

OTR18. SECRETOME ANALYSIS OF CHLAMYDOMONAS REINHARDTII MICROALGAE IN RESPONSE TO AUTOPHAGY INDUCING CONDITIONS.

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INTRODUCTION *Chlamydomonas reinhardtii* (Cr) microalgae secretes extracellular vesicles (EVs) but factors affecting EVs formation and release are unknown. In mammals, these processes are associated with autophagy. In Cr, autophagy is triggered by nitrogen deprivation and exposure to rapamycin. However, the correlation between autophagy and EVs formation in photosynthetic cells is unexplored.

OBJECTIVE Evaluate the effects of nitrogen deprivation and rapamycin exposure on Cr protein secretion.

METHODOLOGY A prediction of Cr proteins potentially secreted in EVs using 23,000 non-redundant protein sequences known to be present in other organisms' EVs (EVpedia database) to interrogate the Cr proteome (19,526 sequences from Phytozome v5.5). 3,600 (18.4% of Cr proteome) positive blast hits (Cr EVs-predicted proteins, Cr-EVs-PP) were obtained. To verify the actual secretion of the Cr-EVs-PP, mid-log phase cc-124 cells were cultured (5.5×10^6 cells/mL, n=3) in TAP medium (+N), TAP containing 5 μ M rapamycin (+R) or TAP without nitrogen (-N). After 17 hours, a portion of each culture supernatants obtained by centrifugation were freeze-dried and the other part were ultracentrifuged (EVs secretome) and evaluated by Nanoparticle Tracking Analysis (NTA) technique.

RESULTS Cr derived-proteins accumulated in -N (25 mg/mL) and +R (3 mg/mL) compared to +N (< 1 mg/mL). 10% SDS-PAGE protein separation and Sypro Ruby staining revealed complex electrophoretic profiles of proteins ranging from 250 to 20 kDa. Preliminary *in gel* trypsin protein digestion and LC-MS/MS allowed the confident (at least 2 peptides, P < 0.05, FDR < 1%) identification of 24

proteins in +R, and 38 proteins in -N samples, out of which 13 and 12, respectively, are Cr-EVs-PP including the EVs' marker HSP70A. The NTA revealed a higher number of 90 to 100 nm particles in the supernatant of +R (3.8×10^8 part/mL) compared to -N (5.8×10^7 part/mL) and +N (6.0×10^7 part/mL) cultures.

CONCLUSION The supernatant of Cr cultures deprived of nitrogen or supplemented with rapamycin presented more proteins than control cultures. The NTA suggested more exosomes (90 to 100 nm particles) in the supernatant of +R than -N and + N cultures. The correlation between autophagy and EVs formation have been indicating proteins, which can open up new possibilities for biotechnological research in *Chlamydomonas*. The proteomic analysis of TAP, TAP+Rap and TAP-N is still in progress.

KEYWORDS algae, secretome and vesicles.