MICRORNA-23b NEGATIVELY REGULATES UROKINASE AND c-MET AND INHIBITS MIGRATION OF HUMAN HEPATOCELLULAR CARCINOMA CELLS.

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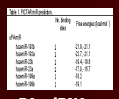
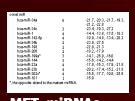
Urokinase (uPA) and c-met take part in the invasion and metastasis processes of several malignancies and their mRNA overexpression has been assessed as unfavourable prognostic marker of hepatocellular cacrinoma (HCC) (Cancer Res. 1998, In J Cancer 2000). Previously siRNAs and plasmid-based shRNAs stable expression were used to target uPA and c-met in HCC cells (SK-Hep1C3). Consequently the malignant properties of SKHep1C3 cells resulted inhibited *in vitro* and *in vivo* (Mol Cancer Ther 2004, Tumour Biology 2007, Int J Oncol 2007). In the present work we verified the possibility of a miRNA-mediated coregulation mechanism of uPA and c-met in HCC cells.

PicTar miR prediction

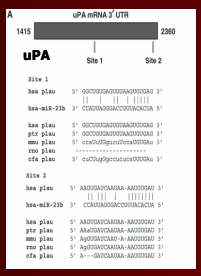


uPA miRNAs

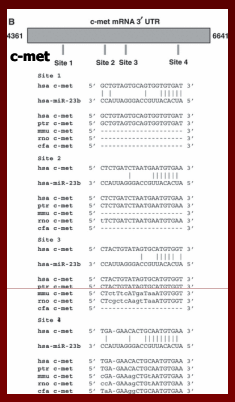


MET miRNAs

By bioinformatic tools, we predicted that miRNA-23b could recognize 2/4 sites in the 3'UTR of uPA/MET respectively.

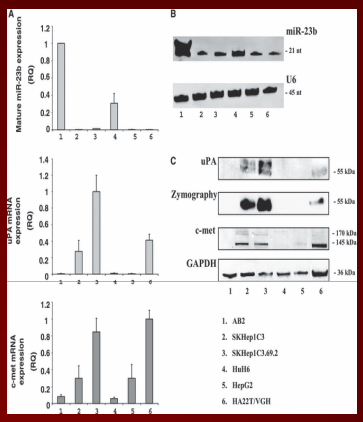


The *uPA* 3'-UTRs harbors two putative binding sites for miR-23b. (A) The location of sites 1 and 2 in the *uPA* 3'-UTR, and complementarity between miR-23b and the putative *uPA* 3'-UTR target sites. The conserved bases of the putative miR-23b target sequence are also shown.

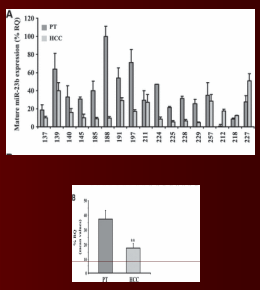


(B) The *MET* 3'-UTRs harbors four putative binding sites for miR-23b. The location of sites 1, 2, 3 and 4 in the *c-met* 3'-UTR, and complementarity between miR-23b and the putative *c-met* 3'-UTR target sites. Conserved bases of the putative miR-23b target sequence present in the *c-met* 3'-UTR are also shown. has, *Homo sapiens*; ptr, *P. troglodytes* (chimpanzee); mmu, *M. musculus* (mouse); rno, *R. norvegicus* (rat); cfa, *C. familiaris* (dog).

miR-23b expression shows an inverse trend with uPA and MET expression in human cells

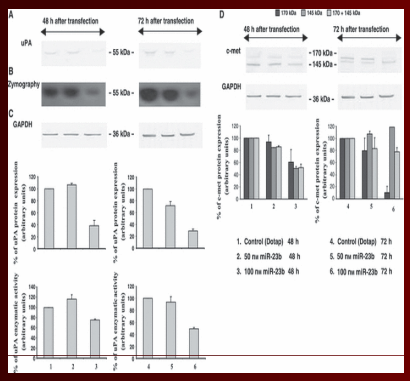


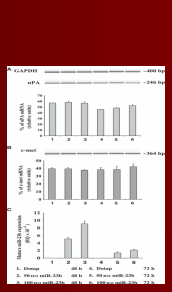
and in HCC bioptic fragments.

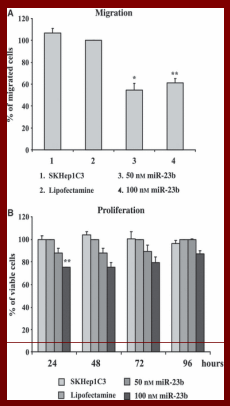


Among the HCC patients there are two subgroups: one (14/17, 82%) showing miR-23b downregulation, as compared with peritumoral tissues; another 3/17 (18%) with miR-23b upregulation.

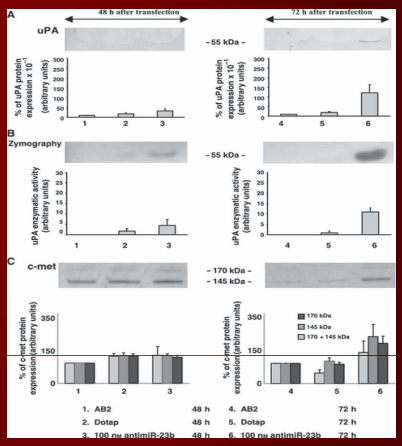
miR-23b inhibits uPA and c-met protein expression in SKHep1C3 and decreases the migration and proliferation abilities of miR-23b transfected SKHep1C3 cells.

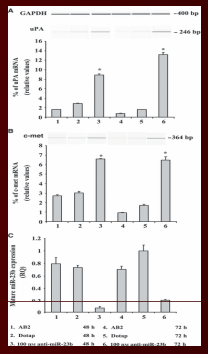






Anti-miR-23b enhances uPA and c-met protein and mRNA expression in AB2 cells.





Results and Conclusions.

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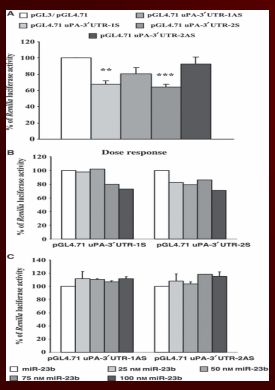
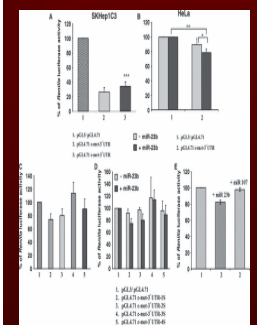
miR-23b interacts with uPA 3'UTR and with c-met 3'UTR





-A subset of HCC (14/17, 82%) showed a miR-23b downregulation as compared with PT tissues with an average expression level of miR-23b about twofold lower than in PT tissues ((P<0.01).

-The miR-23b analysis revelead and inverse trend with uPA and MET expression in human tumour and normal cells.

-Transfection of miR-23b in HCC cells led to inhibition of target gene protein expression and to a decrease in cell migration/proliferation capabilities.

-AntimiR-23b transfection in human AB2 cells fibroblasts upregulated the uPA and MET endogenous expression at protein and at mRNA levels.

-Cotransfection experiments in HCC cells of the miR-23b with luciferase reporter gene constructs, containing the uPA and MET 3'UTR target sites, indicated that miR-23b can translationally repress the

uPA and MET expression in HCC cells *via* direct interaction miR::mRNAs. Both target sites of uPA 3'UTR (1S, 2S) resulted to be effective as well as the 1S, 2S and 4S c-met 3'UTR constructs.The whole

c-met 3'UTR construct decreased luciferase activity, in the absence/presence of miR-23b; thus indicating that the c-met 3'UTR may be an important target for control of protein expression.

These findings suggest a negative regulatory mechanism of gene expression involving molecules,

miR-23b (as negative regulator), uPA and MET (as target genes), until now not Known to interact and an inhibitory effect of this miR in the migration ability of HCC cells.

As pharmacological regulation of uPA and MET has proved to be a challenge in cancer , this regulatory mechanism may be potentially be a new tool with which to alter the expression of uPA and MET as well as shRNA, uPA inhibitors, TPK inhibitors...