TUSC2 Suppresses Tumorigenic Properties in Malignant Pleural Mesothelioma Cells

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Introduction

Malignant Pleural Mesothelioma (MPM) is a rare, highly aggressive, asbestos-associated neoplasm with a median survival of 10 -12 months. TUSC2, a tumor suppressor gene located in the 3p21.3 chromosomal region, is frequently deleted in multiple cancers, and at least one allele is absent in 36% of MPM. TUSC2 protein resides mostly in mitochondria and plays an important role in energy metabolism. When the TUSC2 gene is absent, an autoimmune disorder with an inflammatory background develops, resulting in tumors such as hemangiosarcoma. Since MPM is clearly linked to chronic inflammation induced by asbestos, we investigated whether TUSC2 overexpression could modulate MPM's aggressive properties.

Scientific Rationale

Pre-clinical studies demonstrate that systemic delivery of TUSC2 plasmid DNA complexed with DOTAP-Cl:-Cholesterol liposome solution (quaratusugene ozeplasmid; Quar Oze) suppresses tumor growth, inhibits experimental lung metastasis and prolongs animal survival. Moreover, clinical trials in lung cancer are ongoing using Quar Oze. These studies provide the scientific rationale to study the anti-tumorigenic role of TUSC2 re-expression in MPM.

Materials and Methods

Quar Oze and control liposomes were provided by Genprex.

Four MPM cell lines (H2595, HP1, H2373, and H2591) and tert-transformed normal mesothelial LP9 cells were treated with Quar Oze and control liposomes for 48h. Post treated cells were then evaluated for:

- ❑ TUSC2 gene expression by semi quantitative RT-PCR
- ❑ TUSC2 protein by Western Blot analysis
- ❑ Pooled populations of transfected cells were then evaluated for tumorigenic functional assays such as
  - Cell proliferation
  - Invasion
  - Apoptosis

Statistical Analysis

The Quar Oze dependent changes for all of the functional assays were analyzed for statistical differences using the standard Student's *t*-test, with a *p*-value less than 0.05 considered as significant. All of the experiments were done in triplicates.

Results

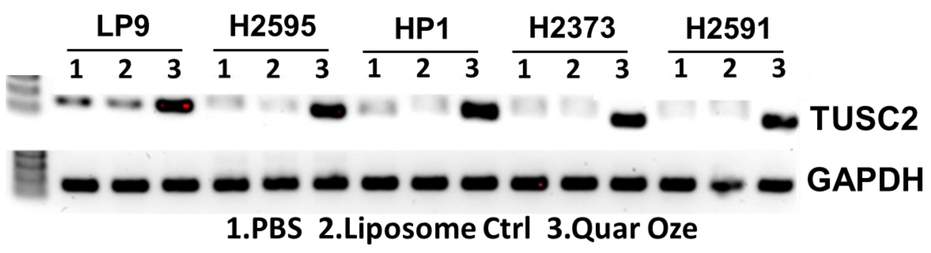

Fig.A: Semi-Quantitative RT-PCR analysis validated overexpression of TUSC2 in Quar Oze transfected cells. GAPDH was used as a house-keeping gene.

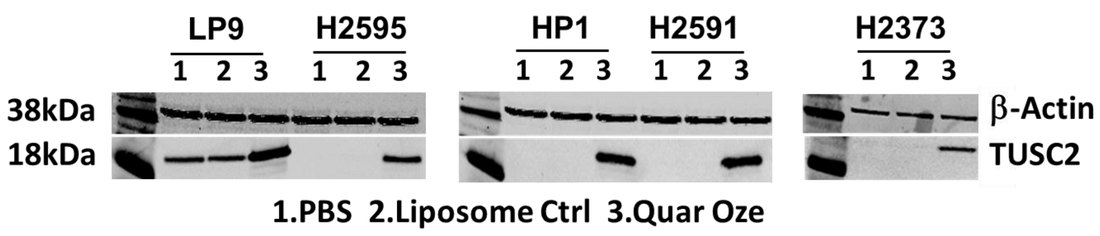

Fig.B: Western Blot analysis demonstrates overexpression of TUSC2 protein in Quar Oze transfected cells. Beta-Actin was used as a loading control.

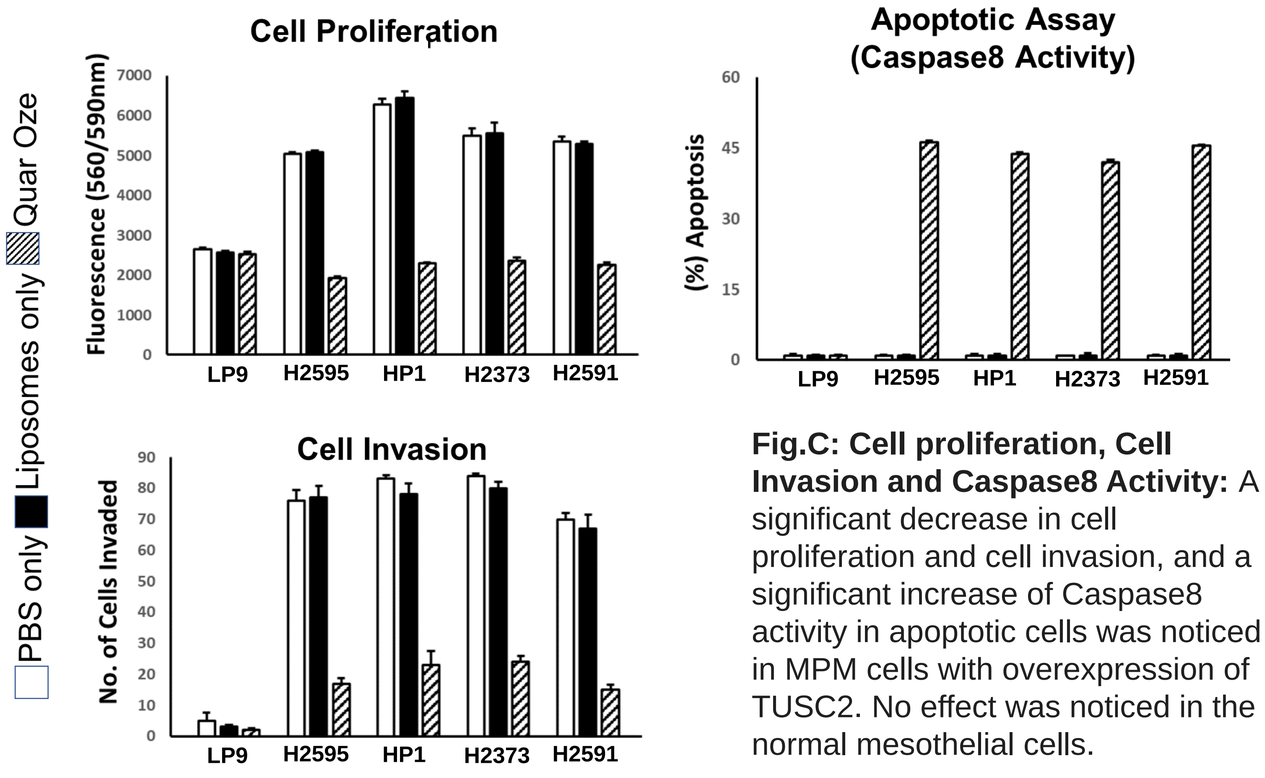

Fig.C: Cell proliferation, Cell Invasion and Caspase8 Activity: A significant decrease in cell proliferation and cell invasion, and a significant increase of Caspase8 activity in apoptotic cells was noticed in MPM cells with overexpression of TUSC2. No effect was noticed in the normal mesothelial cells.

Table: Functional assays

	(%) Proliferation	p Value	(%) Invasion	p Value	(%) Apoptosis	p Value
H2595	-62	2.57E-08	-78	1.65E-06	+47	8.31E-07
HP1	-63	1.08E-06	-75	5.43E-06	+43	1.26E-06
H2373	-57	2.40E-05	-71	1.77E-06	+42	8.27E-05
H2591	-59	1.23E-06	-78	2.16E-05	+45	1.99E-05

Conclusion

Our data demonstrate the potent tumor-suppressive activity of the TUSC2 gene delivered by Quar Oze and its re-expression could serve as a potential therapeutic strategy for the treatment of MPM.

References

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