Repository Text

Methods

Cell lines and Transfections

IL-6R expressing Cos-7 (Origene) cells and U3A cells (provided by G. Stark, Cleveland Clinic, Ohio), were used. The cells were maintained in complete Dulbecco modified Eagle medium (DMEM; Life technologies, NY) supplemented with 10% FCS, 2mM L-glutamine, HEPES and antibiotics. Transient transfections were carried out using either electroporation (Lonza, Germany) or Lipofectamine 2000® transfection reagent (Life technologies, NY) following manufacturers' protocol.

Plasmids and constructs

Plasmid encoding Myc-DDK tagged wild type STAT3 transcript variant 1 was purchased from Origene Technologies, MD, USA (RC215836). Point mutations were introduced in the wild type sequences using point mutagenesis PCR and the resulting PCR products were cloned into the pCM6-XL4 background (BioInnovatise, Rockville, MD). Endotoxin-free plasmid preparations were made using Endo Free plasmid kits purchased from Qiagen (Germany).

STAT3 phosphorylation assay

Cells transfected with plasmids encoding wild type or mutant *STAT3* were stimulated with oncostatin M (OSM; 50ng/ml) or IL-6 in increasing concentrations (20ng, 100ng, 200ng, 1,000ng) for 30 min at 37°C. Cells were then fixed with 4% paraformaldehyde (Electron Microscopy Sciences, PA), permeabilized with 100% methanol and stained for STAT3 (BD APC) and phosphoSTAT3 (BD AF 488, pY705). Fold induction of phosphoSTAT3 (Y705) in OSM or IL-6 stimulated cells over non-stimulated cells was calculated using the geometric mean

of the fluorescent values and the fluorescent values are denoted as MFI values (Mean Fluorescent Intensity).

All data were acquired on either FACS Calibur or BD LSR Fortessa (BD Biosciences). A minimum of 50,000 counts were acquired within the live cell population gate for each tube. Data were analyzed using FlowJo version 9 (Tree Star Inc.).

Protein Models

The 3D models of STAT3 were generated using I-TASSER server. The electrostatic potential was calculated with APBS and displayed using UCSF Chimera.

References

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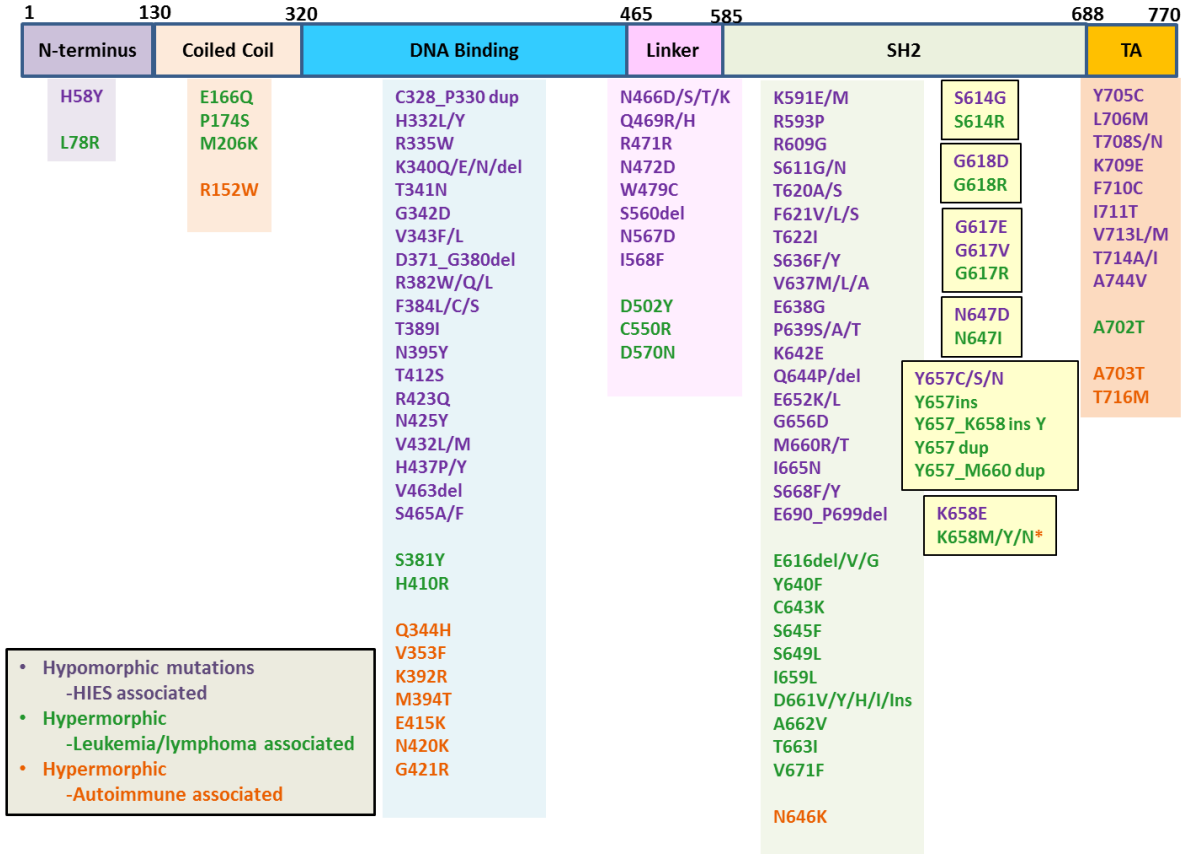
Supplementary figure legend

Figure E1

Previously reported *STAT3* mutations by STAT3 domain. Hypomorphic dominant negative mutations found in HIES (Job's) patients are indicated in purple; hypermorphic gain of function mutations found in leukemia or lymphoma patients are in green; hypermorphic gain of function mutations found in autoimmune disorders are in orange.

Figure E1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585
688
770
N-terminus
Coiled Coil
DNA Binding
Linker
SH2
TA
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M206K
R152W
C328_P330 dup
H332L/Y
R335W
K340Q/E/N/del
T341N
G342D
V343F/L
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K709E
F710C
I711T
V713L/M
T714A/I
A744V
A702T
A703T
T716M
• Hypomorphic mutations
-HIES associated
• Hypermorphic
-Leukemia/lymphoma associated
• Hypermorphic
-Autoimmune associated