



Background

Mesothelioma is an aggressive cancer that develops from mesothelial cells, the protective cells that cover many internal organs¹. The primary risk factor is asbestos and it takes 30~40 years for mesothelioma to develop after asbestos exposure⁶. During the long developing period, an accumulation of multiple genetic alterations results in a very heterogeneous tumor at the molecular level. Clinical data indicates mesothelioma is resistant to almost all conventional cancer therapies. Chemotherapy combinations for mesothelioma can offer median survival of only one year⁴. The poor therapeutic outcome is due to the lack of predictive biomarkers and variability in tumor biology at the molecular level among the patients. Previous researches suggested that PI3K/AKT/mTOR pathway is constitutively activated in many tumor and identified as an oncogenic driver³. It is an intracellular pathway that plays essential roles in cell function including protein synthesis and growth control. Recent studies indicated genetic variants in this pathway predict the response to chemotherapy of solid tumor and tumor recurrence of head and neck cancer patients³. PI3K/AKT/mTOR pathway also serves as a drug target for cancer treatment. The dual PI3K/mTOR inhibitor PKI-587 has been tested *in vitro*, showing preclinical efficacy in 50 diverse human tumor cell lines². Based on these developments the hypothesis below was formed.

Hypothesis

Genetic variability of the key molecules involved in PI3K/mTOR pathway will predict the response to standard therapy for mesothelioma and serve as predictive biomarkers for using PI3K/mTOR pathway inhibitors as combination therapy with standard treatment. And the dual inhibitor PKI-587 in this pathway can be a promising drug for mesothelioma treatment.

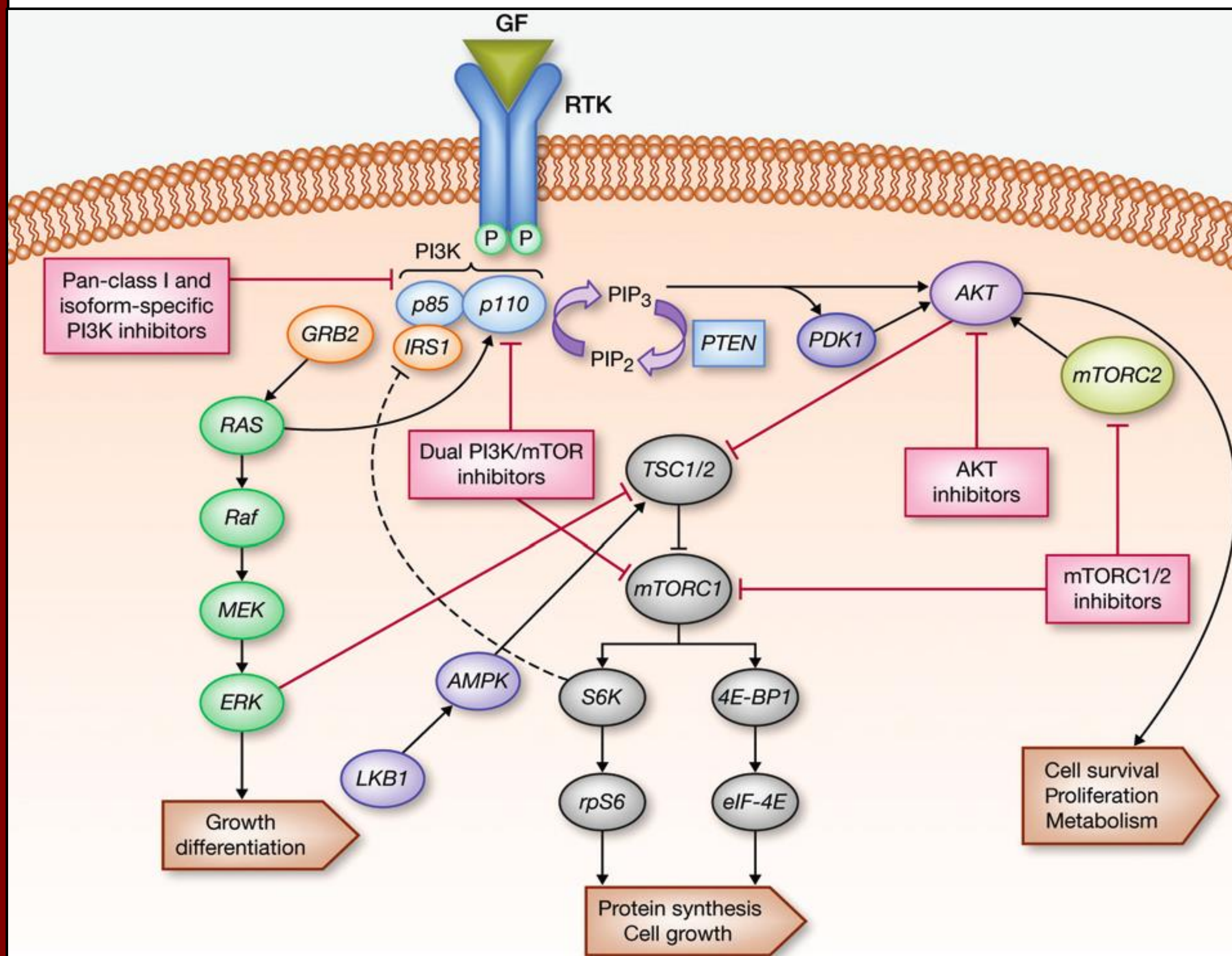


Figure 1: Overview of the PI3K/AKT/mTOR pathway and drug targets. PI3K serves as dual inhibitor, which inhibits both PI3K and mTOR, thus limits the activity of downstream effectors.

Method

- DNA isolation was performed employing QIAamp DNA FFPE tissue kit (cat. No. 56404) from paraffin-embedded tissues of mesothelioma patients.
- SNP selection and analysis: tagging SNPs within each gene (coding region, 5'UTR and 3' UTR) were selected with a minor allele frequency greater than 5% using samples from European ancestry (CEPH) genotyped by the HapMap project.
- Cell culture: H513, H2373, H2461 and H2596 mesothelioma cell lines were maintained on RPMI 1640 media containing 10% calf serum and 1% antibiotics.
- Cell viability assay: Cell viability was measured by cell count kit-8 (CCK-8) after 72 h drug (PKI-587) treatment.
- Western blot: Cells were exposed to 400 nM PKI-587 for 0, 24h, 48h respectively. Antibodies were from Cell signaling Technology. Total and phosphorylation forms of each protein are visualized from western blots.

Result

Table 1: Characteristics of SNPs in the PI3K/AKT/mTOR pathway, which showed the best association between minor allele and survival.

SNP	Gene	Protein	Position	Type of mutation	Minor allele	Minor allele frequency	Hazard ratio (95% CI)	P-value
rs1003857	RPS6KA2	Ribosomal protein S6.K	coding	Nonsense	C	0.21	0.4 (0.2-0.7)	0.004*
rs2230730	RPS6KA2	Ribosomal protein S6.K	coding	Nonsense	G	0.38	1.7 (1.1-2.7)	0.027*
rs2498804	AKT1	AKT1	3'UTR	-	T	0.30	1.8 (1.0-3.3)	0.058
rs1130214	AKT1	AKT1	5'UTR	-	T	0.27	1.3 (0.9-1.8)	0.240

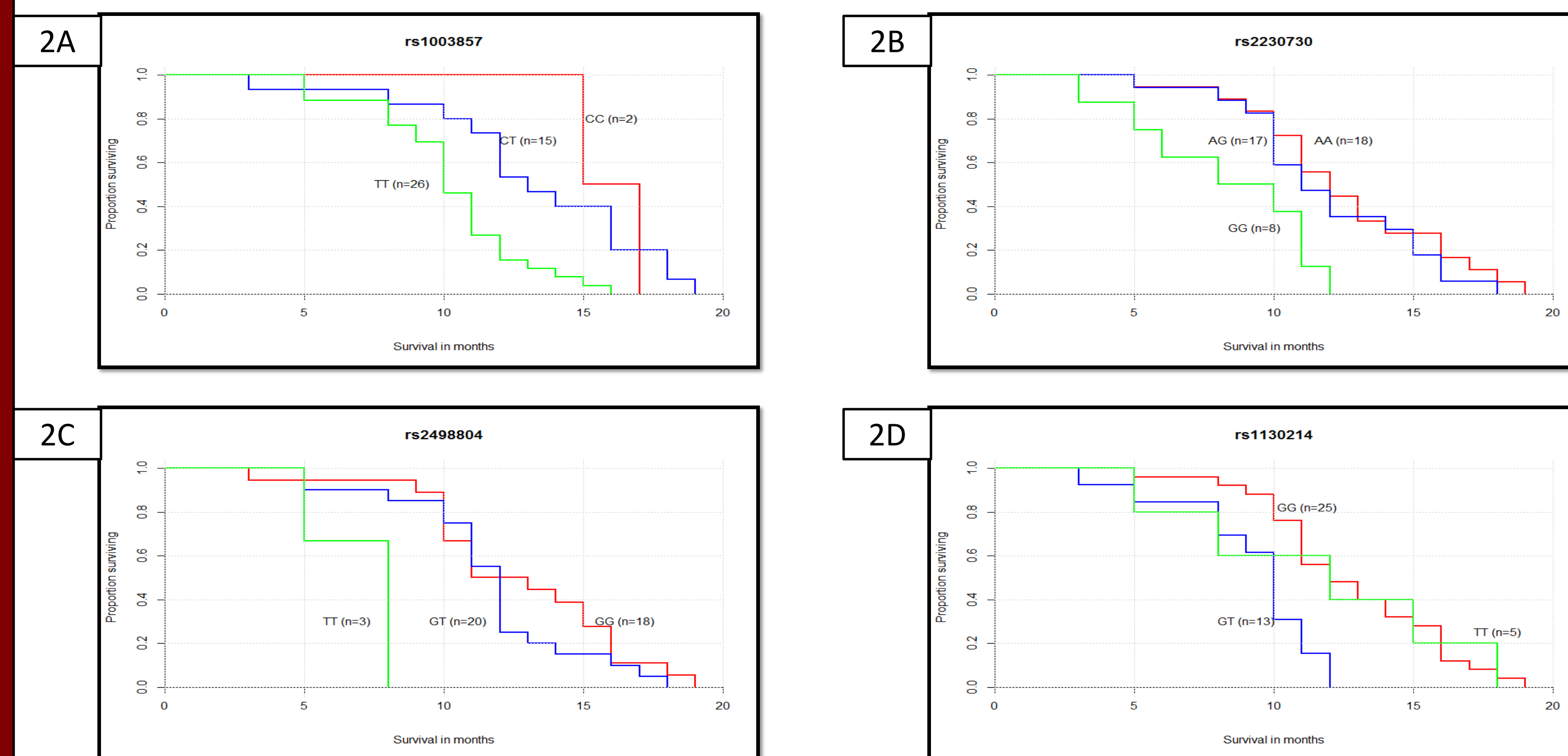


Figure 2: Association analysis between minor allele and survival. SNPs- rs1003857, rs2230730, rs2498804, rs1130214 are associated with overall survival in forty mesothelioma patients under standard treatment.

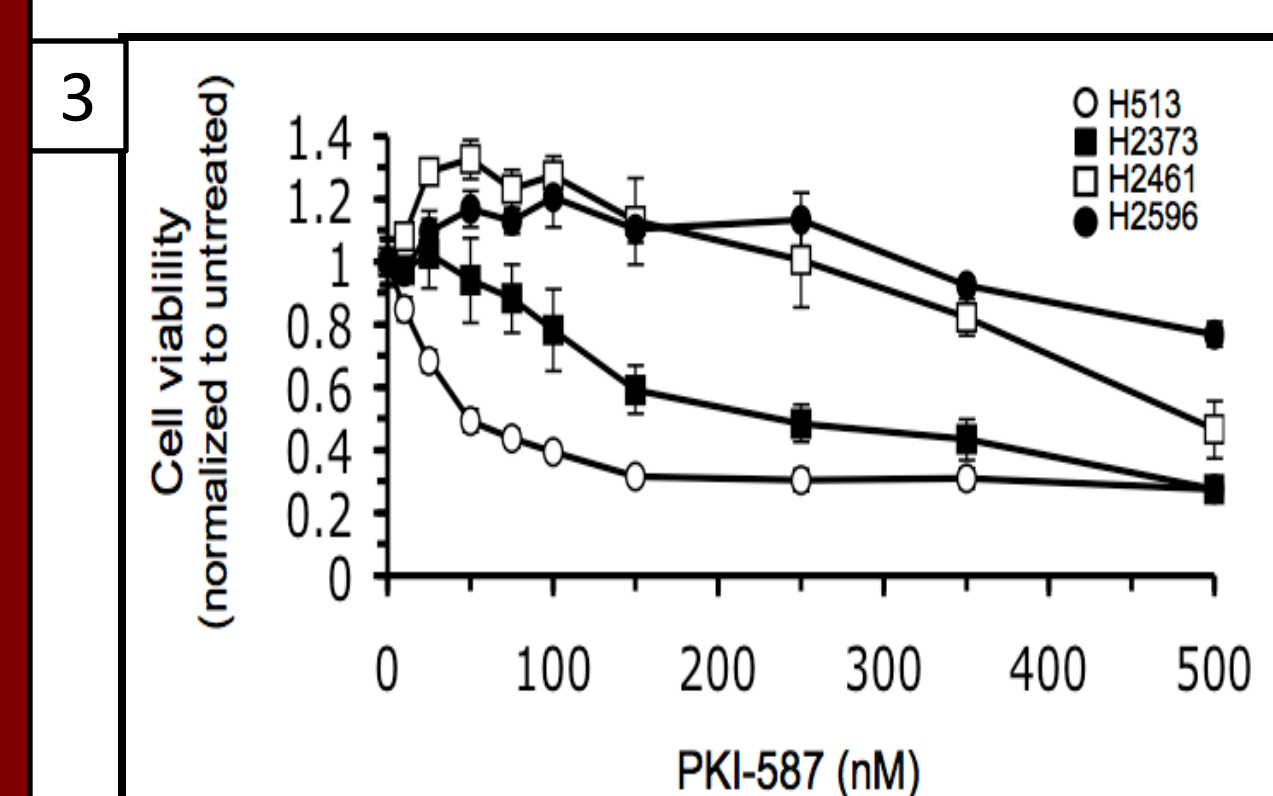
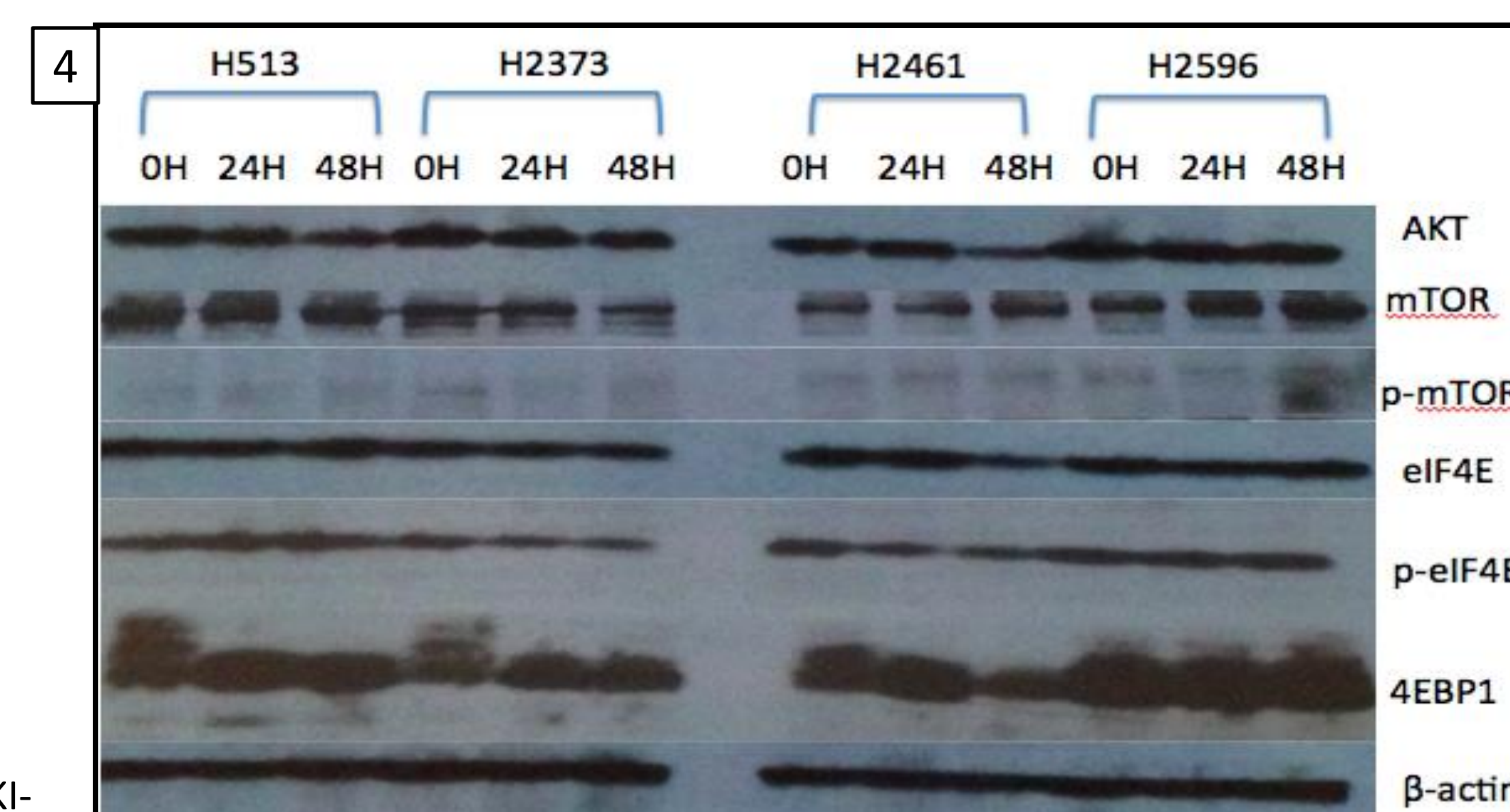


Figure 3: Cell viability for mesothelioma cell lines following PKI-587 treatment.

Figure 4: Phosphorylation status after PKI-587 treatment. Protein phosphorylation changes are revealed after 24, 48 h treatment with 400nM PKI-587 in mesothelioma cells.



Conclusion

- rs1003857, rs1130214, rs2230730 and rs2498804 in the PI3K pathway may serve as potential biomarkers to predict the therapeutic outcomes of mesothelioma patients to standard treatment.
- Rs1003857 (T>C) is significantly associated with overall survival of mesothelioma patients with a hazard ratio 0.4, indicated that patients have a longer survival with at least one minor allele (C). rs1003857 is located in the coding region of RPS6KA2, which encodes ribosomal protein S6 kinase. Even though rs1003857 doesn't alter the amino acid sequence, it may decrease the stability of mRNA or create splicing variants, thus affect the RPS6KA2 expression.
- Rs2230730 (A>G) minor allele is significantly associated with shorter survival with the hazard ratio 1.7. rs2230730 is also located in the coding region of RPS6KA2, however it may increase the stability of mRNA, which leads to overexpression of ribosomal protein S6 kinase.
- Patients with minor allele of rs2498804 (G>T) have a shorter survival based on the hazard ratio 1.8. This SNP is located in 3' UTR of AKT1 gene, which may alter the AKT1 expression by changing the miRNA binding sites and result in the overexpression of AKT protein. Previous research also showed rs2498804 is potential biomarker for brain metastases in non-small cell lung cancer⁸.
- Survival data showed heterozygote patients in rs1130214 have shorter survival than homozygote patients. This SNP is located in 5' UTR of AKT1 gene, which may enhance the binding affinity between promoter and DNA, leading to overexpression of AKT1.
- Based on the hazard ratio, patients with TT form of rs1003857, GG form of rs2230730, TT form of rs249804 and GT form of rs1130214 may need to use PI3K/AKT pathway inhibitor combined with standard treatment to obtain a better clinical outcome in mesothelioma.
- PKI-587 is a promising PI3K pathway inhibitor for mesothelioma patients, which inhibit phosphorylation of 4EBP1 to hinder the cell proliferation.
- Combination therapy with PKI-587 and standard care can be a potential treatment strategy for selected mesothelioma patients, who are supposed to have a worse response to standard care according to the SNPs analysis.

Further direction

- Ribosomal protein S6 kinase and AKT expression level in the mesothelioma patients should be studied.
- The mechanism that these SNPs can alter the gene expression should be further studied.
- Explore the mechanism of resistance to PKI-587 in mesothelioma resistance cell line (H2596).
- Study the combination therapy with other pathway inhibitor (MAPK inhibitor) to overcome the PKI-587 resistance in mesothelioma.

Reference

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