TUSC2 suppresses energy metabolism in lung cancer cells with opposite effects in normal bronchial epithelial cells

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Introduction

TUSC2 protein is a mitochondrial resident that plays a critical role in mitochondrial activities, such as mitochondrial respiration/energy metabolism, ROS production, Ca^{2+} flux to/from mitochondria, lipid metabolism, etc. Dysregulation of these mitochondrial functions profoundly affects cellular metabolism, multiple signaling pathways, cell proliferation, apoptosis, mitophagy, immune responses, DNA damage response, etc. Preclinical studies on tumor-bearing mice treated with TUSC2-containing plasmid encapsulated in lipid nanoparticles (**quar**atusugene **oze**plasmid) and Phase I and II clinical trials of quar oze in lung cancer provides a strong rationale to further study the tumor-protective mechanisms of TUSC2 protein.

Main scientific question

The main scientific question of our study is if and how human lung adenocarcinoma cells, A549 and H358, that have ~80-90% decrease in TUSC2 expression, change their energy metabolism in response to re-introduction of TUSC2 to these cells as compared to Beas2B, a normal human bronchial epithelial cell line. It is well known that cancer cells rely heavily on anaerobic glycolysis, while normal epithelial cells use mitochondrial respiration as a primary way of energy production.

Methods

Transient transfection of TUSC2-expressing plasmid to A549, H358, and Beas2b cell lines; qPCR for the presence of plasmid TUSC2 in cells at 72h post-transfection; Seahorse metabolic analysis of energy production by these cells at 72h post-transfection using Mitochondrial Stress and Glycolysis tests. Based on the captured Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) under different conditions, the analyzer calculates main metabolic parameters, i.e. basal and maximal respiration, ATP production, spare respiratory capacity, coupling efficiency, glycolysis, glycolytic capacity and reserve.

Drugs used in the study to interrogate mitochondrial respiration and anaerobic glycolysis

1. **Oligomycin**: an inhibitor of ATP synthase (Complex V) , which significantly reduce electron flow through the electron transport chain and prevents ATP production
2. **FCCP**: an uncoupling agent that disrupts ATP synthesis by transporting protons (H+) through the mitochondrial membrane before they can be used to provide the energy for oxidative phosphorylation. Evaluates maximal respiratory capacity of the cell.
3. **Antimycin + Rotenone**: inhibit complex III and I of the mitochondrial electron transport chain (mETC), thus effectively mitochondrial respiration.
4. **2-DG , 2-Deoxy- D-glucose,** non-metabolizing glucose molecule, used to shut down glycolysis

Main tool used in the study

Seahorse Analysis. Agilent Seahorse XF 96-well analyzer (Fig 1A) measures in real life and reports the oxygen consumption rate (OCR), proton efflux rate (PER) or extracellular acidification rate (ECAR), as well as ATP production rates of live cells in a 96-well format Fig 1A, red arrow . Cells are seeded into a 96-well XF culture plate with test-specific media. Drugs are automatically injected at specific time points by the analyzer. The drug cartridge (Fig 1b) contains sensor sleeves (Fig 1b, red circle) with polymer embedded fluorophores on the tip; one measures oxygen consumption rate (OCR) and the other measures protons for extracellular acidification rate (ECAR). Drugs are loaded into the 4-wells surrounding the sensor sleeve and are released into media one by one during analysis. After drug injection into culture media, Sensor probes slide into the sleeve to mix the drug and emit light to activate the fluorophores (Fig 1C). Fluorophores are activated depending on the concentration of oxygen or protons in the chamber (space between cells and sensor, Fig 1D, red arrow).Using these measurements, the instrument then calculates OCR and ECAR.

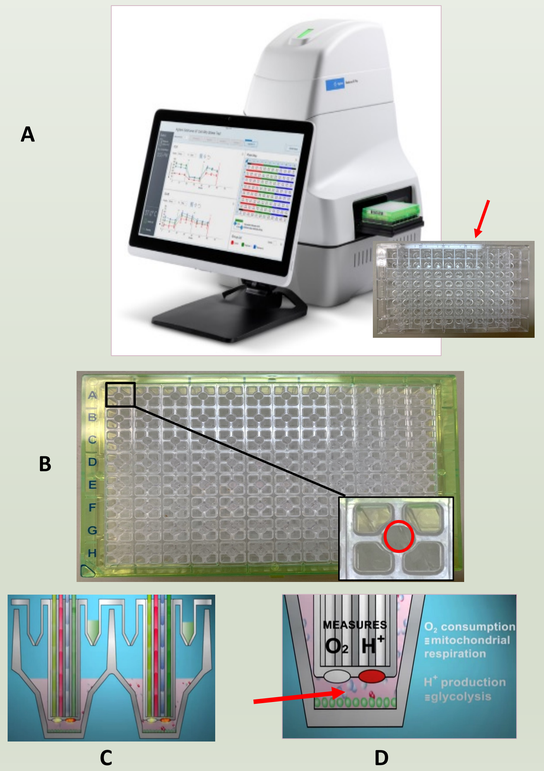
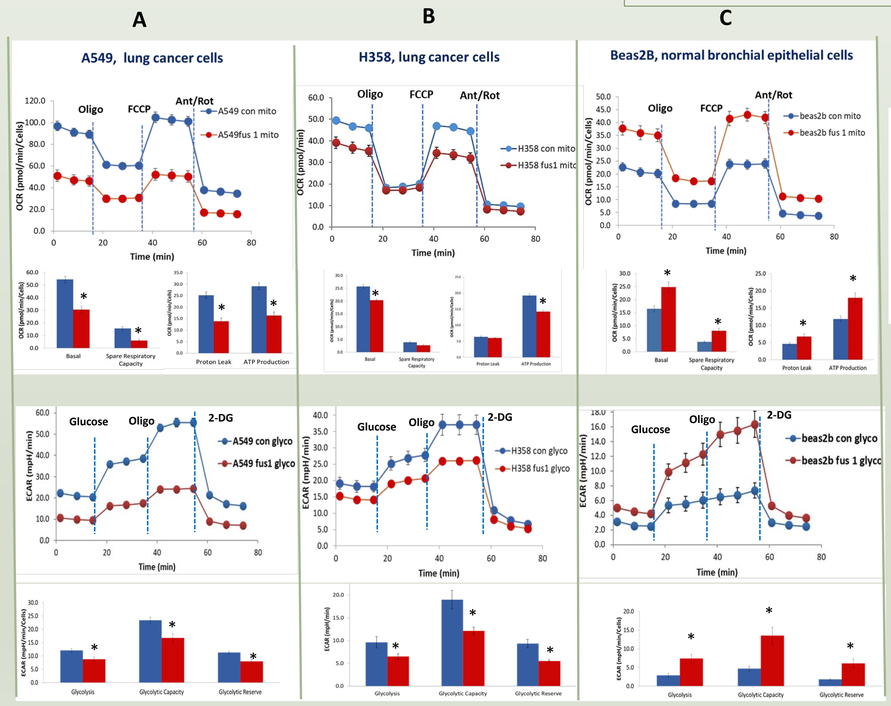
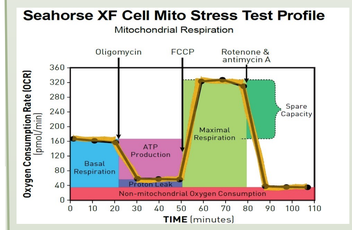


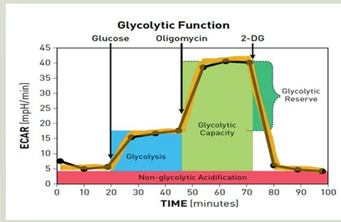
Figure 1. The schematics and principle of Seahorse analyzer

Results

All three cell lines demonstrated high transfection efficiency, confirmed by qPCR analysis. Seahorse analysis revealed that TUSC2 re-introduction to TUSC2-deficient cancer cells consistently **suppressed** both glycolytic and mitochondrial ATP production at 72h after transfection, thus leaving cells without sufficient energy to support their vital functions (Fig. 2A and B). We found a significant decrease in basal respiration, maximal respiration, spare respiratory capacity, ATP production, glycolysis, glycolytic capacity, and glycolytic reserve in both cancer cell lines transfected with TUSC2-expressing plasmid Unlike cancer cells, both glycolytic and mitochondrial metabolism of normal epithelial cell line Beas2B were significantly **strengthened** after the introduction of TUSC2 (Fig. 2C), suggesting a beneficial role of TUSC2 for the metabolic health of normal cells. The experiments were repeated four times; Student T-test was used to calculate statistical significance.







Discussion

TUSC2/FUS1(Tumor Suppressor Candidate 2/*FUSion1*) is a tumor suppressor gene (TSG) from the human 3p21.3 chromosomal region frequently deleted in lung cancers. Independently of deletion, reduced expression of TUSC2 is observed in up to 80% of lung cancers, mesothelioma, breast, head-and neck, osteosarcoma, glioblastoma, and other cancers, suggesting a critical anti-tumor role of TUSC2.
Our findings indicate that one mechanism through which TUSC2 promotes its anti-tumorigenic activities in lung cancer cells is by regulating energy homeostasis in epithelial cells.

Therapeutic Significance

These experiments suggest that TUSC2 plasmid delivery to cancer patients, and thus quar oze therapeutic use, may target and disrupt the metabolism of cancer cells, triggering either senescence or apoptotic pathways, while on the other hand, supporting the metabolism of normal epithelial cells. These data have high therapeutic significance, suggesting that one of the anti-tumor mechanisms of quar oze action in patients is the suppression of cancer cell metabolism resulting in cancer cell death.

Figure 2. Mitochondrial stress test (upper panel) and Glycolysis test (bottom panel) applied to two lung cancer cell lines (A and B) and normal bronchial epithelial immortalized cell line (C) . Two panels on the right show schematics of drug injection and mitochondrial parameters calculated upon test completion.

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