null epithelium, TGF- β was not able to inhibit ductal extension. In addition, TGF- β did not inhibit branching in Wnt5a-null organoids grown in 3-D culture. Together the results suggested that Wnt5a is required for TGF- β 's effects on branching morphogenesis [18]. Subsequently it was shown the Wnt5a has similar effects on prostate development; however, a link to TGF- β signaling was not investigated [40, 41].

TGF- β , Wnt5a and the Mammary Stem Cell Population

Mammary and Cancer Stem Cells

Mammary gland development is a good model to study basic questions in cancer biology including mechanisms of growth, differentiation, and invasion. [42–44]. At the onset of puberty the ductal epithelium responds to hormones and growth factors and begins to ramify through the fat pad guided by TEBs, specialized structures where active proliferation, invasion, and differentiation occur. The epithelium continues to grow until it reaches the limit of the fat pad where the TEBs disappear and the ductal tree remains relatively quiescent until the onset of pregnancy. During pregnancy another round of proliferation and

differentiation occurs leading to the formation of lobuloalveolar structures. After removal of the pups, involution via apoptosis and remodeling occurs returning the gland to a quiescent state. Multiple rounds of regulated growth and differentiation through the lifetime of the mouse, in addition to initial organogenesis, necessitate a population of cells that can expand and differentiate into all of the mammary epithelial cell types. It has been proposed that the cells underlying these processes are mammary stem cells (MaSCs), which have the capacity to self-renew and generate daughter cells that can form cells of any of the three mammary epithelial lineages: luminal, myoepithelial, and alveolar. Evidence for the existence of MaSCs was first demonstrated by transplanting fragments of mouse mammary epithelium into a cleared fat pad to generate functional mammary epithelial outgrowths of luminal, basal, and alveolar cells [45–47]. The search for cell surface differentiation markers became essential to conclusively demonstrate MaSCs existence and characterize their cellular and molecular mechanisms. Two groups provided this major step forward in the field by observing that Lin-CD29^{hi} or CD49^{hi}CD24⁺ cells could regenerate an entire mammary gland from a single MaSC [48, 49]. Currently, a differentiation hierarchy within the mammary gland that originates as a self-renewing stem cell capable of asymmetrically dividing leading to transit amplifying or progenitor populations that give rise to all three epithelial lineages is being uncovered (Reviewed in [50, 51]).

Heterogeneous pathology and molecular profiles found within breast cancer suggested the existence of cancer stem cells. The cancer stem cell hypothesis was first proposed by Dick and colleagues when studying acute myelogenous leukemia and states that mutations within the stem cell population may result in unregulated self-renewal, resulting in more differentiated progeny that give rise to cancer [52]. Recent studies utilized a similar approach to isolating tumor initiating cells in solid human breast tumors and demonstrated only a small population of cells within the tumor expressing Lin-CD24^{low} CD44⁺ may initiate new tumor growth. Consistent with the hypothesis, this subset of cells generated a tumor that maintained identical heterogeneity as the original tumor [53], indicating stem cell-like properties of self-renewal and the ability to differentiate into multiple cell types. The relationship between normal mammary stem cells and tumor stem cells is not yet clear, but evidence is accumulating that the tumor stem cell is derived from normal mammary stem or transient amplifying progenitor cells [51]. An important pathway shown to play a role in carcinogenesis and maintenance of stem cells in multiple cell types is Wnt signaling [54].

Canonical Wnt Signaling Regulates Maintenance and Proliferation of Stem Cells

Canonical Wnt signaling has been implicated in maintaining regulation of the stem cell microenvironment. As a key paracrine secreted factor, it controls stem cell fate by suppressing differentiation and promoting self-renewal as seen in skin, intestine, and other tissues including breast [54]. Knockout studies of the Tcf4 transcriptional factor demonstrated prevention of stem cell activity in the small intestine [55]. Furthermore, hematopoietic stem cell self-renewal was promoted upon addition of exogenous canonical Wnt protein, Wnt3a, in vitro and in vivo [56]. Together, these studies suggest that canonical Wnt signaling mediates the maintenance or expansion of stem cell populations in multiple tissues.

An extensive amount of evidence has indicated that canonical Wnt signaling plays a significant role in maintaining stem cell activity in mouse mammary glands. MaSCs have been shown to reside within the basal epithelial compartment of the mammary gland [48, 49] where expression of the Wnt receptor, Lrp5, can also be found [57]. In the absence of Lrp5, stem cell activity within the mammary gland is lost, thereby, preventing its regenerative capability [57]. Secretion of the Wnt ligand, Wnt4, is stimulated by progesterone receptors found within the mammary gland, which has been shown to activate

β -catenin signaling and increase stem cell activity [58–60]. Inhibition of β -catenin signaling in the mammary gland resulted in disruption of lobuloalveolar development [61], suggesting that MaSCs require signaling from Wnt to maintain the stem cell niche and the cells required for development during pregnancy. Supporting this model, elevated levels of exogenous Wnt3a resulted in an expansion of the MaSC population in vitro and after transplantation [62]. Gain-of-function mutations of β -catenin signaling using $\Delta N89\beta$ -catenin mice also demonstrated increased MaSC self-renewal [63]. Collectively, these studies demonstrate the importance of Wnt signaling in normal MaSC function. In addition, activation of canonical Wnt signaling in the mammary gland has been suggested to induce tumorigenesis from stem and luminal progenitor populations. Isolated cells from MMTV-Wnt1 mammary tumors support this concept by demonstrating an expansion of stem and Sca-1 cell populations as well as heterogeneity within the tumors [48, 63–66].

Regulation of Stem and Progenitor Cells by TGF- β and Wnt5a

As mentioned above, members of the TGF- β family have a complex and important role in mediating tumor progression and metastasis (Reviewed in [6, 15]). TGF- β acts as a tumor suppressor in early stages of breast cancer. The mechanism by which TGF- β acts as a tumor suppressor is not completely known; however, when overexpressed, TGF- β can limit the life-span of mammary stem cells as measured by reduced ability to regenerate a mammary gland after serial transplantation [14]. This loss of stem cell activity correlated with reduced tumor formation [12, 13]. In addition, both RNA and protein levels of Sca-1, a marker for luminal progenitor cells [67, 68], were increased in DNIIR glands suggesting that loss of responsiveness to TGF- β results in an increase in this population of progenitor cells present in the mammary gland [69]. Furthermore, expression of the DNIIR in MMTV-PyVmT tumors resulted in redirection of tumors to a more basal phenotype suggestive of a stem or progenitor cell origin [69]. Recently, it was shown that TGF- β could reduce the Hoechst dye stained side population, which is thought to be enriched in luminal progenitor cells, in breast cancer xenografts again suggesting TGF- β could regulate stem or progenitor populations [70]. Another study supporting a role for TGF- β in regulating the stem or progenitor cell population in the mammary gland involved determining the global gene expression pattern in CD24 + CD44+ breast cancer stem cells. Serial Analysis of Gene Expression data indicated that TGF- β signaling was silenced within this population by chromatin modification and promoter methylation [71]. Collectively, these results support a role for TGF- β in limiting the stem cell population. More importantly, these findings suggest a novel mechanism for TGF- β 's tumor suppressive role.

Loss of Wnt5a has been shown to associate with poor prognosis in breast cancer patients [20–22]. Furthermore, a screen of Wnt expression in various established tumor cell lines showed that, in general, canonical Wnts were up-regulated in cancer cell lines relative to normal human mammary epithelial cells while the expression of non-canonical Wnts, including Wnt5a, was down-regulated suggesting that like TGF- β , Wnt5a can act as a tumor suppressor [72]. In support of this observation, suppression of Wnt5a expression leads to transformation similar to that induced by Wnt1. Likewise, Wnt1 transformed epithelial cells regain normal morphological properties upon induction of Wnt5a expression [73–76].

There is also evidence that Wnt5a can limit the stem or progenitor cell populations in the mammary gland. Since loss of Wnt5a is perinatal lethal [77] and no floxed Wnt5a mouse is available, mammary buds from E16.5–E18.5 day Wnt5a null embryos were rescued and transplanted into cleared fat pads of SCID mice [18]. Wild type tissue was transplanted as a control. Sca-1 expression was visibly increased in lysate from Wnt5a null epithelial cells, suggesting that loss of Wnt5a resulted in an increase in this mammary progenitor population [69]. It was also shown that Wnt5a-null, MMTV-PyVmT tumors demonstrated redirection of the mammary tumor phenotype to that resembling a more heterogenous and basal type

tumor suggesting a stem or progenitor origin. The phenotype of these tumors was reminiscent of tumors generated by activation of canonical β -catenin signaling [78, 79].

It has been shown in other model systems that Wnt5a can antagonize the canonical Wnt pathway [35–37, 80, 81]. Since canonical Wnt signaling is involved in stem cell maintenance, it was proposed that Wnt5a contributes to the regulation of stem cells through suppression of canonical signaling. Indeed, β -catenin localized more strongly to the nucleus in TGF- β and Wnt5a deficient tumors than in control tumors [69]. In addition, transcriptional targets of β -catenin signaling, including Axin2, were up-regulated in the Wnt5a-deficient tumors. Non-tumorigenic ductal tissue from DNIIR or Wnt5a deficient glands also demonstrated increased activation of canonical Wnt signaling. Taken together, the data suggests that TGF- β and Wnt5a can inhibit canonical Wnt signaling within the mammary epithelium and redirect mammary tumor phenotype to more basal characteristics. It was proposed that TGF- β acting through Wnt5a antagonizes β -catenin thereby limiting the stem or progenitor cell populations providing a novel mediator for TGF- β 's tumor suppressive effects (Fig. 2).

Wnt5a in the Regulation of Migration and Metastasis

Wnt5a as an Inhibitor of Migration and Metastasis

Apart from the tumor suppressive role of Wnt5a on initiation of breast cancer, other studies have focused on the effects of Wnt5a on the progression of invasive breast cancer. Loss of Wnt-5a protein is associated with poor differentiation and shortened recurrence-free survival in invasive ductal breast cancer because of higher frequency of distant metastases, suggesting a suppressive role of Wnt5a in breast cancer metastasis [21]. Branching involves both regulated cell proliferation and migration activities that are unregulated during metastasis. It was shown that when cells of a human mammary epithelial cell line, HB2, were grown on plastic in monolayer, Wnt5a expression was very high [82]. When the cells were grown in 3-dimensional culture under conditions that promote branching, Wnt5a expression was significantly reduced and down-regulation of Wnt5a preceded branching. In addition, Hepatocyte Growth Factor (HGF), which promotes branching, was shown to down-regulate Wnt5a expression [82]. Similar results were found in a spontaneously immortalized human epithelial cell line, MCF10A suggesting an inverse correlation between Wnt5a expression and branching [83]. In a separate study, over-expression of Wnt5a in HB2 cells inhibited HGF-induced branching in 3-dimensional culture while antisense inhibition of Wnt5a expression promoted branching under the same conditions [73]. Reduced Wnt5a expression also resulted in decreased adhesion to collagen and increased migration in monolayer cultures. In support of the in vitro studies, levels of Wnt5a were higher in the mammary stroma of C57BL/6 mice, which have low levels of endogenous branching, when compared to that of the 129 strain suggesting an inverse correlation between branching and Wnt5a expression in vivo [84]. This correlation was not found in F2 crosses between C57BL/6 and 129 mice, where branching is highly variable, making the role of Wnt5a in vivo less clear.

It has been shown that Wnt5a is necessary for activation of the Discoidin Domain Receptor 1 (Ddr1) by collagen [73]. Ddr1 and Ddr2 are receptor tyrosine kinases that act as collagen receptors [85, 86]. In tumors, Ddr1 is primarily expressed on epithelial cells while Ddr2 is primarily expressed in the surrounding stroma [87]. Although Ddr1 and integrins can both act as receptors for collagen, they exert their effects via distinct intracellular signaling pathways [88]. Ddr1 is highly expressed in several human breast tumors [89]. Previously, it was shown that repression of Wnt5a in HB2 cells results in enhanced cell motility and impaired binding to collagen [73]. Alterations in binding and migration after depletion of Wnt5a were correlated with impaired phosphorylation of Ddr1. Activation and subsequent

phosphorylation of Ddr1 was shown to require both collagen and Wnt5a, neither alone was sufficient to activate the receptor. Activation of Ddr1 in vitro was independent of β -catenin and required G-protein activity suggesting Wnt/Ca²⁺ signaling was involved [90]. A binding partner for Ddr1, Darp32 is a phospho-dependent antimigratory molecule. Wnt5a triggers Frizzled-3/Gas/cAMP signaling that results in PKA-dependent phosphorylation of Darp32. Furthermore, phospho-Darp32 increases Wnt5a-mediated CREB activity eliciting the anti-migratory response [91]. It was proposed that elevated metastatic ability, including altered adhesion and increased migration, in breast cancers that lack Wnt5a is caused by alterations in Ddr1/Darp32 activity [39, 91] (Fig. 3). Based on the studies suggesting Wnt5a acts to inhibit metastasis, a Wnt5a peptide agonist, named FOXY-5, was developed and tested [39, 92, 93]. In vitro assays were used to show that both recombinant Wnt5a and FOXY-5 could inhibit cell migration and invasion without affecting cell growth or apoptosis. Metastasis of breast cancer cells injected into the mammary fat pad was also inhibited by i.p. injection of FOXY-5 into mice. The results suggest that Wnt5a can be used as a therapeutic target to inhibit breast cancer metastasis.

Wnt5a Can Also Promote Metastasis

Depending on different cell/tissue contexts, Wnt5a acts as either a promoter or suppressor of aggressiveness in different types of cancer. In thyroid and colorectal cancer, Wnt5a has inhibitory effects on cell growth, invasion and migration [74, 94, 95]. On the other hand, in the aggressive skin cancer, melanoma, Wnt5a directly promotes cell invasion and motility through inhibition of metastasis-associated genes (KISS1 and CD44) and induction of Epithelial-Mesenchymal-Transition (a key step in metastasis) in a PKC-dependent manner [96–98]. This positive role of Wnt5a in melanoma metastasis could be inhibited by a Wnt5a-derived antagonist and is mediated by Ror2 [98, 99]. Ror2 is a known co-receptor for Wnt5a [100]. Ror2 plays important roles in enhancing cell invasion and migration in multiple cancer types, including osteocarcinoma, prostate carcinoma, and renal cell carcinoma [101–103]. Several lines of evidence have also indicated that Wnt5a-Ror2 signaling can induce selective expression of Matrix Metalloproteinases (MMPs), which provides fundamental mechanisms to connect Wnt5a and microenvironment in tumor metastasis, and suggests potential targets for therapeutic strategies in those types of cancer [102–104].

One example of Wnt5a promoting malignant progression in breast cancer is dependent on the microenvironment. Wnt5a signaling is essential for macrophage-induced invasion of the breast cancer cell line, MCF-7 [105]. It was previously shown that co-culture of MCF-7 breast cancer cells with macrophages enhanced MMP-dependent invasion and resulted in up-regulation of Wnt5a in macrophages. The study showed that non-canonical signaling via JNK in the tumor cell was necessary for invasion as was Wnt5a induced MMP expression in the macrophages. The authors concluded that Wnt5a mediated cell migration in tumor cells as well as proteolytic activity of the macrophages (Fig. 3). Macrophages play a critical role in extracellular-matrix breakdown and remodeling, angiogenesis, as well as tumor invasion and were considered obligate partners in the microenvironment leading to metastasis [106]. It was proposed that specific intracellular signaling pathways, as well as intercellular interactions, modulate whether Wnt5a suppresses or promotes tumor progression [105].

Potential Interactions of TGF- β and Wnt5a in Migration and Metastasis

A role for Wnt5a in mediating some of the effects of TGF- β on metastasis is not at all clear. The roles for TGF- β in regulating metastasis appear to be distinct from the currently known actions of Wnt5a. As tumors accumulate mutations, TGF- β is thought to promote EMT, migration and metastasis in the late stage tumors. However, in some cases TGF- β can inhibit metastasis (reviewed in [15, 107]). In mouse models with complete loss of Tgfr2 through Cre-mediated deletion, pulmonary metastasis in MMTV-PyVmT generated tumors in

increased [108]. This result was hard to reconcile with previous reports until the effects on the surrounding microenvironment and immune system were considered [107–109]. It was shown that loss of *Tgfb2* increased infiltrating immune cells at the edge of the tumor. This population of cells was enriched for myeloid derived suppressor cells that are known to promote metastasis in other systems through activation of MMPs and stimulation of angiogenesis. The results suggested TGF- β can inhibit the recruitment of these cells to the tumor thereby blocking metastasis. Whether or not Wnt5a has a role in recruitment of myeloid cells to the tumor has not been addressed. Nevertheless, as described above, co-culture of breast cancer cells and macrophages promotes invasion of cancer cells through up regulation of Wnt5a in the macrophage population [105]. It has been documented that many late stage tumors express very high levels of TGF- β ligands and TGF- β promotes recruitment and differentiation of macrophages to tumor sites (Reviewed in [110]). TGF- β also alters the gene expression profile of tumor-associated macrophages altering the expression of Tumor Necrosis Factor alpha as well as various chemokines contributing to tumor progression. It is not known if TGF- β stimulates Wnt5a in this context to promote tumor invasion.

Another target of TGF- β , the transcription factor CUTL1, enhances cancer cell migration and invasion. CUTL1 is expressed at high levels in high-grade tumors and its expression is inversely correlated to survival in breast cancer patients [111]. It was subsequently shown that one of the transcriptional targets of CUTL1 is Wnt5a [112]. It was also shown that Wnt5a mediated a large proportion of the invasive effects of CUTL1 in pancreatic tumors. The roles of TGF- β , CUTL1 and Wnt5a have not been addressed in breast cancer.

Potential mechanisms to inhibit migration and invasion include activation of Ddr1, a collagen receptor. Germline deletion of Ddr1 has significant effects on the development of the mammary gland [113, 114]. Ddr1 null mice demonstrated defects in ductal extension through the fat pad and lateral branching in adults that are both distinct and overlapping to what is seen in DNIIR mice and Wnt5a null glands. At 3-weeks of age, ductal extension was delayed relative to control mice; however, the endbuds were greatly enlarged. In contrast, in adult mice at 3-months of age, an increase in the number and diameter of ducts was detected along with an increase in cell proliferation as measured by staining with Ki67 [113]. Increased proliferation and branching observed at 3-months in Ddr1-null mice is similar to that observed in DNIIR mice and Wnt5a-null tissue suggesting that TGF- β , Wnt5a, and Ddr1 may interact to regulate branching in adult mammary gland. In cell culture, treatment with Wnt5a results in increased phosphorylation of Ddr1 and reduced migration in primary mouse mammary epithelial cells [18, 90]. When primary cells on collagen were treated with TGF- β , activation of Ddr1, as measured by phosphorylation of the protein, was observed by 30 h of treatment while the level of total Ddr1 protein remained constant. Likewise, loss of TGF- β signaling in DNIIR mice resulted in reduced phospho-Ddr1 although the level of total Ddr1 protein remained constant between wild type and DNIIR mice. When primary Wnt5a-null cells were grown on collagen and treated with TGF- β , activation of Ddr1 was not observed indicating Wnt5a was required for the response. Since activation of Ddr1 inhibits migration of breast cancer cells, the results present a potential mechanism for TGF- β and Wnt5a to inhibit metastasis under certain circumstances.

Concluding Remarks

In summary, development of the mammary gland and tumor progression are complex processes that require coordination and deregulation of many signaling pathways. Data suggest that TGF- β and Wnt5a have similar functions. Both TGF- β and Wnt5a have similar expression patterns during mammary development. It has been shown that TGF- β and Wnt5a play important roles in regulating ductal elongation and lateral branching during

adolescence. Cell culture and in vivo studies indicate that Wnt5a mediates the effects of TGF- β on branching. Both proteins are thought to act as tumor suppressors with complex roles in mediating invasion and metastasis. Tumor suppression may involve antagonism of Wnt/ β -catenin signaling and limiting the stem cell population. Metastasis is more complex, and interactions between TGF- β and Wnt5a may depend on specific cues from the microenvironment as well as activation of specific intracellular signaling pathways. Nevertheless, the data combined suggest a potential functional interaction between TGF- β and Wnt5a in mammary development and cancer.

Abbreviations

Ddr1	Discoidin Domain Receptor 1
DNIIR	dominant-negative mutation of Tgfr2
Dsh	Dishelved
GSK-3β	Glycogen Synthase Kinase-3 β
HGF	Hepatocyte Growth Factor
JNK	c-Jun N-terminal kinase
LEF/TCF	Lymphoid Enhancer Factor/T-Cell-Specific Transcription Factor
MaSCs	mammary stem cells
MMP	Matrix Metalloproteinases
PKC	Phospho Kinase C
Ror2	receptor tyrosine kinase-like orphan receptor 2
TEBs	terminal end buds
TGF-β	Transforming Growth Factor -beta
Tgfr1	TGF- β receptor type I
Tgfr2	TGF- β receptor type II
Wnt5a	Wingless-related MMTV Integration Site 5A

Acknowledgments

Breast cancer research in R. Serra's laboratory was supported by NIH R01 CA126942. S.E. Baxley was supported by the UAB Cancer Prevention and Control Training Program, NCI R25 CA047888 and MSTP training grant T32 GM008361.

References

1. Roberts, AB.; Sporn, MB. The transforming growth factor- β s. In: Sporn, MB.; Roberts, AB., editors. Peptide growth factors and their receptors. Heidelberg: Springer; 1990. p. 419-472.
2. Massague J. TGF- β signal transduction. Annu Rev Biochem. 1998; 67:753–791. [PubMed: 9759503]
3. Hogan BLM. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev. 1996; 10:1580–1594. [PubMed: 8682290]
4. Kingsley DM. The TGF- β superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev. 1994; 8:133–146. [PubMed: 8299934]
5. Serra R, Crowley MR. TGF- β in mammary gland development and breast cancer. Breast Dis. 2003; 18:61–73. [PubMed: 15687689]

6. Serra R, Crowley MR. Mouse models of transforming growth factor beta impact in breast development and cancer. *Endocr Relat Cancer*. 2005; 12(4):749–760. [PubMed: 16322320]
7. Ewan KB, Shyamala G, Ravani SA, Tang Y, Akhurst R, Wakefield L, et al. Latent transforming growth factor-beta activation in mammary gland: regulation by ovarian hormones affects ductal and alveolar proliferation. *Am J Pathol*. 2002; 160(6):2081–2093. [PubMed: 12057913]
8. Robinson SD, Silberstein GB, Roberts AB, Flanders KC, Daniel CW. Regulated expression and growth inhibitory effects of transforming growth factor-b isoforms in mouse mammary gland development. *Development*. 1991; 113:867–878. [PubMed: 1821856]
9. Strange R, Li F, Saurer S, Burkhardt A, Friis RR. Apoptotic cell death and tissue remodelling during mouse mammary gland involution. *Development*. 1992; 115:49–58. [PubMed: 1638991]
10. Nguyen AV, Pollard JW. Transforming growth factor beta3 induces cell death during the first stage of mammary gland involution. *Development*. 2000; 127(14):3107–3118. [PubMed: 10862748]
11. Joseph H, Gorska A, Sohn P, Moses HL, Serra R. Over-expression of a kinase-deficient transforming growth factor- β type II receptor in mouse mammary stroma results in increased epithelial branching. *Mol Biol Cell*. 1999; 10:1221–1234. [PubMed: 10198068]
12. Boulanger CA, Smith GH. Reducing mammary cancer risk through premature stem cell senescence. *Oncogene*. 2001; 20(18):2264–2272. [PubMed: 11402321]
13. Boulanger CA, Wagner KU, Smith GH. Parity-induced mouse mammary epithelial cells are pluripotent, self-renewing and sensitive to TGF- β 1 expression. *Oncogene*. 2005; 24(4):552–560. [PubMed: 15580303]
14. Kordon EC, McKnight RA, Jhappan C, Hennighausen L, Merlino G, Smith GH. Ectopic TGF[β]1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. *Dev Biol*. 1995; 168(1):47–61. [PubMed: 7883078]
15. Baxley S, Serra R. Inhibiting breast cancer progression by exploiting TGF signaling. *Curr Drug Targets*. 2010; 11:1089–1102. [PubMed: 20545612]
16. Crowley MR, Bowtell D, Serra R. TGF- β , c-Cbl, and PDGFR- α in the mammary stroma. *Dev Biol*. 2005; 279(1):58–72. [PubMed: 15708558]
17. Gorska AE, Joseph H, Derynck R, Moses HL, Serra R. Dominant-negative interference of the transforming growth factor beta type II receptor in mammary epithelium results in alveolar hyperplasia and differentiation in virgin mice. *Cell Growth Differ*. 1998; 9:229–238. [PubMed: 9543389]
18. Roarty K, Serra R. Wnt5a is required for proper mammary gland development and TGF- β -mediated inhibition of ductal growth. *Development*. 2007; 134(21):3929–3939. [PubMed: 17898001]
19. Katoh M. Transcriptional mechanisms of WNT5A based on NF- κ B, Hedgehog, TGF β , and Notch signaling cascades. *Int J Mol Med*. 2009; 23(6):763–769. [PubMed: 19424602]
20. Dejmek J, Leandersson K, Manjer J, Bjartell A, Emdin SO, Vogel WF, et al. Expression and signaling activity of Wnt-5a/Discoidin domain receptor-1 and syk plays distinct but decisive roles in breast cancer patient survival. *Clin Cancer Res*. 2005; 11(2):520–528. [PubMed: 15701836]
21. Jonsson M, Dejmek J, Bendahl PO, Andersson T. Loss of Wnt-5a Protein Is Associated with Early Relapse in Invasive Ductal Breast Carcinomas. *Cancer Res*. 2002; 62(2):409–416. [PubMed: 11809689]
22. Leris ACA, Roberts TR, Jiang WG, Newbold RF, Mokbel K. WNT5A Expression in Human Breast Cancer. *Anticancer Research*. 2005; 25(2A):731–734. [PubMed: 15868903]
23. Gavin BJ, McMahon AP. Differential regulation of the Wnt gene family during pregnancy and lactation suggests a role in postnatal development of the mammary gland. *Mol Cell Biol*. 1992; 12(5):2418–2423. [PubMed: 1373817]
24. Weber-Hall SJ, Phippard DJ, Niemeyer CC, Dale TC. Developmental and hormonal regulation of Wnt gene expression in the mouse mammary gland. *Differentiation*. 1994; 57(3):205–214. [PubMed: 7988795]
25. Chu EY, Hens J, Andl T, Kairo A, Yamaguchi TP, Briskin C, et al. Canonical WNT signaling promotes mammary placode development and is essential for initiation of mammary gland morphogenesis. *Development*. 2004; 131(19):4819–4829. [PubMed: 15342465]

26. Kouros-Mehr H, Werb Z. Candidate regulators of mammary branching morphogenesis identified by genome-wide transcript analysis. *Dev Dyn*. 2006; 235(12):3404–3412. [PubMed: 17039550]
27. Veeman MT, Axelrod JD, Moon RT. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev Cell*. 2003; 5(3):367–377. [PubMed: 12967557]
28. Kikuchi A, Kishida S, Yamamoto H. Regulation of Wnt signaling by protein-protein interaction and post-translational modifications. *Exp Mol Med*. 2006; 38(1):1–10. [PubMed: 16520547]
29. Katoh M. WNT/PCP signaling pathway and human cancer (review). *Oncol Rep*. 2005; 14(6): 1583–1588. [PubMed: 16273260]
30. Brennan KR, Brown AM. Wnt proteins in mammary development and cancer. *J Mammary Gland Biol Neoplasia*. 2004; 9(2):119–131. [PubMed: 15300008]
31. Widelitz R. Wnt signaling through canonical and non-canonical pathways: recent progress. *Growth Factors*. 2005; 23(2):111–116. [PubMed: 16019432]
32. Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT. The Wnt/Ca²⁺ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet*. 2000; 16(7):279–283. [PubMed: 10858654]
33. Kuhl M. The WNT/calcium pathway: biochemical mediators, tools and future requirements. *Front Biosci*. 2004; 9:967–974. [PubMed: 14766423]
34. Kohn AD, Moon RT. Wnt and calcium signaling: beta-catenin-independent pathways. *Cell Calcium*. 2005; 38(3–4):439–446. [PubMed: 16099039]
35. Westfall TA, Brimeyer R, Twedt J, Gladon J, Olberding A, Furutani-Seiki M, et al. Wnt-5/pipetail functions in vertebrate axis formation as a negative regulator of Wnt/beta-catenin activity. *J Cell Biol*. 2003; 162(5):889–898. [PubMed: 12952939]
36. Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y. 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]
37. 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]
38. Oishi I, Suzuki H, Onishi N, Takada R, Kani S, Ohkawara B, et al. The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. *Genes Cells*. 2003; 8(7):645–654. [PubMed: 12839624]
39. Sahlholm A, Leandersson K, Dejmeck J, Nielsen CK, Villoutreix BO, Andersson T. A formylated hexapeptide ligand mimics the ability of Wnt-5a to impair migration of human breast epithelial cells. *J Biol Chem*. 2006; 281(5):2740–2749. [PubMed: 16330545]
40. Huang L, Pu Y, Hu WY, Birch L, Luccio-Camelo D, Yamaguchi T, et al. The role of Wnt5a in prostate gland development. *Dev Biol*. 2009; 328(2):188–199. [PubMed: 19389372]
41. Allgeier SH, Lin TM, Vezina CM, Moore RW, Fritz WA, Chiu SY, et al. WNT5A selectively inhibits mouse ventral prostate development. *Dev Biol*. 2008; 324(1):10–17. [PubMed: 18804104]
42. Imagawa, W.; Yang, J.; Guzman, R.; Satybrata, N. Control of mammary gland development. In: Knobil, E.; Neil, JD., editors. *The physiology of reproduction*. 2nd ed.. New York: Raven; 1994. p. 1033-1063.
43. Hennighausen L, Robinson GW. Signaling pathways in mammary gland development. *Dev Cell*. 2001; 1(4):467–475. [PubMed: 11703938]
44. Silberstein GB. Postnatal mammary gland morphogenesis. *Microsc Res Tech*. 2001; 52(2):155–162. [PubMed: 11169863]
45. DeOme KB, Faulkin LJ, Bern HA, Blair PB. Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer Res*. 1959; 19(5):515. [PubMed: 13663040]
46. Daniel CW, De Ome KB, Young JT, Blair PB, Faulkin LJ. The in vivo life span of normal and preneoplastic mouse mammary glands: a serial transplantation study. *Proc Natl Acad Sci USA*. 1968; 61(1):53–60. [PubMed: 4301594]
47. Kordon EC, Smith GH. An entire functional mammary gland may comprise the progeny from a single cell. *Development*. 1998; 125(10):1921–1930. [PubMed: 9550724]

48. Shackleton M, Vaillant Fo, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat M-L, et al. Generation of a functional mammary gland from a single stem cell. *Nature*. 2006; 439(7072):84–88. [PubMed: 16397499]
49. Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, et al. Purification and unique properties of mammary epithelial stem cells. *Nature*. 2006; 439(7079):993–997. [PubMed: 16395311]
50. Stingl J. Detection and analysis of mammary gland stem cells. *J Pathol*. 2009; 217(2):229–241. [PubMed: 19009588]
51. Visvader JE, Smith GH. Murine mammary epithelial stem cells: discovery, function, and current status. *Cold Spring Harbor Perspectives in Biology*. 2010
52. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997; 3(7):730–737. [PubMed: 9212098]
53. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA*. 2003; 100(7):3983–3988. [PubMed: 12629218]
54. Alonso L, Fuchs E. Stem cells in the skin: waste not, Wnt not. *Genes Dev*. 2003; 17(10):1189–1200. [PubMed: 12756224]
55. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet*. 1998; 19(4):379–383. [PubMed: 9697701]
56. Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature*. 2003; 423(6938):409–414. [PubMed: 12717450]
57. Badders NM, Goel S, Clark RJ, Klos KS, Kim S, Bafico A, et al. The Wnt receptor, Lrp5, is expressed by mouse mammary stem cells and is required to maintain the basal lineage. *PLoS ONE*. 2009; 4(8):e6594. [PubMed: 19672307]
58. Briskin C, Heineman A, Chavarria T, Elenbaas B, Tan J, Dey SK, et al. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. *Genes Dev*. 2000; 14(6):650–654. [PubMed: 10733525]
59. Briskin C, Park S, Vass T, Lydon JP, O'Malley BW, Weinberg RA. A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proc Natl Acad Sci USA*. 1998; 95(9):5076–5081. [PubMed: 9560231]
60. Joshi PA, Jackson HW, Beristain AG, Di Grappa MA, Mote PA, Clarke CL, et al. Progesterone induces adult mammary stem cell expansion. *Nature*. 2010; 465(7299):803–807. [PubMed: 20445538]
61. Tepera SB, McCrea PD, Rosen JM. A beta-catenin survival signal is required for normal lobular development in the mammary gland. *J Cell Sci*. 2003; 116(Pt 6):1137–1149. [PubMed: 12584256]
62. Zeng YA, Nusse R. Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. *Cell Stem Cell*. 2010; 6(6):568–577. [PubMed: 20569694]
63. Liu BY, McDermott SP, Khwaja SS, Alexander CM. The transforming activity of Wnt effectors correlates with their ability to induce the accumulation of mammary progenitor cells. *Proc Natl Acad Sci USA*. 2004; 101(12):4158–4163. [PubMed: 15020770]
64. Li Y, Welm B, Podsypanina K, Huang S, Chamorro M, Zhang X, et al. Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. *Proc Natl Acad Sci USA*. 2003; 100(26):15853–15858. [PubMed: 14668450]
65. Rosner A, Miyoshi K, Landesman-Bollag E, Xu X, Seldin DC, Moser AR, et al. Pathway pathology: histological differences between ErbB/Ras and Wnt pathway transgenic mammary tumors. *Am J Pathol*. 2002; 161(3):1087–1097. [PubMed: 12213737]
66. Cho RW, Wang X, Diehn M, Shedden K, Chen GY, Sherlock G, et al. Isolation and molecular characterization of cancer stem cells in MMTV-Wnt-1 murine breast tumors. *Stem Cells*. 2008; 26(2):364–371. [PubMed: 17975224]
67. Welm BE, Tepera SB, Venezia T, Graubert TA, Rosen JM, Goodell MA. Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. *Dev Biol*. 2002; 245(1):42–56. [PubMed: 11969254]

68. Sleeman KE, Kendrick H, Robertson D, Isacke CM, Ashworth A, Smalley MJ. Dissociation of estrogen receptor expression and in vivo stem cell activity in the mammary gland. *J Cell Biol.* 2007; 176(1):19–26. [PubMed: 17190790]
69. Roarty K, Baxley SE, Crowley MR, Frost AR, Serra R. Loss of TGF-beta or Wnt5a results in an increase in Wnt/beta-catenin activity and redirects mammary tumour phenotype. *Breast Cancer Res.* 2009; 11(2):R19. [PubMed: 19344510]
70. Tang B, Yoo N, Vu M, Mamura M, Nam JS, Ooshima A, et al. Transforming growth factor-beta can suppress tumorigenesis through effects on the putative cancer stem or early progenitor cell and committed progeny in a breast cancer xenograft model. *Cancer Res.* 2007; 67(18):8643–8652. [PubMed: 17875704]
71. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, et al. Molecular definition of breast tumor heterogeneity. *Cancer Cell.* 2007; 11(3):259–273. [PubMed: 17349583]
72. Benhaj K, Akcali KC, Ozturk M. Redundant expression of canonical Wnt ligands in human breast cancer cell lines. *Oncol Rep.* 2006; 15(3):701–707. [PubMed: 16465433]
73. Jonsson M, Andersson T. Repression of Wnt-5a impairs DDR1 phosphorylation and modifies adhesion and migration of mammary cells. *J Cell Sci.* 2001; 114(Pt 11):2043–2053. [PubMed: 11493640]
74. Kremenevskaja N, von Wasielewski R, Rao AS, Schofl C, Andersson T, Brabant G. Wnt-5a has tumor suppressor activity in thyroid carcinoma. *Oncogene.* 2005; 24(13):2144–2154. [PubMed: 15735754]
75. Olson DJ, Gibo DM. Antisense wnt-5a mimics wnt-1-mediated C57MG mammary epithelial cell transformation. *Exp Cell Res.* 1998; 241(1):134–141. [PubMed: 9633521]
76. Olson DJ, Gibo DM, Sagers G, Debinski W, Kumar R. Reversion of uroepithelial cell tumorigenesis by the ectopic expression of human wnt-5a. *Cell Growth Differ.* 1997; 8(4):417–423. [PubMed: 9101087]
77. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development.* 1999; 126(6):1211–1223. [PubMed: 10021340]
78. Teissedre B, Pinderhughes A, Incassati A, Hatsell SJ, Hiremath M, Cowin P. MMTV-Wnt1 and -DeltaN89beta-catenin induce canonical signaling in distinct progenitors and differentially activate Hedgehog signaling within mammary tumors. *PLoS ONE.* 2009; 4(2):e4537. [PubMed: 19225568]
79. Li Y, Hively WP, Varmus HE. Use of MMTV-Wnt-1 transgenic mice for studying the genetic basis of breast cancer. *Oncogene.* 2000; 19(8):1002–1009. [PubMed: 10713683]
80. Miller JR, Moon RT. Signal transduction through beta-catenin and specification of cell fate during embryogenesis. *Genes Dev.* 1996; 10(20):2527–2539. [PubMed: 8895655]
81. Ishitani T, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, et al. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca2+ pathway to antagonize Wnt/{beta}-catenin signaling. *Mol Cell Biol.* 2003; 23(1):131–139. [PubMed: 12482967]
82. Huguet EL, Smith K, Bicknell R, Harris AL. Regulation of Wnt5a mRNA expression in human mammary epithelial cells by cell shape, confluence, and hepatocyte growth factor. *J Biol Chem.* 1995; 270:12851–12856. [PubMed: 7759542]
83. Bui TD, Tortora G, Ciardiello F, Harris AL. Expression of Wnt5a is downregulated by extracellular matrix and mutated c-Ha-ras in the human mammary epithelial cell line MCF-10A. *Biochem Biophys Res Commun.* 1997; 239(3):911–917. [PubMed: 9367869]
84. Naylor MJ, Ormandy CJ. Mouse strain-specific patterns of mammary epithelial ductal side branching are elicited by stromal factors. *Dev Dyn.* 2002; 225(1):100–105. [PubMed: 12203726]
85. Shrivastava A, Radziejewski C, Campbell E, Kovac L, McGlynn M, Ryan TE, et al. An orphan receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors. *Mol Cell.* 1997; 1(1):25–34. [PubMed: 9659900]
86. Vogel W, Gish GD, Alves F, Pawson T. The discoidin domain receptor tyrosine kinases are activated by collagen. *Mol Cell.* 1997; 1(1):13–23. [PubMed: 9659899]

87. Vogel W. Discoidin domain receptors: structural relations and functional implications. *FASEB J*. 1999; 13(Suppl):S77–S82. [PubMed: 10352148]
88. Vogel W, Brakebusch C, Fassler R, Alves F, Ruggiero F, Pawson T. Discoidin domain receptor 1 is activated independently of beta (1) integrin. *J Biol Chem*. 2000; 275(8):5779–5784. [PubMed: 10681566]
89. Barker KT, Martindale JE, Mitchell PJ, Kamalati T, Page MJ, Phippard DJ, et al. Expression patterns of the novel receptor-like tyrosine kinase, DDR, in human breast tumours. *Oncogene*. 1995; 10(3):569–575. [PubMed: 7845682]
90. Dejmek J, Dib K, Jonsson M, Andersson T. Wnt-5a and G-protein signaling are required for collagen-induced DDR1 receptor activation and normal mammary cell adhesion. *Int J Cancer*. 2003; 103(3):344–351. [PubMed: 12471617]
91. Hansen C, Howlin J, Tengholm A, Dyachok O, Vogel WF, Nairn AC, et al. Wnt-5a-induced phosphorylation of DARPP-32 inhibits breast cancer cell migration in a CREB-dependent manner. *J Biol Chem*. 2009; 284(40):27533–27543. [PubMed: 19651774]
92. Safholm A, Tuomela J, Rosenkvist J, Dejmek J, Harkonen P, Andersson T. The Wnt-5a-derived hexapeptide Foxy-5 inhibits breast cancer metastasis in vivo by targeting cell motility. *Clin Cancer Res*. 2008; 14(20):6556–6563. [PubMed: 18927296]
93. Ford CE, Ekstrom EJ, Howlin J, Andersson T. The WNT-5a derived peptide, Foxy-5, possesses dual properties that impair progression of ERalpha negative breast cancer. *Cell Cycle*. 2009; 8(12):1838–1842. [PubMed: 19448401]
94. Dejmek J, Dejmek A, Safholm A, Sjolander A, Andersson T. Wnt-5a protein expression in primary duks B colon cancers identifies a subgroup of patients with good prognosis. *Cancer Res*. 2005; 65(20):9142–9146. [PubMed: 16230369]
95. McDonald SL, Silver A. The opposing roles of Wnt-5a in cancer. *Br J Cancer*. 2009; 101(2):209–214. [PubMed: 19603030]
96. Weeraratna AT, Jiang Y, Hostetter G, Rosenblatt K, Duray P, Bittner M, et al. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell*. 2002; 1(3):279–288. [PubMed: 12086864]
97. Dissanayake SK, Wade M, Johnson CE, O'Connell MP, Leotlela PD, French AD, 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]
98. O'Connell MP, Fiori JL, Xu M, Carter AD, Frank BP, Camilli TC, et al. The orphan tyrosine kinase receptor, ROR2, mediates Wnt5A signaling in metastatic melanoma. *Oncogene*. 2010; 29(1):34–44. [PubMed: 19802008]
99. Jenei V, Sherwood V, Howlin J, Linnskog R, Safholm A, Axelsson L, et al. A t-butyloxycarbonyl-modified Wnt5a-derived hexapeptide functions as a potent antagonist of Wnt5a-dependent melanoma cell invasion. *Proc Natl Acad Sci USA*. 2009; 106(46):19473–19478. [PubMed: 19901340]
100. Nishita M, Enomoto M, Yamagata K, Minami Y. Cell/tissue-tropic functions of Wnt5a signaling in normal and cancer cells. *Trends Cell Biol*. 2010; 20(6):346–354. [PubMed: 20359892]
101. Enomoto M, Hayakawa S, Itsukushima S, Ren DY, Matsuo M, Tamada K, et al. Autonomous regulation of osteosarcoma cell invasiveness by Wnt5a/Ror2 signaling. *Oncogene*. 2009; 28(36):3197–3208. [PubMed: 19561643]
102. Slaton JW, Inoue K, Perrotte P, El-Naggar AK, Swanson DA, Fidler IJ, et al. Expression levels of genes that regulate metastasis and angiogenesis correlate with advanced pathological stage of renal cell carcinoma. *Am J Pathol*. 2001; 158(2):735–743. [PubMed: 11159211]
103. Yamamoto H, Oue N, Sato A, Hasegawa Y, Matsubara A, Yasui W, et al. Wnt5a signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase. *Oncogene*. 2010; 29(14):2036–2046. [PubMed: 20101234]
104. Westermarck J, Kahari VM. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J*. 1999; 13(8):781–792. [PubMed: 10224222]

105. Pukrop T, Klemm F, Hagemann T, Gradl D, Schulz M, Siemes S, et al. Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA*. 2006; 103(14):5454–5459. [PubMed: 16569699]
106. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell*. 2006; 124(2):263–266. [PubMed: 16439202]
107. Bierie B, Moses HL. Gain or loss of TGFbeta signaling in mammary carcinoma cells can promote metastasis. *Cell Cycle*. 2009; 8(20):3319–3327. [PubMed: 19806012]
108. Forrester E, Chytil A, Bierie B, Aakre M, Gorska AE, Sharif-Afshar AR, et al. Effect of conditional knockout of the type II TGF-beta receptor gene in mammary epithelia on mammary gland development and polyomavirus middle T antigen induced-tumor formation and metastasis. *Cancer Res*. 2005; 65(6):2296–2302. [PubMed: 15781643]
109. Cheng N, Bhowmick NA, Chytil A, Gorska AE, Brown KA, Muraoka R, et al. Loss of TGF-b type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-a, MSP-, and HGF-mediated signaling networks. *Oncogene*. 2005 In Press.
110. Bierie B, Moses HL. Transforming growth factor beta (TGF-beta) and inflammation in cancer. *Cytokine Growth Factor Rev*. 2010; 21(1):49–59. [PubMed: 20018551]
111. Michl P, Ramjaun AR, Pardo OE, Warne PH, Wagner M, Poulsom R, et al. CUTL1 is a target of TGF(beta) signaling that enhances cancer cell motility and invasiveness. *Cancer Cell*. 2005; 7(6): 521–532. [PubMed: 15950902]
112. Ripka S, Konig A, Buchholz M, Wagner M, Sipos B, Kloppel G, et al. WNT5A-target of CUTL1 and potent modulator of tumor cell migration and invasion in pancreatic cancer. *Carcinogenesis*. 2007; 28(6):1178–1187. [PubMed: 17227781]
113. Vogel WF, Aszodi A, Alves F, Pawson T. Discoidin domain receptor 1 tyrosine kinase has an essential role in mammary gland development. *Mol Cell Biol*. 2001; 21(8):2906–2917. [PubMed: 11283268]
114. Faraci-Orf E, McFadden C, Vogel WF. DDR1 signaling is essential to sustain Stat5 function during lactogenesis. *J Cell Biochem*. 2006; 97(1):109–121. [PubMed: 16167341]

Figure 1.

Wnt signaling pathways. Wnt signaling can be broadly divided into two categories: canonical, β -catenin dependent signaling and non-canonical, β -catenin independent signaling. The most well characterized of the non-canonical signals are the Planar Cell Polarity (PCP) pathway and Wnt/Ca + 2 pathway. PCP and Wnt/Ca signaling have been shown to regulate cell migration

Figure 2.

Antagonism of Wnt/β-catenin signaling by TGF-β and Wnt5a. Wnt is a secreted factor that binds to frizzled receptors on basal cells, enabling β-catenin to escape destruction and translocate into the nucleus to associate with the transcription factors, TCF/LEF. Upon association, transcription of genes responsible for proliferation and stem cell maintenance, such as c-myc, tcf4, and axin2, is stimulated. We propose that TGF-β acting through Wnt5a antagonizes Wnt-β-catenin signaling through a yet unknown mechanism thus limiting the stem cell population

Figure 3.

Roles of Wnt5a in tumor metastasis. Wnt5a is a secreted protein, which could act both in autocrine and paracrine ways to affect tumor cells and their microenvironment. Wnt5a may act to inhibit metastasis through increased activity of Ddr1 and inhibition of migration. Wnt5a can also promote metastasis, depending on the context of the microenvironment. In this case tumor cells regulate expression of Wnt5a in tumor associated macrophages. Wnt5a then regulates activities in both the tumor cell and the macrophage resulting in increased invasion. F: Fibroblast, L: Lymphocyte, M: Microphage