Conclusion: Intrarterial injection chemotherapy using WOW emulsion will be applied to the first line treatment for HCC.

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P-081 DYSREGULATION OF THE LYSOSOMAL COMPARTMENT WITH VERTEPORFIN POTENTIATES THE ANTI-TUMOR EFFECT OF SORAFENIB IN HEPATOCELLULAR CARCINOMA

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Introduction: Hepatocellular carcinoma (HCC) is one of the most common malignant cancers worldwide and the FDA-approved oral multikinase inhibitor Sorafenib (SF) is still the first-line treatment for patients with advanced HCC. Alteration in the autophagic flux and in lysosomal compartment are often linked to carcinogenesis and chemoresistance development.

Methods: Here we study the effect of the photosensitizer Verteporfin (VP), without prior light activation, by using in vitro and in vivo translational preclinical models of patient-derived HCC samples, focusing particularly on VP mechanism of action alone and in combination with SF.

Results: Combining in vitro VP with SF led to a synergistic (HuH7) and additive (HepG2) reduction of tumor cell proliferation. Similar results have been obtained by treating 3D HCC patient-derived tumoroids (PDT). Interestingly, VP/SF treatment strongly decreased SF-induced autophagic flux, which was distinctly and differently activated between the two HCC cell lines tested and the PDT. Immunoblot analysis showed that VP inhibits the formation of newly forming autophagosomes (LC3-I) and induces an accumulation of high-molecular weight complexes of proteins (HMW-p62). Furthermore, VP specifically targets lysosomes, increasing their number, altering their shape, size and inducing a strong alkalinization of their intraluminal pH (assessed by flow cytometry), which finally leads to lysosomal membrane permeabilization (LMP) and cell death. VP-induced lysosomal compartment instability synergistically contrasts the inert lysosomotropism of SF, which gives rise to its drug-resistance development. Similar results were obtained in a subcutaneous HCC-cell line and patient-derived xenograft (PDX) mouse model. Here VP potentiated the antitumor effect of SF, by synergistically decreasing tumor cell proliferation (Ki67), tumor angiogenesis (CD31) and the SF-induce autophagic flux, followed by a decreased accumulation of lysosomes (LAMP-1).

Conclusion: Taken together, these findings suggest that VP, without prior light activation, can significantly potentiate the anti-tumor effect of SF in a translational HCC preclinical model. Verteporfin inhibits the formation of newly forming autophagososmes and - by specifically targeting the lysosomal compartment stability - concurrently induces their dysregulation and permeabilization. Targeting these pathways with Verteporfin, might constitute a novel strategy to overcome chemoresistance development and to improve cancer therapy in the setting of advanced HCC.

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P-082 VEGFR-2, HSP90 AND GRP78/BIP EXPRESSION AND HCC RECURRENCE AFTER LIVER TRANSPLANTATION

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Introduction: Liver transplantation (LT) for hepatocellular carcinoma (HCC) is a satisfactory therapeutic choice in patients with "early HCC" selected according to Milan criteria. However, the risk of HCC recurrence after LT is about 7-20% at five years and molecular markers which can predict recurrence are still lacking. We investigated in HCC samples and LC surrounding tissues the significance of VEGFR-2, HSP90, and GRP78/BiP expression in patients with HCC who underwent LT in a western transplantation center and their possible role as molecular markers of recurrence.

Methods: 42 patients (35 M, 7 F) with early HCC who underwent LT between 2012 and 2013 were enrolled. The tumor recurrence rate was analyzed after a minimum follow-up of 36 months. Immunohistochemical expression of VEGFR-2, HSP90, and GRP78/BiP in HCC tissues and LC surrounding tissues, was correlated with clinicopathological variables and recurrence-free post-LT survival. At statistical analysis t Student's or Mann Whitney U test were used where appropriate. To assess which variable measured at baseline was predictive of HCC recurrence, the univariate Cox proportional hazards model (Hr) was fitted to each variable. All variables with a $P<0.05$ underwent multivariate analysis to assess their value as independent predictors.

Results: Microvascular invasion was observed in 23 of 42 evaluated patients (45.2%). The expression levels of GRP78, HSP90 and VEGFR-2 in the tumor tissue (TT) compared to non-tumor surrounding tissue (NT), analyzed by the Mann Whitney U test detected significantly higher values of expression for HSP90 and VEGFR-2 in the TT compared to NT ($p<0.0001$ and $p<0.001$, respectively). Moreover, the correlation between the expression of these molecules and the presence of microvascular invasion was analyzed through a multiple logistic regression analysis and the presence of VEGFR-2 overexpression in tumor tissues positively correlated with the presence of microvascular invasion in HCC ($p < 0.03$). Finally, on uni- and multivariate analyses the only parameters which were related to the presence of post LT recurrence were MELD score and microvascular invasion ($p < 0.02$ and $p < 0.04$, respectively).

Conclusion: In conclusion, the finding that over-expression of HSP90 and VEGFR-2 in the tumor compared to non-tumor surrounding HCC samples confirm the likely involvement of these molecules in liver carcinogenesis, and particularly of VEGFR-2 in neoplastic angiogenesis, however these results need to be extended to a wider population, to be used as markers of HCC recurrence after LT in addition to traditional ones like MELD score and microvascular invasion.

Disclosure of Interest: None Declared

P-083 STUMBLING BLOCKS FOR SORAFENIB IN THE TREATMENT OF ADVANCED-STAGE HEPATOCELLULAR CARCINOMA

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Introduction: Based on two phase III trials that reveal an overall survival benefit of three months compared to placebo[1,2], sorafenib represents the current standard of care for patients with advanced stage hepatocellular carcinoma (HCC). However, its use is hampered by the frequent occurrence of drug resistance and up to 80% of patients treated with sorafenib suffer from side effects necessitating therapy interruption or termination[3]. In order to improve sorafenib therapy, the aim of the study was to elucidate the molecular basis for this drug resistance. In addition, we studied the effect of sorafenib withdrawal as rebound phenomena have been reported in the context of anti-anglogenic therapy abrogation[4].

Methods: To investigate the molecular mechanisms involved in sorafenib resistance we developed a sorafenib resistant human liver cancer cell line in which we studied morphology, protein expression as well as proliferative and migratory potential under sorafenib exposure and withdrawal conditions. The effects of sustained sorafenib exposure were additionally investigated at the protein and lipid level using a mass spectrometry-based proteomics and lipidomics approach as well as metabolic parameters by a glycolytic stress test.

Results: The sorafenib resistant human liver cancer cells changed their morphology to elongated spindle-shaped cells, lost E-Cadherin and showed high expression of N-Cadherin and Vimentin, indicating epithelial-to-mesenchymal transition with altered migratory potential. Further they showed increased apoptosis, a G1-phase cell cycle arrest and evasive PI3K (phosphatidylinositol 3-kinase)/Akt pathway activation. Remarkably, following withdrawal of sorafenib, the resistant cells undergo rebound growth proliferation, a phenomenon discussed to occur in patients. A whole-cell proteomics screen revealed on the one hand decreased receptor and cell cycle activity of the sorafenib-resistant cells compared to their rebound analogues and their parental HCC cell line. On the other hand major alterations of metabolic processes especially lipid synthesis were observed. As the sorafenib-resistant cells obtain broad cross-resistance to the chemotherapeutics commonly used in the clinic, this cell model was used to investigate potential alternative, combination or second-line therapies.

Conclusion: Our work demonstrated that several mechanisms are involved in the acquired resistance to sorafenib, such as crosstalks involving the PI3K/Akt pathway and epithelial-to-mesenchymal transition. Therefore, acquired sorafenib-resistance not only leads to a generalized chemotherapeutic treatment failure, but also accelerates tumor progression after therapy termination. This rapid tumor growth rebound influences the clinical application of sorafenib with regard to dosing schedules and presurgical intervention strategies.

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