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 tumor invasion. TGFβ-triggered signaling pathway, p38/ERK/PLA2, and its downstream target, PPARG, 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 PPARG and cell survival. The up-stream regulators (p38, ERK, pPLA2α/COX-2) of PPARG were also activated (phosphorylated) by TGFβ at early time points (1−6 h). To further confirm the role of PPARG in TGFβ-induced EMT and cell invasion in H460, we added PPARG 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 PPARG 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 PPARG 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 PPARG inhibitor (GW9662) in the treatment of NSCLCs.

Materials and Methods:

Growth inhibitory effects of monastrol and STLC expression of EMT and invasion were determined by MTT assay in MKN1, MKN45, MKN74, NUGC3, NUGC4, NCI-N87 gastric cancer cell lines.

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, LDL1, 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 ranging from 0.16 to 0.89 μM except for AsPC3 cells, while monastrol showed almost no effect at concentrations up to 10 μM. 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.