**Tumor suppressor TUSC2 immunogene therapy is synergistic with anti-PD1 in syngeneic mouse models of lung cancer**

Ismail M Meraz, Mourad Majidi, Xiaobo Cao, David Rice, Boris Sepesi, Lin Ji, Jack A Roth

Section of Thoracic Molecular Oncology, Department of Thoracic and Cardiovascular Surgery

The University of Texas MD Anderson Cancer Center, Houston, Texas

**OBJECTIVES**

TUSC2, a tumor suppressor gene whose expression is reduced or is deleted in 83% of non-small cell lung cancers (NSCLC) and 100% of small cell lung cancers (SCLC). We previously showed that restoration of TUSC2 significantly inhibits tumor growth and metastasis in mouse human xenograft NSCLC models through induction of apoptosis and inhibition of tyrosine kinase signaling pathways. Abrogation expression of a wide spectrum of cytokines has been observed after forced expression of TUSC2 in NSCLC cells. Protracted complete response observed in TUSC2 clinical trials suggest that TUSC2 may mediate the immune response. Checkpoint blockade immunotherapy against PD1 and PD-L1 yields durable antitumor activity in a subset of NSCLC patients and has recently been approved in the United States for the treatment of this cancer. Here we investigated the immune response to TUSC2 in immune cell populations and the synergistic antitumor effect of TUSC2 in combination with anti-PD1 checkpoint blockade in syngeneic mouse NSCLC models.

**METHODS**

The TUSC2 gene was injected intravenously in NCI-1 (U3-dedicated/ppl enumeration/NUbxplumlymphocytosis (DDOTATHP) nanovesicles encapsulating a TUSC2 expression plasmid. Two syngeneic mouse models were used for this study: (1) C57BL6 mice subcutaneously injected with murine lung adenocarcinoma CMT167-luc cells (Kras12V mutation) and (2) ICR mice with 34424 adrenocarcinoma with a Kras12V allele and a knock-in Trp53172insG allele, which metastasize to the lung. Tumor growth was monitored by imaging ex-vivo immunostaining using the IVIS Imaging System 200. Multicolor flow cytometry was used for immune profiling of circulating immune cells after intravenous injection of an extracellular TUSC2. Cytokine gene expression in response to TUSC2 in murine immune populations was determined by real-time PCR. A multiplex luminescence assay was performed to analyze serum cytokines and chemokines. The NanoLuminx Pan-Cancer Immune Panel (78 immune-related genes) was used to investigate the expression of immune response-related genes in the microenvironment of tumors treated with TUSC2 in combination with checkpoint blockade.

**RESULTS**

*Antitumor efficacy of TUSC2 in combination with anti-PD1 in a syngeneic mouse model of NSCLC. TUSC2+anti-PD1 exhibited greater antitumor activity than either single agent or control (Cont). (Left) Tumor volume curve (N=10). (Middle) IHC imaging of subcutaneous tumors (Right) Tumor volume curve (N=9).*

Effect of TUSC2 alone or in combination with anti-PD1 on immune cell populations in peripheral blood. Multicolor flow cytometry showed that TUSC2 significantly upregulated NK and cytotoxic T cells, and downregulated regulatory T cells, myeloid-derived suppressor cells (MDCs), and B lymphocytes in tumor-bearing mice. The plot of the upper left shows that TUSC2 upregulated NK cells by 2-fold in tumor-free mice. All analyses were done 2 weeks after tumor cell implantation.

TUSC2 antitumor activity is dependent on NK cells. The antitumor effect of TUSC2 was abolished when NK cells were depleted by NK1.1 antibody (top panel). A T-cell-mediated tumor response was shown in mice treated with TUSC2 or TUSC2+anti-PD1, and this response was diminished when NK cells were depleted. High levels of cytokines IL-15 and IL-18 were detected in the serum of mice treated with TUSC2 or TUSC2+anti-PD1. These cytokines are important for NK proliferation, and their levels were absent in NK-depleted mice. The right panel graphs shows that multiple immune-related genes significantly changed by TUSC2 are associated with the antitumor response.

**CONCLUSIONS**

1. The antitumor activity of TUSC2 alone is significantly greater than that of control or anti-PD1 treatment alone. The combination of TUSC2+anti-PD1 shows a greater antitumor effect than either single agent.
2. Immune profiling of peripheral blood revealed significant upregulation of NK cells by TUSC2 or TUSC2+anti-PD1. Increased numbers of CD8 cells and decreased numbers of CD4 cells were found in the circulation after treatment with TUSC2 or TUSC2+anti-PD1.
3. Significantly higher numbers of cytokines: T cells infiltrated into the tumor microenvironment in mice treated with TUSC2 or TUSC2+anti-PD1 combination. This was associated with reduction in numbers of CD11c-positive and FoxP3-positive cells, which was strongly associated with antitumor response.
4. The antitumor activities of TUSC2 and TUSC2+anti-PD1 were completely lost when NK cells were depleted, suggesting that the antitumor immune response induced by TUSC2 is mediated by NK cells.
5. Serum analysis revealed strong Th1-mediated immune responses induced by TUSC2 or TUSC2+anti-PD1, which were diminished in NK cell depletion. Increased IL-15 and IL-18 serum levels were associated with NK upregulation.

**REFERENCES**


