Phosphoproteome of ECM-treated trypomastigotes from *Trypanosoma cruzi.*

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Introduction: Signaling events triggered by ECM (extracellular matrix) in mammalian cells is well studied. In contrast, scarce information is available on the events triggered in trypomastigotes (the infective stage from *T. cruzi)* upon incubation with ECM. One first report showed changes in the phosphorylation level of several trypomastigote proteins, which may be key elements during parasite adhesion to laminin and fibronectin. In fact, phosphorylation and dephosphorylation events may have an important role in parasites as can be inferred from different phosphoproteomes described in literature. **Objective:** This work aims to identify the phosphoproteome of *T. cruzi* trypomastigotes upon incubation with ECM from the host cells. **Materials and methods:** Trypomastigotes were incubated with ECM for two hours and collected for further protein extraction and digestion. Peptides were labeled with TMT-sixplex: 126, 127 and 128 Da tags bind to three replicates of trypomastigotes alone and 129, 130 and 131 Da tags to trypomastigotes incubated with ECM triplicates. The peptides were separated by HILIC fractionation and 5% of each fraction was selected for total proteome and 95% for TiO_2 – phosphoenrichment for further proteome analysis. All fractions were analysed by LC-MS/MS. Raw files were analyzed by COMPASS software for protein and phosphopeptide identification. **Results and discussion:** From the 303 phosphopeptides identified, 69 were phosphorylated while 234 were dephosphorylated when ECM-treated or untreated parasites were compared, indicating that trypomastigotes adhesion to ECM induces a general protein dephosphorylation in the parasite. 67% corresponded to hypothetical proteins, in addition to phosphopeptides from cytoskeletal proteins (34), heat shock proteins (5), kinase and phosphatases (15) and proteins from the metabolism, including carbohydrate metabolism. **Conclusion:** Adhesion of trypomastigotes from *T.cruzi* to ECM results in significant changes in the phosphorylation level of proteins that may prepare the parasite for mammalian cell invasion.

Key-words: *Trypanosoma cruzi*, extracellular matrix, phosphorylation, metabolism
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