Membrane-proximal binding of STAT3 revealed by cancer-associated receptor variants

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ABSTRACT
In cancer biology, somatic mutations in the extracellular (ligand binding) and cytosolic (functional/catalytic) domains are pursued with great interest. However, in our recent publication we report that germline mutations in the membrane-proximal region of type I receptors are able to modulate the amplitude of signal transducer and activator of transcription 3 (STAT3) signaling in cells. This unexpected finding has implications for the prognosis of heritable cancer.

Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the general population. These genetic differences among individuals predict an individual’s response to certain drugs, susceptibility to environmental factors, and risk of developing particular diseases. Most importantly, SNPs play a significant role in complex diseases such as autoimmune diseases, metabolic disorders, cardiovascular diseases, and cancer. Approximately 30% of non-synonymous SNPs located in coding regions are estimated to impact protein functions, and risk of developing particular diseases. Most importantly, SNPs play a significant role in complex diseases such as autoimmune diseases, metabolic disorders, cardiovascular diseases, and cancer. Approximately 30% of non-synonymous SNPs located in coding regions are estimated to impact protein functions, and risk of developing particular diseases. Most importantly, SNPs play a significant role in complex diseases such as autoimmune diseases, metabolic disorders, cardiovascular diseases, and cancer. Approximately 30% of non-synonymous SNPs located in coding regions are estimated to impact protein functions, and risk of developing particular diseases. Most importantly, SNPs play a significant role in complex diseases such as autoimmune diseases, metabolic disorders, cardiovascular diseases, and cancer. Approximately 30% of non-synonymous SNPs located in coding regions are estimated to impact protein functions, and risk of developing particular diseases. Most importantly, SNPs play a significant role in complex diseases such as autoimmune diseases, metabolic disorders, cardiovascular diseases, and cancer.
the transmembrane segments of all type I membrane proteins in humans indicated that such motifs (YXXQ/C) occurring proximal to the inner membrane are far from rare. Remarkably, such motifs are particularly enriched in human cluster of differentiation (CD) molecules, which are generally considered surface markers for immune cells. Extensive analysis of coding region variants using publicly available SNP data sets obtained from the National Heart, Lung and Blood Institute (NHLBI) Grand Opportunity (GO) Exome Sequencing Project (ESP) Exome Variant Server, the Ensembl variation database release 79, and the Catalog of Somatic Mutations in Cancer (COSMIC) database led to the identification of many similar germ-line variants that introduce cryptic STAT3 binding sites proximal to the membrane. Intriguingly, such germ-line mutations were found to be co-localized with somatic mutations in the COSMIC cancer dataset, thus questioning the de

enhanced STAT3 activation dependent on the FGFR4 p. Gly388Arg variant results from a direct interaction with the Y390RGQ motif proximal to inner cell membranes and is not due to increased tyrosine kinase activity. Several independent assays led us to this conclusion. First, truncated FGFR4 constructs (in which the cytoplasmic segment from the 398th to 802nd amino acids was replaced by YFP [FGFR4 Gly388ΔYFP & FGFR4 Arg388ΔYFP]) co-localized with STAT3. Second, using mass spectrometry we identified the phosphate-modified tyrosine-390 (Y-390), a prerequisite for an SH2 domain interaction, in purified recombinant full-length and truncated FGFR4 Arg388 protein variants. Third, we observed a significant increase in fluorescence resonance energy transfer (FRET) signals in cell membranes co-expressing STAT3-CFP and FGFR4 Arg388ΔYFP compared to cells co-expressing STAT3-CFP and FGFR4 Gly388ΔYFP. Fourth, the FGFR4 Arg388 variant lacking the tyrosine kinase domain was still able to enhance endogenous STAT3 activation. Finally, pull-down of biotin-conjugated transmembrane peptide sequences corresponding to FGFR4 p.Gly388Arg (rs351855-G/A and macrophase stimulating 1 receptor (MST1R) p.Arg983Gln (rs375697146-C/T) germ-line receptor variants from cell membrane extracts of peptide transfectants exhibited increased binding of YXXQ motif containing peptides to endogenous STAT3 compared to peptides lacking the motif, although similar amounts of FGFR were associated with membrane extracts. In the transfectants, YXXQ-containing peptide variants not only colocalized with STAT3, but also enhanced tyrosine phosphorylation and STAT3-dependent promoter activity. Thus, we established the biological function of the membrane-proximal STAT3 binding site in type I receptor proteins independent of its extracellular or intracellular kinase domains. Finally, we validated our mechanistic findings in vivo by assessing the pSTAT3 (Y705) levels
in whole organs and tumors extracted from \( Fgfr4^{G/G} \) and \( Fgfr4^{A/A} \) mice.

We also addressed the eminent question of whether mere recruitment of STAT3 to the inner membrane is sufficient to mediate STAT3 phosphorylation and enhance STAT3 signaling activity. Upon membrane targeting of STAT3 to the plasma membrane using lipid-modified sequence motifs, the tyrosine-705 phosphorylation and activation of STAT3 dramatically increased. Intriguingly, membrane-proximal STAT3 tyrosine phosphorylation events depended on the function of EGFR, although the direct components involved in membrane-proximal phosphate transfer reactions remain to be identified. The preponderance of genetic variations affecting the membrane-proximal STAT3 binding site in immune cell surface markers suggests a function of such germline variations in modulating immune cell responses. Thus, our work demonstrates that membrane-proximal STAT3 interaction sites modulate the amplitude of the STAT3 signaling pathway.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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