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ROLE OF cMYC IN CELL DORMANCY DISRUPTION IN LATENT TUMORS ESTABLISHED FROM HUMAN HEPATOCELLULAR CARCINOMA (HCC) CELL LINES

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Quiescence is a strategy that cancer stem cells (CSCs) adopt in stressful environments such are those triggered by antineoplastic treatments. CSCs form micrometastases that stay dormant for variable time periods and regain proliferation after unknown stimuli. *cMYC* induces pluripotency in differentiated cells and may have a role in dormant cells activation.
Aim: To analyze *cMYC* role in the switch from dormant (generated by HCC line BCLC5) to growing tumors formed by BCLC5 cells transfected with *cMYC* (BCLC5-cMYC), compared to BCLC9 already expressing *cMYC*.
Methods: *Creation of a stable BCLC5 cell line expressing cMYC by transfection of pCMV6-cMYC,* selection of cells expressing *cMYC* was done by G418 treatment. cMYC levels were confirmed by PCR/Western Blot (WB). *Subcutaneous (s.c.) injection of cells into SCID mice:* 10^6 BCLC9, BCLC5 and BCLC5-cMYC cells were s.c. injected. *Stable cell lines derived from xenograft tumors:* eight lines (t1 to t8) were derived from a BCLC5-cMYC tumor and 3 cell lines (c1, c2, c4) from tumors produced by injection of BCLC5-cMYC t7 cells. *Gene expression* of proangiogenic genes (*VEGFA*, *CD13*), epithelial–mesenchymal markers (*CDH1, VIM*), invasion markers (*NM23-H1, cMET, ITGB1, MMP2, MMP9)* and genes associated with cell pluripotency (*OCT4, SOX2, NANOG, CD133, EPCAM)* were assessed by real-time PCR, immunohistochemistry and WB.
Results: BCLC9 cells produced tumors in SCID mice while BCLC5 cells developed viable tumors <1–2 mm in size that remained stable after 6 months of injection. BCLC5-cMYC cells generated tumors with high proliferation and cell lines showed increased expression of *VEGFA, CD13* and *CDH1* and decreased *VIM, cMET* and *MMP2* compared to BCLC5. Tumor derived cell lines keep expressing pluripotency related genes and acquire *EPCAM's*, which was originally expressed only by BCLC9. Second generation of tumors BCLC5-cMYC and their cell lines showed increased expression of proangiogenic markers, genes associated to epithelial phenotype and those related to CSC while markers associated with invasive behavior decreased.
Conclusion: *cMYC* activation induces angiogenesis and epithelial markers while preserving pluripotent phenotype and reducing invasive potential. These changes consolidate along tumor evolution explaining the aggressive behavior of residual disease after antineoplastic treatment.

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INTRAHEPATIC ULTRASOUND-MEDIATED GENE DELIVERY

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Background and Aims: Transient ultrasound-induced increase in permeability of cell membrane and in some cases may enhance gene transfer. The objective of this work is to evaluate transfection efficiency and safety for intrahepatic gene delivery by sonoporation.
Methods: Polyplexes of expression vector DNAs complexed to galactose-bearing polyethylenimine have been used. Expression vectors are two plasmid containing expression cassette for full-size human preproinsulin gene controlled with CMV promoter and flanked by inverted terminal repeats of AAV (pTRhins) and the same cassette for marker gfp gene (pTRegfp). Animals were injected under US guidance with polyplexes in a dose of 40 μg pTRegfp/0.7 ml (rat) and of 15 μg pTRhins/0.15 ml (diabetic mouse) into the liver parenchyma of subdiaphragmal segments using 31 G needles. Afterwards injection locus in depth of 1 cm during 180 sec was insonated by 130 Db ultrasound using multifrequency 3–8 MHz probe.
Results: Sonoporation is able to enhance polyplex intrahepatic gene delivery to transfecting liver cells in vivo. Flow cytometry analysis of primary hepatocytes isolated from the liver of experimental rats showed that ultrasound-enhanced polyplex gene transfer was highly localized, and was superior to all controls. At least 42% of the liver cells *in vivo* can be transfected in this way with ultrasound exposure versus 1.2% without it. Hypoglycemic effect of the insulin gene delivery followed by 3 min US exposure was observed on the third day: glucose level of diabetic mice (hyperglycemic 6-week) decreased on average by 30%. A week after the procedure serial sections from rat liver injected with polyplexes containing 40 μg marker plasmid or saline solution alone exposed to US and without it were analysed for the presence of inflammation. Pathomorphological and histological analysis of experimental livers revealed no inflammatory processes in tissues, and any detectable side effects of US-enhanced gene delivery were seen. Experimental mice and rat liver DNAs were positive in transgene PCR for 1 month after gene delivery.
Conclusions: Our results demonstrate that polyplex gene transfer by US exposure is effective, robust and feasible and can become the new relevant technology for gene therapy.

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CELL CYCLE DEREGULATION BY HCV PROTEIN EXPRESSION, A POTENTIAL HEPATOCARCINOGENIC TRIGGER

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Chronic infection by hepatitis C virus (HCV) is a major risk factor for the onset and progression of hepatocellular carcinoma (HCC), which appears to be principally related to chronic local inflammation and fibrosis. Nevertheless, *in vitro* studies have shown that HCV proteins can directly interact with cell cycle regulators which might trigger carcinogenic processes.
Our goal was to assess *in vivo* hepatocyte cell cycle perturbation(s) by HCV proteins after an acute liver injury (CCl_4) using the FL-N/35 transgenic mouse model expressing the full HCV-ORF.
Early after CCl_4 challenge, no differences in the expression of immediate-early genes, growth factors or cytokines were observed between FL-N/35 mice and wild-type littermates (wt), suggesting that cell cycle initiation steps are not perturbed by HCV protein expression.
However, cyclin-A expression and BrdU incorporation at cell S-phase entry were delayed in FL-N/35 mice compared to wt. At cell S-phase entry, Retinoblastoma protein (Rb) phosphorylation was reduced in FL-N/35 mice, suggesting a G1/S transition impairment in the liver of these mice.
We recently published that FL-N/35 mouse livers displayed high levels of DNA-damage. It has been established that the ATM pathway is activated by DNA double-strand breaks and leads to cell cycle arrest. We observed that Chk2 and p53 phosphorylation and $p21^{waf1/cip1}$ expression, three actors of the ATM pathway, were significantly higher in FL-N/35 mice than in wt mice at G1/S transition. These results suggest that HCV-induced DNA-damage