Referee report for the project "Protection against physical and chemical stresses with tardigrade proteins (TARDISS)"

This project aims to study the protective properties of the tardigrade proteins both in a model system and *in vitro*. A number of classical and most modern methods of molecular genetics and biophysics will be used in these studies. A number of leading scientific groups abroad are studying the properties of proteins obtained from extremophile organisms, but research in this area is at an early stage, which enables the authors of the project to make a significant contribution to the development of this area.

Scientific significance and novelty of the project

In the described project, the authors set ambitious goals for studying the radio- and crioprotective properties of the Dsup protein in living systems and *in vitro*, creating model living systems with induced expression of the Dsup protein, and creating high-tech materials modified with the Dsup protein. The study of the characteristics of proteins from radioresistant organisms is one of the topical and important areas of world science. Among organisms resistant to ionizing radiation, tardigrades occupy a special place, since they are leaders among multicellular organisms in terms of their ability to withstand high doses of radiation. The resistance of tardigrades to various influences was studied by several foreign teams, during the work of which world-class results were obtained. The gene for the Dsup protein was isolated from the most radiation-resistant species of tardigrades. Dsup protein is capable of binding nonspecifically to nucleic acids, thereby protecting the genetic apparatus of the cell from exposure to ionizing radiation. In the course of 2021-2023, a team of authors did significant work to create a transgenic line of a model organism *D. melanogaster* producing the Dsup protein, as well as a human cell line HEK239, into which the Dsup protein gene was inserted. The model systems showed an increased radioresistance of the lines producing the Dsup protein compared to the control ones. The results obtained earlier are of world significance and open the way to the creation of high-tech radioprotectors. Solving the tasks stated in the project for 2024-2028 will make it possible to make significant progress in the field of studying the properties of the Dsup protein, which will be of high both fundamental and applied importance.

The scientific content of the project

The objectives of the project are clearly described and make it possible to assess the potential of the practical application of the Dsup protein from different angles.

At the previous stage of the project, the authors obtained lines of genetically modified model organisms – fruit fly *D.melanogaster,* that stably produce the Dsup protein. An increase in the radioresistance of the modified lines compared to the control ones was shown. In addition to increasing radioresistance, the constant production of the Dsup protein also had a number of side effects, and therefore one of the tasks of the current project is to create a model system with regulated expression of the Dsup protein in response to an increase in the concentration of reactive oxygen species that occurs due to the action of ionizing radiation. It should be noted that such model systems have not been created before in the world.

For a deeper understanding of the mechanisms of the influence of the Dsup protein on gene activity, a quantitative analysis of open chromatin regions will be carried out using the modern ATAC sequencing method and transcriptomic analysis. The use of these methods will make it possible for the first time to evaluate the effect of radioprotective proteins on chromatin packing in the nucleus and to deeply understand the processes occurring at this moment in the cell.

The next task, which has a high potential for applied science, is the assessment of the resistance of a Dsup protein to high temperatures and ionizing radiation in vitro. To assess the influence of these factors on the protein structure, a number of high-tech methods will be used, such as SAXS, DLS, SDS-PAGE, circular dichroism and Western blotting. The described approach will make it possible to evaluate the potential of using the Dsup protein as a stabilizer for DNA/RNA-containing drugs and vaccines, as well as a protector in radiotherapy and chemotherapy. The relevance and practical significance of the task is extremely high for the world community, since modern technologies in the field of radio protection of living systems are actively developing and have a number of shortcomings that need to be improved.

The fourth task that the authors described is the creation of high-tech materials modified with the Dsup protein for the selective accumulation of nucleic acids from chemical solutions and biological fluids. The possibility of creating such materials is beyond doubt, since the Dsup protein has the ability to nonspecifically bind DNA and RNA. Protein fixation on sorbents and porous membranes will be carried out using covalent bonds and non-covalent interactions. The creation of high-tech materials based on tardigrade proteins was proposed by the authors for the first time in the world. Potentially, such materials can be used in medical and environmental research, the creation of smart filters, biotechnological and pharmaceutical industries.

In summary, the tasks set are fully consistent with the stated goal and cover a wide range of areas. The described project will allow a comprehensive assessment of the prospects for using the Dsup protein to solve problems in radiobiology, molecular genetics, astrobiology, biotechnology, and ecology, which is of great importance in the context of world research in this area.

Funding and employees

The requested funding is fully justified and necessary to fulfill the stated objectives. The instrumental base of DLNP JINR and the announced partners (FLNP JINR, Moscow Institute of Physics and Technology) fully complies with the methods described in the project. The experience and qualifications of the employees involved in the implementation of the project correspond to the required level for the successful implementation of the project. During the implementation of the project, one PhD and one master's theses will be defended.

Conclusion

The declared project is relevant and corresponds to the world level of research. The tasks set, the chosen methods and the qualifications of the participants indicate that the project will be implemented in full. I recommend assigning the highest priority (A) to the project. I believe that the declared project is worthy to be fully funded.

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