Abstract #138

Novel PAK4 allosteric modulators provide potential therapeutic options in human gastric cancer

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Background

Gastric cancer is one of the leading cancers worldwide with more than 950,000 cases diagnosed and 723,000 deaths in 2012[1]. Expression of PAK4 (p21-activated serine/threonine kinase 4) is associated with gastric tumorigenesis[2]. PAK4 promotes cell proliferation[3] and regulates cell migration[4]. Overexpression of PAK4 is correlated with metastasis of gastric cancer and in part confers cisplatin resistance in gastric cancer patients[5]. Novel PAK4 allosteric modulators (PAMs) were evaluated for their potential therapeutic use for gastric cancer.

Methods

A panel of human gastric cancer cell lines was screened with four PAMs (KPT-9274, -9307, -9331, -8752). MTS and soft agar assay were used to analyze cell growth. Protein expression was detected by Western blot analysis. Wound assay was used to examine effects of PAMs on gastric cancer cell migration. Synergistic effect between PAM and cisplatin was investigated.

Results

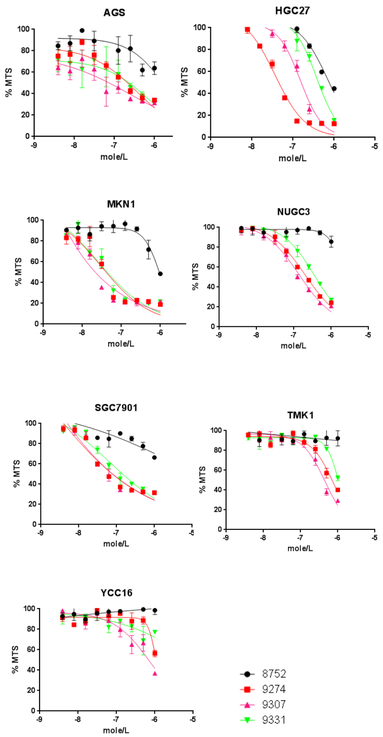


Figure 1. PAMs exhibited anti-tumor activity in gastric cancer cell lines. Seven gastric cancer cell lines were treated with four PAMs (KPT-8752, -9274, -9307, -9331) for 72 hr and cell viability was detected by MTS assay. KPT-9274 was the most growth-inhibitory PAM and 6 cell lines had IC50 < 1 μM.

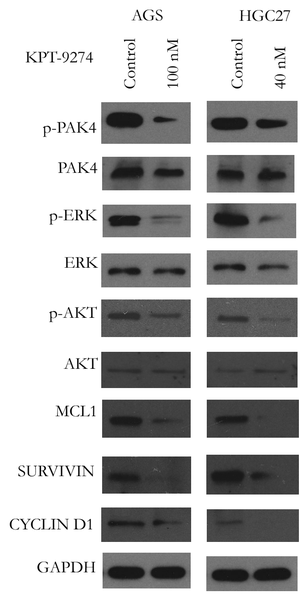


Figure 2. KPT-9274 suppressed PAK4 signaling pathway. Two human gastric cancer cell lines (AGS, HGC27) were treated with KPT-9274 at IC50 for 72 hr. Protein lysates were prepared for Western blot analysis. Treatment of KPT-9274 reduced phosphorylation levels of PAK4 and its signaling to AKT and ERK, regulated apoptosis-related proteins MCL1 and survivin, and reduced cell-cycle protein cyclin D1.

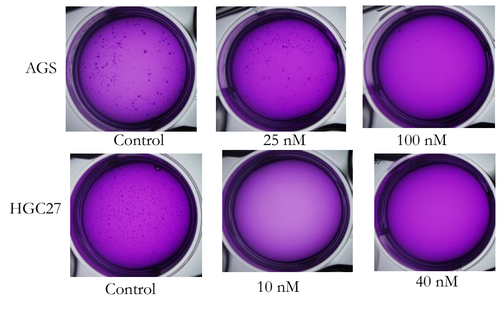


Figure 3. KPT-9274 suppressed anchorage-independent cell growth. Colony formation of human gastric cancer cell lines (AGS, HGC27) were evaluated two weeks after 3,000 cells were seeded on top layer of soft agar with or without KPT-9274. Number of giemsa-stained colonies showed cytotoxic activity of KPT-9274 was dose-dependent.

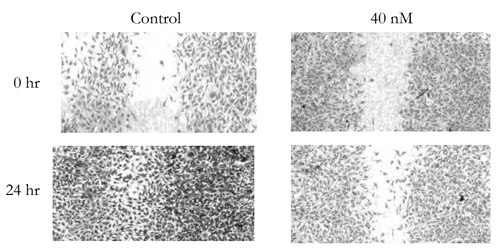
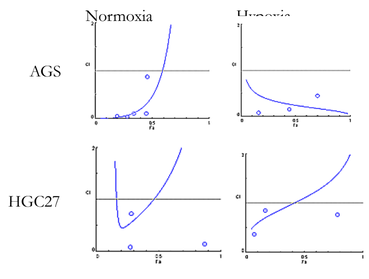


Figure 4. KPT-9274 repressed wound healing of gastric cancer cells. *In vitro* scratch wound healing assay was performed in HGC27 gastric cancer cell line. Impaired wound healing was observed in the cells treated with KPT-9274 (40 nM, 24hr).



KPT-9274 : Cisplatin (1 : 1 , nM)	CI (Normoxia)	CI (Hypoxia)
1000	0.14	2.13
100	21.44	0.77
10	3.88	2.12
1	0.73	0.85
0.1	0.08	0.37

KPT-9274 : Cisplatin (1 : 1 , nM)	CI (Normoxia)	CI (Hypoxia)
1000	0.87	3.66
100	0.11	0.45
10	0.10	0.15
1	0.04	0.08
0.1	0.05	40.2

Figure 5 . Synergistic interaction between KPT-9274 and cisplatin in human gastric cancer cell lines. Two cisplatin-resistant gastric cancer cell lines (AGS, HGC27) were treated with KPT-9274 and cisplatin simultaneously for 72 hr at normoxic (20% O_2) or hypoxic (1% O_2) condition. Combination index (CI) was calculated based on MTS assay. Fa (fraction affected)-CI plots generated with Calcusyn[6] indicated synergy between KPT-9274 and cisplatin in these cell lines . (CI <1 : synergistic; =1: additive; >1: antagonistic).

Conclusions

The PAMs (KPT-9274, -9307, -9331, -8752) exhibited anti-tumor activity in gastric cancer cell lines. KPT-9274 suppressed both anchorage-dependent and -independent cell growth. KPT-9274 induced cell cycle arrest and apoptosis. PAK4 signaling pathway was inhibited by KPT-9274. Gastric cancer cells had impaired wound healing when treated with KPT-9274. Remarkably, KPT-9274 in combination with cisplatin induced cell death in cisplatin-resistant gastric cancer cells in a synergistic manner. This study demonstrates PAMs have anti-tumor activity against gastric cancer cells and suggests their potential use for the treatment of gastric cancer.

References

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