Clinicopathological Implications of Wingless/int1 (WNT) Signaling Pathway in Pancreatic Ductal Adenocarcinoma

Mitsuhiro Nakamoto and Masanori Hisaoka*

Abstract: Pancreatic cancer is still one of the most lethal malignancies in the world, and a more thorough understanding of its detailed pathogenetic mechanisms and the development of more effective therapeutic strategies are urgently required. Pancreatic ductal adenocarcinoma (PDA), the most common type of pancreatic cancer, is characterized by consistent genetic abnormalities such as point mutations in the Kirsten rat sarcoma viral oncoprotein homolog (KRAS) and in the tumor suppressor protein p53 (TP53) genes. Alterations in intracellular core signal pathways have also been shown to induce the development or progression of PDA. The Wingless/int1 (WNT) signal pathway plays a pivotal role in embryonic development, cellular proliferation and differentiation, and dysregulation of WNT signaling can lead to neoplastic transformation in a variety of organ systems, including the pancreas. Recent studies have shown that altered WNT signaling is associated with a poor prognosis in patients with PDA, suggesting that the pathway is a predictor of patients' survival and a potential therapeutic target of PDA. In this review, the clinicopathological implications of WNT signaling in PDA are highlighted.

Keywords: WNT signaling pathway, pancreas, cancer, pathology.

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Introduction

Pancreatic cancer is the 12th most prevalent type of cancer, and more than 330,000 patients in the world suffered from pancreatic cancer in 2012 [1]. Japan had the seventh largest number of cases of pancreatic cancer, and the 5-year overall survival rate of patients with this cancer is still lower than 30% [1, 2]. Such a high mortality rate is probably due to difficulties in detecting the cancer in its early stage and an unfavorable response to systemic therapeutic treatments in the later stages [3]. Although smoking and obesity are thought to be the major risk factors for pancreatic cancer [4, 5], other plausible causal factors need to be further elucidated.

Pancreatic ductal adenocarcinoma (PDA), the most common histological type of pancreatic cancer, is characterized by frequent mutations in the genes, such as the Kirsten rat sarcoma viral oncogene homolog (KRAS) (>90% of cases), the tumor suppressor protein p53 (TP53) (60–70%), the cyclin-dependent kinase inhibitor 2A (CDKN2A) (>50%), and mothers against decapentaplegic (DPP) homolog 4/deleted in pancreatic cancer locus 4 (SMAD4/DPC4) (50%) genes and oth-
ers (adenine-thymine (AT) rich interactive domain 1A (ARID1A), mixed lineage leukemia-2 (MLL2), ataxia telangiectasia mutated (ATM), etc.) [3, 6]. Some of the genetic mutations, including those affecting KRAS, can be identified not only in fully developed PDA but also in its precursor lesion known as pancreatic intraepithelial neoplasia (PanIN) [7], indicating that such genetic alterations are requisites in the initial stage of pancreatic carcinogenesis. In addition, a natural course of PDA may be modulated by other factors, such as alterations in cellular metabolism, microenvironment and immunity [8]. Moreover, aberrantly activated intracellular core signal pathways (e.g. epidermal growth factor receptor (EGFR), caudal type homeobox 2 (CDX2), interleukin-6R/Janus kinase/signal transduction and activator of transcription (IL-6R/Jak/Stat) and Notch signaling pathways) have been shown to be involved in the development or progression of PDA [9–12].

Wingless/int1 (WNT) signaling is a major determinant of developmental processes and functions as a pivotal regulator of cell proliferation, morphogenesis and differentiation [13]. In addition, it has been suggested that dysregulated WNT signaling is one of the most important mechanisms of neoplastic transformation in a variety of cancers, including PDA [14, 15]. The currently available information on WNT signaling in PDA is summarized in this review in order to better understand the biology of PDA.

WNT signaling pathways in the development of PDA

WNTs are a family of secreted proteins that bind to receptors of the Frizzled protein family and co-receptors, which transduce signals through distinct pathways (i.e. canonical and non-canonical pathways) into nuclei (Fig. 1) [16]. The ligand binding in the canonical pathway inactivates a complex of cytoplasmic proteins, including those encoded by adenomatous polyposis coli (APC) and axis inhibition protein 1 (AXIN1) genes, which leads to degradation of β-catenin by the ubiquitin-proteasome system, resulting in its cytoplasmic accumulation and subsequent nuclear translocation. β-catenin can bind to T-cell factor/lymphoid enhancer binding factor (TCF/LEF) and activate its target genes, such as cyclin D1 (CCND1) gene and cellular myelocytomatis (MYC) oncogene, in the nuclei. WNT signaling is also transduced in a β-catenin-independent manner through two distinct non-canonical pathways, either of which is mediated by activation of small guanosine triphosphatases (GTPases) of the ras homolog gene (Rho) family, which is also known as the G protein family, and their downstream kinases such as jun N-terminal kinase (JNK) and Rho-associated protein kinase (Rock), or by Ca²⁺ that is released from the endoplasmic reticulum that activates calcium-dependent enzymes or proteins, including calmodulin, calcium/calmodulin-dependent kinase (CAMK) and calcineurin. The non-canonical WNT signaling induces activation and subsequent nuclear accumulation of c-jun and nuclear factor of activated T cells (NFAT), inducing the expression of their target genes. Thus, dysregulated WNT signaling through its canonical and/or non-canonical pathways can result in enhanced or aberrant expression of the WNT target genes [13, 15].

It has been shown that a minimal threshold of ligand-mediated canonical WNT signaling is potentially required for the development of PanIN [17]. Besides, nuclear accumulation of β-catenin, a hallmark of the activated canonical WNT signaling pathway, is a common phenomenon in PDA but not in normal pancreatic tissue [18,19]. Suppression of β-catenin by siRNA substantially compromises cell proliferation of PDA and increases apoptosis [19]. Moreover, increased levels of cytoplasmic and nuclear expression of β-catenin correlate with PanIN grade and the development of invasive PDA [20]. Thus, the activated canonical WNT signaling pathway is assumed to play an important role in pancreatic carcinogenesis. However, genomic alterations of APC, AXIN1 and the catenin beta 1 (CTNNB1) gene that encodes β-catenin are only rarely detectable in PDA [21, 22], and the precise molecular mechanisms responsible for the activated canonical WNT signaling pathway in PDA are poorly understood. Canonical WNT ligands such as WNT2 and WNT7B, which are highly expressed in PDA [23–25], may be potential mechanisms inducing the enhanced WNT activation in autocrine and/or paracrine manners, although the molecular basis of the highly expressed WNT ligands in PDA remains to be investigated. In addition, some epigenetic mechanisms, such as DNA methylation, may also be involved in the al-
tered WNT/β-catenin signaling in PDA, as suggested by a recent unbiased global epigenetic analysis [26]. It has recently been suggested that hypoxia inducible factor (HIF) 2α is a molecule that can modulate WNT signaling and is required in early pancreatic carcinogenesis [27]. Information regarding the biological roles of non-canonical WNT signaling pathways in PDA is relatively limited compared to that on the canonical pathway. Expression of WNT5A, which classically stimulates

**Fig. 1. Schematic representation of the Wingless/int1 (WNT) signaling pathway.** Canonical Wingless/int1 (WNT) signaling is transduced through Frizzled family receptors (FZD) and low density lipoprotein receptor-related protein (LRP) 5/6 to the β-catenin signaling cascade that activates WNT target genes by nuclear translocation of β-catenin. WNT molecules can also trigger intracellular signaling by activating a variety of functional molecules such as Rho-associated protein kinase (Rock), jun N-terminal kinase (JNK) and nuclear factor of activated T cells (NFAT) in a non-β-catenin-independent manner (i.e. non-canonical pathway).

the non-canonical pathway, is commonly upregulated in PDA and has been shown to protect tumor cells from apoptosis [28]. Besides, WNT2, which also functions as a member of the non-canonical WNT family, can induce increased survival of circulating PDA cells in mice by suppressing anoikis [29]. We recently demonstrated frequent immunohistochemical expressions of both WNT2 and WNT5A in PDA (Fig. 2) [23]. High expressions of NFATc1 and NFATc2, which are downstream targets of the non-canonical pathway, have been described in PDA (Fig. 2) [23, 30]. Taken together, potentially enhanced activation of the non-canonical pathway is also assumed to be involved in pancreatic carcinogenesis and tumor progression.

**Table 1. Representative inhibitors of canonical Wingless/int1 (WNT) pathway**

<table>
<thead>
<tr>
<th>Properties/functions</th>
<th>Inhibitors</th>
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<tbody>
<tr>
<td>Wingless/int1 (WNT) soluble frizzled cysteine rich domain</td>
<td>OMP-54F28</td>
</tr>
<tr>
<td>Frizzled antibody</td>
<td>OMP-18R5</td>
</tr>
<tr>
<td>Dishevelled antibody</td>
<td>3289-8625</td>
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<tr>
<td></td>
<td>FJ9</td>
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<td></td>
<td>NSC668036</td>
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<tr>
<td>β-catenin interactors</td>
<td>PFK115-584</td>
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<tr>
<td></td>
<td>CGP049090</td>
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<tr>
<td></td>
<td>iCRT3/5/14</td>
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<tr>
<td>Tankyrase inhibitors</td>
<td>IWR-1</td>
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<td></td>
<td>XAV939</td>
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<tr>
<td>β-catenin transcription inhibitors (a cyclic adenine monophosphate response-element binding protein-binding protein (CBP)-dependent)</td>
<td>ICG-001</td>
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<td></td>
<td>PRI-724</td>
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<td>Retinoids</td>
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**Fig. 2. Immunohistochemical expressions of molecules involved in the WNT signaling pathways in pancreatic ductal adenocarcinoma.** In the activated canonical pathway, nuclear expressions of β-catenin (A) and its molecular target, cyclin D1 (C), can be seen, whereas WNT2 is not expressed (B). The expression of WNT2 (E) and the nuclear localization of NFATc1 (F) were identified in the activated non-canonical pathway, where the expression of β-catenin is retained mainly in a membranous manner (D). bars=100 μm.
Clinical impact of WNT signaling in PDA

Apart from the above oncogenic roles, studies focusing on the clinical implication of aberrantly activated WNT signaling in PDA are limited. Some previous retrospective studies based on small case series suggested a correlation between high WNT/β-catenin activation and poor survival of patients with PDA [31, 32]. This association was reinforced by our cohort study of 101 PDA cases examined immunohistochemically, which demonstrated that nuclear β-catenin expression is an independent unfavorable prognostic factor, although activation of the non-canonical pathway did not seem to correlate with reduced survival of patients [23]. Consequently, WNT signaling is considered to be one of the putative molecular targets for the therapy of PDA, particularly through its canonical pathway. Some studies have shown that inhibition of WNT signaling by an endogenously secreted inhibitor of the canonical pathway, such as the Dickkopf WNT signaling pathway inhibitor 1 (Dkk1), or anti-β-catenin siRNA, can inhibit proliferation and induce apoptosis of cultured PDA cells [19, 33]. The activation of the Ca²⁺-dependent non-canonical pathway can interfere with the WNT/β-catenin signaling through the canonical pathway. These findings can endorse the potential clinical application of a variety of so far available inhibitors, particularly of the canonical WNT pathway, including naturally occurring compounds, small-molecule inhibitors, blocking antibodies and peptide antagonists (Table 1). As a clinical trial, PRI-724, a derivative of ICG-001 that is a cyclic adenosine monophosphate (cAMP)-response element-binding protein (CBP) and a potent specific inhibitor of the canonical WNT signaling pathway [34], is currently being examined in combination with gemcitabine, a nucleotide analog widely used as adjuvant chemotherapy for PDA, in the second line treatment of metastatic pancreatic cancer (NCT01764477) [35]. Incidentally, a recent study has demonstrated that PDA cells are sensitized to the WNT inhibitor ICG-001 by galectin 4 (Gal-4), a member of the family of galactoside-binding soluble lectins that can reduce cytoplasmic β-catenin levels [36]. Thus, ICG-001 and PRI-724 may be better candidates as a treatment option for PDA expressing Gal-4. Recently, other agents, such as potent and selective inhibitors of serine/threonine protein phosphatases 2A (PP2A) and 5-azacytidine, have also been shown to inhibit the WNT signaling in PDA cells [37, 38], and need further investigations to address their potential clinical applications.

Conclusion

PDA is the most lethal and common cancer in the pancreas and so far lacks effective therapies at its advanced stages, when PDA is frequently detected or diagnosed. Recent studies have shown that aberrantly activated WNT signaling, which is normally involved in cell fate, differentiation and proliferation, can induce the development and progression of PDA. Such oncogenic mechanisms can be exerted through abnormal nuclear accumulation of β-catenin, which may be an adverse prognostic determinant for PDA. Thus, the WNT signaling pathway is implicated as a major therapeutic target of PDA. Molecules or compounds targeting the WNT signaling pathway will be a promising future strategy in the treatment of PDA, although additional studies are mandatory to attest their effectiveness and safety.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


膵管癌におけるWingless/int1（WNT）シグナル伝達経路の臨床病理学的意義について

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要 旨: 肝管癌は世界的に依然としてヒトでのもっとも致死的な癌の一つであり、その発生のメカニズムのさらなる解明とより効果的な治療法の開発が望まれている。膵管癌は肝管癌の中でもっとも代表的な組織型であり、Kirsten rat sarcoma viral oncogene homolog (KRAS) や tumor suppressor protein p53 (TP53) などにおける突然変異などの遺伝子異常が高頻度に認められることが特徴である。また、膵管癌ではいくつかの細胞内のシグナル伝達系の異常によりその発生や進展が誘導されることも知られている。それらの中で Wingless/int1 (WNT) シグナル伝達系は、胎児発生や細胞増殖、分化において主軸的な役割を演じており、その異常は臓器を含む様々な臓器において腫瘍発生を惹起する。最近の研究では、WNT シグナル伝達系の異常が膵管癌患者の不良な予後と相関することも示されており、このシグナル伝達系は膵管癌の予後予測因子であると共に、今後の治療標的として有力な候補であることが示唆されている。本総説では、膵管癌において WNT シグナル伝達系が臨床病理学的に意義深いことに焦点をあてて解説する。

キーワード: WNT シグナル伝達系, 膵臓, 癌, 病理.