The role of CacyBP/SIP phosphatase in regulation of NPM1 function in the nucleus

Research project objectives / hypothesis:

Proteins are the main effector molecules in the cell ensuring the proper course of all biological processes. Proteins often work in teams and for that they interact with each other and form multiprotein complexes. Furthermore, they are subject to many chemical modifications, which usually alter their activity, affinity towards other proteins, cellular localization and other important parameters. NPM1 (also called nucleophosmin or B23) is a protein involved in a broad range of cellular processes especially in the nucleus and the nucleolus, the smallest and most specialized part of the nucleus, where it plays a role in biogenesis of ribosomes and cell growth/proliferation. The activity of NPM1 depends on whether it is phosphorylated i.e. has a covalently bound phosphate moiety; in such a state NPM1 is active and able to change its localization from the nucleus to the cytoplasm and vice versa. Several kinases i.e., enzymes responsible for NPM1 phosphorylation/activation have been identified so far, however, nothing is known about the enzyme(s) (molecular switches), which could de-phosphorylate or, in other words, turn off NPM1. The aim of our studies is to find out whether a protein discovered in our laboratory, called CacyBP/SIP, can regulate NPM1 function in the nucleus. Interestingly, CacyBP/SIP is a novel phosphatase, which means that dephosphorylates other proteins and could possibly dephosphorylate NMP1. Our preliminary results clearly indicate that NPM1 is able to bind CacyBP/SIP. What is more, both CacyBP/SIP and NPM1 can be found in the nucleus. It has been shown also, that both proteins are abundantly expressed in cancer cells and that they both influence cell proliferation. Accelerated cell proliferation is an inherent property of cancer cells making their eradication extremely difficult. Taking into considerations all those facts we assume that the role of the CacyBP/SIP-NPM1 interaction and its implications for NPM1 activity are interesting to study. Thus, in order to prove the hypothesis that CacyBP/SIP may affect NMP1 activity and nuclear functions we plan to answer 3 main questions:

1) Are CacyBP/SIP and NPM1 able to bind directly?
2) Can CacyBP/SIP, earlier identified as ERK1/2 phosphatase, dephosphorylate NPM1?
3) Does CacyBP/SIP influence the NPM1 subcellular/nuclear localization and ability to bind RNA?

Research methodology:

In order to accomplish the project the experiments will be performed using two approaches: cell line cultures and purified proteins. Such methodology, combined of two models, is always more reliable. We will obtain purified CacyBP/SIP from bacterial cultures induced to produce this protein in large quantities. Then, we will analyze the CacyBP/SIP-NPM1 interaction in cells using methods commonly used in biochemistry and molecular biology: co-immunoprecipitation, Western blot and, to examine co-localization of both proteins in the same cellular compartments, confocal microscopy. In order to confirm that NMP1 and CacyBP/SIP interact directly with each other we will use purified proteins and methods such as: affinity chromatography, ELISA and chemical cross-linking. To check whether CacyBP/SIP dephosphorylates NPM1, we will apply two-dimensional electrophoresis and Western blot methods, initially using cell lysates and, subsequently, purified proteins. Finally, we will investigate how interaction with CacyBP/SIP, or dephosphorylation, affects NPM1 function. Because of the fact, that NPM1 is able to bind RNA and can translocate between the nucleus and the cytoplasm depending on its phosphorylation state, the effect of CacyBP/SIP on NPM1 localization and its activity towards RNA will be analyzed by microscopy and by the EMSA-RNA assay, respectively.

Reasons for choosing the research topic:

So far, nobody has shown that CacyBP/SIP interacts with NPM1. Also, only one phosphatase responsible for NPM1 dephosphorylation has been described. Thus, the results obtained during realization of this project concerning a possible interaction between CacyBP/SIP-NPM1 and the influence of CacyBP/SIP on NPM1 dephosphorylation, subcellular localization and RNA binding, will significantly widen our knowledge about the cellular role of both proteins. Since NPM1 has been suggested as a new target of cancer therapy, the obtained results might have practical implications in the future. That is why we assume that investigation of the CacyBP/SIP-NPM1 interaction is worthwhile and important.