Activating point mutations in the common β subunit of the human GM-CSF, IL-3 and IL-5 receptors: implications for receptor function and role in disease

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Summary

Human granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3 and IL-5 are a group of cytokines that regulate the proliferation, differentiation and functional activation of the various cell lineages that constitute the haemopoietic and immune systems. These cytokines mediate their diverse biological activities by binding to specific receptors expressed on the surface of target cells. The high-affinity receptors for human GM-CSF, IL-3 and IL-5 are heterodimeric complexes consisting of cytokine-specific α subunits and a common signal-transducing β subunit (hβc).

The constitutive activation of this group of receptors has previously been demonstrated by the identification of mutations in hβc, including two point mutations, able to confer factor independence on the factor-dependent murine haemopoietic cell line, FDC-P1 (Jenkins *et al.*, 1995). One of these point mutants is located in the extracellular, membrane-proximal domain and resulted in the substitution of Ile^{374} to Asn (I374N), whereas the other is located in the transmembrane domain and resulted in the replacement of a Val residue with Glu at position 449 (V449E). Interestingly, only V449E was able to confer factor independence on murine factor-dependent BAF-B03 cells, suggesting that I374N and V449E activate hβc by different mechanisms.

Although the above study successfully isolated activating point mutations in hβc, the screen for mutations was relatively insensitive and only covered about one third of hβc, thus raising the possibility that other constitutive point mutations may be present in the remainder of hβc. To address this issue, random mutagenesis was combined with a rapid retroviral expression cloning strategy and a more sensitive screen to comprehensively screen the entire hβc molecule. This led to the identification of additional constitutive point mutations, all of which are clustered exclusively in a central region of hβc that encompasses the extracellular membrane-proximal domain, transmembrane domain and membrane-proximal region of the cytoplasmic domain. Although all of the hβc mutants confer factor independence on murine FDC-P1 cells, most exhibited cell type-specific constitutive activity, with only two transmembrane domain mutants also able to confer factor independence on murine BAF-B03 cells. Examination of the biochemical properties of these mutants in FDC-P1 cells indicated that ERK MAP kinase and STAT signalling molecules were constitutively activated by all mutants. In contrast, only some of the mutant β subunits were constitutively tyrosine

phosphorylated. Taken together, these results highlight key regions involved in hβc activation, dissociate hβc tyrosine phosphorylation from MAP kinase and STAT activation, and suggest the involvement of distinct mechanisms by which proliferative signals can be generated by hβc.

One of the questions raised by the isolation of the I374N mutation and the other extracellular mutations is how they alter hβc structure and lead to receptor activation. To address this question, site-directed mutagenesis was employed to explore predictions, based on a structural model of hβc, suggesting the possibility of interactions between Ile^{374} and other hydrophobic residues in its vicinity. Replacement of two such residues, Leu^{356} and Trp^{358}, with Asn resulted in constitutive activation of hβc. The prediction that these two residues are in close proximity to Ile^{374} was further supported by the synergistic effect of combining weakly activating hydrophobic substitutions at Leu^{356}/Trp^{358} and Ile^{374}, since factor-independent cells expressing these double mutants showed substantially higher growth rates than the 'parental' single mutants. These results suggest that Ile^{374} normally interacts with Leu^{356} and Trp^{358}, and that disruption of these interactions results in a conformational change in the extracellular membrane-proximal domain of hβc that leads to constitutive activity, possibly by promoting interaction with another receptor subunit (see below).

The observation that I374N and most other hβc mutants confer factor independence on FDC-P1 cells, but not BAF-B03 cells, implicates the involvement of cell type-specific molecules in signalling by these mutants. Retroviral expression cloning was used to demonstrate that introduction of the mouse (m) GMRα subunit into BAF-B03 cells expressing the I374N mutant conferred factor independence on these cells. Most other hβc mutants also required mGMRα for constitutive activity in BAF-B03 cells, and this correlated with the ability of these mutants to constitutively associate with mGMRα. The observation that mGMRα facilitated the constitutive activity of I374N in BAF-B03 cells contrasted with the failure of human (h) GMRα to perform this function. Importantly, expression of I374N in human GM-CSF/IL-3/EPO-dependent UT7 cells, which express endogenous hGMRα, led to factor-independent proliferation, and the expression of GMRα on the surface of these cells was significantly upregulated. Taken together, these observations suggest a species-specific role for GMRα in the constitutive activity of I374N, and this is most likely dependent upon the species of origin in which the mutant is being tested.

In contrast to its activity in human UT7 cells, I374N was unable to confer factor independence on human GM-CSF/IL-3-dependent TF-1.8 cells, which also express hGMRα: this implied that in human cells, another cell type-specific molecule, in addition to hGMRα, was required for the constitutive activity of I374N. Intriguingly, this molecule was identified as the human erythropoietin receptor (hEPOR), since co-expression of hEPOR with I374N in TF-1.8 cells conferred factor-independent proliferation. Furthermore, hEPOR and I374N were constitutively tyrosine phosphorylated in factor-independent UT7 cells. Importantly, the bi-directional cross-phosphorylation between hEPOR and hβc was also observed in 'uninfected' UT7 cells stimulated with either GM-CSF or EPO. Notably, this is the first report whereby activation of hβc, either by a constitutive mutation or GM-CSF-binding to the normal GMR, leads to the cross-phosphorylation of EPOR. Although the biological significance of this receptor 'cross-talk' is not yet known, it does imply that receptors can utilize other non-related receptors, via tyrosine phosphorylation, as downstream docking molecules. Presumably, these could then attract other signalling molecules to link the receptor to certain signalling pathways, and in doing so, may provide receptors with a novel mechanism by which to positively or negatively regulate the strength and/or duration of signal transduced.

In addition to furthering our understanding of both the oncogenic potential of GMR/IL-3R/IL-5R and how they and, by implication, other cytokine receptors generate intracellular signals, the above studies also provide a map of the location of activating point mutations in hβc. As one of these mutations (I374N) confers factor independence on human haemopoietic cell lines, such a map should provide a rational basis for screening human haemopoietic disorders for alterations to hβc.