Wnt1 inducible signalling pathway protein-2 (WISP-2/CCN5): Roles and regulation in human cancers (Review)

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Received August 7, 2013; Accepted September 27, 2013

DOI: 10.3892/or.2013.2909

Abstract. Wnt1 inducible signalling pathway protein-2 (WISP-2), also known as CCN5, CT58, CTGF-L, CTGF-3, HICP and Cop1, is one of the 3 WNT1 inducible proteins that belongs to the CCN family. This family of members has been shown to play multiple roles in a number of pathophysiological processes, including cell proliferation, adhesion, wound healing, extracellular matrix regulation, epithelial-mesenchymal transition, angiogenesis, fibrosis, skeletal development and embryo implantation. Recent results suggest that WISP-2 is relevant to tumorigenesis and malignant transformation, particularly in breast cancer, colorectal cancer and hepatocarcinoma. Notably, its roles in cancer appear to vary depending on cell/tumour type and the microenvironment. The striking difference in the structure of WISP-2 in comparison with the other 2 family members may contribute to its difference in functions, which leads to the hypothesis that WISP-2 may act as a dominant-negative regulator of other CCN family members. In the present review, we summarise the roles, regulation and underlying mechanism of WISP-2 in human cancers.

Contents

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Key words: Wnt1 inducible signalling pathway protein-2, CCN5, epithelial-mesenchymal transition, angiogenesis, cellular migration, metastasis

1. Introduction

WISP proteins [WNT1 (wingless-type MMTV integration site family, member 1)-inducible signalling pathway proteins] are a subfamily of the CCN family (1). WNT1 is a member of a family of cysteine-rich, glycosylated signalling proteins that mediate diverse developmental processes (2). The CCN family of proteins is a crucial group of signalling molecules found in eukaryotic organisms, and its nomenclature is based on the first 3 members of the family: cysteine-rich protein 61 (CYR61), connective tissue growth factor (CTGF) and nephroblastoma overexpressed gene (NOV) (3), which are now designated as CCN1, CCN2 and CCN3; and 3 other family members WISP-1 WISP-2 and WISP-3 are designated as CCN4, CCN5 and CCN6 (4).

rCop-1 (CCN5) was first identified as being downregulated following transformation of rat embryo fibroblasts by inactivation of p53 and concomitant activation of H-ras (5). At a similar time, WISP-1 and -2 were identified as an indirect response to WNT1 but not WNT4 induction in C57MG mouse mammary epithelial cells. After sequence alignment, human WISP-1, -2 and -3 were found to be homologous and were cloned in 1998 (1). These WISP proteins exhibit the modular structure of the CCN family, characterised by 4 conserved cysteine-rich domains and are believed to be homologous to the CTGF family of proteins of the CCN family. Another research group analysed a human osteoblast cDNA library and identified an EST that contained an IGF binding domain, and based on sequence homology to the CCN family member CTGF, the authors named the predicted gene product as CTGF-like protein or CTGF-L (6). CTGF-L (CCN5), encoding a 250-amino acid single-chain polypeptide of 26 kDa, lacks the C-terminal domain implicated in dimerisation and heparin binding. These early discoveries led to further research into their roles in cell signalling, proliferation, adhesion, invasion,

wound healing, fibrosis, skeletal development, implantation, epithelial-mesenchymal transition and angiogenesis as well as in cancers. WISP-2 has been particularly well investigated in human cancers and is the main subject of the present review.

2. Structure of WISP-2

Human *WISP-2* is located at chromosome 20q13.12 and consists of 3 exons. Full-length cDNA clones of human WISP-2 are 1,293 bp in length and encode a protein of 250 amino acids, with a predicted relative molecular mass of 26 kDa. Mouse and human WISP-2 are 73% identical at the amino acid level and homologous to the rat gene, rCop-1 (5). The nucleotide and protein sequence of WISP-2 shares a 30-40% sequence homology with other family members.

WISP proteins exhibit the modular architecture of CCN family members that are characterised by 4 conserved and discrete cysteine-rich domains that act both independently and in concert; the N-terminal domain, which includes the first 12 cysteine residues, contains a consensus sequence (GCGCCXXC) conserved in most insulin-like growth factor (IGF)-binding proteins (BP) (IGFBPs). This sequence is conserved in WISP-2 and it has been found that a truncated *nov* protein lacking the IGFBP domain in chicken embryo fibroblasts was sufficient to induce their malignant transformation (7). The von Willebrand factor type C module (VWC) covers the next 10 cysteine residues, and is thought to participate in protein complex formation and oligomerisation (8). A short variable region closely following the VWC domain is highly susceptible to proteolytic degradation by matrix metalloproteases (MMPs) (9). It has been shown that a wide variety of MMPs (MMP-1, -3, -7, -9 and -13) targets this central linker region, and additional proteases such as elastase and plasmin could attack linkers that connect domains 1 and 2 or domains 3 and 4 (10). The third domain, the thrombospondin domain (TSP), is implicated in binding with sulfated glycoconjugates and contains 6 cysteine residues and a conserved WSxCSxxCG motif first identified in thrombospondin (11) and necessary for the regulation of endothelial cell proliferation and promotion of cell attachment (3,12). The C-terminal cystine knot-like (CT) domain is present in all CCN family members described to date while WISP-2-encoded protein lacks this domain which is implicated in receptor binding and dimerisation (13). These receptors include heparin (14), matrix molecules, integrins and signalling molecules such as Notch-1 and LRP1 (15).

Due to the importance of the CT domain in receptor binding, WISP-2 may bind its receptor through other domains, such as the IGFBP domain (6). Heparin-binding growth factor (HBGF) from pig uterine luminal flushings was identified as a highly truncated form of CTGF and showed that the N-terminal 2/3 of the CTGF primary translation product is not required for mitogenic activity or heparin binding, and mitogenic activity of the 10-kDa truncated form of CTGF is heparin-dependent (14). Growth factors, such as platelet-derived growth factor, TGF-β and nerve growth factor, which contain a cystine knot motif, also exist as dimers. This has led to speculation that WISP-1 and -3 may exist as dimers, whereas WISP-2 exists as a monomer (1). This has further led to the hypothesis that WISP-2 may act as a dominant-negative regulator of other CCN family members (16). Furthermore, the existence of a putative signal sequence in front of the N-terminal IGFBP domain and the absence of a transmembrane domain suggest that WISP-2 is a secreted protein (Fig. 1).

3. WISP-2 expression and clinical significance in human cancers

Following the identification of WISP-2 as a Wnt1 inducible protein, a number of researchers have focused on the roles and regulation for this molecule in human disorders, particularly in cancers.

Clinical studies have shown different expression profiles and roles of WISP-2 in cancers. The inconsistency between the results in multiple cancers has raised uncertainty concerning the role of WISP-2 in carcinogenesis. For example, induction of WISP-2 by IGF-1 or EGF is required for the mitogenic action in oestrogen receptor-positive non-invasive breast tumour cells (17-19), while it acts as a growth arrest-specific (gas) gene in vascular smooth muscle cells and prostate cancer cells (20). In addition, it is likely that WISP-2 plays a preventive role in the progression of pancreatic cancer as it participates in morphological alterations from mesenchymal to epithelial transition (MET) of pancreatic adenocarcinoma (21) and breast cancer cells (22).

The first study concerning tumour cells was reported in 2000. WISP-2 was found to be markedly increased in 17 β-estradiol-treated MCF-7 human breast cancer cells compared with control cells and was directly regulated by the oestrogen receptor (23). The induction of secreted WISP-2 protein by oestrogen in the culture supernatant was dose-dependent (24,25). It was therefore believed to be an oestrogen response gene. A number of other studies have since reported the relationship between WISP-2 and oestrogen, mostly in breast cancer.

Breast cancer. More than 20 studies from several laboratories suggest that elevated WISP-2 has a particular relevance to human breast disease *in vitro* and *in vivo* (21,22,25-28), and WISP-2 has been indicated as a useful indicator of breast cancer progression (29). In these studies, WISP-2 mRNA and protein levels were found to be elevated in different human breast tumour-derived cell lines, such as MCF-7, ZR-75, T-47D and SKBR2 (26,30), in node-positive breast tumours with metastatic potential and in breast tumours from patients with a poor prognosis (28). These studies also showed that WISP-2 was either undetectable or minimally detectable in normal breast epithelial cells.

Similar reports from Banerjee *et al* showed that WISP-2 was upregulated in non-invasive MCF-7 cells by epithelial growth factor, and was believed to be linked to poor prognosis in breast cancer (25). Silencing of the function of the *WISP-2* gene minimized serum-induced breast tumour cell proliferation (17). Banerjee *et al* found that WISP-2 expression in breast samples was biphasic; a marked increase was noted in non-invasive breast lesions but a significant decrease was found as cancers progressed from a non-invasive to an invasive type (22). WISP-2 became almost undetectable in poorly differentiated cancers when compared with moderately or well-differentiated samples including testing with

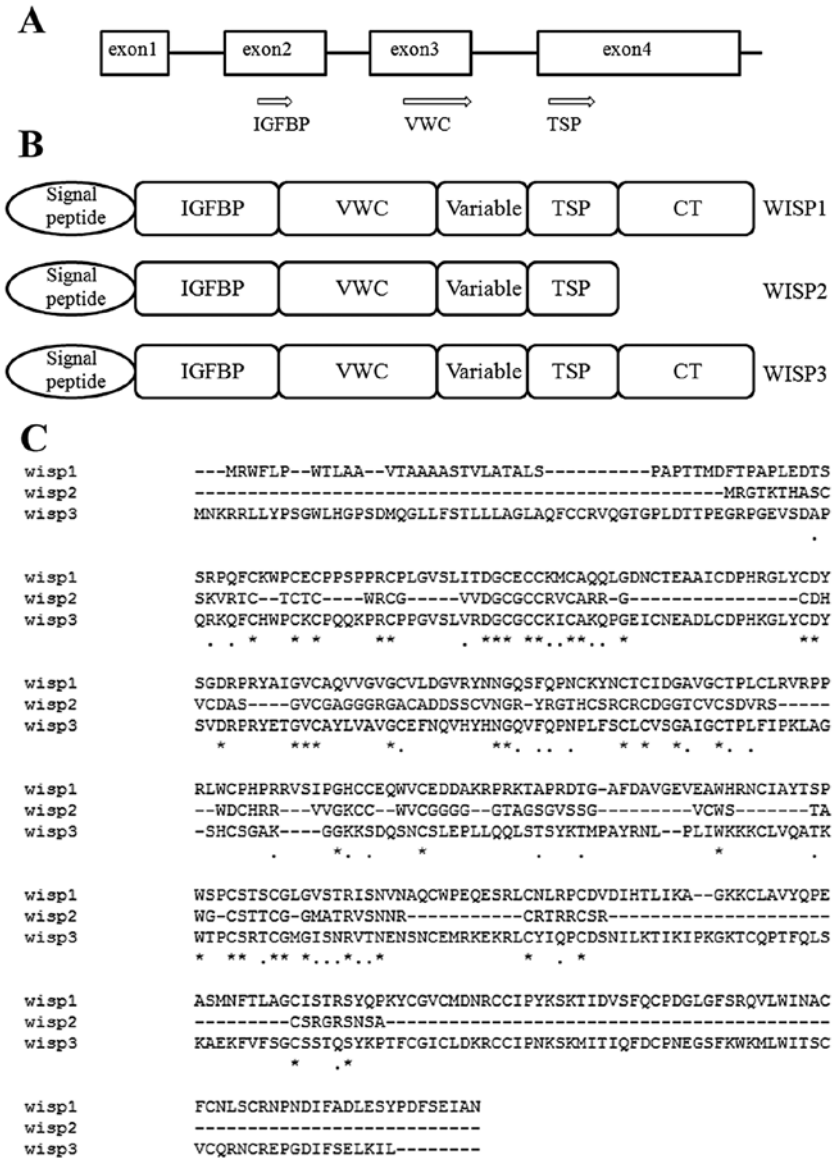

Figure 1. Structure of WISP-2. (A) Exon-intron structure and domain location of the WISP-2 gene. IGFBP, 37 bp; VWC, 61 bp and TSP, 44 bp. (B) Schematic representation of the WISP proteins showing the domain structure. WISP-2 lacks the CT domain. (C) Sequence alignment of WISP proteins; identity = 33.68%, with Dnaman software. WISP-2, Wnt1 inducible signalling pathway protein-2; IGFBP, insulin-like growth factor binding proteins; CT, cystine knot-like; VWC, von Willebrand factor type C; TSP, thrombospondin.

microdissected sections (22). This indicated a possible protective function of WISP-2 in non-invasive breast tumour cells. In contrast, the same group also reported that in hormone-related tumours, including breast cancer, the activation of WISP-2 expression by oestrogen promoted cancer progression, and disruption of WISP-2 signalling by use of antisense oligomers in MCF-7 cells caused a significant reduction in tumour cell proliferation (25).

Pancreatic adenocarcinoma. Pancreatic adenocarcinoma exhibits greatly decreased levels of WISP-2 expression compared with adjacent normal pancreatic tissue and chronic pancreatitis, and the loss of WISP-2 mRNA was associated with overexpression of p53 which was similar to that in breast carcinoma. Dhar *et al* revealed a strong correlation between the degree of differentiation and progression of pancreatic adenocarcinoma and decreased expression of the WISP-2 signalling protein, which indicated that the development of pancreatic adenocarcinoma was associated with the silencing of WISP-2 signalling (21). Treatment of the highly aggressive pancreatic cancer cell line, MIA-PaCa-2, with recombinant WISP-2 protein reduced the expression of the mesenchymal cell marker vimentin and altered the morphological appearance of the cells to a cobble stoned, epithelial-like phenotype. These results suggest that WISP-2 may have a role in maintaining an epithelial-like phenotype in pancreatic adenocarcinoma cells thereby decreasing their invasive potential.

Colorectal cancer. Research has shown that WISP-2 is a potential tumour-suppressor in colorectal cancer. WISP-2 DNA was amplified in colon tumours, but its transcription was significantly reduced in the majority of tumours when compared with that in paired normal colonic mucosa (1). The gene for human WISP-2 is localised to chromosome 20q13, in a region

frequently amplified and associated with poor prognosis in node-negative breast cancer and colon cancers, suggesting the existence of 1 or more oncogenes at this locus (31,32). It is possible that the apparent amplification observed for WISP-2 may be caused by another gene in this amplicon (1). In another cohort which was comprised of 94 human colorectal tumours and 80 normal colorectal tissues, WISP-2 showed a significantly lower level of expression in colorectal cancer cells when compared with that in normal cells. Although no significant differences were found within the cancer group when indices of a more aggressive tumour were compared with the normal tissue, a significant reduction in expression was associated with Dukes' stage, poor differentiation, lower TNM stage and node-positive disease (33).

Hepatocarcinoma. Research found that the WISP-2 transcript was not expressed in the 4 hepatocellular carcinoma-derived cell lines HepG2, HuH-6, HuH-7 and HA22T/VGH (34). However, overexpression of the hepatitis C viral core protein in Huh-7 cells caused upregulation of Wnt-1 and WISP-2 and increased proliferation of the cells (35). As 1 of the 13 genes activated by Wnt/β-catenin signalling pathway T-cell transcription factor 4J isoform in HCC cells, WISP-2 was also upregulated in HCC tumours when compared with that in adjacent peritumour tissues (36).

Skin cancer. WISP-2 is one of the most abundantly expressed mRNAs of the CCN family members in normal human skin. Following exposure to UV irradiation, WISP-2 expression was found to be decreased by 50% at 24 h and returned to a basal level at 48 h (37).

Pituitary tumours. WISP-2 was found to be overexpressed in adrenocorticotropic hormone (ACTH)-secreting pituitary tumours when compared to its expression in normal pituitaries, non-secreting pituitary tumours and growth hormone (GH)-secreting tumours (38). There was otherwise no reported association between WISP-2 and gender, age at diagnosis, tumour size, altered visual field, remission of the disease, or tumour progression in any subtype of pituitary tumours.

Gastric cancer. The expression of the 3 WISP molecules in a cohort of 316 cases of human gastric cancers and normal gastric tissues were analyzed using q-PCR and IHC, respectively, and were correlated with the clinicopathological features and outcome of the patients by our group (39). Knockdown of WISP-2 in human gastric cancer cell lines HGC27 and AGS was further carried out. The WISP family of proteins, in particular WISP-2, was a significant independent prognostic indicator for gastric cancer patients. WISP-2 knockdown resulted in significant changes in the growth rate and *in vitro* invasiveness, with little effect on the adhesive capability, when compared with its transfection controls. This was found to be linked to the MMP activities, mediated by the JNK pathway.

4. Signalling regulation of WISP-2 in cancers

WISP-2 expression can be regulated by various factors. For example, WNT1 was found to regulate WISP-2 in the mouse mammary epithelial cell line C57MG (1) and WNT signalling-activated s/αβ-catenin in synovial fibroblasts (40). In cancer cells, much effort has been focused on the role of Wnt signalling, oestrogen signalling, serum and hormones (41).

Wnt signalling. The wingless (wg)/Wnt family of secreted signalling molecules and the downstream components of Wnt signal transduction are highly conserved among animal species. Canonical and non-canonical pathway transductions result in tissue-specific cell fate decisions during embryogenesis and regulate cell proliferation, proper alignment and bundling of actin filaments in adult tissues (42). WISP-2 has been found to be one of such downstream components. Expression of HCV core protein by transient transfection in the human HCC-derived cell line Huh-7 increased cell proliferation, DNA synthesis, and cell cycle progression by upregulation of Wnt1 and WISP-2 (35). Wnt-PKA and Wnt-aPKC are both non-canonical Wnt signalling pathways (43). Treatment with protein kinase A (PKA) activators CT/IBMX induced WISP-2 mRNA expression in the MCF-7 human breast cancer cell line by a direct mechanism. Simultaneous treatment with protein kinase C (PKC) activators, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and oestrogen E2 completely prevented WISP-2 induction by E2 (44). The Wnt/β-catenin signalling pathway regulates genes involved in cell proliferation, survival, migration and invasion through regulation by T-cell factor (TCF)-4 transcription factor proteins. WISP-2 was 1 of the 13 genes found to be activated by T-cell transcription factor 4J isoform in HCC HAK-1A cells, and it was also found to be upregulated in HCC tumours when compared with adjacent peritumour tissues (36).

Oestrogen signalling. Several studies revealed that WISP-2 is oestrogen inducible in human breast cancer MCF-7 cells and is implicated in tumour cell proliferation (23-25). Inadera *et al* found that WISP-2 induction was highly specific for hormones that interact with the oestrogen receptor in MCF-7 cells (24). The oestrogen receptor α (ER-α) appears to be directly responsible for oestrogen induction of WISP-2 expression, as cultured human mammary epithelial cells that lack ER-α do not respond to oestrogen stimulation. However, stable transfection of ER-α into these cells rendered the ability of oestrogen to induce WISP-2 expression (25). There is some evidence to suggest that oestrogen may also function to stabilize WISP-2 mRNA. Banerjee *et al* reported that WISP-2 was upregulated by progesterone through a PR-dependent mechanism in MCF-7 cells, although the induction of progesterone was rapid and transient. When used in combination with oestradiol, progesterone acted as an antagonist to inhibit the expression of WISP-2, indicating a dual action of progesterone (25). In addition, PLK1, a key regulator of cell division, was found to be overexpressed in many types of human cancers, mediating ER-regulated gene transcription by coactivating WISP-2 and suggesting a mechanism for the tumour-suppressive role of PLK1 in MCF7 cells as an interphase transcriptional regulator of WISP-2 (45).

Signalling pathway crosstalk within oestrogen/WISP-2 signalling has also been the subject of several investigations. Treatment of MCF-7 cells with TPA completely blocked oestrogen-induced WISP-2 mRNA transcription (44). Epidermal growth factor (EGF) has been shown to induce

expression of WISP-2 mRNA in MCF-7 cells in a dose- and time-dependent manner and can act synergistically with oestrogen to raise WISP-2 expression levels, possibly through the PI3K and MAPK signalling pathways (17). A similar study was carried out by the same group using IGF-1 and reported a similar result. IGF-1 induced WISP-2 mRNA expression in a dose- and time-dependent manner, and knockdown of WISP-2 abrogated the ability of IGF-1 to stimulate MCF-7 cell proliferation. The IGF-1 induction of CCN5 expression was blocked by a pure anti-oestrogen drug, but unlike EGF the signalling crosstalk appeared to function through PI3K/AKT signalling (18).

Other regulators: serum, hormone and transcription factors. WISP-2 is serum-inducible during the process of mitogen-induced tumour cell proliferation (26). WISP-2 was found to be overexpressed in ACTH-secreting pituitary tumours when compared to that in normal pituitaries, NS pituitary tumours and GH-secreting tumours (38). However, there were no differences in expression of genes in the canonical and non-canonical Wnt pathways between all studied subtypes of pituitary tumours and normal pituitaries. It has been suggested that the elevated glucocorticoid levels observed in ACTH-secreting pituitary tumours activate WISP-2 transcription since WISP-2 has a glucocorticoid-responsive region in its promoter (46). The same phenomenon was found in ER-negative breast cancer cells. MDA-MB-231 cells exposed to glucocorticoids underwent morphological alterations, decreased invasiveness and attenuated expression of mesenchymal markers. These results thus indicate that the induction of the WISP-2 gene promoter probably requires the agonist-activated glucocorticoid receptor. Taken together, these results indicate that glucocorticoid treatment of ER-negative breast cancer cells induces high levels of WISP-2 expression and is accompanied by a more differentiated and less invasive epithelial phenotype. These findings propose a novel therapeutic strategy for high-risk breast cancer patients (46). In addition, Stiehl *et al* found that amphiregulin (AREG) and WISP-2 expression was strongly dependent on hypoxia inducible factor (HIF)-2α and their promoters were particularly responsive to HIF-2α in breast cancer. A strong correlation among HIF-2α/AREG/WISP-2 protein levels in breast cancer samples provides evidence that the HIF-2α-specific transcriptional pathway could have an important role in maintaining a non-invasive phenotype (47).

WISP-2 in other physiopathologic processes. WISP-2 is also essential in other physiopathological processes including apoptosis, anti-proliferation and osteogenic differentiation. Retroviral overexpression of rCop-1 (WISP-2) was found to induce apoptosis in transformed rat fibroblasts, but was unable to affect normal fibroblasts (48). Cop-1 mRNA was expressed at high levels in quiescent vascular smooth muscle cells (VSMCs) and in heparin-treated VSMCs but was found at low levels in proliferating VSMCs, indicating that COP-1 may play a role in the anti-proliferative mechanism of action of heparin (48). Another report provided functional evidence that WISP-2 is a growth arrest-specific gene that is temporally and spatially expressed and can inhibit VSMC proliferation, motility and invasiveness; however, adhesion and apoptosis were unaffected by WISP-2 in VSMCs (20). In addition, WISP-2 was found to be relevant to the low osteogenic differentiation capacity of placental mesenchymal stromal cells when compared to mesenchymal stromal cells from bone marrow (49). Large-scale analysis of transcripts in non-familial, isolated ACTH-independent macronodular adrenal hyperplasia (AIMAH) confirmed clinical heterogeneity and revealed that WISP-2 can be used as a clinical index of GIP-dependent AIMAH (50).

5. Roles of WISP-2 in proliferation, motility, invasiveness, adhesion and epithelial-mesenchymal transition

In vitro and *in vivo* studies have indicated the potential roles of WISP-2 in regulating cell proliferation, motility, invasiveness, adhesion and EMT.

Proliferation. Overexpression of WISP-2 has been shown to inhibit serum-induced proliferation of highly invasive ER-negative breast cancer cell line MDA-MB-231 (51). However, in the less invasive ER-positive MCF-7 cell line, the effect of WISP-2 is not consistent. Some have suggested an inhibitory role in serum-induced proliferation of MCF-7 cells (51). Others have suggested a promoting role in MCF-7 cell proliferation (25) or no effect (22). Moreover, the ability of PMA, EGF or IGF-1 alone to induce MCF-7 cell proliferation was blocked by WISP-2 knockdown (17,19,21). Knockdown of WISP-2 in MCF-7 cells was found to eliminate the oestrogen-dependent growth requirement of these cells. More studies are needed to clarify the biochemical and biological basis of the contrasting role of WISP-2 in these cells.

Motility, invasiveness and metastasis. Overexpression of WISP-2 was found to inhibit both motility and invasiveness in the highly aggressive breast carcinoma cell line, MDA-MB-231 (51). The inhibitory effect of WISP-2 on motility was also observed in MCF-7 cells where knockdown of WISP-2 expression increased the IGF-1-induced motility of MCF-7 cells. WISP-2 knockdown in MCF-7 cells also induced expression of pro-motility enzymes such as MMP-2 and -9 (22,51). Mutant p53 overexpression induced in MCF-7 cells exhibiting increased invasiveness was inhibited by treatment with recombinant WISP-2 protein (52).

Adhesion. Little is known concerning the role of WISP-2 in cell adhesion. Kumar *et al* observed that 3 different osteoblastic cell lines, primary human osteoblasts, osteosarcoma MG63 cells, and rat osteoblast-like osteosarcoma Ros 17/2.8 cells, attached to immobilized CCN5 in a dose-dependent manner (6). Recent data from our laboratory revealed that WISP-2 knockdown in gastric cancer cells resulted in little effect on the adhesive capability, compared with its transfection controls (39).

EMT. Phenotypical alterations including EMT are a hallmark of the progression of cancer and provide a new basis for understanding the progression of cancer toward a more malignant state. Mesenchymal cells are also implicated in the formation of epithelial organs through mesenchymal-epithelial transition (MET). Cellular plasticity, the ability to undergo EMT and subsequently MET in the appropriate microenvironments are key features of a successful metastatic cell (53). The process

of EMT plays an important role during foetal, postnatal development, invasion and metastases and is regulated by transcription factors such as Twist1, Snai1 and Slug, which inhibit E-cadherin expression (54).

Current evidence suggests that WISP-2 may suppress EMT in different cancers and that EMT in turn can suppress WISP-2 expression. Human pancreatic adenocarcinoma is associated with the silencing of WISP-2/CCN5 signalling. Functional analysis studies demonstrated that exposure of pancreatic cancer MIA-PaCa-2 cells to WISP-2 recombinant protein for 48 h markedly altered the phenotype of these cells from a spindle shape (mesenchymal type) to a cobblestone-like shape (epithelial type) and also markedly reduced the expression of vimentin, a mesenchymal marker, in these cells, suggesting that WISP-2 may play a critical role in reversing EMT (or inducing MET) (21). Although mitogen-induced upregulation of WISP-2 participates in cell proliferation events of ER-positive breast cancer cells, the basal level of WISP-2 does not exhibit a mitogenic response in these cells (17-19). Instead, it protects cells from gaining invasive phenotypes. For example, silencing of WISP-2 in MCF-7 non-invasive carcinoma cells significantly enhanced motility and EMT, and it modulated the expression of several genes associated with invasive phenotypes of cancer cells (22,51,52,55), while ectopic Snail expression suppressed WISP-2 transcripts and downregulated WISP-2 gene promoter expression in transfected cells (55). In WISP-2-knockout ER-α-positive breast cancer cells, IGF-1 and EGF lost their mitogenic effect (17,18) but possibly gained aggressive phenotypes. Sabbah *et al* showed that WISP-2 silencing promoted EMT via activation of the TGF/β signalling cascade known to promote EMT in breast cancer (56). Recently, Ferrand *et al* discovered that glucocorticoid treatment of ER-negative breast cancer cells induced high levels of WISP-2 expression and this was accompanied by marked changes in the cellular morphology. Cells were found to grow as groups of flattened cells consistent with a normal epithelial cell phenotype. This morphological change was correlated with a reduction in cell motility and invasion, characteristic of a more differentiated and less invasive epithelial phenotype. Meanwhile, WISP-2 expression repressed cadherin 11, vimentin and ZEB1 expression (46).

6. Perspectives

WISP-2 is a unique member of the CCN family that lacks the CT domain and exhibits different functions in multiple cellular processes. However, similar to the other CCN family members, WISP-2 is a protein with important roles in embryonic development, normal cell function and disease, particularly in cancers. The functions of WISP-2 in human cancers include effects on cell proliferation, adhesion, motility, invasiveness, metastasis and epithelial-mesenchymal transition (EMT); however these functions are dependent upon the cell and tissue type and the microenvironment. Several independent studies have shown the expression pattern of WISP-2 and a link with patient clinical course in breast cancer, pancreatic cancer, hepatocarcinoma, colorectal and gastric cancer. However, the results are inconsistent and somewhat conflicting in certain tumour types. Further clinical research requires studies using larger clinical cohorts and scientific investigation into the cellular functions in more than 2 cell lines together, which would allow for more powerful statistical conclusions and further insight into the action of WISP-2. Studies to decipher the myth of its domain binding in relation to the differential response in different cell types to different stimuli would also be important. Together, WISP-2 is a potential regulator and a novel therapeutic target in cancer and warrants further investigation at the cellular and clinical levels.

Acknowledgements

The authors wish to thank the Cancer Research Wales and the Albert Hung Foundation for supporting their study. S.J. and K.J. are recipients of the China Medical Scholarship of Cardiff University.

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