Nuclear export dynamics in the presence and absence of Hop

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Introduction: Heat shock proteins Hsp70 and Hsp90 are important proteins in the molecular chaperone network, working in complex with Hsp70/Hsp90-organising protein (Hop/STIP1), a co-chaperone of the dynamic multichaperone heterocomplex. Through proteomic analysis, we identified proteins interacting with Hsp70 in Hop expressing and Hop depleted cells, and analyzed the biological processes associated with the interactomes via gene ontology (GO) analysis. This analysis identified a putative relationship between Hop and proteins of the nuclear-cytoplasmic export machinery, NXF1 (export factor for mRNA), CRM1 (protein and mRNA export factor), ALYREF, RAN and NXT1 (export co-adaptors) and selected nucleoporins of the nuclear pore complex NPC).

Methods: Subcellular localization of the export factors NXF1, CRM1 and co-adaptors ALYREF, RAN and NXT1 and nucleoporins in HEK293T and HeLa wild type (WT) and Hop CRISPR knockout (KO) cells was observed using immunofluorescence staining. Protein levels of the nuclear export factors and selected nucleoporins were studied via immunoblotting. The export of intronless polyA mRNA in WT and HOP KO cells and the export of intron containing RNA was studied using the retroviral constitutive transport element (CTE) RNA, which hijacks the human NXF1 export machinery to be exported through the NPC. Transcripts levels of specific mRNA exported specifically by NXF1 and CRM1 export pathway was studied using quantitative Reverse Transcription - Polymerase Chain Reaction (qRT-pPCR).

Results: Our results show that Hop depletion influences the nuclear to cytoplasmic distribution of nuclear export factors and NPC nucleoporins depending on the cell type. RNA export dynamics were affected by the depletion of Hop and selected transcripts of the NXF1 and CRM1 export pathway showed significant changes in nuclear to cytoplasmic distribution in Hop KO cells. Changes in protein levels of the nuclear export factors and nucleoporins were observed with loss of Hop and Hsp70 inhibition. Taken together, these data suggest that Hop is involved nuclear to cytoplasmic transport via regulation of the levels and localization of the nuclear export machinery via Hsp70.

Conclusions: Our results offer the first insight into the role of Hop on mRNA and protein nuclear export.

Keywords: *Hop, heat shock proteins, nuclear export, protein export, mRNA export, nucleoporins, nuclear pore complex*