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Increased Cancerous Inhibitor ofPP2A (CIP2A) Expression in Hepatocellular Carcinoma Cells Following Co-Culture With Anti-Inflammatory Macrophages

Introduction: Major regulators of the tumour microenvironment are macrophages with two function classes: pro-inflammatory M1 and anti-inflammatory M2 macrophages. Pro-tumourigenic macrophages are known as tumour associated macrophages (TAMs) and have been shown to increase matrix metalloproteinasessecretion whilealtering tumour oncogenic signalling (1). PP2A is a major tumour suppressor and its activity is often altered in cancers. In recent years, its cellular inhibitor, Cancerous Inhibitor of PP2A (CIP2A) has been identified as a contributory factor in the oncogenic transformation of cells.Thus, this study aims to investigate whether TAMs alter PP2A function in tumour cells and the implications of CIP2A on the tumour microenvironment.

Methods: THP-1 cells were differentiated to a macrophage (Mφ) phenotype by exposure to PMA (320nM) for 24 hours. M1 phenotype was generated by differentiating THP- 1 cells with PMA (320nM) for 24h during which time LPS (100ng/ml) and IFN-γ (20ng/ml) were added for the last 18 h.THP-1 cells were differentiated to the M2 phenotype following exposure to PMA (320nM) for 24 h, along with IL-4 (20ng/ml) and IL-13 (20ng/ml) for the last 18 h.THP-1 macrophages were pre-polarised before being co-cultured with Hep3B cells using a Transwell apparatus (0.4 μM pore). For CIP2A overexpression, Hep3B cells were transfected with a CIP2A overexpression plasmid.PP2A activity was determined using a PP2A immunopercipitation phosphatase assay. Gene expression was determined by RT-PCR. PP2Ac and CIP2A protein expression was determined by western blot. MMP-9 activity was determined by zymography and tumour cell lactate production measured using a lactate assay kit.

Results: PP2A activity in Hep3B cells co-cultured with Mϕ cells was 25 ± 1.48% lower than mono-cultured Hep3B cells ($P< 0.05$). Co-culture with pro-inflammatory M1 macrophages did not alter PP2A activity in Hep3B cells. However, co-culture with anti-inflammatory M2 cells reduced activity by 29± 2.58% compared to Hep3B cells alone ($P< 0.05$). PP2Ac protein expression was unaffected by macrophage co-culture, however, co-culture with M2 macrophages increased Hep3B CIP2A mRNA and protein expression. M2 macrophages increased Hep3B MMP-9 activity by 3.66 ± 0.63 fold ($P < 0.05$). MMP-2 activity was unaltered in Hep3B cells. M2 macrophages increased VEGFA, IL-8 and IL-10 mRNA expression in Hep3B cells by 5.8 ± 0.98, 2.37 ± 0.74 and 1.88 ± 0.53 fold respectively ($P < 0.05$). LDHA and HIF1A mRNA expression increased by 1.02 ± 0.39 and 2.53 ± 0.59 fold respectively in Hep3B cells co-cultured with M2 macrophages ($P < 0.05$). PP2A inhibition with okadaic acid andHep3B CIP2A overexpression increased lactate release from tumour cells (15 mM; $P < 0.05$), consistent with concentrations found in M2/Hep3B co-cultures. Loss of PP2A activity and M2 macrophage co-culture increased the invasion potential of Hep3B cells($P < 0.05$).

Conclusion: Anti-inflammatory macrophages decrease PP2A activity in Hep3B cells, promoting CIP2A expression. Loss of PP2A activity promotes pro-metastatic factors such as MMP-9, VEGFA, IL-8 and IL-10 and LDHA expression in Hep3B cells. The resulting acidosis of the tumour microenvironment stimulates cell invasion. These data indicate a novel role for loss of PP2A function and increase in CIP2A resulting from smouldering inflammationin hepatocellular carcinoma.

Pollard, J. W. (2004). *Nat. Rev. Cancer***4**: 71-78.