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Combined deletion of the fibronectin-type III domains and the stalk region results in ligand-independent, constitutive activation of the Interleukin 6 signal-transducing receptor gp130

Larissa Lamertz, Doreen M. Floss, Jürgen Scheller*

Institute of Biochemistry and Molecular Biology II, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany

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ABSTRACT

Gp130 is the common receptor within the Interleukin 6 cytokine family. Gp130 consists of 6 extracellular domains followed by a small stalk region connecting the last extracellular domain with the trans-membrane domain. Whereas the first three extracellular domains bind to IL-6-type cytokines, the domains 4–6 are needed for correct positioning of the intracellular domains to facilitate Janus kinase activation after cytokine binding. Interestingly, deletion within the cytokine-binding domain resulted in cytokine-independent constitutive activation of mutant gp130 receptors. Here, we tested the hypothesis, if deletions of the stalk region and/or domains 4–6 of gp130 might also result in constitutive receptor activation. Shortening of the stalk region of gp130 alone did, however, not result in constitutive receptor activation, whereas a gp130 receptor deletion variant only consisting of the three N-terminal cytokine binding domains but lacking all FNIII domains was biologically inactive. Importantly, combined deletion of the three FNIII domains plus shortening of the stalk region of gp130 resulted in ligand-independent, constitutive receptor activation of gp130.

1. Introduction

Cytokine-induced receptor homo- and hetero-dimerization combined with subsequent conformational receptor rearrangements are common features of signal initiation. Interestingly, several reports have shown that ligand-independent dimerization of naturally occurring and synthetic cytokine receptors results in autonomous, prolonged activation of signal transduction. Naturally occurring short in-frame oncogenic deletions in the second of six extracellular domains within the Interleukin (IL)-6 signal-transducing receptor chain glycoprotein 130 (gp130) led to ligand-independent, autonomous activation of the IL-6/IL-11 signaling pathway contributing to inflammatory hepatocellular adenomas [17]. Previously, we have generated synthetic, constitutively active gp130 receptor variants in which the extracellular domain of gp130 was replaced by leucine zippers or the IL-15/sushi system [22,23]. The IL-15/sushi system also allowed to synthetically generate ligand-independent, autonomous heterodimeric cytokine receptor complexes consisting of gp130, LIF, OSMR, WSX-1 and GPL [23].

For signaling, IL-6 normally binds to the non-signal transducing IL-6 receptor (IL-6R), followed by complex formation with the signal-transducing glycoprotein 130 (gp130) co-receptor. IL-6 signaling activates downstream signaling pathways such as Janus kinases/signal transducers and activators of transcription (Jak/STAT), the phosphatidyl-inositol-3-kinase (PI3K) cascade and the mitogen activated protein kinase (MAPK) cascade, through gp130 homodimer activation [6]. Apart from receptor activation, constitutive activation of the gp130-dependent transcription factor STAT3 has been shown to be involved in many human neoplastic malignancies, including multiple myeloma [3,15,16], prostate cancer, melanoma, ovarian cancer, and renal carcinoma [1], as well as gastric cancer [13]. Synthetically dimerized STAT3 proteins exhibits oncogenic potential, and STAT3 was therefore defined as an oncogene [2]. Since the IL-6/gp130 signaling pathway is involved in constitutive activation of STAT3 in tumors [9], also gp130 can be defined as an oncogene [17].

Gp130 is a shared cytokine signaling receptor of the IL-6 family and the founding member of the 'tall' class of cytokine receptors. In these receptors, the cytokine binding domains are connected with the transmembrane region by three large fibronectin type III (FNIII) domains, which are crucially involved in structural conformational changes after cytokine binding to enable activation of intracellular associated Janus kinases (Jaks) [10]. In opposite to gp130, the IL-23 receptor (IL-23R) of the shared IL-6/IL-12 cytokine family has a different domain architecture. The human IL-23R is composed of an N-terminal immunoglobulin (Ig) like domain (D1), a cytokine binding module (CBM)

* Corresponding author.
E-mail address: jscheller@uni-duesseldorf.de (J. Scheller).

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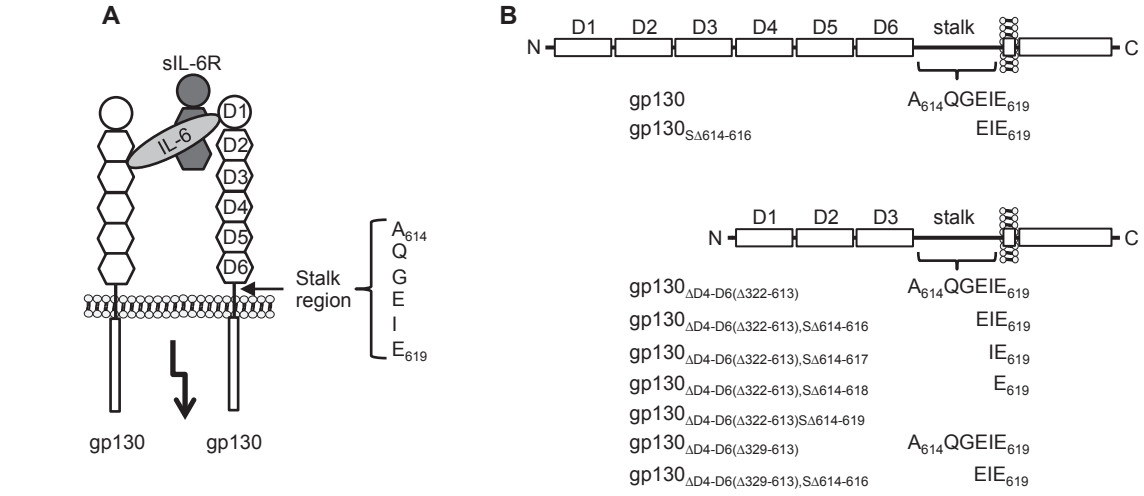

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  1 MLTLQTWLVQ ALFIFLTTES TGELLDPCGY ISPESPVVQL HSNFTAVCVL KEKCMDYFHV D1
 61 NANYIVWKTN HFTIPKEQYT IINRTASSVT FTDIASLNIQ LTCNILTFGQ LEQNVYGITI
121 ISGLPPEKPK NLSCIVNEGK KMRCEWDGGR ETHLETNFTL KSEWATHKFA DCKAKRDTPT D2
181 SCTVDYSTVY FVNIEVWVEA ENALGKVTSD HINFDPVYKV KPNPPHNLSV INSEELSSIL
241 KLTWTNPSIK SVIILKYNIQ YRTKDASTWS QIPPEDTAST RSSFTVQDLK PFTEYVFRIR D3
301 CMKEDGKGYW SDWSEEASGI TYEDRPSKAP SFWYKIDPSH TQGYRTVQLV WKTLPPFEAN D4
361 GKILDYEVTL TRWKSHLQNY TVNATKLTVN LTNDRYLATL TVRNLVGKSD AAVLTIPACD
421 FQATHPVMDL KAFPKDNMLW VEWTTPRESV KKYILEWCVL SDKAPCITDW QQEDGTVHRT D5
481 YLRGNLAESK CYLITVTPVY ADGPGSPESI KAYLKQAPPS KGPTVRTKKV GKNEAVLEWD
541 QLPVDVQNGF IRNYTIFYRT IIGNETAVNV DSSHTEYTLS SLTSDTLYMV RMAAYTDEGG D6
601 KDGPEFTFTT PKFAQGEIEA IVVPVCLAFL LTTLLGVLFC FNKRDLIKKH IWPNVPDPSK
661 SHIAQWSPHT PPRHNFNSKD QMYSDGNFTD VSVVEIEAND KKPFPEDLKS LDLFKKEKIN
721 TEGHSSGIGG SSCMSSSRPS ISSSDENESS QNTSSTVQYS TVVHSGYRHQ VPSVQVFSRS ICD
781 ESTQPLLDSE ERPEDLQLVD HVDGGDGILP RQQYFKQNCS QHESSPDISH FERSKQVSSV
841 NEEDFVRLKQ QISDHISQSC GSGQMKMFQE VSAADAFGPG TEGQVERFET VGMEAATDEG
901 MPKSYLPQTV RQGGYMPQ*
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Fig. 1. Design of gp130 deletion variants. (A) Schematic illustration of gp130 in complex with IL-6 and sIL-6R. IL-6 binds via site I to sIL-6R, via site II to gp130-D2-D3 and via site III to gp130-D1. Gp130 consists of six extracellular domains, domains 1–3 are responsible for cytokine binding followed by FNIII domains 4–6, the 6 amino acid long stalk region, the transmembrane and intracellular domain. (B) Schematic illustration of gp130 and variants thereof analyzed in this study. (C) Amino acid sequence of human gp130: Grey/black: extracellular domains 1–6 and the intracellular domain. Purple/red box: linker peptide spanning the last β-sheet of domain 3 with the first β-sheet of domain 4 as deduced from [24]. Blue: stalk region. Red: trans-membrane region. Myc tag was cloned c-terminal and the amino acid sequence was EQKLISEEDLNGAVE (Nucleotide sequence: GAACAAAAACTCATCTCAGAAGA GGATCTGAATGGGGCCGTCGAGTGA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

formed by the domains 2 and 3 (D2, D3) that carry binding sites for IL-23 [18], but the cytokine binding domains are connected with the transmembrane region by a most likely unstructured stalk region of 37 amino acids followed by the transmembrane domain and the intracellular region. This is of particular importance because the IL-23R receptor complex consists of the IL-23R and the IL-12Rβ1, the latter of which has the typical composition of the tall cytokine receptor family with two cytokine binding domains followed by three FNIII domains. How the functional interaction of the stalk region of the IL-23R with the FNIII domains of the IL-12Rβ1 looks like, has not been addressed so far.

Recently, we demonstrated that extensive deletion of the stalk region of the IL-23R resulted in ligand-independent, constitutive activation of IL-23R homodimers [12]. Moreover, this deletion results in assembly and activation of homodimeric IL-23R complexes [12].

Here, we demonstrate that also the combined deletion of all three FNIII domains of gp130 and complete or partial deletion of the stalk region resulted in constitutive activation of gp130.

2. Results and discussion

We have previously shown that a gp130 variant lacking all extracellular domains was not constitutively active, demonstrating that only the transmembrane domain and intracellular domain alone cannot activate signal transduction [22].

Here, we hypothesized that in analogy to the IL-23R, deletion of the stalk region and/or the FNIII domains might also result in constitutive activation of gp130. First of all, we deleted all FNIII domains of gp130 directly after the last amino acid of domain 3 resulting in gp130ΔD4-D6(Δ322-613) but leaving the original short stalk region intact (Fig. 1A–C). Ba/F3 cells stably expressing gp130ΔD4-D6(Δ322-613) on the cell surface (Fig. 2A and B) were stimulated with Hyper-IL-6 which did not result in any STAT3 phosphorylation (Fig. 3A). As a control for this and all following STAT3 phosphorylation experiments, we showed that STAT3 phosphorylation in Ba/F3 cells expressing wild-type gp130 react with a rapid increase of STAT3 phosphorylation within 5 min and a decline of STAT3 phosphorylation after 120 min due to negative feedback inhibition mediated by SOCS3 [11] (Fig. 3A). Hyper-IL-6 is a fusion protein of IL-6 and the soluble IL-6R and able to stimulate gp130 receptors without the need of the membrane bound IL-6R [4]. This data demonstrates that deletion of D4-D6 resulted in biologically inactive gp130 receptors. This finding was not surprising because truncation of individual FNIII domains already resulted in inactive gp130 molecules without signaling capacity [14]. Later it has been speculated that the functional role of the FNIII domains is the assembly of the transmembrane and intracellular domains in close proximity to each other to allow activation of gp130-associated intracellular Jaks [20,21].