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Pathways for selecting ribosomal proteins for packaging into exosomes

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Motivation and Aim: Many ribosomal proteins are “moonlighting” ones having various functions beyond their canonical role as constituents of the ribosome (extra-ribosomal functions). These functions often relate to human pathologies, primarily, to carcinogenesis, via their participation in various signaling pathways, e. g., in the p53-MDM2 pathway, which regulates keystone tumor suppressor p53. Besides, expression of many ribosomal proteins is considerably changed (upregulated or downregulated) in malignant cells. On the other hand, exosomes derived from both malignant and normal cells often contain specific sets of ribosomal proteins. These extracellular vesicles are generally considered to be responsible for the intercellular communication by transporting specific molecular cargo from the donor cell to the recipient one. Thus, one can assume that oncogenic (or onco-suppressor) properties of ribosomal proteins could be, at least in part, transferred from one cell to another by exosomes. In the case of ribosomal protein uS3 such kind transfer has been documented experimentally. In particular, it has been demonstrated that transport of uS3 by exosomes from the gastric cancer cells resistant to an antitumor drug cisplatin to the non-resistant ones makes the recipient cells cisplatin-resistant [1]. However, mechanisms of sorting of ribosomal proteins to exosomes remain unknown. One can suggest that this process is mediated by proteins of the endosomal sorting complex ESCRT, namely the HGS and/or Alix proteins, which trigger a cascade of reactions for the formation of exosomes and the packaging of various biopolymers in them [2]. The purpose of this work was identification of partners of ribosomal proteins from the ESCRT complexes, which might be the most likely participants in their packaging.

Methods and Algorithms: The work was carried out with the triple-negative human breast cancer cell line BT-549. In this study, we used and optimized the co-immunoprecipitation technique to better detect interactions between the human ribosomal protein uS3 and transport proteins HGS and Alix. Binding partners of uS3 were identified with the use of Western blotting with the use of specific antibodies against proteins under investigation/ Besides, we used recombinant ribosomal protein uS3 to simulate cellular ribosomal stress conditions, upon which the level of free ribosomal proteins considerably increase.

Results: We did not find interactions between uS3 and HGS or Alix proteins at normal physiological conditions. Moreover, such interactions were not detected even in the presence of cross-linking agent formaldehyde that fix protein-protein complexes. On the other hand, we clearly demonstrated co-immunoprecipitation of uS3 with HGS or Alix proteins in cell lysates supplied with recombinant His-tagged uS3. Obviously, a low level of ribosome-free uS3 in cells under normal conditions does not allow detection of its interaction with transporting proteins. However, when concentration of uS3 considerably increases (which imitate cellular ribosomal stress, when ribosomal assembly is impaired), the interactions become well detectable. Under these conditions, we indeed demonstrated the ability of HGS and Alix proteins to bind uS3 in lysates of breast cancer cells.
Conclusion: The results obtained indicate that ESCRT components – proteins HGS and Alix can interact with ribosomal protein uS3 when the latter is present at a concentration considerably higher than that in normal breast cancer cells. These results support the suggestion on the role of these proteins in sorting of uS3 to exosomes, but they can play this role only under conditions of abnormally high level of free ribosomal protein in the cell, which could take place, for example, under stress conditions when ribosomal subunits assembly is impaired. Several questions remain unresolved: is there an interaction between other ribosomal proteins and the transporting proteins of the ESCRT complex? Is this kind interaction typical for other cell malignant lines and for non-malignant cells? Finally, it would be essential to understand whether intercellular transfer of ribosomal proteins by exosomes contribute to cancer progression and metastasis. All this is the next frontier of investigations.
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References

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