Anti-cancer effects of artesunate in a panel of chemoresistant neuroblastoma cell lines

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Peroxisome proliferator-activated receptor Gamma responsible for TGFβ-induced epithelial mesenchymal transition (EMT) and tumor invasion of NSCLC cells (H460)

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We have previously demonstrated that non-small cell lung cancer cell, H460, had strong drug resistance to TGFβ and can grow and metastasize successfully in animal model. Despite the fact TGFβ can suppress the growth of gastric carcinoma and hepatoma cells, it also promotes the epithelial mesenchymal transition (EMT) and/or metastasis of liver and breast cancers. We therefore were interested to know would TGFβ also promote the EMT and invasion in NSCLC cells (H460) and exacerbate tumor metastasis. TGFβ-triggered signaling pathway, P^{38}/ERK/cPLA$_2\alpha$/COX-2, and its downstream target, PPARγ were also analyzed in H460 with or without TGFβ to link with the induced EMT and/or tumor invasion. According to our early observation, PPARγ was already known to play a critical role in the early development of TGFβ resistance of H460. Our current results showed that TGFβ-induced cell scattering of H460 first appeared at day 3 after the TGFβ treatment followed by a morphological shift (from round to fibroblast or spindle-like shape) at day 7 and 14. The results clearly demonstrated a TGFβ-induced EMT in H460. Seven days after TGFβ treatment, the migration and invasion of H460 were significantly increased in accompany with the induced expression of PPARγ and cell survival. The up-stream regulators (P^{38}, ERK, cPLA$_2\alpha$ and COX-2) of PPARγ were also activated (phosphorylated) by TGFβ at early time points (1−6 h). To further confirm the role of PPARγ in TGFβ-induced EMT and cell invasion in H460, we added PPARγ inhibitor (GW9662) into TGFβ-treated H460 and found that not only survival of H460 was decreased, TGFβ-induced EMT and cell invasion were also interrupted. The results suggested that PPARγ was critical in the protection of H460 from TGFβ-mediated growth inhibition and also promoted TGFβ-induced EMT and cell invasion in H460. In overall, TGFβ-induced EMT and cell invasion in H460 have been confirmed and proved to be PPARγ dependent. Results from the study not only provided information about the drug resistance and metastasis of H460 in response to TGFβ treatment but also implied the therapeutic value of PPARγ inhibitor (GW9662) in the treatment of NSCLCs.

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A novel mitotic kinesin Eg5 inhibitor exerts the growth inhibitory effect on cancer cells in a manner independent of neither Eg5 expression level nor induction of monoastral formation

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Background: Mitotic kinesin Eg5 plays an important role in mitosis, as it is critical for proper bipolar spindle assembly. After the discovery of the first Eg5 inhibitor monastrol, a number of Eg5 inhibitors have been developed as anticancer drugs. We have synthesized a series of S-trityl-L-cysteine (STLC) derivatives as Eg5 inhibitors and showed potent growth inhibitory effect on cancer cells and induction of monoastral formation.
Materials and Methods: Growth inhibitory effects of monastrol and STLC derivatives, Compound1, Compound2 and Compound3 were evaluated by MTT assay in MKN1, MKN45, MKN74, NUGC3, NUGC4, NCI-N87 gastric cancer cell lines, C170, DLD1, HCT15, COLO205 colon cancer cell lines and AsPC1, BxPC3, SUIT2 pancreatic cancer cell lines. Cell cycle analysis and immunocytochemistry were carried out to evaluate the induction of mitotic arrest and monoastral formation, respectively. Expression levels of Eg5, BubR1 and MAD2 were evaluated by western blot for a predictive biomarker study.
Results: Among the derivatives, Compound3 showed the most potent growth inhibitory effect with IC50 s ranging from 0.16 to 0.89 mM except for AsPC3 cells, while monastrol showed almost no effect at concentrations up to 10 mM. Compound3 induced G2/M arrest as early as 8 hours after the treatment at a concentration of IC50. Induction of monoastral formation was modestly observed in BxPC3 and HCT15 cells which are sensitive to, Compound3, while it was also observed in AsPC3 cells which are not sensitive to Compound3 with an IC50 of around 10 mM. There was no correlation observed between the growth inhibitory effect and the Eg5 expression level and no significant change was observed in the BubR1 or MAD2 expression level after the treatment, either.
Conclusions: It is suggested that Compound3 should be considered for further exploration and development and that induction of monoastral formation may not work as a predictive biomarker. Taken together, a novel mitotic kinesin Eg5 inhibitor Compound3 may have other mechanisms of action for its growth inhibitory effect on cancer cells and further investigation on alternative biomarkers is necessary to develop Eg5 inhibitors as an anticancer drug.

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Plumbagin induces ROS-mediated apoptosis in human myeloid leukaemia cells in vivo

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Plumbagin, a naphtoquinone from the roots of Plumbago zeylanica, known to possess anticancer and antibacterial activity. Based on the former finding in vitro, we further investigated the effects of Plumbagin on the growth of human myeloid leukemia NB4 cells that had been transplanted subcutaneously into NOD/SCID mice.
Material and Methods: In order to elucidate the molecular mechanism involved in plumbagin-induced apoptosis. we studied the effect of Plumbagin with IC50 (9 uM) by monitoring the activity of the caspase-3 and caspase-9, the change of mitochondrial membrane potential ($\Delta\Psi$m), the expression of the Bcl-2 family as well as ROS change in plumbagin-induced apoptosis. The efficacy of Plumbagin in vivo was evaluated with intraperitoneal injection of plumbagin (2 mg/kg body weight) daily for three weeks using subcutaneous NB4 xenograft in NOD/SCID mice, comparing with the vehicle and Doxorubicin (1 mg/kg thrice a week). The tissue sections were applied to hematoxylin and eosin histological staining as well as TUNEL assay.
Results: We revealed that plumbagin triggered the mitochondrial apoptotic pathway, as indicated by the increase in Bax/Bcl-2 ratios, resulting in mitochondrial membrane potential loss, and corresponding caspase activation. We also found that the generation of ROS was a critical mediator in plumbagin-induced cell apoptosis, which would be abrogated completely by the antioxidant, NAC. Furthermore, compared with the control, Plumbagin presented a ~60% reduction of tumor volume and marked increase in tumor apoptosis; There was no overt manifestation of toxicity such as weight loss, tissue damage and behavior change as showed in Doxorubicin -treated mice.
Conclusion: Our data support that Plumbagin has potential as a novel therapeutic agent for myeloid leukemia with minimal side-effects.

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Anti-cancer effects of artesunate in a panel of chemoresistant neuroblastoma cell lines

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Background: Artemisinin derivatives are well-tolerated anti-malaria drugs that also exert anti-cancer activity. Here, we investigated artemisinin and its derivatives dihydroartemisinin and artesunate in a panel of chemosensitive and chemoresistant human neuroblastoma cells as well as in primary neuroblastoma cultures.
Materials and Methods: Cell viability was determined by MTT assay or by determination of ATPase activity. Apoptosis was examined by staining for activated caspase-3 and detection of cells with low DNA content (sub-G1) by flow cytometry. Bioinformatic analysis of gene microarray data was used to identify genes relevant fr neuroblastoma cell response to artesunate.
Results: Only dihydroartemisinin and artesunate affected neuroblastoma cell viability with artesunate being more active. Of 16 cell lines and two primary cultures, only UKF-NB-3^{r}CDDP^{1000} showed low sensitivity to artesunate. Artesunate induced apoptosis and reactive oxygen species in neuroblastoma cells. L-Buthionine-S,R-sulfoximine, an inhibitor of GCL (glutamate-cysteine ligase), resensitised in part UKF-NB-3^{r}CDDP^{1000} cells to artesunate. This finding together with bioinformatic analysis of expression of genes involved in glutathione metabolism showed that this pathway is involved in artesunate resistance.
Conclusion: These data indicate that neuroblastoma represents a artesunate-sensitive cancer entity including chemoresistant cells. Characteristic gene expression signatures based on a previous analysis of artesunate resistance in the NCI60 cell line panel clearly separated UKF-NB-3^{r}CDDP^{1000} from the other cell lines.