Oncopig and Human Hepatocellular Carcinoma Cell Lines Exhibit Similar Response to Liver Cancer Chemotherapy Agents

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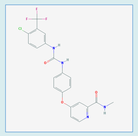
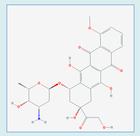
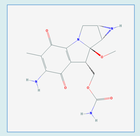
Introduction

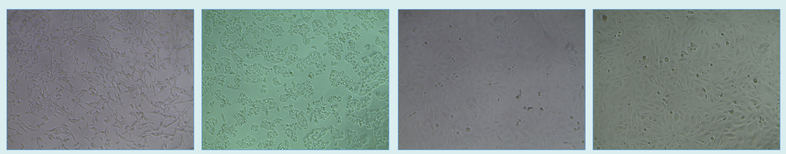
- Many therapeutic agents showing promise in mouse studies fail to translate into successful human clinical trials
- Pigs share many genetic, physiological, & metabolic characteristics with humans
- The Oncopig Cancer Model (OCM) is a novel, inducible large animal model to study human cancer & bridge the preclinical gap
- The OCM has Cre-inducible porcine transgenes encoding *KRAS*^{G12D} & *TP53*^{R167H}, which represent a commonly mutated oncogene & tumor suppressor in human cancers

Do porcine HCC (pHCC) & human HCC cell lines share similar drug responses to commonly used chemotherapy agents?

Methods

- pHCC cell line produced from pig liver resection, hepatocyte isolation & AdCre transformation
- Commonly used chemotherapy agents added to pHCC & human HCC cell lines

Sorafenib	Doxorubicin	Mitomycin C	Cisplatin
			
Cytostatic anti-angiogenic agent	Cytotoxic DNA intercalating agent	Cytotoxic DNA cross-linking agent	Cytotoxic DNA cross-linking agent



pHCC | HepG2 | SNU-387 | SNU-475

- MTT assay—which assesses oxidoreductase enzymatic activity & reflects the number of viable cells—performed at 0, 24, 48, & 72 h

Add MTT to cells → MTT converted to insoluble Formazan in live cells → Solubilizing agent allows for absorbance readout at 570 nm

Sorafenib is cytostatic at clinically relevant concentrations

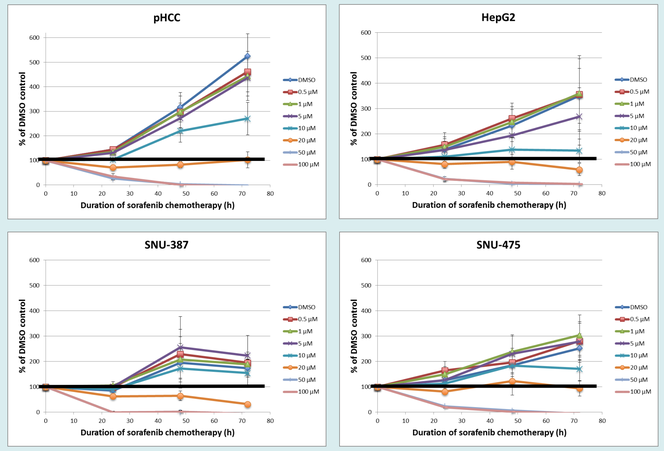


Figure 1. Cell growth in the presence of increasing concentrations of sorafenib, when compared to number of cells seeded at time 0 h (100%), after 24, 48 & 72 h, as determined by an MTT assay. Negative control was 1% DMSO. n = 3; error bars are S.E. Clinical relevant concentration = 2-10 µg/mL = 3-15 µM

Doxorubicin leads to comparable IC_{50} in pHCC, HepG2, & SNU-387

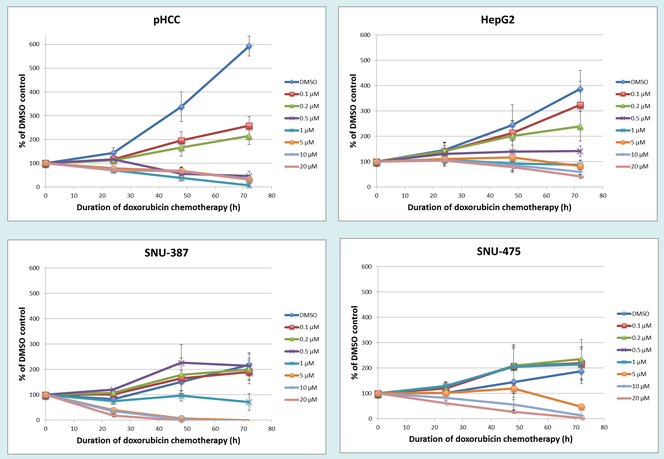


Figure 2. Cell growth in the presence of increasing concentrations of doxorubicin, when compared to number of cells seeded at time 0 h (100%), after 24, 48 & 72 h, as determined by an MTT assay. Negative control was 1% DMSO; n = 3; error bars are S.E. Clinical relevant concentration = 0.6-2.9 µg/mL = 1-5 µM

Table 1. Half maximal inhibitory concentration (IC_{50}) after 72 h exposure to doxorubicin. MTT assay results at 72 h were normalized to DMSO only control (100%); a trend line was fitted to the results & line equations determined; IC_{50} corresponds to 50% growth; n = 3

Cell Line	IC_{50} Dox (µM)
pHCC	0.19
HepG2	0.45
SNU-387	0.94
SNU-475	3.31

Mitomycin C has similar IC_{50} in all tested lines

Effect of Cisplatin in pHCC & HepG2 is similar

Table 2. Half maximal inhibitory concentration (IC_{50}) after 72 h exposure to mitomycin C or cisplatin. MTT assay results at 72 h were normalized to DMSO (MMC) or media (cis-Pt) only control (100%); a trend line was fitted to the results & line equations determined; IC_{50} corresponds to 50% growth; n = 3

Cell Line	IC_{50} MMC (µM)	IC_{50} cis-Pt (µM)
pHCC	1.77	7.54
HepG2	1.73	8.34
SNU-387	7.91	25.89
SNU-475	2.93	16.57

Chemotherapy response is consistent across different pHCC cell lines

Table 3. Cytostatic sorafenib concentrations & doxorubicin half maximal inhibitory concentration (IC_{50}) after 72 h exposure to these agents across 6 distinct OCM pHCC cell lines derived from different animals. MTT assay results at 72 h were normalized to DMSO only control (100%); P > 0.05 for all comparisons; n = 3

Cell Line	Sorafenib inhibitory concentration (µM)	IC_{50} Dox (µM)
$pHCC_1$	10	0.15
$pHCC_2$	10-20	0.14
$pHCC_3$	10	0.18
$pHCC_4$	10-20	0.18
$pHCC_5$	10-20	0.18
$pHCC_6$	10	0.16

Conclusions & Future Work

- pHCC & human HCC lines display comparable responses to the tested chemotherapy agents, suggesting that the OCM can be used to screen promising chemotherapy agents
- pHCC responses are most similar to HepG2, which is among the most widely used HCC cell lines
- Observed differences may be explained by different mechanisms of action across compounds & genetic differences among human cell lines
- OCM offers benefit of *in vitro* screening to *in vivo* testing, & future work may aim to determine if the OCM is more accurate than other platforms (e.g. mouse) in predicting clinical trial success

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