Molecular and cellular studies examining the biological significance of different isoforms of the receptor tyrosine kinase, c-Kit

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Contents

Abstract

c-Kit is a member of the Receptor Tyrosine Kinase Type III family and has four naturally occurring isoforms. The work presented in Chapter 3 utilised full-length human or murine c-Kit cDNA expressed in murine cells. Over-expression of normal c-Kit was capable of contributing to oncogenic transformation. The analysis of human c-Kit isoforms demonstrated dissociation of various indicators of transformation (anchorage independence, loss of contact inhibition, tumourigenicity) in the NIH3T3 cell model.

Biochemical analysis of the c-Kit signalling revealed qualitative and quantitative differences between the GNNK+ and GNNK- c-Kit isoforms. The GNNK- isoform was hyperphosphorylated more extensively and rapidly, and was also more efficiently ubiquitinated and degraded than the GNNK+ counterpart. PI3-K was recruited and activated equally by both isoforms. Phosphorylation of MAPK paralleled that of the c-Kit isoform's phosphorylation.

In Chapter 4, a new model was developed using a chimaeric human extracellular c-Kit/murine transmembrane + intracellular c-Kit. This new molecule, in conjunction with a murine Myb Immortalised Haemopoietic Cell (MIHC) line was used to investigate a number of biological outcomes stimulated by SCF simultaneously. A MIHC line lacking Lyn was also analysed.

Chimaeric c-Kit displayed the same signalling characteristics exhibited by its' full-length human counterpart. The model showed that the GNNK- isoform was superior

in its survival stimulus to GNNK+, but both were equivalent in promoting proliferation. The absence of Lyn reduced the ability of both isoforms to promote survival.

The aim of work in Chapter 5 was to elucidate the expression patterns of the c-Kit isoforms in subsets of normal human haemopoietic cells. Methodology was developed to detect GNNK+/- c-Kit mRNA from rare subsets of cells from bone marrow. As c-Kit is known to be down-modulated in mobilised peripheral blood stem cells, mobilised CD34+ cells were also investigated. In all haemopoietic cells analysed, there was no significant difference in expression patterns of the c-Kit isoforms, with all samples expressing approximately 90% of total c-Kit transcripts as the GNNK- isoform. c-Kit downmodulation observed in mobilisation of CD34+ cells was not influenced at the level of transcription, but at the protein level.