Extracellular substrates of apoptotic and inflammatory caspases in cancer

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Programmed cell death describes several mechanisms that maintain homeostasis and consequently, impairments in these mechanisms are detrimental to the organism. Our research focused on caspases activated during apoptosis and pyroptosis. In the case of apoptosis, insufficient clearance of apoptotic bodies leads to the progression to secondary necrosis, which is morphologically similar to pyroptosis. In both cases, membrane integrity is disrupted and intracellular proteins can get released to the extracellular space. Caspase-3-like DEVDase activity has already been detected in the extracellular space of apoptotic cells and similarly, some evidence exists of caspase-1 activity in supernatants of pyroptotic cells. We checked whether released active caspases could, similarly to cathepsins, perform selective proteolysis of membrane proteins from cancer cells.

To investigate the extracellular role of caspases-3, -7 and -1 we treated breast cancer cells with recombinant human caspases and used the mass-spectrometry proteomic platform to identify the proteins released from the cell surface. Furthermore, several cleavages were additionally confirmed using immunoblotting. Target analysis revealed that caspases can mostly cleave membrane proteins that act as cell adhesion molecules (e.g. CD44) or cell transmembrane receptors (e.g. NRP-1), but the exact consequences of caspase cleavages remain unknown. Additionally, we confirmed the presence of DEVDase activity in extracellular space during progression from apoptosis to secondary necrosis. Using immunoblotting we showed the presence of caspase-3 in the supernatants from apoptotic cells. As for caspase-1 activity, additional tests are needed to determine whether it can be detected in the supernatants from pyroptotic cells.

In the future, we will try to validate the effect of extracellular caspase cleavages on the properties of cancer cells and try to determine the physiological relevance of caspases as potential sheddases.