The role of MIF in melanoma progression

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Statement of Originality

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Abstract

There is currently no effective treatment for melanoma once the tumour has spread beyond the primary site. Unlike many other cancers, metastatic melanoma is frequently resistant to all conventional forms of anti-cancer treatment. This inherent resistance of melanoma cells is in large part due to their hyper-activation of survival signalling pathways, most notably ERK and Akt. This often results from mutations of key proteins such as in BRAF, NRAS and PTEN, and consequently these molecules have been the subject of intensive investigation. These efforts have led to the revolutionary new treatments such as those targeting mutated BRAF that occurs in ~50% of melanomas. However, while these agents demonstrate a high initial response rate, their clinical benefit has been plagued by the development of acquired drug resistance. In any case this treatment is not applicable to those patients not presenting with the BRAF mutation and finding other therapeutic targets is urgent.

Another important mechanism driving survival signalling pathways in melanoma is the aberrant production of growth factors that act in an autocrine manner. The work presented in this thesis fits within this area with studies focused on the role of macrophage migration inhibitory factor (MIF). MIF is an atypical cytokine for which a number of diverse roles have been described including those of both hormone and enzyme. In the context of cancer, MIF is believed act as the autocrine factor driving activation of survival pathways. MIF signalling is known to be initiated by binding to the cell surface CD74/CD44 receptor complex or to the chemokine receptors CXCR2 and CXCR4. Although MIF signalling has been implicated in several tumours, the role of MIF in melanoma had not been previously studied in great detail.

This thesis first investigated the expression of MIF in melanocytic tumours in vivo using a combination of in silico analysis of microarray data and immunocytochemistry staining of ex vivo tumour sections. The results presented herein show that MIF expression generally increases with disease progression and in advanced tumours it preferentially localises to the nucleus of cancer cells. Analysing the available survival data it was shown that MIF was a significant prognostic factor for patients with metastatic melanoma, with higher expression levels predicting poorer outcome since patients underwent faster relapse. MIF expression was only important in the context of secondary tumours since the analysis of MIF levels in primary
melanoma samples failed to show outcome differences. Similar prognostic analyses of the known MIF receptors, CD74, CD44 that acts as a co-receptor, and CXCR2 and CXCR4 were also performed. Only CD44 expression appeared to be associated with prognosis since high CD44 levels in tumours were also predictive of shorter survival in metastatic disease. The conclusion from these data suggested that high levels of MIF expression in metastatic melanoma are associated with tumour aggressiveness.

Further experiments undertaken in a large panel of 20 human melanoma cell lines showed that MIF, CD44 and CXCR4 were ubiquitously expressed. CD74 was only expressed in 50% of cell lines and CXCR2 in none. The function of MIF was then examined in six of these cell lines using siRNA to deplete MIF. With respect to control treated cells, MIF siRNA significantly decreased cell proliferation in 4 out of 6 cell lines. Further analysis showed that MIF also influenced cell survival and anchorage-independent growth. The sensitivity of cells to MIF depletion appear to be associated with the presence of MIF in the cell nucleus, but it was independent of BRAF mutational status. Analysis of signalling pathways showed that MIF acts to regulate the Akt pathway in a high proportion of melanoma cell lines and this finding is highly significant with respect to targeting survival signalling in this disease.

The receptor systems that MIF likely utilises in melanoma were also investigated. It was noticeable that MIF effects on melanoma cell lines were independent of CD74 expression since cells not expressing CD74 were also sensitive to MIF knockdown. Analyses focussed on the nuclear localisation of MIF and the presumed involvement of CD44 in this process, since CD44 had been previously shown to translocate to the nucleus. Extensive imaging and biochemical analyses failed to demonstrate this was the mechanism of MIF nuclear translocation in melanoma.

In conclusion, the work presented here implicates MIF in melanoma progression and reveals MIF as a potential prognostic factor for metastatic melanoma. MIF actions are likely to involve the activation of Akt signalling pathway to regulate the cell-cycle, a key finding that has implications for melanoma proliferation and progression. Taken together, these results indicate MIF as a potential new therapeutic target for melanoma and one that is potentially independent of - and complementary to - current therapies.