Nucleophosmin interactome in acute myeloid leukemia

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Nucleophosmin (NPM), one of the most abundant nucleolar phosphoproteins, is involved in initiation and/or development of acute myeloid leukemia (AML). AML-associated *NPM1* gene mutations lead to altered aminoacid sequence at the C-terminal part of NPM and to aberrant cytoplasmic localization of the mutated protein (NPMmut). NPM serves as a chaperone, and majority of its interactions are mediated by its N-terminal oligomerization domain, although proteins interacting with regions near the NPM C-terminus were also reported. In any case, NPM broad interaction network is significantly affected by the mutation: whereas some interactions are disrupted, others are retained, the interacting proteins being dislocated along with the NPMmut. By combination of standard biochemical methods and time-resolved fluorescence techniques, we established a robust experimental system for analysis of NPM interactome in both cell lysates and live cells. We focused in detail on impact of NPM mutations on NPM interaction with tumor suppressor p53 and we analyzed an effect of nuclear-export-inhibitor, selinexor, on this complex. We confirmed the presence of p53 in cytoplasm of NPMmut co-transfected cells suggesting the NPM-p53 interaction is not disrupted by the AML-associated NPM mutation. N-terminal NPM oligomerization domain was also found to be non-essential for the interaction. These results were confirmed both in cell lysates by immunoprecipitation and in live cells by FLIM-FRET measurements. Another NPM variant, lacking N-domain and concurrently mutated at the C-terminus, caused extensive p53-cytoplasmic localization, but its interaction with p53 was substantially attenuated. We monitored time-course of selinexor-induced NPM/p53 nuclear relocalization and we found that individual proteins revert into the nucleus with different rates. These results indicate highly dynamic character of the NPM-p53 interaction. The work was supported by the Czech Science Foundation grant No. 19-04099S.