Mass spectrometry-based secretome analysis of melanocyte-keratinocyte-fibroblast co-culture systems

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Melanoma is an aggressive form of skin cancer in the later stages of the disease. Despite recent advances in targeted therapies and immunotherapies, the relapse rate among melanoma patients remains significant. The tumour microenvironment (TME) has been shown in other cancers and melanoma to provide a protective niche where the malady can reside and resist treatment. It has been shown that the increase in secreted fibroblasts growth factors can in turn lead to melanoma cell growth and further para/autocrine signalling, leading to increased survival of the tumour. This altered TME can have a negative effect on the efficacy of treatment and may be a contributing factor to the relapse rate amongst melanoma patients. A better understanding of the interplay between the secreted factors of the melanoma TME would be beneficial to therapy.

To investigate the secretome a combination of melanoma cells with keratinocytes in a transwell and either normal human dermal fibroblasts (NHDF) or patient-derived cancer-associated fibroblasts (CAFs) were seeded below, separated by a semipermeable membrane. This combination of cells simulates the disease allowing us to analyse, via mass spectrometry, the secretome of the two co-cultures.

Our preliminary data suggests a fundamental difference between the secretomes. Of the co-cultures secreted proteins, 66% of proteins are common to the CAFs and NHDF co-cultures, 18% and 17% of CAFs and NHDF co-cultures respectively are unique. This difference between the secretome is evident in the higher expression of metalloproteases inhibitors present in the NHDF co-culture, which indicates potential factors responsible for the prevention of metastasis early in the disease. There are also a higher number of receptors involved in cell adhesion in the NHDF co-culture than the CAFs co-culture, again suggesting factors whose expressions may need to be lost for the cells to metastasize. We also observed an increase in metalloproteases and secreted factors associated with apoptosis evasion in the CAFs co-culture. These preliminary findings highlight the need for further investigation into the role of the TME in melanoma, and the potential of simulating the TME using a transwell co-culture system.