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POSTER ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Gsdme Promotes PAD4 Activation and DNA Externalization from Apoptotic Neutrophils

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Introduction

Neutrophil extracellular traps (NETs) neutralize bacterial and fungal pathogens but can also promote thrombosis, autoimmunity, and sterile inflammation. The presence of citrullinated histones, generated by the peptidylarginine deiminase 4 (PAD4), is synonymous with NETosis and is considered independent of apoptosis. However, non-apoptotic cell death signaling pathways mediated by MLKL and GSDMD can also engage PAD4 in neutrophils to trigger NET formation. Additionally, while PAD4 is hypothesized to neutralize charge on histones to facilitate chromatin decondensation, a genetic deficiency in *Padi4* fails to prevent chromatin decondensation but does prevent NET formation following MLKL activation. Together, these data suggest more complex roles for PAD4 in the extrusion of nuclear DNA. Here we explored the role of GSDME - another pore-forming protein activated by apoptotic caspases - in controlling PAD4 activation, DNA externalization, and NET formation in apoptotic neutrophils.

Methods

Cell viability was quantified by flow cytometry and live-cell imaging using Hoechst, Cell Tracker Green, Annexin V, and propidium iodide. Histone citrullination was studied at the population level by immunoblot, at the single cell level by lattice SIM super-resolution microscopy, and at the molecular level by an *in-situ* ChIP-Seq methodology CUT&Tag. Calcium signaling linked to PAD4 activation was monitored by flow cytometry. Ultrastructural changes occurring in the absence of PAD4 and GSDME were investigated by transmission electron microscopy and super-resolution microscopy.

Results

Intrinsic and extrinsic apoptosis promote GSDME-dependent calcium mobilization and membrane permeabilization, leading to histone H3 citrullination (H3Cit), nuclear DNA extrusion, and cytoplast formation. H3Cit distribution on chromatin is developmentally controlled by PAD4 in neutrophils, with H3Cit found concentrated at promoter regions during neutrophil development. Lattice SIM super-resolution microscopy revealed clustered distribution of H3Cit in the nuclei of neutrophils, reflecting a unique spatial organization and coordination of multiple promoter elements in neutrophils. Following apoptotic stimulation, H3Cit redistributes in a coordinated process from promoter to intergenic and intronic regions. Loss of GSDME prevents nuclear and plasma membrane disruption of apoptotic neutrophils, but also blocks calcium signaling that activates PAD4, ultimately preventing NET formation. During apoptosis in GSDME-deficient neutrophils, early-apoptotic cellular changes to the chromatin and cytoplasmic granules are prolonged, generating highly atypical cellular states that fail to extrude nuclear DNA.

Conclusion

Apoptotic signaling engages PAD4 in neutrophils, establishing a cellular state that is primed for NETosis, but that occurs only upon membrane disruption by GSDME, thereby redefining the end-of-life for neutrophils.

Disclosures No relevant conflicts of interest to declare.

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