Introduction:

TUSC2, a novel tumor suppressor gene in the human chromosome 3p21.3 region, is deleted in many cancers. A phase I clinical trial assessing TUSC2-mediated molecular therapy has reported antitumor activity in lung cancer patients. Previous studies showed that TUSC2 regulates the activation of multiple oncogenic kinases. MK2206 is a highly selective non-ATP-competitive allosteric inhibitor of AKT currently being evaluated in early-phase clinical trials for treatment of patients with lung cancer.

Abstract:

In this study, we evaluated the combined effects of the tumor suppressor gene TUSC2 and MK2206 on tumor cell growth and apoptosis induction in NSCLC cells and explored the molecular mechanism of their mutual action. We found that exogenous expression of TUSC2 sensitized the response of NSCLC cells to MK2206, resulting in a marked increase in growth suppression and apoptosis in LKB1-mutant NSCLC cells. However, TUSC2 had no effect on the response of LKB1-wild-type NSCLC cells to MK2206. Systemic treatment with a combination of TUSC2-nanoparticles and MK2206 in an LKB1 mutant H322 lung cancer subcutaneous xenograft mouse model enhanced the therapeutic efficacy of MK2206. The mice receiving the combination of MK2206 and TUSC2-nanoparticles showed a significantly reduced mean tumor volume compared with mice receiving empty vector/MK2206, MK2206 alone, or empty vector by day 21 (P<0.01 for all three comparisons).

Results:

Figure 1. Western blot analysis of expression of TUSC2 in NSCLC cell lines.

Figure 2. Combined effect of exogenous expression of TUSC2 and MK2206 treatment in various NSCLC cell lines by (A) Cell viability assay and (B) Clonogenic assay.

Figure 3. (A) The inhibition effects of TUSC2/MK2206 combination on tumor growth in H322 xenograft mouse model and (B) the pharmacodynamic effects.

Figure 4. Combination Effect of TUSC2/MK2206 on phosphorylation of AMPK, AKT, mTOR and their direct substrates.

Figure 5. The effect of AMPK-specific small interfering RNA (siRNA) on TUSC2/MK2206-induced cell death.

Table 1. Fold decrease in IC50 of MK2206 when combined with TUSC2-nanoparticles and gene mutation status

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50 (MK2206 alone)</th>
<th>IC50 (MK2206+TUSC2)</th>
<th>Fold reduction</th>
<th>Braf</th>
<th>EGFR</th>
<th>PI3KCA</th>
<th>LKB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>H322</td>
<td>20.39</td>
<td>1.24</td>
<td>16.4</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>mutant</td>
</tr>
<tr>
<td>HCC366</td>
<td>18.4</td>
<td>2.17</td>
<td>8.5</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>mutant</td>
</tr>
<tr>
<td>H2887</td>
<td>16.53</td>
<td>1.28</td>
<td>12.9</td>
<td>unknown</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>A549</td>
<td>2.86</td>
<td>0.56</td>
<td>5.1</td>
<td>mutant</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
</tr>
</tbody>
</table>

Conclusions:

- Exogenous expression of TUSC2 by nanoparticle-mediated gene transfer sensitized the response of NSCLC cell lines to AKT inhibitor, MK2206.
- TUSC2-increased sensitivity to MK2206 is associated with the down-regulation of AKT and mTOR phosphorylation and up-regulation of AMPK phosphorylation.
- Combination of TUSC2 gene therapy and AKT inhibitor MK2206 may be an efficient treatment strategy for human lung cancer treatment.