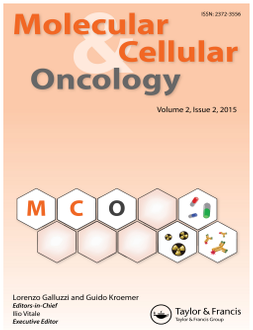

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AUTHOR'S VIEW

Paracrine WNT5A signaling in healthy and neoplastic mammary tissue

David Kusner[a,b,*], Nicholas Borcherding[a,c,d,*], and Weizhou Zhang[a,b,c,d,e]

[a]Department of Pathology, Carver College of Medicine, University of Iowa, Iowa City, USA; [b]Holden Comprehensive Cancer Center, Carver College of Medicine, University of Iowa, Iowa City, USA; [c]Medical Science Training Program, Carver College of Medicine, University of Iowa, Iowa City, USA; [d]Molecular and Cellular Biology Program, Carver College of Medicine, University of Iowa, Iowa City, USA; [e]Immunology Program, Carver College of Medicine, University of Iowa, Iowa City, USA

ABSTRACT

Paracrine signaling between mammary epithelial cells has long been appreciated. Recently, we found that Wnt5a, a novel noncanonical Wnt ligand of luminal origin, counteracts canonical Wnt signaling in basal mammary epithelial cells through a paracrine pathway, inhibits the expansion of Erbb2-induced tumor-initiating cells, and suppresses tumor incidence and metastasis.

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Breast cancer; mammary development; Noncanonical WNT; WNT5A signaling

In cellular biology, one usually looks to understand the inner workings of individual cells, thereby building up to an understanding of tissues, organs, and whole body systems. It is important, however, to recognize that tissues are complex networks of cells with autocrine, paracrine, and endocrine interactions. This is critically important within mammary glands where there are unique populations that interact in developmental and pathophysiologic contexts. Cells within the mammary gland include luminal cells, such as committed luminal progenitors and mature luminal cells, and basal cells, such as bipotent mammary stem cells and myoepithelial cells (Fig. 1). Understanding how these cell populations work together in the normal mammary gland and how their dysregulation leads to tumorigenesis can lead to greater insights into breast cancer.

Adult mammary glands are tightly regulated by 2 ovarian hormones, 17-β-estradiol (E2) and progesterone, via their respective nuclear receptors estrogen receptor α (ERα) and progesterone receptor (PR). These steroid receptors are expressed only in the inner, luminal layer of mammary ducts. Mammary stem cells lack expression of ERα and PR, and thus have to receive paracrine signals from ERα- and/or PR-positive luminal cells for self-renewal and ductal morphogenesis. Using cell surface markers, it has become feasible to separate and purify different mammary epithelial lineages by flow cytometry using protocols developed in 2 independent laboratories.[1,2] Follow-up studies from the same laboratories further found that progesterone promotes self-renewal of mammary stem cells via paracrine signal transduction, by promoting the PR-mediated expression of Wingless (Wnt) ligands and receptor activator for nuclear factor κ-B ligand (Rankl, official name Tumor Necrosis Factor Superfamily, Member 11, TNFSF11).[3,4] Another laboratory confirmed the requirement for Wnt4, but not Rankl, in the progesterone-mediated regeneration capacity of the mammary epithelium.[5] Rankl, on the other hand, is induced by progesterone during pregnancy and leads to the subsequent expansion and differentiation of mammary stem cells into alveolar epithelial cells.[6] All of the factors mentioned above, including progesterone, WNT signaling, and RANKL, are involved in the progression of different steps of human breast cancer. Much less is known about whether paracrine signaling plays important roles in breast cancer.

In addition to these positive regulators for the regeneration of mammary epithelium, negative regulators, such as transforming growth factor β (Tgfβ) and Wnt5a, a noncanonical Wnt ligand, are also reported to inhibit the development of mammary glands.[7,8] Wnt5a has been shown to be a downstream factor of Tgfβ and mediates the suppressive role of Tgfβ in mammary gland growth and morphogenesis.[8] In addition, Tgfβ and Wnt5a are known to suppress tumor initiation and early progression in several models of breast cancer, including the Erbb2-induced model.[9] Less is known, however, about the cell types that produce these negative regulators and their effector cells during mammary gland morphogenesis and breast cancer.

In our recently published paper, we described novel paracrine Wnt5a signaling during Erbb2-induced mammary tumorigenesis.[10] We found that Tgfβ and Wnt5a form a feed-forward activating mechanism whereby Tgfβ induces Wnt5a expression in mammary epithelium, likely from luminal cells. Wnt5a, in turn, leads to the activation of Smad2 in Erbb2-expressing basal mammary epithelial cells (referred to as basal tumor-initiating cells, or basal TIC Fig. 1). We believe that the expansion of basal TICs is critical for Erbb2-induced mammary cancer; this is supported by the fact that heterozygous deletion of *Wnt5A* led to an increased incidence and metastasis of Erbb2-induced murine cancer. In human breast cancer, we found that close to 30% of breast cancer specimens have heterozygous deletions of *WNT5A* alleles, which correlates with decreased *WNT5A* expression and shortened patient survival.

CONTACT Weizhou Zhang weizhou-zhang@uiowa.edu
*These authors equally contributed to this work.

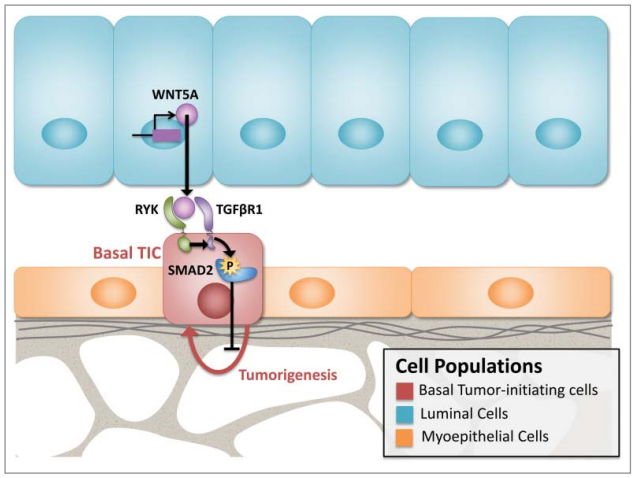

Figure 1. Paracrine Wnt5a signaling from luminal epithelial cells inhibits the expansion of basal tumor-initiating cells. The expression of Wnt5a in luminal cells is likely a result of Tgfβ signaling. Secreted Wnt5a inhibits the expansion of Erbb2-expressing basal tumor-initiating cells by inducing a feed-forward activation of Smad2, in a Ryk- and Tgfβr1-dependent mechanism. Basal TIC, basal tumor-initiating cell.

WNT5A has a number of receptors: receptor tyrosine kinase-like orphan receptor 1 and 2 (ROR1 and ROR2), Frizzled 2 and 4 (FZD2 and FZD4), and receptor-like tyrosine kinase (RYK). Although many of the Wnt5a receptors show differential expression between purified human luminal and basal epithelial cells, *RYK* expression is significantly higher in basal cells compared to luminal cells. Furthermore, we show that WNT5A signals through RYK and TGFβ receptor 1 (TGFβR1) to induce SMAD2 phosphorylation, a transcriptional factor activated by the TGFβ pathway. The TGFβ pathway is known for its role in tumor initiation and early progression, and many cell cycle inhibitors, such as cyclin-dependent kinase inhibitor (CDKN) 2B/p15 and CDKN1A/p21, function downstream of SMAD activation. We found that WNT5A induces formation of a complex between TGFβR1 and RYK, a likely mechanism for SMAD2 phosphorylation and activation. Taken together, these results support the finding that WNT5A, secreted from luminal cells, inhibits basal TICs through RYK, TGFβR1, and SMAD2.

Learning how normal tissues suppress cancer initiation is key to the development of novel targeted therapeutics for cancer prevention and early tumor progression, the most important advancement in reducing cancer death over the last several decades. In our paper, we described how luminal cells within the mammary gland could inhibit the expansion of basal TICs, and thus Erbb2-induced tumorigenesis, through the secretion of Wnt5a (Fig. 1). This paracrine regulation may be important for maintaining normal mammary gland homeostasis, as the loss of *Wnt5a* leads to increased growth and alters the morphogenesis of normal mammary glands.[8] In the future, it will be important to examine the impact of other WNT5A receptors on the development of mammary glands and tumorigenesis. It is likely that WNT5A binds to different receptors with different affinity or availability under pathophysiologic contexts, leading to different, and sometimes opposing, roles during cancer progression. In this regard, several recent papers have recently shown that WNT5A is able to mediate cancer progression via various receptors. Understanding how multiple Wnt5a receptors function in the microenvironment of the mammary duct will shed light on the various roles that they play in cancer progression.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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