

## **EARTHWORM COELOMOCYTE DERIVED CYTOTOXICITY IS NOT RESCUED BY ANTI-LYSENIN PRETREATMENT**

Engelmann Péter<sup>1</sup>,

Somogyi Ildikó<sup>2</sup>, Mácsik Levente László<sup>1</sup>, Pollák Edit<sup>2</sup>, Molnár László<sup>2</sup>, Németh Péter<sup>1</sup>

<sup>1</sup>Department of Immunology and Biotechnology, Clinical Center, University of Pécs, Pécs, Hungary

<sup>2</sup>Department of Comparative Anatomy and Developmental Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary

**Objectives:** Coelomocyte subpopulations have essential role in earthworm cellular immunity. In addition to secreted antimicrobial factors of earthworms, there is a firm evidence for certain cytotoxic components. Previously, we observed rapid cell lysis of multiple tumor cell lines caused by coelomocyte lysate or supernatant of *in vitro* cultured coelomocytes. Moreover, we demonstrated that coelomocyte lysate induced a caspase independent apoptotic-like cell death in tumor (Sp2 and HeLa) cell targets using different technical approaches (transmission electron microscopy, TUNEL assay, Annexin V staining, assessment of mitochondrial membrane potential changes, and measurement of Ca<sup>2+</sup> influx).

**Methods:** Our recent aim was to study whether a lytic protein named as lysenin participates in the observed cytotoxic mechanisms. For this attempt we applied our in-house developed anti-lysenin monoclonal antibody (a-EFCC5) to inhibit the coelomocyte lysate evoked toxicity in various assays (TUNEL, Annexin V, MMP, Ca<sup>2+</sup> influx).

**Results:** Indeed, anti-EFCC5 pre-treated coelomocyte lysate caused less TUNEL positivity in HeLa target cells; however there was detectable DNA fragmentation compared to negative controls. Similarly, pretreatment with a-EFCC5 mAb rescued Sp2 target cells from the loss of mitochondrial membrane potential, but it was still significantly different from the control cells. A-EFCC5 pretreated coelomocyte lysate induced an attenuated phosphatidylserine translocation of target cells revealed by Annexin V/propidium iodide staining. In the case of Ca<sup>2+</sup> kinetics, mAb pretreatment caused a fall in lysate-evoked Ca<sup>2+</sup> influx compared to the untreated lysates, but it was clearly noticeable in contrast to the negative controls.

**Conclusion:** Anti-lysenin specific mAb pretreatment rescued the majority but not all target cells from coelomocyte-induced death. These data suggest that factors other than lysenin may also participate in the cytotoxic activity of coelomocytes. Our observations underscore the complexity of cytotoxicity-related immune response in the earthworm.

Preferált prezentáció formája: előadás

Prezentáció besorolása: elméleti