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Role of Tetraspanins in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is characterized by high prevalence, morbidity, and mortality. Liver cancer is the sixth most common cancer worldwide; and its subtype, HCC, accounts for nearly 80% of cases. HCC progresses rapidly, and to date, there is no efficacious treatment for advanced HCC. Tetraspanins belong to a protein family characterized by four transmembrane domains. Thirty-three known tetraspanins are widely expressed on the surface of most nucleated cells and play important roles in different biological processes. In our review, we summarize the functions of tetraspanins and their underlying mechanism in the life cycle of HCC, from its initiation, progression, and finally to treatment. CD9, TSPAN15, and TSPAN31 can promote HCC cell proliferation or suppress apoptosis. CD63, CD151, and TSPAN8 can also facilitate HCC metastasis, while CD82 serves as a suppressor of metastasis. TSPAN1, TSPAN8, and CD151 act as prognosis indicators and are inversely correlated to the overall survival rate of HCC patients. In addition, we discuss the potential of role of the tetraspanin family proteins as novel therapeutic targets and as an approach to overcome drug resistance, and also provide suggestions for further research.

Keywords: hepatocellular carcinoma, tetraspanin, tetraspanin family, tumor proliferation, tumor metastasis, targeted therapy, drug resistance

1 INTRODUCTION

Liver cancer was the sixth most common cancer worldwide in 2018, with an annual death toll of over 782,000. Hepatocellular carcinoma (HCC), also known as hepatoma, constitutes nearly 80% of liver cancer cases and poses a significant burden on global healthcare systems due to its prevalence and high death rate (1). A survey in 2012 revealed that HCC in developing countries accounted for

8.1% of all new cancer cases and 83% of all HCC cases worldwide (2). HCC is also the second most common cause of cancer-related death (2). Chronic liver diseases such as chronic hepatitis B and C, among others, are significant risk factors for HCC (3, 4).

Tetraspanins are a family of transmembrane proteins with four transmembrane domains (TM1, TM2, TM3, and TM4), two extracellular loops (ECL1 and ECL2), and one intracellular loop. ECL2 is essential to the functions of tetraspanins since most of protein–protein interaction sites have been mapped to ECL2. ECL2 consists of a constant domain and a variable domain. While the constant domain facilitates interactions between different tetraspanin molecules, the variable domain accounts for interactions with other non-tetraspanin proteins. The conserved cysteine residues in ECL2 can form a disulfide bond within the same ECL2. TM domains contain many polar residues that can stabilize tetraspanin structure with the help of ECL2 disulfide crosslinks. The structure and function of ECL1 have so far remained unclear (5, 6).

Tetraspanins are widely expressed in metazoans. In *Homo sapiens*, the tetraspanin family has 33 family members (7). Some tetraspanin-like proteins have also been identified in plants (8). Since 65% to 95% of amino acids are highly conserved among the tetraspanin family, it is likely that they share similar functions across different species (9).

Tetraspanins are expressed on the surface of most nucleated cells (10) and play important roles in cell proliferation, differentiation, adhesion, migration, and cell–cell crosstalk (7–11). In terms of tumor biology, tetraspanins are indispensable at all the stages of tumor initiation and progression, showing both tumor-promoting and tumor-inhibiting functions (5, 10, 12–14). Specifically, tetraspanins are involved in viral infection, such as hepatitis virus C (HCV), which is a high-risk carcinogenesis factor for HCC (15). Indeed, recent studies on HCC pathogenesis have accrued evidences to support the vital roles of tetraspanins in HCC development (5, 13).

Tetraspanins take part in different biological processes mainly *via* interacting with different partner molecules. Integrins are the most prominent partner of tetraspanins (5, 13). Through binding to integrins, tetraspanins can influence the distribution and function of integrins. Tetraspanins mainly act as a modulator by controlling the trafficking of their partner (8). Tetraspanins can also make an impact on their partners directly by influencing the function of their partner, without influencing the trafficking of their partner. For instance, CD151 can enhance integrin-mediated adhesion to laminin, resulting in integrin signaling activation (16). CD151 can form a complex with integrin α3β1 and regulate PI3K or PI4K signaling pathway in different cancer cells, which finally impacts cancer cell migration *via* rearrangement of actin cytoskeleton or metalloproteinase secretion. In melanoma cell line, CD151–α3β1/α6β4 integrin complexes recruit small G proteins (Ras, Rac1, and Cdc42) to form integrin–CD151–GTPase complexes, finally leading to GTPase activation (17). Besides integrins, tetraspanins can also form a complex with various other proteins such as G protein-coupled receptors (GPCRs), G proteins, growth factor receptors, or some non-proteins such as cholesterol and gangliosides to form tetraspanin-enriched membrane microdomains (TEMs). They are involved in the crosstalk between growth factor receptors and integrins, mediating growth factor pathway such as hepatocyte growth factor receptor (HGFR) signaling pathway, epidermal growth factor receptor (EGFR) signaling pathway, and transforming growth factor-β (TGF-β) signaling pathway (18).

We herein present the interaction between tetraspanins and HCC and their potential as targets for HCC treatment.

2 TETRASPANINS AS HCC RISK FACTORS

Hepatitis B virus (HBV), HCV, cirrhosis, hereditary hemochromatosis, and non-alcoholic fatty liver disease are all proven risk factors of HCC (3). Tetraspanins are associated with HBV, HCV infection, and cirrhosis and therefore may play a vital role in precancerous disease as well as HCC initiation.

2.1 Role of Tetraspanins in Hepatitis B-Associated HCC

The incidence of HCC positively correlates with serum levels of HBV DNA, which is a marker of HBV infection and viral proliferation. For individuals with a serum level of HBV DNA above 1 million copies per milliliter, the cumulative incidence of HCC could reach as high as 14.9% (19). CD82 is a member of the tetraspanin family and performs a variety of functions in HCC progression and metastasis. CD82 is the only tetraspanin that is proven to be a suppressor of HCC progression. In HCC cell lines, Yu et al. found that HBx, a component of HBV, could induce CD82 promoter methylation and impair CD82 expression at the transcriptional level (20). However, an analysis of clinical samples failed to reveal any statistically significant differences in CD82 expression between HBsAg-positive and HBsAg-negative samples. Nevertheless, the HBV-induced downregulation of CD82 may accelerate HCC progression.

2.2 Role of Tetraspanins in Hepatitis C-Correlated HCC

HCV infection is a critical risk factor for HCC. A prospective cohort study in China reported that 23.73% of HCV-positive

Abbreviations: HCC, hepatocellular carcinoma; TM, transmembrane; ECL, extracellular loop; ADAM10, A Disintegrin And Metalloprotease Domain 10; HSECs, hepatic sinusoidal endothelial cells; VCAM-1, Vascular Cell Adhesion Molecule-1; CTGF, connective tissue growth factor; EpCAM, epithelial cell adhesion molecule; CK19, cytokeratin-19; YAP, Yes-associated Protein; CREB, cAMP-response element-binding protein; KLF4, Krüppel-like factor 4; NET-1, neuroepithelial transforming gene 1; GEF, guanine nucleotide exchange factor; CDK4, cyclin-dependent kinase 4; Rb, retinoblastoma; MMP9, metalloproteinase 9; GPCRs, G protein-coupled receptors; TEMs, membrane microdomains; HGFR, hepatocyte growth factor receptor; EGFR, epidermal growth factor receptor; TGF-β, transforming growth factor-β; HUVECs, human umbilical vein endothelial cells; EMT, epithelial–mesenchymal transition; ceRNAs, competing endogenous RNAs; ADAM12m, A Disintegrin And Metalloproteinase Domain 12; AEG-1, astrocyte elevated gene-1; CRC, colorectal cancer; TSPAN-LEL, TSPAN8 large extracellular loop; TIMP-1, metalloproteinase-1; HSCs, hepatic stellate cells; VEGF, vascular endothelial growth factor; NS, not significant.

men and 16.71% of HCV-positive women may develop HCC during their lifetime (21). CD81, a tetraspanin, binds to E2, the HCV envelop protein, *via* its ECL2 domain and facilitates the entry of HCV into hepatocytes. CD81 also mediates immune reactions against viral infections by inducing the secretion of IFNα in the infected cells. CD81 and CD9 are involved in the recognition of HCV-infected cells by plasmacytoid dendritic cells (pDCs), while direct cell–cell contact is a vital step of IFN induction (22).

2.3 Role of Tetraspanins in Cirrhosis-Associated HCC

Almost all chronic liver diseases cause liver fibrosis. Although reversible at its initial stage, liver fibrosis can eventually progress into cirrhosis, which is a known risk factor of HCC (23, 24).

2.3.1 TSPAN5

TSPAN5 is a tetraspanin that regulates ADAM10, a metalloprotease, and thereby activates Notch signaling (25). The prognosis of patients with cirrhosis correlates with the epigenetic modification of TSPAN5. Lubecka et al. reported that among HBV-negative cirrhosis patients, hypomethylation of TSPAN5 gene is more frequently found in the patients who eventually develop HCC (26). Therefore, the locus-specific DNA methylation may be a useful biomarker for screening at-risk populations, and the expression TSPAN5 may be an indicator for carcinogenesis.

2.3.2 CD151

CD151, another tetraspanin, mediates lymphocyte recruitment during the initiation and progression of chronic inflammation. In patients with chronic liver diseases, the upregulation of CD151 is predominantly on hepatic sinusoidal endothelial cells (HSECs) and neovessels, which in turn upregulates the expression of endothelial adhesion molecule/immunoglobulin superfamily member VCAM-1 and subsequently promote lymphocyte adhesion (27).

3 ROLE OF TETRASPANINS IN HCC CELL GROWTH AND SURVIVAL

The tetraspanin family facilitates hepatoma promotion by activating proliferation and anti-apoptotic properties of HCC cells. Some tetraspanins, such as TSPAN15, can enhance tumor cell proliferation ability (28), while other family members, such as CD9, CD63, TSPAN1, TSPAN7, and TSPAN31, can antagonize apoptosis and facilitate tumor cell survival (29–31). These two effects act together in promoting the development of tumors (**Figure 1**).

3.1 TSPAN15

TSPAN15 exhibits a heterogeneous expression pattern in different HCC patients. In some specific types of HCC, the level of TSPAN15 is upregulated, which positively associates with the stemness of cancer cells and recurrence of cancer significantly (28). Stemness is the core property of stem cells, including self-renewal and the ability to generate differentiated progeny (32). Sidahmed-Adrar et al. found that TSPAN15 could enhance the phosphorylation of ERK, which controls the expression and secretion of connective tissue growth factor (CTGF) and thereby promotes HCC cell proliferation. The pro-proliferation effect may also relate to that TSPAN15 can enhance the metalloprotease ADAM10 secretion to some extent, but with unclear mechanism. TSPAN15 expression is also associated with several stemness markers such as epithelial cell adhesion molecule (EpCAM) and cytokeratin-19 (CK19) (28).

3.2 CD9

CD9 activity alone does not suppress hepatoma cell apoptosis, but it is believed that it can enhance the function of CD166 (33), thus contributing to the survival of HCC cells indirectly. CD166 can promote the transcription of Yes-associated Protein (YAP), a critical anti-apoptosis effector in HCC (34), *via* cAMP-response element-binding protein (CREB) (29). CD166 mainly functions as a CD166–CD166 or CD166–CD6 dimer (33), and CD9 can facilitate CD166–CD166 homophilic interaction, which accentuates CD166 anti-apoptosis effect at the protein level (29).

Paradoxically, CD9 itself displays an opposing function in a recent study. It is shown that CD9 can inhibit HCC cell proliferation *in vitro* and that knockdown of CD9 enhances HCC tumorigenicity *in vivo* (35). CD9 can inhibit the phosphorylation and promoter activity of JNK and c-JUN. Therefore, downregulation of CD9 in HCC can finally lead to JNK signaling pathway activation, which promotes HCC cell proliferation *via* downstream factors cyclin D1 and Bcl-2. It is hard to explain how CD9 can perform contradictory functions. Whether CD9 acts as a suppressor of HCC cell apoptosis or proliferation under different conditions still needs further investigation. What is currently known is that Krüppel-like factor 4 (KLF4), an important inhibitor of tumor growth in various kinds of cancers, is a transcriptional factor of CD9 (35).

3.3 TSPAN1

TSPAN1, commonly known as neuroepithelial transforming gene 1 (NET-1), is a guanine nucleotide exchange factor (GEF) (36). It is upregulated in HCC tissue (37, 38) and identified as a proliferation promoter (39–42). Sun et al. found that after TSPAN1 knockdown, the expression level of some important factors in PI3K/Akt signaling pathway, such as pPI3K, was also decreased, suggesting that TSPAN1 may regulate the proliferation of HCC cells by targeting PI3K/Akt signaling pathway (41).

Based on the fact that TSPAN1 is highly expressed in HCC cells and positively regulating HCC cell proliferation, Wu et al. developed a gene therapy method to specifically silence TSPAN1 gene expression in HCC cells (39, 40, 42, 43). They used targeted nanobubbles to deliver TSPAN1 siRNA to HCC cells, with the aid of ultrasound exposure to increase transfection efficiency. This method could significantly reduce the expression level of TSPAN1 in HCC tissue and prolong the survival interval in mouse model. Considering the role of TSPAN1 in HCC cell proliferation, this gene therapy method can efficiently deliver the

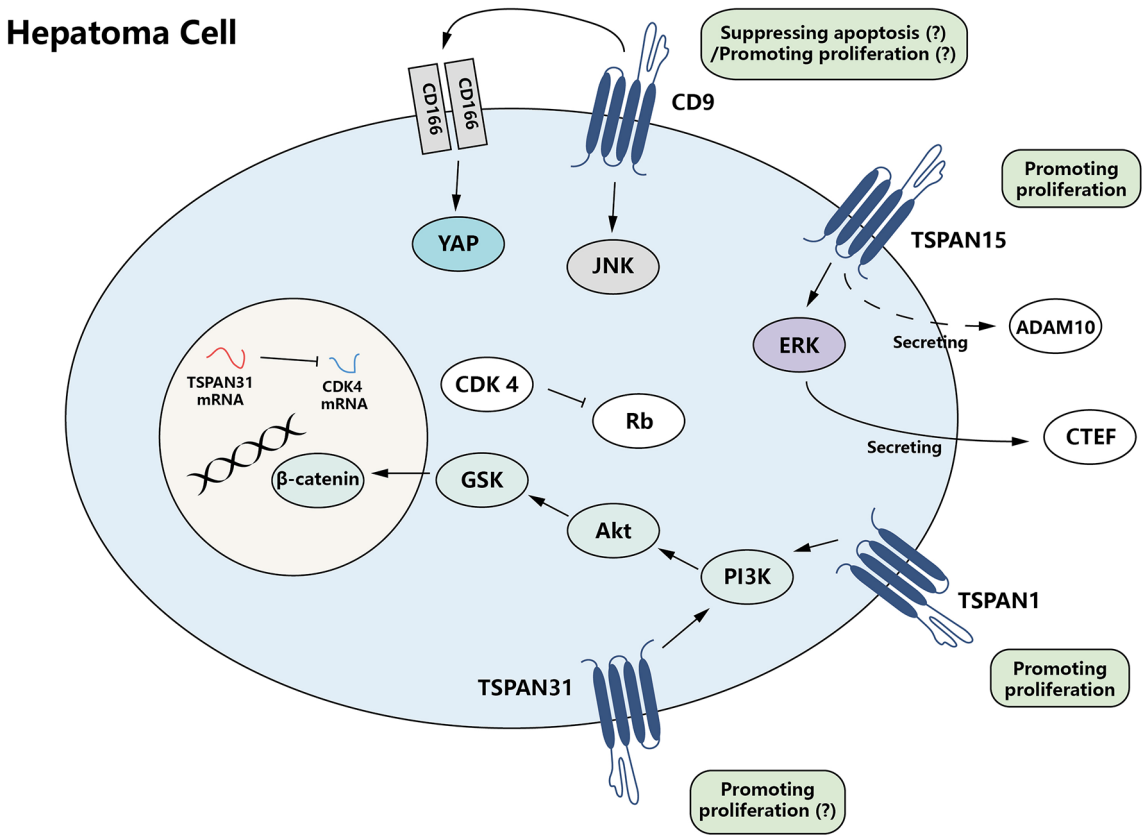

FIGURE 1 | The growth promotion effect of tetraspanin protein family for hepatoma cells. CD9, TSPAN15, and TSPAN31, members of the tetraspanin family, are able to mediate proliferation-promoting and apoptosis-suppressing signals directly or indirectly, which results in modulation of tumor cell growth.

genes to cancer cells, which may be one kind of important precision treatment for HCC in the future.

3.4 TSPAN7

TSPAN7 transcription and expression level are proven to be decreased in different HCC cell lines, and its overexpression can inhibit cell proliferation *in vivo* (44). However, the mechanism involved in the role of TSPAN7 is not investigated. Despite this phenomenon, either its upstream or downstream crosstalk is still unclear.

3.5 TSPAN31

Some evidence suggests that TSPAN31 can also regulate survival and apoptotic signals in HCC cells. TSPAN31 can activate the Akt/GSK-3β/β-catenin signaling pathway, an important pathway for cell survival. However, TSPAN31 mRNA serves as a natural anti-sense transcript of cyclin-dependent kinase 4 (CDK4), a kinase responsible for the phosphorylation of retinoblastoma (Rb) protein, critical in cell cycle regulation. TSPAN31 itself is negatively regulated by tumor suppressor protein p53 (30). Overall, the effects of TSPAN31 on HCC cell proliferation are not significant since TSPAN31 knockdown shows no influence on HCC cell proliferation (30). This may be due to the fact that TSPAN31 exerts bidirectional activity on different pro-proliferation factors simultaneously. Meanwhile, CD63, besides its role in the metastasis of hepatoma (described below), can also favor the survival of hepatoma cells (31).

4 ROLE OF TETRASPANINS HCC NEOANGIOGENESIS

In order to fulfill the high nutrition requirements of tumor cells, tumors often secrete kinds of pro-angiogenic factors or express some membrane-bound proteins that can contribute to neoangiogenesis. CD151 and TSPAN8 are proteins that participate in vascular formation (45, 46). Anti-angiogenesis has already been a key target of cancer therapy, and several drugs have been proven to be effective and adopted in clinical applications (47). Thus, the close relationship between the tetraspanin family and tumor neoangiogenesis, CD151, and TSPAN8 may represent potential therapeutic targets of HCC in the future.

4.1 CD151

CD151 is unique membrane-bound pro-angiogenic factor highly expressed by HCC cells. Its pro-angiogenic effects have been

proven by different laboratories both *in vitro* and *in vivo* (45, 48). The pro-angiogenic effects of CD151 are a result of metalloproteinase 9 (MMP9) secreted by high-CD151 expression HCC cells. As mentioned above, possibly by forming heterodimer receptors with integrin and binding laminin 5 (49), CD151 can simulate downstream PI3K/Akt/GSK-3b/Snail signaling pathway to control MMP9 expression (48). Besides, MMP9 also plays a role in HCC metastasis (as follows).

4.2 TSPAN8

Similar to CD151, human umbilical vein endothelial cells (HUVECs) were co-cultured with TSPAN8 knockdown HCC cells to reduce HUVEC tube formation, which is an indicator of low angiogenic activity, when compared with HUVEC cells co-cultured with control HCC cells (46). This reveals that TSPAN8 may also promote neoangiogenesis of unclear mechanism.

5 ROLE OF TETRASPANIN IN HCC METASTASIS

The members of tetraspanin family can bidirectionally regulate HCC progression. Some members, such as CD151 and TSPAN8 (50, 51), can enhance the invasiveness and mobility of HCC cells, while the metastasis-suppressive effect of some tetraspanins is also evident in various kinds of tumors, including HCC (52). Tetraspanins also function in epithelial–mesenchymal transition (EMT), the process of which contributes to both the initiation and metastasis of HCC. Generally speaking, tetraspanins are involved in multiple steps of hepatoma metastasis (**Figure 2**).

5.1 Role of Tetraspanin in HCC and Epithelial–Mesenchymal Transition

EMT plays an important role in the initiation and progression of the tumor. In HCC, CD82 and CD151 are often associated with EMT.

5.1.1 CD82

It has been reported that CD82 can negatively regulate EMT in several other human cancers, such as non-small cell lung cancer and prostate cancer (53–57). In HCC cells, the levels of CD82 are also correlated with those of EMT markers. When CD82 is reduced through the TGF-β signaling pathway, the level of epithelial marker E-cadherin is also decreased while the level of mesenchymal marker N-cadherin is elevated, which means EMT progression is positively regulated (58).

5.1.2 CD151

CD151 is upregulated in human HCC and various other cancers (59, 60) and exerts a positive effect on EMT (61, 62). CD151 complexes with integrin α6β1 and amplifies α6β1 signaling, which can activate the PI3K signaling pathway and then induce EMT (62). Sterk et al. found in the human erythroleukemic cell line, CD151, regulates α6β1 signals by directly interacting with the α subunits of integrins (63), but whether it employs the same mechanism in HCC cells still needs further research. The association between CD151 and α6 integrin can affect the mode of diffusion of α6 integrins and eventually supports their function, such as adhesion (64).

Since CD151 can favor EMT in HCC cells *via* the PI3K/Akt signaling pathway, Zhang et al. also considered the activation of this pathway relating to sorafenib resistance of HCC cells (61). They found that in high-CD151 expression HCC cells, the sensitivity to sorafenib is inversely correlated to CD151 levels. These findings provide a factual theoretical basis for clinical medication. Clinicians may need to consider whether the expression level of CD151 would influence the chemotherapy plans of HCC patients.

5.2 Role of Tetraspanin in HCC Invasiveness and Migration

The suppressive function of CD82 in metastasis has been validated in various kinds of tumors (65, 66), which means that the downregulation of CD82 can be a characteristic of metastatic tumors (5, 13). Approximately 20 years ago, the role of CD82 in hepatoma metastasis and prognosis has been reported (67, 68). The levels of CD82 are significantly reduced in cancerous tissues of HCC patients, especially in metastatic tumors and satellite metastases (68). This negative relationship between CD82 levels in HCC cells and their invasiveness ability has also proven both *in vitro* and *in vivo* (52, 69).

5.2.1 CD82

Co-culture of HCC cells and vein endothelial cells suggests that CD82 may mediate the communication between cancer cells and endothelial cells (70). However, this study was conducted *in vitro* and was based on cells from two different species and thus may not elucidate the real mechanism in the human body. Through cell biomechanics analysis, measuring the visco-elastic properties of HCC tissues using the micropipette aspiration technique, Yang et al. showed that the adhesion force was significantly higher in CD82 upregulated cell lines compared with control. Meanwhile, the interaction between HCC cells and the extracellular matrix also proved to be enhanced in the xenografted and orthotopic tumors (69). All these results show that CD82 can strengthen the cell–cell and cell–matrix adhesion of HCC cells.

CD82 can suppress metastasis *via* regulating cell signaling pathways such as EGFR signaling pathway, HGFR signaling pathway, and the Wnt-β catenin signaling pathway (5, 13). In HCC, it is clear that CD82 can influence the HGFR pathway (71). CD82 can induce Sprouty2 expression, which acts as a negative regulator of the HGFR pathway (72). The upregulation of Sprouty2 ultimately decreases the activity of SphK1, a vital intracellular second messenger transducing growth factor bioactivity (73). Another suggested mechanism involving CD82 suppression of tumor metastasis is the interaction of CD82 and integrin. It is concomitant with decreased adhesion ability (5). However, since it is found in the other tumor, their relationship in HCC still needs further research.

The levels of CD82 expression are influenced by multiple factors. MiR-197 can bind to the 3′-UTR of the CD82 gene and

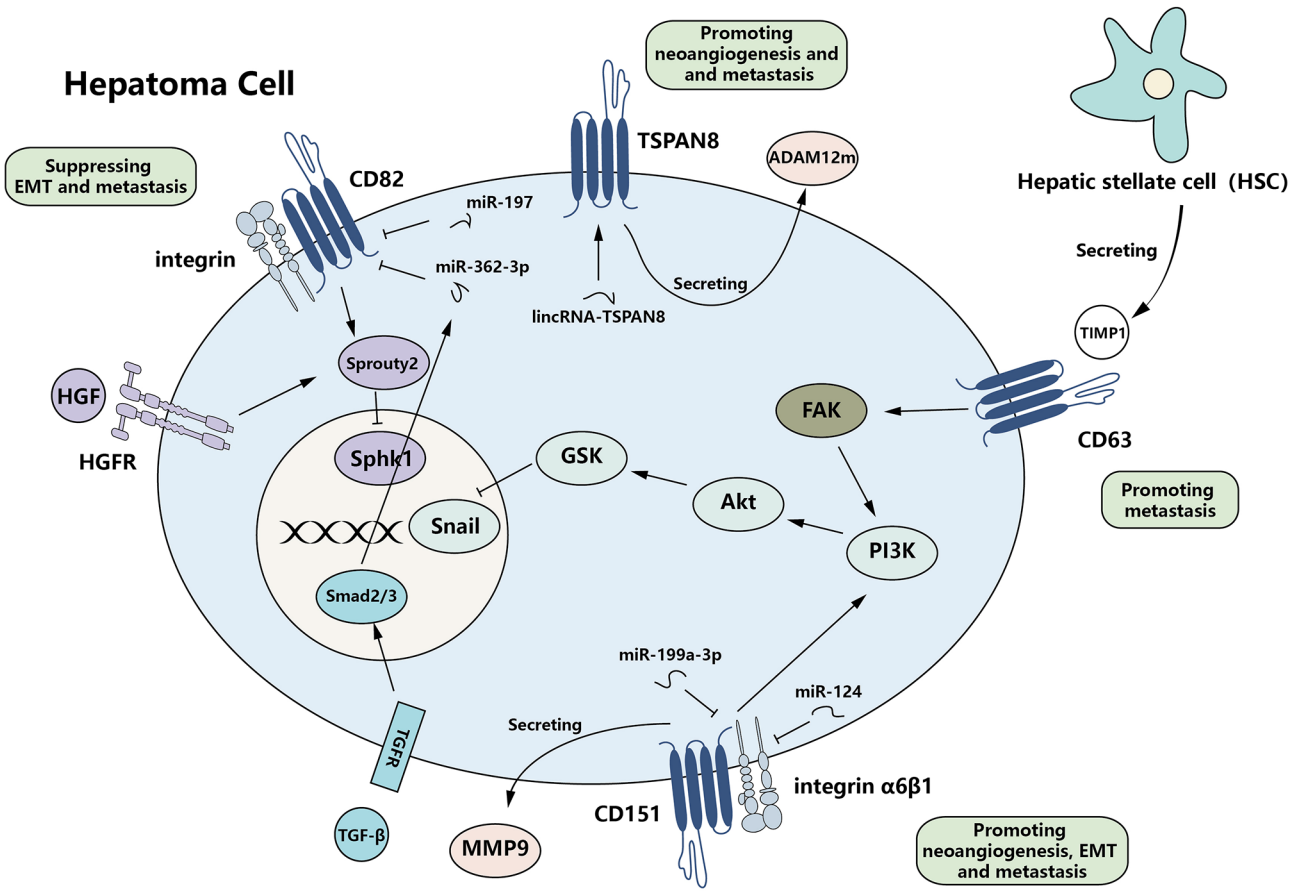

FIGURE 2 | Complexity of the function of tetraspanins in HCC metastasis. Tetraspanins can bidirectionally regulate HCC metastasis by affecting EMT-related protein expression and invasiveness/migration. CD63, CD151, and TSPAN8 serve as promoters of metastasis, while CD82 functions as a repressor. Multiple signaling pathways are active and finally regulate the expression of downstream effectors. Meanwhile, various non-coding RNAs can regulate the expression of tetraspanins. HCC, hepatocellular carcinoma; EMT, epithelial–mesenchymal transition.

subsequently inhibits the transcription of CD82 mRNA. qRT-PCR analysis shows that miR-197 is overexpressed in the cancerous tissue of HCC patients (74). Besides, CD82 is the target gene of p53. Acetylation and overexpression of p53 favor the transcription of CD82 (75). All these molecules influencing the expression of CD82 might be involved in the progression of HCC.

5.2.2 CD151

CD151 is reported to be the first member of the tetraspanin family associated with the promotion of metastasis (76). It plays a pro-metastatic role in multiple cancers (49, 77). The role played by CD151 in HCC has been elucidated in detail from laboratory studies to the effects on clinical prognosis. All these studies suggest that the upregulation of CD151 might be a sensitive predictor of HCC metastasis (50, 78).

Devbhandari et al. showed that CD151 might induce metastasis through an integrin β1-dependent manner (78). Meanwhile, CD151 can induce MMP9 expression and facilitate extracellular matrix degradation and migration of cancer cells, which all contribute to the metastasis of HCC (50).

The crosstalk of CD151 with its upstream effectors is relatively complicated. It is reported that several non-coding RNAs can influence the level of CD151 and, ulteriorly, the metastasis of HCC (**Table 1**). Kim et al. showed that miR-199a-3p could impair the expression of CD151 in HCC cell lines and finally reduce cell migration and invasion *in vitro* (79). MiR-124 also targets CD151 mRNA, serving as the suppressor of HCC cell migration and invasion, sharing a similar effect with miR-199a-3p (80). Further studies suggest that some competing endogenous RNAs (ceRNAs), such as the mRNAs of oncogene PIK3C2A and laminin γ1 LAMC1, are able to bind miR-124 competitively, which reduces the level of miR-124 and then increases the level of CD151 (79, 80).

Based on a previous study indicating that CD151 interacts with integrin β1, Ke et al. generated monoclonal antibody recognizing the integrin β1 binding site of CD151 (82). Consistent with its function in angiogenesis, invasiveness, and migration, the blockade of CD151 suppresses both vascular remodeling and metastasis both *in vitro* and *in vivo*. While the HCC cell proliferation was not slowed down *in vitro*, tumor

TABLE 1 | The non-coding RNAs that regulate the expression of tetraspanin.

	Non-coding RNAs regulating tetraspanin expression	Reference
CD82	MiR-197 (74)	(74)
CD151	MiR-199a-3p (79), miR-124 (80), miR-128 (61)	(61, 79, 80)
TSPAN8	LincRNA-TSPAN8 (81)	(81)

growth inhibition may have been due to inadequate nutrition resulting from its anti-angiogenic effect. No further details about the antibody are currently available. Further studies are needed to assess the efficacy and safety of this antibody for metastatic HCC before it can be put into clinical use.

5.2.3 TSPAN8

As in other tumors such as pancreatic cancer and breast cancer (83–85), TSPAN8 is upregulated at both the mRNA and protein levels, and its level is associated with the potential of the tumor to metastasize (85, 86). Overexpression of TSPAN8 *in vitro* demonstrates that the level of TSPAN8 is not correlated with the potential of proliferation and adhesion, but it is confirmed that its level is positively correlated with intrahepatic metastasis (46, 51, 87). However, Akiel et al. and Fang et al. suggested that TSPAN8 may increase the tumor size by facilitating neoangiogenesis (46), which is contrary to results by Kanetaka et al., indicating that TSPAN8 would not influence the size of the primary tumor (51). This contradiction may be owing to the weak and indirect growth-promoting effects of TSPAN8. Larger tumor size might be a side effect of neoangiogenesis. Thus, the correlation between TSPAN8 level and tumor size is not obvious.

An *in vitro* study showed that TSPAN8 may serve as a promoter of metastasis in HCC cells by inducing ADAM12m, a type of matrix metalloproteinase; and its expression may enhance the degradation of the extracellular matrix (87). It is found that in the *Drosophila*, tetraspanins can directly interact with ADAM10, control its trafficking by regulating its exit from endoplasmic reticulum, and finally decide ADAM10 expression on the surface (88). Maybe in human HCC, TSPAN8 would regulate ADAM12m expression in a similar way. TSPAN8 and α6β4 integrin can form a complex, which is internalized and then re-expressed, acting to ultimately decrease the adhesion to laminin 5 and shifting from an adhesion-supporting stage into a migration-supporting stage in pancreatic adenocarcinoma (84, 89). In resting stage, the complex of TSPAN8 and integrin localize at the trail of cancer cell; but under PKC signaling simulation, the complex will distribute in a different way. This mechanism may also contribute to the metastasis of HCC cells, although no evidence has been found.

It is reported that in HCC the expression of TSPAN8 is regulated by astrocyte elevated gene-1 (AEG-1), an oncogene upregulated in various cancers; and TSPAN8 acts as one essential effector on its pro-metastasis effect (46). Besides, lincRNA-TSPAN8 also leads to a high expression of TSPAN8 (81) (**Table 1**).

Despite the fact that TSPAN8 has not been an available therapeutic target in HCC, there are several antibodies of TSPAN8 that may act as candidates for potential agents for colorectal cancer (CRC) and ovarian cancer. In recent years, Kim et al. found that the TSPAN8 large extracellular loop (TSPAN8-LEL) is a critical domain of its function, and the authors developed a new type of antibody, using phage display technology, targeting TSPAN8-LEL (90). This anti-TSPAN8 antibody has been proven to suppress the invasiveness of HCT116 and LoVo CRC cell lines. Meanwhile, according to the relatively specific distribution of TSPAN8, a radiolabeled anti-TSPAN8 antibody was developed, which could inhibit tumor growth significantly in nude mice carrying HT29 tumors (91). In ovarian cancer, TSPAN8-LEL also performs similar functions in tumor metastasis. TSPAN8-LEL recognized antibody shows inhibition of ovarian cancer cell metastasis both *ex vivo* and *in vivo* without severe *in vitro* cytotoxicity or *in vivo* nephrotoxicity and hepatotoxicity (92). Although in different types of cancers tetraspanins may interact with different partners and function discordantly, with the consistent role of TSPAN8 and its upregulation in HCC, it may also be a promising therapeutic target *via* a highly specific antibody.

5.2.4 Other Tetraspanins

CD9 and CD63 also act as pro-metastatic effectors of HCC. In highly metastatic HCC cell lines, CD9 is upregulated, and inhibition of CD9 expression can reduce its migration and invasion (93). It is believed that CD63 can mediate the TGF-β signaling pathway through binding of inhibitors of metalloproteinase-1 (TIMP-1), a key mediator of TGF-β-mediated crosstalk between hepatic stellate cells (HSCs) and HCC cells, activate the FAK-Akt signaling pathway in HCC cells, and further favor migration and survival of HCC cells (31). TSPAN7, besides its growth-inhibitory effect, Qi et al. also found it acted as a suppressor of metastasis (44). CD81 may be associated with organ-specific metastasis. It has been reported that the level of CD81 mRNA is downregulated in high lymphatic metastatic potential HCC cell lines compared with high lung metastatic potential HCC cell lines, which suggest that some certain tetraspanins might impose a potential impact on the target of HCC metastasis (81).

6 ROLE OF TETRASPANIN IN EXOSOMES IN HCC

Exosome, a nanosized vesicle secreted by both tumor cells and normal cells, often contains proteins, RNA, and even DNA from its host cell. Since it can carry cargos and information between different kinds of cells, it is regarded as a novel intercellular communication mechanism. Exosomes take part in nearly all the processes of the development of HCC such as hepatocarcinogenesis, HCC growth, angiogenesis, and metastasis (94). Tetraspanins are also proven to play a key role in exosome formation, target selection, and uptake in various types of tissues and cancers (95). Current studies have already shown that exosomal tetraspanins are an important marker for

HCC; and tetraspanins such as CD9, CD31, and CD63 were used as collection markers of exosomes derived from HCC cells (96–101). Thus, it is reasonable to believe that in HCC, tetraspanins are also involved in the process of exosome formation and function, although there are still few direct evidences.

Since exosomes can be secreted by HCC cells into peripheral blood, exosomal tetraspanins have the potential to be used as a novel diagnostic target for early detection or treatment monitor of HCC. In other cancers, such as oral cancer, exosomal tetraspanins CD9, CD63, and CD81 can be potential biomarkers for early diagnosis in high-risk patients before any clinical symptoms (102). It is worthwhile to investigate the sensitivity and specificity of these tetraspanin serum levels in HCC early diagnosis or treatment monitoring in the future.

7 CORRELATIONS OF TETRASPANIN WITH HCC CLINICOPATHOLOGICAL PARAMETER AND PATIENT PROGNOSIS

As many tetraspanins are associated with HCC initiation and progression, the expression of some specific tetraspanins can also help to diagnose HCC and predict HCC patients' prognosis. Based on the available evidence, CD81 and CD82, and TSPAN6 can serve as cancer suppressors, while CD151, TSPAN1, and TSPAN8 act as cancer promoters in the diagnosis and prognosis of HCC patients (50, 68, 78, 86, 87, 103–105) (**Tables 2** and **3**).

Few studies have investigated the function of TSPAN6 in HCC; until recently, a study suggests TSPAN6 may associate with the aggregates of CD20+ B cells in tumor. It is also shown that the expression of TSPAN8 positively correlates with a better prognosis (107). However, we still know little about the function of TSPAN6.

8 CONCLUSIONS AND PROSPECTS

Tetraspanins interact with other multiple molecules and form a complicated and vast network participating in numerous essential cell activities. As discussed above, tetraspanins have been shown to be involved in the entire life cycle of HCC. Since the tetraspanin family plays a bidirectional function at each stage of carcinogenesis, the blockade of cancer-promoting tetraspanins or their downstream factors may be successful therapeutic targets in the future. To date, specific antibodies or RNA interference has been used to block tetraspanin-related signals. Some reagents have achieved significant results in animal studies (82). However, as for clinical use, there is still a long way to go. For the tetraspanins that serve as suppressors, some agonists may be used to amplify the protective signals mediated by, for instance, CD82.

Sorafenib, a vascular endothelial growth factor (VEGF) inhibitor, is the first-line medication of HCC. Sorafenib monotherapy has become the gold standard for systemic treatment of advanced HCC. Since it has been proven that sorafenib resistance is strongly correlated with the levels of CD151 in HCC cells, CD151 and its downstream pathway might be the key to combat antineoplastic drug resistance. However, how CD151 attenuates sorafenib resistance still needs further research. As high CD151 levels predict high malignancy and low sorafenib sensitivity simultaneously, this underlines the substantial heterogeneity between high and low CD151 levels in HCC and the importance of assessing the molecular type of tumor when choosing a treatment regimen.

TABLE 2 | The expression level of tetraspanins and their relationship with tumor characteristics.

	Expression		Tumor characteristics				Reference
	Expression level in HCC tissue	Expression level in serum	Tumor size	Tumor number	Tumor encapsulation	Differential grading	
CD81			NS (104)			Negative (*) (103)	(103, 104)
CD82 (KAI1)	Downregulated (**, compared with adjacent cancerous tissues and distant cancerous tissues) (105); downregulated (*, compared with adjacent non-tumorous tissues) (86)	Downregulated (**, compared with cirrhosis, hepatitis, and normal control samples) (105)	NS (68, 105)			NS (68, 105)	(68, 86, 105)
CD151	Upregulated (*, compared with adjacent non-tumorous tissues) (50)		Positive (*) (50, 78)	Positive (**) (50) (*) (78)	NS (50, 78)	Positive (*) (50); NS (78)	(50, 78)
TSPAN1	Upregulated (**, compared with both adjacent non-tumorous tissues and normal tissues from control group) (106); upregulated (*, compared with adjacent non-tumorous tissues) (37)					Positive (*) (106)	(37, 106)
TSPAN8 (CO-029)	Upregulated (*, compared with adjacent non-tumorous tissues) (85, 86)		NS (86, 87)	NS (87)	NS (87)	Positive (**) (86) (*) (87)	(85–87)

For tumor characteristics, positive means HCC malignancy is positively correlated with the level of tetraspanins.
HCC, hepatocellular carcinoma; NS, not significant.
*$p < 0.05$.
**$p < 0.01$.

TABLE 3 | The relationship between the level of tetraspanins and tumor metastasis, patient prognosis.

	Metastasis				Prognosis		Reference
	TMN stage	Intrahepatic metastasis	Vascular metastasis	Lymph node or peritoneal metastasis	Overall survival rate	Cumulative recurrence	
CD81			NS (104)	Negative (*) (104)	NS (multivariate analysis) (104)		(104)
CD82 (KAI1)	Negative (*) (68, 105)	Negative (*) (68) (**) (105)	Negative (**) (105)	Negative (*) (68) (**) (105)			(68, 105)
CD151	Positive (**) (50) (*) (78)		Positive (**) (50) (*) (78)		Negative (**, univariate analysis; *, multivariate analysis) (50) (*, univariate analysis; NS, multivariate analysis) (78)	Positive (**, univariate analysis; *, multivariate analysis) (50) (**, univariate analysis; *, multivariate analysis) (78)	(50, 78)
TSPAN1	Positive (**) (38)			Positive (*) (106)	Negative (*, univariate analysis; *, multivariate analysis) (106)		(38, 106)
TSPAN8 (CO-029)	Positive (*) (87)	Positive (*) (86)	Positive (*) (87)		Negative (*, univariate analysis; *, multivariate analysis) (87)		(86, 87)

For metastasis, positive means HCC malignancy is positively correlated with the level of tetraspanins. For prognosis, positive means the overall survival rate and cumulative recurrence rate are positively correlated with the level of tetraspanins.
NS, not significant.
*$p < 0.05$.
**$p < 0.01$.

Nevertheless, there is no perfect treatment regimen for HCC. As more detailed information about the relationship between tetraspanins and hepatoma becomes available, we hope that the tetraspanin family can be a promising anticancer target that provides an approach for curing HCC.

AUTHOR CONTRIBUTIONS

SC and YD designed and wrote all the parts of the manuscript. HP and JS supervised and revised the manuscript. All authors contributed to the article and approved the submitted version.

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