Role of Wnt/β-catenin and CXCL12/CXCR4 signalling axes in the damage and recovery of the bone marrow microenvironment following methotrexate chemotherapy

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Abstract

The bone marrow microenvironment is home to mesenchymal and haematopoietic stem cells and their respective progeny. Mesenchymal stem cells are multipotent and have the capacity to differentiate into a number of cell types, namely osteoblasts, adipocytes and chondrocytes. These cells and cells of the haematopoietic lineage maintain close interactions within the marrow cavity and are responsible for bone and bone marrow maintenance throughout life. Disruptions to cell populations and steady-state interactions within the bone marrow such as that seen following cancer chemotherapy treatment are associated with bone-related complications in later life such as osteoporosis. However, the underlying mechanisms of these defects and the subsequent recovery potential remain unclear. The studies presented herein have investigated the effects of the commonly used antimetabolite methotrexate (MTX) on the damage and recovery of the bone marrow microenvironment and potential signalling pathways involved, focusing on Wnt/β-catenin and CXCL12/CXCR4 signalling axes. Using a short-term rat MTX model of 5 consecutive daily doses at 0.75mg/kg, histological techniques were employed to assess bone/fat formation and cell culture techniques were used to investigate differentiation potential of bone marrow mesenchymal and haematopoietic cells. These investigations were further supported by protein expression and quantitative RT-PCR analyses of associated genes over the MTX time-course.

The bone marrow cavity was observed to undergo a number of changes when assessed histologically, with damage obvious on days 6 and 9 and recovery apparent by day 14. This was identified by an increased adipogenic marrow and reduced trabecular bone volume, parallel to a reduction in mineralising potential yet increased adipogenic potential of isolated marrow stromal cells. This was further supported by changes in bone marrow stromal cell gene expression, whereby adipogenic transcription factor PPARγ was increased concurrent to a reduction in osteogenic transcription factor Osterix, indicating a switch in lineage commitment. In order to characterise molecular mechanisms

underlying such altered lineage commitment, the role of Wnt/β-catenin signalling was investigated, known to critically function in mesenchymal stem cell differentiation. Interestingly, MTX induced notable changes in Wnt signalling-associated genes assessed in the stromal cell population. Concurrent administration of the synthetic GSK-3β inhibitor BIO abrogated the above transient changes in bone/fat volumes, osteogenic/adipogenic commitment and gene expression. This demonstrates a potential role for Wnt/β-catenin signalling in MTX chemotherapy-induced changes to osteogenic/adipogenic commitment and a therapeutic potential for preventing bone loss and marrow adiposity by promoting Wnt signalling via GSK-3β inhibition.

Furthermore, to clarify the mechanisms associated with the recovery response of the bone marrow microenvironment, the current project also examined the CXCL12/CXCR4 signalling axis, known to be involved in mobilisation, homing and maintenance of a quiescent stem cell pool, enabling reestablishment of a functioning marrow in response to damaging conditions. After MTX, coinciding with the reduction of marrow cellularity, CXCL12 protein expression was observed to decrease on day 9, accompanied by an increase in CXCL12-degrading metalloproteinase MMP-9. *In vitro* studies confirmed that recombinant MMP-9 was able to degrade CXCL12 protein. In addition, changes in gene expression of CXCL12 and its receptor CXCR4 in the bone marrow stromal cell population as well as the non-adherent fraction were observed following MTX treatment. This further suggests the CXCL12/CXCR4 axis is deregulated over the MTX damage/repair time-course and is potentially involved in the regulation of bone marrow damage and recovery.