Nuclear Receptors as Potential Pharmacological Targets for Colorectal Cancer Therapy via Regulating Wnt/β-catenin Signaling

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Abstract
Hyperactivation of the Wnt/β-catenin signaling pathway due to mutations in its components initiates the majority of colorectal cancer (CRC) cases and promotes CRC development. Unphosphorylated β-catenin accumulates in the nuclear and interacts with TCF/LEF factors to stimulate the transcription of the downstream target genes of Wnt/β-catenin signaling. Therefore, the suppression of dysregulated Wnt/β-catenin signaling is considered as a promising strategy for CRC therapy. In the past decade, accumulating evidence revealed that nuclear receptors (NRs) modulated Wnt/β-catenin signaling activity via binding to diverse members of this pathway. In this review, we mainly focus on the regulation and the underlying mechanisms of Wnt/β-catenin signaling by NRs and their ligands or pharmacological modulators. Their potential in the precise treatment and individualized therapy for colorectal cancer is also discussed.
Introduction

Nuclear receptors (NRs) are ligand-dependent transcription factors, with wide distributions in biological organisms. NRs regulate gene expression, activating or preventing the transcription of specific target genes, and thus play important roles in cell growth, proliferation and metabolism, etc. (1-4). Till now, 48 members of human NR superfamily have been found (5). The structures of NRs are conservative, mainly composed of four domains with different functions, namely A/ B, C, D and E. The N-terminal A/B domain is cis-activated in a ligand-independent manner. The conserved C domain (DNA-binding domain, DBD) is a characteristic area of NRs, which determines its DNA binding activity and the selection of binding partners. D domain is a flexible hinge area with nuclear location information, which connects C and E domains. E domain is the ligand binding domain (LBD) (6, 7). After their corresponding ligands binding to the LBD, NRs form homologous or heterologous dimers, and transcriptionally regulate the expression of the downstream genes.

Wnt gene was originally found in murine breast cancer research. The activation of Wnt gene depended on the insertion of mouse mammary tumor virus gene, so it was named Int1 gene. The following research demonstrated that the Int1 gene was essential in the mouse embryonic development, which was the equivalent of the Wingless gene (Wingless) in fruit flies. Thus, the names of Int1 and Wingless were harmoniously known as Wnt (8). The Wnt pathways were involved in embryonic development, tissue homeostasis and cellular cancerization (9-11). Hyperactivation of the Wnt signaling pathway due to mutations in its components initiates most cases of colorectal cancer (CRC) and promotes CRC development (12). β-catenin is a key protein of the Wnt pathway. Activated Wnt pathway impedes GSK-3β-induced phosphorylation and the following degradation of β-catenin, resulting in the accumulation of β-catenin in the nucleus, which then binds to the transcription factor TCF/LEF (13, 14) and initiates the transcription of target genes (15, 16). The abnorimity of Wnt pathway promotes the development of cancer as a great majority of these target genes play critical roles in multiple cellular functions, including cell cycle regulation and epithelial-mesenchymal transition (EMT) (17, 18). Therefore, the inhibition of hyperactivated Wnt signaling is regarded as a promising strategy for CRC therapy.

A large number of studies have shown that NRs regulate the Wnt signaling pathway in CRC (19). Some small molecule compounds, ligands or modulators of NRs, have also been identified to regulate Wnt signaling via NRs mediation (20, 21). Recently, our studies also demonstrated that berberine, an isoquinoline alkaloid isolated from traditional Chinese medicine Coptis chinensis, inhibited Wnt/β-catenin signaling pathway via binding to RXRα and thus decelerated the CRC progression. The underlying mechanisms were also revealed, which might provide new strategies for berberine’s application in CRC therapies.

Nuclear receptors and Wnt/β-catenin

NRs are ligand-activated transcription factors that bind to the specific response element on the promoter of their target genes. NRs function mainly in three ways: (i) directly interact with the corresponding DNA response elements and modulate the transcription level of their target genes, (ii) mediate by protein-protein interaction, and (iii) regulate via different subcellular localization (22-24). In recent years, a series of great breakthrough has been made in the mechanism research of NR signal transduction. NRs’ regulatory effects on Wnt/β-catenin have also been further studied. It has been reported that a variety of NRs directly bind with β-catenin or TCF4, restrain β-catenin/TCF/LEF compound formation, resulting in the inhibition of the transcriptional activity of downstream target genes and the
consequent promotion of CRC development.

1. RXRα and Wnt/β-catenin
RXRα is one of the most important members in NRs. RXRα, in the form of homologous or heterologous dimer, is involved in multiple signaling pathways in cells. RXRα has close relationship with many diseases, such as cancer, metabolic syndrome, and so on (25, 26). So far, many RXRα agonists have been found, such as retinol, 9-cis-retinoic acid (9-cis-RA), docosahexaenoic acid (DHA), fatty acids (FAs), arachidonic acid (AA), oleic acid, bexarotene, AGN194204, etc. (7, 27). In recent studies, RXRα and its ligands or pharmacological modulators were found to regulate Wnt/β-catenin pathway. RXRα was found to directly bind to β-catenin to induce the degradation of β-catenin in colorectal cancer cells, thus inhibiting the transcription of Wnt/β-catenin downstream genes and ultimately affecting CRC cell cycle progression (28). This report was further confirmed by the observation that retinol enhanced the interaction between RXRα and β-catenin and promoted the transport of β-catenin into cytoplasm to undergo degradation (29). In APC- and P53-mutated CRC cell lines, RXRα agonist AGN194204 inactivates Wnt/β-catenin signaling via RXRα mediation (20). Besides, RXRα expression decreased while β-catenin expression increased at both mRNA and protein levels in CRC tissues as compared to the adjacent normal colon tissues; moreover, the lower-expression of RXRα was significantly associated with TNM classification of CRC (30). Our recent studies also found that berberine directly bound to RXRα to promote the interaction between RXRα and β-catenin, which led to the degradation of nuclear β-catenin and thus inhibited the transcription of Wnt signaling downstream genes, therefore arrested the cell cycle at G2/M phase and inhibited the proliferation of colon cancer cells (unpublished data).

2. VDR and Wnt/β-catenin
Vitamin D receptor (VDR) plays an important role in the regulation of bone mineral homeostasis, disease prevention and cancer (31, 32). Agonists of VDR include lithocholic acid (LCA), vitamin D3 (1, 25-dihydroxyvitamin D3) and its derivatives, phenyl-pyrrole-based pentane derivatives, etc. (33, 34). Recent researches showed that VDR inhibited CRC progression via targeting to Wnt/β-catenin pathway. VDR agonists, vitamin D3 and LCA, significantly promoted the interaction between VDR and β-catenin, down-regulated the transactivational activity of β-catenin/TCF and finally inhibited the expression of downstream target genes (35). Knocking down VDR accumulated β-catenin in the nucleus and raised the expression of downstream target genes in colon cancer cells; meanwhile, systemic knockout of VDR blocked vitamin D3-mediated β-catenin translation from the nucleus to the cell membrane (35). Tumors of Apoc5-Vdr−/− mice had increased nuclear β-catenin and the volume of these tumors was larger than that in Apoc5-Vdr+/+ mice (36). VDR expression was down-regulated by the transcription factor snail (37, 38); in snail-expressed colorectal cancer cells, vitamin D3-induced Wnt/β-catenin inhibition and E-cadherin expression were eliminated (39). Vitamin D3 promoted the interaction between VDR and β-catenin to suppress the transactivational activity of β-catenin/TCF (21, 40, 41). In Apoc5-Vdr−/+ mice, vitamin D and its analogs down-regulated the nuclear β-catenin, decreased the expression of downstream target genes, and reduced the number of tumors (42, 43).

3. PPAR and Wnt/β-catenin
Peroxisome proliferator-activated receptor (PPAR) is related to fat cell differentiation, metabolism, inflammation and cancer (44, 45). PPAR was named mainly because that it could be activated by fatty acids-like compound peroxisome proliferator. Agonists of PPAR include fatty acids, 15d-PGJ2, thiazolidinedione (TZD), isosilybin A and L312, etc. (46, 47).

It has been reported that PPARγ interacted with β-catenin (48). In the process of fat formation, PPARγ targeted
β-catenin and induced its degradation to suppress the Wnt signaling pathways (49). The agonists of PPARγ inhibited the β-catenin signals independently on the transactivational activity of PPARγ (50). PPARγ decreased the expression of downstream genes of Wnt/β-catenin signaling in gastric cancer cells (51). In mice treated with PPARγ agonist pioglitazone or rosiglitazone, the length of the colon fossae and the proliferation of colon epithelial cell were significantly suppressed. PGI2-activated PPARγ inhibited the transcription mediated by β-catenin/TCF in colon cancer cells (52). PPARγ inhibited β-catenin expression in the Apcmut/1638N mice, and impeded the occurrence and development of colon cancer (53).

4. LXR and Wnt/β-catenin

Liver X receptor (LXR) play important roles in the process of cholesterol, fatty acids and glucose metabolism (54, 55). The agonists of LXR include T0901317, GW3965, GSK9772, etc. (56, 57). The activation of LXR by T0901317 inhibited the cell proliferation of prostate and breast cancer, and the effect of T0901317 directly correlated with LXRα expression levels (58). T0901317 influenced the formation of β-catenin/TCF/LEF complex by activating LXR, and prevented the transactivational activity of this complex (59). LXR agonist GW3965 inhibited cell proliferation and induced apoptosis in colon cancer cells (60). T0901317 or GW3965 binding to LXR promoted the interaction between LXR and β-catenin in HCT116 cells, which did not affect the mRNA expression or the degradation of β-catenin but changed the formation of β-catenin/TCF/LEF complex, leading to the suppression of the transactivational activity of this complex and the inhibition of cell proliferation (61).

5. TR3 and Wnt/β-catenin

Orphan receptor TR3, also called Nur77, regulates tumor development, metabolic disease and cardiovascular disease (62-64). In recent years, some agonists of TR3 were identified, including Csn-B and THPN (65, 66). TR3 was also reported to regulate Wnt/β-catenin pathway. In CRC cells, degradation of β-catenin protein was induced by TR3, which required both the interaction between TR3 and β-catenin and TR3 shuttling from nucleus to cytoplasm (67). TR3 inhibited the development of CRC by down-regulating the Wnt signaling pathways in the Apcmut/1638N mice models, through binding to β-catenin and TCF4 and influencing the assembly of β-catenin/TCF/LEF complex; moreover, Csn-B inhibited the Wnt signal via TR3 (68).

6. HNF4α and Wnt/β-catenin

Hepatocyte nuclear factor 4α (HNF4α), one of the orphan receptors, is involves in the physiological process of cell metabolism, differentiation and cancer development (69-71). The mRNA and protein levels of HNF4α was significantly lower in the clinical samples of colon cancer compared to the adjacent normal colon tissues, and was significantly associated with clinical outcomes. In xenograft tumor experiment in nude mice using CRC cell line HT29, HNF4α inhibited the growth and liver metastasis of xenograft tumor; in CRC cells, HNF4α competed with β-catenin to interact with TCF4, resulting in the suppression of assembly of β-catenin/TCF/LEF complex and cell growth (72). The nuclear β-catenin was increased by 50% in Hnf4αΔmice compared with that in Hnf4αloxP/loxP mice; Wnt downstream target genes were subsequently raised by accumulated nuclear β-catenin (73). Over-expressed HNF4α improved the transcriptional activity of TOP/FOP report gene in HCT116 colorectal cancer cells (73). However, β-catenin protein level in intestinal crypt cell cytoplasm had no significant change in Hnf4α intestinal deleted mice (73).

Discussion

NRs interact with Wnt signaling pathway in colon cancer mainly through two approaches: (i) NRs directly interact with nuclear β-catenin in colon cancer...
cells and β-catenin is guided out of the nucleus and degraded by protease, which inhibit the expression of the Wnt downstream target genes. (ii) NRs interact with TCF4 to inhibit β-catenin/TCF4/LEF complex formation independently on β-catenin degradation. The binding of the corresponding agonists to NRs enhanced the effects of NRs on the regulation of Wnt/β-catenin signaling and development of CRC. Notably, the pharmaceuticals targeting NRs are the second leading approved medicines by FDA, accounting for 13% of all FDA approved 20000 drugs in 2006 (74). Therefore, the exploration of molecular mechanism and optimization of small molecule agonists targeting NRs in Wnt pathway may provide theoretical basis for their future application in the CRC treatment.

Nuclear receptors usually function as homodimers or heterodimers. It was proposed that the combined use of different NR agonists may be significantly superior to single agonist in cancer treatment. It has been reported that the phosphorylated RXRα accumulated in colon cancer cells and tissues (75). The single use of 9-cis-RA (the agonist of RXRα) does not change the phosphorylation levels of RXRα (75). However, the combined use of 9-cis-RA and ciglitazone (an agonist of PPAR) can synergistically reduce the phosphorylation of RXRα, leading to the activation of the promoter of PPARs and the inhibition of the activity of AP-1 promoter and the expression of COX2 and C-JUN, which then inhibited cell growth and induced apoptosis (75). LGD1069 (an agonist of RXRα) in combination with rosiglitazone (PPARγ agonist) synergistically activated the transcription of a serial of genes including S100A2 in A375 (DRO) melanoma cells remarkably (76).

Since multiple NRs and their ligands or pharmacological modulators regulate Wnt pathway, it would make great sense to further explore whether they showed synergistically effect on Wnt/β-catenin signaling, as well as the underlying mechanisms. If so, the combined treatments with NRs agonists would have significant advantages for CRC treatment by targeting Wnt/β-catenin signaling.

Together, the clarification of NR-related molecular mechanisms in CRC, as well as the optimization of ligands or pharmacological modulators targeting NR-Wnt signaling and the exploration for the potential combined use of these agents will provide new strategies for the precise treatment and individualized therapy of colorectal cancer.

Conflict of interest

The authors declare no competing financial interests.

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