TUSC2 Modulates Cancer Immune Responses

MUNA MOHAMMED[1], SALVADOR GONZALEZ OCHOA[1], JANE TONELLO[1], MARK BERGER[3], ALLA IVANOVA[2], ANIL SHANKER[1,2]
[1]Department of Biochemistry, Cancer Biology, Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN 37208, USA.
[2]School of Medicine, Meharry Medical College, Nashville, TN 37208, USA
[3]Genprex, Inc USA.

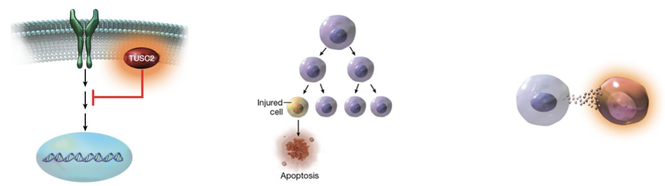
Society for Immunotherapy of Cancer
sitc2024
NOV. 6-10 | HOUSTON

BACKGROUND

- The Tumor Suppressor Candidate2 (TUSC2) is located within the chromosomal region 3p21.3 frequently deleted in patients with non-small cell lung carcinoma (NSCLC), mesothelioma, breast, head-and neck, and other cancers. It has the capability to suppress proliferation and induce apoptosis in cancer cells.
- TUSC2 protein is reduced or absent in about 80% of NSCLC and in 100% of SCLC patients. In patients with NSCLC, the loss of TUSC2 was found to be associated with significantly worse overall survival.
- Our early studies showed that Tusc2 is a mitochondrial protein that regulates cellular calcium homeostasis and modulates immune responses However, it's precise role in immune responses to cancer is unclear. Tumor microenvironment (TME) impacts tumor responsiveness to cancer therapy, in particularly the immunotherapy.

- **Quar**atusugene **oze**plasmid (**quar oze**) is Gene Therapy for NSCLC and SCLC. It consists of the TUSC2 gene expressing plasmid encapsulated in non-viral nanoparticles made from lipid-based molecules in a lipoplex form.
- Quar oze is injected intravenously and specifically targets cancer cells. It is designed to deliver the functioning TUSC2 gene to cancer cells while minimizing their uptake by normal tissue.

How quar oze works



HYPOTHESIS

Since Tusc2 expression is significantly decreased in tissues of aging individuals, Tusc2 KO mice represent proper microenvironment to study the effect Tusc2 supplementation on tumorigenic processes. We hypothesize that TUSC2 nanoparticles modulate the tumor microenvironment (TME) and change the cellular makeup of immune cells infiltrating TME in Tusc2-deficient tissues.

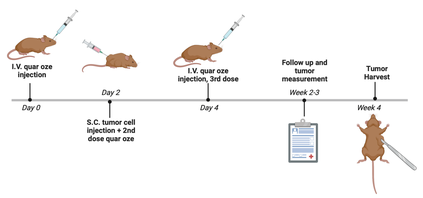
AIM

Characterize the immune cell landscape within tumors of Tusc2 knockout (KO) mice treated with quar oze or control nanoparticles, focusing on identifying specific immune cell populations and their roles in modulating the tumor microenvironment.

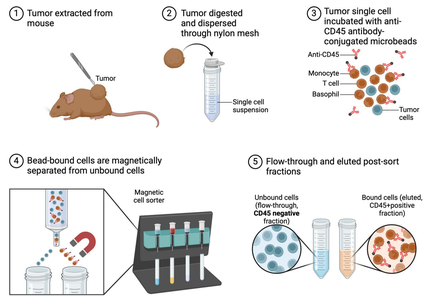
KEY REFERENCES

1. Ivanova AV, Ivanov SV, Pascal V, Lumsden JM, Ward JM, Morris N, Tessarolo L, Anderson SK, Lerman MI. Autoimmunity, spontaneous tumourigenesis, and IL-15 insufficiency in mice with a targeted disruption of the tumour suppressor gene Fus1. J Pathol. 2007 Apr;211(5):591-601. doi: 10.1002/path.2146. PMID: 17318811.
2. Kondo M, Ji L, Kamibayashi C, Tomizawa Y, Randle D, Sekido Y, Yokota J, Kashuba V, Zabarovsky E, Kuzmin I, Lerman M, Roth J, Minna JD. Overexpression of candidate tumor suppressor gene FUS1 isolated from the 3p21.3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells. Oncogene. 2001 Sep 27;20(43):6258-62. doi: 10.1038/sj.onc.1204832. PMID: 11593436.
3. Meraz IM, Majidi M, Cao X, Lin H, Li L, Wang J, Baladandayuthapani V, Rice D, Sepesi B, Ji L, Roth JA. Tusc2 Immunogene Therapy Synergizes with Anti-PD-1 through Enhanced Proliferation and Infiltration of Natural Killer Cells in Syngeneic Kras-Mutant Mouse Lung Cancer Models. Cancer Immunol Res. 2018 Feb;6(2):163-177. doi: 10.1158/2326-6066.CIR-17-0273. Epub 2018 Jan 16. PMID: 29339375; PMCID: PMC5909977.

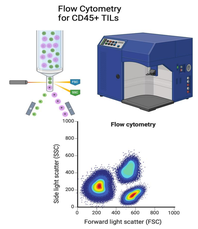
MATERIALS AND METHODS



Purification of Tumor Infiltrating Immune Cells (TILS)



Age-matched female Tusc2 KO mice were injected intravenously with 3 doses of quar oze. The first dose was delivered 2 days prior tumor establishment (344SQ cell line), the second one- at the day of tumor cells injection, and the last dose – 2 days later. 3 weeks later tumor infiltrating cells purified and were analyzed by FACS.



CONCLUSION

- These preliminary results demonstrate that TUSC2 recovery significantly impacts the tumor microenvironment (TME), reducing the tumor growth rate in treated mice by increasing the infiltration of cytotoxic CD8+ T cells, enhancing NK cell cytotoxicity through Granzyme B production, and reducing T regulatory cell presence, which typically suppresses immune responses.
- These changes suggest that TUSC2 nanoparticles could be a powerful tool in boosting the effectiveness of cancer immunotherapy.

FUTURE WORK

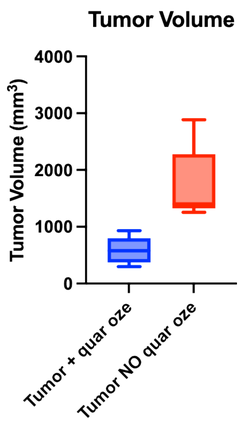
- Characterize other types of immune cells within the tumor microenvironment
- Investigate the TUSC2-specific mechanisms of action on tumor immune cells
- Optimize the injection schedule of Quar Oze to achieve a prolonged effect of the nanoparticles
- Combine nanoparticles with different immune checkpoint inhibitors to harness infiltration of cytotoxic cells
- Study the intergender effect of TUSC2 nanoparticles

ACKNOWLEDGEMENTS

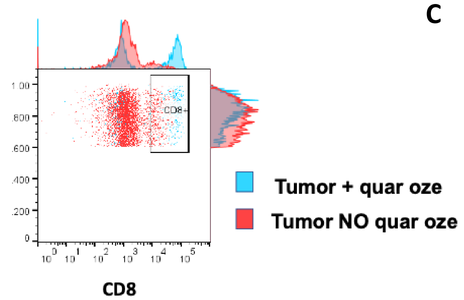
- This project was supported by:
- Microbial Pathogenesis Training Program(T32)
- Meharry Medical College Core Facilities, which are supported by NIH Grants MD007586, CA163069, and S10RR025497".
- Meharry Medical College Animal Care Facility.
- Grant "Glycolytic Metabolism in TUSC2 Deficiency" provided by Geneprex, Inc

RESULTS

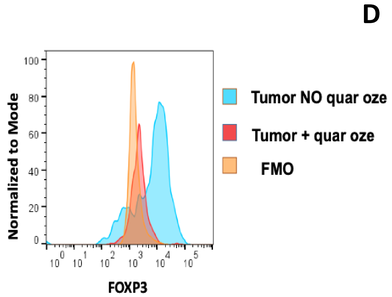
A



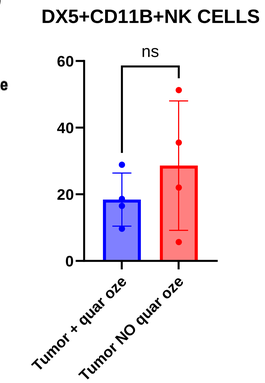
B



C

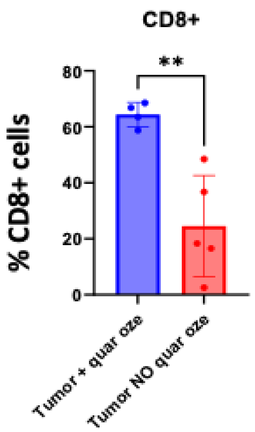


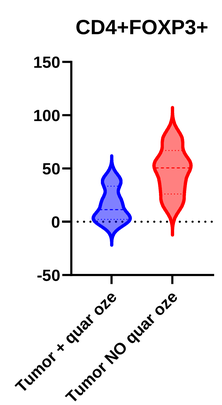
D

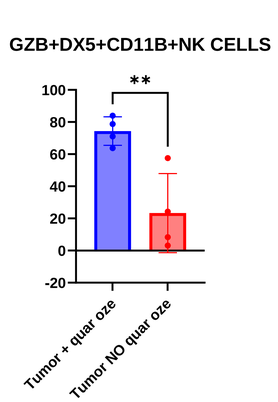


Effects of Tusc2 nanoparticles:

A. The tumor volume is reduced in TUSC2 nanoparticles- injected group
B. The upper and lower panels showing the percentage of $CD8^+$ T cells infiltrate tumor which is higher in TUSC2 nanoparticles-injected group
C. The upper and lower panels showing T reg cells infiltration is reduced in TUSC2 nanoparticles-injected group
D. The upper and lower panels showing the total NK cells infiltration in control and experimental groups is not changed significantly, while the granzyme B expression by these cells is higher in TUSC2 nanoparticles-injected group







N = 5