ABSTRACT

Nucleophosmin 1 (NPM1) is predominantly localized in the nucleolus and occurs mainly in oligomers formed through its N-terminal domain (NTD). As a transport facilitator and chaperone, NPM1 has a wide range of interacting partners including tumor suppressors p53 and p14Arf. Characteristic C-terminal mutations in NPM1 are reported in approximately 30 % of acute myeloid leukemia (AML) cases and cause aberrant cytoplasmic localization of mutated (mut) NPM1. As a result, many NPM1-interacting proteins, including wild type (wt) NPM1, are relocalized to the cytoplasm. In order to analyze interactions and the oligomeric state of NPM1, we have introduced and optimized several *in vitro* techniques – native and semi-native polyacrylamide gel electrophoresis and immunoprecipitation – as well as *in vivo* confocal microscopy and time-resolved fluorescence approaches. Using these methods, we revealed that mutations at the C-terminal domain of NPM1 prevent it from binding nucleolar protein nucleolin (NCL), which has previously been shown to interact with the central part of NPM1, and that drug-induced relocation of mutNPM1 to close proximity of NCL does not induce mutNPM1-NCL complex formation. We proved a lowered stability of mutNPM1-formed oligomers as compared to the wtNPM1 ones, which could be useful for NPM1 oligomer-targeting drugs design. NPM1 oligomerization domain also often serves as an interface for interaction with other proteins. Thus, targeting the NPM1 oligomerization domain might weaken mutNPM1 interaction ability and allow the proper localization and function of misplaced proteins to be restored. We therefore constructed NPM1 variants with mutations or deletions within NTD and analyzed their oligomerization characteristics and binding ability to wtNPM1. While point mutations did not cause significant effects on the NPM1 oligomerization, partial or complete deletion of NTD efficiently prevented NPM1 complex formation. Nevertheless, all the N-terminal mutated variants were found to coprecipitate wtNPM1 to some extent. Finally, we examined the effect of NSC348884, a putative inhibitor of NPM1 oligomerization. When administered in recommended concentrations, NSC348884 induced apoptosis of all AML cell lines, but it did not disrupt oligomer formation. Simultaneously we revealed that NSC348884 interfered with adhesion signaling. Overall, this thesis presents methods to analyze the oligomeric state of NPM1 and evaluates the effects of N- and C-terminal mutations on NPM1 oligomerization and interaction.

Key words: NPM1, oligomerization, mutation, AML, NCL, NSC348884, detection of oligomers