TUSC2 immunogene therapy enhances checkpoint blockade through increased cytotoxic immune activation in chemo-resistant small cell lung cancer (SCLC) in humanized mice

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Abstract

Immunotherapy has been effective but has had limited effect in clinical trials for SCLC. We identified TUSC2, a potent tumor suppressor gene, as a novel immunogene, which mediates both apoptosis in cancer cells and induces a strong antitumor immune response. TUSC2 expression is reduced in almost all SCLC tumors and absent in 41%. We investigated the antitumor immune response to TUSC2 gene therapy in combination with immune checkpoint blockade (ICB) therapy against a chemotherapy resistant inflamed subtype of SCLC tumors in humanized mice. Colony formation was markedly reduced in platinum resistant inflamed H841 cells transfected with TUSC2. Immune-competent humanized mice were generated by transplanting fresh human cord blood derived CD34 stem cells into sub-lethally irradiated NSG mice(hu-NSG). The level of engraftment of human CD45, CD3 T, CD19 B, NK cells was verified before tumor implantation at > 25%. Subcutaneous tumors were developed in hu-NSG mice and treated with intravenous injections of TUSC2 gene expression plasmid loaded cationic lipid nanoparticles (quaratusugene ozeplasmid abbreviated quar oze) with or without pembrolizumab (pembro; anti-PD1). A dramatic antitumor effect was mediated by quar oze treatment, whereas pembro remained ineffective. A synergistic antitumor effect was found when quar oze was combined with pembro. The antitumor effect of the quar oze + pembro combination was associated with increased infiltration of human TILs, CD3 T, cytotoxic T, and NK cells, and a decreased number of human regulatory T (Treg) cells, and PD1 expressing exhausted CD8 T cells in the tumor microenvironment(TME). Quar oze was also combined with atezolizumab (atezo; anti-PD-L1) and the antitumor effect on H841 lung metastases in hu-NSG mice was tested. Metastases were treated with quar oze and atezo alone or in combination. Although quar oze alone and atezo alone showed a strong antitumor effect in controlling metastases, a greater antitumor effect was found when quar oze was combined with atezo. All the humanized mice treated with quar oze + atezo showed no or fewer metastases in the lung compared to the other groups. Infiltration of human CD45, CD3 T, CD8 T and NK cells increased in the quar oze and quar oze +atezo treated groups compared with others. A significantly higher number of CD8 T effector memory and lower number of naïve CD8 T cells were found in the quar oze +atezo group which were associated with increased activated CD8 T cells (CD69+, PD1+ CD8 T cells). The number of HLA-DR+ dendritic cells was increased, whereas myeloid derived suppressor cells were strongly inhibited by the combination. Quar oze was also combined with carboplatin + atezo which showed enhanced antitumor efficacy compared with carbo+atezo or quar oze alone. When quar oze was combined with carboplatin, a greater effect was found in inhibiting H841 cell growth, colony formation, and induction of apoptosis. Quar oze was combined with NPRL2, another tumor suppressor gene, in H841 tumors in humanized mice. Synergistic antitumor efficacy of the dual gene therapy occurred with the quar oze +NPRL2 treatment, and the antitumor effect was associated with augmented immune responses. Taken together, these data suggest that TUSC2 gene therapy in combination with ICB induces strong antitumor activity on a chemo-resistant inflamed subtype of SCLC through cytotoxic CD8 T and NK cell activation.

Background: Chemo-resistant Inflamed SCLC Subtype

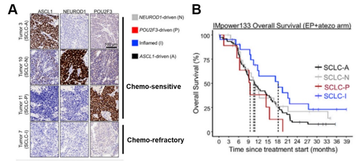

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Fig 1. Background of chemo resistant inflamed SCLC subtype. A) Neuroendocrine markers expression for subtypes of SCLC; B) Overall survival of different subtypes of SCLC treated with immunotherapy.

TUSC2 Inhibited Colony Formation & Cell Growth

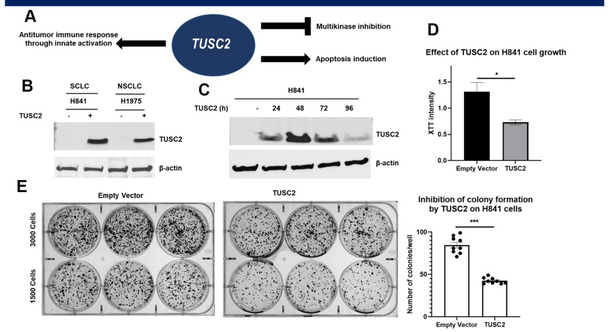

Fig 2. TUSC2 inhibited colony formation & cell growth in chemo-resistant inflamed SCLC. A) Mode of action of TUSC2; B) Transfection of TUSC2 in H841; C) Time course of transient transfection of TUSC2; D) XTT assay shows growth inhibition by TUSC2 at 96h post transfection; E) Colony formation assay and its quantitation on TUSC2 transfected H841. * p < 0.05, *** p<0.0005

Synergistic Antitumor Effect of TUSC2 Immunogene + Pembrolizumab On Chemo-resistant Inflamed Tumors

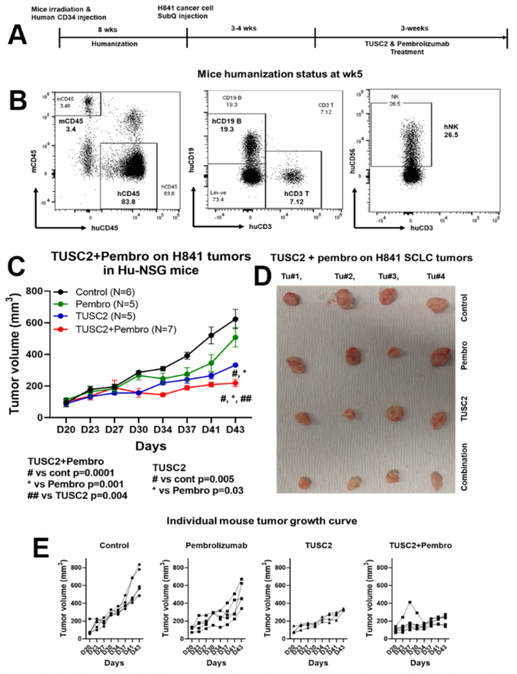

Fig 3. Synergistic effect of TUSC2 + pembrolizumab on chemo-resistant H841 inflamed tumors. A) treatment strategy, B) Humanization status at week 5 post humanization, C) Tumor growth curves, D) Dissected tumors at end of treatment; E) individual mouse growth curves

TUSC2 Combination Induces Antitumor Immune Responses In A Humanized Mouse Model

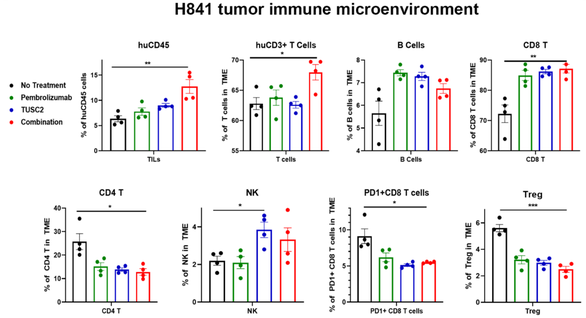

Fig 4. TUSC2+Pembro induces antitumor immune responses in H841 tumors in humanized mice. Subcutaneous tumors developed in humanized mice were analyzed by multicolor flow cytometry. Upper panel shows the alteration of human CD45, CD3 T, B, CTL cells and lower panel shows the level of human CD4 T, NK, PD1+CD8+ T, & Treg cells in TME. * p < 0.05, ** p<0.005, and *** p < 0.0005

Antitumor Immune Responses Of TUSC2 + Atezolizumab On H841 Lung Metastases In A Humanized Mouse Model

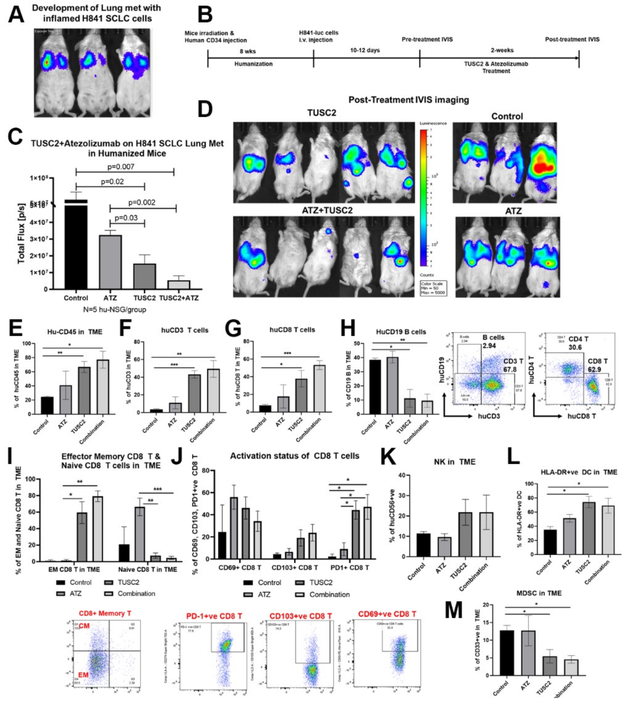

Fig 5. Antitumor immune responses of TUSC2 + Atezolizumab on H841 lung metastases in humanized mice. A) Development of SCLC lung metastases, B) Humanization and treatment strategy, C) Effect of TUSC2+ATZ on lung metastases, D) Post treatment IVIS images show level of lung metastases after treatment, E-M) TME analysis showing the level of infiltrating immune cells CD45, CD3 T, CD8 T, CD19B, memory T cells, activated CD8 T cells, NK cells, DC and MDSC in the tumors. *p < 0.05, **p<0.005, and ***p < 0.0005

TUSC2+NPRL2 Dual Gene Therapy Effect On Chemo-resistant H841 Tumors In a Humanized Mice

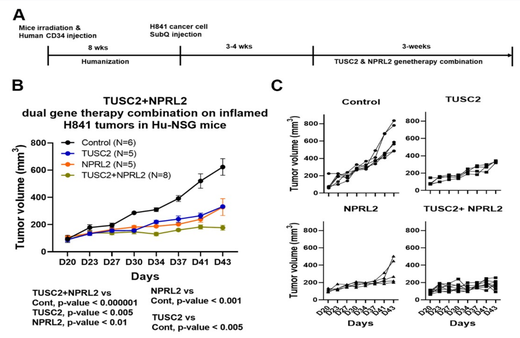

Fig 6. Antitumor effect of TUSC2 + NPRL2 combination on H841 tumors in humanized mice. A) treatment strategy, B) Dual gene therapy combination effects on H841 tumors, C) individual mouse response towards treatments.

Enhanced Antitumor Activity By TUSC2 In Combination With Chemo-immune Therapy On SCLC

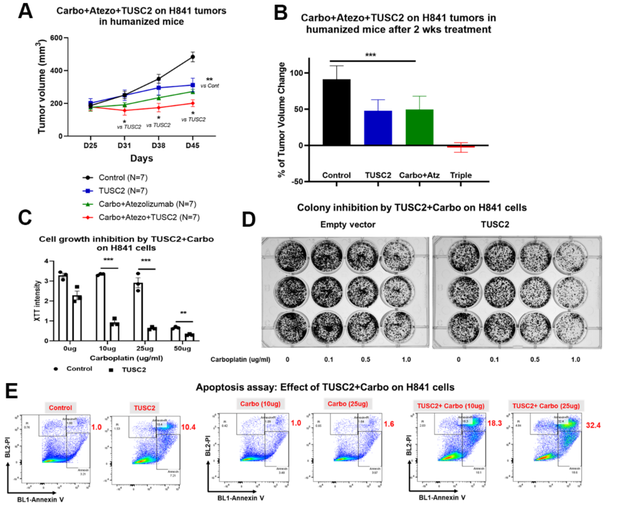

Fig 7. Antitumor effect of TUSC2 in combination with carboplatin + Atezolizumab on chemo resistant H841 model. A) Antitumor effect of TUSC2, Carbo+Atz, and TUSC2+Carbo+Atz in humanized mice, B) Percentage of tumor volume changes after TUSC2 + chemo-immune treatments, C) *In-vitro* cell growth inhibition by TUSC2 with carboplatin, D) Inhibition of colony formation by TUSC2 in combination with different doses of carboplatin, E) Apoptosis assay shown by Annexin V and PI staining on H841 cells after TUSC2 plus carboplatin combination treatment.

Conclusions

- Restoration of TUSC2 in chemo-resistant inflamed H841 SCLC cells significantly inhibited cell growth and colony formation.
- Liposomal delivery of TUSC2 gene therapy (DOTAP-TUSC2) showed significant antitumor effect on chemo-resistant H841subcutaneous tumors in human CD34 stem cell mediated fully humanized mice.
- A dramatic antitumor effect was found when TUSC2 gene therapy was combined with pembrolizumab, a checkpoint blockade aPD1 therapy, although the aPD1 immunotherapy alone showed insignificant effect on H841 inflamed tumors.
- The antitumor effect of the TUSC2 + pembrolizumab combination was associated with increased infiltration of human TILs, CD3 T, cytotoxic T, and NK cells, and a decreased number of human Treg, and PD1 expressing exhausted CD8 T cells in TME.
- A significant antitumor effect of TUSC2 gene therapy was found on H841 lung metastases in humanized mice and a synergistic antitumor activity was noticed when TUSC2 was combined with Atezolizumab (aPD-L1).
- TME analysis in lung metastases showed significantly higher infiltration of human CD45, CD3 T, cytotoxic T, NK, activated T cells, effector memory T cells after treatment with TUSC2 and TUSC2+Atz in humanized mice.
- This combination also increased the number of DC in lung metastases and significantly inhibited Tregs and MDSCs.
- An enhanced antitumor activity was demonstrated when TUSC2 gene therapy was combined with carbo+Atz standard treatment in humanized mice.
- TUSC2 in combination with carbo augmented inhibition of cell growth and colony formation and increased apoptosis in *in-vitro*.
- TUSC2 also showed a synergistic antitumor effect with another gene therapy, NPRL2, in humanized mice.

References & Disclosures

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- *Jack A. Roth is a consultant, stock owner (including pending patent) in Genprex, Inc. All other authors have declared that no competing interests exist*