Overcoming sotorasib-acquired resistance in KRAS^{G12C} mutant NSCLC by TUSC2 gene therapy

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Abstract

Acquired resistance (AR) to sotorasib, an FDA-approved KRAS inhibitor, poses a significant challenge in treating KRAS^{G12C} mutant NSCLC. Despite initial responses, patients invariably develop resistance, necessitating alternative therapeutic strategies. The mechanisms of AR include the emergence of additional mutations in the KRAS gene, reactivation of the KRAS pathway, or activation of alternative signaling pathways. TUSC2, a potent tumor suppressor gene with immunogenic properties, exhibits multifunctional activity by a) inhibiting signaling pathways, including MAPK and mTOR, b) arresting cancer cell growth and proliferation, c) inducing tumor cell death, and d) activating immune responses. This study shows that TUSC2 gene therapy effectively overcomes AR in KRAS^{G12C} mutant non-small cell lung cancer (NSCLC). AR patient-derived xenografts (PDX), PDX-derived organoids (PDXO), and cell lines were generated. Both AR cell lines and PDXOs showed >100-fold resistance over sensitive counterparts. The resistant cell lines also showed resistance to adagrasib, another KRAS^{G12C} inhibitor. Whole Exome Sequencing (WES) was performed for AR PDXs, CDXs, and cell lines and all retained the KRAS^{G12C} mutation with no additional KRAS mutations. Mass spectrometry (MS) on TC303AR and TC314AR PDXs showed significant sets of differentially regulated proteins in AR vs parental. A significant upregulation of MTORC1 signaling in AR PDXs was found. Proteins involved in the PI3K-AKT-mTOR pathway were significantly upregulated in AR PDXs and cells. TUSC2 transfection reduced colony formation and increased the annexin V-positive apoptotic cells in both H23AR and H358AR cells. Re-expression of TUSC2 into AR PDXOs significantly decreased the viability of organoids compared with the empty vector. The H23AR tumors, which exhibited no significant sensitivity to sotorasib, showed robust antitumor responses to quaratusugene ozeplasmid, a lipoplex gene therapy containing the TUSC2 gene for both H23AR and TC314AR PDXs. A synergistic antitumor effect was observed when TC314AR PDXs were treated with the combination of TUSC2 and sotorasib. To evaluate the antitumor immune responses, immune-competent humanized-NSG mice are generated by transplanting fresh human cord blood-derived CD34+ stem cells into sub-lethally irradiated NSG mice to reconstitute a human immune system. The robust antitumor effect of TUSC2 was observed in both PDX and xenograft humanized mice. When sotorasib was combined with TUSC2, synergistic antitumor activity was found. This antitumor effect was correlated with significant infiltration by human CD8 T, NK, DC, and M1 MQ, and downregulation of MDSCs and exhausted T cells. TUSC2 therapy, alone or in combination with sotorasib, induced apoptosis, inhibited colony formation, and showed significant antitumor efficacy in KRAS^{G12C} AR tumors.

TUSC2 inhibits colony formation & induces apoptosis in acquired resistant NSCLC cells

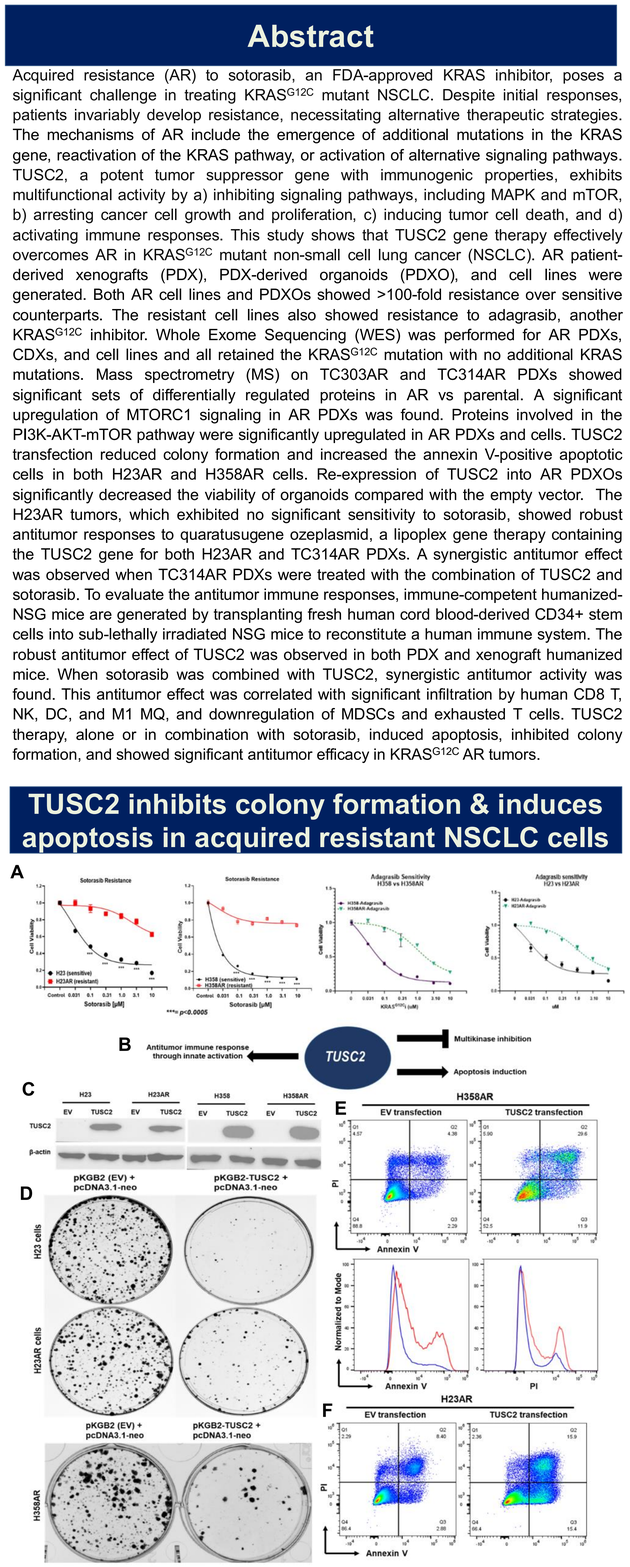

Fig 1. TUSC2 inhibited colony formation and induced apoptosis in resistant cells. A) Degree of resistance to sotorasib and adagrasib for two AR isogeneic cell lines, B) Mode of action of TUSC2; C) Transient transfection of TUSC2 in AR cells; D) Colony formation assay on TUSC2 transfected AR cells; E-F) Apoptosis of acquired resistant H23AR and H358AR cells after TUSC2 transfection.

TUSC2 sensitizes sotorasib-acquired resistant PDX-derived organoids

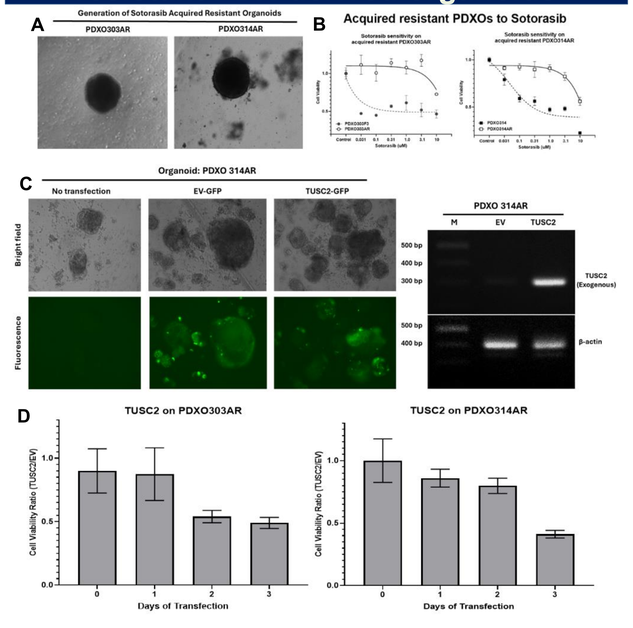

Fig 2. TUSC2 is cytotoxic for AR PDXOs. A) Sotorasib-resistant PDX-derived organoids (PDXOs) were generated; B) Sotorasib sensitivity on AR two isogeneic PDXOs; C) Evaluation of TUSC2 transfection on PDXOs by fluorescence imaging (left) and RT PCR (right); D) Cell viability assay by Glow 3D on AR PDXOs by TUSC2 transfection.

Sotorasib resistance on PDXs and its underlying mechanism

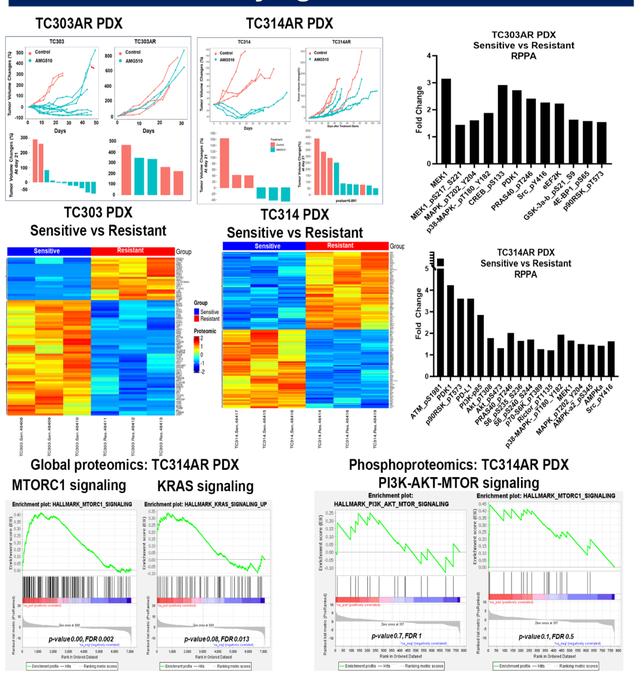

Fig 3. Global proteomics and phosphoproteomics on acquired resistant PDXs. A) Generation of AR TC303AR and TC314AR PDXs; B) Heatmaps showed the upregulation of a distinct set of proteins in AR PDXs (TC303AR & TC314AR) as compared with their sensitive PDXs; C) Enrichment analysis in global (left) and phosphoproteomics (right) in TC314AR showed MTORC1 & KRAS and MTORC1 & PI3K-AKT-MTOR pathways significantly upregulated respectively; D); RPPA analysis on TC303AR (top) and TC314AR PDXs showing upregulation of MAPK and PI3K-AKT-mTOR signaling molecules.

Antitumor effect of TUSC2 on acquired resistant H23AR xenograft and TC314AR PDX models

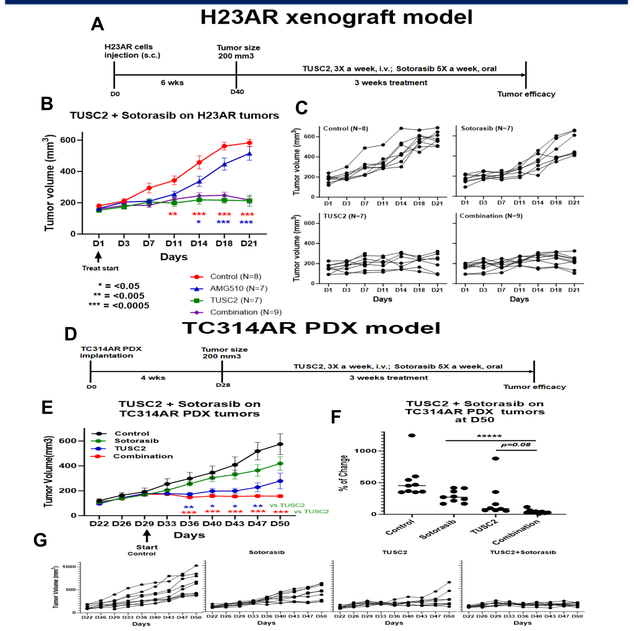

Fig 4. Antitumor effect of TUSC2 gene therapy on H23AR xenografts and TC314AR PDXs. A) Treatment strategy for H23AR model; B) Combination effects on H23AR xenograft tumors; C) Individual mouse response to treatments; D) Treatment strategy for TC314AR PDXs; E) Antitumor effect of TUSC2, sotorasib and their combination; F) Percentage of changes in tumor volume after treatment; G) Individual mouse response to treatments. * means p < 0.05, ** means p<0.005, and *** means p < 0.0005

Antitumor immune response of TUSC2 on H23AR xenograft tumors on a humanized mouse model

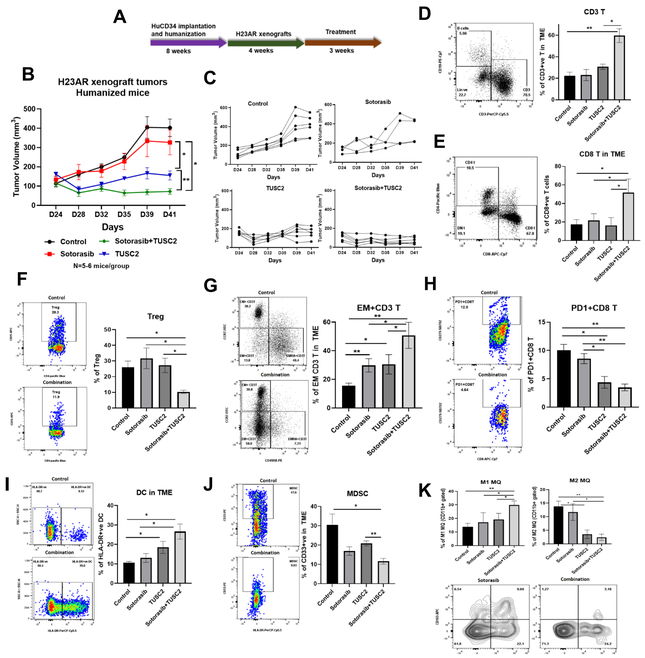

Fig 5. TUSC2 antitumor immune response on H23AR xenografts on humanized mice. A) Exp. Strategy; B) Antitumor effect of TUSC2 in combination with sotorasib; C) individual mouse responses; D-H) Tumor microenvironment analysis for lymphoid cells in humanized mice after treatment; I-K) TME analysis for myeloid cells in humanized mice after treatment. * means p < 0.05, ** means p<0.005

TUSC2 overcomes AR resistance by inducing antitumor immunity in a Humanized Mouse Model

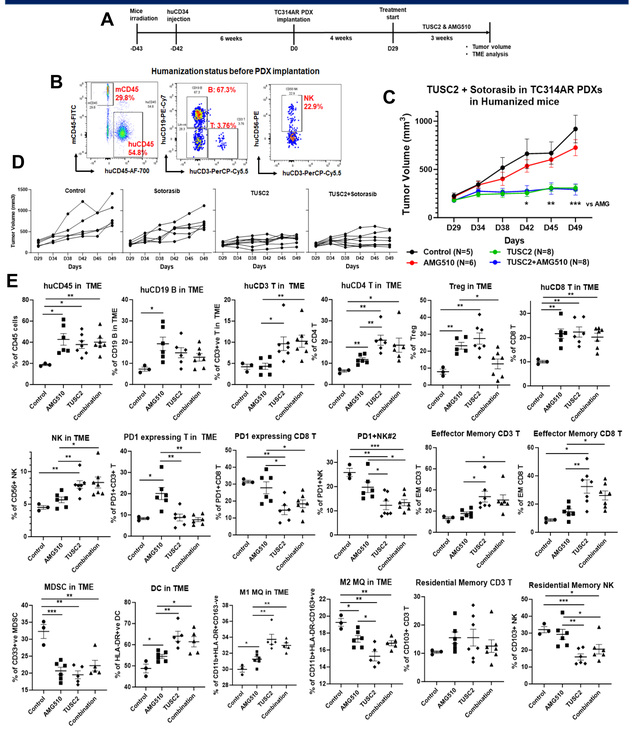

Fig 6. The antitumor immune response of TUSC2 on TC314AR PDXs in humanized mice. A) experimental strategy; B) Humanization status before PDXs implantation; C) Antitumor effect of TUSC2, sotorasib (AMG510) and its combination; D) Individual mouse treratment responses; E) Tumor microenvironment (TME) analysis in humanized mice: (upper panel) effect on human CD45, CD3 T, CD4 T, CD8 T, Treg; (middle panel) effect on human NK, PD1+CD3 T, PD1+CD8 T, PD1+NK, Effector memory CD3 & CD8 T cells; (bottom panel) effect on human MDSC, DC, M1 & M2 MQ, Residential memory CD3 T and residential memory NK cells. * means p < 0.05, ** means p<0.005, and *** means p < 0.0005

Conclusions

- Two isogeneic AR NSCLC cell lines showed >100-fold resistance to sotorasib over their sensitive counterparts, and restoration of TUSC2, a multipotent tumor suppressor, on these AR cells by transient transfection significantly reduced colony formation and induced apoptosis.
- PDXO303AR and PDXO314AR organoids showed over 100-fold resistance as compared to their parental PDXOs, and TUSC2 transfection on those AR PDXOs induced significant cell death.
- Sotorasib-resistant TC303AR & TC314AR PDXs showed no sensitivity to sotorasib, and proteomic analysis revealed the reactivation of the KRAS pathway and upregulation of PI3K-AKT-mTOR signaling in AR PDXs.
- Robust antitumor activity was found in acquired resistant H23AR xenograft tumors when tumors were treated with quaratusugene ozeplasmid, a lipoplex gene therapy containing the TUSC2 gene
- TUSC2 gene therapy also showed a synergistic antitumor effect on acquired resistant TC314AR PDX tumors when PDXs are treated with TUSC2 and sotorasib combination
- Quaratusugene ozeplasmid exhibited a significant antitumor effect on both H23AR xenografts and TC314AR PDXs as compared with sotorasib alone treatment in humanized mice. The antitumor effect of TUSC2 + sotorasib combination was found to be synergistic in H23AR tumors.
- TUSC2 antitumor effect was associated with enhanced antitumor immune response in both H23AR and TC314AR PDX tumors in humanized mice, which was characterized by the significantly increased infiltration of human CD3, CD4, cytotoxic T, and NK cells, and inhibition of human regulatory T cells
- PD1-expressing T and NK cells were significantly downregulated, whereas effector memory CD3 and CD8 T cells were markedly increased by TUSC2 treatment.
- TUSC2 activated innate immunity by enhanced infiltration of DC and M1 macrophages, with significant inhibition of MDSC and M2 macrophages.

Disclosures

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