

Received: 2016.10.20
Accepted: 2016.12.08
Published: 2016.12.22

Multiple Roles of WNT5A in Breast Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCEF 1 Ruolan Zeng
A 1 Junhui Huang
AD 1 Mei-zuo Zhong
D 1 Li Li
B 2 Guorong Yang
F 3 Li Liu
CF 4 Yin Wu
B 1 Xiaoyi Yao
B 1 Jing Shi
B 1 Zhifu Wu




1 Department of Oncology, Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China
2 Department of Oncology, The First People's Hospital of Chenzhou, University of South China, Chenzhou, Hunan, P.R. China
3 32th Department, Hunan Tumor Hospital, The Affiliated Tumor Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, P.R. China
4 Department of Cardiothoracic Surgery, The Second Xiangya Hospital, Central South University, Changsha, Hunan, P.R. China

Corresponding Author: Junhui Huang, e-mail: 808003@csu.edu.cn
Source of support: Self financing

Breast cancer is one of the most common malignant tumors of women. Modern combinatorial therapeutic regimens can reduce patient tumor burdens to undetectable levels, yet in many cases these tumors will relapse. Understanding of breast cancer biology, developing more potent therapeutic approaches, and overcoming resistance are of great importance. WNT5A is a non-canonical signaling member of the WNT family. Its role in breast cancer still remains unclear. Most of the evidence shows that WNT5A is a suppressor in breast cancer and loss of its expression is associated with poor prognosis, while some evidence suggests the tumorigenicity of WNT5A. WNT signaling molecules are potent targets for treatment of cancer. Therefore, understanding the role of WNT5A in breast cancer may provide new ideas and methods for breast cancer treatment. We review the evidence concerning WNT5A and breast cancer involving the signaling pathways and the molecular-targeted therapy of WNT5A. Our results show that the role WNT5A plays depends on the availability of key receptors and intercellular interactions among different cell types.

MeSH Keywords: **Breast Neoplasms • Molecular Targeted Therapy • Wnt Signaling Pathway**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/902022>

 4224  1  1  63



Background

Breast cancer is one of the most common malignant tumors of women and is the second leading cause of cancer death in women. In the United States, about 231,840 new cases of invasive breast cancer and 40,290 breast cancer deaths are expected to occur in 2015. Breast cancer has become one of the biggest threats to woman's health [1].

WNT genes encode a large family of secreted cysteine-rich glycoproteins with lipid modifications [2]. WNT family proteins (also known as Wingless-related/MMTV-integration family) comprise at least 19 highly conserved members shown to be present in humans and mice [3], and play essential roles in development and homeostasis of the body [4]. WNT signaling can be broadly divided into 2 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 [5]. The human WNT5A gene is located at 3p14.2-p21.1 [6], with over 99% homology at amino acid level to mouse WNT5A [7], and 90% with *Xenopus* WNT5A [8]. This protein maintains certain features: a hydrophobic signal sequence, the WNT-1 family "signature sequence" (CKCHGvSGSC), and a number of other conserved amino acid residues consisting of 24 cysteine residues, 4 asparagine-linked oligosaccharide consensus sequences, and a tyrosine sulfation site [7]. WNT5A plays an important role in developmental processes [9] and is strongly implicated in a number of diseases, including cancer [10,11], diabetes [12], and inflammatory diseases such as atherosclerosis [13] and rheumatoid arthritis [14,15]. Moreover, WNT5A activates the canonical WNT signaling pathway in mouse embryonic stem cells to maintain self-renewal and prevent differentiation [16,17].

Expression and Function of WNT5A in Breast Cancer

WNT5A, which is capable of inhibiting ductal extension and lateral branching in the mammary gland, is required for proper mammary gland development [18]. However, its role in breast cancer is still unclear.

Decreased WNT5A expression is associated with tumorigenesis and enhanced cell motility and is a poor prognostic factor

By testing on cell lines established from normal breast duct luminal epithelium, benign epithelial proliferation, and *in situ* or invasive components of breast epithelium, Lejeune et al. found that levels of WNT5A expression in a panel of human breast cell lines measured by nuclease protection assays were low. One cell line from luminal epithelium (MTSV1-7) had higher levels than the others. Cell lines established from malignant pleural

effusions and metastasis had lower levels of WNT5A expression than the luminal cell line MTSV1-7 in all but 1 case (BT20) [8].

However, Fernandez-Cobo found that expression of WNT5A in MTSV1-7 is quite low [19]. MacMillan et al. found that WNT5A is expressed at highest levels in 21MT-1 cells (invasive mammary carcinoma) and at lowest levels in 21PT (atypical ductal hyperplasia) and 21NT (ductal carcinoma *in situ*) cells [20].

By analyzing the mRNA levels of WNT5A from 120 malignant breast tumors and 33 normal breast tissues, Leris et al. [21] founded that levels of WNT5A mRNA were lower in tumors than in normal tissue. In patients with ER-negative disease, lower levels of WNT5A are significantly associated with worse clinical outcome. These results are in accordance with those of Trifa et al. in Tunisian patients [22].

It appears that there is a trend for WNT5A mRNA levels to be lower in cancerous tissue and lower still in those showing more aggressive behavior. This fits with the hypothesis that WNT5A is a tumor-suppressor gene in breast cancer.

By detecting WNT5A expression of 90 triple-negative breast cancer specimens in an immunohistochemistry study, Zhong et al. found out that negative WNT5A expression correlated with positive lymph node metastasis, Ki67 proliferation, and significantly worse recurrence-free survival. Multivariate Cox regression analysis revealed that decreased WNT5A expression is an independent prognostic factor for RFS [23].

Moreover, Borchering et al. found that heterozygous loss of WNT5A was correlated with shorter survival of breast cancer patients. In a mouse model of ErbB2-induced breast cancer, WNT5A heterozygosity promoted tumor multiplicity and pulmonary metastasis [24].

The work of Gavin et al. showed that the C57MG mouse mammary epithelial cells were transformed in the presence of ectopic WNT-1 or WNT-2, but not WNT5A or WNT-4, which was endogenously expressed in this cell line [25]. In accordance with the Gavin et al. study, Wong et al. found that WNT-4 and WNT5A failed to induce transformation in C57MG cells [26]. Transfecting C57MG cells with a mammalian expression vector carrying antisense WNT5A results in a cell phenotype that mimics cell transformation by ectopic WNT-1 or WNT-2. Correspondingly, WNT-1-transformed cells are partially reverted by overexpressing sense WNT5A. Together, these results support the proposed hypothesis that WNT5A is a factor inhibiting cell transformation. WNT-1 and WNT-2 transform C57MG cells by down-regulating the endogenous expression of WNT5A. However, overexpressed WNT5A appears to be capable of antagonizing WNT-1 function in C57MG cells [27].

C57MG cells transfected with antisense WNT5A continued to grow after reaching confluence, while soft agar colony formation did not occur. This means that WNT-1-transformed cells are not tumorigenic and do not grow in soft agar [26]. Furthermore, transfecting C57MG cells with neu T, which can down-regulate WNT5A to undetectable levels, formed colonies in soft agar assays [28]. These studies indicate that WNT5A is required for normal growth of C57MG cells and that loss of WNT5A expression leads to cell transformation, loss of contact growth inhibition, and tumorigenesis.

In accordance, the experiment by Prieve [29] supports that gain of function of WNT-1 in C57MG mouse mammary epithelial cells leads to their morphological transformation, while loss of function of WNT5A leads to the same transformation.

Huguet et al. did an experiment to further understand the function of WNT5A, showing that the regulation of WNT5A mRNA expression in the human mammary epithelial cell line HB2 was up-regulated 30-fold at confluence. WNT5A was down-regulated 3-fold by changes in cell shape associated with the transition from growth on a two-dimensional surface (flat cell morphology) to growth in three-dimensional gels (spherical cell morphology). In addition, cytoskeletal disruption with non-toxic doses of colchicine also induced a spherical morphology and brought about a dose-dependent down-regulation of WNT5A. WNT5A was also down-regulated 10-fold during the hepatocyte growth factor-induced branching of HB2 cell aggregates in collagen gels. Together, these results suggest that the down-regulation of WNT5A initiates the branching process. A possible explanation for its up-regulation at confluence is that it may be a mechanism for inhibition of cell migration [30].

In agreement with the above, Jonsson and Andersson showed that overexpression of WNT5A protein enhanced cell-to-collagen binding and abolished hepatocyte growth factor-stimulated migration of HB2 transfectants through collagen matrices. Branch formation was reduced 6 times more effectively in the WNT5A-overexpressing cells than in the antisense cells. The expression of WNT5A protein in MCF-7 cells enhanced contact inhibition at cell confluence and tight association between the cells. Transfecting MCF-7 cells with antisense WNT5A led to cell scattering, impaired cell-collagen interaction, and enhanced cell motility [31].

Furthermore, Medrek et al. showed that WNT5A stimulates human breast epithelial cell-enhanced Ca^{2+} -dependent cell-cell adhesion. The cells with loss of WNT5A protein have a significantly lower level of membrane-associated beta-catenin. Down-regulation of WNT5A expression and subsequent reduction of membrane-associated beta-catenin can therefore contribute to decreased cell-cell adhesion and results in metastasis [32].

By detecting the expression of WNT5A protein in 96 primary invasive breast carcinomas, Jonsson et al. found that loss of WNT5A protein expression in the invasive ductal carcinomas was significantly associated with higher histological grade and absence of estrogen receptors. In addition, they analyzed the expression of WNT5A protein in 83 additional invasive primary ductal carcinomas from patients with a longer follow-up time. WNT5A expression was lost in tumors from 78% of the patients with recurrent disease compared with 35% of the recurrence-free patients, and recurrence-free survival was significantly shorter in the WNT5A -negative group. By multivariate analysis, loss of WNT5A expression proved to be an independent and powerful predictor of recurrence after adjustment for lymph node status and tumor size. The results above show that loss of WNT5A increases the risk of early relapse and death in breast cancer [33].

In brief, down-regulation or even loss of WNT5A expression prompts cell scattering, impaired cell-collagen interaction, enhanced cell motility, promoted the branching process, led to tumorigenesis, and was associated with worse clinical outcome.

Increased WNT5A expression is associated with tumorigenesis, aggressiveness, and immunosuppression

Although much evidence supports the hypothesis that WNT5A is a tumor-suppressing factor in breast cancer, there are some disputes.

Using nuclease protection, Lejeune et al. found that WNT5A was expressed at low levels in human normal breast tissue. Benign proliferations and invasive cancer showed 10-fold and 4-fold, respectively, higher WNT5A mRNA levels than normal breast tissues. Levels in benign tumors were significantly higher than those in normal tissue and malignant tumors. The up-regulation of WNT5A was not due to gene amplification, since Southern blot analysis of the high-expression cases showed no evidence of this [8]. The up-regulation in benign conditions suggests a role in aberrant differentiation or, on the contrary, an adaptive mechanism for inhibition of cell differentiation. WNT5A expression is decreased in breast cancer compared to benign tumors, indicating that loss of WNT5A is important in the progression of events leading to the development of cancer, as reported by Olson et al. [27].

Interestingly, Iozzo et al. [34] found that breast carcinomas showed a 4-fold increase in WNT5A expression, a finding that is in full agreement with the work of Lejeune et al.

In addition, Fernandez-Cobo et al. detected the mRNA levels of WNT5A in normal breast tissue, metastatic tissue, and breast cancer cell lines. In agreement, they found overexpression of WNT5A in metastasis-derived breast cancer cells in comparison with normal tissues and to breast cancer cell lines [19].

Table 1. Different expressions of WNT5A in human breast cancer.

Experimental material	Detection method	Detection substance	WNT5A expression levels	References
Human breast cell lines	Nuclease protection assays	mRNA	low	Lejeune et al. [8]
Human normal breast tissue, Benign proliferations and invasive cancer tissue	Nuclease protection	mRNA	invasive cancer tissue is higher than normal breast tissues but lower than benign proliferations	Lejeune et al. [8] Fernandez-Cobo et al. [19] Iozzo et al. [34]
Human normal breast tissues and malignant breast tumor tissues	Real-time PCR	mRNA	lower in tumors than in normal tissue	Leris et al. [21] Trifa et al. [22]
Human breast cell lines	Western Blot	Protein	cells from invasive mammary carcinoma is higher than hyperplasia and carcinoma <i>in situ</i>	MacMillan et al. [20]
Human triple-negative breast cancer specimens	Immunohistochemistry	Protein	negative WNT5A expression correlated with positive lymph node metastasis, Ki67 proliferation and shorter survival	Zhong et al. [23] Borcherding et al. [24]
Human primary invasive breast carcinoma tissues	Immunohistochemistry	Protein	loss of WNT5A associates with early relapse and death	Jonsson et al. [33]
Human breast cancer biopsies obtained pre- and post-chemotherapy	Immunohistochemistry	Protein	WNT5A was found significantly overexpressed in breast cancer biopsies after chemotherapeutic treatment than before	Hung et al. [44]

Bergenfelz et al. found that WNT5A induces CD163(+) immunosuppressive macrophages. The suppressive phenotype induced by WNT5A is associated with induction of IL-10 and inhibition of classical TLR4-NF-kappaB signaling [35]. Moreover, they found that WNT5A is expressed at a higher level in human monocyte-derived myeloid dendritic cells (Mo-mDCs) than in monocytes and macrophages, and it inhibits cell generation by stimulating human breast cancer cells producing IL-6 [36].

WNT5A regulates breast cancer cell proliferation, migration, metastasis, and cancer stem cell (CSC)-like properties

Down-regulating WNT5A promotes proliferation of breast cancer cells [28]. WNT5A also enhances cell-to-collagen binding and tight association between the cells in MCF-7 breast cancer cells [31] and inhibits breast cancer cell migration [37,38]. On the contrary, some evidence shows the opposite role of WNT5A in regulating cell migration of breast cancer cells. WNT5A was reported to inhibit stress fiber formation and promote migration of MDA-MB-231 breast cancer cells [39]. Another study found that WNT5A promotes breast cancer cell migration in MCF-7 breast cancer cells [40].

Existing evidence concerning breast cancer metastasis is also unclear, showing that WNT5A either inhibits or promotes

metastasis [41,42]. Moreover, WNT5A regulated by Twist-BRD4 influences cancer stem cell (CSC)-like properties [43].

WNT5A plays a role in breast cancer drug resistance

WNT5A was found to be significantly overexpressed in breast cancer biopsies after chemotherapeutic treatment. In addition, WNT5A was found to be significantly up-regulated in drug-resistant MCF7/ADR2 cells, consistent with clinical chemoresistance. By being treated with WNT5A antibody, cell viability of the drug-resistant cancer cells was also reduced by doxorubicin treatment [44].

In brief, whether WNT5A is a tumor suppressor or promotor remains uncertain (Table 1). In the next section of this article, by sorting the possible signaling pathways of WNT5A, we show why it plays multiple roles.

Signaling Pathways of WNT5A in Breast Cancer

The process of mammary gland development and breast cancer is complex, and it is likely that coordination of signaling by many factors is involved.

In the well-established “canonical” WNT signaling pathway, WNT binding to Frizzled receptors induces beta-catenin protein stabilization and entry into the nucleus, where it complexes with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to affect the transcription of target genes such as c-myc [45].

WNT5A is a non-canonical signaling member of the WNT family, and a wide variety of hypotheses about how it affects the cellular signal transduction have been published. Here, we review the possible signaling pathways of WNT5A in breast cancer, and try to determine a signaling network.

WNT5A signals through DVL1 to suppress rRNA transcription

WNT5A rapidly represses rDNA gene transcription in breast cancer cells and generates a chromatin state with reduced transcription of rDNA by RNA polymerase I (Pol I). These effects are mediated by Dishevelled1 (DVL1) accumulating in nucleolar organizer regions (NORs) and binding to rDNA regions of the chromosome. Upon DVL1 binding, the Pol I transcription activator and deacetylase Sirtuin 7 (SIRT7) releases from rDNA *loci*, concomitant with disassembly of Pol I transcription machinery at the rDNA promoter. This provides a novel mechanism for how WNT5A signals through DVL1 to suppress rRNA transcription and exerts tumor-suppressive effects [46].

WNT5A mediate effect on DDR1 phosphorylation partly through PI3K and G-proteins

By searching for modified collagen receptors in WNT5A antisense cells, Jonsson and Andersson discovered that the collagen-binding discoidin domain receptor 1 (DDR1) failed to undergo phosphorylation. The DDR1 protein was endogenously expressed in parental HB2 cells. Repression of the WNT5A expression by antisense technology led to significantly lower tyrosine phosphorylation of DDR1 receptors. Furthermore, by detecting in MCF-7, Jonsson and Andersson found that only the WNT5A-expressing MCF-7 cells showed a significant tyrosine phosphorylation of DDR1 receptors in the presence of collagen. These results suggest that the expression of WNT5A is required for the phosphorylation of DDR1 receptors (Figure 1A) [31].

In accordance with the above, Dejmek et al. found that direct mastoparan-induced activation of G-proteins in WNT5A-deficient MCF-7 cells enabled collagen-induced phosphorylation of DDR1 and enhanced their adhesion. Pertussis toxin inhibited the recruitment of the cytoskeletal regulator phosphatidylinositol 3-kinase (PI3K) to DDR1, as well as its phosphorylation. In agreement with this, the PI3K inhibitor wortmannin significantly impaired adhesion of normal WNT5A-expressing HB2 cells but had little effect on adhesion of WNT5A-antisense HB2 cells (Figure 1A) [47].

Two years later, Dejmek et al. found that the expressions of WNT5A and Syk were correlated in 4 of 5 tumor cell lines. However, they found no evidence of a WNT5A/DDR1-mediated activation of Syk. Instead, β_1 -integrins initiate the adhesion-induced activation of Syk. WNT5A/DDR1 and Syk protein lack signaling interaction [48].

WNT5A-induced phosphorylation of DARPP-32 inhibits breast cancer cell migration in a CREB-dependent manner

Acting through autocrine or paracrine, WNT5A ligand activates Frizzled-3 receptors, and then stimulates cAMP formation by adenylyl cyclases (AC) via $G\alpha_s$. cAMP activates PKA, which phosphorylates DARPP-32 at Thr-34 and CREB (a well-known PP1 substrate) at Ser-133. pThr34-DARPP-32 inhibits migration of breast cancer cells. pThr34-DARPP-32 suppresses filopodia formation, in part by reducing CDC42 activity. In addition, pThr34-DARPP-32 inhibits PP1, which in turn leads to enhanced CREB phosphorylation. CREB mediates transcription of genes that also regulate cell migration by an as yet unidentified mechanism (Figure 1B) [37].

WNT5A induces ROR1-dependent signaling and enhances cell growth

Receptor-tyrosine-kinase-like orphan receptor 1 (ROR1), which is expressed during embryogenesis and in the neoplastic cells of human breast cancers, is a receptor of WNT5A. Treatment of MDA-MB-231 cells with recombinant WNT5A enhanced the phosphorylation of AKT and cAMP-response-element-binding protein (CREB), and induced higher expression of CREB-target genes, but not in cells silenced for ROR1. ROR1 interacts with CK1 ϵ to activate PI3K/AKT, leading to activation of CREB and consequently regulating tumor cell growth (Figure 1C) [49].

Luminal cell-produced WNT5A induces a feed-forward loop to limit the expansion of basal TIC

Borcherding et al. found out that luminal cell-produced WNT5A induced a feed-forward loop to activate SMAD2 in a RYK- and TGF β 1-dependent manner to limit the expansion of basal tumor-initiating cells (TIC) in a paracrine fashion (Figure 1D) [50].

A non-canonical WNT5A-Frizzled2 pathway regulates EMT and metastasis

Gujral et al. found that the WNT receptor Frizzled2 (Fzd2) and its ligands WNT5A are elevated in metastatic breast cancer cell lines and in high-grade tumors, and that their expression correlates with markers of epithelial-mesenchymal transition (EMT). Fzd2 drives EMT and cell migration through a previously unrecognized, non-canonical pathway that includes Fyn and Stat3 (Figure 1E) [41].

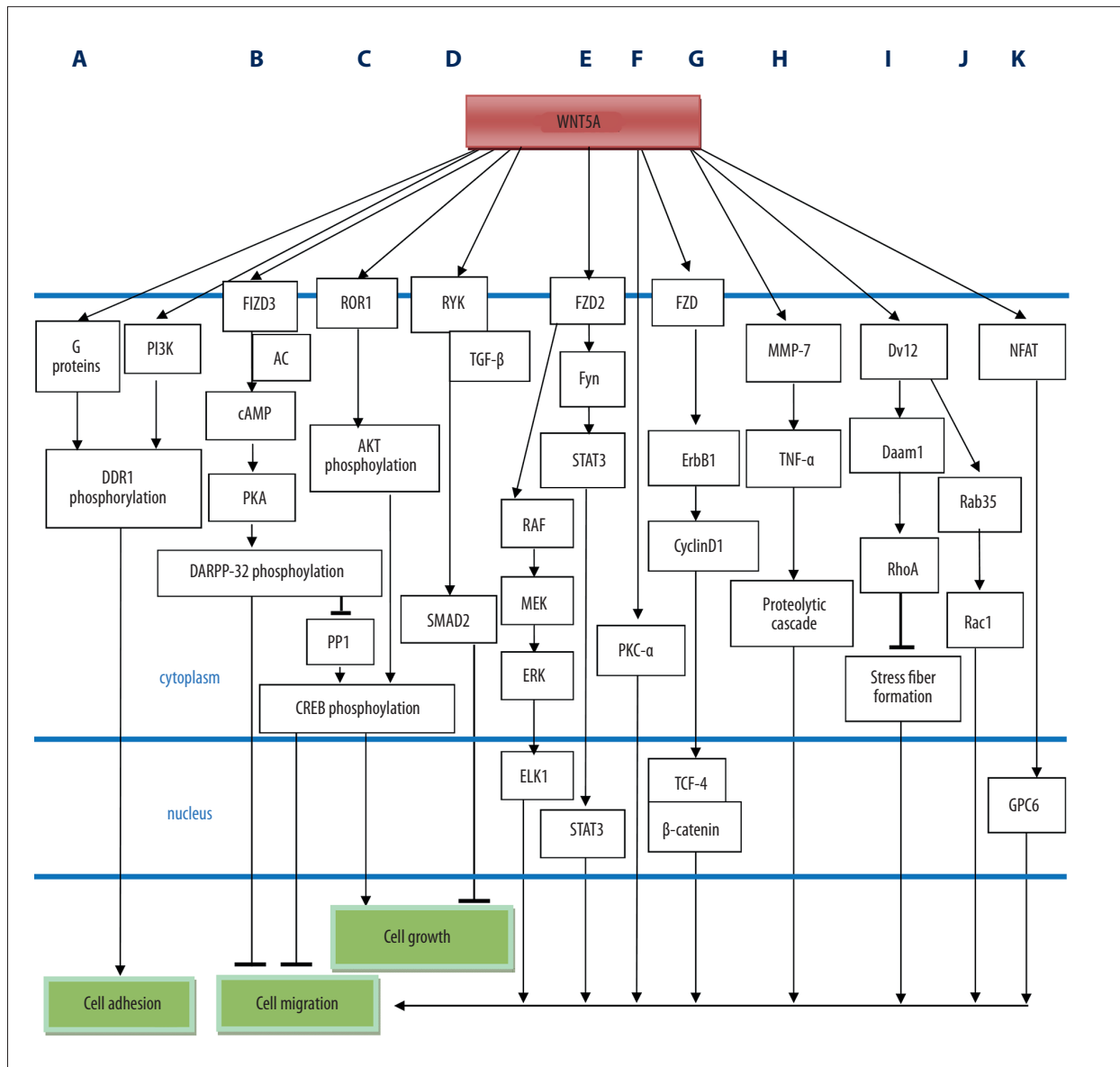


Figure 1. Signaling pathways of WNT5A in breast cancer.

WNT5A, along with the enhanced expression of PKC-alpha, promotes cell migration

Kim et al. found that short-term exposure (3 days) of MCF-7 cells to TPA (an activator of PKC) exhibited significant induction of WNT5A expression, along with the enhanced expression of PKC-alpha, to promote cell migration (Figure 1F) [51].

WNT5A activates the classic β -catenin/TCF pathway by inducing cyclin D1 expression through ErbB1 transactivation

Civenni et al. transfected HC11 mammary epithelial cells with vectors conferring puromycin resistance and containing a TCF

luciferase reporter (TopTK). After antibiotic selection, cell lines were divided into WNT1-HC11, WNT5A-HC11, and C-HC11 (control) groups. In comparison to C-HC11 cells, cytosolic fractions from WNT1-HC11 and WNT5A-HC11 cells contained high levels of β -catenin and display enhanced Top-TK luciferase activity. Thus, in HC11 cells, both WNT1 and WNT5A activate the classic β -catenin/TCF pathway. In mammary cells, WNT binds to its receptor, Frizzled (Fz), and then transactivates ErbB1. PKI166, an ErbB1-selective kinase inhibitor, reduced cyclin D1 to control levels in WNT1-HC11 and WNT5A-HC11 cells, suggesting that ErbB1 is responsible for the increased levels of cyclin D1 present in the WNT-expressing HC11 cells (Figure 1G) [52].

WNT5A triggers production of MMP-7 and TNF- α by macrophages, and then induces invasiveness

Pukrop et al. co-cultured MCF-7 cells and macrophages, which led to up-regulation of WNT5A in the latter. Recombinant WNT5A mimicked the co-culture effect. WNT5A was also detectable in tumor-associated macrophages in primary breast cancers and lymph node metastases. DKK-1 (a member of the dickkopf family) inhibits co-culture- and WNT5A-mediated induction of matrix metalloproteinase-7 (MMP-7) as well as production of TNF- α in macrophages.

They hypothesized that co-culture leads to up-regulation of macrophage-WNT5A, which, in turn, induces MMP-7. MMP-7 then releases TNF- α , which is followed by activation of the proteolytic cascade (Figure 1H) [53].

Dvl2-dependent activation of Daam1 and RhoA regulates WNT5A-induced breast cancer cell migration

WNT5A triggers the Dishevelled (Dvl) and Dishevelled-associated activator of morphogenesis 1 (Daam1) pathway, which regulates cellular polarity during development and tissue homeostasis. The experiment by Zhu et al. showed that WNT5A activated Dvl2, subsequent Daam1 and RhoA, inhibited stress fiber formation, and promoted migration of MDA-MB-231 breast cancer cells (Figure 1I) [39].

WNT5A promotes breast cancer cell migration via the Dvl2/Rab35/Rac1 signaling pathway

RhoA activation was not enhanced by WNT5A in breast cancer cells MCF-7. WNT5A dose-dependently activates Dvl2, Rab35, and Rac1 and subsequently promotes the migration of MCF-7 cells. Additionally, by blockade with WNT5A siRNA, Dvl2 siRNA, Rab35 shRNA, and Rac1 siRNA, WNT5A promotes breast cancer cell migration via the Dvl2/Rab35/Rac1 signaling pathway (Figure 1J) [40].

WNT5A/Ca²⁺-induced NFAT activity promotes breast carcinoma invasion through induction of GPC6

NFAT (nuclear factor of activated T-cells), a transcription factor involved in breast cancer metastasis, is activated by WNT5A through a Ca²⁺ signaling pathway in human breast epithelial cells. Simultaneously, this activation was counteracted by a WNT5A-induced Yes/Cdc42 signaling pathway. The inhibition of the WNT5A/Yes/Cdc42 signal prolonged the duration of ionomycin-induced NFAT1 activation. Therefore, WNT5A/Ca²⁺-induced NFAT activity is hard to detect [54].

NFAT promotes breast carcinoma invasion through induction of GPC (glypican) 6, a cell-surface glycoprotein. Silencing GPC6

with shRNA (small-hairpin RNA) markedly blocks the migration effect (Figure 1K) [55].

Twist recruits BRD4 to direct WNT5A expression in basal-like breast cancer

The diacetylated Twist binds the second bromodomain of BRD4, whose first bromodomain interacts with acetylated H4, thereby constructing an activated Twist/BRD4/P-TEFb/RNA-Pol II complex at the WNT5A promoter and enhancer. Pharmacologic inhibition of the Twist-BRD4 association reduced WNT5A expression and suppressed invasion, cancer stem cell (CSC)-like properties, and tumorigenicity of basal-like breast cancer cells [43].

WNT5A signaling impairs breast cancer cell migration and invasion via decreased CD44 expression

WNT5A-expressing cells demonstrated reduced *in vitro* migration and *in vivo* metastasis relative to controls. WNT5A signaling impaired CD44 expression and its downstream signaling via AKT in breast cancer cells. Moreover, knocking down CD44, an adhesion molecule, in breast cancer cells using siRNA impaired cell migration and invasion [38,42].

PKC upregulates WNT5A expression and causes changes in cell morphology

Calphostin C, which selectively blocks protein kinase C (PKC), markedly reduces the level of WNT5A expression in HB2, a normal breast epithelial cell line. Protein kinase C (PKC), which is activated by phorbol 12-myristate 13-acetate, up-regulated WNT5A, partly through prolongation of WNT5A mRNA half-life. Cytoskeleton reorganization following cytochalasin D treatment causes an induction of WNT5A in HB2, which is associated with changes in cell morphology. Calphostin C does not block these effects. However, cancer cell lines treated with cytochalasin D showed no changes in cell morphology or WNT5A induction. Elevation of WNT5A in HB2 is linearly correlated with cell density, but this does not occur in cancer cell lines [56].

c-Ha-ras is an upstream inhibitory regulator of WNT5A

Expression of WNT5A is down-regulated by extracellular matrix and mutated c-Ha-ras in the human mammary epithelial cell line MCF-10A. In the presence of activated c-Ha-ras, the level of WNT5A mRNA expression is markedly decreased and cell growth rate is elevated. When treated with p21ras inhibitor (BZA-5B), the abnormal cell growth induced by activated H-ras is reversed. In addition, there was a moderate increase in the WNT5A mRNA level in BZA-5B treated MCF-10A cells compared to untreated cells [57].

HuR inhibits the translation of WNT5A mRNA

Leandersson et al. proposed that the lack of WNT5A expression in invasive human breast tumors was caused by HuR-mediated suppression of WNT5A mRNA translation. They found that WNT5A was regulated at the post-transcriptional level. This regulation was mediated by the Embryonic Lethal Abnormal Vision (ELAV)-like protein HuR, which inhibited translation of WNT5A mRNA when bound to highly conserved AU-rich sequences in the 3'-untranslated region (3'-UTR) of the WNT5A mRNA molecule, as shown by both HA-tagged WNT5A- and Luciferase-WNT5A-3'-UTR reporter assays. The HuR-dependent inhibition of WNT5A was supported by the fact that the active HuR is located in the cytoplasm in invasive human breast tumors and that hypoxia-induced activation of HuR inhibits translation of both Luciferase- WNT5A -3'-UTR and endogenous WNT5A [58].

WNT5A as an effector of TGF- β in mammary development and cancer

TGF-beta is a pleiotropic cytokine that regulates all phases of postnatal mammary gland development, including branching morphogenesis, lactation, and involution [59]. TGF- β s involve 3 isoforms that have been detected in the terminal end buds: TGF- β 1, TGF- β 2, and TGF- β 3. 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 3 isoforms of TGF- β is dramatically reduced during lactation. TGF- β 1 expression then rises during involution [60].

WNT5A is required for TGF- β -mediated effects on mammary development. TGF-beta and WNT5A regulate WNT/beta-catenin activity. Loss of TGF-beta or WNT5A signaling resulted in stabilization of nuclear beta-catenin and expression of WNT/beta-catenin target genes, which resemble tumors induced by activation of WNT/beta-catenin [61].

WNT5A-CKI α signaling promotes β -catenin/E-cadherin complex formation

WNT5A/casein kinase I α (CKI α)-specific Ser-45 phosphorylation of β -catenin is associated with increased complex formation of β -catenin/E-cadherin. Mutation of Ser-45 decreases the β -catenin/E-cadherin association. Also, the inhibitory effect of WNT5A on breast epithelial cell invasion is reduced upon mutation of beta-catenin-Ser-45 [32].

Taken together, we can explain why WNT5A plays multiple roles, in which the role WNT5A plays depends on the availability of key receptors. The function of WNT5A either as a suppressor or as a promoter of malignant progression is determined not only by intracellular conditions, but also by intercellular interactions among different cell types.

WNT5A-Relevant Molecular-Targeted Therapy

An antibody against WNT5A reversed chemoresistance of the breast cancer cell lines

WNT5A was found to be significantly up-regulated in drug-resistant MCF7/ADR2 cells. Cell viability of the drug-resistant cancer cells was reduced by treatment with WNT5A antibody [62].

An antibody against WNT5A receptor inhibited the migration and invasion of the breast cancer cell lines

As mentioned above, Gujral et al. found that WNT5A- Frizzled2 (Fzd2) drives EMT and breast cancer cell migration through a previously unrecognized, non-canonical pathway that includes Fyn and Stat3. They developed an antibody to Fzd2 that reduces cell migration and invasion and inhibits tumor growth and metastasis in xenografts. They proposed that targeting this pathway could benefit patients with tumors expressing high levels of Fzd2 and WNT5A [45].

Mohammadi et al. designed a specific single-chain variable fragment (scFv) against the WNT5A binding site of the MUC18 receptor. The antibody inhibited the migration and invasion of a MUC18-positive cell line. The results suggest the specific anti-MUC18 scFv as an effective antibody for breast cancer immunotherapy [63].

There has been less research on WNT5A-relevant molecular-targeted therapy, perhaps because, in some ways, WNT5A plays multiple roles in breast cancer, and the role depends on the availability of key receptors. We preliminarily conclude that it is necessary to detect the WNT5A expression levels and the receptor type of breast cancer tissues before any WNT5A-relevant molecular-targeted therapy is designed and performed.

Conclusions

WNT5A is the first member of the WNT family to be demonstrated to be overexpressed in human breast cancer. However, its role in breast cancer still remains unclear. Most of the evidence shows that WNT5A is a suppressor in breast cancer and loss of its expression is associated with poor prognosis, while some evidence suggests the tumorigenicity of WNT5A. The role WNT5A plays depends on the availability of key receptors and intercellular interactions among different cell types.

The signaling mechanism of WNT5A in breast cancer has not been fully established. We have used the existing evidence to determine a signaling network. WNT5A-related molecular-targeted therapy is still in its infancy. Protracted and unremitting efforts are required to improve therapeutic efficacy and prolong the survival of breast cancer patients.

Disclosure of conflict of interest

None.

References:

- DeSantis CE, Fedewa SA, Goding SA et al: Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. *Cancer J Clin*, 2016; 66(1): 31–42
- Wodarz A, Nusse R: Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol*, 1998; 14: 49–88
- Kikuchi A, Yamamoto H, Sato A: Selective activation mechanisms of Wnt signaling pathways. *Trends Cell Biol*, 2009; 19(3): 119–29
- Agholme F, Aspenberg P: Wnt signaling and orthopedics, an overview. *Acta Orthop*, 2011; 82(2): 125–30
- Serra R, Easter SL, Jiang W, Baxley SE: WNT5A as an effector of TGFbeta in mammary development and cancer. *J Mammary Gland Biol Neoplasia*, 2011; 16(2): 157–67
- Danielson KG, Pillarsetti J, Cohen IR et al: Characterization of the complete genomic structure of the human WNT-5A gene, functional analysis of its promoter, chromosomal mapping, and expression in early human embryogenesis. *J Biol Chem*, 1995; 270(52): 31225–34
- Clark CC, Cohen I, Eichstetter I et al: Molecular cloning of the human proto-oncogene Wnt-5A and mapping of the gene (WNT5A) to chromosome 3p14-p21. *Genomics*, 1993; 18(2): 249–60
- Lejeune S, Huguet EL, Hamby A et al: WNT5A cloning, expression, and up-regulation in human primary breast cancers. *Clin Cancer Res*, 1995; 1(2): 215–22
- Moon RT, Campbell RM, Christian JL et al: Xwnt-5A: A maternal Wnt that affects morphogenetic movements after overexpression in embryos of *Xenopus laevis*. *Development*, 1993; 119(1): 97–111
- Weeraratna AT, Jiang Y, Hostetter G et al: Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell*, 2002; 1(3): 279–88
- Kurayoshi M, Oue N, Yamamoto H et al: Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. *Cancer Res*, 2006; 66(21): 10439–48
- Fuster JJ, Zuriaga MA, Ngo DT et al: Noncanonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion. *Diabetes*, 2015; 64(4): 1235–48
- Fujio Y, Matsuda T, Oshima Y et al: Signals through gp130 upregulate Wnt5a and contribute to cell adhesion in cardiac myocytes. *FEBS Lett*, 2004; 573(1–3): 202–6
- Sen M, Lauterbach K, El-Gabalawy H et al: Expression and function of wingless and frizzled homologs in rheumatoid arthritis. *Proc Natl Acad Sci USA*, 2000; 97(6): 2791–96
- Sen M, Chamorro M, Reifert J et al: Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synovioocyte activation. *Arthritis Rheum*, 2001; 44(4): 772–81
- Sato N, Meijer L, Skaltsounis L et al: Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med*, 2004; 10(1): 55–63
- Hao J, Li T G, Qi X et al: WNT/beta-catenin pathway up-regulates Stat3 and converges on LIF to prevent differentiation of mouse embryonic stem cells. *Dev Biol*, 2006; 290(1): 81–91
- Roarty K, Serra R: Wnt5a is required for proper mammary gland development and TGF-beta-mediated inhibition of ductal growth. *Development*, 2007; 134(21): 3929–39
- Fernandez-Cobo M, Zammarchi F, Mandeli J et al: Expression of WNT5A and Wnt10B in non-immortalized breast cancer cells. *Oncol Rep*, 2007; 17(4): 903–7
- MacMillan CD, Leong HS, Dales DW et al: Stage of breast cancer progression influences cellular response to activation of the WNT/planar cell polarity pathway. *Sci Rep*, 2014; 4: 6315
- Leris AC, Roberts TR, Jiang WG et al: WNT5A expression in human breast cancer. *Anticancer Res*, 2005; 25(2A): 731–34
- Trifa F, Karray-Chouayekh S, Jmal E et al: Loss of WIF-1 and Wnt5a expression is related to aggressiveness of sporadic breast cancer in Tunisian patients. *Tumour Biol*, 2013; 34(3): 1625–33
- Zhong Z, Shan M, Wang J et al: Decreased Wnt5a expression is a poor prognostic factor in triple-negative breast cancer. *Med Sci Monit*, 2016; 22: 1–7
- Borcherding N, Kusner D, Kolb R et al: Paracrine WNT5A signaling inhibits expansion of tumor-initiating cells. *Cancer Res*, 2015; 75(10): 1972–82
- 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): 2418–23
- Wong GT, Gavin BJ, McMahon AP: Differential transformation of mammary epithelial cells by Wnt genes. *Mol Cell Biol*, 1994; 14(9): 6278–86
- Olson DJ, Gibo DM: Antisense wnt-5a mimics wnt-1-mediated C57MG mammary epithelial cell transformation. *Exp Cell Res*, 1998; 241(1): 134–41
- Olson DJ, Papkoff J: Regulated expression of Wnt family members during proliferation of C57mg mammary cells. *Cell Growth Differ*, 1994; 5(2): 197–206
- Prieve MG, Moon RT: Stromelysin-1 and mesothelin are differentially regulated by Wnt-5a and Wnt-1 in C57mg mouse mammary epithelial cells. *BMC Dev Biol*, 2003; 3: 2
- Huguet EL, Smith K, Bicknell R, Harris AL: Regulation of WNT5AmRNA expression in human mammary epithelial cells by cell shape, confluence, and hepatocyte growth factor. *J Biol Chem*, 1995; 270(21): 12851–56
- 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): 2043–53
- Medrek C, Landberg G, Andersson T, Leandersson K: Wnt-5a-CKI{alpha} signaling promotes {beta}-catenin/E-cadherin complex formation and intercellular adhesion in human breast epithelial cells. *J Biol Chem*, 2009; 284(16): 10968–79
- Jonsson M, Dejmeck 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): 409–16
- Iozzo RV, Eichstetter I, Danielson KG: Aberrant expression of the growth factor Wnt-5A in human malignancy. *Cancer Res*, 1995; 55(16): 3495–99
- Bergenzel C, Medrek C, Ekstrom E et al: Wnt5a induces a tolerogenic phenotype of macrophages in sepsis and breast cancer patients. *J Immunol*, 2012; 188(11): 5448–58
- Bergenzel C, Janols H, Wullt M et al: Wnt5a inhibits human monocyte-derived myeloid dendritic cell generation. *Scand J Immunol*, 2013; 78(2): 194–204
- Hansen C, Howlin J, Tengholm A 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): 27533–43
- Prasad CP, Chaurasiya SK, Guilmain W et al: WNT5A signaling impairs breast cancer cell migration and invasion via mechanisms independent of the epithelial-mesenchymal transition. *J Exp Clin Cancer Res*, 2016; 35(1): 144
- Zhu Y, Tian Y, Du J et al: Dvl2-dependent activation of Daam1 and RhoA regulates Wnt5a-induced breast cancer cell migration. *PLoS One*, 2012; 7(5): e37823
- Zhu Y, Shen T, Liu J et al: Rab35 is required for Wnt5a/Dvl2-induced Rac1 activation and cell migration in MCF-7 breast cancer cells. *Cell Signal*, 2013; 25(5): 1075–85
- Gujral TS, Chan M, Peshkin L et al: A noncanonical Frizzled2 pathway regulates epithelial-mesenchymal transition and metastasis. *Cell*, 2014; 159(4): 844–56
- Jiang W, Crossman DK, Mitchell EH et al: WNT5A inhibits metastasis and alters splicing of Cd44 in breast cancer cells. *PLoS One*, 2013; 8(3): e58329
- Shi J, Wang Y, Zeng L et al: Disrupting the interaction of BRD4 with diacetylated Twist suppresses tumorigenesis in basal-like breast cancer. *Cancer Cell*, 2014; 25(2): 210–25

44. Hung TH, Hsu SC, Cheng CY et al: Wnt5a regulates ABCB1 expression in multidrug-resistant cancer cells through activation of the non-canonical PKA/beta-catenin pathway. *Oncotarget*, 2014; 5(23): 12273–90
45. Mikels AJ, Nusse R: Purified WNT5A protein activates or inhibits beta-catenin-TCF signaling depending on receptor context. *PLoS Biol*, 2006; 4(4): e115
46. Dass RA, Sarshad AA, Carson BB et al: Wnt5a Signals through DVL1 to repress ribosomal DNA transcription by RNA polymerase I. *PLoS Genet*, 2016; 12(8): e1006217
47. 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): 344–51
48. Dejmek J, Leandersson K, Manjer J 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 Pt 1): 520–28
49. Zhang S, Chen L, Cui B et al: ROR1 is expressed in human breast cancer and associated with enhanced tumor-cell growth. *PLoS One*, 2012; 7(3): e31127
50. Borcherding N, Kusner D, Kolb R et al: Paracrine WNT5A signaling inhibits expansion of tumor-initiating cells. *Cancer Res*, 2015; 75(10): 1972–82
51. Kim S, Chun SY, Kwon YS et al: Crosstalk between Wnt signaling and Phorbol ester-mediated PKC signaling in MCF-7 human breast cancer cells. *Biomed Pharmacother*, 2016; 77: 114–19
52. Civenni G, Holbro T, Hynes NE: Wnt1 and WNT5A induce cyclin D1 expression through ErbB1 transactivation in HC11 mammary epithelial cells. *EMBO Rep*, 2003; 4(2): 166–71
53. Pukrop T, Klemm F, Hagemann T et al: Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA*, 2006; 103(14): 5454–59
54. Dejmek J, Safholm A, Kamp NC et al: Wnt-5a/Ca2+-induced NFAT activity is counteracted by Wnt-5a/Yes-Cdc42-casein kinase 1alpha signaling in human mammary epithelial cells. *Mol Cell Biol*, 2006; 26(16): 6024–36
55. Yiu GK, Kaunisto A, Chin YR, Toker A: NFAT promotes carcinoma invasive migration through glypican-6. *Biochem J*, 2011; 440(1): 157–66
56. Jonsson M, Smith K, Harris AL: Regulation of WNT5A expression in human mammary cells by protein kinase C activity and the cytoskeleton. *Br J Cancer*, 1998; 78(4): 430–38
57. Bui TD, Tortora G, Ciardiello F, Harris AL: Expression of WNT5As 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): 911–17
58. Leandersson K, Riesbeck K, Andersson T: Wnt-5a mRNA translation is suppressed by the Elav-like protein HuR in human breast epithelial cells. *Nucleic Acids Res*, 2006; 34(14): 3988–99
59. Parvani JG, Taylor MA, Schiemann WP: Noncanonical TGF-beta signaling during mammary tumorigenesis. *J Mammary Gland Biol Neoplasia*, 2011; 16(2): 127–46
60. Nguyen AV, Pollard JW: Transforming growth factor beta3 induces cell death during the first stage of mammary gland involution. *Development*, 2000; 127(14): 3107–18
61. Roarty K, Baxley SE, Crowley MR et al: 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
62. Hung TH, Hsu SC, Cheng CY et al: WNT5A regulates ABCB1 expression in multidrug-resistant cancer cells through activation of the non-canonical PKA/beta-catenin pathway. *Oncotarget*, 2014; 5(23): 12273–90
63. Mohammadi M, Nejatollahi F, Ghasemi Y et al: Anti-metastatic and anti-invasion effects of a specific anti-MUC18 scFv antibody on breast cancer cells. *Appl Biochem Biotechnol*, 2016 [Epub ahead of print]