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Research paper

Utilization of imaging flow cytometry to define intermediates of megakaryopoiesis in vivo and in vitro

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ABSTRACT

Imaging flow cytometry is a particularly powerful analytical approach for the study of megakaryopoiesis. It can utilize well-defined immunophenotypic markers as well as assess maturation of megakaryocytes by their increasing ploidy as they endoreplicate. Imaging flow cytometry can also assess morphometric cell characteristics of size and nuclear to cytoplasmic ratio, which are informative indications of maturation. However, megakaryopoiesis is challenging for flow cytometric analysis, particularly in vivo, because megakaryocytes are very rare in the bone marrow and their odd shape, high DNA content and cell size are similar to clumps of cells. Additionally, both megakaryocytes and immunophenotypically similar platelets are frequently found associated with other cells. Due to these challenges, imaging flow cytometry of megakaryopoiesis exemplifies several strengths of this approach in utilizing fluorescent signal's shape, texture and overlap with other fluorescent signals to distinguish megakaryocytes from a variety of contaminants and to restrict analysis to megakaryocytes, even when associated with other cells. Presented here is a strategy for imaging flow cytometric analysis of rare murine megakaryocytes directly from the bone marrow as well those grown in vitro and analyzed as live cells, or after fixation and permeabilization.

1. Introduction

Platelets are small enucleate cells in our bloodstream with important roles in preserving vascular integrity as well as in mediation of innate and reactive immunity (Morrell et al., 2014 for review). Adult humans have over one trillion circulating platelets whose short lifespan requires synthesis of over two million platelets every second. Ironically, this smallest of blood cells is derived from the largest blood cell in the bone marrow, the megakaryocyte (Mk). Megakaryopoiesis begins with specified progenitors that can be functionally defined using colony-forming assays and prospectively identified by immunophenotype (Pronk et al., 2007; Weissman and Shizuru, 2008). Mk progenitors generate maturing Mks, which undergo incomplete cell divisions that result in cells with increased ploidy. During maturation, Mk cytoplasm grows dramatically and includes production of platelet-specific granules and excess membrane needed for platelet generation. Ultimately, it is this cytoplasm that is pinched off as pro-platelets during thrombopoiesis, leaving behind the "exhausted megakaryocyte" consisting of the nucleus surrounded by a thin layer of cytoplasm.

In many ways, the features of megakaryopoiesis make it well suited for analysis by flow cytometry. Mks are very rare in the bone marrow, only 0.1 to 0.5% of bone marrow cells in the mouse, and benefit from analysis of large numbers of cells (Corash et al., 1989). There are many flow cytometric markers associated with Mk lineage and maturation (Tomer, 2004). Furthermore, quantitation of ploidy is a powerful tool to gauge the maturational status of Mks. However, analysis of megakaryopoiesis also poses significant challenges for flow cytometry. The large size of maturing MKs, their frequent amorphous shape, and their high ploidy result in light scatter characteristics that are similar to cell clumps, preventing

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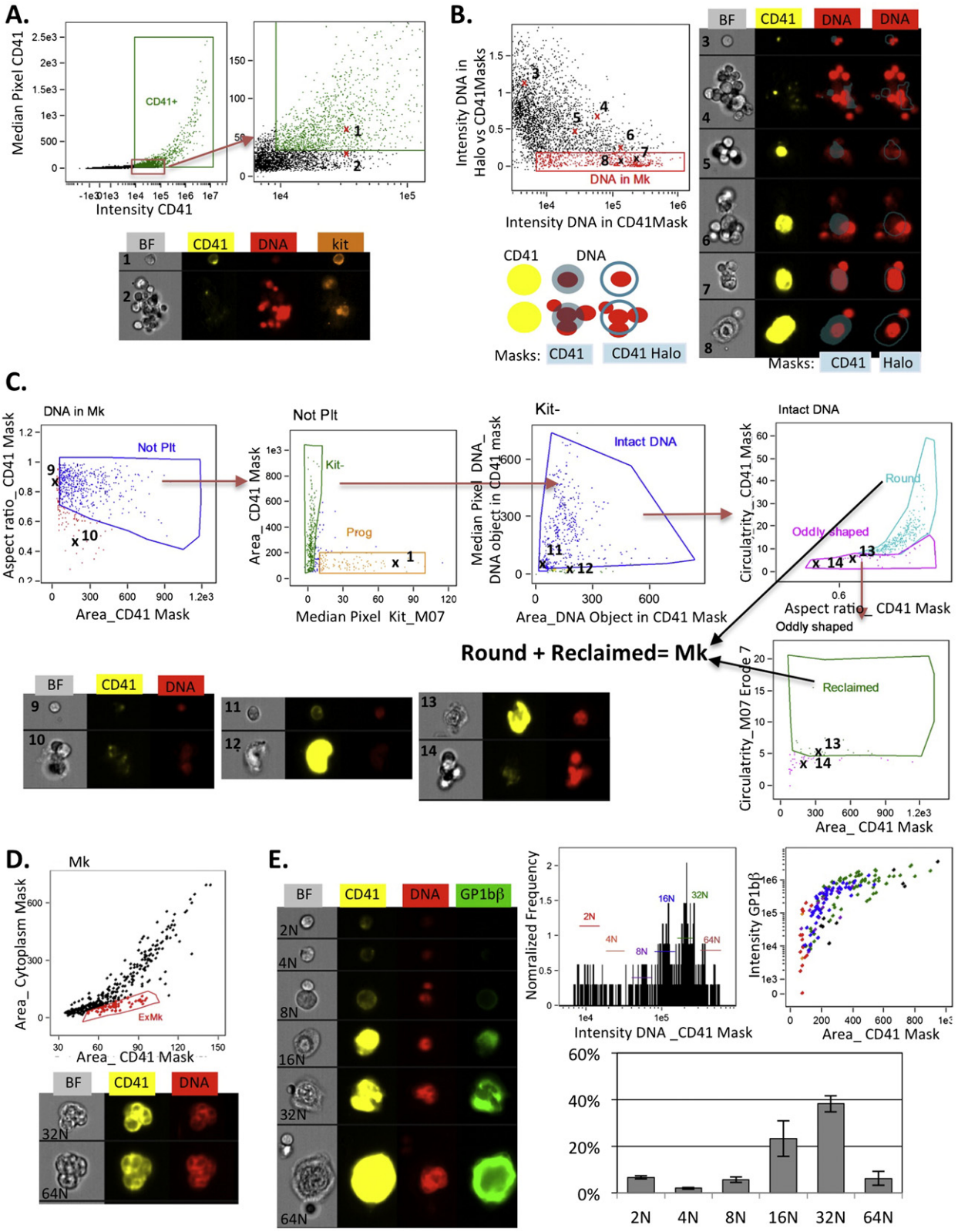
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