Integrated Nuclear Interactomics and Phosphoproteomics Identify RNA-Binding Proteins as Functional Targets of NEK2 in triple negative breast cancer

Mohammad Salik Zeya Ansari1,2, Tobias Longin Haas3 , Uwe Warnken4 , Claudio Sette1,2* and Chiara Naro1,2* 1 Department of Neuroscience, Section of Human Anatomy, Catholic University of the Sacred Heart, 00168 Rome, Italy 2 GSTeP-Organoids Research Core Facility, IRCCS Fondazione Policlinico A. Gemelli, 00168 Rome, Italy 3 Department of Translational Medicine and Surgery, Catholic University of the Sacred Heart, Rome, Italy. 4 Functional Proteomic Analysis, German Cancer Research Center (DKFZ), Heidelberg, BW, Germany.

Background: NEK2 is a cell cycle regulated serine/threonine kinase, controlling centrosome duplication at mitosis. In cancer, NEK2 upregulation promotes aneuploidy, as well as its aberrant localization in the nucleus, which enables interaction with splicing factors and oncogenic dysregulation of alternative splicing (AS). High nuclear levels of NEK2 are displayed by triple-negative breast cancer (TNBC) cells, whose transcriptome was found to be sensitive to NEK2 depletion. However, a comprehensive map of its nuclear partners is yet to be defined. Method: To dissect the nuclear interactome of NEK2, we performed a targeted two-step co-immunoprecipitation (TIP) coupled with mass spectrometry (IP-MS) on nuclearlysates of MDA-MB-231 TNBC cells. In parallel, quantitative LC-MS/MS phosphoproteomics was conducted on nuclear extracts of MDA-MB-231 cells acutely treated with the selective NEK2 inhibitor CMP3A, to capture its direct substrates. Results: Interactome profiling identified 162 high-confidence nuclear partners, enriched for RNA-binding proteins (RBPs), involved in multiple steps of RNAmetabolism, including spliceosomal core factors. Concurrently, phosphoproteomic analysis revealed 293 downregulated and 172 upregulated phosphopeptides upon NEK2 inhibition. Downregulated phosphopeptides were enriched for proteins related to RNA transcription, whilst proteins related to RNA splicing and translation were enriched among those upregulated. However, minimal overlap was observed between NEK2 nuclear interactome and the phosphoproteome sensitive to its inhibition, with only 8 interacting proteins showing reduced phosphorylation following CMP3A treatment. Conclusion: Mapping the nuclear interactome of NEK2 identifies numerous proteins, yet very few are direct kinase targets sensitive to its acute chemical inhibition. This suggests that NEK2 nuclear interactome is primarily composed of cofactors and functional partners, rather than canonical substrates. Furthermore, the prominence of RNA binding proteins within the NEK2 nuclear interactome suggests that its oncogenic activity in TNBC cells is partly mediated by a direct functional impact on RNA metabolism.