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Modulation of MMP-9 and TIMP-1 expression by Protein Phosphatase in Hep3B and LX-2 cell lines

MP Ward, JP Spiers. Trinity College Dublin, Dublin, Ireland

Matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases, TIMPs) are involved in tissue remodelling associated with hepatitis and liver cancer (1). While kinase-dependent signalling pathways are involved, little is known regarding the function of serine/threonine protein phosphatases (PSP) and protein tyrosine phosphatases (PTP) in MMP regulation. The aim of the present study was therefore to investigate the effects of inhibition of PSPs and PTPs on expression of MMPs and key proteins associated with hepatic remodelling.

Human hepatic stellate cells (LX-2) and hepatocellular carcinoma cells (Hep3b) were cultured in serum-free media and exposed to inhibitors: okadaic acid (40nM, PP2A); tautomycetin (10nM, PP1); cyclosporin A (10nM, PP2B); BVT 948 (4-hydroxy-3,3-dimethyl-2*H*-benzo[*g*]indole-2,5(3*H*)-dione, 10nM, PTP). Effects of the inhibitors (0.1-100nM) on cell viability were assessed by MTT assay. MMP-2 and MMP-9 activity was determined by gelatin zymography and quantified by densitometry. The mRNA expression profile of 15 genes involved in fibrosis/remodelling were analysed by real-time RT-PCR and expressed as fold increase following normalisation to GAPDH. Data are presented as mean ± S.E.M (n=5) and analysed by one-way ANOVA with *post hoc* analysis (Bonferroni). $P<0.05$ indicates statistical significance.

The protein phosphatase inhibitors did not affect cell viability at any concentration studied. Okadaic acid increased MMP-9 activity by 1.23 ± 0.05 and 1.16 ± 0.30 fold ($P<0.05$) in LX-2 and Hep3B cells, respectively. Tautomycetin also increased MMP-9 activity by 1.22 ± 0.03 fold ($P<0.05$) in LX-2 cells, but had minimal effect in Hep3B cells (0.74 ± 0.21 fold increase). In contrast, BVT 948 did not alter MMP-9 in LX-2 cells, but increased it by 1.59 ± 0.35 fold ($P<0.05$) in Hep3B cells.Okadaic acid, but not tautomycetin, increased expression of MMP-9 mRNA in both cell lines. None of the phosphatase inhibitors affected MMP-2 activity or mRNA expression in either cell lines. Okadaic acid produced a 1.74 ± 0.31 fold increase in TIMP-1 mRNA levels in Hep3B cells while decreasing its expression by 2.14 ± 0.48 fold in LX-2 cells. Tautomycetin and BVT 984 did not affect TIMP-1 mRNA expression in either cell line. Cyclosporin A did not affect MMP-2 or MMP-9 activity/expression in either cell line. None of the phosphatase inhibitors altered mRNA expression of the other fibrogenic markers investigated.

In conclusion, MMP-9 and TIMP-1 activity are modulated via selective protein phosphatases. In Hep3B cells, while MMP-9 is modulated by PP2A, PP1 and PTP, TIMP-1 is specifically altered through PP2A. In LX2 cells, PP2A and PP1 similarly contribute to alterations in MMP-9 activity. However, differential regulation of TIMP-1 via PP2A signalling appears to shift LX-2 cells towards a degradative phenotype (increased MMP-9/TIMP-1 ratio) over that achieved in Hep3B cells. As hepatic stellate cells are the major matrix producing cells in the liver, our data would

indicate that targeting PP2A could be developed as an antifibrotic therapy.

(1) Page-McCaw A et al, Nat Rev Mol Cell Biol 8:221, 2007