New and Notable

Gathering Support for Critical Mass: Interleukin 4 Receptor Signaling Requires Clustering in Endosomes

Stefan N. Constantinescu[1,*]
[1]Ludwig Institute for Cancer Research, Brussels Branch, and de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

The signaling paradigm for cytokine receptors consists of ligand binding to the extracellular domains of cell-surface-exposed receptors. This first even leads to either dimerization or conformational change of a preformed inactive dimer (1,2), which in turn leads to reciprocal activation of Janus kinase proteins (JAK) that are appended to the cytosolic juxtamembrane regions of receptors. Once JAKs become activated they phosphorylate tyrosines on cytosolic domains of receptors and on JAKs themselves. Phosphorylated tyrosines attract SH2-containing proteins such as members of the signal transducer and activator of transcription (STAT) proteins, which becomes themselves substrates of JAKs (3). In phosphorylated form, STATs dimerize and translocate to the nucleus where they regulate gene expression (3). In addition to JAKs, cytokine receptors attract adaptors that connect to the MAP-kinase and ras/PI-3′-kinase/Akt pathways (2). This process, which physiologically occurs only in the presence of cytokines, can be activated constitutively if JAKs or receptors are mutated and therefore signal in the absence of ligand, as is the case for several cancers, most notably the myeloproliferative neoplasms (MPNs) (4).

In this model, cytokine receptor signaling starts from the cell surface, and its duration critically depends on internalization of receptors, first to endosomes and eventually JAK-stimulated transport to lysosomes for degradation. Internalization is regarded as one major path to signal termination. Certain receptors can be cleaved in the cytosolic domains by proteasomes (5), either on the surface or while en route to lysosomes. In revised signaling models it has been shown that prolonged signaling via certain pathways requires internalization, suggesting that once initiated at the cell surface, certain pathways continue to be activated in intracellular compartments. This was the case for G-CSFR, where certain signaling pathways, as well as lysine ubiquitination and redox-controlled phosphatase activities, are linked to the signaling endosome (6).

A provocative and elegant biophysical study by Gandhi et al. (7) in this issue of the *Biophysical Journal* brings forward data that shift this paradigm. By using fluorescence cross-correlation spectroscopy on functional, but truncated receptors in HEK293 cells, authors show that heteromeric complexes involving interleukin 4 receptor (IL4R) subunits (especially Type-II complexes of IL4Rα and IL13Rα1 coupled to either IL4 or IL13) exhibit very low (100-fold lower than conventional receptors) intersubunit affinity. An elegant approach was used whereby one subunit was fused to green fluorescence protein and the other contained a His tag to which a fluorescent chemical can bind, independently from cytokine binding (7). This system allowed the determination of intramembrane dissociation constants, which are highly relevant for receptor function and cannot be extrapolated from the different affinities of ligands for soluble recombinant extracellular domains. The weak affinity for receptor subunit dimerization makes it impossible for cell surface complexes, which authors show can be formed by ligand, to induce significant levels of signaling at the surface and implies that mechanisms must exist for concentration of active receptors to reach a critical mass for signaling. Authors show that this mechanism is represented by internalization in cortical endosomes, which are closely located to the plasma membrane. Of interest, internalization is constitutive, not requiring ligand binding.

Thus, a novel model is introduced where ligand-binding and conformational changes (or hetero-dimerization changes) at the cell surface do not suffice for IL4R signaling via any of the types of complexes that are formed. Constitutive internalization into cortical adjacent endosomes places ligand-activated receptors in clusters that then can trigger sufficient signaling to induce biologic effects. Remarkably, the conformational changes induced by ligand must be stable during internalization into endosomes, leading to strong JAK-STAT activation on the cytosolic side of the endosomal membrane due to receptor clustering. In other receptor systems such as erythropoietin or growth hormone receptors, which are preformed dimers in the membrane, it has been shown that conformational changes leading to activation require both rotation- and scissorlike movements (8–10). In contrast, IFN receptor subunits appear to be essentially monomeric (11), similar to IL4R receptor subunits (12), but activation requires ligand-induced heteromerization and possibly conformational changes leading JAK activation. It is not clear whether ligand binding in the case of IL4R subunits only induces the required conformational changes and JAK activation in cortical endosomes, or to a low extent below the threshold of signaling at the cell surface as well, where heteromerization is shown to occur.

There are certain potential caveats with this work. One is that IL4Rα needed to be truncated in the cytosolic domain for getting sufficient cell

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*Correspondence: stefan.constantinescu@bru.licr.org

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surface localization for measurements. The truncation preserves the cytosolic box-1, required for JAK binding and activation, but eliminates the cytosolic tyrosine that upon phosphorylation, recruits STAT6. In the physiological setting, binding of STAT6 might stabilize weak dimers and therefore could increase intersubunit affinity at the surface. Another potential caveat is that certain transfected receptors might not have been coupled to JAK proteins and it would be necessary to assess the effect of different levels of JAK proteins on intersubunit affinity. Nevertheless, the pathway presented shows that endosomal clustering can drive signaling for IL4R complexes. This opens new perspectives for the study of cytokine receptor signaling and points again to endosomes as major hubs of signaling. It will be very interesting to assess whether endosomal signaling is also essential for modified IL4 cytokines (superkines) that have been designed to exhibit increased affinity for recruiting IL2Rγ or IL13Rα1 second-chain subunits (13). Finally, changes in ligand-receptor affinity in endosomes have been created by mutagenesis to enhance biologic potency of cytokines such as IL2 or G-CSF. This is done by taking advantage of residues that, when mutated, only decrease affinity for receptors at acidic pH and not at the neutral pH at the cell surface. They then stimulate recycling of ligands, especially because acidic pH appears to stabilize IL2 and G-CSF (14). It will be of major interest to test the precise pH of these cortical signaling endosomes, to determine whether ligand recycling occurs in vivo and investigate how cortical endosomal clustering might regulate signaling by other heteromeric and homomeric cytokine receptors.

One implication of this work also concerns the efforts to inhibit pathologic JAKs in cancer. Starting with the discovery of JAK2 V617F as a highly prevalent somatic acquired mutation in human MPNs, several JAK2 inhibitors have been tested and one has been approved for treatment of MPNs (4). All available JAK inhibitors in the clinic are type-I ATP competitive, targeting JAK kinase domains in the active conformation, which allows rapid recovery for short half-life compounds. Although it is expected that many cytokine receptors would signal from both the cell surface and endosomes, having cortical endosomes cluster receptor-JAK complexes could make inhibition less efficient, given that not all JAKs in such clusters need to be active for signaling to occur.

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