A WNT7B-m6A-TCF7L2 positive feedback loop promotes gastric cancer progression and metastasis

Signal Transduction and Targeted Therapy
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Dear Editor,

Signal transduction takes the responsibility of translating extracellular information into specific cellular activities, enabling cells, including cancer cells, to respond exquisitely to extracellular guidance cues. N6-methyladenosine (m6A), the most pervasive and abundant modification within eukaryotic mRNAs, is known to have specific effects on cellular activities relevant to cancer.1 Despite the fact that m6A methylation is a dynamic event involving a series of enzymes, it remains unknown whether signal transduction, much less extracellular signaling molecules, uses m6A methylation as an effector mechanism in cancer.

Gastric cancer carries a poor prognosis, mainly due to its high proclivity to metastasize and the lack of bona fide biomarkers for early diagnosis and precision-targeted therapy. In addition to upregulated m6A methylation,2 gastric cancer harbors hyper-activated Wnt/β-catenin signaling, whose underlying mechanism is unclear.3 Given that cross talk with other mechanisms often determines Wnt/β-catenin signaling activation, here we investigated whether Wnt/β-catenin signaling synergized with m6A methylation in gastric cancer.

Transcription Factor 7 like 2 (TCF7L2), a core component binding to nuclear β-catenin to transduce Wnt signaling,4 was upregulated in gastric cancer tissues (Fig. 1a). High TCF7L2 expression was associated with aggressive clinical features, poor overall survival, and high recurrence rate of patients (Supplementary Fig. S1). Subsequently, TCF7L2 was either knocked down by shRNAs in N87 and 44As3 cells or overexpressed in SGC7901 cells to evaluate the correlation between TCF7L2 expression and malignant phenotypes of gastric cancer cells (Supplementary Fig. S2). Our results showed that TCF7L2 endowed gastric cancer cells with the advantage of proliferation and migration, and induced the gastric cancer stem cell phenotype by strengthening the self-renewal capacity and upregulating the expression of stem cell markers. The notable role of TCF7L2 in promoting gastric cancer growth and metastasis has also been demonstrated in heterotopic and orthotopic mouse models. Accordingly, TCF7L2 could promote metastatic progression of gastric cancer.

To probe the mechanisms underlying TCF7L2’s essential role in gastric cancer metastasis, we performed KEGG pathway enrichment analysis of differentially expressed proteins in TCF7L2-depleted N87 cells, and found that Wnt/β-catenin signaling was the most significantly enriched pathway (Supplementary Fig. S3a). Furthermore, TCF7L2 utilized the oncogenic target genes of Wnt/β-catenin signaling to exacerbate gastric cancer (Supplementary Fig. S3b–f). Interestingly, among Wnt signaling-related genes that encode secreted proteins, WNT7B had the largest expression change during modulation of TCF7L2 expression (Supplementary Table S1, S2, and Supplementary Fig. S4a), implying that its expression was under tight regulation of TCF7L2, which has not been reported so far. This was further validated by the results showing that both WNT7B mRNA levels of gastric cancer cells and WNT7B protein-released amount in gastric cancer cell culture medium were positively correlated with TCF7L2 expression (Supplementary Fig. S4b, c). Furthermore, with primer sets covering the putative binding sites provided by the JASPAR database, ChIP analysis revealed that TCF7L2 directly bound to the region (−1777 to −1668) of the WNT7B promoter (Supplementary Fig. S4d). TCF7L2 knockdown attenuated WNT7B promoter activity in N87 (Fig. 1b) and 44As3 cells (Supplementary Fig. S4e). Disruption of TCF7L2/β-catenin transcriptional activity by iCRT-14 dose-dependently reduced WNT7B abundance (Supplementary Fig. S4f). Moreover, WNT7B resembled TCF7L2 in enhancing the metastatic properties of gastric cancer cells (Supplementary Fig. S4g–j). Together, our results identify TCF7L2 as a transcriptional activator of WNT7B in gastric cancer cells.

m6A methylation has been shown to play a critical role in regulating gene expression,1 while gastric cancer displays elevated m6A levels.5 The SRAMP online tool predicted that five m6A sites with high confidence were located in the UTR of TCF7L2 mRNA (Supplementary Fig. S5a). Thus, we analyzed the m6A methylation of TCF7L2 mRNA to explore the mechanism underlying TCF7L2 upregulation. A noticeable enrichment in m6A levels of TCF7L2 mRNA was detected in 3’UTR close to the stop codon (Supplementary Fig. S5b), while the levels were even higher in WNT7B-stimulated cells (Fig. 1c). The dynamic m6A methylation of TCF7L2 mRNA was further validated by m6A level changes induced by modulating the expression of m6A methyltransferase METTL3 and inhibiting FTO, which was the first identified demethylase of m6A modification (Supplementary Fig. S5c, d). Furthermore, METTL3 knockdown decreased TCF7L2 mRNA levels (Supplementary Fig. S5e). Inhibiting FTO-mediated demethylation by MA2 increased TCF7L2 mRNA levels along with WNT7B mRNA levels (Fig. 1d), indicating that m6A methylation positively regulates TCF7L2 expression. Consistently, the TCF7L2 mRNA (Supplementary Fig. S5f) and protein (Fig. 1e) levels of WNT7B-stimulated cells dramatically increased and remained elevated throughout the entire stimulation period (96 h). To our surprise, the mRNA (Supplementary Fig. S5f) and protein (Fig. 1e) levels of FTO exhibited an opposite trend in changes upon WNT7B stimulation, revealing a novel role for WNT7B in negatively regulating FTO. WNT7B stimulation could not obviously change the mRNA levels of METTL3, which has been implicated in gastric cancer progression (Supplementary Fig. S5f). Nevertheless, global m6A levels in mRNA were remarkably elevated by WNT7B (Fig. 1f). Therefore, by downregulating FTO and thereby enhancing m6A methylation, WNT7B increased TCF7L2 expression in gastric cancer cells.

In line with the previous report that WNT7B was involved in Wnt/β-catenin signaling activation,5 WNT7B stimulation elevated nuclear β-catenin levels in gastric cancer cells (Fig. S5g). With upregulation of TCF7L2 abundance and nuclear translocation of β-catenin, WNT7B effectively activated the transcriptional activity of TCF7L2/β-catenin (Supplementary Fig. S5h). Accordingly, there...
was a reciprocal regulatory loop between WNT7B and TCF7L2. In this setting, treating N87 cells with WNT7B protein induced a continuous increase in WNT7B mRNA during the 96-h period examined (Fig. 1g). Collectively, our findings reveal a WNT7B-m6A-TCF7L2 positive feedback loop wherein TCF7L2 enhances WNT7B expression by transducing Wnt/β-catenin signaling and WNT7B elevates TCF7L2 abundance via upregulating m6A methylation (Fig. 1h).

Fig. 1 The role of the WNT7B-m6A-TCF7L2 positive feedback loop in gastric cancer metastatic progression. a Immunohistochemical staining of TCF7L2 protein in gastric tissues from patients with gastric cancer (Cancer) and chronic gastritis (Control) was quantitatively measured using Image J software. b Luciferase reporter assay for WNT7B promoter in N87 cells transfected with TCF7L2 shRNA. c m6A methylation of TCF7L2 mRNA in N87 cells treated with WNT7B protein (200 ng/mL) for 48 h was analyzed by gene-specific m6A-RIP-qPCR. HPRT1 served as a negative control. d mRNA levels of TCF7L2 and WNT7B in N87 cells treated with MA2 for 48 h were analyzed by qRT-PCR. e Expression of TCF7L2 and FTO at the indicated time points (two biological replicates for each) was analyzed by immunoblotting in N87 cells treated with WNT7B protein (200 ng/mL). f ELISA-based assay for quantification of global m6A levels in N87 cells treated with WNT7B for 48 h. g Levels of WNT7B mRNA at indicated time points were analyzed by qRT-PCR in N87 cells treated with WNT7B protein (200 ng/mL). h Proposed working model of the WNT7B-m6A-TCF7L2 positive feedback loop. i Correlation between WNT7B and TCF7L2 mRNA levels in cancer tissues from patients with gastric cancer. j Levels of WNT7B protein in the serum of healthy people undergoing health check-ups (Normal), gastric cancer patients without lymph node metastasis (LN−), and those with lymph node metastasis (LN+) were analyzed by ELISA. k PDX tumor growth curves for vehicle-, iCRT-14-, and WNT7B-treated mice are shown. N=6 mice per group. l mRNA levels of WNT7B, FTO, and TCF7L2 in PDX tumors from vehicle-, iCRT-14-, and WNT7B-treated mice were analyzed by qRT-PCR. P values were calculated with the unpaired Student t test (a-c, g), one-way ANOVA with post hoc LSD test (d, f, j, l), Pearson’s correlation test (i), or two-way repeated-measure ANOVA with post hoc LSD test (k).
The significance of the positive feedback loop was further investigated in clinical samples. Consistent with the observations in gastric cancer cells, FTO expression was reduced in gastric cancer tissues (Supplementary Fig. S6a), whereas WNT7B expression was upregulated and positively correlated with the recurrence rate (Supplementary Fig. S6b–d), which was similar to the TCF7L2 expression pattern. More importantly, a positive correlation between WNT7B and TCF7L2 mRNA levels was manifested in gastric cancer tissues (Fig. 1f). As shown in Fig. 1j, a progressive increase in serum WNT7B levels between healthy people, gastric cancer patients without lymph node metastasis, and those with lymph node metastasis indicated that serum WNT7B was positively correlated with metastatic progression ($r = 0.646$, $P < 0.001$, Spearman’s Rho), potentially facilitating noninvasive diagnosis and monitoring of gastric cancer.

To evaluate the therapeutic potential of targeting the positive feedback loop, we established patient-derived xenograft (PDX) models of gastric cancer. Tumor fragments from one patient with gastric cancer were implanted subcutaneously and treated via intratumoral injection with WNT7B, iCRT-14, or vehicle twice a week. Tumor growth was effectively suppressed by iCRT-14, but promoted by WNT7B (Fig. 1k and Supplementary Fig. S6e, f). Moreover, iCRT-14 treatment resulted in a significant reduction in mRNA levels of WNT7B and TCF7L2 but an increase in FTO mRNA levels in PDX tumors, whereas WNT7B treatment had the opposite effect (Fig. 1f), consistent with our in vitro data. METTL3 expression remained unchanged (Supplementary Fig. S6g). These results collectively suggest that targeting the WNT7B-m6A-TCF7L2 positive feedback loop suppresses gastric cancer in vivo, which may serve as a novel therapeutic strategy.

In summary, our study identifies a WNT7B-m6A-TCF7L2 positive feedback loop whereby Wnt/β-catenin signaling and m6A methylation mutually reinforce each other to promote gastric cancer progression and metastasis, and holds promise for the development of effective diagnosis and treatment strategies against gastric cancer.

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AUTHOR CONTRIBUTIONS
G.S. and X.Y. conceived and coordinated the study. Q.G. and L.Y. designed, performed, and analyzed most experiments. A.S. and Y.L. collected the clinical gastric cancer samples. Y.L. participated in molecular biology experiments. S.H., R.Y., and X.W. performed statistical data analysis. All authors reviewed the results and approved the final version of the manuscript.

REFERENCES
1. Liu, J., Harada, B. T. & He, C. Regulation of gene expression by N(6)-methyladeneine in cancer. Trends Cell Biol. 29, 487–499 (2019).
2. Wang, Q. et al. METTL3-mediated m6A modification of HDGF mRNA promotes gastric cancer progression and has prognostic significance. Gut 69, 1193–1205 (2020).
3. Yang, X. Z. et al. LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/β-catenin pathway. Mol. Cancer 17, 126 (2018).
4. Nusse, R. & Clevers, H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 169, 985–999 (2017).
5. Yang, D. D. et al. METTL3 promotes the progression of gastric cancer via targeting the MYC pathway. Front Oncol. 10, 115 (2020).

ADDITIONAL INFORMATION
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