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Consequences of C-terminal nucleophosmin mutations in acute myeloid leukemia

Nucleophosmin (NPM) is an abundant nucleolar protein playing multiple roles in many cellular processes, including ribogenesis, cell division, or DNA-damage repair, and it also functions as a chaperone. Mutations of nucleophosmin gene (*NPM1*) occur in one-third of patients with acute myeloid leukemia (AML). Due to numerous interactions with other nucleolar proteins and ribosomal RNAs, the wild-type NPM (NPMwt) is localized mainly in the nucleolus, a protein-rich membraneless high-density complex formed inside the cell nucleus. Characteristic AML-related mutations of NPM C-terminus cause aberrant localization of the mutated protein (NPMmut). The most frequent mutation type A (mut A) is a result of incorrect doubling of 4-bp sequence leading to frameshift and synthesis of alternate C-terminal aminoacid sequence. The NPMmutA is detected in 75-80% of patients with mutated *NPM1*. So far, more than one hundred NPM mutations were described. Their impact is not clearly elucidated, however, all the mutations are manifested by delocalization of the resulting protein into the cytoplasm. The presence of isolated *NPM1* mutation is a favorable prognostic factor for AML therapy. We aim to solve consequences of the NPMmut cytoplasmic localization for AML and to describe the effect of anticancer drugs on NPM and on its interaction partners.
We monitor the expression and localization of NPM and of other nucleolar proteins, nucleolin (NCL) and fibrillarin (FBL), both *in vitro* by immunofluorescence and *in vivo* using confocal microscopy and time-resolved fluorescence methods [1]. With help of fluorescent protein tags, we also perform a time-lapse measurement of live cell response to treatment with cytostatics. We have found, that whereas the NPMmut is always present in the cell cytoplasm, it may also reside in the nucleolus, together with the NPMwt, dependently on the cell line (Fig. 1) [2]. We observed a substantial fraction of nucleoli-localized NPMmut in particular in HeLa cell line, which is characterized by high endogenous NPM expression and, simultaneously, by low production of the exogenous protein. Thanks to wt-mut heterooligomer formation, a part of the exogenous NPMmut is retained in the nucleolus. On the other hand, in the HEK-293T cell line with high expression of exogenous proteins, the nucleolar localization of NPMmut is found only in cells concurrently transfected with vector coding for NPMwt.

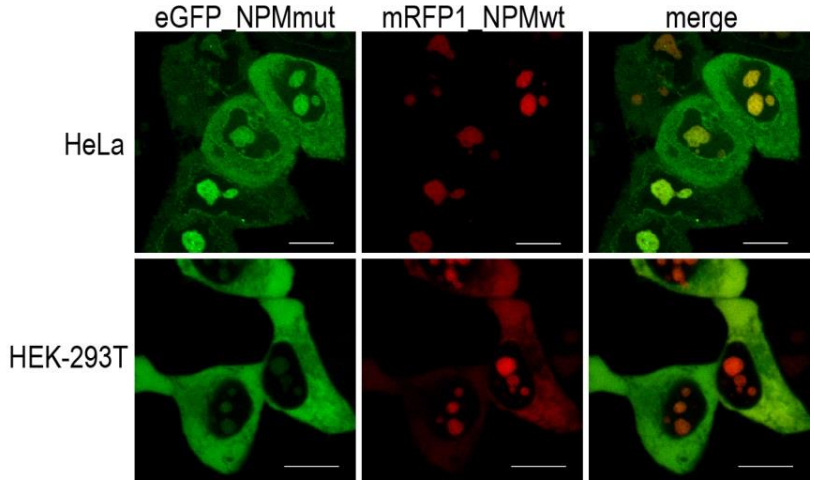

Fig. 1: Localization of mutated (eGFP_NPMmut, green) and wild-type (mRFP1_NPMwt, red) NPM in different cell lines transfected with plasmids coding for fluorescently tagged NPM variants. Upper line: HeLa, lower line: HEK-293T. Bars represent 10 μm.

We demonstrated that NPM mutation impedes NPM interaction with other nucleolar proteins [3]. We also documented that although NPM N-terminal domain, which is responsible for its oligomerization, is indispensable for its interaction with numerous other proteins, the truncated NPM lacking this oligomerization domain binds the NCL and FBL with higher affinity compared to full-length NPM [4]. Our recent results proved NPM interaction with the tumor suppressor p53, and we described persistence of this interaction also in the presence of NPM mutation, which leads to p53 cytoplasmic delocalization (Fig.2) [5]. Cytoplasmic p53 is probably more prone to degradation by proteasome. On the other hand, the p53 in cytoplasm is more easily accessible for mitochondrial apoptotic pathway. The processes requiring the p53 activity are thus altered in the cells containing NPMmut. Currently, we analyze human analog of Mdm2 protein, a ubiquitin ligase significantly regulating the p53 level and activity. Mdm2 was reported to interact also with NPM under stress conditions. Our experiments indicate that aberrant cytoplasmic Mdm2 localization in cells expressing NPMmut is mediated by mutual interactions of both the Mdm2 and the NPMmut, with p53.

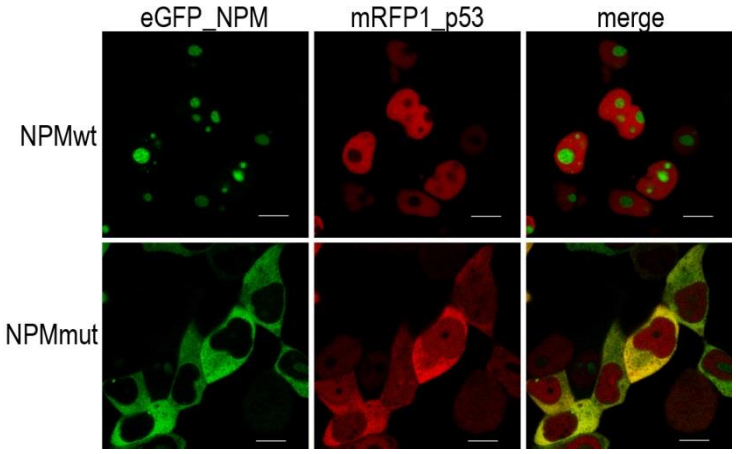

Fig. 2: Localization of mRFP1-tagged tumor suppressor p53 (red) in HEK-293T cells co-transfected with eGFP_NPMwt or eGFP_NPMmut (green). Bars represent 10 μm.

We elaborated a method enabling to monitor the NPM oligomerization and interactions with other proteins directly in live cells [6]. In cooperation with the Institute of Physics (Charles University), we use methods of fluorescence lifetime measurement (FLIM) to detect protein-protein interactions with help of fluorescence energy transfer (FRET) between two fluorophores, eGFP and mRFP1. During these experiments, we uncovered a unique characteristic of eGFP - fluorescence lifetime shortening after defined illumination with excitation beam [7]. We utilised this photoconversion to follow the protein dynamics after cell treatment with cytostatics. Using this method, we proved that the nuclear relocalization of the NPMmut and p53, induced by a specific inhibitor of nuclear export, have different dynamics, despite the mutual interaction of the proteins [5]. Currently, we apply the FRET method on the complex of three differently labeled proteins to simultaneously monitor interactions among individual complex members. This approach allows us to follow Mdm2 localization changes in cells expressing NPMmut treated with inhibitors of p53/Mdm2 interaction (Nutlin-3a, RITA). Together with experiments on cell lysates we analyze the role of specific NPM phosphorylations, in particular of Serine 4 and Threonine 199, on mechanism of action of these inhibitors.

We also established an immunofluorescence method for the detection of NPM mutation in AML patient samples (Fig. 3), which correlates well with the results of genetic analysis.

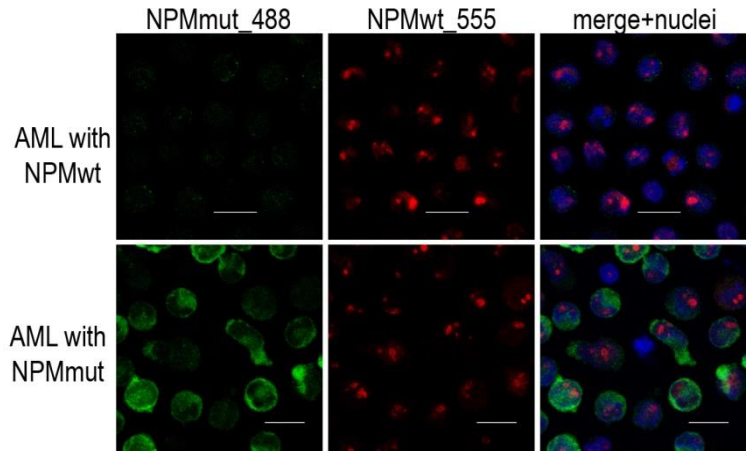

Fig. 3: Immunofluorescence staining of NPMwt (AlexaFluor555, red) and NPMmut (AlexaFluor488, green) in cells isolated from the peripheral blood of AML patients without (upper line) or with the C-terminal NPM mutation (lower line).The nuclei are counterstained with Hoechst 33342 (blue). Bars represent 10 μm.

References:

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