UNRAVELING THE MEMBRANE PERMEABILIZING BEHAVIOR OF GASDERMIN E USING A PHOTOPORATION- BASED EFFLUX STRATEGY

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Intro. The role of Gasdermin E (GSDME) in cell death and its potential role in cancer immunotherapy has been a topic of intensive research. When activated by caspase-3 cleavage, this protein promotes necrosis, either primary or following a primary apoptotic phase (secondary necrosis). Owing to the explosive nature of necrosis, cell permeabilization by GSDME may induce a more immunogenic environment, which could aid in eliminating cancer cells. Although GSDME is assumed to be a pore-forming protein, little is known about its membrane permeabilizing function. This is why we aimed to study its membrane destabilizing behavior by monitoring over time the cytosolic leakage of molecules of different sizes. Meth. We first delivered fluorescent dextrans of distinct molecular weights in live cells using photoporation as a new intracellular delivery technique that minimally perturbs the cell's homeostasis. Upon initiation of cell death with aFas, an apoptotic trigger, we monitored efflux of the cytosolic dextrans in function of time. Res. We observed that all molecules (up to 2000 kDa) were eventually released from dead cells. Nevertheless, smaller dextrans could leak out early on in the cell permeabilization process, whereas for larger molecules this is more likely to happen later on when cells are completely permeabilized. These observations were independent of GSDME expression. However, aFas treatment of GSDME-positive cells resulted in a higher cell death rate compared to GSDME-knockout cells. Concl. Our observations show complete disintegration of the plasma membrane after aFas treatment independent of GSDME expression. We were not able to establish a cut-off size for GSDME pores, questioning distinct pore formation by GSDME instead of other membrane destabilizing mechanisms.