

Perspective

Self-renewal and circulating capacities of metastatic hepatocarcinoma cells required for collaboration between TM4SF5 and CD44

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Tumor metastasis involves circulating and tumor-initiating capacities of metastatic cancer cells. Hepatic TM4SF5 promotes EMT for malignant growth and migration. Hepatocellular carcinoma (HCC) biomarkers remain unexplored for metastatic potential throughout metastasis. Here, novel TM4SF5/CD44 interaction-mediated self-renewal and circulating tumor cell (CTC) capacities were mechanistically explored. TM4SF5-dependent sphere growth was correlated with CD133⁺, CD24⁺, ALDH activity, and a physical association between CD44 and TM4SF5. The TM4SF5/CD44 interaction activated c-Src/STAT3/ Twist1/ Bmi1 signaling for spheroid formation, while disturbing the interaction, expression, or activity of any component in this signaling pathway inhibited spheroid formation. In serial xenografts of less than 5,000 cells/injection, TM4SF5-positive tumors exhibited locally-increased CD44 expression, suggesting tumor cell differentiation. TM4SF5-positive cells were identified circulating in blood 4 to 6 weeks after orthotopic liver-injection. Anti-TM4SF reagents blocked their metastasis to distal intestinal organs. Altogether, our results provide evidence that TM4SF5 promotes self-renewal and CTC properties supported by CD133⁺/TM4SF5⁺/CD44^{+(TM4SF5-bound)}/ALDH⁺/CD24⁻ markers during HCC metastasis. [BMB Reports 2015; 48(3): 127-128]

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Keywords: Biomarkers, Circulating tumor cells, Epithelial-mesenchymal transition, Hepatic cancer, Self-renewal**Abbreviations:** TM4SF5, Transmembrane 4 L6 family member 5; ALDH, Aldehyde dehydrogenase; CSC, Cancer stem cells; CTC, Circulating tumor cells; HCC, Hepatocellular carcinoma**Perspective to:** Doohyung Lee (2015) Interaction of tetraspan(in) TM4SF5 with CD44 promotes self-renewal and circulating capacities of hepatocarcinoma cells. *Hepatology* Accepted online 2015.01.27, doi: 10.1002/hep.27721 Accepted online 2015.01.27

Although hepatic cancer has been aggressively studied with regards to stemness and metastasis, it remains largely unknown how (a direct link between) the cells' tumor-initiating self-renewal and circulating properties can be mechanically regulated. Different biomarkers for the self-renewal capacity of HCC have been suggested. However, their metastatic potentials were not consistently explored in enough detail to support the mechanistic aspects, in which certain biomarkers cause signaling transduction for the self-renewal of HCC, leading to their circulation in blood streams and distal metastasis.

Epithelial-mesenchymal transition (EMT) is the process by which epithelial cells lose their polarity, gain mesenchymal traits, and generate stem cells in mammary epithelial models. EMT has been suggested to play roles in the formation of circulating tumor cells (CTCs), which can eventually form metastatic tumors. Metastatic cells that have undergone EMT are thought to resemble cancer stem cells (CSCs), because they can initiate tumor growth after colonization at a distant region, and are enriched for genes associated with stemness as well as drug resistance. Although previous reports suggested that CSCs may arise from the transformation of normal cells, more recent reports have suggested that these cells are derived from fully differentiated cells through adaptive trans-differentiation mechanisms, such as EMT. Therefore, CTCs may acquire self-renewal capacity during cancer metastasis. However, the molecules responsible for the acquisition of these CTCs characteristics are not fully known.

As a membrane protein with 4 transmembrane domains, transmembrane 4 L six family member 5 (TM4SF5) is highly expressed in hepatic cancers and causes metastasis by enhancing cellular migration. Although tumor metastasis, a life-threatening complicated process, occurs through circulation of tumor cells, the mechanistic aspects of self-renewal and circulating capacities of tumor cells remain largely unknown. Here, hepatocarcinoma cells expressing TM4SF5 correlated with biomarkers including CD133 and CD44. They also transduced signaling pathways responsible for tumor-initiating property and circulation through blood streams, eventually leading to intestinal metastasis of liver-injected tumor cells. Such TM4SF5-dependent phenotypes were abolished by inhibition or suppression of TM4SF5 and/or -related signaling molecules.

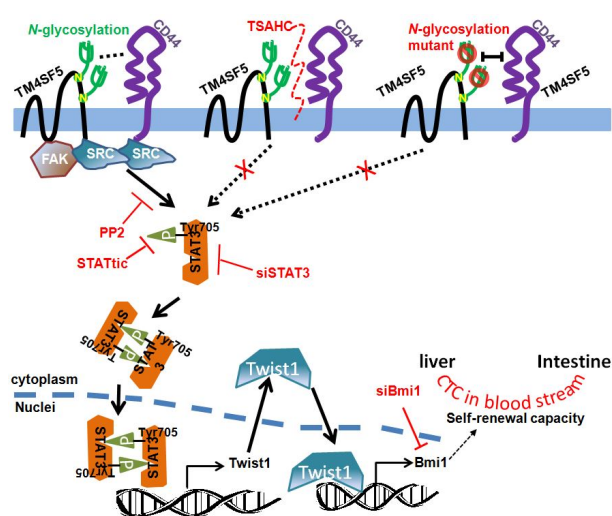


Fig. 1. Schematic model for TM4SF5-induced self-renewal capacity leading to CTC and liver-to-intestine metastases. TM4SF5 at the TM4SF5-enriched microdomain of the cell surface (T₅EM) can have interfaces to bind many other membrane proteins and receptors, including CD44, through its N-glycosylation moiety. Thus, the interaction between TM4SF5 and CD44 can be disrupted by mutations of N-glycosylation residues or treatment with the anti-TM4SF5 reagent, TSAHC (Lee SA et al., *Hepatology*. 2009 Apr; 49(4): 1316-25. doi: 10.1002/hep.22777). The association-mediated c-Src activity can cause activation of STAT3, which causes induction of Twist1 and leads to expression of Bmi1 for self-renewal. Thus, the cells with enhanced Bmi1 expression can circulate through blood streams for metastatic colonizations with self-renewal capacity.

CD44 is a well-known general stemness marker for different cancer types. TM4SF5 is highly expressed in hepatic cancers and causes epithelial-mesenchymal transition for enhanced cellular migration. However, the roles of TM4SF5 in promoting self-renewal and circulating properties have never been explored. We found that the interaction of TM4SF5 with CD44 is required for self-renewal (such as sphere growth, serial xenografts, and tumor cell differentiation) and circulation of tumor cells via blood streams (observed using an endomicroscopy), eventually leading to metastasis to distant organs like the intestines. Such TM4SF5-mediated properties were supported by CD133/TM4SF5/CD44^(bound to TM4SF5)/c-Src/STAT3/Twist1/ Bmi-1 signaling pathways. Suppression of CD133, TM4SF5, or CD44, or disruption of the interaction between TM4SF5 and CD44 abolished the self-renewal and circulating tumor cell properties. Further, disturbance in the expression or phosphorylation of any component of the pathway inhibited the TM4SF5-mediated effects (Fig. 1).

In conclusion, our study reveals systemic evidence for the unique roles of TM4SF5 and CD44 in promoting circulation and tumor-initiating capacities of HCC cells, which are potential therapeutic targets for blocking TM4SF5-mediated cancer stem cell-like properties of hepatocarcinoma cells.

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