ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Author: Michaela Hudáková

Supervisor: RNDr. Klára Konečná, Ph.D.

Consultant: doc. PharmDr. Ivona Pávková, Ph.D.

Title of master's thesis: Isolation of exosomes produced by macrophages infected with *F. tularensis*

Background: The main objective of the presented work was to establish and optimize a model for isolating exosomes from infected BALB/c mouse macrophages using the virulent strain of the bacterium *Francisella tularensis* subsp. *holarctica* FSC200, followed by the basic characterization and identification of the isolated proteins.

Methods: The model setup for the exosome isolation method was carried out using light and fluorescence microscopy, during which the experimental conditions were established — a two-hour interval for infection, and cultivation and infection of cells in a serum-free environment. For the actual isolation of exosomes, a method based on differential centrifugation and ultracentrifugation was chosen. Selected analytical methods were used for the basic characterization of the isolated proteins – protein determination using bicinchoninic acid modified for small volumes and and analysis by dynamic light scattering. The final identification of these proteins was carried out by performing mass spectrometry.

Results: Based on the selected methodological approach, it was possible to isolate and characterize two mouse proteins in the control sample (proteins isolated from uninfected macrophages) and thirteen mouse proteins and two proteins originating from *F. tularensis* in the infected sample (proteins isolated from infected macrophages). Mass spectrometry showed zero or undetectable amount of proteins typical for exosomes (e.g., CD9, CD81, TSG101, Alix).

Conclusions: The conditions chosen for the exosome isolation model proved to be inadequate due to the low exosomal yield. A possible cause might be the short time interval selected for macrophage infection, during which not enough exosomes are produced.

Keywords: exosomes, macrophages, isolation, *Francisella tularensis*