Apoptosis-inducing Factor and Autophagy-mediated Cell Death of *Entamoeba histolytica* in Response to Stresses

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Abstract

Entamoeba histolytica, which is a endoparasite to human being, is one of the earliest eukaryote belonging to the phylum protozoa. In case of symptomatic disease, it causes enteric amoebiasis and abscesses in different organs. Owning a biphasic life cycle, it alters between two functionally and morphologically distinct forms: the invasive, motile trophozoite form and the infective, non-motile cyst form. Being an obligatory parasite, their survival is dependent on and challenged by the host body. It is continuously exposed to different stresses imposed by the host body as a result of which a major fraction of the protozoan die within the host body. The aim of this study was to find out the mechanisms by which death is executed in this unicellular organism. To that end, the putative Apoptosis-inducing factor (AIF) was identified in *Entamoeba histolytica* (ammoebadb ID EHI_153000). The protein is 447 aā long with estimated molecular weight 49,755 Da. Bioinformatical analyses predict the presence of bipartite nuclear localization sequence, three transmembrane domains and a FAD/NADH oxidoreductase domain in the putative EhAIF. The Expression profiling of the EhAIF suggests that its expression increases during the oxidative stress and heat stress but decreases in serum and glucose deprived conditions. The putative EhAIF was cloned and heterologouly expressed in bacterial system with His-tag and GST-tag separately. The His-EhAIF was purified in denatured form and used to develop antibody against it. The GST-EhAIF, in native form was used for functional characterizations. The putative cytoplasmic EhAIF migrates to the nucleus on receiving stresses. The EhAIF is capable of binding with DNA, RNA and plasmid in vitro with a preference towards the linear DNA and genomic DNA in vivo. It can also bind and degrade isolated intact nuclei. EhAIF knockdown attenuates the apoptotic features of insulted cells and increases the survival potency in terms of cell viability and vitality as well as the total cellular DNA content of the trophozoites, whereas over-expression of the same effectively enhances the phenomena. *Entamoeba histolytica* also displays autophagic cell death under oxidative stress. Oxidative stress leads to nucleophagy and ultimately the autophagosed nuclei are digested within the lysosomal chamber. The formation of the nucleophagosome depends on the apoptosis-inducing factor (AIF) that migrates to the nucleus from cytoplasm upon oxidative stress. The interaction between ATG8 and AIF has been proved by in silico, in vitro

and in vivo experiments. Surprisingly, the most-widely used drug to treat amoebiasis, metronidazole was also found to induce AIF-mediated and autophagic cell death. AIF expression enhanced with nuclear translocation under metronidazole treatment. Down-regulation and upregulation of AIF increased and decreased the survival potency of the metronidazole-treated cells respectively. Metronidazole was found to increase oxidative stress in the *Entamoeba* trophozoites. Therefore, it also imparts the similar effect i.e. nucleophagy-mediated death in it.