MPN CALR MUTANTS PROMOTE CELL-SURFACE LOCALIZATION OF TPOR WHICH IS OBLIGATORY FOR ONCOGENESIS: NOVEL THERAPEUTIC AVENUES AND RESCUE OF CONGENITAL THROMBOCYTOPENIA TPOR MUTANTS

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Abstract: S153

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Background

Mutant calreticulins are major drivers of myeloproliferative neoplasms (MPNs) by activating TpoR/MPL and JAK2 signaling. However how exactly this interaction occurs, and in which cell compartment this abnormal TpoR activation will lead to an oncogenic signaling remains unknown.

Aims

Our objectives were: 1) to determine the precise sequences of CALR mutant and TpoR required for interaction, traffic and activation, 2) whether disease-inducing TpoR signaling occurs from intracellular compartments or cell-surface, 3) whether CALR mutants can also interact and activate defective TpoR mutants, such as TpoR mutants in congenital amegakaryocytic thrombocytopenia (CAMT) and 4) whether CALR mutants are secreted.

Methods

Engineered CALR and TpoR mutants were analyzed by a combination of biochemical approaches (thermal shift assay in cells and on protein), functional assay (cell growth assay, luciferase assay, flow cytometry, primary megakaryocytic clonogenic assay) and cell imaging (confocal microscopy).

Results

1) We map the required sequences for interaction between mutant CALR and TpoR extracellular domain. A minimum of 10 proximal aminoacids of the new CALR tail and sequences of the N-terminal globular domain, but not the P-domain are required for TpoR activation. We also isolated a complex between extracellular domain of TpoR and CALR del52 produced in insect cells and mapped the required N-glycosylation profile required for complex formation. 2) We identify a specific region of 8 aminoacids in D1 TpoR that can confer the ability to activate JAK2-STAT5 to another receptor (EpoR) after binding of mutant CALR. Mutation to alanines of this TpoR region (TpoR 8A) abolishes response to CALR mutant. 3) As a direct consequence of mutant CALR-TpoR interaction, the thermal stability of TpoR was found to significantly increase in the presence of CALR del52. Such an increase was not observed with EpoR. However, engineered EpoR that can induce CALR del52 dependent JAK2-STAT5 activation also showed increased thermal stability with CALR del52. Concurrently, neither TpoR mutated at key asparagine residues involved in N-glycosylation nor TpoR with Alanine mutations could show enhanced thermal stability with CALRdel52. 4) We provide genetic evidence that the cell-surface TpoR-CALR mutant complexes are obligatory for hematopoietic cell transformation. Mutant CALRs and TpoR must pass via the Golgi apparatus to transform, and they co-localize in the Golgi compartments. The cell-surface complexes of active tyrosine phosphorylated forms of TpoR-JAK2 induced by mutant CALRs are internalized and can be detected in early endosomes, suggesting prolonged signaling in that compartment. 5) We show that both CALR del52 and ins5, but not wild type CALR are able to rescue traffic and cell-surface localization and signaling by the CAMT TpoR R102P mutant. Mutant CALRs promote folding and stability of TpoR R102P. This effect can also be seen with TpoR P106L and other defective engineered TpoRs. 6) Mutant CALRs are secreted to levels of 0.1-0.2 µg/ml in patients.

Conclusion

We show that mutant CALRs act as rogue chaperones, leading to folding and cell-surface localization of TpoR, which is obligatory for hematopoietic cell transformation. Mutant CALRs can rescue defective TpoR in CAMT to cell-surface localization and activation. Moreover our data indicate that cell-surface mutant CALR is crucial for oncogenicity. We discuss avenues of targeting mutant CALRs on the cell-surface and potential effects of secreted mutant CALR.

Session topic: 15. Myeloproliferative neoplasms – Biology & Translational Research
Keyword(s): Myeloproliferative disorder, Signaling, Thrombopoietin (TPO)