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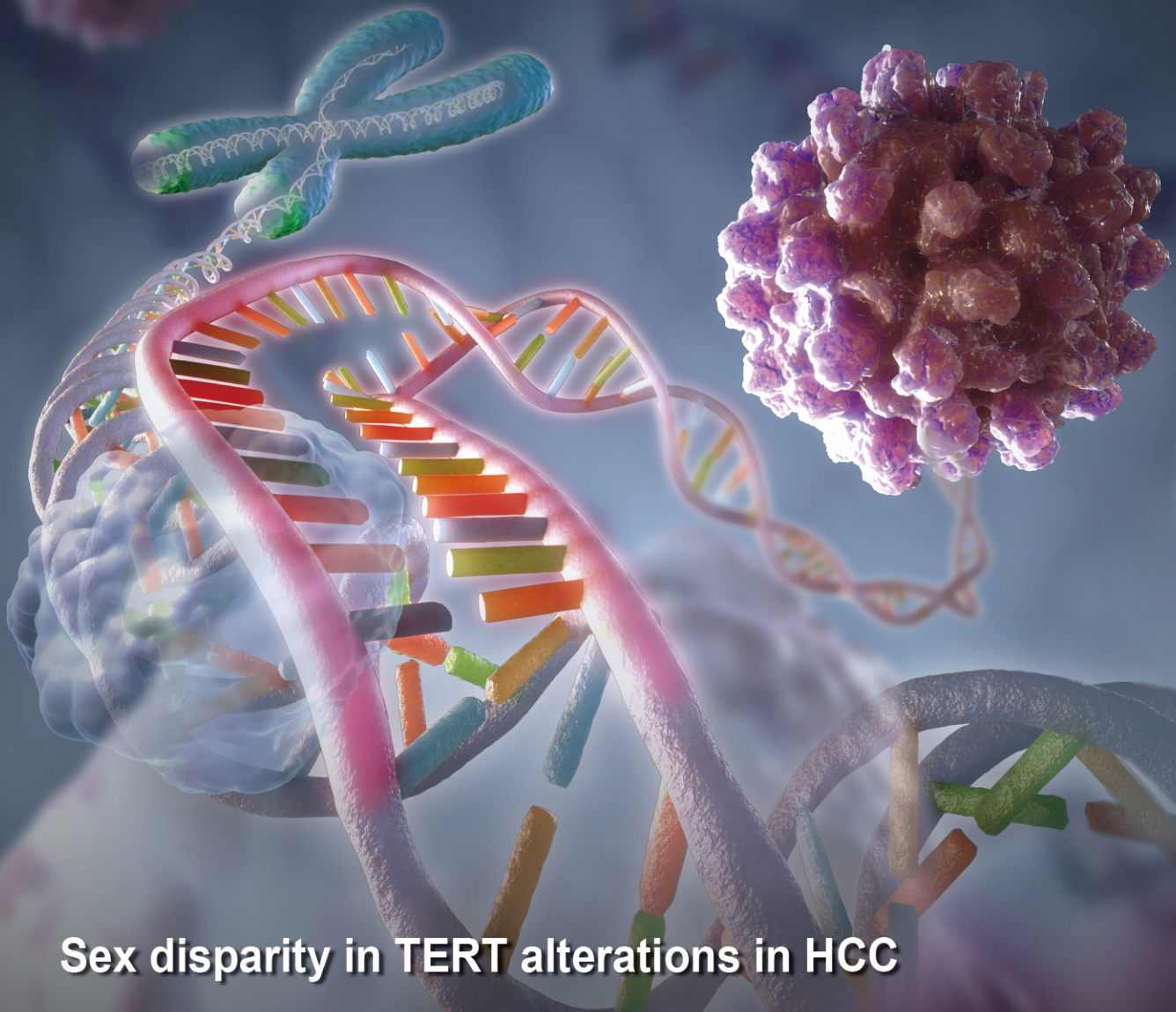

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Editorial

TM4SF1 - A new immune target for treatment of hepatocellular carcinoma: Editorial on "Targeting TM4SF1 promotes tumor senescence enhancing CD8+ T cell cytotoxic function in hepatocellular carcinoma"

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Hepatocellular carcinoma (HCC) is one of the most lethal malignancies and ranks as the fourth leading cause of cancer-related mortality worldwide.[1] While immunotherapies such as immune checkpoint inhibitors (ICIs) show promising potential for treating HCC, their efficacy is limited, with response rates less than 20%.[2] Therefore, it is crucial to identify an immune-related factor that can be targeted to complement ICI treatment with the aim of improving survival rates among HCC patients. In an issue of *Clinical and Molecular Hepatology*, Zeng et al.[3] reported that transmembrane 4 L six family member 1 (TM4SF1), also known as tumor-associated antigen L6, functions as a novel immune modulatory molecule in the HCC tumor microenvironment (TME) and that targeting TM4SF1 with an adeno-associated virus (AAV) complements anti-PD-1 therapy in a preclinical HCC mouse model.

TM4SF1 directly interacts with various cellular components, including integrins, receptor tyrosine kinases, and collagen. These associations contribute to the formation of tetraspanin-rich microdomains, which subsequently facilitate tumor cell proliferation, motility, and angiogenesis.[4,5] In line with this physiological role, Zeng et al.[3] reported that TM4SF1 was overexpressed in HCC at both the mRNA and protein levels in publicly available datasets and their in-house HCC patient cohort. These data are consistent with recent findings by Yang et al.[6] demonstrating the up-regulation of TM4SF1 expression in 90 HCC patient samples. These findings, in conjunction with the significant correlation between the expression level of TM4SF1 and the overall and disease-free survival of HCC patients, highlight the need for functional characterization of TM4SF1 and elucidation of its mechanism of action. Genetic alteration of TM4SF1 by overexpression and knockdown approaches revealed that TM4SF1 modulates HCC cell proliferation via cell cycle regulation. Further transcriptome sequencing of

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TM4SF1-knockdown and control cells revealed significant enrichment of the senescence pathway upon TM4SF1 knockdown. These results were further confirmed by the observed upregulation of cellular senescence markers, including p16 and p21, positive senescence-associated beta-galactosidase staining, and enrichment of cells in the G1/S phase. Notably, TM4SF1 was observed to regulate nonsecretory senescence, as the expression of secretory factors and their regulators, such as p38, p65, and STAT3, was not altered upon its knockdown. This result contrasts with that of a previous study by Wu et al.[7], which demonstrated a change in STAT3 expression upon alteration of TM4SF1 in colon cancer. The observed discrepancy may be attributed to cancer type-specific effects. Nonetheless, the role of TM4SF1 was clinically relevant, as there was a negative correlation between TM4SF1 levels and cellular senescence marker levels in HCC samples.

To further clarify the mechanism underlying TM4SF1 regulates cellular senescence, immunoprecipitation–mass spectrometry was performed. Notably, TM4SF1 physically interacts with AKT1 and PDPK1, forming a complex that activates the AKT signaling pathway. Further analysis confirmed that AKT1 binds to the N-terminus of TM4SF1, while PDPK1 binds to the C-terminus. In line with previous findings on the role of the AKT pathway and PDPK1 in cellular senescence,[8] Zeng et al. demonstrated that TM4SF1 regulates AKT1-mediated cellular senescence, a process that necessitates the involvement of PDPK1. In a hydrodynamic tail vein (HTVi) model involving MYC and CCND1 administration, in vivo suppression of Tm4sf1 inhibited tumor growth and cellular senescence while significantly increasing the proportion of activated granzyme B+ and IFN-γ+ T cells. This was accompanied by a reduction in PD-1+ T cells, indicating enhanced cytotoxic function and reduced exhaustion. This study represents the first report to clearly demonstrate the role of TM4SF1 in reshaping the HCC TME. They further demonstrated that TM4SF1 regulates PD-L1 and MHC1 expression in an AKT1-dependent manner in both a mouse HCC model and HCC clinical samples, which aligns with previous findings indicating the role of TM4SF1 in PD-L1 and MHC1 expression.[9,10] Consistent with this finding, TM4SF1 was observed to induce T-cell exhaustion by directly impairing the function of CD8+ T cells, as demonstrated with an *in vitro* coculture system involving the incubation of both HCC cells and T cells. These data further support the putative role of the cell cycle regulator p16 in promoting T-cell exhaustion.[11]

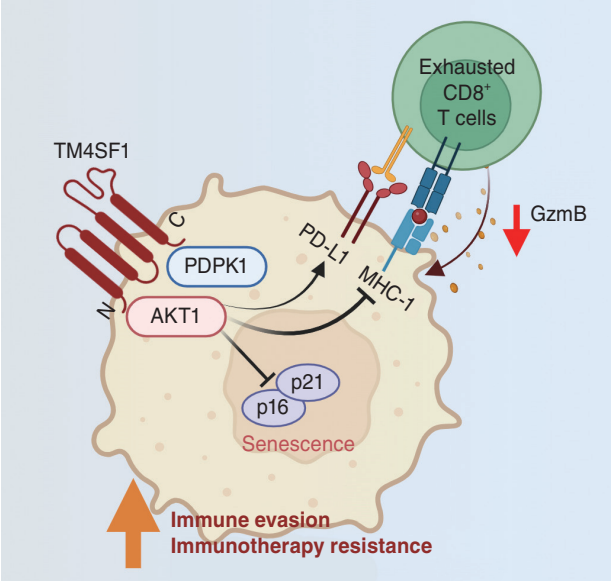

Figure 1. Schematic representation of the role of TM4SF1/AKT1/PDPK1 in immune evasion and immunotherapy resistance in HCC. HCC, hepatocellular carcinoma; TM4SF1, transmembrane 4 L six family member 1.

To further translate the above findings, Zeng et al. examined the effect of AAV targeting TM4SF1 in both immune-deficient and immune-competent HCC models. In an orthotopic mouse HCC model, they demonstrated that suppression of TM4SF1 inhibited tumor growth, concomitantly decreasing p-AKT levels while promoting cellular senescence with decreased PD-L1 expression. Using the same HTVi HCC mouse model, they examined the combination effect of AAV targeting TM4SF1 with anti-PD-1 therapy. Strikingly, TM4SF1-targeted AAV treatment synergized with anti-PD-1 treatment to increase the infiltration of $CD8^+$ T cells into the HCC TME and decrease the percentage of $PD\text{-}1^+CD8^+$ T cells. To further investigate these promising findings, they conducted a prospective analysis to examine the clinical significance of TM4SF1 expression in HCC pa-

Abbreviations:

AAV, adeno associated virus; HCC, hepatocellular carcinoma; HTVi, hydrodynamic tail vein injection; ICIs, immune checkpoint inhibitors; TM4SF1, transmembrane 4 L six family member 1; TME, tumor microenvironment

tient samples in relation to the anti-PD-1 response. They reported that TM4SF1 and its associated downstream pathway components exhibited reduced expression in responder groups, whereas the opposite phenomenon was noted in nonresponder groups. Furthermore, PD-L1 and MHC-1 were consistently altered in response to PD-1 treatment. On the basis of these findings, targeting both TM4SF1 and PD-1 may represent a novel strategy for HCC treatment.

In conclusion, this study provides novel mechanistic insights (Fig. 1), as well as clinical and therapeutic implications. First, this study revealed the role of TM4SF1 in regulating cellular senescence. Second, TM4SF1 was observed to interact with AKT1 and PDPK1, forming a complex that facilitates AKT activation. Third, TM4SF1 was found to regulate the expression of PD-L1 and MHC-1 in an AKT-dependent manner. Fourth, TM4SF1 was found to suppress antitumor immunity by directly modulating $CD8^+$ T-cell function. Finally, the results indicated that TM4SF1 may serve as a potential predictive biomarker for the PD-1 response in HCC patients, and targeting this molecule may represent a novel strategy for HCC treatment. Despite the encouraging current *in vitro*, *in vivo* animal, and clinical data obtained by Zeng and colleagues, this study has several limitations. The mechanism by which TM4SF1 is upregulated in HCC remains unclear. Furthermore, it is unclear whether the immunomodulatory effect on MHC-1 and PD-L1, as well as T-cell exhaustion, is attributable to TM4SF1-mediated cellular senescence. Additionally, investigating the effects of targeting TM4SF1 in diverse HCC mouse models that mimic cold tumors would be valuable. Given the potential involvement of β-catenin in TM4SF1-induced cancer stemness observed in previous research,[12] further investigation is necessary to elucidate the potential immunomodulatory effect of TM4SF1 through the Wnt/β-catenin pathway. Finally, given that the therapeutic efficacy of the TM4SF1 antibody has been demonstrated,[13] further investigations to explore targeting TM4SF1 via an antibody approach in combination with PD-1 treatment are warranted. Nevertheless, this study is crucial for exploring TM4SF1 as a novel target for HCC immunotherapy.

Authors' contribution

C.R.Y drafted the manuscript. T.K.L reviewed and finalized the manuscript.

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Conflicts of Interest

The authors have no conflicts to disclose.

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