Uncovering “hidden” mutations in hepatocellular carcinoma: the use of droplet digital PCR to detect TERT promoter mutations

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Introduction and aim: Activating somatic mutations in the promoter region of telomerase reverse transcriptase (TERT) gene are common events in hepatocellular carcinoma (HCC). Sanger nucleotide sequencing of end-point PCR amplified products is frequently used for mutation detection despite the limited sensitivity. Droplet digital PCR (ddPCR) is a highly sensitive technique and provides the absolute quantification of mutant alleles. We have developed and validated a probe-based ddPCR assay for detection of the TERT promoter mutation -124 G>A in a series of viral hepatitis related HCC.

Materials and methods: One-hundred-ten HCC cases, previously analyzed for TERT promoter mutations by PCR and Sanger sequencing, were tested by ddPCR using a probe-based assay detecting the common hot spot mutation -124 G>A within the TERT promoter region.

Results: Overall, 75 HCC samples out of 110 (68.2%) were found mutated at position -124 in the TERT promoter region by ddPCR. Eighty-three percent concordance was observed between ddPCR and Sanger sequencing. The use of ddPCR allowed to identify 18 previously unrecognized mutated HCC. Notably, the mutated cases identified only by the use of ddPCR showed a mean mutant allele frequency (MAF) of 13.4%. Overall, the MAF observed in our analysis ranged from 0.10% to 73%. The majority of mutated cases (n = 51) showed a MAF lower than 45% suggesting a heterogeneous composition of the tumour tissue. Fourteen TERT promoter mutated cases showed a MAF ranging from 55% to 73%, suggestive of loss of heterozygosity of TERT gene, which was associated with advanced stage of HCC.

Conclusion: The detection of TERT promoter mutation -124 G>A by ddPCR showed a remarkable high prevalence (68.2%) in our series of HCC confirming that such mutation represents a specific signature of liver cancer and that such strategy may be used as reliable biomarker for the early identification of neoplastic nodules in cirrhosis.

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Nuclear orphan receptor COUP-TF2 induces anoikis resistance, amoeboid migration and metastatic potential in hepatocellular carcinoma (HCC)

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Introduction: HCC is the second cause of cancer-related death worldwide. In the last years, the role of nuclear receptors in hepatocarcinogenesis has received great attention. COUP-TF2 regulates important biological processes and, studies indicate that is a pro-oncogenic factor but its role in HCC is still controversial. Aim of this study was to evaluate the role of COUP-TF2 in HCC.

Materials and methods: Results COUP-TF2, evaluated by immunohistochemistry, is over-expressed in primary HCC samples and Kaplan–Meier and Cox regression analysis show that it may be an independent prognostic factor of worst outcome. Overexpression of COUP-TF2, through stable transfection with pcR3.1/COUP-TF2, has no significant effects on cell proliferation. The migration and the ability to colonize sites distant from the growth front were evaluated by Time-Laps microscopy showing that COUP-TF2 induces a pro-metastatic phenotype characterized by an increased anoikis resistance and amoeboid migration. Western blot and immunofluorescences show that proteins involved in the organization of the cytoskeleton, cell-cell or cell-substrate, were differently modulated in COUP-TF2 overexpressing versus control cells. After we studied the role of COUP-TF2 in an in vivo models of mouse carcinogenesis (TgN(Alb1HBV)44Bri) realizing a triple transgenic animal where the liver-specific Cre expression deletes COUP-TF2 in hepatocytes (Tg[HBV]CreKOCOUP-TF2). COUP-TF2 deletion reduces tumour growth. Finally, for to evaluate the role of COUP-TF2 in metastasis formation we used a xenograft model where mice were inoculated with human sMMC7221 hepatocarcinoma cells stable transfected with COUP-TF2 or silenced by specific short hairpin. COUP-TF2 overexpression induces an increase, number of lung metastasis whereas its deletion reduces pulmonary metastasis compared to control animals.

Conclusion: In the light of our data, COUP-TF2 appears to play a role in the metastatic progression of HCC. The evidence that COUP-TF2 transcriptional activity could be potentially regulated by ligands, indicates that this nuclear receptor is a promising therapeutic target for HCC.

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