Quaratusugene Ozeplasmid mediated TUSC2 upregulation in EML4-ALK bearing Non-Small Cell Lung Carcinoma can induce cellular apoptosis

Ananya Banerjee[1], Neeke Busette[1], Mark S. Berger[3], Matthew B. Soellner[2], Angel Qin[1], Sofia D. Merajver[1], Nathan M. Merrill[1]

[1] Department of Internal Medicine, University of Michigan, Ann Arbor, MI,
[2] Departments of Medicinal Chemistry and Chemistry, University of Michigan, Ann Arbor, MI
[3] Genprex, Inc. Austin, TX

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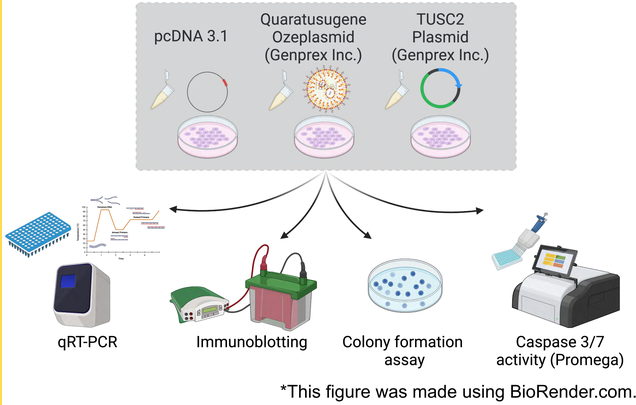
ABSTRACT

Anaplastic Lymphoma Kinase (ALK), a potent oncogenic driver in Non-Small Cell Lung Carcinoma (NSCLC), is found to be rearranged and fused to Echinoderm Microtubule-associated protein-Like 4 (EML4), contributing to approximately 5% of NSCLC. Tumors bearing this fusion are sensitive to ALK Tyrosine Kinase Inhibitors (TKIs). However, emerging resistance to ALK TKIs demands new treatment strategies. Quaratusugene Ozeplasmid (QO), developed by Genprex, is an immunogene therapy that upregulates Tumor Suppressor Candidate 2 (TUSC2) expression in cancer cells by delivering the functional TUSC2 gene in non-viral lipid nanoparticles. We evaluated TUSC2 expression in three ALK+ cell lines, both before and after exposure to QO and to a TUSC2-containing plasmid. Controls were non-ALK+ cell lines and transfection with a plasmid containing no insert. Our studies reveal that overexpressing TUSC2 using QO in ALK+ lung cancer cell lines can suppress colony formation by 50%, the effect being more significant than using a TUSC2-containing plasmid. Furthermore, we have observed a robust pro-apoptotic response to TUSC2 expression in ALK+ NSCLC, as both QO and TUSC2-containing plasmid induced an increase in caspase 3/7 activity in the cancer cells, accompanied by an increase in cleaved PARP expression. Taken together, our data indicate that overexpression of TUSC2 in ALK+ NSCLC cell lines with QO or with TUSC2-containing plasmid is effective in decreasing growth and proliferation through the activation of apoptotic pathways and warrants further investigation as an anti-ALK NSCLC strategy.

MATERIALS AND METHODS

- Cell lines: EML4-ALK+ NSCLC cell lines NCI-H2228, NCI-3122, DFCI-032; Non-ALK NSCLC cell lines A549, NCI-H2170.



*This figure was made using BioRender.com.

- All Western Blot images were analyzed using ImageJ and all data were plotted using GraphPad Prism.

RESULTS

TUSC2 overexpression:

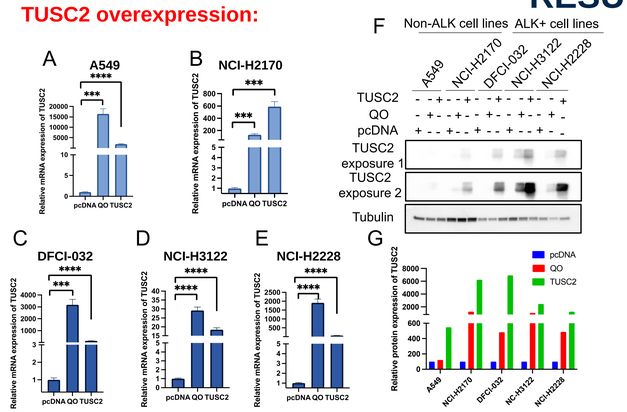


Fig 1: Quaratusugene Ozeplasmid can overexpress TUSC2 in EML4-ALK+ NSCLC and non-ALK cell lines. A-B,F, TUSC2 is overexpressed in non-ALK lung cancer cell lines, C-F, and is significantly overexpressed in EML4-ALK+ NSCLC cell lines (transcriptional and translational levels), G, The protein expressions have been quantified and normalized to the housekeeping control. For all qPCR, data have been analyzed using t-test. *** represents 0.0001 ≤ p ≤ 0.001, **** represents p < 0.0001.

Apoptosis in parental cells:

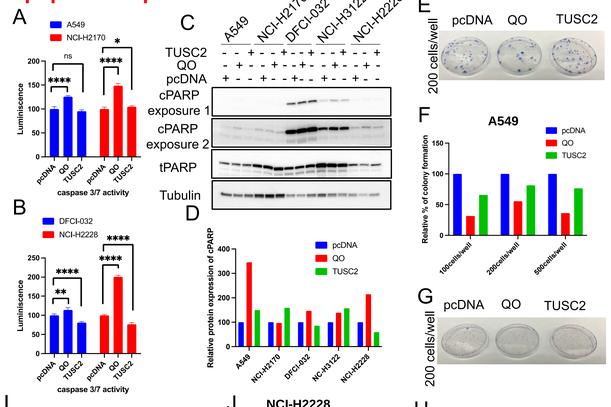


Fig 2: Quaratusugene Ozeplasmid mediated TUSC2 overexpression can induce apoptosis in EML4-ALK+ NSCLC cell lines. A, QO mediated overexpression of TUSC2 increases the caspase 3/7 activity in non-ALK NSCLC cell lines B, and in EML4-ALK+ NSCLC cell lines. C, QO mediated overexpression of TUSC2 increases the expression of cleaved PARP. D, The protein expressions have been quantified and normalized to the housekeeping control. E-J, The same combination decreases the colony formation ability of the cells. All data have been analyzed using t-test. ****:p< 0.0001,**:0.001≤p≤0.01,*:0.01≤p≤ 0.05, ns: p ≥ 0.05.

Generation of Alectinib Resistant cells:

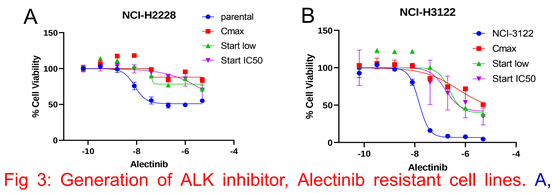


Fig 3: Generation of ALK inhibitor, Alectinib resistant cell lines. A, NCI-H2228 and B, NCI-H3122 cell lines were made resistant to the ALK inhibitor, Alectinib by 3 independent methods. The cells were treated for a prolonged period with their Cmax values, IC50 values and a range of drug doses starting from a low value and gradually escalating the dose, to establish these cell lines, and were respectively labelled as Cmax, Start IC50 and Start low. Each method was generated in triplicates.

Apoptosis in Alectinib Resistant cells:

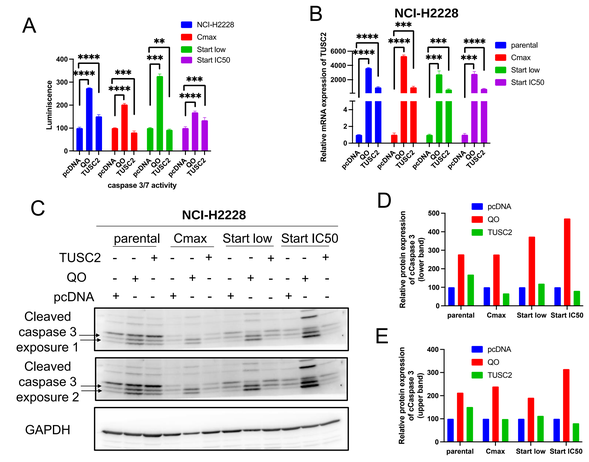


Fig 4: Quaratusugene Ozeplasmid mediated TUSC2 overexpression can induce apoptosis in Alectinib resistant EML4-ALK+ NSCLC cell lines. A,B, QO mediated overexpression of TUSC2 in Alectinib resistant NCI-H228 cells significantly increases the caspase 3/7 activity. C, QO mediated overexpression of TUSC2 increases the expression of cleaved caspase 3 in these cells. D,E, The protein expressions have been quantified and normalized to the housekeeping control. All data have been analyzed using t-test. ****:p< 0.0001, *** : 0.0001≤ p≤ 0.001 **:0.001≤p≤0.01.

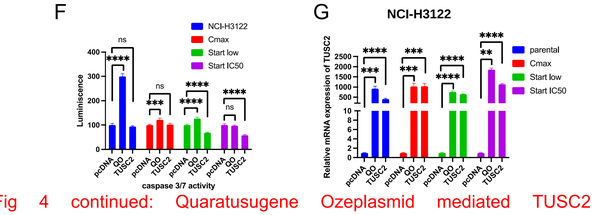


Fig 4 continued: Quaratusugene Ozeplasmid mediated TUSC2 overexpression can induce apoptosis in Alectinib resistant EML4-ALK+ NSCLC cell lines. F,G, QO mediated overexpression of TUSC2 in Alectinib resistant cells significantly increases the caspase 3/7 activity in these cells. All data have been analyzed using t-test. ****:p< 0.0001, ***:0.0001≤ p≤ 0.001 **:0.001≤p≤0.01, ns: p ≥ 0.05.

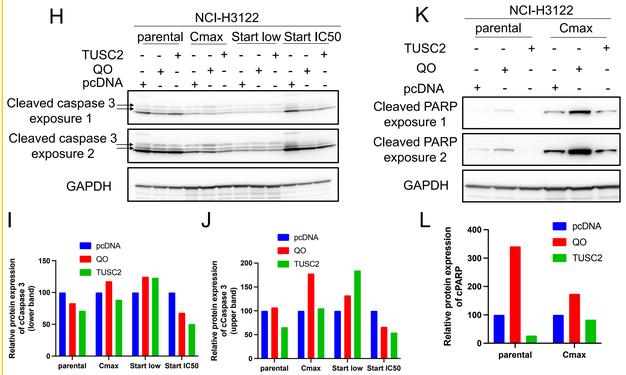


Fig 4 continued: Quaratusugene Ozeplasmid mediated TUSC2 overexpression can induce apoptosis in Alectinib resistant EML4-ALK+ NSCLC cell lines. H,K, QO mediated overexpression of TUSC2 increases the expression of cleaved caspase 3 and cleaved PARP in the resistant cells. I,K,L The protein expressions have been quantified and normalized to the housekeeping control.

Pathway:

Fig 5: TUSC2 regulates apoptosis in EML4-ALK+ NSCLC.

[1] Rimkus et. al., *DiscovMed,* 2017
[2] Ji et. al., *J. Thorac. Oncol.*, 2008
[3] Papageorgiou et. al., *Cancers, 2022.*

*This figure was made using BioRender.com.

CONCLUSION

- QO mediated upregulation of TUSC2 can induce cellular apoptosis in ALK+ NSCLC cells, and in the corresponding Alectinib resistant cell lines, as seen by increased caspase 3/7 activity, increased protein expressions of cPARP and cCaspase 3 and decreased colony formation ability.
- Interestingly, QO mediated overexpression of TUSC2 has been found to be more effective in promoting apoptosis in comparison to the TUSC2 plasmid alone in ALK+ lung cancer and thus emerges as a promising treatment approach in NSCLC dominated by ALK.
- However, it is important to understand the mechanism by which TUSC2 governs ALK in regulating apoptosis via the cell signaling network, and hence warrants further research.

ACKNOWLEDGEMENT

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