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Potential role of Tenascin C (TNC) in human lung adenocarcinoma progression

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Introduction

Tenascin C (TNC) is an extracellular matrix protein and a potential biomarker affecting progression of different tumor types, such as pancreatic, bladder and lung cancer. In this study, we investigated the role of TNC in a cohort of adenocarcinomas of the lung as well as a possible impact of TNC in essential tumor cell properties.

Methods

TNC expression was evaluated by immuno-histochemistry using a cohort of 137 lung adenocarcinomas (LUAD) with 32 tumor-adjacent normal lung tissues and metastasis. In addition, univariate Kaplan-Meier (KM) survival analysis of adenocarcinoma patients showing a beneficial overall survival with low TNC protein level. Subsequently, LUAD cell lines (with and without *EGFR* mutation) were tested for crucial cell properties like invasion capabilities during exposure to medium collected from human lung fibroblast (HLF) after TNC downregulation by siRNA. The measurement of invasion was ensured by using culture inserts with 8µm pore size. Moreover actin cytoskeleton remodeling was investigated using Alexa488-linked Phalloidin. Furthermore, Western Blot analysis of HLF treated with different media was used to investigate a linkage between HLF and LUAD cells. Finally, *EGFR* mutated LUAD cells were treated with specific RAC/RHO/CDC42 inhibitors to examine the reduced invasion capability due to TNC downregulation and a associated putative signaling pathway.

Conclusion

Increased TNC protein level is observed in the peritumoral stroma of lung adenocarcinoma samples and correlates with *EGFR* aberrations. Inhibition of TNC in the lung stromal cells leads to reduced invasiveness of LUAD cells harboring *EGFR* mutation. This study provides evidence that TNC expression might be a biological relevant factor in human lung adenocarcinoma progression in an EGFR-dependent manner and regulates tumor cell invasion by reinforcement of the Rac/CDC42 signalling.

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Results

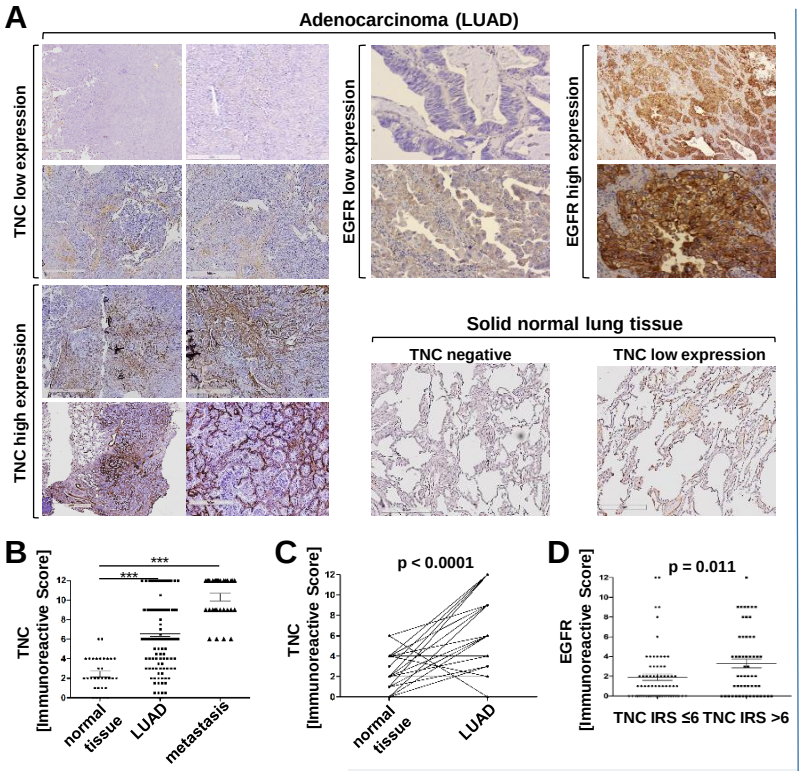

Figure 1: Increased Tenascin C (TNC) expression in primary lung tumor tissue and metastasis. **(A)** Immunohistochemistry shows a TNC positive staining in tumor associated stromal cells of primary lung adenocarcinoma (n=137). **(B and C)** Scatter plot analysis demonstrating a significant increase of TNC protein level in primary LUAD tissue, which is verified by the comparison of matched pairs (n=32). **(D)** Scatter plot analysis displaying a significant higher total EGFR level in samples with higher TNC protein expression. **(E)** Univariate Kaplan-Meier (KM) survival analysis of adenocarcinoma patients showing a beneficial overall survival with low TNC protein level, suggesting TNC as possible prognostic marker.

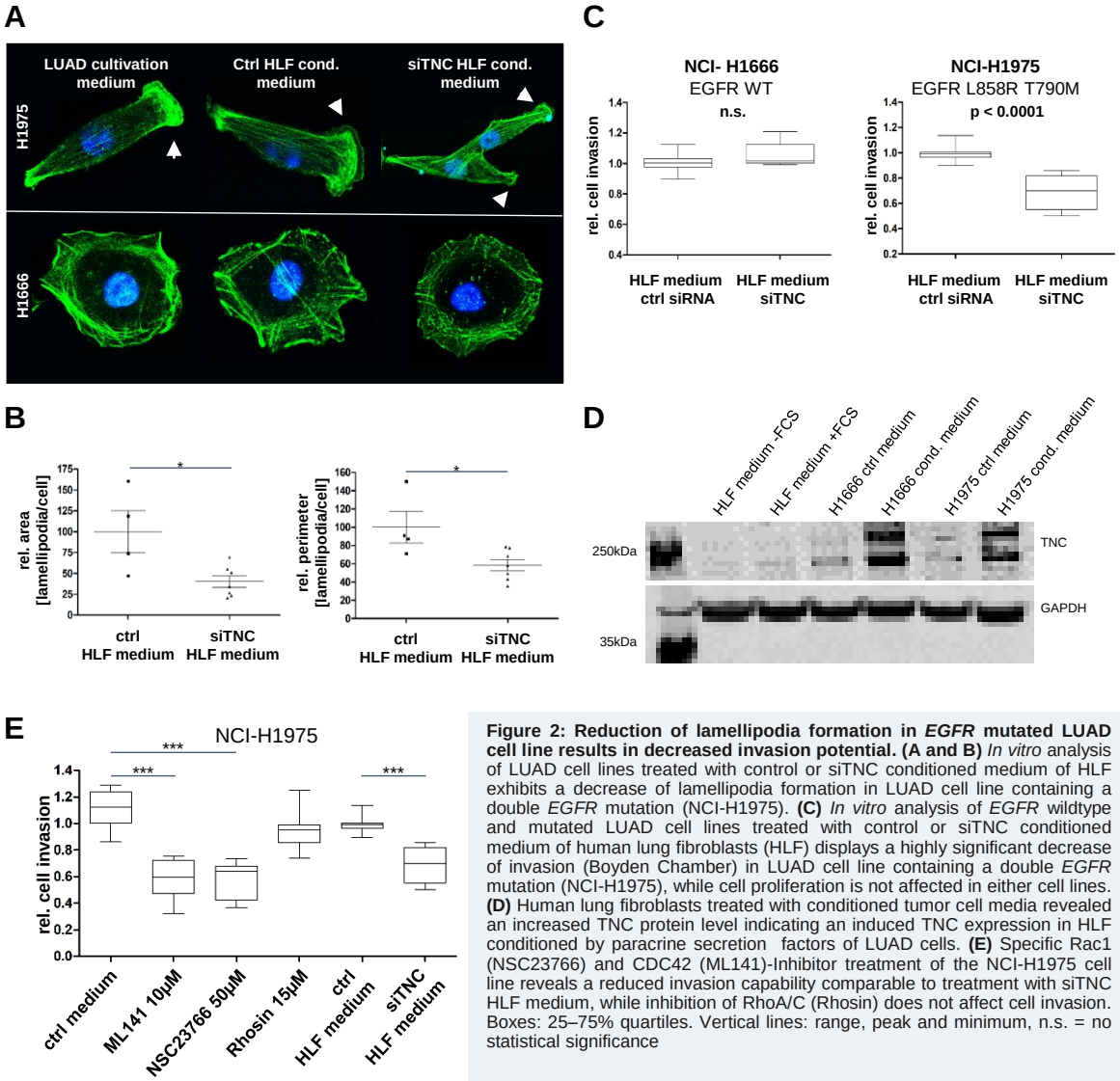

Figure 2: Reduction of lamellipodia formation in *EGFR* mutated LUAD cell line results in decreased invasion potential. (A and B) *In vitro* analysis of LUAD cell lines treated with control or siTNC conditioned medium of HLF exhibits a decrease of lamellipodia formation in LUAD cell line containing a double *EGFR* mutation (NCI-H1975). **(C)** *In vitro* analysis of *EGFR* wildtype and mutated LUAD cell lines treated with control or siTNC conditioned medium of human lung fibroblasts (HLF) displays a highly significant decrease of invasion (Boyden Chamber) in LUAD cell line containing a double *EGFR* mutation (NCI-H1975), while cell proliferation is not affected in either cell lines. **(D)** Human lung fibroblasts treated with conditioned tumor cell media revealed an increased TNC protein level indicating an induced TNC expression in HLF conditioned by paracrine secretion factors of LUAD cells. **(E)** Specific Rac1 (NSC23766) and CDC42 (ML141)-Inhibitor treatment of the NCI-H1975 cell line reveals a reduced invasion capability comparable to treatment with siTNC HLF medium, while inhibition of RhoA/C (Rhosin) does not affect cell invasion. Boxes: 25–75% quartiles. Vertical lines: range, peak and minimum, n.s. = no statistical significance