Synergistic Antitumor Activity of MK2206 and TUSC2/FUS1-nanoparticle in NSCLC

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Instroduction:

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 , *TUSC2* alone, or empty vector by day 21 (P<0.01 for all three comparisons).

Results:

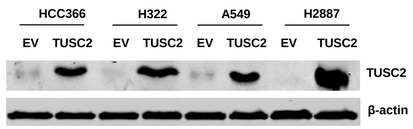

Figure 1. Western blot analysis of expression of TUSC2 in NSCLC cell lines.

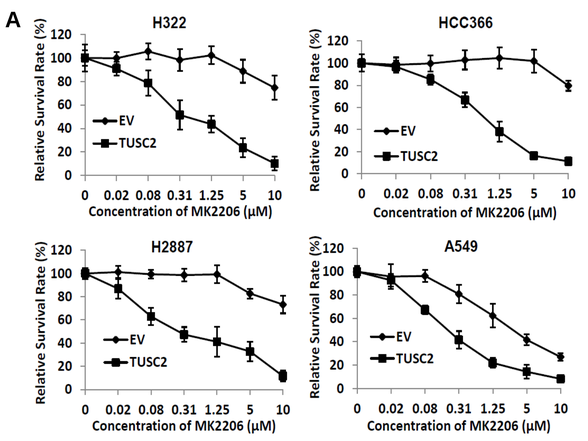

Figure 2. Combined effect of exogenous expression of TUSC2 and MK2206 treatment in various NSCLC cell lines by (A) Cell viability assay and (B) Clonogenic assay.

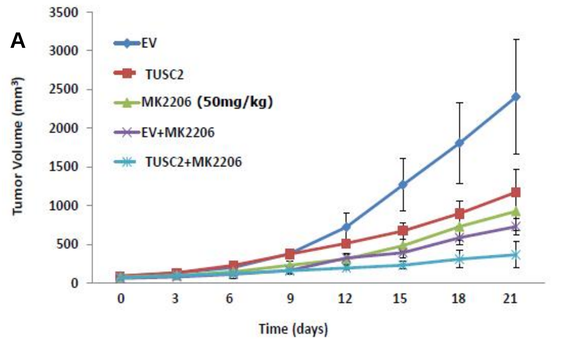

Figure 3. (A) The inhibition effects of TUSC2/MK2206 combination on tumor growth in H322 xenograph mouse model and (B) the pharmacodynamic effects.

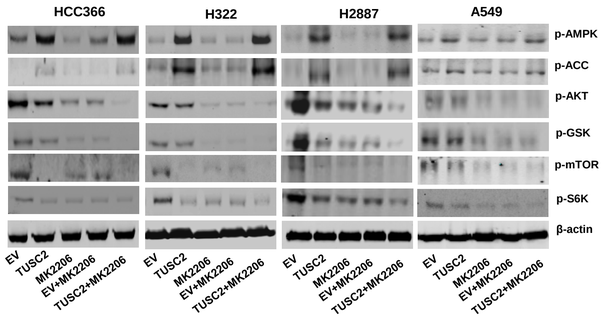

Figure 4. Combination Effect of TUSC2/MK2206 on phosphorylation of AMPK , AKT, mTOR and their direct subtracts.

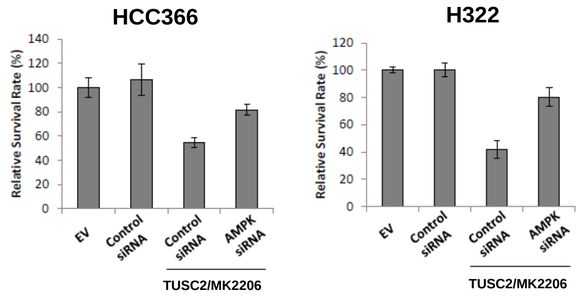

Figure 5. The effect of AMPK-specific small interfering RNA (siRNA) on TUSC2/MK2206-induced cell death.

Table 1. Fold decrease in IC_{50} of MK2206 when combined with TUSC2-nanoparticles and gene mutation status

Cell line	IC_{50} (MK2206 alone)	IC_{50} (MK2206+TUSC2)	Fold reduction	kras	Braf	EGFR	PIK3CA	LKB1
H322	20.39	1.24	16.4	wt	wt	wt	wt	mutant
HCC366	18.4	2.17	8.5	wt	wt	wt	wt	mutant
H2887	16.53	1.28	12.9	mutant	wt	wt	wt	unknown
A549	2.86	0.56	5.1	mutant	wt	wt	wt	mutant

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.