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Review

Midbody Formation and Fate

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Abstract: Faithful cell division is crucial for successful proliferation, differentiation and development of cells, tissue homeostasis, and preservation of genomic integrity. Cytokinesis is a terminal stage of cell division, leaving two genetically identical daughter cells connected by an intercellular bridge (ICB) containing the midbody (MB), a large protein rich organelle, in the middle. Cell division may result in symmetric or asymmetric abscission of the ICB. In the first case, the ICB is severed on the one side of the MB, and the MB is inherited by the opposite daughter cell. In the second case, the MB is cut from both sides, expelled into the extracellular matrix, and later it can be engulfed by surrounding cells. Cells with lower autophagic activity, such as stem cells and cancer stem cells, are inclined to accumulate MBs. Inherited MBs affect cell polarity, modulate intra- and intercellular communication, enhance pluripotency of stem cells, and increase tumorigenic potential of cancer cells. In this review, we briefly summarize the latest knowledge on MB formation, structure, and degradation and, in addition, present and discuss our recent findings on the electrical and chemical communication of cells connected through the MB-containing ICB.

Keywords: cytokinesis; intercellular bridge; midbody; conductance; permeability; tumorigenicity

1. Introduction

It is known that tetraploidy causes chromosomal aberrations and development of aneuploid cancer [1, 2]. One of the mechanisms of tetraploid formation is related to the defects of cytokinetic proteins resulting in cytokinesis failure [2]. An abnormal separation of chromosomes, when both copies of a chromosome agglomerate in the same daughter cell, can promote cleavage furrow regression and development of tetraploidy *in vitro* [3]. Therefore, faithful cell division is crucial for successful proliferation, differentiation, development, communication, and preservation of genomic stability. Cytokinesis is a terminal stage of cell division leaving two daughter cells connected through the intercellular bridge (ICB). During the abscission of the ICB, the central spindle microtubules are packed into a structure named the midbody (MB) [4]. There are two possible ways of final cell division: asymmetric and symmetric ICB abscission. In the first case, the MB can be incorporated by one of the daughter cells. In case of symmetric ICB abscission, the MB can be ejected into the extracellular milieu and later engulfed by the surrounding cells. Then MBs undergo encapsulation by autophagic membranes and degradation via selective macroautophagy. Cells with lower autophagic activity, such as stem cells and cancer stem cells, are inclined to accumulate MBs [5]. Inherited MBs affect cell polarity, modulate intracellular signaling and intercellular communication, enhance pluripotency of stem cells, and increase tumorigenic potential of cancer cells. In this review, we summarize the main steps of MB formation and inheritance, its structure, mechanism of degradation and, in addition, present our own new data on the conductance and permeability of the MB-containing ICB that has not been sufficiently described in the scientific literature yet.

2. Midbody Formation

During metaphase, duplicated chromosomes accumulate at the midzone of the dividing cell (Figure 1, step 2). Cytokinesis starts during anaphase and is the final step of cell division. During anaphase, two sets of chromatids translocate to the opposing spindle poles (step 3). Overlapping microtubules of the mitotic spindle compose the central spindle, and the further assembly of it is regulated by the heterotetrameric centralspindlin complex, which is composed of two subunits of MKLP1, a kinesin-6 motor protein, and CYK-4, a Rho-family GTPase-activating protein [6]. Activated RhoA coordinates the assemblage of the actomyosin contractile ring, which constricts the cell forming an ingression of the cleavage furrow (step 4). The contractile ring is built of formin-nucleated actin filaments, bipolar filaments composed of the motor, myosin II, membrane-associated septin filaments, and anillin cross-linking the actin filaments [7]. The further constriction of the contractile ring results in a formation of the ICB, which connects two daughter cells (step 5). The ICB is ~2 μm in thickness and contains residuals of the contractile ring and central spindle microtubules situated in anti-parallel *overlapping* bundles. The overlapping plus-ends of these microtubules shape the MB, a structure located at the center of the ICB (Movie 1). More than 100 years ago Walter Flemming was the first who noticed and described this structure, which was then named after his name (Fleming body), recently known as the midbody (MB) [8]. Abscission of the ICB starts from VAMP8-dependent fusion of FIP3 positive endosomal vesicles with the plasma membrane of the ICB, causing gradual bridge narrowing, which forms the secondary ingression [9]. The abscission of the ICB proceeds when endosomal sorting complexes required for transport (ESCRT -0, -I, -II, and –III filament system) are recruited to the constriction zone and interacting with MB-severing proteins, such as spastin, catalyze membrane scission on the one (step 5A) or both (step 5B) sides of the MB to separate the two daughter cells (steps 6A and 6B). In the first case, MBs may be asymmetrically internalized by one of the daughter cells (steps 6A and 7A) (Movie 2), possibly with the older centrosome [5]. In the second case, the MB is released into the extracellular matrix (step 6B), but later can be engulfed by the same or other surrounding mobile cells (step 7B) (Movie 3). In stem cells, the MB release occurs more frequently than in cancer-derived cells [10]. Inside the cells, the MB is encapsulated by the FYCO1 (FYVE domain-containing protein)-containing isolation membrane [11] and degraded by autophagy (insert in Fig. 1-7B) (detailed below).

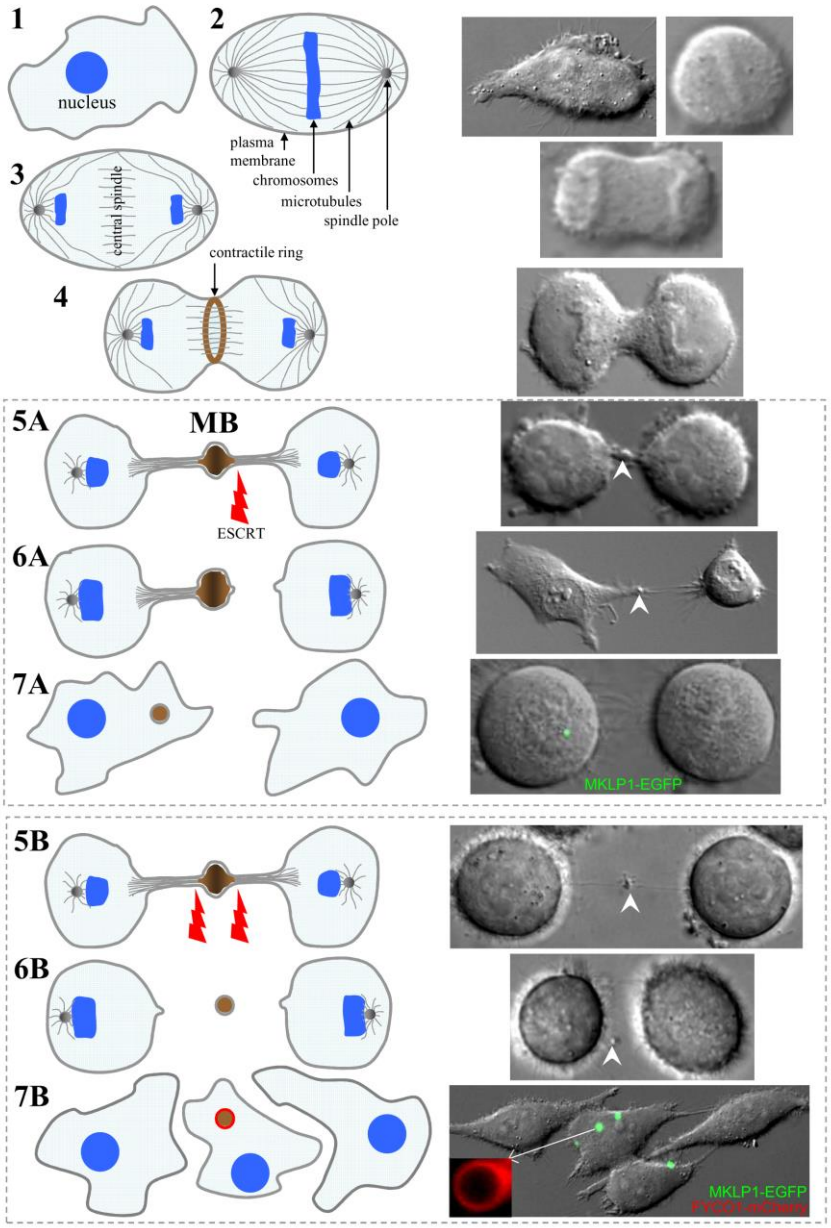

Figure 1. Midbody formation and inheritance during cytokinesis (detailed in the main text).

3. Midbody Structure

Endosomal recycling controls the surface expression of proteins during cell division. The vesicles derived from endosomes then fuse with the ICB membrane, generating a secondary ingression [9]. More than 100 proteins have been identified in the MB by proteomic analysis ([12] and our unpublished data); however, the functional role of only few of them has been defined by now. MB proteins are divided into three groups following their localization (Figure 2A): the central bulge (brown), central dark zone (green), and peripheral flanking region (yellow) [13]. The central bulge contains centralspindlin with interacting proteins and few microtubules. All this makes a ring-shaped bulge. Its function is not clear, but the presence of RhoA and anillin in it suggests that it may serve as a signaling center to coordinate cortical alterations in late cytokinesis. The central dark zone where antiparallel microtubules overlap determines the architecture of the midzone. Presumably, PRC1, a protein regulator of cytokinesis 1, and KIF4, chromosome-associated kinesin, localize there to stabilize the overlap and to inhibit plus-end elongation, respectively. The flanking zones bind Aurora B, a member of the Aurora family protein kinases, and several other microtubule-interacting proteins. Mission of this zone is to position the sites of abscission which occurs at the outer margin of the

Aurora A-binding region. Two other proteins that define the specialized cortex of the furrow, RhoA and anillin, also are found at the presumptive abscission sites and may guide the ESCRT complexes to these sites, providing the first molecular clue to the abscission mechanism

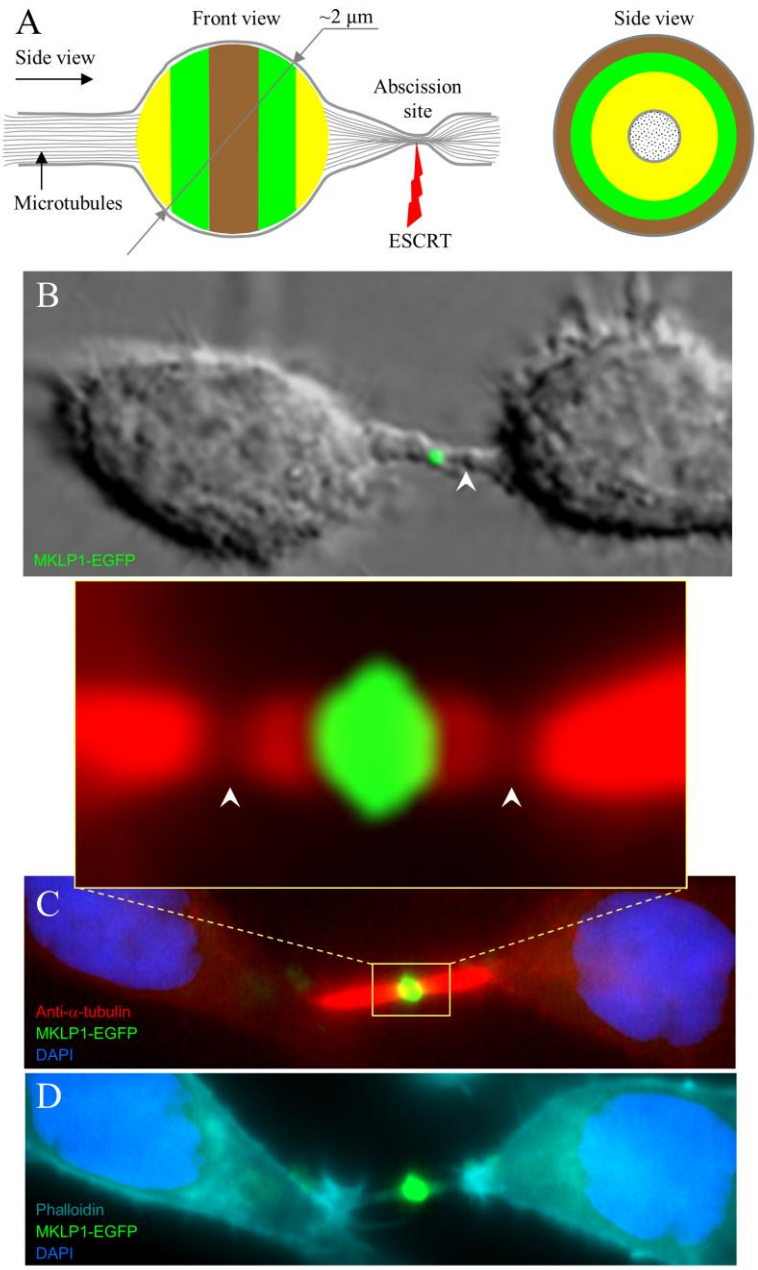

Figure 2. Structure of the midbody. (A) The MB is formed from overlapping plus-ends of central spindle microtubules, which deliver different proteins to compose 3 zones of the MB: central bulge (brown), central dark zone (green), and peripheral flanking regions (yellow). (B) The MB is located in the middle of the ICB. Abscission of the MB includes changes in actin and microtubule cytoskeleton, and ESCRT complex-mediated membrane scission (white arrowheads) that may occur at one (A and B) or both (insert in C) sides of the MB. These changes lead to asymmetric abscission followed by inheritance of the MB by one of the daughter cells or to symmetric abscission when the MB is separated from the cells.

4. Midbody Degradation

Differentiating and normally dividing cells that exhibit high autophagic activity do not accumulate MBs; however, cells with lower autophagic activity, such as stem cells and cancer stem cells, are prone to accumulate MBs by evading autophagosome encapsulation [5, 10]. The accumulation of MBs correlates with the aggressiveness of cancer cells; however, the mechanism of post-mitotic MB accumulation has not been completely elucidated yet. Post-mitotic MBs can be internalized during asymmetric abscission by one of the daughter cells or, in case of symmetric abscission, separated from the cells, the MB later can be engulfed by the same or other surrounding cells (Fig. 1) [4]. Inside the cells, the MBs are encapsulated by autophagic membranes and degraded [4, 5]. Macroautophagy plays a major role in preventing the accumulation of MBs. During the process of degradation, MBs like other protein complexes and organelles are encapsulated within an isolation membrane to form an autophagosome. The autophagosome then fuses with lysosomes to form a phagolysosome, where its content is degraded. When the MB is recognized and destined for degradation, the cell starts forming an isolation membrane around the MB by the recruitment of a specialized membrane structure, known as a phagophore. The phagophore is formed by the recruitment of LC3, a microtubule-associated protein-1 light chain 3 that stably associates with the membrane of autophagosomes. The formation of the autophagic isolation membrane is also dependent on the extension of the phagophore by the delivery of additional membrane. These membranes also deliver Atg9, an autophagy-related protein 9, the only Atg protein that has a transmembrane domain and functions in delivering membrane to the expanding phagophore [14]. Endosome delivery and fusion with a phagophore is required for autophagic degradation [11].

Importantly, in cells with low autophagic activity, MBs can avoid degradation [5]. Such cells can even accumulate MBs which then modify various cellular functions, including proliferation, differentiation, and colony formation [11]. MB degradation by autophagy is a highly regulated process that requires targeted endocytic membrane delivery during the extension of autophagic isolation membrane and involves specific regulatory proteins. For instance, in our recent study, we presented evidence that FYCO1, known to be implicated in autophagy [15], was also mediating MB degradation [11] (insert in Fig. 1-7B). FYCO1 depletion led to MB accumulation and increased cancer cell invasiveness [11]. This, in turn, suggests that accumulation of MBs may promote cancer invasiveness and promote metastasis.

5. Conductance and Permeability of Midbody-Containing Intercellular Bridges

Gap junction (GJ) channels and membranous tunneling tubes (MTTs) or nanotubes provide a direct pathway for electrical, metabolic, and genetic communication between cells [16, 17]. MTTs are much longer structures than ICBs formed during cytokinesis. The permeability properties of MTTs are relatively well characterized (see, for example [18] and [16]). However, MTTs do not contain MBs. It is insufficiently addressed by now whether ICB electrical conductance as well as permeability for larger molecules is affected by the presence of the MB. To examine these possibilities, we employed fluorescence microscopy, time-lapse imaging, and dual whole-cell patch-clamp techniques [18, 19]. The last one allowed us to understand whether ICBs containing MBs (Fig. 3A) preserved electrical conductance between the cells, and if so, whether these ICBs contained GJs. To test this, we measured the I_j response in cell-2 (Fig. 3B, lower panel) to the voltage ramp from 0 to 120 mV in cell-1 (Fig. 3B, upper panel). Depending on the stage of abscission, the ICBs exhibited electrical conductances (obtained from ratio $\Delta I_j/\Delta V_j$) from less than 1 nS to 70 nS (n=15). The absence of voltage gating suggested that these ICBs were open-ended channels not containing GJs. However, at the end of abscission, we occasionally observed the appearance of single channels (Fig. 3C) typical of connexin 45 (Cx45)(γ_j = ~30 pS) [20]. HeLa cells express low levels of endogenous Cx45 that occasionally may form low transjunctional conductance GJs [21]. Single-channel currents were measured in the cell-2 as a response to voltage step of –80 mV in the cell-1, and the single-channel conductance γ_j was obtained from ratio i_j/V_j. This observation suggests that when cells come into a contact at the end of cytokinesis, electrical coupling between them, first of all, is initiated by forming GJs at the abscission site.

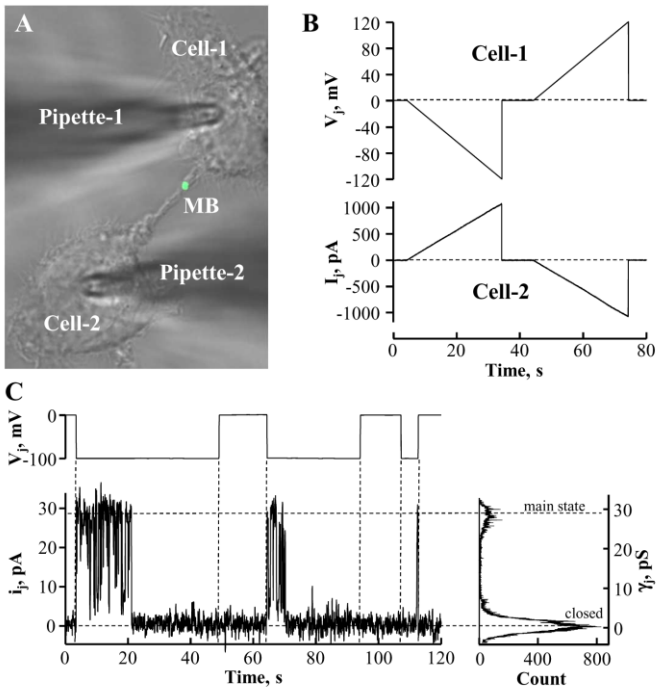

Figure 3. Characterization of MB-containing ICB electrical properties. (A) The MB-containing ICB connecting a pair of HeLa cells. (B) Electrical properties of TTs were evaluated by applying a voltage ramp from 0 to ±120 mV (B, upper panel) to the cell-1 and measuring junctional current in the cell-2 (B, lower panel). Absence of voltage gating implies that the ICB did not contain GJ. (C) Abscission may result in the formation of GJ at the abscission site composed of ~30 pS single channels typical of endogenous Cx45.

Further, we examined the permeability of MB-containing ICBs to fluorescent dyes of different molecular weight and net charge. We used the following dyes (molecular mass of the fluorescent ion; valence): Alexa Fluor-350 (AF350) (326; –1), Lucifer Yellow (LY) (443; –2), Alexa Fluor-546/10000 dextran (AF546/10000) (10000; –1), DAPI (279; +2), and Hoechst-33342 (562; +3). To measure the permeability of the MB-containing ICB, the pipette filled with the dye was attached to the cell-1 (Fig. 4). After opening patch by electrical pulse, the dye diffused to the cell-1 followed by dye transfer through the ICB to the cell-2. Kinetics of dye accumulation in both cells is shown in the respective panels of Fig. 4. These results demonstrate that MB-containing ICBs were permeable to dyes of negative net charge such as AF350 (Fig. 4A) and LY (Fig. 4B) as well as even such a large molecule as AF546/10000 (Fig. 4C). However, ICBs were virtually impermeable to dyes of positive net charge such as DAPI (Fig. 4D) and Hoechst-33342. At the end of the experiment, the patch in the cell-2 was opened with the second pipette to measure electrical conductance of ICBs in a dual whole-cell patch-clamp mode, i.e., to confirm that ICBs were open. These results indicate that MBs may play a role of selectivity filter for positively charged molecules, because tunneling nanotubes that do not contain MBs allowed the passage of molecules independent of their charge polarity, but rather dependent on charge size [18]. GJs allow the passage of molecules up to ~1 kDa, such as cAMP, ATP, ADP, AMP, adenosine, IP_3, glutamate, glutathione, etc. (reviewed in [17]). Family of connexins (Cxs) composing GJ channels consists of 21 genes in the human genome. Some of them are better permeable to positively charged, others to negatively charged molecules, and in general, GJ permeability is inversely dependent on charge size [22]. Low concentrations of Ca^{2+} can permeate GJ channels; however, higher concentrations close the channels directly or in calmodulin-dependent manner [23]. Mg^{2+} and H^{2+} also gate GJs in concentration- and Cx type-dependent manner [24, 25].

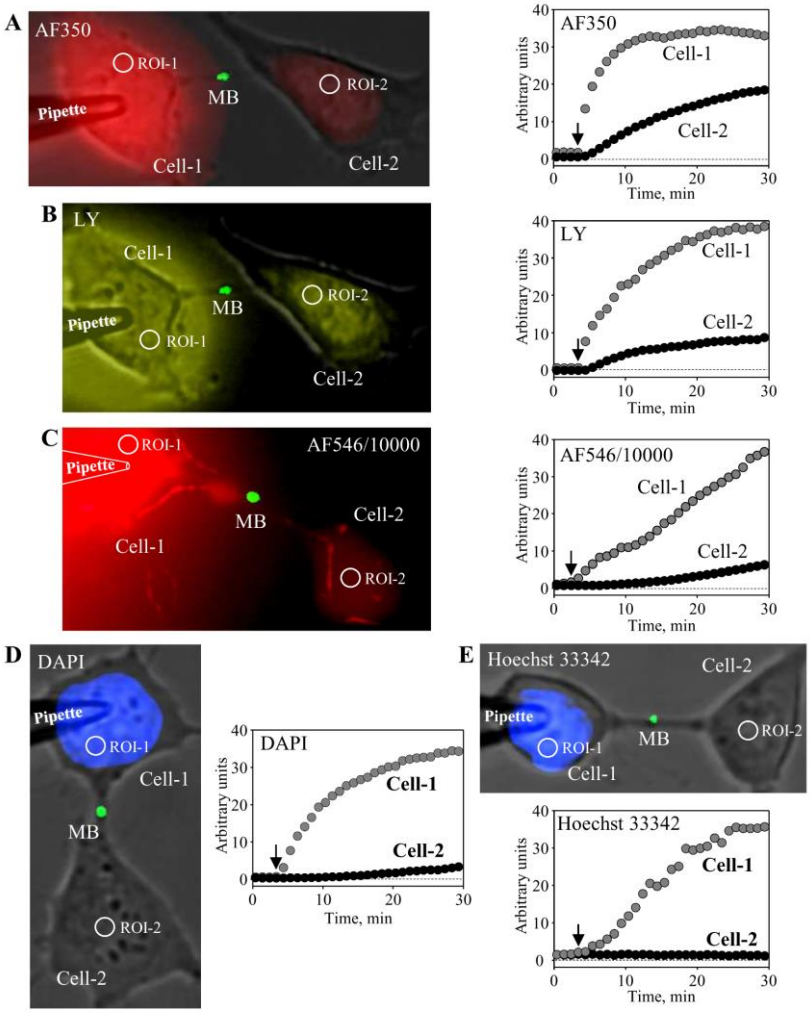

Figure 4. Characterization of MB-containing ICB permeability. To measure the permeability of MB-containing ICB, the pipette filled with the dye was attached to the cell-1. After opening patch by electrical pulse, the dye diffused to the cell-1 followed by dye transfer through the ICB to the cell-2, and kinetics of dye accumulation in both cells was measured by time-lapse imaging. MB-containing ICBs were permeable to negatively charged dyes AF350 (A), LY (B), and AF546/10000 (C) and impermeable to positively charged dyes DAPI (D) and Hoechst-33342 (E).

Our observations that GJ channels appear between daughter cells at the end of ICB abscission and that MB-containing ICBs have a strong preference for negatively charged molecules suggest that one of the properties of MBs might be to play a GJ role in attenuating Ca^{2+}, Mg^{2+}, and H^{+} fluctuations between cells until they establish GJ-dependent communication. However, further studies are required to verify this hypothesis and understand its physiological significance.

6. Concluding Remarks

MB inheritance and accumulation in cells lead to reprogramming of cell fate and conversion to highly proliferative stem cell-like phenotypes. However, the mechanisms that regulate asymmetric MB inheritance and post-mitotic degradation remain completely unknown. Proteomic analysis of the MB identified hundreds of proteins pointing out that this organelle has powerful and sophisticated machinery capable of regulating fate of stem cells and cancer stem cells. Therefore, the major future challenges should include the determination of putative proteins or their complexes related to specific cellular mechanisms regulating their proliferation, differentiation, development, intra- and intercellular communication, tumorigenicity, and other properties. Moreover, the discovery of

symmetric IBC abscission and following ejection of the MB into the extracellular matrix, where it can be later engulfed by surrounding cells, suggests that genetically modified MBs may provide new biotechnological solutions for cancer treatment.

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure S1: title, Table S1: title, Video S1: title.

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Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "X.X. and Y.Y. conceived and designed the experiments; X.X. performed the experiments; X.X. and Y.Y. analyzed the data; W.W. contributed reagents/materials/analysis tools; Y.Y. wrote the paper." Authorship must be limited to those who have contributed substantially to the work reported.

Conflicts of interest: The authors declare no conflicts of interest.

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