Survivin: a new player during erythroblast maturation

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Enucleation of erythroblasts is a phenomenon unique to mammals. During their terminal stage of differentiation, mammalian erythroblasts exit the cell cycle and enucleate. They complete their terminal differentiation and enucleation in "erythroid niches" composed of erythroblastic islands nested in extracellular matrix proteins.[1-6] It has been recently reported that, in the very last stage of enucleation, not only proteins but also specialized regions of the lipid bilayer, known as lipid rafts, as well as organelles participate in the extrusion of the nucleus.[7] Approximately 120 million reticulocytes are generated each minute in our body. Macrophages phagocyte and recycle a similar number of extruded nuclei and other organelles, such as mitochondria. In addition, wastes in the reticulocyte are digested through autophagy and extruded via exosomes. Keerthivasan and colleagues shed new light on the functional relationship between enucleation and vesicle trafficking by identifying a novel role for the apoptosis inhibitor, survivin.[8]

Is enucleation actually a type of cytokinesis ?

One popular postulate is that enucleation is a type of asymmetric cytokinesis.[1-6] Although "cytokinesis" generally refers to the phenomenon that occurs as the cell divides into two symmetric daughter cells, in the case of erythroblast enucleation, it entails an asymmetric division into a nucleus (pyrenocyte) and a reticulocyte.[5] The occurrence of an asymmetric cytokinesis is supported by the observation of a concentration of actin in the region extending between the extruding nucleus and the nascent reticulocyte, reminiscent of the actomyosin ring observed during cytokinesis. Furthermore, it has been shown that enucleation requires functional actin and Rac GTPases that participate in the formation of the actin ring. In fact, the actomyosin is likely the main player during enucleation[1] as it has been demonstrated that non-muscle myosin IIB plays also an important role in the enucleation of human erythroblasts.[9]

One controversial theory is that microtubules may also participate in some phases of the enucleation process because *in vitro* and *in vivo* studies in rats show that microtubule-depolymerizing agents inhibit nuclear extrusion. Hence investigations so far mainly offer hints as to the players involved in enucleation but do not present any well-delineated molecular mechanism driving the sequence of events leading to successful enucleation. To address this important issue, Keerthivasan and colleagues have comprehensively investigated a model of asymmetric cytokinesis. Based on their and other groups' experimental data, it is shown that widely used inhibitors of cytokinesis, including blebbistatin, hesperadin or nocodazole, have no effect on postmitotic primary murine erythroblasts. Instead, they propose a novel hypothesis based upon earlier electron micrographs that described an accumulation of vesicles in the region extending between the extruding nucleus and the nascent reticulocyte.[10]

Characterization of vesicle trafficking during enucleation

Keerthivasan and colleagues elegantly examined different components of the vesicle trafficking pathway, by evaluating the impact of a diverse set of molecules on the enucleation of adult spleen and fetal liver mouse erythroblasts. The inhibition of endocytosis by either dynamin inhibitors or sucrose, which blocks the formation of clathrin-coated pits, prevents enucleation. Monensin, which disrupts trafficking between endosomes and lysosomes, also inhibits enucleation. Importantly, these small molecules had little effect on cell differentiation or viability. In contrast, neither treatment with brefeldin A, a blocker of endoplasmic reticulum and Golgi transport, nor with A5, an inhibitor of trafficking between the trans-Golgi network and endosomes, interfere with enucleation. These results clearly show that intact endocytic vesicle trafficking and the endosome/lysosome secretory pathway are important components of nuclear extrusion. Further evidence of the importance of vesicle trafficking is provided by the inhibition of enucleation in cultured human $CD34^+$ cells in which clathrin has been silenced, and by the increased enucleation observed after induction of vacuole formation mediated by vacuolin-1. Using this multifaceted experimental strategy, the authors conclude that endocytosis and coalescence of vesicles play a key role in nuclear extrusion.[10]

Survivin in erythroblasts

In 2007, Leung *et al.*, led by Professor Crispino, found that an inhibitor of the apoptosis (IAP) family of proteins, survivin, is highly expressed in non-dividing erythroblasts.[11,12] Heterozygous deletion of survivin causes defects in erythropoiesis in animal models, with a reduction in enucleated erythrocytes and the presence of immature megaloblastic erythroblasts. Their studies demonstrate that survivin is necessary for steady-state hematopoiesis and survival of the adult and, furthermore, that survivin expression is important for proper erythroid differentiation. As evidenced for a functional role of survivin in erythrocyte maturation, they observed that a subset of survivin heterozygous mice exhibits a decrease in the percentage of enucleated cells when compared to wild-type littermates.[10]

What is survivin?

Survivin is a member of the inhibitor of apoptosis protein (IAP) family. This 16.5 kDa protein adopts a unique dimer structure (Figure 1).[13] In addition to its function as an apoptosis inhibitor, survivin is a chromosomal passenger protein

that mediates spindle assembly checkpoint and cytokinesis.[14-19] In addition to normal proliferating cells, survivin is also frequently over-expressed in cancer cells where it likely also serves as an inhibitor of apoptosis.[14-19] As such, survivin has been proposed as an attractive target for anticancer therapies and, in some cases, has even been proposed as a cancer-specific gene. Survivin plays an essential role in cell division as a component of the chromosome passenger complex (CPC), which is comprised of survivin, aurora B kinase, INCENP (inner centromere protein) and borealin. This complex fulfills essential functions at multiple steps and in multiple cellular locations during mitosis. This complex binds first along the length of chromosomes, then relocalizes to centromeres, and finally migrates towards the central spindle in anaphase and resides in the midbody and cleavage furrow during cytokinesis. Loss of any of the CPC proteins results in delocalization of the partner proteins and multiple cell division defects. For example, silencing or knockout of survivin leads to G1 arrest, polyploidy, and death as a result of mitotic catastrophe.

Involvement of a novel survivin-based protein complex in enucleation

Keerthivasan and colleagues report that, unlike in other cell types,[14-16] survivin does not co-localize with inner centromere protein or aurora B in enucleating erythroblasts. Instead, it is part of a multi-protein complex with epidermal growth factor receptor substrate15 (EPS15) and clathrin,[20] two proteins that mediate endocytic vesicle trafficking.[3] In

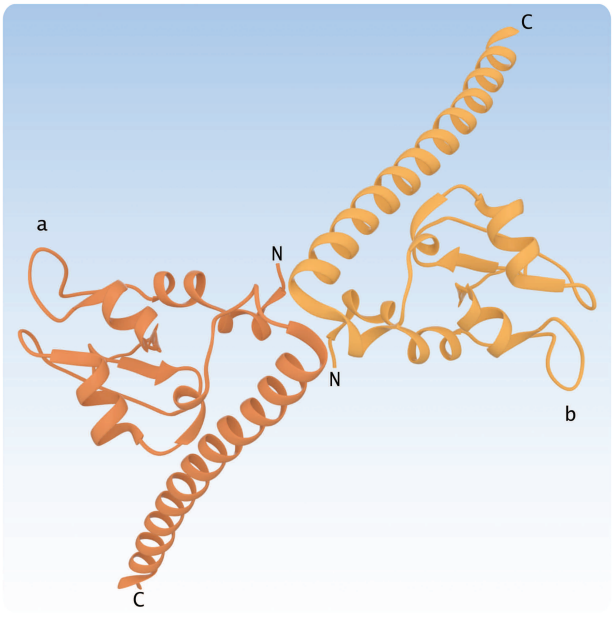

Figure 1. 3D structure of survivin (PDB accession No.1e31). The structure of full-length human survivin determined by X-ray crystallography at 2.7Å.[13] The protein adopts a very unusual bow tie–shaped dimer structure (shown as *a* and *b*). Chantalat *et al.* reported that survivin does not dimerize through a C-terminal coiled-coil (shown as *C*), contrary to sequence analysis prediction.[13] The C-terminal helices contain hydrophobic clusters that may mediate protein-protein interactions. *N* indicates the amino terminal region of each surviving monomer.

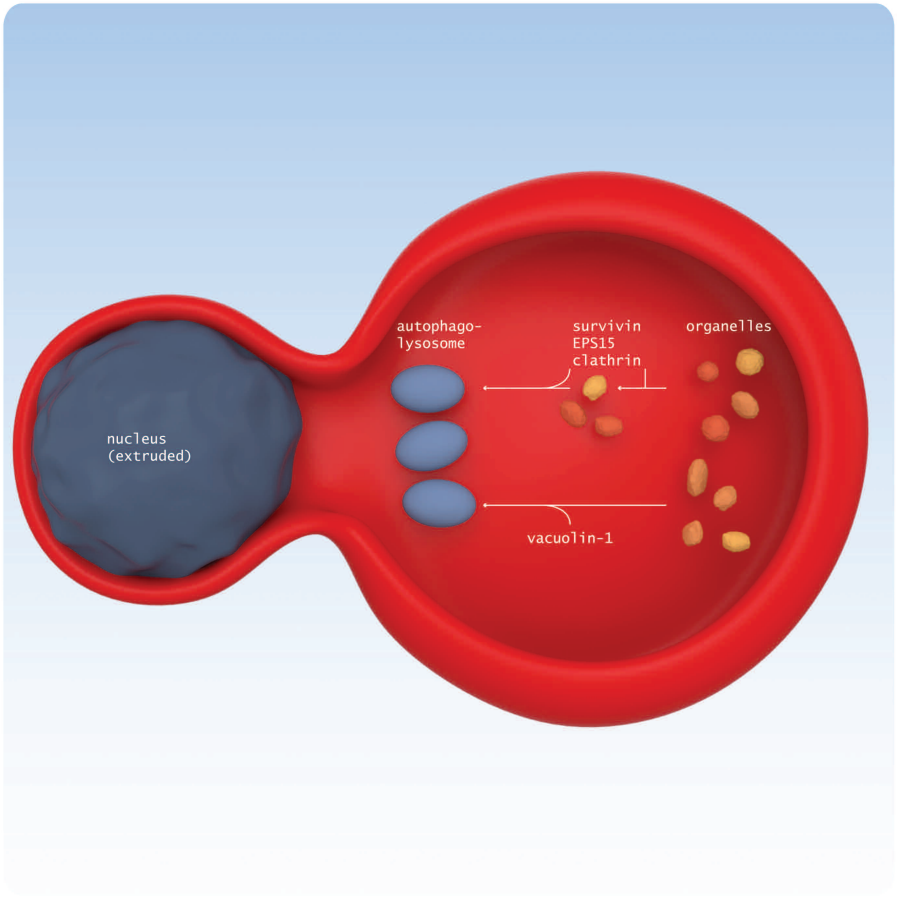

Figure 2. Model of erythroblast enucleation. During the last stage of erythroblast enucleation, organelles, vesicles/vacuoles, endosomes, lysosomes and autophagosomes migrate to the border of reticulocytes and are excluded along with the nucleus. A complex consisting of survivin, EPS15 and clathrin is proposed to act cooperatively in the formation, movement and/or fusion of vesicles, endosomes or lysosomes while vacuolin-1 induces the formation of vacuoles by homotypic fusion of endosomes and lysosomes. Konstantinidis *et al.* suggested that the fusion of lipid rafts upon docking of autophagosomes with the cytoplasmic membrane is an important process during the terminal phase of enucleation.[7] This cartoon is based on Figure 6C in the study of Keerthivasan *et al.*[8]

support of a direct role of this latter complex in enucleation, the authors found that knockdown of the genes reduces the efficiency of enucleation of primary human erythroblasts. Based on their own findings, they confirm that overexpression of survivin in cells dramatically increases the ratio of enucleated cells. They then provide evidence for a direct interaction between survivin and two membrane trafficking proteins, i.e. EPS15 and clathrin, in well-established murine (MEL) and/or human (K562) erythroleukemia cell lines using a combination of size exclusion chromatography and mass-spectroscopy analyses. They also confirm the survivin-EPS15 interaction by performing endogenous EPS15 immunoprecipitation in lysates from Day 12 primary human enucleating erythroblasts (Figure 2).[3] Finally, they observe that a loss of survivin in murine erythroblasts inhibits enucleation and that survivin-deficient cells harbor smaller cytoplasmic vacuoles. Of particular note, vacuolin-1, a small molecule that induces vacuole fusion, rescues the defective enucleation caused by survivin deficiency (Figure 2). In the light of these observations, the authors suggest that, in addition to providing required membrane material for the dividing daughter cells, an intact vesicle trafficking pathway is important for the completion of cytokinesis and that the function of the survivin-EPS15-clathrin complex may be highly relevant in dividing cells.

Keerthivasan and colleagues conclude that vacuole fusion with endosomes or lysosomes can occur in at least two ways in reticulocytes (Figure 2). Further investigation will clarify the mechanism of vacuole formation and its relationship to enucleation. The findings in this study provide novel mechanistic insights into the enucleation process in relationship to vesicle trafficking, in particular vacuole and vesicle fusion with autophagolysosomes. Studies extending the present findings will hopefully further clarify the mechanisms underlying the selection between the two pathways of vacuole fusion with endosomes or lysosomes during enucleation.

One must keep in mind that vesicle trafficking is not a requirement restricted to enucleation, but that it is a general phenomenon during the final process of cell division. Although the importance of membrane trafficking in enucleation has now been demonstrated and key proteins in this process, such as survivin, have been identified, the biological significance of this biological process still needs to be further investigated. There is speculation that membranes from organelles may contribute to the formation of cytoplasmic membrane after enucleation. Such speculations will hopefully be validated through the use of inhibitors of enucleation.

Finally, although many data have been generated about the enucleation process in mammalian erythroblasts, the burning question of why enucleation is essential for mammalian erythroblasts still remains unanswered

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