

UNIVERSIDADE ESTADUAL DE CAMPINAS  
SISTEMA DE BIBLIOTECAS DA UNICAMP  
REPOSITÓRIO DA PRODUÇÃO CIENTÍFICA E INTELLECTUAL DA UNICAMP

**Versão do arquivo anexado / Version of attached file:**

Versão do Editor / Published Version

**Mais informações no site da editora / Further information on publisher's website:**

<https://www.sciencedirect.com/science/article/pii/S075333222200988X>

**DOI: 10.1016/j.biopha.2022.113599**

**Direitos autorais / Publisher's copyright statement:**

©2022 by Elsevier. All rights reserved.

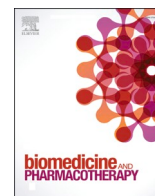
DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

<http://www.repositorio.unicamp.br>



## Review

## WNT5A in tumor development and progression: A comprehensive review

Maura Lima Pereira Bueno<sup>\*</sup>, Sara Teresinha Olalla Saad, Fernanda Marconi Roversi<sup>\*</sup>

Hematology and Transfusion Medicine Center - University of Campinas/Hemocentro - UNICAMP, Campinas, São Paulo, Brazil

## ARTICLE INFO

## Keywords:

WNT5A  
Tumor suppressor  
Oncogene  
Tumor microenvironment  
Prognostic factor  
Target therapy

## ABSTRACT

The investigation of tumor microenvironment (TME) is essential to better characterize the complex cellular crosstalk and to identify important immunological phenotypes and biomarkers. The niche is a crucial contributor to neoplasm initiation, maintenance and progression. Therefore, a deeper analysis of tumor surroundings could improve cancer diagnosis, prognosis and assertive treatment. Thus, the WNT family exerts a critical action in tumorigenesis of different types of neoplasms due to dysregulations in the TME. WNT5A, an evolutionary WNT member, is involved in several cellular and physiopathological processes, in addition to tissue homeostasis. The WNT5A protein exerts paradoxical effects while acting as both an oncogene or tumor suppressor by regulating several non-canonical signaling pathways, and consequently interfering in cell growth, cytoskeletal remodeling, migration and invasiveness. This review focuses on a thorough characterization of the role of WNT5A in neoplastic transformation and progression, which may help to understand the prognostic potentiality of WNT5A and its features as a therapeutic target in several cancers. Additionally, we herein summarized novel findings on the mechanisms by which WNT5A might favor tumorigenesis or suppression of cancer progression and discussed the recently developed treatment strategies using WNT5A as a protagonist.

## 1. Background

Signaling of the wingless-related integration site family (WNT) plays a critical role in tumor development and advancement, and is implicated in different neoplasms due to WNT pathway dysregulations [1].

The WNT gene family was first reported by Nusse and Varmus in the description of mouse mammary tumor virus carcinogenesis, in which a carcinogenic proviral insertion at the "integration site", referred to as INT1, was detected [2]. The WNT-family secreted lipid-modified glycoproteins comprise 350–400 cysteine-rich amino acids and its signaling depends on two different pathways, namely the canonical and the non-canonical pathways. In the canonical pathway, an accumulation of stable  $\beta$ -catenin occurs, caused by the binding of WNT proteins to the transmembrane receptor Frizzled (FZD). In the non-canonical pathway (WNT/planar cell polarity (PCP), WNT proteins can bind to the FZD receptor, tyrosine kinase-like orphan receptor (ROR) and receptor-like tyrosine kinase (Ryk), transmitting signals to Dishevelled, leading to the activation of downstream target genes or releasing intracellular  $\text{Ca}^{2+}$ , triggering  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase II (CamKII) and protein kinase C (PKC) [3].

At least 19 WNT members have been identified in mammals,

including WNT1, WNT2, WNT2b/13, WNT3, WNT3a, WNT4, WNT5A, WNT5b, WNT6, WNT7a, WNT7b, WNT8a/d, WNT8b, WNT10a, WNT10b/12, WNT11, WNT14, WNT15, and WNT16 [4]. WNT5A, specifically, is an evolutionary conserved WNT protein, involved in several crucial physiopathological processes. Abnormal WNT5A activity encompasses important effects in carcinogenesis. The tumor suppressive action has been observed in cell proliferation, migration and invasion of thyroid and colorectal cancer (CRC) [5] by activating the non-canonical WNT/ $\text{Ca}^{2+}$  pathway through Ryk, Frizzled or ROR receptors and regulating cell migration, fate determination, axon guidance, and contributing to tissue polarity control through the PCP pathway [6].

In different cancer types, WNT5A can either act as a tumor suppressor and an oncogene [7]. In the present review, we delineate the behavior of WNT5A in several cancers based on recent findings in order to evaluate the functional mechanisms highlighting the emerging importance of this protein in neoplasia.

## 2. WNT5A gene and protein

Clark et al. cloned and characterized the first cDNA sequence of WNT5A (GenBank L20861) from human fetal fibroblast samples from

<sup>\*</sup> Correspondence to: Hematology and Transfusion Medicine Center, Unicamp, Rua Carlos Chagas, 480, Barão Geraldo, Campinas, São Paulo CEP: 13083-878, Brazil.

E-mail addresses: [mauralimabueno@gmail.com](mailto:mauralimabueno@gmail.com) (M.L.P. Bueno), [fermr@unicamp.br](mailto:fermr@unicamp.br) (F.M. Roversi).

<https://doi.org/10.1016/j.bioph.2022.113599>

Received 8 July 2022; Received in revised form 18 August 2022; Accepted 21 August 2022

Available online 9 September 2022

0753-3322/© 2022 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

patients with perinatal lethal osteogenesis imperfecta [8]. The entire *WNT5A* gene has approximately 4.1 kb (start: 55,465,715 bp, end: 55,487,306 bp; orientation: Plus strand), it is located at 3p14.2-p21.1 and contains 5 exons. The mature form of human *WNT5A* has over 93 % of homology compared to sequences of other *WNT5A* proteins and is over 99% homologous to murine *WNT5A* [9].

In 2013, Bauer et al. described the existence of two *WNT5A* protein isoforms, a short isoform (*WNT5A-S*), which generates a 319 amino acid protein, and a long isoform (*WNT5A-L*), which generates a 337 amino acid protein [10]. Both isoforms are products of alternative transcription of the *WNT5A* gene, positioned at chromosome 3p14-p21 with their promoters, namely promoters A and B show comparable transcriptional potential and could exert different signaling activities, depending on the cell type. *WNT5A-S* and *WNT5A-L* proteins carry lipid-modified and glycosylated characteristics with an N-terminal hydrophobic signal. For maturation, cleavage of the N-terminal signal sequence of *WNT5A-S* occurs at the 46th amino acid, and *WNT5A-L* at the 43rd, originating the mature these isoforms [11] (Fig. 1).

### 3. Biological functions of *WNT5A* in cancer

*WNT5A* has been a subject of interest in various tumor types regarding its abnormal modulation. This member of the *WNT* family has been described as playing a key role in carcinogenesis, with oncogenic or tumor suppressive actions, that promote several biological alterations, such as reactive oxygen species (ROS) production, proliferation, differentiation, autophagy, chemotaxis, migration, invasion, and adhesion [12]. Thus, the action of *WNT5A* in various tumors may represent not only a significant indicative to successful therapy but also a possible prognosis marker. In this setting, diverse experimental studies have been performed to better understand *WNT5A* in cancer development, as detailed below (Table 1).

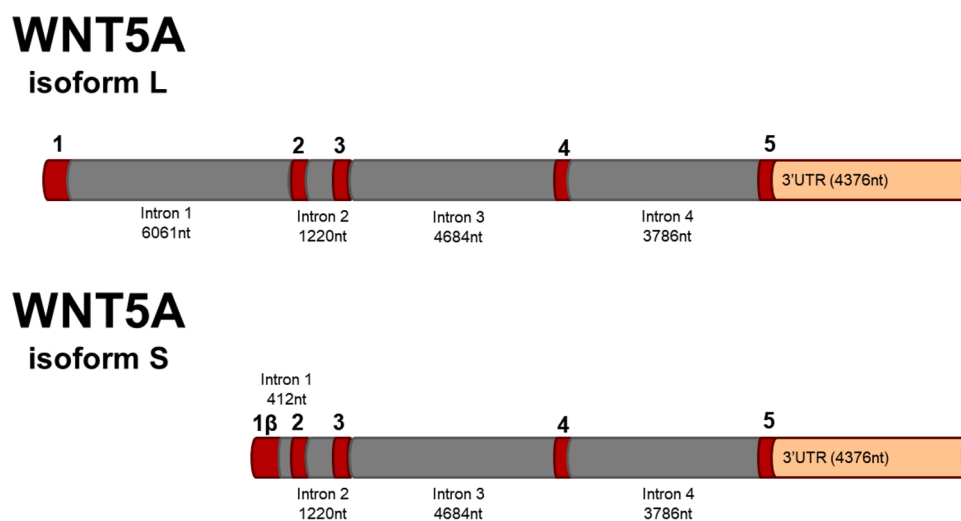
*WNT5A* was associated to cancer progression and development [38, 41] through modulation of several processes. In gastric cancer, the knockdown of *WNT5A* protein using an antibody suppressed cell migration in vitro and invasion and inhibited metastasis in vivo [42]. Similarly, in a specific gastric cell line, MKN-7, *WNT5A* depletion induced downregulation of some genes involved in intracellular signaling (IL1A/B and *WNT4*), chemokine-cytokine interaction (CXCL1/2/6) and focal adhesion (LAMC3 and ITGA2/B2), inhibiting epithelial cell migration. In the colorectal cell line HCT116, endogenous downregulation of the *WNT5A-S* mRNA isoform inhibited growth and induced apoptosis through activation of FASLG and reduction of

TNFRSF11B [26]. In PANC-1 pancreatic cells, after knockdown of *WNT5A* gene, cell cycle modulation was observed [75]. In PC3 prostate cancer cells, silencing of *WNT5A* gene and protein expression by siRNA decreased the migration ability of PC3 cells [81]. In MCF-7 breast cell, the expression of *WNT5A* is related to migration, which is activated or reduced by time-dependent treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent tumor inducer: the short exposure significantly induced *WNT5A* expression to promote cell migration while the chronic exposure suppressed *WNT5A* expression to stimulate cell migration and an EMT [15]. In NSCLC, underexpression of *WNT5A* reduced clone formation, migration, invasion and metastatic potential through modulating epithelial-to-mesenchymal transition process [58] and was associated with a poor clinical prognosis. Similarly, an in vitro study with EGFR-mutant NSCLC cells (HCC827 and H1975) showed that *WNT5A* gene depletion increased cell viability, migration and invasiveness, suggesting that *WNT5A* suppresses cellular proliferation [56]. In a NSCLC transgenic animal model, *WNT5A* knockdown in myeloid-derived suppressor cells (MDSC) led to inhibition of lung metastatic spread [61]. In melanoma cells (HTB63), downregulation of *WNT5A* expression by lentiviral particles impaired the invasive cell capacity [62] and resulted in inhibiting the invasion of melanoma cells [77,85]. In an acute lymphoblastic leukemia cell line (MOLT4), *WNT5A* silencing by lentivirus was able to reduce migration and invasion induced by CCL25 treatment [49]. *WNT5A* can be demonstrated to induce invasion and proliferation of a range of cancer cells types [18], depending on promoter methylation and the receptor activated.

*WNT5A* was further associated with immunomodulatory functions. In melanoma, the *WNT5A* knockdown decreased the immunosuppressive nature of myeloid-derived suppressor cells (MDSC) and regulatory T cell (Treg) as well as reduced the expression of TGFβ1, arginase 1, PD-1 and LAG3 in tumor-infiltrating lymphocytes, suggesting a less exhausted phenotype and an important modulator of melanoma proliferation rate [61]. In colorectal cancer, *WNT5A* led to M2 macrophage polarization of tumor-associated macrophages through activation of the CaMKII-ERK1/2-STAT3 pathway and IL-10 expression, enabling proliferation, migration and invasion and, consequently neoplasm progression [23]. In ovarian cancer cell lineage (SKOV-3), *WNT5A* protein acted as an immunomodulator [71].

#### 3.1. *WNT5A* and signaling pathway

*WNT5A* regulates diverse non-canonical signaling pathways. The binding of *WNT5A* to the Frizzled (FZD) or ROR1/2 receptors can



**Fig. 1. *WNT5A* gene.** Structure of the human *WNT5A-L* and *WNT5A-S* isoforms. Numbered boxes indicate the exons, with coding regions in red and 3' untranslated (UTR) regions in red. The intron regions are in gray. The different transcription initiation sites in exon 1 and exon 1β and splicing of exon 1- and exon 1β-initiated transcripts. nt: nucleotides, kb: kilo base can be verified.

**Table 1**

Summary of WNT5A expression and methylation in cancer.

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Breast cancer	RT-PCR, WB, IHC.	HUVEC.	WNT5A promoted VEGF-independent angiogenesis in HUVEC.	1085 cases of primary tumors. 291 cases of normal tissue samples.	WNT5A was upregulated in breast tumor tissues compared with adjacent non tumor tissues (public database). WNT5A serum levels were higher detected in breast cancer patients and correlated with microvessel density.	[13]
Breast cancer	RT-PCR, WB, IF.	SKBR3, MDA-MD453, HCC1954, and JIMT1.	Recombinant human WNT5A increased invasiveness in breast cancer cells.	None.	None.	[14]
Breast cancer	RT-PCR, IF, FC.	MCF-7.	WNT5A expression was higher after short term 12-O-tetradecanoylphorbol-13-acetate (TPA) and promoted cell migration.	None.	None.	[15]
Breast cancer	IHC.	None.	None.	90 triple-negative breast cancer (TNBC) tissue samples.	Patients with negative WNT5A expression had significantly poorer recurrence-free survival (RFS) compared with patients with positive WNT5A expression.	[16]
Breast cancer	RT-PCR.	None.	None.	120 malignant breast tumors. 33 normal breast tissue samples.	WNT5A mRNA levels were lower in tumors.	[17]
Cervical, Lung, Gastric cancer	RT-PCR, ELISA.	HeLaS3, A549, KKLS, Calu-6, KYSE-70, and TE-11.	WNT5A stimulated invasion and proliferation of cervix, lung, and GC.	None.	None.	[18]
Cervical cancer	RT-PCR, IHC.	None.	None.	8 surgically resected cervical cancer. 8 adjacent normal cervical tissue samples. 94 cervical cancer biopsies	WNT5A was overexpressed in cervical cancer tissue samples compared with adjacent normal cervix	[19]
Colorectal cancer	RT-PCR, WB, IHC.	HT-29, Caco-2, and MDA-MB-468.	WNT5A mimicking peptide increased 15-PGDH, JNK and cyclin-D1 expression in CRC cell lines and led to decreased tumor volume in the xenographic model using MDA-MB-468 cells.	66 CRC patient tissue samples.	WNT5A expression was absent or reduced in approximately 40% of the primary tumor samples from CRC patients	[20]
Colorectal cancer	RT-PCR, IF, ChIP.	SW620, and SW480.	WNT5A expression was decreased in a highly metastatic human colon cancer cell line compared with the non-metastatic human colon cancer cell.	None.	None.	[21]
Colorectal cancer	IHC.	None.	None.	217 CRC patient tissue samples.	WNT5A was diminished in the majority of the primary colon cancers and negatively related with EMT biomarkers.	[22]
Colorectal cancer	RT-PCR, IHC, IF, FC, ELISA.	SW480, HT29, HCT116, DLD-1, and THP-1.	WNT5A induced M2 macrophage polarization via IL-10 and promoted CRC proliferation, migration and invasion.	63 CRC patient tissue samples. 20 normal tissue samples.	WNT5A expression was markedly elevated in CRC compared with normal tissue. WNT5A+ Tumor-associated macrophages expression was significantly associated with poor recurrence-free survival and overall survival.	[23]
Colorectal cancer	MSP, bisulfite genomic sequencing, RT-PCR, WB.	HCT116, HT29, SW620, HCT8, LoVo, SW480, and Rko.	WNT5A was silenced by methylation in CRC cell lines.	126 sporadic adenocarcinomas patient tissue samples.	Patient response to 5-Fluorouracil chemotherapy was associated with WNT5A methylation. Patients with WNT5A methylation were	[24]

(continued on next page)

Table 1 (continued)

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Colorectal cancer	RT-PCR, WB, IHC.	SW620, RKO, Caco-2, HT29, SW480, HCE8693, DLD-1, and Lovo.	TrpC5 was an independent adverse prognostic factor for death in CRC, reducing differentiation through the $Ca^{2+}$ /WNT5A signaling pathway.	None.	associated with longer progression-free survival. None.	[25]
Colorectal cancer	RT-PCR, WB, IHC.	HCT116, CRL-1459, Lovo, SW480, DLD1, HC165, WiDr, and HT-29.	WNT5A-S isoform transcript expression levels were relatively higher in CRC cell lines and specimens. WNT5A-L isoform transcript expression levels were relatively lower in CRC cell lines and specimens.	None.	None.	[26]
Gallbladder squamous/adenosquamous carcinomas and adenocarcinomas.	IHC.	None.	None.	46 SC/ASC patient tissue samples. 80 AC patient tissue samples.	Positive ROR2 and WNT5A expression was significantly lower in SC/ASC or AC. Positive ROR2 and WNT5A expression levels in highly differentiated AC were significantly lower than in poorly differentiated AC. WNT5A mRNA was decreased in endometrial cancer.	[27]
Endometrial cancer	RT-PCR, IHC.	None.	None.	RT-PCR 8 unchanged endometrial control. 28 EC patient tumor samples. IHC 6 unchanged endometrial controls. 19 EC patient tumor samples.	WNT5A immunoreactivity was visualized in the cytoplasm and nuclei of endometrial cancer cells. In normal endometrium, WNT5A protein was expressed in the luminal and glandular epithelial cells.	[28]
Epithelial ovarian cancer	MSP, bisulfite genomic sequencing, RT-PCR, IHC.	HOSE, PEO1, OVCAR3, OVCAR5, OVCAR10, A1847, A2780, UPN289.	WNT5A was expressed at significantly lower levels in human EOC cell lines due to gene promoter hypermethylation.	130 EOC patient tissue samples. 31 normal ovarian surface epithelium or fallopian tube epithelium tissue.	WNT5A was expressed at significantly lower levels in primary human EOCs compared with normal human ovarian surface epithelium or fallopian tube epithelium due to gene promoter hypermethylation and contributed to tumor stage and predicted shorter overall survival.	[29]
Epithelial ovarian cancer	RT-PCR, WB, IHC.	None.	None.	623 EOC patient tissue samples.	WNT5A gene expression was found to be upregulated in serous, endometrioid, clear cell and mucinous of EOC compared to borderline tumors and benign controls. WNT5A protein expression in tissues was located predominantly in the cytoplasm.	[30]
Epithelial ovarian cancer	IHC.	OVCAR3, and SKOV3.	WNT5A enhanced the vasculogenic capacity, the motility and invasiveness of OvCa cells. WNT5A also promoted OvCa EMT and vasculogenic mimicry via the PKC $\alpha$ pathway.	79 EOC patient tissue samples.	WNT5A staining was significantly correlated with metastasis in EOC and associated with vasculogenic mimicry.	[31]
Epstein-Barr virus-associated gastric carcinoma	MSP, bisulfite genomic sequencing, RT-PCR.	GT38, GT39, PT, YCCEL1, AGS, KatoIII, MKN45, SNU1, SNU16, SNU719, YCC1, YCC2, YCC3, YCC6, YCC7, YCC9, YCC11, YCC16,	WNT5A expression was downregulated in EBVaGC cell lines by promoter hypermethylation.	None.	None.	[32]

(continued on next page)

Table 1 (continued)

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Epstein-Barr virus-associated nasopharyngeal carcinoma	RT-PCR, WB, IHC.	SGC-7901, HGC-27, and Akata. NP69, NP460, TW01, TW04, HONE1, SUNE1, HK1, CNE1, CNE2, and C666-1.	WNT5A promoted cell growth, cell migration and invasion of NPC cell lines.	16 snap-frozen nasopharyngeal biopsies. 14 undifferentiated EBER-positive NPC. 2 histologically normal nasopharynx epithelial cells with no evidence of malignancy and EBER-negative.	WNT5A mRNA levels were increased in NPC tissue when compared to two non-malignant controls.	[33]
Nasopharyngeal carcinoma	RT-PCR, IHC.	SUNE-1, CNE-2 and CNE-2 clones (S18, S22, and S26).	WNT5A promoted tumorigenesis, migration, invasion, and metastasis and up-regulated stem-like cell markers in NPC cell lines.	220 nasopharyngeal carcinoma patient tissue samples.	WNT5A expression was increased in metastatic NPC tissues.	[34]
Esophageal Adenocarcinoma	RT-PCR, WB, IHC.	EPC1 (EPC1-hTERT), EPC2 (EPC2-hTERT), CP-A, and OE33.	WNT5A mRNA expression was increased in EPC1 and EPC2 cells compared to Barrett esophagus derived cells. WNT5A expression was reduced and acted as tumor suppressor in OE33 cells.	11 human esophageal biopsies of esophageal adenocarcinoma patients 15 human esophageal biopsies from patients with Barrett esophagus 10 healthy esophageal biopsy samples.	Lower level of WNT5A in esophageal adenocarcinoma samples compared with squamous mucosa, Barrett esophagus biopsy samples and healthy esophageal mucosa	[35]
Esophageal squamous cell carcinoma	MSP, bisulfite genomic sequencing RT-PCR.	Het-1A, NE1, NE3 and NE083.	WNT5A promoter CpG methylation was detected in the downregulated ESCC cell lines. Aza treatment induced WNT5A expression by promoter demethylation.	None.	None.	[36]
Ewing sarcoma	RT-PCR, WB, IHC.	A-673, RD-ES, SK-N-MC, and SK-ES-1.	WNT5A promoted cell migration through JNK and PKC signaling.	15 Ewing sarcoma patient tissue samples.	WNT5A and CXCR4 mRNA levels were significantly higher in samples from ES patients with metastasis compared with samples from ES patients without metastasis.	[37]
Gastric cancer	RT-PCR, WB, IHC, IF.	MKN-28, MGC80-3, AGS, AZ521, and SGC7901.	WNT5A/b-catenin signaling pathway regulated differentiation.	108 gastric carcinoma patient tissue samples.	HEF1 upregulation increased WNT5A expression and the nuclear translocation of $\beta$ -catenin, resulting in poor differentiation in GC.	[38]
Gastric cancer	IHC.	None.	None.	27 GC tissue samples. 26 with early-stage GC tissue samples. 1 GC with <i>H. pylori</i> infection tissue samples. 20 tissue samples without <i>H. pylori</i> .	WNT5A expression was significantly lower in the after the eradication group compared to the <i>H. pylori</i> infection group.	[39]
Gastric cancer	RT-PCR, ELISA.	BGC-803, HGC-27, and MKN-45.	WNT5A stimulated MCP-1 and IL-1 $\beta$ expression in GC cells WNT5A-conditioned medium promoted macrophage chemotaxis and cytoskeletal changes.	50 primary GC specimens with matched adjacent non-malignant tissues.	WNT5A was overexpressed in GC tissues and correlated with monocyte chemotactic protein 1 (MCP-1) and interleukin 1 $\beta$ (IL-1 $\beta$ )	[40]
Gastric cancer	RT-PCR, IHC, Microarray.	MKN-7, MKN-74, HSC-45, HSC-57, and TMK-1.	WNT5A transduction did not increase tumor activity in GC cells.	42 cases of various histological subtypes of human GC tissues surgically removed at Kobe University Hospital (Kobe, Japan)	WNT5A expression was correlated with aggressiveness and poor prognosis of GC. High levels of WNT5A expression were observed in 29 samples (66 %).	[41]
Gastric cancer	Migration and invasion assays.	KKLS, MKN-1, MKN-45, and TMK-1. Six-week-old male BALB/cAnNCrj-nu mic.	WNT5A induces gastric-cancer migration and invasion by binding to their receptors.	None.	None.	[42]
Glioblastoma	ELISA.	U87MG, U251, and T98MG.	WNT5A stimulated GBM cell invasion via Daam1 activation. WNT5A/Daam1/RhoA signaling sustained the formation of stress fibers.	9 GBM patient tissue samples.	WNT5A was upregulated in invasive GBM tissues, stimulating cell invasion.	[43]

(continued on next page)

Table 1 (continued)

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Glioblastoma	IHC.	None.	None.	175 GBM patient tissue samples.	WNT5A protein expression was higher in human GBM. WNT5A was strongly stained in perikarya of neurons with a marginal accentuation in glial cells	[44]
Glioblastoma	IHC.	None.	None.	12 GBM patient tissue samples.	WNT5A protein immunoreactivity was increased in GBM.	[45]
Glioblastoma	IHC.	Astroglial and oligodendroglial lineages.	WNT5A expression was upregulated in astroglial compared to oligodendroglial lineages.	9 grade II glioma patient biopsy samples. 10 grade III glioma patient biopsy samples. 72 grade IV glioma patient biopsy samples.	WNT5A, WNT3a, FZD2 and beta-catenin were increased in GBM.	[46]
Hematological neoplasm/Acute Lymphoblastic Leukemia	MSP, bisulfite genomic sequencing RT-PCR.	Molt4, Jurkat, T-ALL1, and RPMI 8402.	WNT5A was methylated in the cell lines. The treatment with Aza restored WNT5A expression.	86 childhood ALL patient samples. 6 healthy bone marrow of transplantation donors. 10 normal peripheral blood. Healthy and patient thymus tissue samples.	WNT5A expression was downregulated both in B-ALL and T-ALL patients compared with specific control cell populations. WNT5A expression was lower in T-ALL patients compared to B-ALL patients due to promoter methylation.	[47]
Hematological neoplasm/Acute myeloid leukemia (AML)	RT-PCR, WB, pyrophosphate sequencing, ChIP.	None.	None.	95 treatment-naive acute leukemia patients: - 62 AML - 33 ALL (11 T-ALL; 22 B-ALL) 58 bone marrow transplant donors None.	WNT5A, HDPR1, DKK1 and DKK3 expression was lower in bone marrow cells from AML patients compared to normal controls due to promoter methylation.	[48]
Hematological neoplasm/T-cell Acute Lymphoblastic Leukemia	MSP, bisulfite genomic sequencing, RT-PCR, WB.	Molt4.	CCL25 induced WNT5A expression in adult T-ALL cells.	None.	None.	[49]
Hematological neoplasm	FC, WB.	Cells from E17 FL wild-type, WNT5A null and 6-week-old wild-type mouse bone marrow.	Downregulation of WNT5A increases B cell proliferation in a cell-autonomous manner.	None.	None.	[50]
Hematological neoplasm/Hodgkin lymphoma	RT-PCR, Microarray, WB.	L428, KM-H2, L1236, BL-2, BL-30, Carnaval, Karpas-422, JeKo-1, Mino, OciLy1, and OciLy3.	WNT5A expression was increased in primary HL cells compared to normal B-cell subsets and other lymphomas.	None.	None.	[51]
Hematological neoplasm/AML, ALL, CLL, NHL, CML	RT-PCR, WB.	K562, U937, Jurkat, and CD34+ cells.	The treatment with demethylating agent Aza restored WNT5A expression.	68 leukemia patients: 18 ALL, 12 CLL, 10 NHL, 16 AML, 12 CML and 35 patients in complete remission (CR) (8 ALL-CR, 5 CLL-CR, 6 NHL-CR, 12, AML-CR, 4 CML-CR).	WNT5A expression level was higher in non-malignant hematopoietic (NMH) cases. WNT5A expression level was lower in leukemia compared to CR-cases due to WNT5A promoter methylation in leukemia cases but not in NMH and CR cases.	[49]
Hematological neoplasm leukemia	RT-PCR, MSP, sequencing	MUTZ-3, F-39P, TF-1, HL-60, NOMO-1, KG-1, MV4-II, MOLM-13, KASUMI-1, EOL-1, and HEL	WNT5A gene expression in AML-derived cell lines was regulated by promoter hypermethylation.	252 de novo non-M3 AML patients	WNT5A gene expression was decreased in AML samples by hypermethylation and correlated with upregulation of CYCLIN D1 expression. Relapse and mortality rates were lower for patients in the non-methylated group.	[52]
Hepatocellular carcinoma	RT-PCR, IHC.	None.	None.	26 fresh HCC samples and corresponding para-carcinoma tissue samples. 85 HCC and	WNT5A mRNA levels were significantly higher in HCC tissue compared to para-carcinoma tissue.	[40]

(continued on next page)



Table 1 (continued)

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Hepatocellular carcinoma	RT-PCR.	SNU182, SNU-387, SNU-398, SNU-449, HCC, Hep3B, Huh7, and HepG2.	WNT5A expression was elevated in poorly-differentiated HCC cells.	corresponding paracarcinoma tissue samples. 15 hepatic cirrhosis tissue samples. None.	None.	[53]
Hepatocellular carcinoma	IHC.	None.	None.	87 HCC patient tissue samples.	WNT5A expression was significantly lower in the HCC tissue samples compared to their paracarcinoma tissues. WNT5A expression was associated with HCC progression and poor prognosis.	[54]
Non-small-cell lung cancer	RT-PCR.	Normal HBE cells, transformed (1198) and tumorigenic (1170-I) HBE cells	WNT5A was overexpressed in smoking-related HBE cells and lung cancer tissues.	None.	None.	[55]
Non-small-cell lung cancer	RT-PCR, WB, IHC.	A549, HCC827, H1299, and H1975.	WNT5A protein expression was significantly downregulated in BM tissues and EGFR-mutant samples.	None.	None.	[56]
Non-small-cell lung cancer	IHC.	None.	None.	205 patients submitted to surgical resection for lung cancer - 79 squamous cell carcinoma tissue samples, - 75 adenocarcinoma tissue samples - 51 large cell lung cancer tissue samples.	WNT5A expression level was upregulated in the majority of NSCLC tissues, especially in squamous cell carcinoma, while its expression level in adenocarcinoma was the lowest. WNT5A was more frequently expressed in male patients compared with female patients.	[57]
Non-small-cell lung cancer	IHC, WB.	A549, H1299, H1975, H1650, and BEAS-2B.	WNT5A increased clone formation, migration, and invasiveness of NSCLC cells and induced EMT in these cells.	20 ADC NSCLC patient tissue samples. 20 SCC NSCLC patient tissue samples. 20 tumor-adjacent tissue samples.	WNT5A and ROR2 expression levels were higher in NSCLC tissue samples compared to adjacent non-tumor tissues.	[58]
Medulloblastoma	RT-PCR, IHC.	None.	None.	76 Medulloblastoma patient tissue samples.	WNT5A and Ror2 were immunopositive in most Medulloblastoma cases.	[59]
Melanoma	WB, IHC.	BRAFi-R, BRAFi-Mutant HTB63, A375, and A2058.	Inhibition of WNT5A signaling inhibited cell migration and invasion of BRAFi-R melanoma cells.	None.	None.	[60]
Melanoma	IHC, WB, ELISA.	Mice myeloid-derived suppressor cells (MDSC), YUMM1.7, BSC9AJ2.	MDSC-derived WNT5A contributes to metastatic spread and decreases melanoma proliferation in vivo, supporting the phenotype switching phenomenon.	None.	None.	[61]
Melanoma	RT-PCR, Microarray, WB.	WM852, HTB63, A375, and A2058	WNT5A-IL-6 positive feedback loop effectively impaired melanoma cell migration and invasion. IL-6 increases the expression of WNT5A independently of STAT3.	None.	None.	[62]
Melanoma	RT-PCR, WB.	MS1, Mewo, SKmel28, A2058, A375. and HTB63.	WNT5A increased secretion of IL-6, IL-8 and VEGF in cell culture supernatants and induced exocytosis-dependent on the small RhoGTPase Cdc42.	None.	None.	[63]
Melanoma	IHC.	UACC-1273EV, UACC1273-4-3, UACC1273-4-7,	Cells with endogenously high WNT5A showed PKC $\beta$ II expression predominantly at the membrane.	45 melanoma patient tissue samples.	WNT5A was highly expressed in the more metastatic Cohort C, but	[64]

(continued on next page)



Table 1 (continued)

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Melanoma	PCR, WB, IHC.	UACC647, M93-047, and UACC903 SKMel2, SKMel5, SKMel28, A375P, and A375M1.	WNT5A expression was high in metastatic CM, in which motility is a requirement for cells to reach distant sites.	102 melanoma tissue samples. 59 primary melanomas with matched metastases.	not in the less metastatic Cohorts A and B. Cytoplasmic WNT5A showed a trend of increasing expression with melanoma progression whereas there was diminishing p16(ink4a) expression.	[65]
Melanoma		None.	None.	11 squamous cell carcinoma tissue samples. 9 basal cell carcinoma tissue samples.	WNT5A was expressed in both squamous cell carcinoma and basal cell carcinoma relative to its expression level in the basal layer of the epidermis.	[66]
Merkel Cell Carcinoma	RT-PCR, Microarray, WB, IHC.	Mkl-1 (MCV+), UISO (MCV-), BroLi, MaTi.	WNT5A expression was very low in MCCs of differing metastatic or MCV status cell lines.	32 primary MCC patient tissue samples. 17 metastatic MCC patient tissue samples.	WNT5A expression was very low in MCC.	[67]
Oral squamous cell carcinoma	WB.	SCC9, ND, and SCC25.	WNT5A expression was absent in SCC9 and SCC25 cells.	None.	None.	[68]
Oral squamous cell carcinoma	IHC.	None.	None.	60 OSCCs patient tissue samples: - 30 lymph node metastases. - 30 non-metastatic. 10 healthy controls.	WNT5A expression was increased in metastatic OSCC when compared to non-metastatic OSCC.	[69]
Osteosarcoma	RT-PCR.	SaOS-2, and U2O2.	WNT5A promoter B activity was reduced in osteosarcoma cell lines	1 humerus parosteal osteosarcoma patient tissue sample. 1 metastatic osteosarcoma patient tissue sample. 1 recurrent osteosarcoma patient tissue sample. 1 normal human osteoblast tissue sample.	WNT5A promoter B activity was reduced in primary tumor tissue in comparison to normal osteoblasts	[70]
Ovarian cancer	RT-PCR, WB.	SKOV-3.	WNT5A and ROR2 expression are induced by LPS, LTA and IL-6 and activates NF-kB and STAT3 signaling pathways, controlling migration of the OvCa cells.	None.	None.	[71]
Ovarian cancer	RT-PCR, WB.	Kuramochi, SKOV-3, A2780, OVCAR-3, CAOV-3, IGROV1	WNT5A-induced signaling promoted metastatic stem cell-like behaviors of HGSC cells.	20 ascitic fluid samples from patients with aggressive high-grade serous carcinoma (HGSC) of the ovary	Patients with WNT5A protein high levels in their ascites showed a worse course of disease than patients with low levels.	[72]
Ovarian cancer	MSP, bisulfite genomic sequencing RT-PCR, IHC.	SKOV3.	WNT5A was partially methylated in SKOV3 cells. The treatment with Aza restored WNT5A expression.	30 normal tissue samples. 79 human EOC tissue samples.	WNT5A expression was reduced or lost in EOC. WNT5A expression was correlated with histological grade, FIGO stage, and lymph node metastasis due to promoter methylation.	[37]
Ovarian cancer	IHC.	None.	None.	63 EOC patient tissue samples. 18 normal ovarian tissue samples. 30 benign ovarian tumor tissue samples.	WNT5A expression was significantly higher in OvCa compared to benign tumors and normal ovaries.	[73]
Pancreatic cancer	IHC, WB.	PANC-1, and BXP-3.	WNT5A increases pancreatic cancer cells migration, invasiveness and induces EMT in pancreatic cancer cells.	134 PDCA. 134 adjacent normal pancreatic patient tissue samples.	Patients with WNT5A-positive tumors had a slightly higher median cancer-specific survival than those with negative WNT5A expression.	[74]
Pancreatic cancer	RT-PCR, WB, IHC, FC.	None.	None.	32 pancreatic cancer and paracarcinoma patient tissue samples.	WNT5A expression was higher in normal pancreatic tissue	[75]

(continued on next page)

Table 1 (continued)

Cancer type	Techniques	Cell lines		Primary tissues		Publications
		Type	Notes	Type	Notes	
Pancreatic cancer	IHC.	None.	None.	PDAC tissues following surgical pancreatic resection.	compared to tumors and tumor-derived-stroma. WNT5A expression was higher in tumors and normal pancreatic tissue. WNT5A expression was higher in tumor cells when compared to stromal tissue.	[76]
Pancreatic cancer	RT-PCR, WB, ELISA.	UACC647, UACC1273EV, M93-047, BXP-3, PANC-1, HEK 293, WRO 82-1, and ARO 81-1.	WNT5A RNA levels are constitutively expressed in human pancreatic cancer and melanoma cell lines.	None.	None.	[77]
Pancreatic cancer	IHC.	None.	None.	134 pancreatic adenocarcinoma tissue samples. 134 adjacent normal pancreatic patient tissue samples.	WNT5A positive expression percentage showed a bell-shaped pattern in pancreatic cancer tissues, peaking in well-differentiated carcinomas, mainly in glandular luminal border, and negatively associated with tumor histological grade, aggressive tumor phenotype and poor prognosis, probably by promoting EMT and metastasis.	[74]
Prostate Cancer	RT-PCR, WB, IHC.	DU145, and PC-3.	WNT5A gene and protein expression were higher in prostate cancer cells.	None.	None.	[78]
Prostate Cancer	RT-PCR, WB, IHC.	PC3, LNCaP derivative C42B, MDA-PCa-2b, MCF-7, and MDA-MB-231	WNT5A overexpression increased apoptosis and decreased proliferation.	397 prostatectomy patient tissue samples.	WNT5A and FZD5 mRNA expression were significantly higher in the prostate tissue from patients with prostate cancer compared with healthy controls.	[79]
Prostate Cancer	IHC.	None.	None.	312 prostate cancer patient tissue samples.	WNT5A protein expression was higher in prostate cancer and associated with longer relapse-free time after radical prostatectomy.	[80]
Prostate Cancer	RT-PCR.	LNCaP, PC3, and DU145	WNT5A mRNA expression was significantly higher levels in the PC3 cells compared to LNCaP and DU145 cells.	None.	None.	[81]
Prostate Cancer	IHC.	None.	None.	503 prostate patient tissue samples.	WNT5A protein expression was significantly higher in cancer compared to benign cores from the same patients.	[80]
Prostate Cancer	MSP, bisulfite genomic sequencing, RT-PCR.	1542-NPTX (NP) and 1542-CP3TX (CP).	WNT5A was methylated in the cell lines.	None.	None.	[82]
Testicular Cancer	IHC	None.	None.	47 patients with stage I pure testicular seminoma	ROR2/Wnt5a analysis showed no significant protein expression.	[83]
Urothelial carcinoma	RT-PCR, IHC.	HTB-1 <sup>TM</sup> (J82), and HTB-4 <sup>TM</sup> (T24)	WNT5A was underexpressed in UC tissue samples due to hypomethylation.	33 UC patient tissue samples.	WNT5A protein immunoreactivity was increased in UC patient tissue samples. WNT5A protein immunoreactivity was correlated positively with the cancer histological grade and pathological stage.	[84]

**Abbreviations:** PCR – polymerase chain reaction; RT-PCR – real time PCR; WB – western blot; IHC – immunohistochemistry; IF – immunofluorescence; FC – flow cytometry; ELISA – enzyme-linked immunoassay; ChiP – chromatin immunoprecipitation; MSP – methylation specific PCR; CRC – colorectal cancer; EMT – epithelial-

mesenchymal transition; GBC – gallbladder squamous; SC – adenosquamous carcinomas; AC – adenocarcinomas; EC – endometrial cancer; mRNA – messenger ribonucleic acid; EOC – epithelial ovarian cancer; EBVaGC – Epstein-Barr virus-associated gastric carcinoma; NPC – nasopharyngeal carcinoma; ESCC – esophageal squamous cell carcinoma; ES – ewing sarcoma; GC – gastric cancer; GBM – glioblastoma; ALL – acute lymphoblastic leukemia; T-ALL – T cell ALL; B-cell ALL; AML – acute myeloid leukemia; HL – Hodgkin's lymphoma; NHL – Non-Hodgkin Lymphoma; CLL – chronic lymphocytic leukemia; CML – chronic myeloid leukemia; Aza – 5-Aza-2'-deoxycytidine; HCC – hepatocellular carcinoma; NSCLC – non-small-cell lung cancer; HBE – human bronchial epithelial; MPNST – Malignant peripheral nerve sheath tumors; MCC – Merkel cell carcinoma; OSCC – Oral squamous cell carcinoma; OvCa – ovarian cancer; PDCA – pancreatic ductal adenocarcinoma; UC – urothelial carcinoma.

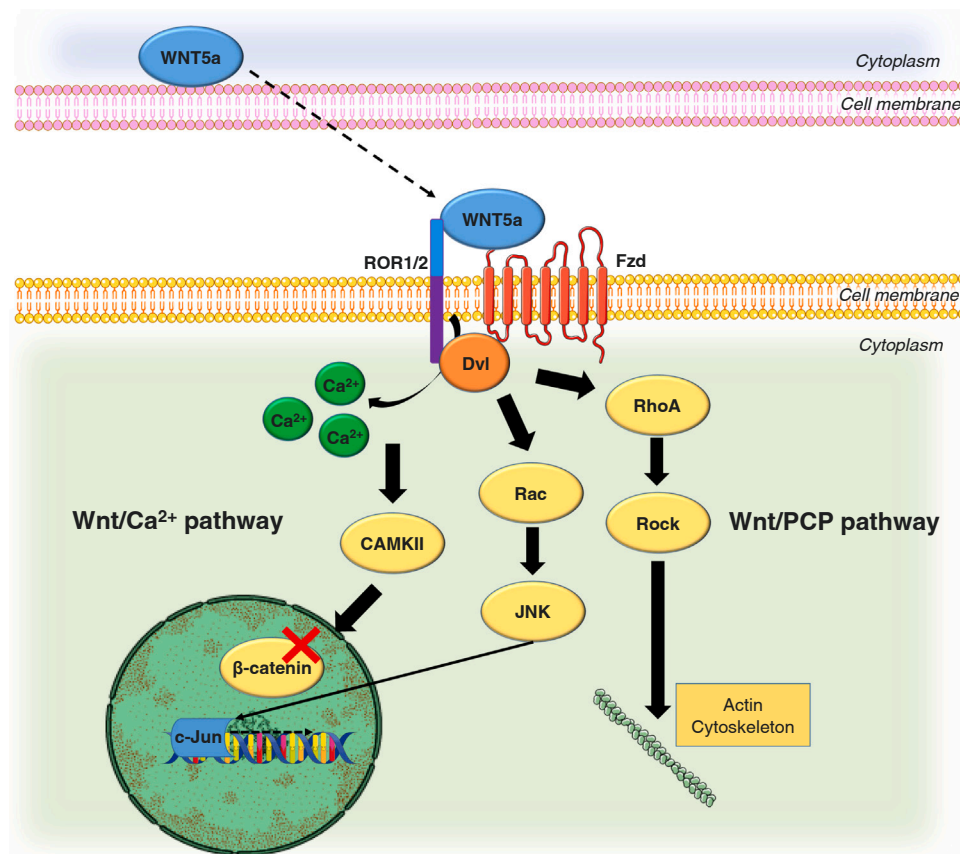
activate the WNT/PCP or the WNT/ $\text{Ca}^{2+}$  pathways. The modulation of distinct WNT5A signaling pathways depends on the diversity of WNT5A receptors, the origin cell and/or tissue and the presence of WNT5A isoforms [5].

In the WNT/PCP pathway, WNT5A forms a complex with FZD, ROR2, and Ryk, which triggers the Disheveled (Dvl), consequently activating DVL-associated activator of morphogenesis 1 (DAAM1), RhoA and then Rho-associated protein kinase (ROCK) and mitogen-activated protein kinase (MRLC). Through Dvl, the Rac1 protein can also be activated, which triggers the Filamin A protein and Jun N-terminal protein kinase (JNK), which phosphorylates c-JUN, resulting in its translocation to the nucleus. The WNT/PCP downstream protein activation is associated with cell migration and invasion events as well as F-actin cytoskeleton remodeling (Fig. 2). In the WNT/ $\text{Ca}^{2+}$  pathway, WNT5A also activates FZD2 or ROR1/2 receptors, resulting in the activation of Dvl and consequently phospholipase C (PLC), which cleaves phosphatidylinositol-4,5-bisphosphate (PtdInsP2) into diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (InsP3). InsP3 binds to inositol-1,4,5-trisphosphate receptors (InsP3Rs) on the cell membrane and causes the opening of calcium channels for  $\text{Ca}^{2+}$  and mobilization of free intracellular calcium. DAG and  $\text{Ca}^{2+}$  are capable of activating signaling molecules that are calcium-dependent, mainly CAMKII and protein kinase C (PKC) [5]. Therefore, a degradation of  $\beta$ -catenin independent of GSK3 $\beta$  [7] and a phosphorylation of STAT3 occur, reducing the expression of several tumor associated-antigens,

such as MART-1, PAX3, SOX10 and MITF and regulating important cellular processes, as actin cytoskeleton remodeling and cell migration (Fig. 2).

### 3.2. WNT5A and the TME cells

WNT5A has an important role in the regulation of TME due to the paracrine function and activation of different oncogenic pathways. Lopez-Bergami and Barbero described that WNT5A has the potential of inducing the autocrine secretion of chemotaxis-related cytokines, as IL-8 (CXCL8), CXCL1, and CCL2, which has a paracrine action onto immune cells through WNT5A/NF- $\kappa$ B/IL-6/STAT3 positive feedback and enables the shift of leukocytes to TME, contributing to an immunosuppressive TME [86]. Furthermore, WNT5A induced tumor-associated macrophages (TAMs) secretion of IL-10 by stimulating a CaMKII - ERK1/2 - STAT3-dependent pathway. In an autocrine manner, IL-10 induced M2 polarization of these TAMs and in a paracrine manner to promote neoplastic cell growth, migration and invasion [23]. Additionally, WNT5A produced by the TME enables the activation of CDC42, a small GTPase of the Rho family, in hematopoietic stem cells and results in the exosome release. These exosomes contain the IL-6 cytokine and the IL-8, VEGF and MMP2 factors, favoring the angiogenesis [63], impairing F-actin polarization, modulating adhesion, migration, homing and engraftment [87]. Interestingly, fetal liver vascular niche endothelial cells (ECs) secreted WNT5A that interacts with the Ryk protein of



**Fig. 2.** WNT5A signaling pathways. Secreted WNT5A protein binds to the transmembrane receptor Frizzled (FZD) and ROR1/2 activating the WNT/ $\text{Ca}^{2+}$  pathway in which intracellular  $\text{Ca}^{2+}$  release triggers  $\text{Ca}^{2+}$ -CamKII and protein kinase C (PKC). After binding to FZD and ROR1/2, WNT5A was able to activate the WNT/PCP pathway through signals that are transmitted through Disheveled to trimeric G proteins leading to the activation of downstream proteins as Rac and JNK or RhoA and Rock. This figure was created using Servier Medical Art tools (<http://www.servier.com>).

hematopoietic stem cells membrane, supporting maturation and expansion for human fetal liver hematopoiesis by modulating PI3K and MAPK pathways [88]. Moreover, through the release of WNT5A, neoplastic cells tend to change their behavior in order to exert beneficial function for the tumor. In the prostate cancer niche, bone marrow mesenchymal stromal cells secrete WNT5A, which acts as a chemo-attractant onto prostate cancer cells by activation PKC/NFkB/BMP-6 pathway and enhancing the migration of prostate cancer cells to stromal cells [81]. In colon cancer, increase of WNT5A expression and secretion by mesenchymal stromal cells in mouse intestinal tumors promotes adhesion and triggers migration and invasion of colon cancer cells [89]. In gastric carcinoma, CXCR4+ gastric innate lymphoid cells (ILCs) (WNT5A secreted) in the perivascular gastric niche augmented the colony formation of MIST1+ gastric stem cells by activating ROR2 and RhoA [90]. Furthermore, the 3D-coculture PDAC cells with adipocytes induced the secretion of WNT5A protein by PDAC cells, which acts in an autocrine manner on PDAC cells as well as in a paracrine manner on adipocytes cells, intensifying the c-JUN/AP1 pathway and reprogramming adipocytes to fibroblast-like cells to produce several cytokines that sustain tumor growth [91]. In ovarian neoplasm, cancer associated fibroblasts (CAFs), the main constituent of the ovarian cancer TME, secretes WNT5A that triggers onto FZD receptor in CSCs, a small subpopulation in the tumor, resulting in the initiation and maintenance of neoplasm cells [31].

#### 4. Expression and methylation in cancer

WNT5A is expressed in a variety of human cancers and the protein and gene expression and methylation patterns have been widely investigated and associated with carcinogenesis. While the overexpression of WNT5A is associated to the promotion of tumor invasion and metastasis, underexpression may also regulate tumor cell characteristics. Epigenetic alterations of WNT5A, including both DNA methylation and histone modifications, also play an important part in cancer development, contributing to the differential expression of the *WNT5A* gene and, consequently, the protein, leading to opposing actions in different neoplasms. Additionally, WNT5A carries two protein isoforms that provoke diverging effects in cancer. While WNT5A-S stimulates cell proliferation probably by overexpression, WNT5A-L inhibits cell proliferation due to downregulation expression through the methylation of the promoter [10]. Recent findings are summarized in Table 2.

##### 4.1. Blood neoplasms

WNT5A is found throughout the hematopoietic system and is increased in progenitors and mature blood cells, suppressing tumorigenesis in these hematopoietic tissues [50]. The *WNT5A* gene expression level is increased in non-malignant hematopoietic (NMH) diseases and decreased in leukemia when compared to complete remission (CR) patients. WNT5A protein immunoreactivity is positive in NMH, CR patients and CD34-positive cells, and negative in leukemia patients. The downregulation of WNT5A in leukemia has been correlated with promoter methylation in patients when compared to controls samples [49].

In a cohort of 95 cases of acute leukemia (AL) at diagnosis, 62 patients with acute myeloid leukemia (AML) and 33 with acute lymphocytic leukemia (ALL), WNT5A gene and protein expressions were lower in bone marrow cells of AL patients than in normal controls. AL patients also showed higher DNA methylation rates than normal controls [48]. Similarly, in de novo AML (n = 252) patients, WNT5A was downregulated in AML patients compared to the control group due to a promoter hypermethylation. Relapse and mortality rates were diminished in non-methylated AML patients when compared to the methylated group [52]. Furthermore, in a larger cohort of 86 ALL patients, WNT5A gene expression was downregulated in B-cell-ALL (ALL-B) and T-cell-ALL (ALL-T) patients through a heavily methylated promoter [47].

In primary classical Hodgkin lymphoma patient samples and early

relapsed patients, an increased WNT5A expression was observed when compared with normal B cells, other lymphomas and late relapsed patients [51].

##### 4.2. Bone neoplasms

In Ewing sarcoma (ES), *WNT5A* mRNA levels were more increased in patients with metastasis than in patients without metastasis, which positively correlated to *CXCR4* mRNA expression, indicating that in ES, WNT5A may act as a pro-metastatic factor [93]. Potratz et al. showed an increased WNT5A expression in metastatic Ewing sarcomas compared to non-metastatic Ewing sarcomas [95].

In osteosarcoma, WNT5A promoter B is decreased in comparison to normal osteoblasts, suggesting that this isoform could act as a tumor suppressor [70].

##### 4.3. Brain neoplasms

In medulloblastoma, WNT5A and ROR2 showed immunopositivity in 29.4 % and 51.5 % tissue samples (n = 68), suggesting dysregulation of the non-canonical WNT signaling pathway [59]. Additionally, WNT5A protein expression was higher in human glioblastoma multiforme (GBM) cells when compared with glial cells from non-neoplastic tissue samples (n = 175) [44]. WNT5A levels gradually increased from grade II to grade IV gliomas [44,46]. Kamino et al. verified a grade III WNT5A protein expression in 8 from a total of 12 glioblastoma patients [45]. Furthermore, in invasive glioblastoma tissues, WNT5A secretion is upregulated, comparing to non-invasive glioblastoma tissue samples [43].

##### 4.4. Urogenital neoplasms

###### 4.4.1. Breast neoplasms

In breast cancer, the WNT5A gene and protein were more upregulated than in adjacent non neoplastic tissue samples. Data from 1085 patient breast tumor samples and 291 normal tissue samples, Wan et al. reported high levels of WNT5A in breast cancer-associated fibroblast tissue samples when compared to normal tissue samples, indicating WNT5A may be increased in tumor stroma [13]. Similarly, Kim et al. verified that WNT5A overexpression correlates with a worse prognosis of HER2+ breast cancer and augmented cell invasiveness and metastasis [14]. Conversely, WNT5A was found to be underexpressed in triple-negative breast cancer, which was correlated with positive lymph node metastasis and proliferation [16].

###### 4.4.2. Cervix neoplasms

In primary cervical tumor, increased levels of WNT5A gene were observed and WNT5A immunoreactivity was correlated with an increased frequency of metastasis in lymph node and ovarian (distant metastases), and predicted a worse prognosis, including decreased overall and recurrence-free survival (multivariate analysis) of cervical cancer progression [19].

###### 4.4.3. Endometrial/uterine neoplasms

WNT5A gene underexpression was verified in patients with uterine cancer (endometrial) when compared to unchanged endometrium cancer samples, which was correlated with primary tumor invasion. The two WNT5A isoforms were detected in endometrium cancer patient samples, both in the cytoplasm and in the nucleus, suggesting that the changes in the pattern of WNT5A isoforms may be caused by modifications in the cellular localization of WNT5A. Furthermore, WNT5A protein was observed in the cytoplasm and nucleus of endometrial cancer cells and cytoplasmic WNT5A protein expression and granulocyte/lymphocyte counts were positively correlated [28]. These findings suggest that due to the secretory and immunomodulator roles, WNT5A could represent a potential marker in endometrial cancer

**Table 2**

Summary of functional assays for WNT5A in cancer cells.

Cancer type	WNT5A overexpression	Note	Publication
Breast cancer	Anti-WNT5A antibody (Novus Biologicals).	In vivo: affected the metastatic potential of HER2+ breast cancer by inducing CXCR8, without affecting cell proliferation.	[14]
Cervical cancer	Anti-WNT5A monoclonal antibody - mAb5A16 (Abcam).	In vitro: promoted invasion and proliferation of cancer cells through receptor-mediated endocytosis-dependent/-independent mechanisms	[18]
Colon cancer	Recombinant WNT5A, rWNT5A (R&D Systems). WNT5A-mimicking peptide, Foxy-5 (WNT Research).	In vitro and in vivo: increased the expression of 15-PGDH, sucrose-isomaltase and mucin-2, reduced $\beta$ -catenin signaling and activated JNK/AP-1 signaling in colon and breast cancer cell lines and modulated differentiation of colon cancer cells	[20]
Colorectal cancer	WNT5A-mimicking peptide, Foxy-5 (WNT Research).	In vitro: reduced the expression of stem-cell marker aldehyde dehydrogenase, cortin-like kinase 1, and cyclo-oxygenase 2, and increased the expression of 15-hydroxyprostaglandin dehydrogenase and PGE2-degrading enzyme.	[92]
Epithelial ovarian cancer	Recombinant WNT5A, Ab#86720 (Abcam).	In vitro: decreased cell adhesion and contributed to progression to EMT in ovarian surface epithelial cells.	[30]
Epstein-Barr virus-associated nasopharyngeal carcinoma	Plasmid pLNC-WNT5A TWO4 and HONE1 overexpressing WNT5A.	In vitro: promoted proliferation, migration and invasion of Epstein-Barr virus-associated nasopharyngeal cells.	[33]
Esophageal Adenocarcinoma	Recombinant WNT5A, rWNT5A (R&D Systems).	In vitro: drastically decreased OE33 cell proliferation, cell survival, invasion, and cell migration in a dose- and time-dependent manner	[35]
Ewing sarcoma (ES)	Recombinant WNT5A, rWNT5A (R&D Systems).	In vitro: enhanced CXCR4 expression in ES cells, which was accompanied by increased ES cell migration.	[93]
Gastric carcinoma	PcDNA3.1-WNT5A expression vector (Invitrogen).	In vitro: stimulated macrophage chemotaxis and cytoskeletal changes via MCP-1, which were suppressed by recombinant IL-1 receptor antagonist.	[40]
Gastric carcinoma	Plasmids carrying WNT5A cDNA (pWNT5A; Osaka University).	In vitro: regulated the induction of EMT and the maintenance of CSC properties in MKN-7 gastric carcinoma cells.	[41]
Glioblastoma	Recombinant human WNT5A.	In vitro: stimulated glioblastoma cell invasion via WNT5A/Daam1/RhoA signaling and sustained the formation of stress fibers.	[43]
Hematological neoplasm Adult T-cell acute lymphoblastic leukemia (T-ALL)	Recombinant WNT5A - rWNT5A (R&D Systems).	In vitro: increased CCL25-induced migration and invasion of MOLT4 cells.	[49]
Hematological neoplasm/Hodgkin lymphoma	Wnt-C59 (Selleck Chemicals). pCDNA3.2 plasmids containing corresponding V5-tagged WNT ligands (Addgene).	In vitro: increased L428 cell migration. In vivo: affected cHL lymphoma engraftment.	[51]
Lung cancer	Human E2F1 overexpression vector (GenePharma).	In vitro: inhibited the growth, migration, invasion and aggressiveness of EGFR-mutant NSCLC cells. In vivo: suppressed tumorigenesis and brain metastasis.	[56]
Lung cancer	Recombinant WNT5A, rWNT5A (R&D Systems).	In vitro: activated PKC and Akt in CSC-transformed 1198 cells.	[55]
Lung cancer	pcDNA 6.2 (Control), pcDNA 6.2-si-WNT5A, pcDNA3.1 (Control), pcDNA3.1- WNT5A (Invitrogen).	In vitro: increased clone formation, migration, and invasion, as well as prompted EMT of NSCLC cells.	[58]
Melanoma	Recombinant WNT5A - rWNT5A (R&D Systems).	In vivo: induced melanoma exosomes and increased endothelial cell branching.	[63]
Oral squamous cell carcinoma	Recombinant WNT5A protein (R&D Systems). WNT5A-mimicking peptide - Foxy-5 (WNT Research).	In vitro: promoted tumor cell migration and invasion	[68]
Pancreatic cancer	Plasmids carrying WNT5A cDNA - pBig2r-WNT5A.	In vitro: mediated resistance to apoptosis in pancreatic cancer cell lines.	[94]
Prostate cancer	WNT5A-mimicking peptide - Foxy-5 (WNT Research).	In vitro: significantly reduced prostate DU145 cell line invasion, but it had no effect on prostate PC3 cell line invasion.	[78]
Prostate cancer	Recombinant WNT5A	In vivo: reduced cell invasion. In vitro: promoted cell proliferation and migration.	[81]
Cancer type	WNT5A underexpression	Note	Publication
Breast cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (GenePharma).	In vitro: essential for CAF-promoting angiogenesis.	[13]
Cervical cancer	pLV5IN-EF1 $\alpha$ Neo Vector containing WNT5A plasmid (Oligo Engine).	In vitro: suppressed proliferation of cervical cancer cells and lung cancer cells.	[18]
Colorectal cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Genechem).	In vitro: indirectly promoted tumor proliferation, migration and invasion through M2 macrophages. In vivo: inhibited the pro-tumor effect of tumor-associated macrophages.	[23]
Colorectal cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Purigo Biotech).	In vitro: drastically inhibited growth and ability by inducing apoptosis through induction of FASLG expression and reduction of TNFRSF11B expression. The simultaneous overexpression of WNT5A-L mRNA isoform and knockdown of WNT5A-S mRNA isoform enhanced apoptosis.	[26]
Colon cancer	pCMV6-Myc-DDK-tagged-WNT5A-L isoform plasmid (Origene).	In vitro: impaired proliferation, clonogenicity, motility and invasive capability by manipulating Bax in colon cancer cells.	[22]
Colon cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Genechem).	In vivo: suppressed tumor growth in nude mice, impaired tumorigenicity and inhibited EMT biomarkers in colon mice model.	[22]
Epithelial ovarian cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide - pSingle-shRNA-WNT5A.	In vitro: inhibited EMT, but did not significantly affect cell behavior in ovarian cancer cells.	[30]
Gastric carcinoma	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Sigma).		[41]

(continued on next page)



Table 2 (continued)

Gastric carcinoma	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide	In vitro: downregulated genes involved in intracellular signaling, chemokine-cytokine interaction and focal adhesion in gastric carcinoma cells. In vitro: suppressed gastric cancer cell migration and invasion. In vivo: inhibited metastasis in a six-week-old male BALB/cAnNCrj-nu mice.	[42]
Hematological neoplasm/ Hodgkin lymphoma	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (scr, AM4611; Life Technologies). WNT5A antagonist Box5 (Millipore).	In vitro: disrupted WNT5A-mediated RHOA activation and cell migration. In vitro: reduced migration of cHL cells via FZD5, DVL3 and RhoA.	[51]
Hematological neoplasm Adult T-cell acute lymphoblastic leukemia (T-ALL)	Wnt inhibitor/recombinant sFRP2 (R&D Systems, MN).	In vitro: reduced migration and invasion of MOLT4 cells.	[49]
Lung cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Invitrogen).	In vitro: decreased clone formation, migration, and invasion, as well as prompted EMT of NSCLC cell. In vivo: significantly increased tumor volume and tumor weight, and prompted EMT in A549 tumor-bearing mice as compared with the control.	[58]
Melanoma	C57Bl/6 Mice with WNT5A knockdown (WNT5A <sup>tm1.1Krvl/J</sup> with <sup>Ly2Mtm1((cre)Lox)</sup> (JAX stock).	In vitro: decreased the immunosuppressive nature of MDSC and decreased expression of TGFβ1 and arginase 1. In vivo: decreased numbers of MDSC and T-cell function and proliferation.	[61]
Melanoma	Silencer Select siRNA against WNT5A (Applied Biosystems).	In vivo: decreased endothelial cell co-culture branches.	[63]
Melanoma	WNT5A antagonist, Box5 (Millipore).	In vitro: impaired the elevated invasive and migration of BRAFi-R melanoma cells.	[60]
Melanoma	WNT5A antagonist Box5 (Millipore).	In vitro: inhibited IL-6 induced cell migration in HTB63 and A375 melanoma cells.	[62]
Melanoma	Lentivirus-mediated transduction using pLKO.1-shWNT5A lentiviral vector/WNT5A- (Addgene)	In vitro: inhibited migration and invasion of melanoma cells.	[85]
Oral squamous cell carcinoma	WNT5A antagonist Box5 (Millipore).	In vitro: inhibited WNT5A-induced Ca <sup>2+</sup> signaling and migration of OSCC cells.	[68]
Ovarian cancer	Lentivirus-mediated transduction using small interfering RNA (siRNA; ON-TARGET plus SMART pool human WNT5A, Fisher Scientific AG).	WNT5A protein have been considered tumor-derived immunomodulator	[71]
Pancreatic cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Invitrogen).	In vitro: increased in drug-induced apoptosis in pancreatic cancer cell lines.	[94]
Pancreatic cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide pSingle-shRNA-WNT5A (RiboBio).	In vitro: increased the percentage of cells in G0/G1 phase and decreased in S phase of cell cycle, induced Cyclin D1 expression through phosphorylation of AKT and caused gemcitabine resistance in PANC-1.	[75]
Prostate cancer	Lentivirus-mediated transduction using synthetic short hairpin RNA (shRNA) oligonucleotide (Dharmacon).	In vitro: reduced the migration capacity of prostate cancer PC3 cells.	[81]

Abbreviations: EMT – epithelial-mesenchymal transition; HL – Hodgkin's lymphoma; NSCLC – non-small-cell lung cancer; OSCC – Oral squamous cell carcinoma.

development.

#### 4.4.4. Ovarian neoplasms

In ovarian cancer (OvCa), Bitler et al. detected a WNT5A protein underexpression in primary epithelial ovarian cancer (n = 130) in comparison with the normal ovarian epithelium (n = 31) or the uterine tube epithelium (n = 28), probably due to promoter hypermethylation, resulting in a negative correlation with tumoral stage and reduction in overall survival [29]. Jin et al. further detected that the WNT5A expression was reduced or lost in ovarian cancer (n = 79) through methylation and correlated with histological grade and lymph node metastasis [37]. Conversely, Peng et al. reported a significantly higher WNT5A protein immunoreactivity than benign tumors and normal tissues, suggesting WNT5A could be a predictor of poor clinical outcome in patients with ovarian cancer [73]. In a large cohort of epithelial ovarian cancer patients (n = 623), WNT5A protein immunoreactivity was upregulated in subtypes of epithelial ovarian cancer in comparison to borderline and benign tumors [30]. Moreover, WNT5A staining was also positively correlated with metastasis in epithelial ovarian cancer [31]. Specifically in high-grade serous carcinoma of the ovary, Kotrbová et al. verified that patients that presented high expression of the WNT5A protein were associated to a worse outcome of the disease [72],

#### 4.4.5. Prostate neoplasms

In prostate cancer, patients (n = 503) with elevated endogenous levels of WNT5A protein were associated with a better clinical outcome

when compared to low WNT5A-level cases. Moreover, in low-grade prostate cancer (n = 312), longer relapse-free time after radical prostatectomy was correlated with increased WNT5A protein expression in the primary tumor [80]. WNT5A gene and protein were overexpressed in malignant in comparison to benign prostate tumors [96] and were associated with longer median survival of prostate cancer patients [79]. The regulation of WNT5A expression was related to the epigenetic mechanism hypomethylation in prostate cancer. Interestingly, a higher WNT5A mRNA expression was observed in normal prostate epithelial cells (1542-NPTX) treated with DNA methyltransferase inhibitor 5-Aza-2'-deoxycytidine [82].

#### 4.4.6. Urothelial neoplasms

In urothelial carcinoma, increased WNT5A protein immunoreactivity (n = 33) intensity was increasingly associated with the tumor staging and the cancer histological grade [84], suggesting an important role for the WNT5A signaling pathway in aggressiveness of urothelial carcinoma though the promotion of the EMT and metastases processes [97].

#### 4.4.7. Testicular neoplasms

In testicular seminoma, ROR2/Wnt5a analysis showed no significant protein expression in patients with stage I pure testicular seminoma (n = 47), excluding these antigens as prognostic biomarkers of this cancer type [83].

#### 4.5. Neoplasms of the digestive organs

##### 4.5.1. Oral cavity neoplasms

In patients with oral squamous cell carcinoma (OSCC) ( $n = 60$ ), an overexpression of WNT5A protein immunoreactivity was observed in metastatic OSCC whereas a WNT5A underexpression was verified in non-metastatic OSCC, indicating an association with lymph node metastasis. Furthermore, WNT5A protein levels were also correlated with higher tumor stages, emerging as an important feature in OSCC progression [69].

##### 4.5.2. Esophageal neoplasms

In esophageal adenocarcinoma, WNT5A gene and protein expression was reduced when compared with the corresponding healthy esophageal and squamous mucosa and Barrett esophagus. Additionally, WNT5A expression was downregulated toward the adenocarcinoma formation. The analysis of WNT5A protein expression in healthy esophageal mucosa demonstrated staining in all mucosal layers [35]. Interestingly, detection of WNT5A promoter methylation occurred in primary esophageal adenocarcinoma, resulting in a WNT5A underexpression [36].

##### 4.5.3. Neoplasms of the gastric tissues

Similarly, Li et al. reported overexpression of WNT5A gene in gastric cancer comparing to the adjacent non-malignant tissue. Furthermore, WNT5A mRNA levels in *Helicobacter pylori*-positive tissue were more prominent than in *Helicobacter pylori*-negative samples. WNT5A was further associated with monocyte chemoattractant protein 1 and interleukin 1 $\beta$ , molecules that promote macrophage recruitment [40]. Matsuo et al. verified that WNT5A protein expression was lower in patients that developed the gastric cancer after eradication for *Helicobacter pylori* when compared to patients with *Helicobacter pylori* infection [39]. In addition, Kanzawa et al. observed WNT5A expression in 66 % of gastric cancer patients, with significant histological correlation, being more frequently detected in diffuse-type gastric cancer than in intestinal-type gastric cancer. In intestinal-type gastric cancer, WNT5A expression was detected in the periphery of tumor nests [41]. Interestingly, gastric primary tumor patients with WNT5A expression reduction due to the increased promoter methylation of WNT5A showed increased survival [32].

##### 4.5.4. Liver neoplasms

In hepatocellular carcinoma (HCC), WNT5A mRNA expression was higher in HCC samples when compared to para-carcinoma, while WNT5A protein immunoreactivity was reduced or absent in HCC when compared to para-carcinoma and hepatic cirrhosis tissues [40]. This paradoxical expression of WNT5A gene and protein expression in HCC tissues is similar to expression patterns observed in thyroid carcinoma and malignant melanoma [69]. Wang et al. observed a significantly lower WNT5A protein expression in HCC samples than in their para-cancerous tissue samples. The authors suggested that the decreased WNT5A signaling correlated with malignant transformation of hepatocytes, cell growth and, consequently, poor prognosis [54]. In addition, WNT5A expression was increased in poorly-differentiated HCC samples, suggesting a role in autocrine positive feedback [53].

##### 4.5.5. Neoplasms of the biliary tract

In gallbladder carcinoma (GBC), positive WNT5A protein immunoreactivity was significantly lower in highly differentiated gallbladder squamous carcinoma (SC), adenosquamous carcinoma (ASC) or adenocarcinomas (AC) than in poorly differentiated gallbladder SC/ASC or AC whereas WNT5A expression among patients with SC/ASC had no significant difference compared with the AC patients. Both SC/ASC and AC patients with positive expression of WNT5A had shorter survival than that of patients with negative WNT5A expression [27].

##### 4.5.6. Pancreatic neoplasms

In pancreatic ductal adenocarcinoma (PDAC), increased WNT5A expression was found in approximately 77 % of PDAC samples while an increased WNT5A expression was detected in normal pancreatic tissue when compared to tumors and tumor-stroma, in a cohort of 88 patients with PDA. The authors also suggested the WNT5A/ROR2 signaling pathway may play an important role in pancreatic tissue physiology and PDAC tumor biology [76]. In addition, a positive WNT5A expression was reported in PDAC tissue samples, mainly in the glandular luminal border, which was negatively associated with tumor histological grade, aggressive phenotype and poor prognosis, probably through the promotion of epithelial-to-mesenchymal transition (EMT) and metastatic events [74]. Wei et al. observed WNT5A, p-AKT and Cyclin D1 protein immunoreactivity in pancreatic neoplasms when compared to para-carcinoma (WNT5A was located in intracellular and stromal tissues) [75]. Furthermore, Griesmann et al. showed that WNT5A and its transcriptional target (NFATc2) were increasingly expressed in pancreatic neoplastic tissue, conferring drug resistance and tumor survival, with antiapoptotic modulation [94]. In PDAC, WNT5A expression was related to a prognostic factor.

##### 4.5.7. Colon and rectum neoplasms

WNT5A methylation was correlated with longer progression-free survival [24]. Hence, increased WNT5A-S expression and low expression WNT5A-L were detected and were positively associated with CRC depth [26]. Moreover, Mehdawi et al. observed that WNT5A expression was reduced or absent in at least 39 % of primary tumor samples from CRC and the low WNT5A expression was correlated with reduced survival [20]. In addition, WNT5A was significantly decreased in most primary colon cancers with negative relation with EMT biomarkers [22], with reduced differentiation through the Ca<sup>2+</sup>/WNT5A signaling pathway [25] and inducing M2 macrophages and promoting CRC cells proliferation, migration and invasion [23]. Thus, according to these findings, in colorectal cancer, WNT5A could function as a tumor suppressor by antagonizing the canonical WNT signaling. Additionally, treatment with a demethylating agent may enable reactivation of the methylation-inactivated WNT5A-L and the expression knockdown of the WNT5A-S may enable the reactivation of  $\beta$ -catenin expression mediated by WNT5A-L downregulation. These events together may jeopardize tumor progression, which highlights the WNT5A mRNA isoforms as potential prognostic markers for CRC.

##### 4.6. Nasopharynx neoplasms

In primary nasopharyngeal carcinoma (NPC), using a microarray, Bose et al. verified an upregulation of WNT5A in primary Epstein-Barr virus-associated nasopharyngeal carcinoma (EBV-NPC) samples with concomitant low or absent expression in normal nasopharyngeal epithelium [98]. Similarly, WNT5A mRNA expression was higher in EBV-NPC, primary NPC and NPC with pulmonary metastases tissues than in non-malignant controls [33,34].

##### 4.7. Neoplasms of lung

In non-small-cell lung cancer (NSCLC), increased protein expression of WNT5A and ROR2, especially in squamous cell carcinoma, was associated with stimulating angiogenesis by increasing microvessel density and vasculogenic mimicry, and promoting EMT and metastasis by modulating cadherin protein expression, which results in a poor prognosis [57,58]. Regarding WNT5A and metastasis association, Li et al. demonstrated a downregulation of WNT5A in NSCLC with brain metastases when compared to NSCLC without brain metastases ( $n = 94$ ), which suggests that WNT5A expression may have an inhibitory action toward brain metastasis of NSCLC, mainly in EGFR-mutant NSCLC [56]. Interestingly, cigarette smoke may induce WNT5A/PKC activity in lung carcinogenesis, leading to increased Akt and



anti-apoptotic activity in lung cancer [55]. WNT5A expression corresponds to an important prognostic factor in non-small-cell lung cancer cases.

#### 4.8. Skin neoplasms

In melanoma, *WNT5A* gene expression was increased in cohorts of high metastatic when compared to low metastatic melanoma cell mobility and downregulated the expression of melanoma differentiation antigens via PKC and STAT3 activation [64]. Da Forno et al. showed that increased cytoplasmic WNT5A was correlated with melanoma progression, and could be considered a risk factor for reduced metastasis-free, overall survival and poor prognosis [65].

Pourreya et al. showed that expression of WNT5A at the leading edge of non-melanoma skin cancer indicates that WNT5A signaling could enable tissue invasion by non-melanoma skin cancer [66]. In primary malignant melanomas (n = 223) *WNT5A* mRNA expression and the angiogenesis marker endothelial cell-selective adhesion molecule, a protein expressed in blood vessel endothelial cell and associated with tight and adherens junctions that modulates trans-endothelial cell migration, were positively correlated [63].

In Merkel cell cancer, WNT5A gene and protein expressions were low or absent [67].

The overall compiled data presented reinforce the evidence that WNT5A could be considered an important diagnostic and prognostic marker for several cancers, contributing to clinical outcomes and advancement of novel therapy strategies.

#### 5. WNT5A in cancer therapy

The advances in understanding the genomic alterations and molecular mechanisms related to neoplasia expansion and progression-related

targets enable the development of novel therapy strategies. The use of targeted therapy for the treatment of different types of neoplasms frequently results in the development of drug resistance through several mechanisms, such as molecular target mutations, feedback mechanisms and/or crosstalk between neoplastic cells and the tumor niche [99].

As some cancers showed an overexpression of WNT5A that was related to poor prognosis, a compound, named Box5 was synthesized. Box5 corresponds to a derivative of WNT5A N-butyloxycarbonyl hexapeptide (t-Boc-Met-Asp-Gly-Cys-Glu-Leu), which acts as a WNT5A antagonist [100]. In melanoma cells, Box5 inhibited melanoma HTB63 and A375 migration induced by IL-6 [62] and effectively decreased the migration and invasion rates of BRAFi-R melanoma cells [60]. In classical Hodgkin Lymphoma, the WNT5A antagonist Box5 reduced migration of cells [51]. In Oral squamous cell carcinoma, the use of Box5 enabled tumor cell migration and invasion [68].

On the other hand, as an upregulation of WNT5A was found in neoplasms, recombinant human WNT5A as well a compound, Foxy-5, were produced. In HER2+ breast cancer, recombinant human WNT5A significantly intensified cell invasiveness by inducing CXCL8 [14]. In colon cells (HT-29, CACO-2), Foxy5 was able to reduce the expression of several markers, such as the active  $\beta$ -catenin, achaete scute complex homolog 2 (CSC-preserving transcription factor), stem-cell marker aldehyde dehydrogenase, specific colon cancer stem cell (CSC) marker double cortin-like kinase 1, and cyclo-oxygenase 2. Conversely, this compound intensified the expression of 15-hydroxyprostaglandin dehydrogenase (PGE2-degrading enzyme) [92]. Moreover, rWNT5A and Foxy5 increased expression of 15-PGDH, sucrase-isomaltase and mucin-2 by reducing  $\beta$ -catenin signaling and increasing the signaling of JNK/AP-1 in colon cancer cells, modulating differentiation [20]. In prostate cells, Foxy5 treatment reduced invasion of DU145 cells, but did not modulate PC3 cell invasion [78] (Table 3). Interestingly, after restoring the WNT5A levels throughout the treatment of prostate and

**Table 3**  
Summary of WNT5A functions in cancer.

Cancer type	Expression	Prognosis	Action	Function
Acute lymphoblastic lymphoma	gene downregulated/promoter hypermethylation protein downregulated	Poor	cancer progression	tumor suppressor
Acute myeloid lymphoma	gene downregulated/promoter hypermethylation protein downregulated	Poor	cancer progression	tumor suppressor
Breast cancer	gene downregulated/promoter hypermethylation protein high immunopositive	Poor	pro-metastatic factor	oncogene or tumor suppressor
Cervical cancer	gene upregulated	Poor	pro-metastatic factor	oncogene
Colorectal cancer	gene downregulated/promoter hypermethylation	Poor	pro-metastatic factor	tumor suppressor
Endometrial cancer	gene downregulated	Poor	marker of molecular changes during endometrial cancer development.	tumor suppressor
Esophageal adenocarcinoma	gene downregulated/promoter hypermethylation protein downregulated	Poor	cancer progression and invasion	tumor suppressor
Ewing sarcoma	gene and protein upregulated in ES metastasis	Poor	pro-metastatic factor	oncogene
Gallbladder carcinoma	protein low immunopositive	Poor	cancer progression	tumor suppressor
Gastric cancer	gene and protein upregulated	Poor	pro-metastatic factor	oncogene
glioblastoma	gene and protein upregulated	Poor	cancer progression	oncogene
Hepatocellular carcinoma	gene upregulated protein downregulated	Poor Poor	cancer progression	oncogene oncogene
Medulloblastoma	protein high immunopositive	Poor	pro-metastatic factor	oncogene
Melanoma	protein upregulated	Poor	pro-metastatic factor	oncogene
Merkel cell cancer	gene downregulated/promoter hypermethylation protein downregulated	Poor	cancer progression	tumor suppressor
Nasopharyngeal carcinoma	gene and protein upregulated	Poor	pro-metastatic factor	oncogene
Non-small-cell lung cancer	gene upregulated/high immunopositive	Poor	pro-metastatic factor	oncogene
Oral squamous cell carcinoma	high immunopositive	Poor	pro-metastatic factor	oncogene
Ovarian cancer	protein high immunopositive gene downregulated/promoter hypermethylation protein high immunopositive	Poor Poor	pro-metastatic factor pro-metastatic factor	oncogene oncogene or tumor suppressor
Pancreatic ductal adenocarcinoma	protein high immunopositive	Poor	pro-metastatic factor	oncogene
Prostate cancer	gene downregulated/promoter hypermethylation	Poor	cancer progression	tumor suppressor
Thyroid carcinoma	gene downregulated/promoter hypermethylation protein downregulated	Poor	cancer progression and invasion	tumor suppressor

colon cell lines with Foxy-5, a WNT5A mimetic, a differentiation of colon cells and a reduction of prostate cell invasion occurred [20,78].

Thereby, WNT5A displays important features that may be interesting to the field of oncology due to the importance in cancer progression and expression imbalance in several cancers. Cancers in which the down-regulation of WNT5A expression was associated with hypomethylation, Foxy-5 and the recombinant WNT5A human protein could positively modulate several cellular processes, such as angiogenesis, F-actin polarization, adhesion, migration, homing and engraftment. In neoplasms in which WNT5A is overexpressed, such as melanoma, Hodgkin Lymphoma and oral squamous cell carcinoma, the WNT5A antagonist Box5 inhibited cell migration, metastasis and neoplasm progression [51,60, 62,68]. In this setting, these compounds altogether arise as promising therapeutic targets.

## 6. Conclusion

The WNT5A opposing functions, as well as its interaction with TME, have been described as altered in various cancer types. The oncogenic and tumor suppressive actions of WNT5A in neoplasia development are conditioned by the isoform type, cell/tissue and receptor effects. New compounds are currently being synthesized and gene therapy is currently under development, specifically targeting WNT5A. Therefore, thorough knowledge of the expression of this gene is essential, including its epigenetic regulation and protein levels in each type of cancer. This review, by focusing on the advances in the genomic alterations and molecular mechanisms of neoplasia expansion and progression related to WNT5A, one of the 19 members of the WNT family, provides a better understanding of the WNT5A signaling pathways and mechanisms through which WNT5A suppresses or promotes tumorigenesis. Consequently, it enables investigators to advance in the discovery of novel targeted therapeutic approaches and the improvement of the currently existing therapy strategies. Interestingly, WNT5A studies are undergoing the pre-clinical phase, including in vitro (cell lines), ex vivo (healthy donors and patient cells) and in vivo (transgenic and xenograft mouse models) and have demonstrated potentialities for further clinical exploration.

## CRediT authorship contribution statement

**Maura Lima Pereira Bueno:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Sara Teresinha Olalla Saad:** Writing – review & editing, Funding acquisition. **Fernanda Marconi Roversi:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

## Funding

The authors received fellowship / grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Grants 2017/21801-2, 2019/25247-5 and 2021/05320-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grants 303405/2018-0).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors would like to thank Raquel S Foglio (Hematology and Hemotherapy Center, University of Campinas, São Paulo, Brazil) for the English revision.

The authors thank FAPESP (Grants 2017/21801-2, 2019/25247-5

and 2021/05320-0) and CNPq (Grants 303405/2018-0).

## References

- [1] N. Ghosh, U. Hossain, A. Mandal, P.C. Sil, The Wnt signaling pathway: a potential therapeutic target against cancer, *Ann. N. Y. Acad. Sci.* 1443 (2019) 54–74, <https://doi.org/10.1111/nyas.14027>.
- [2] R. Nusse, H.E. Varmus, Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome, *Cell* 31 (1982) 99–109, [https://doi.org/10.1016/0092-8674\(82\)90409-3](https://doi.org/10.1016/0092-8674(82)90409-3).
- [3] X. Ding, J. Liu, L. Zheng, J. Song, N. Li, H. Hu, X. Tong, F. Dai, Genome-wide identification and expression profiling of wnt family genes in the silkworm, *Int. J. Mol. Sci.* 20 (2019), <https://doi.org/10.3390/ijms20051221>.
- [4] J.R. Miller, The Wnts, *Genome Biol.* 3 (2002), REVIEWS3001, <https://doi.org/10.1186/gb-2001-3-1-reviews3001>.
- [5] M.S. Asem, S. Buechler, R.B. Wates, D.L. Miller, M.S. Stack, Wnt5a signaling in cancer, *Cancers* 8 (2016), <https://doi.org/10.3390/cancers8090079>.
- [6] A. Rodriguez-Trillo, N. Mosquera, C. Pena, F. Rivas-Tobío, A. Mera-Varela, A. Gonzalez, C. Conde, Non-canonical WNT5A signaling through RYK contributes to aggressive phenotype of the rheumatoid fibroblast-like synoviocytes, *Front. Immunol.* 11 (2020), 555245, <https://doi.org/10.3389/fimmu.2020.555245>.
- [7] A. De, Wnt/Ca<sup>2+</sup> signaling pathway: a brief overview, *Acta Biochim. Biophys. Sin.* 43 (2011) 745–756, <https://doi.org/10.1093/abbs/gmr079>.
- [8] C.C. Clark, I. Cohen, I. Eichstetter, L.A. Cannizzaro, J.D. McPherson, J. J. Wasmuth, R.V. Izzo, Molecular cloning of the human proto-oncogene Wnt-5a and mapping of the gene (WNT5A) to chromosome 3p14-p21, *Genomics* 18 (1993) 249–260, <https://doi.org/10.1006/geno.1993.1463>.
- [9] N. Zhu, L. Qin, Z. Luo, Q. Guo, L. Yang, D. Liao, Challenging role of Wnt5a and its signaling pathway in cancer metastasis (review), *Exp. Ther. Med.* 8 (2014) 3–8, <https://doi.org/10.3892/etm.2014.1676>.
- [10] M. Bauer, J. Bénard, T. Gaasterland, K. Willert, D. Cappellen, WNT5A encodes two isoforms with distinct functions in cancers, *PLoS One* 8 (2013) 1–14, <https://doi.org/10.1371/journal.pone.0080526>.
- [11] K. Kumawat, R. Gosens, WNT-5A: signaling and functions in health and disease, *Cell Mol. Life Sci.* 73 (2016) 567–587, <https://doi.org/10.1007/s00018-015-2076-y>.
- [12] K. Kerekes, L. Bányai, L. Patthy, Wnts grasp the WIF domain of Wnt inhibitory factor 1 at two distinct binding sites, *FEBS Lett.* 589 (2015) 3044–3051, <https://doi.org/10.1016/j.febslet.2015.08.031>.
- [13] X. Wan, S. Guan, Y. Hou, Y. Qin, H. Zeng, L. Yang, Y. Qiao, S. Liu, Q. Li, T. Jin, Y. Qiu, M. Liu, FOSL2 promotes VEGF-independent angiogenesis by transcriptionally activating Wnt5a in breast cancer-associated fibroblasts, *Theranostics* 11 (2021) 4975–4991, <https://doi.org/10.7150/thno.55074>.
- [14] S. Kim, D. You, Y. Jeong, S.Y. Yoon, S.A. Kim, S.W. Kim, S.J. Nam, J.E. Lee, WNT5A augments cell invasiveness by inducing CXCL8 in HER2-positive breast cancer cells, *Cytokine* 135 (2020), 155213, <https://doi.org/10.1016/j.cyto.2020.155213>.
- [15] S. Kim, S.Y. Chun, Y.S. Kwon, K.S. Nam, Crosstalk between Wnt signaling and Phorbol ester-mediated PKC signaling in MCF-7 human breast cancer cells, *Biomed. Pharmacother.* 77 (2016) 114–119, <https://doi.org/10.1016/j.biopha.2015.12.008>.
- [16] Z. Zhong, M. Shan, J. Wang, T. Liu, Q. Shi, D. Pang, Decreased Wnt5a expression is a poor prognostic factor in triple-negative breast cancer, *Med. Sci. Monit.* 22 (2016) 1–7, <https://doi.org/10.12659/msm.894821>.
- [17] A.C. Leris, T.R. Roberts, W.G. Jiang, R.F. Newbold, K. Mokbel, WNT5A expression in human breast cancer, *Anticancer Res.* 25 (2005) 731–734, (<https://www.ncbi.nlm.nih.gov/pubmed/15868903>).
- [18] K. Shojima, A. Sato, H. Hanaki, I. Tsujimoto, M. Nakamura, K. Hattori, Y. Sato, K. Dohi, M. Hirata, H. Yamamoto, A. Kikuchi, Wnt5a promotes cancer cell invasion and proliferation by receptor-mediated endocytosis-dependent and -independent mechanisms, respectively, *Sci. Rep.* 5 (2015) 8042, <https://doi.org/10.1038/srep08042>.
- [19] L. Lin, Y. Liu, W. Zhao, B. Sun, Q. Chen, Wnt5A expression is associated with the tumor metastasis and clinical survival in cervical cancer, *Int. J. Clin. Exp. Pathol.* 7 (2014) 6072–6078, (<https://www.ncbi.nlm.nih.gov/pubmed/25337253>).
- [20] L.M. Mehdawi, C.P. Prasad, R. Ehrnström, T. Andersson, A. Sjölander, Non-canonical WNT5A signaling up-regulates the expression of the tumor suppressor 15-PGDH and induces differentiation of colon cancer cells, *Mol. Oncol.* 10 (2016) 1415–1429, <https://doi.org/10.1016/j.molonc.2016.07.011>.
- [21] Q. Li, H. Chen, Silencing of Wnt5a during colon cancer metastasis involves histone modifications, *Epigenetics* 7 (2012) 551–558, <https://doi.org/10.4161/epi.20050>.
- [22] R. Cheng, B. Sun, Z. Liu, X. Zhao, L. Qi, Y. Li, Q. Gu, Wnt5a suppresses colon cancer by inhibiting cell proliferation and epithelial-mesenchymal transition, *J. Cell. Physiol.* 229 (2014) 1908–1917, <https://doi.org/10.1002/jcp.24566>.
- [23] Q. Liu, C. Yang, S. Wang, D. Shi, C. Wei, J. Song, X. Lin, R. Dou, J. Bai, Z. Xiang, S. Huang, K. Liu, B. Xiong, Wnt5a-induced M2 polarization of tumor-associated macrophages via IL-10 promotes colorectal cancer progression, *Cell Commun. Signal.* 18 (2020) 51, <https://doi.org/10.1186/s12964-020-00557-2>.
- [24] G. Jiang, J. Lin, W. Wang, M. Sun, K. Chen, F. Wang, WNT5A promoter methylation is associated with better responses and longer progression-free survival in colorectal cancer patients treated with 5-fluorouracil-based chemotherapy, *Genet. Test. Mol. Biomark.* 21 (2017) 74–79, <https://doi.org/10.1089/gtmb.2016.0162>.

- [25] Z. Chen, C. Tang, Y. Zhu, M. Xie, D. He, Q. Pan, P. Zhang, D. Hua, T. Wang, L. Jin, X. Qi, X. Yao, J. Jin, X. Ma, TrpC5 regulates differentiation through the  $Ca^{2+}$ /Wnt5a signalling pathway in colorectal cancer, *Clin. Sci.* 131 (2017) 227–237, <https://doi.org/10.1042/CS20160759>.
- [26] T.C. Huang, P.T. Lee, M.H. Wu, C.C. Huang, C.Y. Ko, Y.C. Lee, D.Y. Lin, Y. W. Cheng, K.H. Lee, Distinct roles and differential expression levels of Wnt5a mRNA isoforms in colorectal cancer cells, *PLoS One* 12 (2017), e0181034, <https://doi.org/10.1371/journal.pone.0181034>.
- [27] Z.C. Wu, L. Xiong, L.X. Wang, X.Y. Miao, Z.R. Liu, D.Q. Li, Q. Zou, K.J. Liu, H. Zhao, Z.L. Yang, Comparative study of ROR2 and WNT5a expression in squamous/adenosquamous carcinoma and adenocarcinoma of the gallbladder, *World J. Gastroenterol.* 23 (2017) 2601–2612, <https://doi.org/10.3748/wjg.v23.i14.2601>.
- [28] T. Wasniewski, J. Kiezun, B.E. Krazinski, A.E. Kowalczyk, B. Szostak, P. M. Wierzbicki, J. Kiewisz, WNT5A gene and protein expression in endometrial cancer, *Folia Histochem. Cytobiol.* 57 (2019) 84–93, <https://doi.org/10.5603/FHC.a2019.0010>.
- [29] B.G. Bitler, J.P. Nicodemus, H. Li, Q. Cai, H. Wu, X. Hua, T. Li, M.J. Birrer, A. K. Godwin, P. Cairns, R. Zhang, Wnt5a suppresses epithelial ovarian cancer by promoting cellular senescence, *Cancer Res.* 71 (2011) 6184–6194, <https://doi.org/10.1158/0008-5472.CAN-11-1341>.
- [30] C.E. Ford, G. Punnia-Moorthy, C.E. Henry, E. Llamas, S. Nixdorf, J. Olivier, R. Caduff, R.L. Ward, V. Heinzelmann-Schwarz, The non-canonical Wnt ligand, Wnt5a, is upregulated and associated with epithelial to mesenchymal transition in epithelial ovarian cancer, *Gynecol. Oncol.* 134 (2014) 338–345, <https://doi.org/10.1016/j.ygyno.2014.06.004>.
- [31] H. Qi, B. Sun, X. Zhao, J. Du, Q. Gu, Y. Liu, R. Cheng, X. Dong, Wnt5a promotes vasculogenic mimicry and epithelial-mesenchymal transition via protein kinase  $\alpha$  in epithelial ovarian cancer, *Oncol. Rep.* 32 (2014) 771–779, <https://doi.org/10.3892/or.2014.3229>.
- [32] X. Liu, Y. Wang, X. Wang, Z. Sun, L. Li, Q. Tao, B. Luo, Epigenetic silencing of WNT5A in Epstein-Barr virus-associated gastric carcinoma, *Arch. Virol.* 158 (2013) 123–132, <https://doi.org/10.1007/s00705-012-1481-x>.
- [33] L.F. Yap, M. Ahmad, M.M. Zabidi, T.L. Chu, S.J. Chai, H.M. Lee, P.V. Lim, W. Wei, C. Dawson, S.H. Teo, A.S. Khoo, Oncogenic effects of WNT5A in Epstein-Barr virus-associated nasopharyngeal carcinoma, *Int. J. Oncol.* 44 (2014) 1774–1780, <https://doi.org/10.3892/ijco.2014.2342>.
- [34] L. Qin, Y.T. Yin, F.J. Zheng, L.X. Peng, C.F. Yang, Y.N. Bao, Y.Y. Liang, X.J. Li, Y. Q. Xiang, R. Sun, A.H. Li, R.H. Zou, X.Q. Pei, B.J. Huang, T.B. Kang, D.F. Liao, Y. X. Zeng, B.O. Williams, C.N. Qian, WNT5A promotes stemness characteristics in nasopharyngeal carcinoma cells leading to metastasis and tumorigenesis, *Oncotarget* 6 (2015) 10239–10252, <https://doi.org/10.18632/oncotarget.3518>.
- [35] O. Lyros, L. Nie, T. Moore, R. Medda, M. Otterson, B. Behmaram, A. Mackinnon, I. Gockel, R. Shaker, P. Rafiee, Dysregulation of WNT5A/ROR2 signaling characterizes the progression of Barrett-associated esophageal adenocarcinoma, *Mol. Cancer Res.* 14 (2016) 647–659, <https://doi.org/10.1158/1541-7786.MCR-15-0484>.
- [36] J. Li, J. Ying, Y. Fan, L. Wu, Y. Ying, A.T. Chan, G. Srivastava, Q. Tao, WNT5A antagonizes WNT/ $\beta$ -catenin signaling and is frequently silenced by promoter CpG methylation in esophageal squamous cell carcinoma, *Cancer Biol. Ther.* 10 (2010) 617–624, <https://doi.org/10.4161/cbt.10.6.12609>.
- [37] P. Jin, Y. Song, G. Yu, The role of abnormal methylation of Wnt5a gene promoter regions in human epithelial ovarian cancer: a clinical and experimental study, *Anal. Cell. Pathol.* 2018 (2018) 6567081, <https://doi.org/10.1155/2018/6567081>.
- [38] C. Zhang, T. Wang, H. Wu, L. Zhang, K. Li, F. Wang, Y. Chen, J. Jin, D. Hua, HEF1 regulates differentiation through the Wnt5a/ $\beta$ -catenin signaling pathway in human gastric cancer, *Biochem. Biophys. Res. Commun.* 509 (2019) 201–208, <https://doi.org/10.1016/j.bbrc.2018.12.104>.
- [39] T. Matsuo, M. Ito, M. Tatsugami, T. Boda, S. Takata, S. Tanaka, K. Chayama, Gastric cancer development after Helicobacter pylori eradication therapy: a new form of gastric neoplasia, *Digestion* 85 (2012) 61–67, <https://doi.org/10.1159/00035260>.
- [40] P. Li, Y. Cao, Y. Li, L. Zhou, X. Liu, M. Geng, Expression of Wnt-5a and  $\beta$ -catenin in primary hepatocellular carcinoma, *Int. J. Clin. Exp. Pathol.* 7 (2014) 3190–3195, (<https://www.ncbi.nlm.nih.gov/pubmed/25031739>).
- [41] M. Kanzawa, S. Semba, S. Hara, T. Itoh, H. Yokozaki, WNT5A is a key regulator of the epithelial-mesenchymal transition and cancer stem cell properties in human gastric carcinoma cells, *Pathobiology* 80 (2013) 235–244, <https://doi.org/10.1159/000346843>.
- [42] H. Hanaki, H. Yamamoto, H. Sakane, S. Matsumoto, H. Ohdan, A. Sato, A. Kikuchi, An anti-Wnt5a antibody suppresses metastasis of gastric cancer cells in vivo by inhibiting receptor-mediated endocytosis, *Mol. Cancer Ther.* 11 (2012) 298–307, <https://doi.org/10.1158/1535-7163.MCT-11-0682>.
- [43] G. Liu, T. Yan, X. Li, J. Sun, B. Zhang, H. Wang, Y. Zhu, Daam1 activates RhoA to regulate Wnt5a-induced glioblastoma cell invasion, *Oncol. Rep.* 39 (2018) 465–472, <https://doi.org/10.3892/or.2017.6124>.
- [44] Y. Kim, M. Hong, I.G. Do, S.Y. Ha, D. Lee, Y.L. Suh, Wnt5a, Ryk and Ror2 expression in glioblastoma subgroups, *Pathol. Res. Pract.* 211 (2015) 963–972, <https://doi.org/10.1016/j.prp.2015.10.001>.
- [45] M. Kamino, M. Kishida, T. Kibe, K. Ikoma, M. Iijima, H. Hirano, M. Tokudome, L. Chen, C. Koriyama, K. Yamada, K. Arita, S. Kishida, Wnt-5a signaling is correlated with infiltrative activity in human glioma by inducing cellular migration and MMP-2, *Cancer Sci.* 102 (2011) 540–548, <https://doi.org/10.1111/j.1349-7006.2010.01815.x>.
- [46] Á. Nagy, M. Tompa, Z. Krabóth, F. Garzuly, A. Marácz, B. Kálmán, Wnt pathway markers in low-grade and high-grade gliomas, *Ideggyogy. Sz.* 74 (2021) 349–355, <https://doi.org/10.18071/isz.74.0349>.
- [47] Ö. Hattmaz Ng, S. Firtina, I. Can, Z. Karakaş, L. Ağaoğlu, Ö. Doğru, T. Celkan, A. Akçay, Y. Yıldırım, Ç. Timur, U. Özbek, M. Sayitoğlu, A possible role for WNT5A hypermethylation in pediatric acute lymphoblastic leukemia, *Türk. J. Haematol.* 32 (2015) 127–135, <https://doi.org/10.4274/tjh.2013.0296>.
- [48] H.R. Zhou, H.Y. Fu, D.S. Wu, Y.Y. Zhang, S.H. Huang, C.J. Chen, J.G. Yan, J. L. Huang, J.Z. Shen, Relationship between epigenetic changes in Wnt antagonists and acute leukemia, *Oncol. Rep.* 37 (2017) 2663–2671, <https://doi.org/10.3892/or.2017.5509>.
- [49] G. Deng, Z.Q. Li, C. Zhao, Y. Yuan, C.C. Niu, J. Pan, W.K. Si, WNT5A expression is regulated by the status of its promoter methylation in leukaemia and can inhibit leukemic cell malignant proliferation, *Oncol. Rep.* 25 (2011) 367–376, <https://doi.org/10.3892/or.2010.1108>.
- [50] H. Liang, Q. Chen, A.H. Coles, S.J. Anderson, G. Pihan, A. Bradley, R. Gerstein, R. Jurecic, S.N. Jones, Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in hematopoietic tissue, *Cancer Cell* 4 (2003) 349–360, [https://doi.org/10.1016/s1535-6108\(03\)00268-x](https://doi.org/10.1016/s1535-6108(03)00268-x).
- [51] F. Linke, S. Zaunig, M.M. Nietert, F. von Bonin, S. Lutz, C. Dullin, P. Janovská, T. Beissbarth, F. Alves, W. Klapper, V. Bryja, T. Pukrop, L. Trümper, J. Wilting, D. Kube, WNT5A: a motility-promoting factor in Hodgkin lymphoma, *Oncogene* 36 (2017) 13–23, <https://doi.org/10.1038/ncr.2016.183>.
- [52] V. Martín, A. Valencia, X. Agirre, J. Cervera, E. San Jose-Eneriz, A. Vilas-Zornoza, P. Rodriguez-Otero, M.A. Sanz, C. Herrera, A. Torres, F. Prosper, J. Román-Gómez, Epigenetic regulation of the non-canonical Wnt pathway in acute myeloid leukemia, *Cancer Sci.* 101 (2010) 425–432, <https://doi.org/10.1111/j.1349-7006.2009.01413.x>.
- [53] Y.C. Chong, T.E. Lim, Y. Fu, E.M. Shin, V. Tergaonkar, W. Han, Indian Hedgehog links obesity to development of hepatocellular carcinoma, *Oncogene* 38 (2019) 2206–2222, <https://doi.org/10.1038/s41388-018-0585-5>.
- [54] L. Wang, M. Yao, M. Fang, W.J. Zheng, Z.Z. Dong, L.H. Pan, H.J. Zhang, D.F. Yao, Expression of hepatic Wnt5a and its clinicopathological features in patients with hepatocellular carcinoma, *Hepatobiliary Pancreat. Dis. Int.* 17 (2018) 227–232, <https://doi.org/10.1016/j.hbpd.2018.03.005>.
- [55] Y.M. Whang, U. Jo, J.S. Sung, H.J. Ju, H.K. Kim, K.H. Park, J.W. Lee, I.S. Koh, Y. H. Kim, Wnt5a is associated with cigarette smoke-related lung carcinogenesis via protein kinase C, *PLoS One* 8 (2013), e53012, <https://doi.org/10.1371/journal.pone.0053012>.
- [56] H. Li, F. Tong, R. Meng, L. Peng, J. Wang, R. Zhang, X. Dong, E2F1-mediated repression of WNT5A expression promotes brain metastasis dependent on the ERK1/2 pathway in EGFR-mutant non-small cell lung cancer, *Cell Mol. Life Sci.* 78 (2021) 2877–2891, <https://doi.org/10.1007/s00018-020-03678-6>.
- [57] L. Yao, B. Sun, X. Zhao, Q. Gu, X. Dong, Y. Zheng, J. Sun, R. Cheng, H. Qi, J. An, Overexpression of Wnt5a promotes angiogenesis in NSCLC, *BioMed Res. Int.* 2014 (2014), 832562, <https://doi.org/10.1155/2014/832562>.
- [58] B. Wang, Z. Tang, H. Gong, L. Zhu, X. Liu, Wnt5a promotes epithelial-to-mesenchymal transition and metastasis in non-small-cell lung cancer, *Biosci. Rep.* 37 (2017), <https://doi.org/10.1042/BSR20171092>.
- [59] S.E. Lee, S.D. Lim, S.Y. Kang, S.B. Suh, Y.L. Suh, Prognostic significance of Ror2 and Wnt5a expression in medulloblastoma, *Brain Pathol.* 23 (2013) 445–453, <https://doi.org/10.1111/bpa.12017>.
- [60] P. Mohapatra, C.P. Prasad, T. Andersson, Combination therapy targeting the elevated interleukin-6 level reduces invasive migration of BRAF inhibitor-resistant melanoma cells, *Mol. Oncol.* 13 (2019) 480–494, <https://doi.org/10.1002/1878-0261.12433>.
- [61] S.M. Douglass, M.E. Fane, E. Sanseviero, B.L. Ecker, C.H. Kugel, R. Behera, V. Kumar, E.N. Tcyganov, X. Yin, Q. Liu, Y. Chhabra, G.M. Alicea, R. Kuruvilla, D. I. Gabrilovich, A.T. Weeraratna, Myeloid-derived suppressor cells are a major source of Wnt5A in the melanoma microenvironment and depend on Wnt5A for full suppressive activity, *Cancer Res.* 81 (2021) 658–670, <https://doi.org/10.1158/0008-5472.CAN-20-1238>.
- [62] R. Linnskog, P. Mohapatra, F. Moradi, C.P. Prasad, T. Andersson, Demonstration of a WNT5A-IL-6 positive feedback loop in melanoma cells: dual interference of this loop more effectively impairs melanoma cell invasion, *Oncotarget* 7 (2016) 37790–37802, <https://doi.org/10.18632/oncotarget.9332>.
- [63] E.J. Ekström, C. Bergenfelz, V. von Bülow, F. Serfler, E. Carlemalm, G. Jönsson, T. Andersson, K. Leandersson, WNT5A induces release of exosomes containing pro-angiogenic and immunosuppressive factors from malignant melanoma cells, *Mol. Cancer* 13 (2014) 88, <https://doi.org/10.1186/1476-4598-13-88>.
- [64] S.K. Dissanayake, A.T. Weeraratna, Detecting PKC phosphorylation as part of the Wnt/calcium pathway in cutaneous melanoma, *Methods Mol. Biol.* 468 (2008) 157–172, [https://doi.org/10.1007/978-1-59745-249-6\\_12](https://doi.org/10.1007/978-1-59745-249-6_12).
- [65] P.D. Da Forno, J.H. Pringle, P. Hutchinson, J. Osborn, Q. Huang, L. Potter, R. A. Hancox, A. Fletcher, G.S. Saldanha, WNT5A expression increases during melanoma progression and correlates with outcome, *Clin. Cancer Res.* 14 (2008) 5825–5832, <https://doi.org/10.1158/1078-0432.CCR-07-5104>.
- [66] C. Pourreynon, L. Reilly, C. Proby, A. Panteleyev, C. Fleming, K. McLean, A. P. South, J. Foerster, Wnt5a is strongly expressed at the leading edge in non-melanoma skin cancer, forming active gradients, while canonical Wnt signalling is repressed, *PLoS One* 7 (2012), e31827, <https://doi.org/10.1371/journal.pone.0031827>.
- [67] A.T. Weeraratna, R. Houben, M.P. O'Connell, J.C. Becker, Lack of Wnt5A expression in Merkel cell carcinoma, *Arch. Dermatol.* 146 (2010) 88–89, <https://doi.org/10.1001/archdermatol.2009.348>.



- [68] Z. Prigmet, L. Axelsson, P. Lindberg, T. Andersson, Migration and invasion of oral squamous carcinoma cells is promoted by WNT5A, a regulator of cancer progression, *J. Oral Pathol. Med.* 44 (2015) 776–784, <https://doi.org/10.1111/jop.12292>.
- [69] W. Khan, V.C. Haragannavar, R.S. Rao, K. Prasad, S.V. Sowmya, D. Augustine, S. Patil, P-Cadherin and WNT5A expression in assessment of lymph node metastasis in oral squamous cell carcinoma, *Clin. Oral Invest.* 26 (2022) 259–273, <https://doi.org/10.1007/s00784-021-03996-4>.
- [70] H. Vaidya, C. Rumph, K.S. Katula, Inactivation of the WNT5A alternative promoter B is associated with DNA methylation and histone modification in osteosarcoma cell lines U2OS and SaOS-2, *PLoS One* 11 (2016), e0151392, <https://doi.org/10.1371/journal.pone.0151392>.
- [71] S. Arabzadeh, G. Hossein, Z. Salehi-Dulabi, A.H. Zarnani, WNT5A-ROR2 is induced by inflammatory mediators and is involved in the migration of human ovarian cancer cell line SKOV-3, *Cell. Mol. Biol. Lett.* 21 (2016) 9, <https://doi.org/10.1186/s11658-016-0003-3>.
- [72] A. Kotrbová, P. Ovesná, T. Gybel', T. Radaszkiewicz, M. Bednářková, J. Hausnerová, E. Jandáková, L. Minář, I. Crha, V. Weinberger, L. Závěský, V. Bryja, V. Pospíšalová, WNT signaling inducing activity in ascites predicts poor outcome in ovarian cancer, *Theranostics* 10 (2020) 537–552, <https://doi.org/10.7150/thno.37423>.
- [73] C. Peng, X. Zhang, H. Yu, D. Wu, J. Zheng, Wnt5a as a predictor in poor clinical outcome of patients and a mediator in chemoresistance of ovarian cancer, *Int. J. Gynecol. Cancer* 21 (2011) 280–288, <https://doi.org/10.1097/IGC.0b013e31820aaadb>.
- [74] H. Bo, S. Zhang, L. Gao, Y. Chen, J. Zhang, X. Chang, M. Zhu, Upregulation of Wnt5a promotes epithelial-to-mesenchymal transition and metastasis of pancreatic cancer cells, *BMC Cancer* 13 (2013) 496, <https://doi.org/10.1186/1471-2407-13-496>.
- [75] W. Wei, H.H. Sun, N. Li, H.Y. Li, X. Li, Q. Li, X.H. Shen, WNT5A modulates cell cycle progression and contributes to the chemoresistance in pancreatic cancer cells, *Hepatobiliary Pancreat. Dis. Int* 13 (2014) 529–538, [https://doi.org/10.1016/s1499-3872\(14\)60277-0](https://doi.org/10.1016/s1499-3872(14)60277-0).
- [76] L. Remtisch, G. Wiltberger, K. Schierle, M. Yousef, R. Thieme, B. Jansen Winkeln, C. Wittekind, I. Gockel, O. Lyros, The WNT5A/ROR2 signaling pathway in pancreatic ductal adenocarcinoma (PDAC), *J. BUON* 26 (2021) 1595–1606, <https://www.ncbi.nlm.nih.gov/pubmed/34565024>.
- [77] A.L. Schwartz, R. Malgor, E. Dickerson, A.T. Weeraratna, A. Slominski, J. Wortsman, N. Harii, A.D. Kohn, R.T. Moon, F.L. Schwartz, D.J. Goetz, L. D. Kohn, K.D. McCall, Phenylmethimazole decreases Toll-like receptor 3 and noncanonical Wnt5a expression in pancreatic cancer and melanoma together with tumor cell growth and migration, *Clin. Cancer Res.* 15 (2009) 4114–4122, <https://doi.org/10.1158/1078-0432.CCR-09-0005>.
- [78] G. Canesin, S. Evans-Axelsson, R. Hellsten, A. Krzyzanowska, C.P. Prasad, A. Bjartell, T. Andersson, Treatment with the WNT5A-mimicking peptide Foxy-5 effectively reduces the metastatic spread of WNT5A-low prostate cancer cells in an orthotopic mouse model, *PLoS One* 12 (2017), e0184418, <https://doi.org/10.1371/journal.pone.0184418>.
- [79] S. Thiele, A. Göbel, T.D. Rachner, S. Fuessel, M. Froehner, M.H. Muders, G. B. Baretton, R. Bernhardt, F. Jakob, C.C. Glüer, M. Bornhäuser, M. Rauner, L. C. Hofbauer, WNT5A has anti-prostate cancer effects in vitro and reduces tumor growth in the skeleton in vivo, *J. Bone Min. Res.* 30 (2015) 471–480, <https://doi.org/10.1002/jbmr.2362>.
- [80] A.S. Khaja, L. Egevad, L. Helczynski, P. Wiklund, T. Andersson, A. Bjartell, Emphasizing the role of Wnt5a protein expression to predict favorable outcome after radical prostatectomy in patients with low-grade prostate cancer, *Cancer Med.* 1 (2012) 96–104, <https://doi.org/10.1002/cam4.5>.
- [81] F. Jin, X. Qu, Q. Fan, L. Wang, T. Tang, Y. Hao, K. Dai, Regulation of prostate cancer cell migration toward bone marrow stromal cell-conditioned medium by Wnt5a signaling, *Mol. Med. Rep.* 8 (2013) 1486–1492, <https://doi.org/10.3892/mmr.2013.1698>.
- [82] Q. Wang, M. Williamson, S. Bott, N. Brookman-Amissh, A. Freeman, J. Nariculam, M.J. Hubank, A. Ahmed, J.R. Masters, Hypomethylation of WNT5A, CRIP1 and S100P in prostate cancer, *Oncogene* 26 (2007) 6560–6565, <https://doi.org/10.1038/sj.onc.1210472>.
- [83] S.D. Lim, W.S. Kim, G.Y. Kwon, ROR2 and Wnt5a expression in stage 1 pure testicular seminomas, *Anal. Quant. Cytopathol. Histopathol.* 35 (2013) 41–51, <https://www.ncbi.nlm.nih.gov/pubmed/23469623>.
- [84] R. Malgor, S. Crouser, D. Greco, C. Brockett, K. Coschigano, M. Nakazawa, S. Jenkinson, Correlation of Wnt5a expression with histopathological grade/stage in urothelial carcinoma of the bladder, *Diagn. Pathol.* 8 (2013) 139, <https://doi.org/10.1186/1746-1596-8-139>.
- [85] Y. Zhao, Y. Miao, X. Chen, L. Wang, F. Lu, W. Liao, Preparation of the recombinant lentiviral expression vector targeting human Wnt5a gene and its inhibitory effect on melanoma cell invasion, *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 30 (2014) 462–465, <https://www.ncbi.nlm.nih.gov/pubmed/24796738>.
- [86] P. Lopez-Bergami, G. Barbero, The emerging role of Wnt5a in the promotion of a pro-inflammatory and immunosuppressive tumor microenvironment, *Cancer Metastasis Rev.* 39 (2020) 933–952, <https://doi.org/10.1007/s10555-020-09878-7>.
- [87] C. Schreck, R. Istvánffy, C. Ziegenhain, T. Sippenauer, F. Ruf, L. Henkel, F. Gärtner, B. Vieth, M.C. Florian, N. Mende, A. Taubenberger, A. Prendergast, A. Wagner, C. Pagel, S. Grziwok, K.S. Götz, J. Guck, D.C. Dean, S. Massberg, M. Essers, C. Waskow, H. Geiger, M. Schiemann, C. Peschel, W. Enard, R. A. Oostendorp, Niche WNT5A regulates the actin cytoskeleton during regeneration of hematopoietic stem cells, *J. Exp. Med.* 214 (2017) 165–181, <https://doi.org/10.1084/jem.20151414>.
- [88] Y.-J. Choi, A.M. Heck, B.J. Hayes, D. Lih, S.G. Rayner, B. Hadland, Y. Zheng, WNT5A from the fetal liver vascular niche supports human fetal liver hematopoiesis, *Stem Cell Res. Ther.* 12 (2021) 321, <https://doi.org/10.1186/s13287-021-02380-z>.
- [89] E.R. Bakker, A.M. Das, W. Helvensteijn, P.F. Franken, S. Swagemakers, M.A. van der Valk, T.L. ten Hagen, E.J. Kuipers, W. van Veelen, R. Smits, Wnt5a promotes human colon cancer cell migration and invasion but does not augment intestinal tumorigenesis in Apc1638N mice, *Carcinogenesis* 34 (2013) 2629–2638, <https://doi.org/10.1093/carcin/bgt215>.
- [90] Y. Hayakawa, H. Ariyama, J. Stancikova, K. Sakitani, S. Asfaha, B.W. Renz, Z. A. Dubeykovskaya, W. Shibata, H. Wang, C.B. Westphalen, X. Chen, Y. Takemoto, W. Kim, S.S. Khurana, Y. Tailor, K. Nagar, H. Tomita, A. Hara, A.R. Sepulveda, W. Setlik, M.D. Gershon, S. Saha, L. Ding, Z. Shen, J.G. Fox, R.A. Friedman, S. F. Konieczny, D.L. Worthley, V. Korinek, T.C. Wang, Mist1 expressing gastric stem cells maintain the normal and neoplastic gastric epithelium and are supported by a perivascular stem cell niche, *Cancer Cell* 28 (2015) 800–814, <https://doi.org/10.1016/j.cccell.2015.10.003>.
- [91] E. Zoico, E. Darra, V. Rizzatti, S. Budui, G. Franceschetti, G. Mazzali, A.P. Rossi, F. Fantin, M. Menegazzi, S. Cinti, M. Zamboni, Adipocytes WNT5a mediated dedifferentiation: a possible target in pancreatic cancer microenvironment, *Oncotarget* 7 (2016) 20223–20235, <https://doi.org/10.18632/oncotarget.7936>.
- [92] J. Osman, K. Bellamkonda, Q. Liu, T. Andersson, A. Sjölander, The WNT5A agonist Foxy5 reduces the number of colonic cancer stem cells in a xenograft mouse model of human colonic cancer, *Anticancer Res.* 39 (2019) 1719–1728, <https://doi.org/10.21873/anticancer.13278>.
- [93] Z. Jin, C. Zhao, X. Han, Y. Han, Wnt5a promotes ewing sarcoma cell migration through upregulating CXCR4 expression, *BMC Cancer* 12 (2012) 480, <https://doi.org/10.1186/1471-2407-12-480>.
- [94] H. Griesmann, S. Ripka, M. Pralle, V. Ellenrieder, S. Baumgart, M. Buchholz, C. Pilarsky, D. Aust, T.M. Gress, P. Michl, WNT5A-NFAT signaling mediates resistance to apoptosis in pancreatic cancer, *Neoplasia* 15 (2013) 11–22, <https://doi.org/10.1593/neo.121312>.
- [95] J. Potratz, A. Tillmanns, P. Berning, E. Korsching, C. Schaefer, B. Lechtape, C. Schleithoff, R. Unland, K.L. Schäfer, C. Müller-Tidow, H. Jürgens, U. Dirksen, Receptor tyrosine kinase gene expression profiles of Ewing sarcomas reveal ROR1 as a potential therapeutic target in metastatic disease, *Mol. Oncol.* 10 (2016) 677–692, <https://doi.org/10.1016/j.molonc.2015.12.009>.
- [96] Q. Wang, A.J. Symes, C.A. Kane, A. Freeman, J. Nariculam, P. Munson, C. Thrasivoulou, J.R. Masters, A. Ahmed, A novel role for Wnt/Ca<sup>2+</sup> signaling in actin cytoskeleton remodeling and cell motility in prostate cancer, *PLoS One* 5 (2010), e10456, <https://doi.org/10.1371/journal.pone.0010456>.
- [97] M. Saling, J.K. Duckett, I. Ackers, K. Coschigano, S. Jenkinson, R. Malgor, Wnt5a/planar cell polarity signaling pathway in urothelial carcinoma, a potential prognostic biomarker, *Oncotarget* 8 (2017) 31655–31665, <https://doi.org/10.18632/oncotarget.15877>.
- [98] S. Bose, L.F. Yap, M. Fung, J. Starzynski, A. Saleh, S. Morgan, C. Dawson, M. B. Chukwuma, E. Maina, M. Buettner, W. Wei, J. Arrand, P.V. Lim, L.S. Young, S. H. Teo, T. Stankovic, C.B. Woodman, P.G. Murray, The ATM tumour suppressor gene is down-regulated in EBV-associated nasopharyngeal carcinoma, *J. Pathol.* 217 (2009) 345–352, <https://doi.org/10.1002/path.2487>.
- [99] P. Ramos, M. Bentires-Alj, Mechanism-based cancer therapy: resistance to therapy, therapy for resistance, *Oncogene* 34 (2015) 3617–3626, <https://doi.org/10.1038/onc.2014.314>.
- [100] V. Jenei, V. Sherwood, J. Howlin, R. Linnskog, A. Säfholm, L. Axelsson, T. Andersson, A t-butyloxycarbonyl-modified Wnt5a-derived hexapeptide functions as a potent antagonist of Wnt5a-dependent melanoma cell invasion, *Proc. Natl. Acad. Sci. USA* 106 (2009) 19473–19478, <https://doi.org/10.1073/pnas.0909409106>.