TEMSIROLIMUS IS EFFECTIVE AGAINST PATIENT-DERIVED METASTATIC FIBROLAMELLAR HEPATOCELLULAR CARCINOMA IN VITRO AND IN VIVO

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Background: Fibrolamellar hepatocellular carcinoma (FL-HCC) is a rare and distinct subtype of HCC typically limited to children and young adults. Surgical resection is the only curative modality, however most patients succumb to distant metastasis despite curative intent resection with an overall survival of only 30-45% at five years. There are no recommendations for systemic therapeutic options given its rarity and lack of data. Pre-clinical patient-derived models are critical in order to assess efficacy of treatment regimens, particularly to metastatic disease.

Objective: Our aim was to assess treatment efficacy in vitro and in vivo using patient-derived metastatic FL-HCC tumor tissue.

Methods: With IRB and IACUC approval, resected patient metastatic FL-HCC tumor was obtained. A portion of the tumor tissue was dissociated and cells were treated with a variety of chemotherapeutic regimens. Cell viability was determined after 72-hours. Immunoblots were performed to assess levels of proteins of interest in the primary tumor tissue. Patient-derived xenografts (PDX) were generated by implanting patient metastatic FL-HCC tumor tissue into immunodeficient mice. Mice were treated with vehicle or specified chemotherapeutic regimen for 29 days after which they were sacrificed and the tumors were harvested. Treated tumors were again assessed by immunoblot for proteins of interest, as well as proteins associated with autophagy and apoptosis.

Results: Human FL-HCC cells were treated with a multitude of chemotherapeutic regimens and three were found to significantly reduce cell viability in vivo: Gemcitabine + Oxaliplatin, FOLFIRINOX, and temsirolimus (Figure 1a). Given the role of temsirolimus as an mTOR inhibitor, expression of total and phosphorylated mTOR was assessed by immunoblot in four different patient samples. Phosphorylated mTOR was found to be greatly increased in metastatic tissue when compared to the primary tumor, as well as to normal liver tissue (Figure 1b). To test the efficacy of these three treatment regimens in vivo, PDX models were generated from metastatic FL-HCC tissue. Mice were treated with vehicle, Gemcitabine + Oxaliplatin, FOLFIRINOX, or single-agent temsirolimus. After 29 days of treatment, mice treated with temsirolimus and FOLFIRINOX both showed a significant reduction in tumor growth when compared to the vehicle group (Figure 1c). One mouse in the FOLFIRINOX group died with the others having significant weight loss which was not seen in the temsirolimus group. Immunoblots of the treated tumors showed a reduction in phosphorylated mTOR expression in the tumors treated with temsirolimus (Figure 1d). Tumors from the temsirolimus and the FOLFIRINOX group also showed increased expression of LC3 II, a marker of autophagy, as well as cleaved PARP, cleaved caspase-9, and cleaved caspase-3, all markers of apoptosis (Figure 1d).

Conclusion: Recurrent metastatic disease is a significant cause of death in patients with FL-HCC and there are currently no recommended systemic therapies. We have shown an increased activation of mTOR in these metastatic lesions with mTOR inhibition showing
significant efficacy both in vitro and in vivo. This treatment was as effective as the FOLFIRINOX regimen without the significant treatment side effects. Additional investigation is needed into the role the mTOR pathway plays in the pathogenesis of metastatic FL-HCC.

Figure 1: Treatment of metastatic fibrolamellar hepatocellular carcinoma in vitro and in vivo. In vitro cell viability after 72 hours of treatment with three different chemotherapeutic regimens (A). Total and phosphorylated mTOR expression in samples from four different patients. PRKACA-DNAJB1 used to validate FL-HCC tissue and actin used as loading control (B). Change in tumor volume after 29 days of treatment in patient-derived xenografts of metastatic FL-HCC (C). Immunoblot of treated tumor tissue. Actin used as loading control (D).
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