#227 Over-expression of EphB1 leads to Stat3 phosphorylation

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Abstract

The Receptor Tyrosine Kinase (RTK) family comprises some important disease drivers. Over-expressed or mutated RTKs in disease may lead to aberrant phosphorylation of intracellular signaling molecules. Therefore it is desirable to monitor simultaneously the activation levels of RTKs and signaling nodes. Here we used a multiplexed antibody array to monitor the phosphorylation of 28 RTKs and 11 intracellular signaling molecules simultaneously. We have observed a strong signal on a spot corresponding to phospho-Stat3 (Tyr705) with lysates derived from CHO cells transiently over-expressing EphB1. The strong Phospho-Stat3 signal was not seen in non-transfected cells or in lysates made form cells transfected with several other Eph family members. Western blotting with phospho-specific antibodies showed an increase in Stat3 phosphorylation on Tyr705 in CHO cells upon EphB1 ectopic expression. The amounts of Tyr705 Stat3 phosphorylation positively correlated with the levels of EphB1 protein. Co-precipitation experiments showed that EphB1 and Stat3 interact both in CHO cells and MCF-7 breast cancer cells. Phosphorylated Jak2 was not found in these immuno-precipitates suggesting an involvement of another tyrosine kinase in Stat3 phosphorylation in cells over-expressing EphB1. Consistently with these observations, a potent Jak1 and Jak2 inhibitor INCB018424 inhibited the basal but not the EphB1 induced Stat3 Tyr705 phosphorylation in transfected CHO cells. These results suggest that EphB1 over-expression may bypass the requirement in Jak kinase and lead to Stat3 activation either directly or through another receptor-tethered kinase.

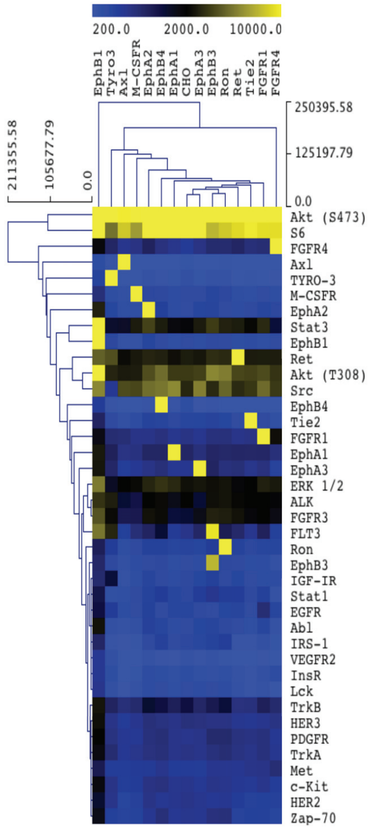

Fig. 1: Heat map display of PathScan® RTK array (fluorescent readout) results. CHO cells were transiently transfected with various RTKs.

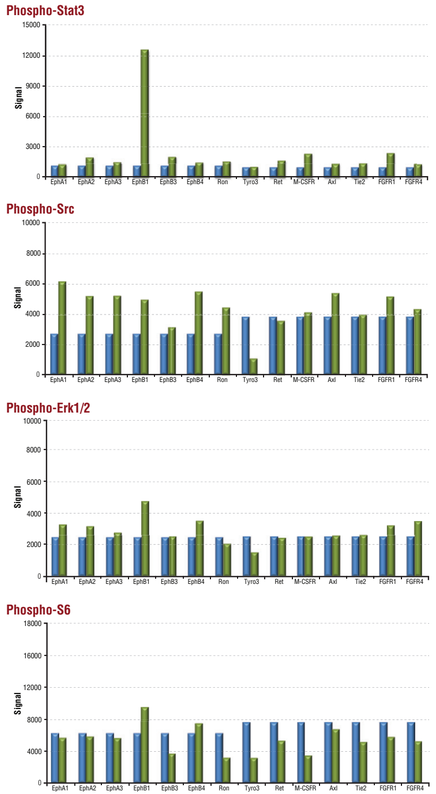

Fig. 2: Specific signal associated with the spot corresponding to Phospho-Stat 3 (Tyr705) is seen upon overexpression of EphB1. CHO Cells were transfected with expression vectors encoding various RTKs. Lysates were prepared and analyzed using PathScan® RTK array (Fluorescent Readout #7949). Signals associated with spots corresponding to p-Stat3, p-Src, p-Erk1/2 and p-S6 are shown.

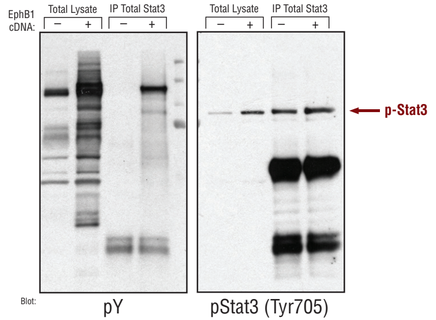

Fig. 3: Evidence for EphB1 and Stat3 interaction in CHO cells transfected with EphB1-Flag. (Stat3 antibodies used in IP #9312 Rabbit polyclonal)

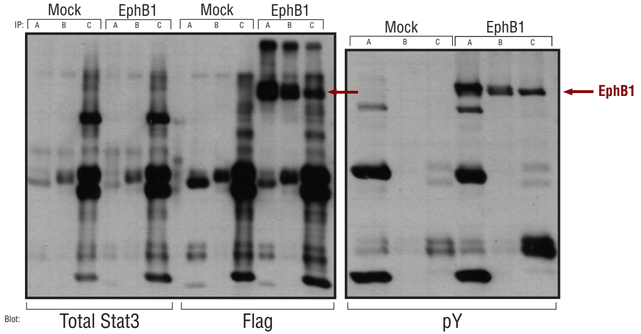

Fig. 4: Co-precipitation of ectopically expressed EphB1-Flag with endogenous Stat3 in CHO cells. IP with EphB1 ectodomain mAb **(A)** Flag antibodies **(B)**, Stat3 Rabbit mAb Antibodies #2358 **(C)**.

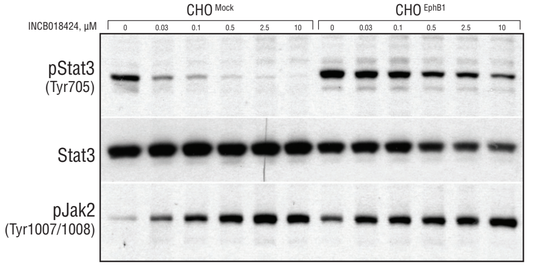

Fig. 5: EphB1 induces Stat3 (Tyr705) phosphorylation. CHO cells were either mock transfected or transfected with EphB1 encoding construct. Cells were treated with various concentrations of the Jak inhibitor compound INCB0184242 for 1.5 hrs. Lysates were analyzed by western blot with various antibodies indicated in the figure.

Conclusion

- Evidence for interaction between Stat 3 and EphB1 was obtained by co-precipitation experiments. EphB1 was overexpressed in CHO cells and was immuno-precipitated using two different Stat3 antibodies directed against two distinct epitopes within Stat3. The interaction between Stat3 and EphB1 could be either direct or indirect (part of a complex).
- Overexpression of EphB1 leads to increase in Stat3 phosphorylation on Tyr705. Increase in the phosphorylation of Stat3 on Tyr705 is typically associated with its activation and translocation to the nucleus to modulate gene expression.
- The tyrosine kinase that is responsible for the increase in Stat3 Tyr705 phosphorylation in cells overexpressing EphB1 is not of the Jak family. This is evidenced by the lack of inhibition of EphB1 induced Stat3 Tyr705 phosphorylation by a Jak inhibitor compound INCB018424.