Analysis of methods for loading therapeutic nucleic acids into horse milk exosomes

P-08.2-77

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Exosomes are natural nanovesicles with a diameter of 40 to 100 nm. Their function is intercellular communication. Since exosomes are biocompatible extracellular vesicles that can efficiently deliver nucleic acid molecules, they are a promising delivery agent for therapeutically valuable drugs. Milk is the only natural source containing exosomes that are commercially available. The possibility and efficiency of loading various therapeutics into exosomes should be evaluated to use exosomes as drug delivery agents.

DNA and RNA oligonucleotides labeled with FAM and ^{32}P-label, and plasmid DNA used as a DNA vaccine were obtained to analyze the efficiency of loading into exosomes. In this work, exosomes were obtained from horse milk by centrifugation, ultrafiltration, ultracentrifugation, and additional purification from co-excreted proteins and other impurities by gel filtration. To load oligonucleotides into exosomes of horse milk, the methods of incubation in the ultrasonic bath, electroporation, and freezing in liquid nitrogen and thawing, as well as loading with the soy lecithin under acidic conditions, components of lipofectamine and its analogs, were used. Loading efficiency was assessed by electrophoresis and PCR.

The exosomes obtained after gel filtration, free of admixtures of co-isolating proteins, showed a higher loading efficiency. In the future, we plan to evaluate the efficiency of the delivery of therapeutic nucleic acids loaded into exosomes to cell cultures and to the organisms of model animals.

The work was supported by the RSF project 18-74-10055.

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