Insights into the role of STAT3 in intrahepatic cholangiocarcinoma (Review)

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Abstract. Intrahepatic cholangiocarcinoma (ICC) is a primary malignant liver tumour whose incidence is second only to that of hepatocellular carcinoma. ICC is a highly heterogeneous disease arising from neoplastic transformation of intrahepatic biliary epithelial cells (cholangiocytes), and it is characterized by a very poor prognosis. Signal transducer and activator of transcription 3 (STAT3) is an important oncogene that is widely expressed in numerous cancers. STAT3 is a candidate target for the treatment of ICC. However, studies on STAT3 and the occurrence and development of ICC require improvements. Therefore, the present review summarized the mechanism of STAT3 in ICC and provided a theoretical basis for STAT3 to become an effective target for determining the prognosis and treatment of ICC.

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1. Introduction

Intrahepatic cholangiocarcinoma (ICC) is a malignant tumour originating from the intrahepatic bile duct epithelium that accounts for ~10-15% of primary liver cancer cases (1,2), and its morbidity and mortality rates are increasing. At present, the molecular mechanism of ICC is not clear. Previous studies have shown that various cytokines produced during chronic inflammation cause abnormalities in oncogenes, DNA mismatch repair genes/proteins, and tumour suppressor genes. Genetic and epigenetic changes in cholangiocytes may promote proto-oncogene activation and tumour suppressor gene inactivation. These cumulative effects eventually lead to malignant transformation. In addition, cytokines also play important roles in promoting cell growth, inhibiting cell apoptosis, increasing cell invasiveness and promoting tumour angiogenesis (3,4). Currently, adjuvant treatments such as radiotherapy and chemotherapy have not significantly improved the overall survival (OS) rate of patients with ICC (5-7). Surgery is the only effective treatment for ICC. However, ICC is characterized by atypical clinical symptoms and early metastasis, leading to the diagnosis of advanced cancer and a lost opportunity for surgery. It is also prone to recurrence after surgery, and the overall 5-year survival rate after surgery is only 14-40% (6). With further research on the pathogenesis of ICC, an increasing number of molecular targets have been discovered. As the convergence point of numerous oncogenic signalling pathways, signal transducer and activator of transcription 3 (STAT3) plays a prominent role in regulating antitumour immune responses. In the tumour ecosystem, STAT3 is extensively overactivated in tumour cells to promote tumor growth. Moreover, STAT3 is also extensively overactivated in non-tumour cells to suppress the expression of key regulators of immune cell activation and promote the production of immunosuppressive factors (8). Therefore, drugs targeting the STAT3 signalling pathway have become a promising therapeutic strategy. Multiple studies (9-11) have shown that STAT3 expression is associated with several clinicopathological features, including tumour size, pathological satellites, vascular invasion, undifferentiated histology, lymph node metastasis and TNM stage. Patients with high STAT3 levels have a poor prognosis in terms of OS and disease-free survival (DFS). A multivariate survival analysis showed that

STAT3 was an independent prognostic factor for OS and DFS. Furthermore, it was observed that STAT3 overexpression promoted the invasion, metastasis and proliferation of ICC cells *in vitro* and *in vivo* and promoted STAT3 phosphorylation (12,13). STAT3 expression may become a new target for the treatment of patients with ICC.

2. Structure and function of STAT3.

STATs are DNA-binding proteins consisting of 750-850 amino acids, and their molecular weight is 84-113 kDa. STATs play a key role in cytokine signal transduction. The STAT family members expressed in mammalian cells mainly include STAT1, STAT2, STAT3, STAT4, STAT5 and STAT6, which are encoded by different genes. As one of the earliest discovered oncogenes, STAT3 has become an important gene that must not be ignored in tumour research and is involved in regulating cell proliferation, differentiation, apoptosis as well as other processes (14). STAT3 is a highly conserved protein consisting of ~770 amino acids (only one amino acid difference exists between mouse and human STAT3), that is expressed as three isoforms: STAT3 'alpha' 'beta' and STAT3 gamma. It contains an amino terminal domain, a DNA binding domain and a C-terminal transcription activation domain. The amino terminal domain forms a coil structure. The structure of the DNA binding domain is an Src homology 2 (SH2) domain. The C-terminal domain adopts the transcription activation domain structure and is located between the two aforementioned domains. The SH2 domain plays an important role in signal transduction and specifically identifies phosphorylated tyrosine residues. It is activated by phosphorylation (15). The key tyrosine associated with dimer formation is located in the SH2 domain. STAT3 activity is regulated by the phosphorylation of serine 727, and phosphorylated STAT3 quickly enters the nucleus in the form of monomers. Homodimers or heterodimers of transcription factors are activated and interact with the promoters of their transcriptional target genes (Fig. 1). STAT3 is widely expressed in certain types of cells and tissues. STAT3 plays an indispensable role in early embryonic development and bone marrow cell differentiation in a STAT3-deficient mouse model (16). Under normal circumstances, STAT3, the main regulator that balances cell proliferation and apoptosis, participates in maintaining the growth and development of embryonic stem cells. Concurrently, it also participates in processes such as antigen tolerance. STAT3 activation is strictly regulated by a negative feedback mechanism, and it is inactivated and transported to the cytoplasm after transducing specific signals. However, upon stimulation with carcinogenic signals, STAT3 is continuously activated, exists in the nucleus in a constant activation state, and continuously activates target genes to promote tumour progression (17).

3. STAT3 signalling pathway is involved in the occurrence and development of ICC

Interleukin (IL)-6/Janus kinase (JAK)/STAT3 pathway. The JAK/STAT pathway is closely related to inflammatory factors. IL-6 binds to the soluble IL-6 receptor (SIL-6R) to activate the IL-6/JAK2/STAT3 signalling pathway (18). Briefly, SIL-6R recognizes and binds to IL-6 to form the SIL-6R/IL-6 complex, and activates glycoprotein 130 (GP130) on the surface of the cell membrane. Activated GP130 activates receptor tyrosine kinase and binds to the STAT3 protein, which phosphorylates and activates the nuclear transcription factor (19). STAT3 enters the nucleus and regulates the expression of inflammatory cytokines. The IL-6 family mainly includes IL-6, IL-11, ciliary neurotrophic factor, leukaemia inhibitory factor, oncostatin M (OSM), cardiac trophic factor 1, cardiac trophic protein-like cytokine and cardiac trophic protein 2 (20). For example, IL-6 expressed in T cells, B cells or macrophages further promotes STAT3 phosphorylation and activation by activating JAK1, and STAT3 subsequently enters the nucleus to initiate downstream gene transcription (8), participating in the malignant process of ICC. Due to the continuous stimulation of upstream molecules, the abnormally and continuously activated IL-6/JAK/STAT3 pathway leads to resistance to apoptosis and further promotes tumour development. A study has shown that almost all cytokines in the IL-6 family activate the STAT3 protein. STAT3 is also considered the most important transcription factor mediating IL-6 function (8). Lipopolysaccharide (LPS) activates the IL-6/STAT3 signalling pathway in normal hepatic bile duct epithelial cells (21). LPS induces activation of the IL-6/STAT3 signalling pathway by not only activating this signalling pathway but also by increasing the expression of C-MYC and MCL-1, suggesting that the IL-6/STAT3 signalling pathway may be an important hub mediating inflammation and ICC (22). Both OSM and IL-11 are IL-6 family cytokines expressed in inflammatory and cancer processes. Tumour-associated neutrophils (TANs) and tumour-associated macrophages (TAMs) produce higher levels of OSM and IL-11 in coculture, respectively (23). Both of these cytokines activate the STAT3 signalling pathway in ICC cells. STAT3 knockout eliminates the tumour-promoting effects of TANs and TAMs on ICC, and increased levels of TANs and TAMs are related to the increased levels of p-STAT3 in tumour samples from patients with ICC (24). Researchers concluded that the effects of TANs and TAMs on ICC mainly depend on OSM- and IL-11-mediated activation of the STAT3 signalling pathway (Fig. 2).

IL-10/JAK/STAT3 pathway. IL-10 is a cytokine encoded by the IL-10 gene. In humans, IL-10 is produced mainly by immune cells, including monocytes, type 2 T helper cells and regulatory T cells (Tregs). IL-10 may play a role by regulating the JAK2/STAT3 signalling pathway and the extracellular signal-regulated kinase 1/2 pathway to alter the expression of downstream genes (25,26). The role of the JAK1/STAT3 pathway in tumours has attracted increasing attention. The JAK1/STAT3 pathway is an important pathway mediating cytokine signal transduction and is involved in various cell functions, such as differentiation, survival, proliferation and apoptosis, as well as pathological immune and inflammatory processes (27). According to previous studies (28,29), IL-10 induces STAT3 phosphorylation in Tregs. Although STAT3-deficient Tregs inhibit the proliferation of $CD4^+$ T cells *in vitro*, their number in inflamed tissues is reduced, and their ability to inhibit the inflammatory activity of TH17 is also reduced (30). Thus, the mechanism by which IL-10 inhibits tumour-associated inflammation may be related to STAT3 phosphorylation and its downstream effects on cytokine

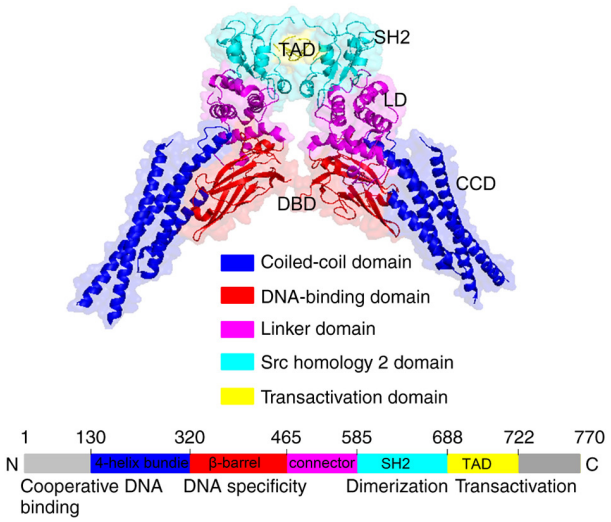

Figure 1. Structure of the STAT3 protein. STAT3, signal transducer and activator of transcription 3.

receptors or subsequent gene expression. After the successful polarization of M2 macrophages *in vitro*, IL-10 levels in the supernatant of M2 macrophages were significantly increased compared with untreated THP1 cells, and IL-10 was suggested to promote ICC cell migration, invasion and epithelial transformation via the STAT3 pathway (31) (Fig. 2).

Epidermal growth factor receptor (EGFR) and STAT3 pathways. EGFR, with a molecular weight of 170 kDa, is a member of the epidermal growth factor receptor family. EGFR is mainly located on the surface of human epithelial cells, fibroblasts, glial cells and other cells, and its signal transduction pathway plays an important role in promoting cell growth, differentiation, as well as other physiological processes. Loss of EGFR protein tyrosine kinase function or abnormal activity of key factors in related signalling pathways may lead to the development of tumours, immune deficiencies and cardiovascular diseases. Upon binding of the ligand to its extracellular ligand binding domain, EGFR is phosphorylated and forms either a homodimer or heterodimer, initiating an extensive intracellular signalling cascade (32-34). STAT3, one of the most important downstream effectors, is phosphorylated at Tyr705 by activated EGFR and is then translocated to the nucleus for transcriptional regulation, contributing to cell proliferation, resistance to apoptosis and angiogenesis (35,36). At present, EGFR overexpression or abnormal expression has been detected in various tumours, leading to the activation of downstream signalling pathways, particularly the continuous activation of STAT3 that causes its nuclear translocation and the transcription of downstream genes. Numerous *in vitro* and *in vivo* experiments have shown that the continuous expression and abnormal activation of the EGFR/STAT3 pathway are closely related to the occurrence and development of ICC (37). The overactivated EGFR/STAT3 signalling pathway is closely related to the development of ICC, based on an immunohistochemical analysis of ICC samples. EGFR-STAT3 overactivation promotes the growth of ICC cells (38,39) (Fig. 2).

Leucine-rich repeat containing G protein-coupled receptor 5 (LGR5) activates the STAT3 pathway. LGR5 is a member of the G protein-coupled receptor subfamily, also known as HG38 and GPR49. It is a large protein composed of 18 leucine-rich repeat units and 7 transmembrane regions. The structure of the protein is characterized by an extracellular region containing a signal peptide, 17 leucine-rich repeats and a highly conserved 7 α-helix transmembrane region (40). Previous studies (41-43) have detected increased expression of LGR5 in gastrointestinal, ovarian, liver, basal cell carcinoma and other tumour tissues to varying degrees (44). IκB kinase α upregulates the expression of LGR5 by activating the STAT3 signalling pathway and accelerates tumour progression in skin basal cell carcinoma cells (45). LGR5 is essential for Wnt signalling-induced activation of β-catenin, and by further activating STAT3, it enhances CSC-like features and the EMT, leading to aggressive tumour progression and a poor prognosis for patients with ICC (Fig. 2).

FAP/STAT3/CCL2 pathway. Fibroblast activation protein (FAP) is a membrane-bound glycoprotein that belongs to the serine protease family. It is a dimer composed of FAPα and β subunits with a molecular weight of 170 kDa (46). It has endopeptidase and weak dipeptidase activity, degrades a variety of dipeptides and type I collagen and is selectively expressed in cancer-associated fibroblasts (CAFs) of a variety of human solid tumours (47). FAP is expressed in embryonic cells, injured tissues and mesenchymal fibroblasts of >90% of malignant epithelial tumours, but it is rarely expressed in benign tumours and normal tissues; it is associated with extracellular matrix remodelling, tumour proliferation and metabolism (48,49).

A previous study showed that FAP induces inflammatory phenotypes and inflation-related gene expression signatures in CAFs (50). Inducing the expression of FAP in normal fibroblasts produces an inflammatory phenotype similar to that of CAF^{FAP+} cells. In addition, FAP continuously activates STAT3 in fibroblasts in mouse liver tumour models, and CAF^{FAP+} is the main source of CCL2. STAT3-CCL2 signalling increases the recruitment of myeloid-derived suppressor cells (MDSCs) and thus promotes tumour growth from CAF^{FAP+} cells. Moreover, FAP, p-STAT3, and CCL2 levels are positively correlated with adverse pathological features of ICC, and increased FAP levels predict low survival rates. Recently, accumulating evidence has shown that CAFs participate in the progression of ICC by affecting tumour cells (51-54). Additionally, a previous study has shown that CAF^{FAP+} is the main source of CCL2 in the ICC microenvironment (55). In addition, the tumorigenic function of FAP mediated by CCL2 in ICC depends on its intracellular activation of STAT3 signalling in CAFs. FAP recruits MDSCs in a CCL2-dependent manner in ICC. In addition to mediating immunosuppression, MDSCs promote tumour progression by enhancing angiogenesis through a paracrine pathway, suggesting that approaches specifically targeting CAF^{FAP+} may be a more effective and safer treatment strategy for ICC (Fig. 2).

4. STAT3-related targets in ICC

Approximately 70% of malignant tumours present abnormally increased STAT3 activity, including acute myeloid leukaemia,

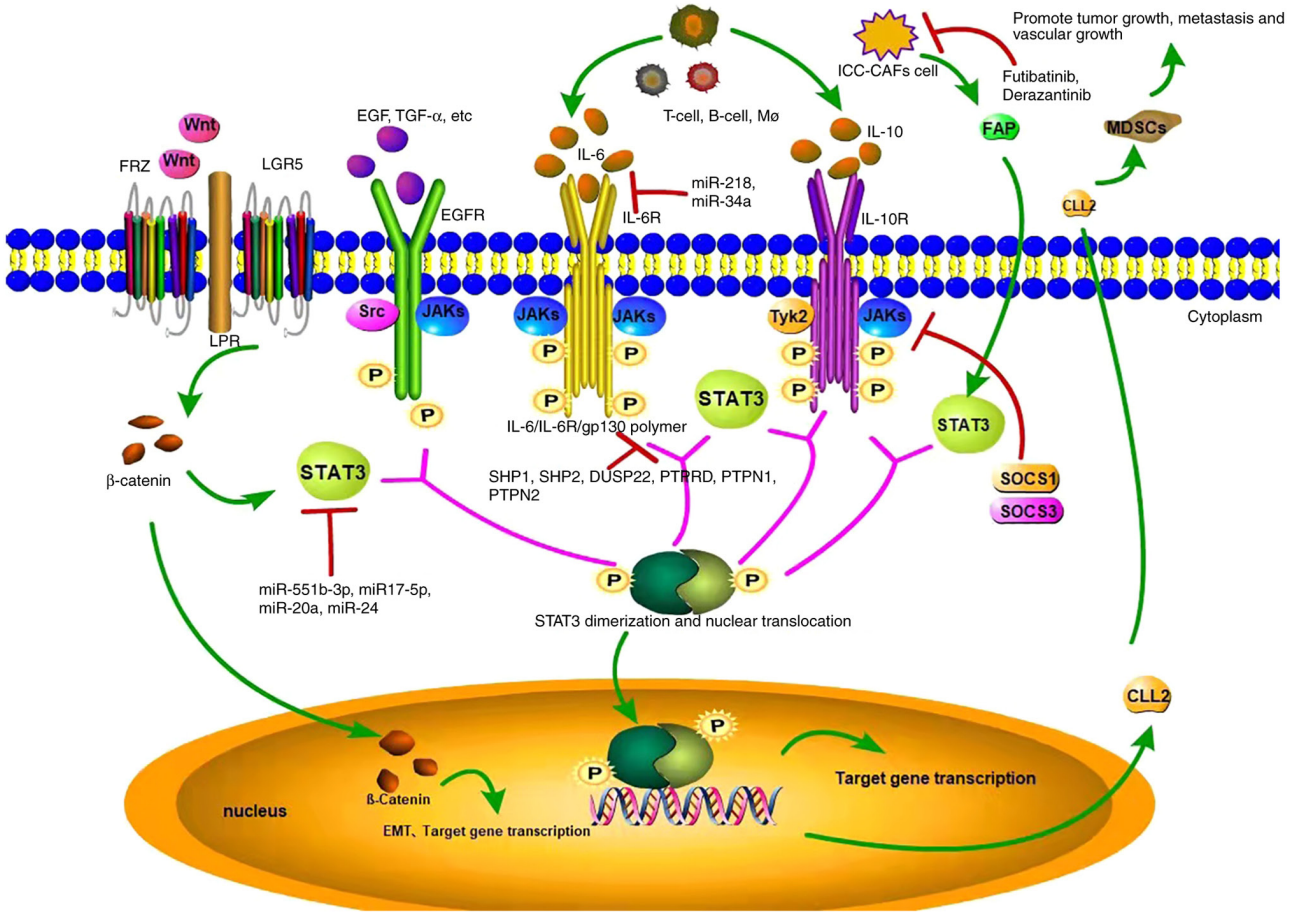

Figure 2. STAT3 is involved in signal transduction pathways associated with intrahepatic cholangiocarcinoma; STAT3 is activated by the cytokine IL-6 and other growth factors, including EGFR, fibroblast growth factor receptor and platelet-derived growth factor receptor, through tyrosine phosphorylation. After dimerization, STAT3 translocates into the nucleus, where it induces gene transcription. STAT3 signalling mediates cell growth, proliferation, inflammatory cytokine production, cell invasion and migration. STAT3, signal transducer and activator of transcription 3; IL, interleukin; EGFR, epidermal growth factor receptor.

multiple myeloma, bladder, breast and colon cancer and ICC (56-64). Phosphorylated STAT3 levels have been revealed to be associated with poor clinical outcomes in patients with these cancers. Therefore, extensive effort has been devoted to identifying and developing STAT3 inhibitors for cancer treatment. However, given the wide range of intracellular functions of STAT3, possible inhibitors have been difficult to develop. However, numerous phase I, phase II, and even phase III trials of drugs targeting STAT3 have been conducted. A number of these treatments are only used as research tools due to their shortcomings, such as limited bio-absorption, utilization, drug resistance, and poor stability, but other drugs achieve favourable effects through oral bio-absorption and by binding to the STAT3 SH2 domain (65-68). Numerous nonpeptide SH2 domain inhibitors have also been identified and shown to inhibit STAT3 activity, including STA-21, IL-6, STAT, TIC, c188-9, OPB31121/51602, WP1066, S3I-201, BP-1-102, STX-0119 and HJC0123 (69-76). The application of these agents in ICC requires further confirmation. In addition to the function of STAT3 inhibitors, another method to inhibit STAT3 activity is to inhibit the interaction of STAT3 with target gene promoter elements. AZD9150 is the second generation of previous iterations optimized by merger, 2', 4' constraints of ethyl STAT3 antisense oligonucleotide-modified residues, which have been shown to prevent STAT3 from binding DNA in a variety of tumours after intravenous injections to inhibit tumour growth (77-83) (Fig. 3). AZD9150 is expected to achieve favourable efficacy in ICC treatment.

5. Summary and prospects

According to previous studies, tumour proliferation, invasion and metastasis, angiogenesis, drug resistance and prognosis are all related to tobacco, alcohol, diet, stress, infection, and chronic inflammation (84,85). Inflammatory factors such as intrahepatic bile duct stones with chronic cholangitis, a high incidence of viral hepatitis B, and biliary parasite infection are considered high-risk factors for ICC (86). STAT3 is located at the intersection of multiple oncogenic signalling pathways and is abnormally activated in malignant tumour tissues, including ICC. STAT3 is mainly activated by various kinases through phosphorylation (87). Activated STAT3 transduces signals from various cytokines and growth factors into the nucleus and participates in regulating the transcription of corresponding target genes, thereby participating in modulating cell survival, proliferation, angiogenesis as well as other processes (88). This inflammatory cascade activates STAT3, leading to the overproduction of bile duct epithelium growth factor, thus

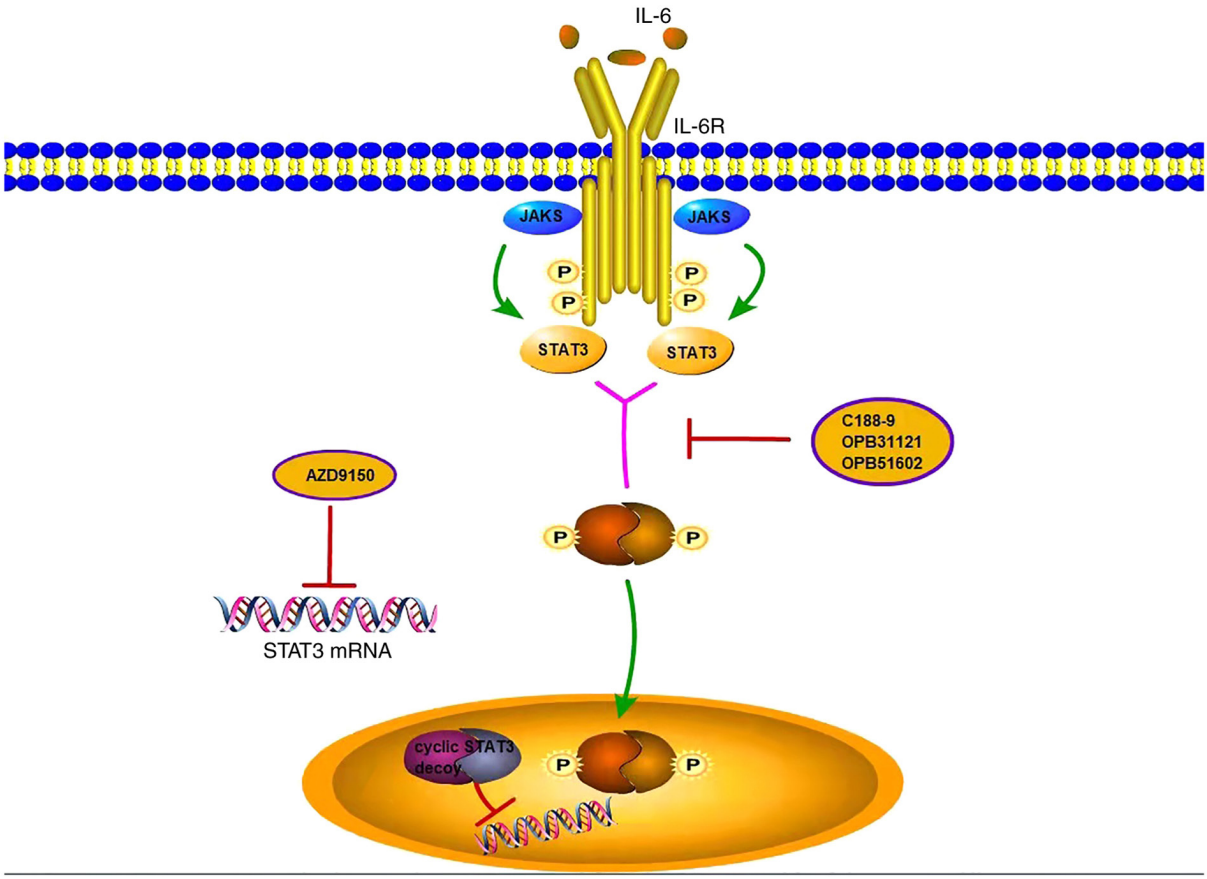

Figure 3. STAT3-associated target proteins in intrahepatic cholangiocarcinoma and numerous domain-specific inhibitors have also been identified. STAT3, signal transducer and activator of transcription 3.

promoting CCA initiation. Due to the role of STAT3 in inflammation and cancer development, targeting STAT3 is a rational treatment strategy for ICC. Numerous studies have shown that STAT3 activation is closely related to the prognosis of patients with multiple myeloma, gastric cancer, hepatocellular carcinoma, lung and laryngeal cancer and ICC (89-92). STAT3 is associated with the development of malignant tumours mainly through STAT3-mediated expression of key target genes that regulate cell proliferation, apoptosis inhibition and the hypoxia response (93). Activated STAT3 also induces the expression of VEGF, which promotes invasive and metastatic angiogenesis (94). In addition, STAT3 binds to the IL-6 promoter, creating a positive feedback loop that leads to increased IL-6 expression. VEGF and IL-6 also exert immunosuppressive effects that may promote the immune escape of tumour cells following STAT3 overactivation, thus forming a vicious cycle of the occurrence, metastasis, and invasion of ICC (95). STAT3 is often used as an important indicator to distinguish ICC from extrahepatic cholangiocarcinoma. Studies (96-98) have shown significantly higher STAT3 expression in ICC than in extrahepatic cholangiocarcinoma. Downregulated STAT3 expression was revealed to significantly reduce the proliferation of ICC cell lines, such as RBE and ICC-9810 cells, and significantly increase the apoptotic rate of RBE and ICC-9810 cells. However, when STAT3 expression was upregulated, the opposite results were obtained. STAT3 promoted the proliferation and inhibited the apoptosis of intrahepatic bile duct cancer cells (99).

STAT3 expression and activation are currently known to be regulated by various mechanisms. Certain cytokines and growth factors activate STAT3 by binding to specific receptors and participate in the pathophysiological process of diseases. Under physiological conditions, STAT3 activation is rapid and transient, lasting only minutes to hours. In the tumour microenvironment, dysregulation of growth factors, cytokines, and co-stimulators leads to continued phosphorylation of STAT3 tyrosine residues. Excessive or constitutive activation of STAT3 alters cell proliferation and apoptosis, promotes invasion and metastasis, and exacerbates immunosuppression in the microenvironment, directly affecting the prognosis and quality of life of patients (100).

Considering the important association between the high STAT3 expression and the malignancy and prognosis of ICC, STAT3 is expected to become a molecular marker for clinical disease staging and may become a new therapeutic target. Based on certain preclinical studies that have identified the potential therapeutic effects of drugs targeting the STAT signal transduction pathway, the development of highly effective and well-tolerated drugs is anticipated in the future. The molecular mechanism of ICC requires further exploration (101), which will facilitate the application of specific genes and signalling pathways to the classification of ICC molecular subtypes and the development of targeted therapeutic drugs. Further studies are required to take advantage of multidisciplinary comprehensive treatment, including surgery, chemotherapy and targeted therapy,

according to the molecular characteristics of ICC in order to improve the quality of life and prolong the survival time of patients.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

RY and YS contributed to the analysis and manuscript preparation. KS revised the review. CP, WY and SL contributed to the conception of the study. YS and SL helped perform the analysis and participated in constructive discussions. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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