

Single-cell analysis of apoptosis associated molecules

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Caspases are proteases that are traditionally associated with apoptosis, the programmed cell death, but novel non-apoptotic functions of caspases were reported as well, especially as modulators of cell differentiation and proliferation. Recently, caspases were reported as molecules partaking on differentiation of mouse osteoblastic cells. Analyzing cell lysates from large sets of cells often obscure the cell-to-cell variations within the cell culture or tissue. It is therefore desirable to analyze the caspases activation at the single-cell level. To address these needs, we have developed a miniaturized device for the single-cell analysis of caspase activation that utilizes commercially available bioluminescence chemistry based on modified aminoluciferin and firefly luciferase. The developed technique was subsequently used for analysis of caspase activation during MC3T3-E1 osteoblastic cell line differentiation.

Fas ligand (FasL) is a transmembrane protein that is also closely associated with apoptosis as it is the initiator of the extrinsic apoptotic pathway. Its polarization on the surface of cells can play a key role in tissue remodeling for example during bone formation. In our work, we have developed functionalized cathodoluminescent nanoparticles based on lanthanide doped LuAG and NaYF₄ that were conjugated with antibodies against the FasL. The nanoparticles provide both fluorescent and cathodoluminescent signal and they have potential for application in correlative light and scanning electron microscopy.

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