Thesis Title: Differential signalling by type I and type II calreticulin (CALR) mutations in myeloproliferative neoplasm

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Abstract

Myeloproliferative neoplasm (MPN) is the disorder of hematopoietic stem cells and progenitor cells with various mutations implicated in its pathogenesis, including Janus kinase 2 (JAK2), thrombopoietin receptor (TpoR), and calreticulin (CALR) mutations. MPN patients harbouring frameshift CALR mutations exhibit megakaryocyte hyperplasia, thrombocytosis, with or without marrow fibrosis. Two of the most prevalent mutations implicated in 80% of CALR-mutated patients are CALR Type I (Δ52) and CALR Type II (ins5) mutations. All the CALR mutations are characterised by a unique C-terminal tail rich in positively charged amino acids and loss of KDEL ER retrieval sequence located in the exon 9 tail of the CALR gene. Mutant CALR binds to the N117 residue in the extracellular domain of the TpoR to dimerise the receptor inducing ligand-independent activation of TpoR. This renders the JAK2/STAT1/3/5 pathway active, promoting megakaryopoiesis, leading to MPN. The CALR mutants are efficient in driving ligand-independent activation of TpoR. Despite this, the difference between the two CALR mutants lie in the prognosis and prevalence. The Type I CALR harbouring patients have higher myelofibrosis cases, whereas younger age at presentation is seen in Type II CALR patients. This difference in patient outcome cannot be explained by the ability of CALR Type I and Type II mutants to activate TpoR. Furthermore, CALR mutations are not restricted to the myeloid lineage but penetrate the lymphoid lineage. This highlights the possible oncogenic capabilities of mutant CALR beyond TpoR and JAK-STAT pathway. Therefore, studying the oncogenic properties of mutant CALR independent of TpoR is imperative. Moreover, KDEL deletion enables the mutant CALR to secrete from the ER and is reported to be found in the Golgi (cis and trans), vesicles, cell membrane, and even in the nucleus. Thus, we hypothesised that mutant CALR might have gained new interacting partners in the various compartments. Hence, we cloned and expressed the recombinant CALR WT and mutant tail. The bead-bound recombinant CALR WT and mutant tails were used as the bait to perform a pulldown followed by mass spectrometry to identify their interacting partners. We identified 21 pulldown partners of the CALR mutant tail, out of which 8 were shared with CALR WT tail. Among the shared pulldown partners of CALR WT and mutant tail, we report ARHGAP21 (a GTPase activating protein (GAP) for CDC42 activity) and USP9X, (a deubiquitinase) interacted with CALR WT and Ins5 exclusively. We showed TGFBR3, co-

receptor for TGF-β signalling, to interact with CALR WT/Δ52 /ins5 tail. TGFBR3, is the third receptor to interact with CALR mutants apart from the already reported and characterized receptors TpoR and Transferrin receptor. Moreover, VPS13C and BORC5, which are involved in the lysosome movement and distribution, were found to interact with CALR WT and mutants. Interestingly, our results suggested low lysosomal count resulting in low mTOR colocalization on the lysosomes in CALR mutants (Δ52 and ins5) compared to the CALR WT. However, the difference between the two CALR mutants was apparent in terms of lysosomal distribution inside the cells. On one hand, CALR Δ52 exhibited majorly perinuclear clustering of lysosomes whereas CALR ins5 showed peripheral localization of the lysosomes, leading to robust activation of mTOR (S2481) by CALR ins5 and diminished mTOR (S2481) activity by CALR Δ52. This differential activation of mTOR (S2481) by the CALR mutants disappears when supplemented with pyruvate-rich medium. Therefore, CALR mutations differentially dictate the lysosomal dynamics impacting their number and distribution by modulating mTOR signalling. Furthermore, we also report downmodulation of basal mTORC2-Akt S473 axis mediated through c-JUN in CALR Type I mutant. Type II CALR mutations rendered robust activation of the mTORC2-Akt S473 axis. Our data supports that Type I CALR mutation compromised mTORC2 assembly which was rescued either by overexpression of mTORC2 assembly subunits RICTOR and mSIN1 or by c-JUN knockdown. We report that CALR Δ52 occupies the promoter and distal enhancers of *jun*, hence, directly regulating c-JUN expression through chromatin immunoprecipitation. Finally, we observed implications of downregulated mTORC2-Akt S473 axis in terms of reduced glucose uptake and ATP levels in cells expressing Type I CALR mutations. This was reversed by c-JUN knockdown. Hence, c-JUN mediated mTORC2-Akt S473 axis interplay was found to be responsible for diminished glucose metabolism in CALR Type I mutant. In contrast, CALR Type II mutations enhanced the glucose uptake in the cells by activating basal mTORC2-Akt S473 signalling, demarking a crucial difference between the two CALR mutations.