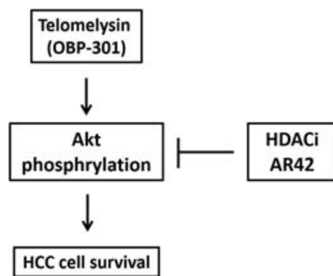
receptor (CAR) on cancer cell membrane was measured by flowcytometry and Western blotting. Human telomerase reverse transcriptase (h*TERT*) was assessed by RT-PCR. The activation of Akt and other signaling pathways were measured by Western blotting. HCC cells with constitutive overexpression of Akt (PLC5-Akt and Hep3B-Akt) were cloned for verification of molecular mechanisms.

Results: Combination of telomelysin and AR42 exhibits synergistic anti-proliferative effects against PLC5 and Hep3B cells. Apoptosis (sub-G1 fractions, cleavage of PARP) induced by telomelysin were significantly enhanced by AR42 in both HCC cells. Expression of CAR and h*TERT*, which may be positively associated with cytotoxicity to telomelysin, was paradoxically attenuated by AR42 treatment. Instead, we found that telomelysin enhanced Akt phosphorylation in HCC cells. Combination treatment of AR42 abolished the telomelysin-induced phospho-Akt activation and enhanced telomelysin-induced apoptosis. The synergistic interaction of telomelysin and AR42 were consistently reversed in HCC cells with constitutive overexpression of Akt.



Conclusions: The anti-HCC efficacy of telomelysin can be facilitated by a histone deacetylase inhibitor AR42 through the inhibition of telomelysin-induced Akt phosphorylation.

SAT-131

AKT inhibitor ARQ 092 and sorafenib additively inhibit progression of hepatocellular carcinoma and improve immune system in cirrhotic rat model

Z.M. Jilkova[1,2], A.Z. Kuyucu[1,2,3], K. Kurma[1,2], S.T.A. Pour[1,2], G.S. Roth[1,2,4], G. Abbadessa[5], Y. Yu[5], V. Leroy[1,2,4], P. Marche[1,2], T. Decaens[1,2,4]. [1]*Université Grenoble Alpes;* [2]*Institute for Advanced Biosciences, Grenoble, France;* [3]*Department of Bioengineering, Izmir Institute of Technology, Izmir, Turkey;* [4]*Clinique Universitaire d'Hépato-gastroentérologie, Grenoble, France;* [5]*ArQule Inc, Woburn, Massachusetts, United States*
E-mail: zuzana.macek-jilkova@inserm.fr

Background and Aims: Hepatocellular carcinoma (HCC) is often diagnosed at advanced stages with limited number of therapeutic options. Longer exposure to classical treatment of advanced HCC, sorafenib, often over-activates AKT pathway, leading to HCC resistance. Moreover, AKT pathway itself is activated in almost half of HCC cases. Therefore, we investigated the efficacy of combination of Sorafenib with allosteric Akt inhibitor ARQ 092 in a DEN-induced cirrhotic rat model with HCC.

Methods: 28 rats were diethylnitrosamine-injured during 14 weeks to obtain cirrhosis with fully developed HCC, then randomized into 4 groups (control, sorafenib, ARQ 092 or combination of ARQ 092 + sorafenib; n = 7/group) and treated for 6 weeks. Tumor progression was followed by MRI every 3 weeks. Pathological analysis and immunohistochemistry were blindly analysed. Flow cytometry analyses, RT-PCR, and western-blot were performed.

Results: Tumor progression was significantly reduced by combination treatment (53%) compared to the control (158%; $p < 0.0001$) sorafenib group (106%; $p = 0.006$) or ARQ 092 group (105%; $p = 0.010$). Mean number of tumors was lower in combination group ($n = 21.2$) when compared to the control ($n = 100.4$; $p < 0.0001$) or sorafenib group ($n = 69.2$; $p = 0.002$). Similarly, tumor mean size was significantly reduced in combination (3.1 mm) compared to the control (9.9 mm; $p < 0.0001$), sorafenib (6.4 mm; $p = 0.019$) or ARQ 092 group (6.3 mm; $p = 0.031$). Tumor decrease was associated with a significant reduction in tumor cell proliferation and an increased apoptosis. CD34 staining showed reduced angiogenesis in combination group compared to the control or sorafenib group and HIF expression in tumor tissues was reduced in combination group. The results from Sirius red staining showed a significant decrease in fibrosis of animals treated with ARQ 092 alone or with combination treatment, accompanied with strong downregulation of TGFB, Collagen1 and ACTA1 expression levels. Granulocyte/T-cells ratio in blood was decreased in all treated groups compared to the control group and accumulation of neutrophils in liver tissue was significantly reduced. Western blot analysis of liver tissues showed a significant reduction of phosphorylation of AKT and its downstream signalling actors mTOR and S6K1 in both ARQ 092 and combination groups.

Conclusions: Combination of ARQ 092 and sorafenib exerted additive effect in controlling tumor progression and improved immune response in blood and liver. Our results confirm the importance of targeting AKT in HCC.

Molecular and cellular biology: Cell cycle control/apoptosis

SAT-415

The nucleoprotein High-mobility group box 1 critically regulates the immune response to bacterial infection

A. Volmari[1], K. Luecke[2], H.-W. Mittruecker[2], A.W. Lohse[1], P. Huebener[1]. [1]*I. Department of Internal Medicine;* [2]*Institute of Immunology, University medical center Hamburg-Eppendorf, Hamburg, Germany*
E-mail: a.volmari@uke.de

Background and Aims: Tissue damage can trigger immune activation even in the absence of infection, yet the molecular links between cell death and inflammation and their disease-specific roles remain elusive. Release of the highly conserved nucleoprotein High-mobility group box 1 (HMGB1) from necrotic hepatocytes was recently shown to initiate neutrophil recruitment and inflammation following sterile liver damage (Huebener *et al.*, JCI 2015).

Aim: To study the role of HMGB1 in the immune response to infection, using newly generated animals with conditional deletions of HMGB1 and its main receptor RAGE (coded by the *Ager* gene).

Methods: Conditional *Hmgb1*- and *Ager*-knockout mice and corresponding control littermates were infected with *Listeria monocytogenes*. HMGB1 serum levels were assessed via ELISA. Bacterial titres were measured in liver homogenates, and immune cell infiltration was assessed via FACS and immunohistochemistry. Hepatic cytokine and chemokine levels were determined by qRT-PCR. Isolated bone-marrow derived macrophages (BMDMs) were incubated with recombinant HMGB1 isoforms in the presence and absence of lipopolysaccharide (LPS).

Results: After infection with *Listeria*, both infiltrating leukocytes and hepatocytes exhibited strong HMGB1 expression, and HMGB1 serum levels were increasingly elevated for at least 72 h following infection. Unlike in sterile liver injury models, hepatocyte-specific HMGB1 ablation (*Hmgb1*Δhep) did not alter neutrophil recruitment or cytokine induction after *Listeria* infection, resulting in similar bacterial burden in *Hmgb1*Δhep and control littermates after 72h. By contrast, myeloid cell-specific HMGB1 ablation (*Hmgb1*ΔlysM) resulted in significantly attenuated IL6 induction and leukocyte infiltration into the liver early after infection, and higher bacterial burden at later time points. While RAGE deletion from dendritic cells resulted in impaired monocyte infiltration into the liver, RAGE deficiency in the myeloid cell compartment (*Ager*ΔlysM) did not alter early leukocyte recruitment, but resulted in significantly attenuated hepatic induction of proinflammatory mediators (CCL2, TNFa, TGFb)