Loss of Secreted Frizzled-Related Protein-1 Expression in Renal Cell Carcinoma Reveals a Critical Tumor Suppressor Function

Jeffrey S. Rubin and Donald P. Bottaro

In this issue of Clinical Cancer Research, Gumz et al. report on the loss of secreted frizzled-related protein-1 (sFRP-1) in clear cell renal cell carcinoma (RCC; ref. 1). sFRP-1 is a member of a family of proteins that bind Wnts and can inhibit Wnt signaling during embryonic development and in the adult homeostasis (2, 3). Inappropriate Wnt signaling is a well-established mechanism of oncogenesis in several malignancies, and in these contexts, sFRPs have been viewed as potential tumor suppressors. Consistent with this hypothesis, the chromosomal loci of sFRPs have been associated with loss of heterozygosity in various tumor types (4, 5). Moreover, several studies have documented the loss of SFRP expression in cancer due to promoter hypermethylation (3). Recent literature, including the article by Gumz et al. (1), indicates that silencing of SFRP expression, particularly SFRP1, is very common in RCC (6, 7). Importantly, Gumz et al. show that restoration of SFRP1 expression not only reduced the expression of Wnt target genes, but also markedly inhibited tumor cell growth in culture, soft agar, and xenografts in athymic nude mice (1). These findings strongly suggest that loss of sFRP-1 has a pivotal role in RCC, and that restoration of its expression could be of therapeutic benefit. Furthermore, analysis of SFRP gene hypermethylation may have diagnostic and prognostic value in the detection and management of RCC (6), which now claims more than 13,000 lives each year in the U.S. alone (8).

Wnts have many diverse functions during embryogenesis, including a critical role in kidney development (9), and they contribute to stem cell renewal in the adult (2). Constitutive activation of the canonical Wnt/β-catenin pathway is common in many cancers, in which it is thought to be an early event in tumorigenesis, particularly in colorectal cancer (2). Stimulation of this pathway inhibits the degradation of cytosolic β-catenin, facilitating its accumulation in the nucleus where it associates with members of the T cell factor/lymphoid enhancer factor (TCF/LEF) family to function as a transcriptional activator (Fig. 1). Although activation of the pathway in cancer is usually attributed to deregulation of downstream effectors (e.g., β-catenin) or suppressors [e.g., adenomatous polyposis coli (APC) protein or Axin], autocrine mechanisms also have been described (10). Maximal stimulation probably involves a combination of both mechanisms (11). Other Wnt signaling cascades have been well-characterized, including a calcium/protein kinase C mechanism implicated in metastatic melanoma (12) as well as a Rac/Rho-associated process that contributes to cell polarity and motility (13). All of these signaling events are mediated by Wnt receptors in the Frizzled (Fzd) family of seven-pass transmembrane proteins. In addition, the lipoprotein receptor-related proteins 5 and 6 (LRP5/6) function as Wnt coreceptors, specifically in the β-catenin pathway (Fig. 1).

sFRPs possess a conserved Fzd-type cysteine-rich domain that binds Wnts and typically antagonize Wnt signaling, presumably by preventing Wnt/Fzd interactions. Four of the five SFRP genes contain dense CpG islands (14), and a growing literature indicates that these genes often are silenced in cancer, primarily by promoter hypermethylation (3). The incidence of gene silencing is particularly high for sFRP-1, both with regard to the diversity of tumor types and the frequency within an individual type (3). Loss of sFRP-1 expression in breast cancer has been associated with decreased survival (15), and restoration of expression in colorectal tumor cell lines resulted in an attenuated tumor phenotype, manifested as diminished anchorage-independent growth in soft agar and increased apoptosis (11). Of note, this inhibition was observed in cells with mutations in APC or β-catenin, providing support for the idea that Wnt stimulation is needed to drive oncogenic signaling by these mutations, and that their full impact may result from the simultaneous activation of β-catenin and noncanonical Wnt pathways. These reports highlighted the potential importance of sFRP silencing in oncogenesis, and provided a strong impetus for the definitive functional experiments done by Gumz et al.

Using a combination of genomic profiling, quantitative PCR, and immunohistochemistry, Gumz et al. documented a remarkably high incidence of sFRP-1 loss in clear cell RCC, with decreases also seen in papillary RCC but not chromophobe or oncocytoma (1). Thirteen Wnt-responsive genes were found to be dramatically up-regulated in RCC specimens. Reintroduction of sFRP-1 into clear cell RCC cell lines via stable cDNA transfection decreased the expression of these genes by 2- to 3-fold. More impressive was the nearly complete inhibition of RCC cell growth in monolayer culture and soft agar assays. Additional experiments indicated that sFRP-1 expression decreased cell proliferation, but did not stimulate apoptosis, in contrast to the proapoptotic effect observed in colorectal tumor cell lines (11). Finally, Gumz and colleagues
have shown, for the first time, the antitumor activity of sFRP-1 in an animal model: complete blockade of tumor xenograft growth in nude mice, using three clonal RCC cell lines stably transfected with sFRP-1 cDNA expression constructs. These results reinforced prior indications of oncogenic Wnt pathway activation in RCC, providing the strongest evidence thus far that loss of sFRP-1 expression is a key event in RCC tumorigenesis, and proof of concept that sFRP-1 restoration is a potential RCC treatment modality.

Other secreted Wnt antagonists have also been implicated as tumor suppressors. The Dickkopfs (DKK) comprise a family of proteins that bind to the Wnt coreceptors, LRP5/6, and specifically block Wnt-dependent, β-catenin transcriptional activity (3). Expression of DKKs is reduced in various tumors, and promoter hypermethylation has been observed in some of these settings (3). This is especially true for DKK-3, originally known as REIC (Reduced Expression in Cancer), which has a high incidence of silencing in RCC (16). Restoration of DKK-3 expression in prostatic carcinoma cells increased apoptosis, apparently through stimulation of c-Jun-amino-terminal-kinase (JNK; ref. 17). It has been suggested that DKK binding to LRP5/6 may shift the balance of Wnt/Fzd signaling away from the β-catenin pathway, which requires the formation of a Wnt/Fzd/LRP complex, to other signaling mechanisms that are mediated by Wnt/Fzd interactions (3).

β-Catenin activity in RCC is influenced by other pathways, in addition to Wnt signaling (Fig. 2). E-cadherin, an adhesion junction protein with tumor suppressor activity, is normally anchored to the cortical actin cytoskeleton via a protein complex that includes β-catenin and the actin cytoskeleton is not thought to be directly affected by Wnt stimulation. The complex is composed of an Axin scaffold, which binds APC, β-catenin, and two enzymes that phosphorylate β-catenin, GSK3β and CK1ε, targeting it for proteosomal degradation. Wnt signaling recruits Dvl to participate in the destabilization of the Axin complex. Consequently, β-catenin accumulates in the cytosol and nucleus, where it associates with members of the DNA binding, TCF/LEF family to promote the expression of specific genes. Dvl is also a mediator of Rac/JNK – and Rho-dependent changes in the actin cytoskeleton that alter cell shape and enhance motility. Dvl may participate in another noncanonical pathway that involves the activation of phospholipase C, PKC, and release of intracellular stores of calcium, increasing the activity of calcium-dependent enzymes such as CaMKII and CALN. The latter dephosphorylates NF-AT transcription factors, which then accumulate in the nucleus to regulate the expression of another set of genes. Heterotrimeric G proteins have been reported to be mediators of the Wnt/calcium and β-catenin pathways. Fzd, Frizzled; sFRP, secreted Fzd-related protein; LRP5/6, LDL receptor-related protein 5 or 6; DVL, disheveled; APC, adenomatous polyposis coli protein; CK1ε, casein kinase 1ε; GSK3β, glycogen synthase kinase 3β; β-caten, β-catenin; TCF/LEF, T cell factor/lymphoid enhancer factor; JNK, c-Jun-amino-terminal-kinase; PLCγ, phospholipase Cγ; PKC, protein kinase C; CaMKII, calmodulin-dependent kinase II; CALN, calcineurin; NF-AT, nuclear factor in activated T cells.

Fig. 1. Wnt signaling pathways. The canonical Wnt/β-catenin pathway is triggered by Wnt binding to Fzd and either LRP5 or LRP6, whereas noncanonical signaling only requires Wnt binding to Fzd. Typically, sFRP inhibits all of these mechanisms of Wnt activity. In the β-catenin pathway, Wnt/receptor interaction results in the dissociation of a multiprotein complex that normally functions to facilitate the turnover of cytosolic β-catenin (β-catenin linked to E-cadherin and the actin cytoskeleton is not thought to be directly affected by Wnt stimulation). The complex is composed of an Axin scaffold, which binds APC, β-catenin, and two enzymes that phosphorylate β-catenin, GSK3β and CK1ε, targeting it for proteosomal degradation. Wnt signaling recruits Dvl to participate in the destabilization of the Axin complex. Consequently, β-catenin accumulates in the cytosol and nucleus, where it associates with members of the DNA binding, TCF/LEF family to promote the expression of specific genes. Dvl is also a mediator of Rac/JNK – and Rho-dependent changes in the actin cytoskeleton that alter cell shape and enhance motility. Dvl may participate in another noncanonical pathway that involves the activation of phospholipase C, PKC, and release of intracellular stores of calcium, increasing the activity of calcium-dependent enzymes such as CaMKII and CALN. The latter dephosphorylates NF-AT transcription factors, which then accumulate in the nucleus to regulate the expression of another set of genes. Heterotrimeric G proteins have been reported to be mediators of the Wnt/calcium and β-catenin pathways. Fzd, Frizzled; sFRP, secreted Fzd-related protein; LRP5/6, LDL receptor-related protein 5 or 6; DVL, disheveled; APC, adenomatous polyposis coli protein; CK1ε, casein kinase 1ε; GSK3β, glycogen synthase kinase 3β; β-caten, β-catenin; TCF/LEF, T cell factor/lymphoid enhancer factor; JNK, c-Jun-amino-terminal-kinase; PLCγ, phospholipase Cγ; PKC, protein kinase C; CaMKII, calmodulin-dependent kinase II; CALN, calcineurin; NF-AT, nuclear factor in activated T cells.
and the acquisition of an invasive cell phenotype (18, 19). Concomitant stimulation of growth factor pathways, such as that of hepatocyte growth factor (HGF) in papillary and clear cell RCC, further enhances cell invasiveness. Activation of the HGF receptor tyrosine kinase, c-Met, leads to tyrosyl phosphorylation of β-catenin, reducing its affinity for E-cadherin and promoting its binding to Bcl9-2 (20, 21). As a result, adherens junctions are disrupted and cytoplasmic β-catenin is more efficiently translocated to the nucleus. In clear cell RCC, frequent loss of von Hippel-Lindau (VHL) tumor suppressor gene function adds a devastating blow to this mix: aberrant expression of a large collection of genes that control cell metabolism, proliferation, motility, extracellular matrix remodeling, and angiogenesis. The VHL gene product (pVHL) forms a stable complex with other proteins possessing E3 ubiquitin ligase activity. This complex is best characterized for its role in targeting hypoxia-inducible factors (HIF) for polyubiquitination and subsequent proteasomal degradation, thereby regulating the cellular response to hypoxia (22). Under normoxic conditions, HIF levels are continuously suppressed by pVHL, whereas under hypoxic conditions or when the VHL gene is mutated or lost, HIFs accumulate, translocate to the nucleus, and activate gene transcription. In addition to HIFs, recent reports reveal that pVHL also regulates β-catenin signaling downstream of c-Met, and that VHL loss in RCC promotes E-cadherin down-regulation and β-catenin–mediated invasiveness (18, 23).

Further convergence of the Wnt/β-catenin and hypoxia pathways occurs at the level of transcription factor/gene promoter binding. The HIF family member HIF-1α directly modulates β-catenin–dependent gene expression by competing with TCF/LEF proteins for binding to β-catenin (24). Modulation of HIF-1α levels determines the distribution of β-catenin at promoter sites, thereby regulating the pattern of gene expression. Under hypoxic conditions or with VHL loss, β-catenin is recruited by HIF-1α to promoters of genes that enhance tumor cell survival, whereas under normoxic conditions in the presence of pVHL, HIF-1α is degraded and β-catenin shifts to TCF/LEF complexes that promote the expression of genes that stimulate tumorigenesis.

Fig. 2. Regulation of β-catenin transcriptional activity in RCC. Wnt binding to Fzd and LRPS/6 stimulates β-catenin activity, whereas sFRP and DKK block it (the latter by binding to LRPS/6). HGF interaction with its cell surface receptor, c-Met, leads to the tyrosyl phosphorylation of β-catenin, which increases the amount of cytosolic β-catenin by reducing its affinity for E-cadherin. pVHL promotes the degradation of cytosolic β-catenin as well as HIF, while also functioning to maintain the expression of E-cadherin. Conversely, VHL loss of function is associated with increased cytoplasmic and nuclear β-catenin, which interacts either with HIF or TCF/LEF to promote the expression of overlapping sets of genes. Stimulation of gene expression contributes to tumor formation, survival, and metastasis. DKK, Dickkopf; HGF, hepatocyte growth factor; HIF, hypoxia-induced factors; pVHL, von Hippel-Lindau protein; for others, see Fig. 1.
tumor cell proliferation. Thus, a variety of factors with important roles in the etiology of RCC affect β-catenin transcriptional activity. Now sFRPs can be added to the picture (Fig. 2).

Despite recent regulatory approval of two new drugs to treat RCC, there is no broadly effective, durable therapy for this disease once it becomes metastatic (25). Although imaging techniques have improved the detection of localized RCC, these tumors are often asymptomatic until they have spread systemically, and patients that present with advanced disease have only an 18% 2-year survival rate (8). Consequently, screening methods to facilitate early detection are urgently needed. The recent findings concerning SFRP gene hypermethylation in RCC and the functional effect of restored sFRP-1 expression are particularly noteworthy in this regard. Demethylating agents or methylase inhibitors that could up-regulate sFRP-1 expression in RCC cells merit consideration as potential new treatment strategies. sFRP promoter methylation analysis of serum and tumor DNA samples might provide diagnostic and prognostic information that would improve the detection and management of RCC (6). We look forward to further progress in this area.

Acknowledgments

The authors regret that not all relevant original reports could be cited due to space limitations.

References

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