1504 EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) CAN BE DRIVEN BY MACROPHAGES THROUGH MULTIPLE SIGNALLING PATHWAYS

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Background EMT is the process whereby epithelial cells acquire mesenchymal characteristics. This process is highly regulated and required in development and tissue regeneration. However, in cancer the process becomes dysregulated leading to increased invasion, metastasis and tumor progression. Macrophages are one of the major infiltrating immune cells in the tumor microenvironment and have been proposed as a primary driver of pathological EMT through interactions with tumour cells. EMT and macrophage infiltration have been correlated to enhance tumor progression in multiple cancer types, making it a potential therapeutic target. In this study we generate and characterise a series of human macrophage subtypes via cytokine polarisation and show that multiple macrophage polarised states enhance the EMT phenotype.

Methods Human macrophages were differentiated using GM-CSF or M-CSF prior to polarisation with individual cytokines (M-CSF + IFN-g; M-CSF + IL-4; M-CSF + IL-1b; M-CSF + IL-10; M-CSF + IL-6) to generate different putative macrophage phenotypes (M1, M2a, M2b, M2c, M2d) as evidenced by differences in cytokine secretion, gene transcription and protein expression. Epithelial cells were either stimulated with macrophage conditioned medium or in co-culture with macrophages in a scratch wound assay (Incucyte) and analysed by immunocytochemistry (Vimentin, SNAIL, E-cadherin). Supernatants of the stimulated macrophages were assessed by Luminex bead array and the macrophages subtypes were also assessed using bulk RNA sequencing for differences in transcriptome.

Results Supernatants derived from macrophages and macrophage co-culture were shown to drive EMT, both in migratory capacity and EMT associated protein expression. Our results demonstrate that even a single cytokine can alter the polarisation and subsequent cytokine production of the macrophage; with IL-12 and TNF-α associated with more pro-inflammatory phenotypes (M1), and IL-10 and VEGFA more anti-inflammatory or tissue remodelling phenotypes (M2). Inhibition of the TGF-βRI by galunisertib can partially reverse the induction of EMT migration and protein expression.

Conclusions The data shows that macrophages are plastic and the cytokines they produce can directly drive the EMT process *in vitro* via multiple mechanisms. This supports the conclusion that individual cytokine inhibition such as TGF-βRI alone is likely to be ineffective as macrophages produce multiple factors that can induce EMT. Using this powerful model, researchers can gain better understanding of complex interactions occurring in the tumor microenvironment, identify molecular targets with potential to block cancer cell metastasis and evaluate candidate drug efficacy.

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