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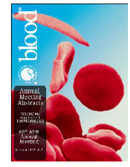

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Cell Surface RNAs Control Neutrophil Function

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Recently, RNAs localizing to the outer cell surface have been reported in mammalian cells, with such RNAs containing glycan modifications (referred to as GlycoRNAs). However, the function of cell surface RNAs are poorly known. In this study, we investigated whether cell surface RNAs exist in neutrophils, what their functions are, and the mechanism by which they function.

We first determined that neutrophils express cell surface GlycoRNAs. We utilized two strategies to assess cell surface GlycoRNAs. First, we utilized a sialic acid homologue to metabolically label glycans inside cells. Labeled glycans can be readily detected in purified total RNAs from primary murine neutrophils. The GlycoRNAs signals were depleted upon RNase digestion but not digestion by proteinase or DNase, supporting the existence of GlycoRNAs in neutrophils. Importantly, treating live neutrophils with RNase extracellularly removed over 90% of GlycoRNA signals, supporting that the majority of GlycoRNAs were located on the surface of neutrophils. Second, we directly visualized cell surface RNAs by labeling cellular RNAs with the nucleoside homologue 5'-bromouridine (BrU) and detecting live cells with an anti-BrU antibody applied extracellularly. These data support the existence of cell surface GlycoRNAs on neutrophils.

We next revealed that cell surface GlycoRNAs play important functions in neutrophils to mediate transendothelial migration both in vivo and in vitro. We utilized an acute peritonitis model in which primarily neutrophils with cell surface GlycoRNAs removed by extracellular RNase were injected into circulation in mice that were treated with thioglycolate, and the migration of these neutrophils into the peritoneal cavity was quantified and compared to mock treated cells in vivo. We observed a 9-fold decrease of migration by neutrophils treated with extracellular RNase. To determine the in vitro function of cell surface RNAs, we tested the ability of neutrophils to migrate toward a chemoattractant in a trans-well assay. While neutrophils treated with extracellular RNase were viable and migrated similarly as control neutrophils, we observed a substantial defect in migration by extracellular-RNase-treated neutrophils when an endothelial layer was present on the tanswell membrane. A similar defect was observed when assaying neutrophil attachment to endothelial cells in vitro. This defect can be replicated using control neutrophils but by pre-blocking endothelial cells with purified neutrophil GlycoRNAs or the glycan fraction of GlycoRNAs. The defect in neutrophil endothelial interaction in vivo was observed by intravital confocal microscopy. These data support the function of neutrophil cell surface GlycoRNAs in helping transendothelial migration.

Lastly, we found that neutrophil GlycoRNAs are bona fide ligands for P-selectin on endothelial surface. Removal of neutrophil surface RNAs with extracellular RNase treatment did not significantly change cell surface integrin levels or reactivity, but reduced recombinant P-selectin binding. Furthermore, recombinant P-selectin, but not recombinant E-selectin, can detect glycoRNAs in purified neutrophil total RNAs. Additionally, blocking endothelial cells with an antibody against P-selectin led to a significant reduction in GlycoRNA binding. These data support that GlycoRNA-P-selectin interaction, at least in part, mediate neutrophil transendothelial migration.

Our data demonstrate a critical role of cell surface RNAs in neutrophils, and reveal a new dimension that regulate the function of hematopoietic cells.

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