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Editorial

CircTUBD1: A Novel Circular RNA Molecule as a Therapeutic Target in Radiation-induced Liver Fibrosis

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Primary liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer mortality worldwide.[1] Liver transplantation and surgical resection are two curative therapeutic options for liver cancer patients at the early stages. However, most patients are diagnosed at advanced stages. For those patients, chemotherapy, targeted therapy, radiotherapy (RT), and combination therapy are employed to treat unresectable liver cancer.[2] Among the treatments, RT has emerged as an effective treatment for patients at an intermediate stage. However, the efficacy of RT is limited due to its radiotoxicity in liver tissues adjacent to tumors, resulting in radiation-induced liver disease (RILD).[3] RILD is an acute response within few weeks or a chronic response that occurs from months to years after RT. Hepatic stellate cells (HSCs),[4] the main fibrogenic cell type, are known to be radiosensitive and to release various profibrotic factors that promote liver fibrosis during RT, resulting in the development of radiation-induced liver fibrosis (RILF). RILF is becoming an increasingly serious problem as it could prevent irradiation dose escalation or terminate repeated irradiation treatment for liver cancer and is associated with a high mortality rate. Efficient treatment options for RILF are limited. Therefore, elucidation of the molecular mechanisms for the development of RILF is urgently needed in order to improve the survival of liver cancer patients treated with RT.

A recent study by Niu *et al*.,[5] titled "CircTUBD1 regulates radiation-induced liver fibrosis response via a circTUBD1/micro-203a-3p/Smad3 positive feedback loop," identified a circular RNA (circRNA) signaling pathway that is critically involved in the development of RILF. That study is based on a previous observation by the same group that circTUBD1 (has_circ_0044897) expression was significantly upregulated, with activation of a human hepatic stellate cell line (LX-2) following irradiation.[6] Based on that interesting lead, Niu *et al*.[5] devised this study to functionally characterize and elucidate the potential role of circTUBD1 in RILF using an *in vitro* 3-dimensional (3D) spheroid model of LX-2 cells as well as an *in vivo* RILF mouse model. Using a knockdown approach, these researchers found that suppression of circTUBD1 reduced the activity of LX-2 cells, as evidenced by a decrease in the expression of fibrosis-related markers at both mRNA and protein levels. Analysis in the CircInteractome database found that circTUBD1 shared response elements with micro-203a-3p. The binding of circTUBD1 and micro-203a-3p was further confirmed by dual-luciferase reporter assays, mutation analysis, and RNA pulldown assays. Functionally, the micro-203a-3p inhibitor not only aggravated radiation-induced activation of LX-2 cells but also partially rescued the suppressive effect of circTUBD1 knockdown in LX-2 cells, revealing the functional role of circTUBD1 as a sponge of micro-203a-3p to regulate RILF in LX-2 cells.

To further elucidate the downstream signaling of circTUBD1/micro-203a-3p, the investigators performed RNA sequencing to compare the genetic profiles between circTUBD1 and control LX-2 cells upon irradiation. The results suggested that the TGF-β signaling pathway was enriched and the expression and phosphorylation of SMAD3, a transcription factor related to the pathway, were repressed upon circTUBD1 knockdown. The observations echo the finding showing that the TGF-β/SMAD3 signaling pathway is a crucial mediator during the process of radiation-induced liver injury.[7] With the TargetScan database, multiple binding sites between micro-203a-3p and SMAD3-3'UTR were identified, and their interactions were further confirmed by dual-luciferase assays and functional characterization. The data show that circTUBD1 suppressed the micro-203a-3p/SMAD3 interaction, resulting in the upregulation of TGF-β signaling.

Additionally, the study provides mechanistic insight into how circTUBD1 expression is upregulated in HSCs following irradiation. Based on the GTAR and JASPAR databases, Niu *et al*.[5] revealed for the first time that SMAD3 potentially binds to multiple binding sites in the promoter region of TUBD1. Further confirmation analysis showed two effective SMAD3 binding sites in the promoter region of TUBD1. Chromatin immunoprecipitation (ChIP) quantitative real-time PCR analysis using a specific anti-SMAD3 antibody confirmed the physical interaction between SMAD3 and the TUBD1 promoter, and that the occupancy of SMAD3 was consistently decreased by knockdown of circTUBD1 in LX-2 cells. The potential positive SMAD3/circTUBD1 feedback loop was further confirmed by a knockdown experiment in which repression of SMAD3 significantly decreased TUBD1, pre-TUBD1, and circTUBD1 levels in LX-2 cells. These excit-

Abbreviations: 3D, 3-dimensional; circRNA, circular RNA; HSC, hepatic stellate cell; RILD, radiation-induced liver disease; RILF, radiation-induced liver fibrosis; RT, radiotherapy.

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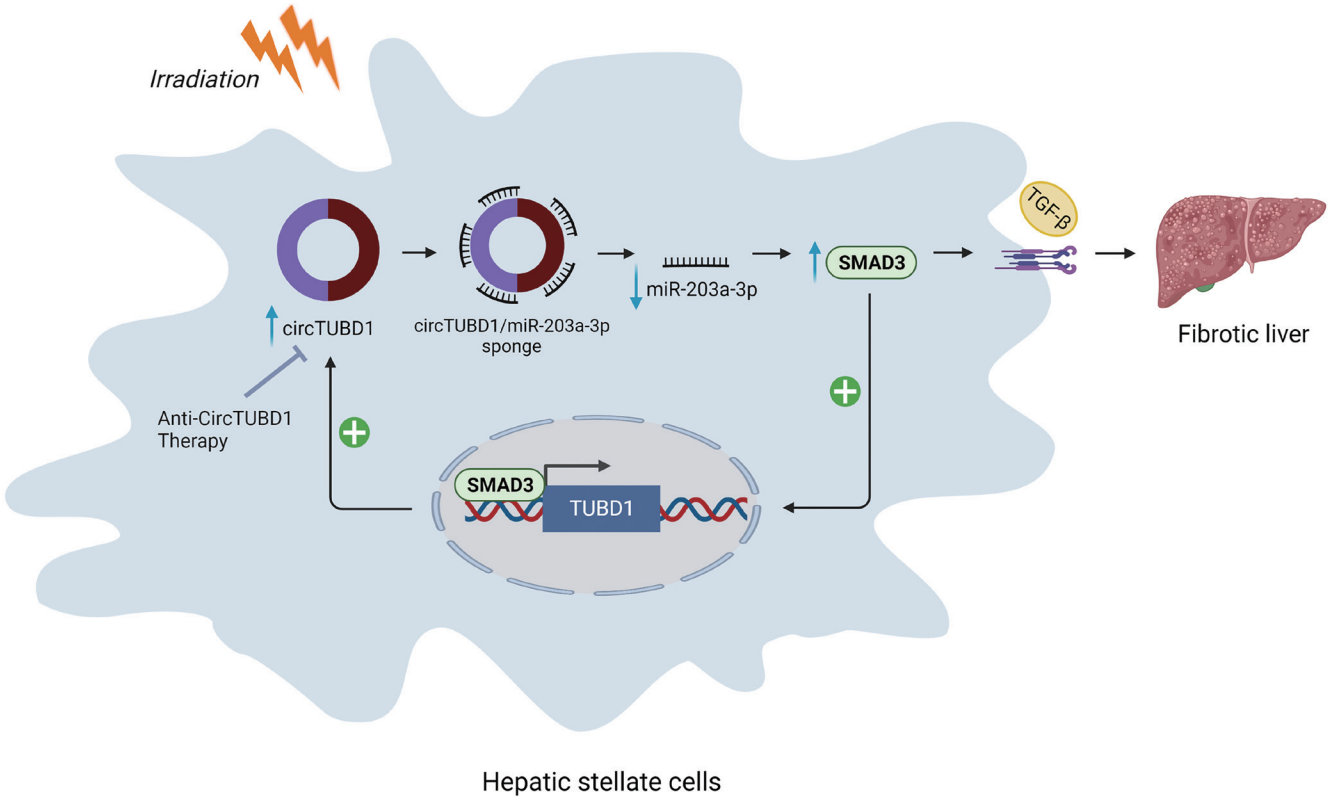

Fig. 1. Schematic diagram of the role of circTUBD1 in the regulation of RILF via a circTUBD1/ micro-203a-3p /Smad3 positive feedback loop.

ing findings indicated that SMAD3 was not only regulated by circTUBD1 but also in turn modulated the endogenous expression of circTUBD1 in LX-2 cells. Targeting the positive feedback loop is a potentially promising therapeutic strategy to alleviate liver fibrosis after irradiation.

To confirm the *in vitro* findings, Niu *et al.*[5] established a RILF mouse model in which the left liver was irradiated with 30 Gy with five fractions, 6 Gy per week, and examined the therapeutic effect of targeting the circTUBD1 signaling cascade by intravenous injection of adenoviral-based sh-circTUBD1 virus. With that RILF model, irradiation resulted in upregulation of the expression of fibrogenic markers including α-SMA, COL1A1, COL3A1, and CTGF. Knockdown of circTUBD1 partially offset the upregulated expression of these markers. Phenotypically, knockdown of circTUBD1 also alleviated the effect of irradiation on inflammatory infiltrates, excess collagen deposition around the vessel, and liver damage.

The study provides novel mechanistic insight into therapeutic strategies for RILF. First, it established a 3D spheroid model of LX-2 cells for the *in vitro* analysis of liver fibrosis upon irradiation, which provides a more physiological system compared to the 2-dimensional cell line approach. Second, it revealed the novel role of circTUBD1 in the regulation of RILF via a circTUBD1/micro-203a-3p/Smad3 positive feedback loop (Fig. 1). Most importantly, the study provides a novel therapeutic strategy to alleviate RILF by targeting the circTUBD1 signaling cascade. Although major progress has been made in circRNA research in recent years, targeting circRNA is still under investigation for potential clinical trials. A recent study showed that the CRISPR/Cas13 system showed promise in knocking down circRNAs with high specificity and efficiency.[8] Further investigation targeting circTUBD1 for RILF is highly warranted to translate the current results to the bedside. Additionally, because of recent advancements in biomedical technology, photoacoustic imaging can be employed to evaluate the therapeutic effect of suppressing circTUBD1 on RILF in mice.[9,10]

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Conflict of interest

TKL has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2021. CYG has no conflicts of interest related to this publication.

Author contributions

Proposing the main opinions and writing the manuscript cooperatively (CYG, TKL), and editing the manuscript (TKL).

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