

## Targeting HIF1 and mTOR expression in normoxic and hypoxic liver cells: towards optimal combination therapy for DEB TACE.

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Transarterial chemoembolisation (TACE) is the treatment of choice for patients with intermediate stage hepatocellular carcinoma. Drug eluting beads (DEB) provide a one-step therapy for occlusion of tumour feeding arteries and sustained local release of chemotherapeutic agents. Whilst embolisation causes cell death by starvation of oxygen and nutrients, an oxygen gradient within the tumour ensues. Under hypoxic conditions, tumour cells activate pro-survival mechanisms which upregulate genes that promote adaptive responses, resulting in a more malignant phenotype and the induction of drug resistance. Central to this response is the transcription factor hypoxia-inducible factor 1 (HIF-1).

**Objectives:** This study investigated the effects of doxorubicin on the viability of HepG2 (human liver cancer) cells in normoxic and hypoxic conditions with respect to expression of HIF-1 $\alpha$  and other pivotal targets. The mTOR inhibitor rapamycin was then investigated. Finally, these agents were studied singly and in combination using DEB in an ectopic HepG2 xenograft mouse model of liver cancer.

**Methods:** *In vitro*, HepG2 cells were grown under normoxic (21% oxygen) and hypoxic (1% oxygen) conditions in the presence of rapamycin, doxorubicin or both in combination. HIF-1 $\alpha$  expression was determined by Western blot. *In vivo* the anti-tumoural effects of dox-loaded DC Beads™ in combination with low dose oral rapamycin (1mg/kg/day) was studied by implanting HepG2 cells into NMRI:nu/nu adult female mice. Doxorubicin-loaded beads were applied adjacent to the tumour by direct injection. Tumour growth was assessed twice weekly by measuring dimensions from 23 to 45 days post-implantation.

**Results:** *In vitro*. 10nM rapamycin inhibited cell proliferation under normoxic but not hypoxic conditions, with 100nM rapamycin proving less effective. 10nM rapamycin significantly inhibited HIF-1 $\alpha$  expression under hypoxia, and was as effective as 100nM. Doxorubicin inhibited cell proliferation in both normoxic and hypoxic conditions, although some resistance was observed under hypoxia. 10 $\mu$ M was observed to be more effective than higher concentrations. Doxorubicin significantly inhibited protein expression of HIF-1 $\alpha$  in a dose dependent manner. Addition of 10nM rapamycin with doxorubicin increased the inhibition of cell proliferation in both normoxia and hypoxia. *In vivo*, doxorubicin-loaded beads in combination with oral rapamycin was found to be the most effective treatment for reducing tumour volume.

**Conclusion:** Our *in vitro* and *in vivo* data both suggest that a combination of doxorubicin-loaded beads with low dose oral rapamycin would make an effective candidate for future DEB-TACE.