Study of the relationship between the proteins Nucleophosmin-1 and Nucleolin and different S6Ks isoforms.

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Abstract
The mTOR signaling pathway has been related to several diseases and metabolic disorders in humans, including obesity, diabetes and various types of cancer. The S6Ks proteins have shown important roles in the signaling of mTOR, acting as effectors of this pathway, but there are still open questions, such as their functional differences and interacting proteins. The aim of this study was to investigate the possible relationship between the proteins Nucleophosmin1 (NPM1) and Nucleolin (NCL), involved with cell proliferation, biogeneses of ribossomes and protein synthesis, and the S6Ks.

Key words: S6K, Nucleophosmin, Nucleolin.

Introduction
The mTOR pathway (mammalian Target Of Rapamycin) is involved with several metabolic disorders, such as obesity, diabetes and cancer1. The main role of the mTOR protein in the cell is to serve as a sensor of nutrients availability, since mTOR is stimulated by high amounts of amino acids, ATP and insulin, all indicators of nutrients abundance2. The mTOR pathway has two important protein effectors, the 4E-BP1 (eIF4E Binding Protein) and the S6Ks (S6 Kinases)3.

The S6K proteins are members of the AGC kinase family, and previous data of our group identified a number of interacting proteins, among them the proteins NPM1 and NCL, that participates in the ribosomal synthesis.

Given the important role of the mTOR/S6K pathway in diseases related to metabolism such as obesity, diabetes and cancer, studies involving this signaling pathway have great demand. Thus, this project aims to study the possible relationship between the proteins NPM1 and NCL and the mTOR/S6K pathway, characterizing the possible interaction between these proteins and the different S6Ks isoforms. We hope to contribute to a better understanding of mTOR/S6K pathway in these metabolic disorders.

Results and Discussion
The cloning of the gene encoding NPM1 was performed in the plasmid pcDNA 3.1 (Invitrogen), fused to the peptide HA, resulting the clone named as pcDNA-HA NPM1. For the NCL, two clones, pGFP-NCL and pFLAG-NCL, were provided by an international research group. Both were transfected in HEK293 cells in order to prove their expression. Also, plasmids for the expression of shRNA against NPM and NCL were transfected in HEK293 cells in order to perform their knockdown. The NPM1 knockdown was effective, it was even observed decreased expression of S6K1.

Immunoprecipitation experiments were performed with NPM1 and NCL. NPM1 had problems in its complete elution, and therefore results were not conclusive. NCL had good elution and was positive for the phosphorylation site shared between the proteins AKT and S6K (RXRXXT/S).

Finally, immunofluorescence assays were performed for NCL in HeLa cells to verify the possible alteration of its localization in the presence of insulin. The results shown that apparently there is no change on its subcellular localization by this stimulus.

Conclusions
Our data show the interaction between NCL and S6Ks, possibly with the involvement of the phosphorylation site RXRXXT/S but further experiments are needed. However we have not detected change in the NCL location when cells were treated with insulin. Besides, the NCL and NPM knockdown appears to be related to the reduction of S6K1 expression. New experiments will be performed to better understand the possible relationship between NCL, NPM and S6K.

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