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Detection of active apoptotic molecules with femtogram sensitivity in single cells: A pilot study

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With an increasing interest in mechanisms of cell death also with regards to therapeutical applications, the research techniques become precised. Particularly at the protein level, where amplification possibilities are limited, novel sensitive approaches are welcome.

Earlier, we demonstrated a novel combination of the laser capture microdissection and flow cytometry of cryopreserved samples (Matalova et al. 2010). Later, a modified chemiluminiscence technology with photon counting detection allowed for detection of active caspase-3 in the femtogram amount (Chlastakova et al. 2012).

Now, a challenging project has been started to detect active caspase-3 in one single cell with precision of femtograms. To achieve the aims, an experimental design of embryonic and stem cells treated by camptothecin to induce apoptosis was used as described in Chlastakova et al. 2012. The developed device for the detection of caspase 3 activity in individual apoptotic cells takes advantage of Luciferin/Luciferase chemiluminescence reaction. The luciferin modified with a tetrapeptide sequence (DEVD), specific to the recognition of caspase-3, is cleaved to form free luciferin immediately reacting with luciferase to produce light. Our detection device consists of a light-proof microfluidic chamber held inside a housing of photomultiplier tube (Hamamatsu R446) with spectral response 185-870 nm. The limit of detection reaches the value lower than 1pg of caspase 3. Thus, the sensitivity of the device proved to be by one order of magnitude better than the commercially available technologies. The results show that about 1.6×10-19 mol (96,000 molecules) of caspase 3 are activated in a single apoptotic cell.

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Chlastakova I et al. 2012: In Vitro Cell Dev Biol - An (DOI 10.1007/s11626-012-9542-8)

Matalova E et al. 2012: Arch Oral Biol 55: 570-575

Biography

Petra Cela graduated in Animal Physiology at Faculty of Science, Masaryk University in 2011. Now, she continues Ph.D. in Laboratory of Animal Embryology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, v.v.i

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