In Vitro and In Vivo Methods to Explore Megakaryopoiesis

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Editorial

Platelets are small, anucleate blood cells, essential for hemostasis, which derive from megakaryocytes (MKs) in the bone marrow (BM). During their differentiation from hematopoietic stem cells, MKs develop into giant cells in a complex process of differentiation and maturation that includes polyploidization and cytoplasmic maturation. In the final stage of development when MKs are in contact with endothelial cells, they achieve their mature stage and extend long cytoplasmic extensions, called proplatelets, into blood vessels, which then fragment to give rise to platelets in the bloodstream^{1,2}. The mechanisms that regulate MK maturation and transendothelial proplatelet formation are still incompletely understood. The goal of this collection is to bring together complementary methods that will contribute to better understand the biology of MKs *in vitro* and *in vivo*.

In vitro expansion and differentiation of MKs is widely used to study the mechanisms underlying platelet biogenesis. In the protocol by Pongerard et al., the authors describe a standardized culture protocol for the differentiation of MKs from human CD34⁺ hematopoietic progenitors. They especially emphasize that leukocyte filters available at blood centers are an exceptional source for CD34⁺ cells collection. Their article contains several essential guidelines on how

to extract CD34⁺ cells, how to assess their purity and the importance of their seeding density. The authors also propose a calibrated pipetting method, five times in succession, to efficiently release functional platelets. Interesting applications of this method are the possibility to evaluate pharmacological compounds or to genetically manipulate CD34⁺ cells using a CRISPR-Cas9 genome editing method^{3,4}. For mouse studies, Kimmerlin et al. present a cell sorting strategy to purify MK progenitors in sufficient numbers compatible with molecular and cellular assays. The authors discuss the merits of using the iliac crest as a source of bone marrow containing a great number of hematopoietic progenitors and of performing a two-step magnetic depletion of unwanted cells prior to cell sorting using a combination of cell surface markers. A major advantage of this method is the reduction in the number of animals required to obtain highly purified MK progenitors which, for example, may be of interest to researchers studying hematopoietic progenitors' hierarchy and their differentiation. In this context, Boscher et al. describe a method to grow progenitors in a 3D medium composed of 2% methylcellulose to better mimic the *in vivo* environment. In fact, these authors have previously demonstrated that MKs grown in a 3D hydrogel achieve a greater maturation state and a greater ability to form

progenitors compared to those grown in a liquid medium⁵. Critical steps are highlighted in this protocol such as the appropriate volume of methylcellulose to obtain optimal medium stiffness, the number of cells that should be equivalent in each experimental condition, and the risk of contamination. For functional verification of the maturation level, the authors explain how to resuspend the gel-cultured cells in liquid medium on the third day of culture and how to fix the cells for future analysis by immunostaining, flow cytometry or electron microscopy. Transmission electron microscopy (TEM) is the imaging approach of choice to provide high resolution ultrastructure of MKs. Here, Scandola et al. share a protocol to analyze the ultrastructure of MKs in their natural BM environment, and to quantify their cell density and score the different maturation stages. The authors use TEM to analyze the cellular events that occur when MKs interact with the sinusoidal wall, such as their short invasive podosome-like protrusions penetrating the endothelium⁶. A limitation of the method is that the initial phase of MK engagement cannot be analyzed because of the lack of specific ultrastructural features of these immature cells. As noted, this imaging approach has the advantage of using small BM samples allowing to perform multiple studies with a single mouse. As an example, the other bone marrow samples can be used for the bone marrow explant technique which is presented in this collection by Guinard et al. to study the dynamic extension of proplatelets from MKs. The strength of this method lies in the use of MKs that have developed in the BM, thus avoiding any potential misinterpretation resulting from artifacts of cell differentiation in culture. Indeed, this has been previously documented in mice with MK-restricted *MYH9* inactivation, where divergent results were found *in vitro* and *ex vivo*⁷. The authors also highlight the simple, reproducible, and rapid nature of this approach. An interesting note of their protocol is the ability to use microscopes equipped with navigation

software to facilitate tracking/quantification of MKs extending proplatelets. A complementary method of the explant model is the two-photon imaging of MKs in the mouse skull. The uniqueness of this technique is the ability to study *in vivo* the dynamic behavior of fluorescently labeled MKs in the bone marrow and to visualize proplatelet extension. This protocol has the advantage of being minimally invasive, with limited surgical interventions, thus avoiding inflammatory reactions that have been reported to impact proplatelet formation. As Bornert et al. note, one limitation of this method is the narrow depth that can be imaged due to the density of the bone, which also imposes the use of younger mice. This technique can be applied to genetically modified mice to study the role of genes of interest in *in vivo* platelet formation. For instance, the authors used this technique to demonstrate that the role of microtubules in the elongation of proplatelets differs between *in vitro* and *in vivo* conditions⁸.

Abnormal MK development and platelet production can lead to thrombocytopenia or thrombocythemia, increasing the risk of bleeding or thrombosis, respectively. Continuing efforts to dissect the molecular mechanisms governing platelet biogenesis will increase our capacity to develop novel strategies for the treatment of thrombocytopenia and thrombocythemia. In the field of transfusion research, these efforts will lay the foundation to produce cultured platelets as a transfusional alternative in selected situations⁹.

Disclosures

The authors have nothing to disclose.

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