tion of biomarkers of treatment response or tumor escape is still an unmet clinical need.
Aims: The aims of this study are to investigate miR-22 contribution to HCC tumorigenesis and sorafenib response, and to analyze its role as a biomarker of drug response.
Materials and Methods: Serum and tissue miR-22 levels were analyzed by qPCR in HCC patients and DEN-HCC rats. Proliferation and apoptosis assays and live imaging analysis evaluated miR-22 influence on HCC phenotype in vitro. A xenograft mouse model was used to determine the role of miR-22 on HCC tumorigenesis. Functional analysis elucidated the regulation of HIF-1A pathway following miR-22 modulation in different settings.
Results: MiR-22 was downregulated in human and rat HCCs and associated with microvascular invasion, tumor grade, and a worse overall survival. In vitro assays revealed that miR-22 inhibits cell growth in normoxic and hypoxic conditions and blocked HIF-1A pathway in HCC cells. Regarding *in vivo* tumorigenesis, miR-22 silencing gave rise to bigger and more vascularized tumor masses in xenograft mice. Lower miR-22 tissue levels associated with sorafenib resistance and correlated with apoptotic markers in the rat model while serum levels showed the opposite. In sorafenib-treated patients, a positive correlation between circulating miR-22 levels and days of treatment was observed. In line, lower miR-22 basal levels were detected in non-responder HCCs.
Conclusion: Low miR-22 levels favor HCC tumorigenesis and associate with a poor prognosis. MiR-22 influences sorafenib sensitivity and deserves attention as a possible biomarker of treatment response.

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Liposomal doxorubicin targeted with the Fab' of atezolizumab exerts an immunomodulatory effect in in vitro models of hepatocellular carcinoma

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Introduction: PD-L1, one of the most studied immune checkpoints, represents a key pharmacological target for HCC therapy. Atezolizumab, a monoclonal antibody inhibiting PD-L1 and restoring T-cell–mediated antitumor activity, is approved for advanced HCC therapy in combination with bevacizumab. Liposomal doxorubicin (DXR) targeted with the Fab' of atezolizumab (SIL treatment) reduced tumor growth in preclinical models of HCC.
Aim: unravel the effect of PD-L1 targeted liposomal DXR on the immune phenotype of tumor associated macrophages and liver cancer cells, and on their invasiveness, using untargeted liposomal DXR as a control (SL treatment), in 2D and 3D cellular HCC models.
Materials and Methods: The effect of SIL and SL treatments on PD-L1 expression was evaluated in HepG2 2D cultures and spheroids treated with INF-gamma to induce its overexpression. Both liposomal formulations were tested to assess the effect on macrophage polarization, clonogenicity and invasiveness on HepG2 spheroids co-cultured with THP-1 monocytic cells. The effect of SL and SIL on the epithelial-to-mesenchymal transition (EMT) of HepG2 cells was evaluated on the epithelial marker Eâ€'cadherin and the mesenchymal marker Vimentin by immunocytochemistry.
Results: Only SIL significantly decreased the INF-gamma-induced PD-L1 overexpression in HepG2 2D cultures and spheroids ($p<0.01$ and $p<0.001$ respectively), showing immunomodulatory activity. In THP-1/HepG2 spheroids, SL and SIL decreased clonogenicity and invasiveness of HepG2 cells ($p<0.0001$) and pro-tumoral CD163-expressing macrophages ($p<0.0001$). Only SIL caused a significant downregulation of PD-L1 expression ($p<0.01$). Accordingly, they significantly increased E-cadherin expression and decreased Vimentin one, downregulated and upregulated by TNF-α, respectively, at variance to SL ($p< 0.001$). Furthermore, SIL downregulated PD-L1 expression also in the TNF-α model ($p<0.001$ vs SL).
Conclusion: PD-L1-targeted liposomal DXR enhances the cytotoxic effect of liposomal doxorubicin and plays an immunomodulatory activity by decreasing PD-L1 expression and prompting macrophage polarization towards an antitumoral phenotype, showing promising results in preclinical models of HCC.

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RUVBL1 correlates with chaperones expression in HCC and its activity is required for HSF1-mediated stress response

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RuvBL1 is an AAA+ ATPase involved in multiple cellular activities including proliferation, chromatin remodeling, gene expression and translation. High RuvBL1 expression correlates with a worse prognosis in HCC and other human tumors. Emerging data suggests that RuvBL1 might exert co-chaperone functions.

Aim of this study is to investigate the relations between RuvBL1 and molecular chaperones in HCC.

Gene expression analysis of the human HCC samples in the TCGA_LIHC cohort shows that RuvBL1 significantly correlates with the expression of dozens of HSPs family members, with all the TCP-1 ring complex (TRiC) genes and with the transcription factor HSF1. Combining gene expression data from the LIHC_TCGA with publicly available CHIP-seq dataset (CHIP-Atlas and HSF1base) we identified a subset of potential common targets of RuvBL1 and HSF1, which includes several HSPs and all the TRiC genes. Reactome analysis revealed that regulation of cytosolic and mitochondrial translation were among the top enriched pathway regulated by shared RUVBL1 and HSF1 targets. The expression of selected shared targets was evaluated by qPCR in AML-12 and Huh7 cells treated with the RUVBL1/2 ATPase inhibitor CB6644 under basal and stressed conditions. The Heat Shock (HS)- or mitochondrial UPR (mtUPR)-induced expression of HSP90AA1(Hsp90alpha), HSPE1(Hsp10), HSPH1(Hsp110) was abrogated by treatment with CB6644. HSPA8 (Hsp70a8) was induced by mtUPR but not by HS, HSPA4(Hsp70a4) and all the TRiC genes were not induced by either stresses but were nonetheless downregulated by CB6644. HSF1 transcriptional activity, measured in AML-12 clones stably expressing a HSE-Nanoluc reporter, was readily induced by HS or mtUPR and completely abrogated by CB6644. Finally, proximity ligation assay revealed a close interaction of RuvBL1 and Hsf1 proteins in the nucleus of Huh7 cells.

In conclusion, RUVBL1 and HSF1 appears to coordinate the expression of several chaperone genes involved in cytosolic and mitochondrial translation. Targeting RuvBL1/2 activity impairs HSF1-mediated stress-response.

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