Abstract

Trichomonas vaginalis is a causative agent of the most common non-viral sexually transmitted disease with approximately 275 mil new cases annually. Virulence of this parasitic depends on at least four factors: cell shape transformation, cytoadherence, secretion of cysteine proteases, and presence of endosymbionts. Over the past decades, extracellular vesicles appeared being another important player in the host-parasite interaction. It was discovered that *T. vaginalis* is one of the protists that can shed the extracellular vesicles such as exosomes and ectosomes. These vesicles are possibly involved in host-parasite communications, however limited information is available about their function. To investigate a possible role of exosomes in *T. vaginalis* virulence, we first selected suitable strain, which is free of endosymbionts (TV 17-2MI). Next we prepared six clones of TV 17-2MI strain to test whether the strain is homogenous concerning the virulence, or there are differences in virulence among individual cells. Mouse intraperitoneal virulence tests revealed that the clones displayed significant differences in virulence level, particularly in abscess formation and mortality of infected animals. Thus, for the first time we demonstrated heterogeneity of cells derived from a single *T. vaginalis* strain in their virulence. Observed heterogeneity is not based on endosymbiont presence or absence.

Next, we established a protocol for exosome isolation. First step was to select suitable exosomal protein marker. We selected three candidates (tetraspanin 1, SNARE protein and multivesicular body protein - MBP) that were expressed with hemagglutinin tag in *T. vaginalis*. Immunofluorescence microscopy shown the localization of all three proteins inside of the cell, however only tetraspanin 1 (TSP1) was detected on the membrane of vesicles inside of the cell that could be multivesicular bodies. In the preliminary isolation of exosomes, we verified presence of exosomes in the microvesicular fraction using immunoblotting. To determine the protein composition of exosomes, we isolated exosomes from two *T. vaginalis* strains (TV T1 that expressed tagged-TSP1, and TV 17-2MI) and analyzed them using mass spectrometry. Our proteomic analyses showed significantly higher number of identified proteins (for TV T1 exosomes 2305 and for TV 17-2MI 1335 proteins) than in previous study (215 protein, Twu et al., 2013). Most of proteins from all studies significantly overlapped and all three marker proteins (TSP1, SNARE and MBP) were detected. The possible reasons for higher number of identified proteins in our experiments are discussed.