

# **Functional analysis of neutral sphingomyelinases of *Entamoeba histolytica***

## **Análisis funcional de esfingomielinasas neutras de *Entamoeba histolytica***

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**Functional analysis of neutral sphingomyelinases of *Entamoeba histolytica***

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### **Abstract:**

The virulence of *Entamoeba histolytica* is a multifactorial process, in which both host and amoeba factors are involved. Among the factors of the parasite are amebapores, cysteine proteases and lectin; however, the participation of other molecules is not ruled out. Some of these candidate molecules are sphingomyelinases (SMases), enzymes that catalyze the hydrolysis of sphingomyelin (SM) producing ceramide, which is involved in different cellular processes such as cell proliferation, apoptosis and cell lysis. In the working group it has been reported that the genome of *E. histolytica* has three genes that code for neutral SMases, which are expressed and produce proteins with enzymatic activity. The objective of the present is the functional analysis of the neutral SMases, for which transfectant trophozoites were obtained to silence or overexpress the genes that code for these enzymes. It was found that the activity of neutral SMase diminishes with the silencing of the genes and increases with its overexpression, which allowed to evaluate its participation in the virulence of the parasite. NSMasa3 is associated with cell death and lysis, while nSMasa1 and nSMasa2 with the destruction of cellular monolayers. By means of immunodetection and confocal microscopy tests, it was found that these proteins are mainly associated with the plasma membrane, which suggests that the cytotoxic and cytolytic effect is contact-dependent.

The activity of nSMase increased in amoebae exposed to H<sub>2</sub>O<sub>2</sub>, UV-C radiation or heat shock, however, the over-expressing strains did not show changes in their activity. This is the first time that the participation of the nSMases in the virulence of *E. histolytica*, mainly of the Ehnm3, has been demonstrated.

### **Reference**

Gandhi, B.M., Gupta, H., Irshad, M., Chawala, T.C., Tandon, B.N., 1986. Cell-mediated immunity in amoebic liver abscess. *J. Trop. Med. Hyg.* 89, 163—166.