Platelet induced hepatocellular carcinoma HepG2 cell proliferation and angiogenic potential is integrin $a_{IIb}b_3$ dependent

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Background: Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world, leading to an estimated one million deaths annually. Although several treatment options are available, the prognosis for HCC patients remains poor, largely due to rapid metastasis. Liver cancers are often highly vascularized, and both experimental and clinical data indicate that the progression of HCC is associated with increased angiogenesis, aiding in their metastatic potential. The vascular nature of HCCs gives them ample opportunity to recruit and interact with platelets. Platelets bind to cancer cells through a range of receptors including the integrin $a_{IIb}b_3$, which is upregulated by platelet derived ADP and thromboxane A2 (TxA2). When activated, platelets release a large array of cytokines and growth factors that may induce angiogenesis and aid in the migration, invasion and proliferation of a range of tumour cells, but it is unclear if they support HCC progression. Identifying the roles platelets play in enhancing HCC proliferation and metastatic potential could provide novel treatment strategies to target HCC.

Aims: To determine the importance of platelet integrin receptor $a_{IIb}b_3$ and released mediators ADP and TxA2 in platelet adhesion to the HCC cell line HepG2. To evaluate if $a_{IIb}b_3$ dependent platelet adhesion enhances HCC proliferation and HCC induced angiogenesis.

Methods: Platelet adhesion to HepG2 cells was measured fluorescently. Activation of $a_{IIb}b_3$ on adherent platelets was measured by fluorescent microscopy with PAC-1 antibody. HepG2 proliferation was determined using CellTitre-Glo and the upregulation of cyclins required for cellular growth were investigated by western blotting. The release of angiogenesis mediators from HepG2 cells treated with platelets was analysed by a R&D systems proteome profiler. The ability of conditioned media from HepG2 cells treated with and without platelets to induce endothelial cell proliferation and angiogenesis was determined with CellTitre-Glo and matrigel assays respectively. Results: Platelets robustly adhered to HepG2 cells in a time dependent fashion. Pre-treatment with RGDS to inhibit $a_{IIb}b_3$ reduced adhesion to 40 ± 7% of control. Combined treatment with apyrase and indomethacin, to block the effects of ADP and TxA2 respectively, reduced adhesion to 60 ± 3%. Combined inhibition of $a_{IIb}b_3$, ADP and TxA2 did not significantly further reduce adhesion. Platelet binding to HepG2 cells induced the activation of platelet $a_{IIb}b_3$ as shown by upregulated PAC-1 binding, which was blunted by treatment with apyrase and indomethacin. Adhesion of platelets increased HCC proliferation by 180 ± 30% and increased the expression of cyclin B1 and D1, both key regulators of cellular proliferation. RGDS reduced proliferation to 103 ± 8%. Conditioned media from HepG2 cells treated with platelets was enhanced with several angiogenesis regulatory cytokines including angiogenin, amphiregulin, Il-8 and VEGF. The conditioned media from HepG2 cells treated with platelets, but not with platelets pre-incubated with RGDS, robustly induced endothelial cell tube formation and branching.

Conclusions: Platelet adhesion to HepG2 cells is largely $a_{IIb}b_3$ dependent, with released ADP and TxA2 potentiating this response. $a_{IIb}b_3$ mediated platelet adhesion induces HepG2 cell

proliferation, associated with upregulated cyclin expression. Additionally HepG2 cells treated with platelets release a raft of angiogenesis mediators and induce endothelial cell angiogenic responses.