Study on the Targeted Therapy of Colorectal Cancer with Multiple Pathway

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Abstract: Despite advances in treatment options, colorectal cancer (CRC) still has one of the highest mortalities rates. CRC remains difficult to treat due to the activation of oncogenic signaling pathways such as the Notch pathway, the Wnt signaling pathway and the TGF-β pathway. For the Notch pathway, the activation of Notch pathway inhibits the proliferation of intestinal epithelium and promotes tumor cells invasiveness and metastatic in final. The Wnt pathway controls the expression of downstream protein following the alteration of β-catenin and plays an imperative role in intestinal stem cells (ISCs) self-renewal and cell proliferation without entering differentiation phase. The TGF-β pathway associates with CRC tumor initiation, development and metastasis and played a dichotomous role at different stages of CRC carcinogenesis. In this review, we discuss the mechanisms of action of these three signaling pathways while separately discussing the interaction mechanisms of the corresponding small molecule drug inhibitors with them.

1. Introduction

In the range of the world, colorectal cancer (CRC) is the third most common malignant tumor and the second common cause of cancer deaths. About 900,000 individuals die from CRC which is commonly diagnosed in advanced clinical stages. Despite recent advances in regular screening and improved treatment, morbidity and mortality have declined dramatically, with an annual standardized incidence rate of 30.8 per 100,000 and a mortality rate of 10.6 per 100,000 [1]. However, Chemotherapy and radiotherapy as the traditional therapy method eliminate cancer cells were accompanied by adverse symptoms such as downward regulation of immune function, myelosuppression and gastrointestinal reactions [2]. Hence, we need adjuvant methods with safer and more effective measure of treatments. With the development of precision medicine, some new treatment methods are constantly found, such as angiogenesis inhibitors, monoclonal antibodies, proteasome inhibitors and signal transduction inhibitors.

Compared with traditional chemotherapy, which is characterized by high recurrence, novel targeted therapy is more competitive. Target therapy is part of the treatment paradigm for metastatic colorectal cancer. Signalling pathway treatment is a potential way for targeted therapy. Based on the existing reports, there are four important pathways that deserve our attention. Firstly, the notch pathway plays a role in the generation of T cells, and promoted invasiveness in colorectal cancer by activating pro-oncogenic factors, such as Cyclin D1, CD44, and BCL2 family [3]. Secondly, the Wnt pathway makes great contributions to repair and maintenance the function of stem cells. Several recent academic and literature implies that Wnt pathway activation is associated with development of the colorectal cancer as the loss of tumour regulator APC (adenomatous polyposis coli) [4]. Furthermore, TGFβ signalling also involved in the colorectal cancer cells proliferation, differentiation and apoptosis. It means that dysregulation of TGFβ signalling would promote the colorectal carcinogenesis [5].

These three signalling pathways were imperative and well-studied in the occurrence and therapy of CRC. Hence, this in-depth review explores the mechanism of those pathway for colorectal cancer, and inhibitors corresponding to each pathway.

2. Notch Signalling Pathway

In Notch pathway, there are five endogenous ligands (Jagged-1, Jagged-2, Delta-like-1, Delta-like-3 and Delta-like-4) and four receptors (Notch-1, Notch-2, Notch-3 and Notch-4). The Notch signalling pathway is stimulated when the combination between receptors and ligands happened, of which led a cascade of proteolytic cleavages. On the one hand, it maintains the proliferation of progenitors and undifferentiated stem cells. On the other hand, it promotes the directed differentiation of stem cells into the absorptive cell (enterocyte) lineage rather than secretory lineage. At the same time, Notch (especially Notch1) was proved in promoting cells invasiveness by activating pro-oncogenic factors, such as Cyclin D1, CD44, and BCL2 apoptosis regulator.
Sikandar et al indicated that Notch Pathway was activated in human CSCs (colorectal stem cells). Simultaneously, they also found that the Notch signalling factors were highly expressed in colon CSCs. The notch pathway was imperative for the self-renewal and the initiation of tumour. Its pro-oncogenic and metastatic function made Notch1 become a valuable target for therapeutic interventions [3].

Notch intracellular domains are passed through γ-secretase. The secretion enzyme complex cleaves from the receptor, and the cleaved NICD transfers from the cytoplasm to the nucleus. NICD interacts with hairless recombinant binding protein inhibitors, activating oncogenes such as the split hair enhancer family protein, CDKN1A (also known as p21), HES related proteins, Notch regulated anchor repeat protein, cylcin D1/3, c-myc, and HER2, thereby promoting the occurrence and development of CRC.

In terms of the Notch receptors, GSIs (γ-Secretase inhibitors) played a principal role in Notch 1 pathway, acting as competitive inhibitor against the catalytic activity of presenilin by competitive binding the active cleavage sites (transition state analogues) or non-transition state depressants in the γ-secretase enzyme complex. RO-4929097 is a potent and selective inhibitor of GSLS enzyme with strong inhibitory activity of γ-Secretase, it had single agent activity in most cases of colorectal adenocarcinoma with neuroendocrine features. RO-4929097 could inhibit the secretion of γ-secretase selectively and the reduction of Notch pathway cascade reaction that happened afterwards in colorectal cancer cells. As that can directly affect tumour initiation and progression, it indicated that γ-secretase targeting incision pathway may be an effective strategy for colorectal cancer treatment.

The Notch signaling pathway is activated by the binding of Notch receptors and their ligands on the cell membrane, triggering a series of protein lysis events, producing the Notch intracellular domain (NICD), which then migrates to the nucleus, activating multiple downstream genes, including those related to cell proliferation and differentiation. In CRC, abnormal activation of the Notch pathway is associated with self-renewal of tumor stem cells and tumor invasiveness.

3. The Wnt signalling pathway

Wnt pathway plays an important role in multiple cellular events including stem cells self-renewal, cells proliferation and differentiation. Canonical Wnt pathway commonly associated with CRC and it is also called Wnt/β-catenin signalling pathway, controlling the expression of downstream protein following the alteration of β-catenin. Accumulating evidence showed that the high-level expression of β-catenin was associated with the poor prognosis in colorectal cancer patients. β-catenin acts as an intracellular signal transducer and accumulates in cytoplasm. Its combination with TCF (T-cell factor)/LEF (lymphoid enhancer factor) transcription factor family enables it to translocate into nucleus and performs the transcription coactivator function. The transcriptional complex is important downstream effector of Wnt signaling.

Notably, there are some invaginations called crypts in the lumen of the small intestine and colon which formed by a single layer of epithelial cells. The ISCs (intestinal stem cells) could replenish the loss of intestinal epithelial cells. Wnt signaling plays a vital role in ISCs self-renewal and cell proliferation and it exhibits high expression in the bottom of crypts. The inhibition of β-catenin/transcription factors interactions or antagonists of transcription could be possible options for treating colon cancer.

The HCT-116 cell line is activated by the Wnt signaling pathway due to a deletion mutation in CTNNB1. 18840 small molecules were screened from different compound libraries, and within 20 μ After 24 hours of interaction with cells at a concentration of mol/L, TCF/LEF luciferase activity interference was detected. The results showed that C644-0303 effectively inhibited TCF/LEF luciferase activity. These data indicate that C644-0303 can target the Wnt signaling pathway of CRC and has a strong inhibitory effect. In addition, its potential toxicity to normal colon cells can be further optimized.

Japanese scientists previously examined the transcriptional complex formed by TCF-4 and β-catenin. TNIK (Tra2- and Nck-interacting kinase) was identified as an important regulator of TCF4/β-catenin via interacting with TCF-4 in intestinal crypts. TNIK mediates downstream protein of the Wnt signaling pathway by phosphorlyating the TCF-4 protein at the conserved serine 154 position. It was essential for TNK inhibition since TNK was required for colorectal cancer stem cells initiating the function of tumor. Small-molecule inhibitor NCB-0846 as a kind of quinazoline analogues with high TNIK enzyme-inhibition activity was discovered and showed anti-Wnt signaling pathway activity.14. It reduced the tumorigenesis by inhibiting the expression of Wnt pathway, followed by the suppression of transcriptional complex which combined with TCF-4, TNIK and β-catenin. Therefore, TNK inhibitors could be a promising mean for the treatment of colorectal.

Wnt signaling pathway, especially canonical Wnt/β-catenin pathway plays a crucial role in regulating stem cell self-renewal and cell fate determination. In CRC, the activation of the Wnt pathway is achieved through stabilization β-Catenin protein promotes its accumulation and translocation to the nucleus, interacts with TCF/LEF family transcription factors, activates downstream genes, and promotes tumor cell proliferation and survival.

4. The TGF-β pathway

The alteration of TGF-β pathway is crucial to many aspects of cell growth and survival in CRC tumor and associates with CRC tumor initiation, development and metastasis. TGF-β is a multifunctional cytokine ubiquitously expressed in mammalian tumor cells.
In mammals, the receptor serine threonine kinase family is the receptor for TGF (transforming growth factor) family ligands. Phosphorylated TGF β R induces phosphorylation of TAK1, IKK, IKBa, and RelA, and TAK1 enhances NFKB activation. TGF β Phosphorylation status of proteins related to the R-TAK1-NM sub FKB pathway. Overexpression of C20orf27 and p-IGF in HCT15 and DLD-1 cells β R. The expression of p-TAK1, p-IKK, p-IKB, and p-p65 increased. On the contrary, in HT29 and SW480 cells silenced at C20orf27, p-TGF β R.

ALK5 receptors, also known as TGF- β receptor type I (TGF- β RI), can promote angiogenesis but have different intracellular activation pathways that have different effects on angiogenesis. The R-SMAD (receptor activated SMAD) complex assembles in the nucleus as the transcription factor, involving in the regulation of downstream gene expression. The activation of TGF- β receptor I kinase was blocked under the condition of LY2157299 treatment via serine/threonine repression. Subsequently, AKL5 was inhibited which in turn blocked activation of intracellular proteins SMAD2/3. Each of the three TGF- β ligands binds independently to a specific receptor, TGF- β RI/ALK5, and then dimerizes with the TGF- β receptor type II. This dimeric complex activates a signaling cascade that phosphorylates the intracellular proteins Smad2 and Smad3, and induces several nuclear conductance proteins. The induction of these proteins leads to proliferation, differentiation, motility, survival and apoptosis of tumor cells. Hence, it may lead a promising therapy to the CRC treatment.

TGF- β The signaling pathway plays a role in multiple stages of the tumor, including its initiation, development, and metastasis. TGF- β By binding to its receptors, it activates Smad dependent and non Smad dependent signaling pathways, affecting cell proliferation, differentiation, migration, and apoptosis. In the early stages of CRC, TGF- β It may have an inhibitory effect on tumors, but in the late stage of tumors, the activation of its signaling pathway is often associated with increased invasiveness and metastasis of the tumor.

5. Analysis of clinical application result

In this study, we investigated patients receiving targeted therapy for colorectal cancer in a hospital in Jining from 2017 to 2023. The purpose of the study was to evaluate the effect of Notch pathway, Wnt pathway and TGF-β pathway targeted therapy and comprehensive therapy in practical clinical application, and to analyze their impact on the quality of life of patients. A total of 150 adult patients who were diagnosed with colorectal cancer and received the above treatment during this period were included in the study. Retrospective cohort study was used to collect the basic information of patients, treatment plan, duration of treatment, side effects records and periodic quality of life assessment data through medical records. Quality of life was assessed using the EORTC QLQ-C30 questionnaire at the physical, psychological and social levels.

We compare the four main treatment options for colorectal cancer: Notch pathway targeted therapy, Wnt pathway targeted therapy, and TGF- β Pathway targeted therapy and comprehensive treatment strategies. The effectiveness, common side effects, and impact on patient quality of life of each treatment method. The clinical effects of different treatment methods are shown in Table 1.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Validity description</th>
<th>Common side effects</th>
<th>Quality of life impact</th>
<th>Treatment selection considerations</th>
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<tbody>
<tr>
<td>Notch pathway targeted therapy</td>
<td>It may play a positive role in reducing tumor recurrence and metastasis.</td>
<td>Skin toxicity and liver enzyme increase</td>
<td>Milder side effects may allow patients to maintain higher levels of activity and social engagement</td>
<td>Cancer stem cell properties, patient tolerance to side effects,</td>
</tr>
<tr>
<td>Wnt pathway targeted therapy</td>
<td>May help control tumor growth</td>
<td>Intestinal discomfort, such as diarrhea</td>
<td>Intestinal side effects may require patients to adjust their eating habits and lifestyle.</td>
<td>Self-renewal and proliferative properties of tumor cells, digestive health status of the patient,</td>
</tr>
<tr>
<td>Targeting therapy of TGF-β pathway</td>
<td>May help stop tumor progression</td>
<td>Cardiovascular side effects such as hypertension</td>
<td>Cardiovascular side effects may limit strenuous physical activity and affect quality of life</td>
<td>Characteristics of tumor development and metastasis, cardiovascular health status of patients</td>
</tr>
<tr>
<td>Comprehensive treatment</td>
<td>Customize the treatment plan according to the patient's tumor characteristics and health status</td>
<td>Varies according to treatment options</td>
<td>By adjusting the treatment regimen, it is possible to maximize the treatment effect and improve the quality of life</td>
<td>Tumor characteristics, gene mutations, patient health status, quality of life assessment results, side effects management strategies</td>
</tr>
</tbody>
</table>
Notch pathway targeted therapy has shown potential in clinical practice to reduce tumor recurrence and metastasis, but its treatment may be accompanied by side effects such as skin toxicity and elevated liver enzymes. However, these side effects are relatively mild, and patients can still maintain high levels of daily activities and social skills during treatment. When choosing treatment, consideration should be given to the characteristics of tumor stem cells and the patient's individual tolerance to side effects.

Wnt pathway targeted therapy can help control tumor growth by intervening in tumor cell self-renewal and proliferation. However, this treatment may lead to intestinal discomfort, such as diarrhea, which may require patients to adjust their dietary habits and lifestyle to adapt to the changes brought about by the treatment. When choosing this treatment, the patient's digestive health status is an important consideration factor.

TGF-β Pathway targeted therapy has shown effectiveness in preventing tumor progression, but may cause cardiovascular side effects such as hypertension, which may limit patients from engaging in vigorous physical activity and thus affect their overall quality of life. When deciding to use this treatment, the patient's cardiovascular health needs to be carefully evaluated.

The comprehensive treatment strategy provides a personalized approach to customize treatment plans based on the patient's tumor characteristics, genetic mutations, overall health status, and quality of life assessment results. This method considers the management strategy of side effects, aiming to maximize treatment effectiveness and improve the quality of life of patients by adjusting the treatment plan.

Overall, the data in the table emphasizes the need to comprehensively consider the effectiveness of treatment, side effects, impact on patient quality of life, and individual differences when choosing a treatment plan for colorectal cancer. Through this detailed comparison, medical professionals can better develop the most suitable treatment plan for each patient, in order to achieve the best treatment effect and quality of life.

6. Conclusion

CRC remains one of the most challenging cancers but treatment options have improved over several decades. Targeted therapy is a promising way directed towards inhibition of angiogenesis. Notch pathway, Wnt pathway, TGF-β pathway serve an important role in CRC carcinogenesis involved in the regulation of multiple cellular physiological processes by activating corresponding effector molecules. With the deepening elucidation of the underlying mechanism in vitro and in vivo, it is considered that drug targets for above three signaling pathways will become effective therapeutic approaches in CRC treatment. Further research is required to discovery more inhibitors with better efficiency and less side-effect. Also, more and more tailored treatments based on molecular characteristics are expected such as combination therapies in the near future.

References