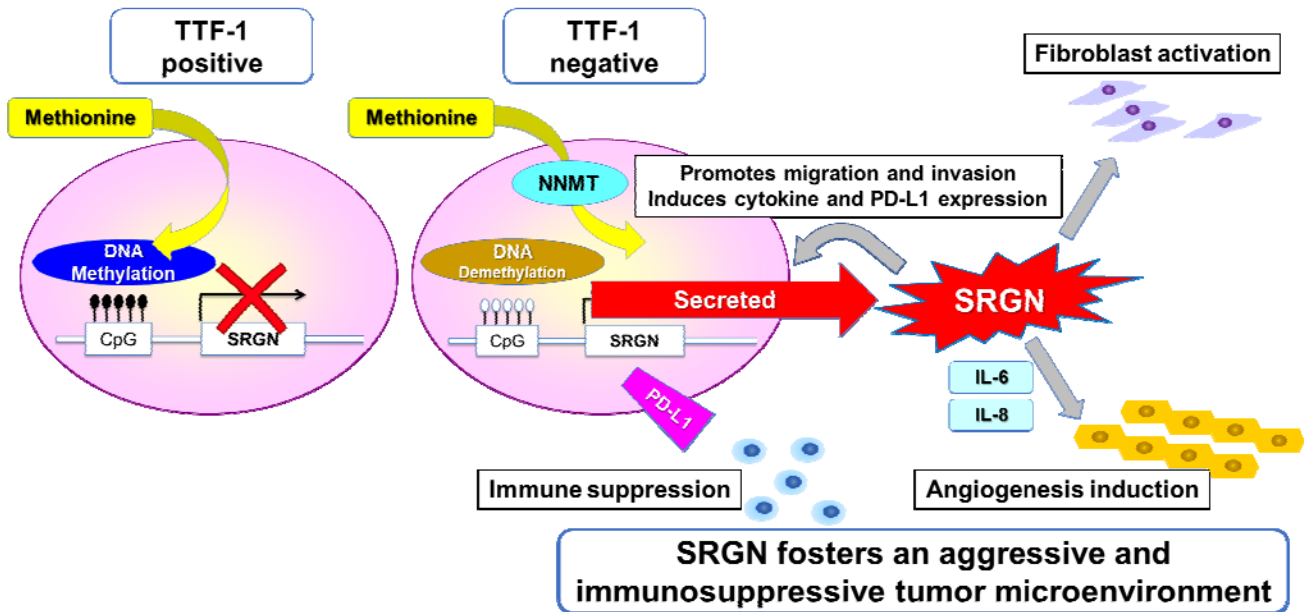## Title

SRGN fosters aggressive and immunosuppressive phenotype in TTF-1–negative lung adenocarcinomas

## Key Points

・ A proteoglycan SRGN was markedly overexpressed in TTF-1–negative lung adenocarcinoma and associated with poor clinical outcome.

・SRGN was induced by DNA de-methylation resulting from NNMT-mediated impairment of methionine metabolism.

・SRGN regulated expression of proinflammatory cytokines, as well as PD-L1, and increased migratory and invasive properties of lung adenocarcinoma cells and fibroblasts, and enhanced angiogenesis.

## Summary

About 20% of lung adenocarcinoma (LUAD) is negative for the lineage-specific oncogene Thyroid Transcription Factor-1 (TTF-1) and exhibits worse clinical outcome. A research team led by Dr. Ayumu Taguchi, M.D., Ph.D. (Division of Molecular diagnostics, Aichi Cancer Center; Division of Advanced Cancer Diagnostics, Nagoya University Graduate School of Medicine), in collaboration with Dr. Ichidai Tanaka, M.D., Ph.D. and Dr. Yoshinori Hasegawa, M.D., Ph.D. (Department of Respiratory Medicine, Nagoya University Graduate School of Medicine), Dr. Toyofumi Chen-Yoshikawa, M.D., Ph.D. (Department of Thoracic Surgery, Nagoya University Graduate School of Medicine), and MD Anderson Cancer Center, demonstrated that a proteoglycan SRGN plays a pivotal role in tumor-stromal interaction and reprogramming into an aggressive and immunosuppressive tumor microenvironment in

TTF-1–negative LUAD.

Through analyzing the genomic and proteomic profiles of 41 LUAD cell lines, the research team identified SRGN as a markedly overexpressed gene in TTF-1–negative LUAD. SRGN was induced by DNA de-methylation resulting from NNMT-mediated impairment of methionine metabolism. SRGN regulated expression of proinflammatory cytokines including IL-6, IL-8, and CXCL1, as well as PD-L1 in LUAD cells. SRGN increased migratory and invasive properties of LUAD cells and fibroblasts, and enhanced angiogenesis. Expression of SRGN in LUAD tumor tissues was associated with poor outcome, and with higher expression of PD-L1 in tumor cells and higher infiltration of PD-1–positive lymphocytes. These findings indicate the potential of SRGN as a therapeutic target and a biomarker for predicting clinical outcome as well as response to immune checkpoint blockade in TTF-1–negative LUAD.

## Research Background

Thyroid transcription factor-1 (TTF-1), also known as NKX2-1, is a homeodomain transcription factor required for lung morphogenesis and epithelial cell differentiation. TTF-1 is amplified in 10-15% of lung adenocarcinoma (LUAD) and plays a crucial role as a lineage-survival oncogene in LUAD. On the other hand, TTF-1 also possesses tumor suppressive functions such as inhibition of metastasis and tumorigenesis induced by oncogenic KRAS, as well as regulation of epithelial differentiation status. TTF-1–negative LUAD, accounting for about 20% of LUAD, is associated with worse clinical outcomes and exhibits a low frequency of actionable genomic alterations including EGFR mutation. As loss of TTF-1 in murine lung tumors is not sufficient to induce metastasis, the mechanisms underlying acquisition of aggressive properties of TTF-1–negative LUAD are not well understood.

## Research Results

Analysis of gene and protein expression profiles of 41 LUAD cell lines revealed that SRGN was significantly overexpressed in TTF-1–negative LUAD cell lines. While TTF-1 overexpression decreased SRGN expression at both mRNA and protein levels, TTF-1 knockdown did not induce SRGN expression in TTF-1–positive cell lines, suggesting the occurrence of additional regulatory mechanisms for suppressing SRGN expression in TTF-1–positive LUAD. Interestingly, the research team found overexpression of NNMT in TTF-1–negative LUAD cell lines. NNMT is a cytosolic enzyme that can impair the methylation potential by consuming S-adenosyl methionine (SAM), the primary methyl group donor for DNA methylation in the methionine cycle. Analysis of stable-isotope tracer studies demonstrated that NNMT consumed the SAM pool available for DNA methylation in TTF-1–negative LUAD, resulting in impaired methionine metabolism and induction of SRGN gene expression through loss of DNA methylation in the promoter region of the SRGN gene.

SRGN regulated expression of proinflammatory cytokines including CXCL1, IL-6, and IL-8, as well as PD-L1, and SRGN knockdown reduced cell migration and invasion in LUAD cell lines. Cancer Cell-derived SRGN promoted activation of fibroblasts and SRGN-regulated

IL-6 and IL-8 enhanced angiogenesis, suggesting that SRGN plays a crucial role in the occurrence of aggressive and pro-metastatic tumor microenvironment. Expression of SRGN in LUAD tumor tissues was associated with lower TTF-1 expression, poor outcome, higher expression of PD-L1 in tumor cells, and higher infiltration of PD-1–positive lymphocytes. PD-1 blockade significantly inhibited growth of Srgn-overexpressing tumors in an immunocompetent syngeneic tumor mouse model, suggesting the possible involvement of SRGN in resistance to immunotherapy and the potential of SRGN as a predictive biomarker for immune checkpoint blockade.

## Research Summary and Future Perspective

In conclusion, transcriptomic and proteomic characterization of LUAD cell lines identified SRGN as a key molecule in reprogramming the tumor microenvironment in TTF-1–negative LUAD, suggesting the potential of SRGN as a therapeutic target and a biomarker for predicting clinical outcome as well as response to immune checkpoint blockade.

## Publication

SRGN-Triggered Aggressive and Immunosuppressive Phenotype in a Subset of TTF-1–Negative Lung Adenocarcinomas

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