The thrombopoietin receptor P106L mutation functionally separates receptor signaling activity from thrombopoietin homeostasis

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c-Mpl, what is known?

- Phosphorylation of JAK2 activates downstream pathway
- Gain-of-function mutations at positions 39, 106 (adjacent to 102 and 104 l.o.f. mutations), 505, 515
- Active as dimer*

c-Mpl dimerization: Two existing models
Dimerization upon ligand binding on the surface?

Downstream pathway activation (incl. Stat 3, Stat 5)
Dimerization before ligand binding and before reaching the cell surface?


Clinical impact of c-Mpl gain-of-function mutations

Essential Thrombocytosis

Different propensity to induce malignancies?

- c-Mpl S505N (germline and somatic)
- W515K/L (somatic)
- c-Mpl P106L (frequent in Arabic countries) (germline)


c-Mpl P106L (germline) gain-of-function mutation
Model system and proof-of-concept

- Transient expression in HeLa cells
- Stable expression in Ba/F3 (hematopoietic cell line)

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Proper glycosylation is not a prerequisite for c-Mpl receptor function

**double** band, fully glycosylated form (75+85 kD):
- c-Mpl wildtype
- c-Mpl F104S

**single** band, impaired glycosylation (75 kD):
- c-Mpl R102P
- c-Mpl P106L

Full glycosylation does not predict a functional receptor!

loss-of-function
gain-of-function
c-Mpl R102P and P106L mutants cannot be processed to the normal, tunicamycin sensitive, strongly glycosylated form.
The impaired processing is not THPO dependent
Impaired glycosylation correlates with impaired subcellular distribution

Full glycosylation requires proper golgi processing

loss-of-function

gain-of-function
Impaired glycosylation and golgi expression correlates with impaired cell surface expression
Quantification by FACS

C

HA-c-Mpl WT

HA-c-Mpl R102P

HA-c-Mpl F104S

HA-c-Mpl P106L

D

n.s. p=0.84

***p=0.00039

***p=0.00026
Impaired glycosylation and surface expression does not correlate with impaired receptor function!
THPO uptake from cell culture supernatants

THPO uptake is reduced in c-Mpl mutants
Serum THPO levels cannot predict a loss-of-function or a gain-of-function mutation

How does THPO enter the cell if the receptor is not present on the cell surface?

- trace amounts are sufficient?
- other receptors?
Outlook: How does P106L differ from other gain-of-function mutations?

I. HA-cMpl-wildtype-GFP
   - no treatment (la)
   - anti-HA w/o triton (lb)
   - overlay (lc)

II. HA-cMpl-P106L-GFP
   - no treatment (ld)
   - anti-HA with triton (le)
   - overlay (lf)

III. HA-cMpl-S505N-GFP
   - no treatment (lld)
   - anti-HA with triton (lle)
   - overlay (llf)

IV. HA-cMpl-W515K-GFP
   - no treatment (iv)
   - anti-HA with triton (ive)
   - overlay (ivf)

V. HA-cMpl-W515L-GFP
   - no treatment (v)
   - anti-HA with triton (ve)
   - overlay (vf)
Hypotheses

- THPO and platelet homeostasis depends on correct processing and surface expression of the receptor whereas downstream signaling does not.
- c-Mpl dimerization can occur before cell surface expression.
- Differential dimerization in c-Mpl mutants might explain the different propensity to induce myeloproliferative malignancies.
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