Preclinical study on the efficacy of Panobinostat (LBH589) in hepatocellular carcinoma (HCC)

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Background

Aberrant regulation of histone deacetylases (HDACs) is known to play a pivotal role in HCC pathogenesis as well as other human malignancies. Panobinostat (LBH589) is a pan-HDAC inhibitor covering a wide range of HDACs (Class I, II and IV) with high inhibitory activity at nanomolar concentration. It has been approved by FDA for treating multiple myeloma and has demonstrated promising anti-proliferative and cytotoxic activity in breast, prostate, colon and pancreatic cancer cell lines. This study investigated *in vitro* and *in vivo* effect of Panobinostat in HCC cell lines.

Methods

Basal expressions of HR23B and HDACs of 7 HCC cell lines (HepG2, PLC/PRF/5, Huh-7, Hep3B, SNU-182, SNU-398 and SNU-449) were determined by western blotting. Their corresponding IC_{50} for 24, 48 and 72 hours towards Panobinostat were determined by cell viability assay. Huh-7, Hep3B and SNU-449 were selected for further *in vitro* experiments. Their cell cycle distribution after Panobinostat treatment was evaluated by flow cytometry. Apoptosis was detected by Cell Death Detection ELISA. Huh-7 and Hep3B xenograft model were used for *in vivo* investigation. Cells were inoculated subcutaneously into the flanks of 3-4 week old male athymic nude mice. When tumors were established, Panobinostat was administrated intraperitoneally at 7.5mg/kg and 15mg/kg five days per week for 2 weeks.

Results

All cell lines were able to achieve nearly 100% growth inhibition and had displayed a dose- and time-dependent manner towards Panobinostat. Maximum growth inhibition was 20-70% at 24hr compared to over 90% at 72hr. There was significant reduction in cell viability at low nanomolar concentrations (IC_{50} at 48hr: HepG2=8.81±0.72nM, PLC/PRF/5=18.9±0.74nM, Huh-7=14.01±1.12nM, Hep3B=25.00±3.69nM, SNU-182=73.33±15.52nM, SNU-398=12.86±3.25nM, SNU-449=73.01±9.09nM). Flow cytometry analysis showed Panobinostat induced accumulation of cells at G0/G1 phase in Huh-7 and SNU-449. Meanwhile, an increase in sub G1 population was detected in Hep3B after exposure to 25nM Panobinostat for 48h. Apoptotic induction was further confirmed by cell death detection ELISA and western blotting. Panobinostat promoted apoptosis more remarkable in Hep3B than other 2 cell lines as evidenced by a stronger cleaved PARP expression level. Panobinostat treatment delayed tumor growth in Hep3B ($p<0.0005$) and Huh-7 ($p<0.0005$) xenografts compared to vehicle control. The overall weight loss was less than 20% despite of greater drop during middle of the treatment.

Conclusion

Panobinostat has been demonstrated to inhibit *in vitro* and *in vivo* HCC cell growth. Further study on the mechanism behind Panobinostat sensitivity is warranted.