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On the origin of giant cells in Hodgkin lymphoma

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Multinucleated giant tumor cells are frequently observed in tissue sections of lymphoma patients. In Hodgkin lymphoma (HL), these cells are pathognomonic for the disease and named Reed-Sternberg (RS) cells. Despite the well-described disease-promoting functions of RS cells, their development has remained obscure. We addressed this open question by continuous live cell imaging to observe the generation of RS cells. Single-cell tracking of HL cell lines revealed that RS cells develop from mononucleated progenitors that divide and subsequently re-fuse, before they grow and become multinucleated giant cells. Thus, RS cell generation is neither due to cell fusion of unrelated Hodgkin cells nor to endomitosis, as previously suggested. In the majority of cases, re-fusion of daughter cells was preceded by an incomplete cytokinesis, visualized by a persistent microtubule bridge connecting the cells. This surprising finding describes a novel mechanism for the formation of multinuclear giant cells with potential relevance beyond HL.

Keywords: Hodgkin lymphoma, Reed-Sternberg cells, giant cells, re-fusion, cell fusion, endomitosis, acytokinetic mitosis, incomplete cytokinesis, single-cell tracking, time-lapse microscopy

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Multinucleated giant tumor cells are frequently observed in lymphoid malignancies.[1,2,3] However, for most lymphoma entities the clinical impact of this subpopulation of tumor cells as well as their development remains obscure. Importantly, diagnosis of Hodgkin lymphoma (HL) relies on the presence of giant and mostly multinucleated tumor cells within affected tissue.[4] These cells with diameters of up to 100 µm are referred to as Hodgkin and Reed-Sternberg (HRS) cells, representing the mononucleated and multinucleated subtype, respectively.[5,6,7] HL presents with a unique histological pattern compared with other lymphomas, as small and highly proliferating tumor cells are almost not detectable. Moreover, less than 1% of the cellular infiltrate consists of HRS cells embedded in a reactive infiltrate dominated by T lymphocytes.[8]

Rearrangement analysis of the immunoglobulin genes indicated a B-cell origin of HRS cells,[2,9] although they lost typical B-cell surface markers and signatures.[7,10,11] In addition, crippling mutations within the variable region of the B-cell receptor implies a post-germinal center B-cell phenotype of HRS cells.[7,12,13] Importantly, Reed-Sternberg (RS) cells represent the most prominent HRS-cell subtype in biopsy specimens and were defined as differentiated end-state of HRS cells playing a pivotal role in the interaction with the tumor microenvironment in situ.[14,15,16,17] However, the development of these giant tumor cells was controversially discussed for a long time.[18]

Re-Fusion of Hodgkin Cells Leads to Formation of RS Cells

Cell fusion of Hodgkin cells with each other or with other cells (e.g., macrophages) has been suggested as a potential mechanism for RS cell generation.[18] The latter could be excluded by molecular analysis of primary HRS cells and hence, endomitosis was postulated as the most favorable mechanism.[19,20] However,

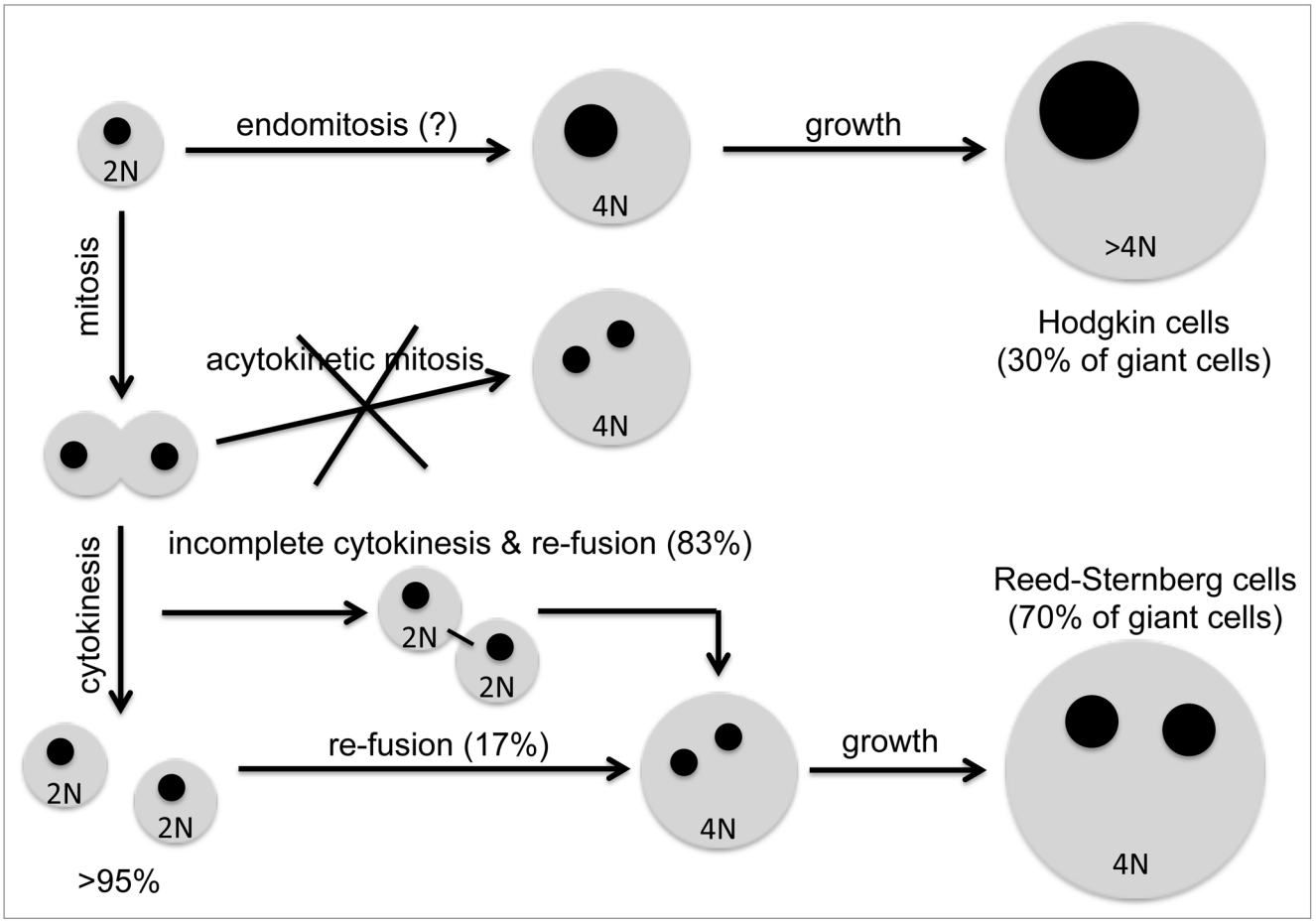

Figure 1. Re-fusion leads to giant multinuclear RS cells. During mitosis a diploid cell divides into two identical daughter cells. The last stage of mitosis is the complete separation of both daughter cells called cytokinesis. Dividing cells display the far majority (> 95%) in HL cell lines that also contain a rare population of giant cells (< 5%). Giant multinucleated RS cells develop from mononuclear Hodgkin cells that undergo mitosis into two separate daughter cells followed by subsequent re-fusion (70% of giant cells). In the majority of cases, the two daughter cells were still connected by the mid-body for hours after mitosis indicating incomplete cytokinesis. Additionally, giant mononuclear Hodgkin cells might develop via endomitosis (30% of giant cells). Acytokinetic mitosis, defined as mitosis without cell division leading to multinuclearity, was not observed.

endomitosis by definition describes mitosis without nuclear division leading to polyploidy (> 4N), but not to multinuclearity (**Fig. 1**). Therefore, the proposed mechanism should have been called acytokinetic mitosis, which is defined as mitosis with nuclear division, but without cellular division (**Fig. 1**).

In a recent study performing continuous single-cell tracking of HL cell lines by long-term time-lapse microscopy, we intriguingly found that re-fusion of daughter cells is the main route to giant HRS cell formation (**Fig. 1**).[21,22,23,24] Of note, HL cell lines contain about 5% of giant HRS cells and we focused on the development of this rare subpopulation at single-cell resolution in real-time. We observed that the majority of giant cell progenitors divided into two daughter cells that often remained separated for many hours, before they subsequently re-fused and developed into giant cells over time. Thereby, re-fused cells tremendously increased in cell size accompanied by a highly prolonged lifetime.

Moreover, we monitored nuclear morphology in real-time by combining time-lapse microscopy with lentivirus-mediated fluorescence labeling of HRS cells. As acytokinetic mitotic events were not observed, it became obvious that only re-fusion leads to multinuclearity and therefore to the formation of giant cells of the RS-type. On the contrary, approximately 30% of giant cells developed without a preceded re-fusion event. These cells stayed mononuclear representing giant HRS cells of the Hodgkin-type. As the nuclear mass increased by time in these giant Hodgkin cells, it might be speculated that these cells undergo endomitosis during their development. Current analyses using labeled chromosomes will further elucidate this issue.

Incomplete Cytokinesis Precedes Re-Fusion of HRS Cells

The study was extended to single-cell tracking of HRS cells expressing RFP-Tubulin to answer the question, if the re-fusing sister cells are completely separated. In the majority of cases, re-fusion was preceded by an incomplete cytokinesis, visualized by a microtubule bridge between the daughter cells

(**Figs. 1** and **2**).[21] Thus, RS cell generation is neither due to cell fusion of unrelated Hodgkin cells nor to endomitosis, but is mediated by re-fusion of daughter cells that underwent mitosis. Moreover, by single-cell tracking of nuclear fluorescently labeled HRS cells, we were able to identify that multinucleated cells are able to undergo multi-daughter divisions also followed by re-fusion.[21] In most cases, only 2 of the daughter cells re-fused, whereas the remaining cell died. However, also re-fusion of multiple daughter cells could be observed.

Alpha-Tubulin was chosen as marker to determine complete cytokinesis, because the midbody, which is derived from the mitotic spindle, displays the last connection between dividing cells.[25] The midbody develops after mitosis and during late cytokinesis by condensation of microtubules that pass the area of the former metaphase plate (**Fig. 2**). Disassembly of the midbody represents the final step of cytokinesis. Importantly, the midbody is only visible for minutes in proliferating cells undergoing mitosis,[21] but in case of the studied re-fusion events of RS-cell progenitors, the midbody persisted for several hours until re-fusion of daughter cells.[21]

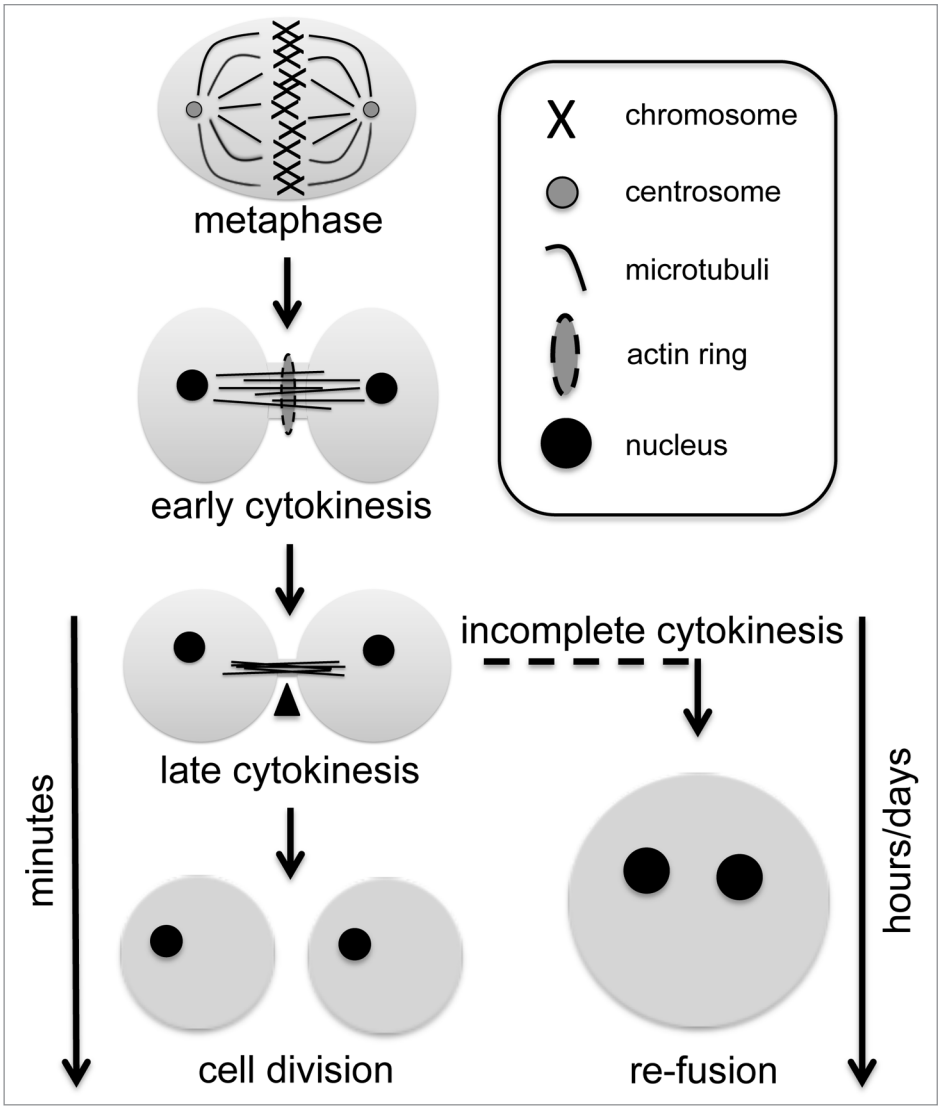

Figure 2. Incomplete cytokinesis precedes re-fusion in RS-cell formation. Proliferating cells duplicate their DNA content before entering mitosis. After the nuclear membrane is disintegrated, microtubules attach to the sister chromatids to form the mitotic spindle that splits the chromatids aligned at the metaphase plate to form two identical nuclei. Next, cytokinesis separates the dividing cell into two daughter cells. Thereby, a contractile actin ring invaginates at the site of the previous metaphase plate. This leads to condensation of spindle-derived microtubules, which form a so-called midbody (marked with an arrowhead) as last connection between dividing cells. Disassembly of the midbody completes cytokinesis and thereby the cellular division. During formation of giant RS cells, the midbody connection between dividing cells persists for many hours indicating an incomplete cytokinesis, which in turn leads to re-fusion of daughter cells.

Conclusions

The presented study unraveled a novel route for the generation of multinucleated RS cells from mononucleated Hodgkin cells. RS cells neither develop by endomitosis nor acytokinetic mitosis, but by re-fusion of daughter cells. As visualization of the microtubule network revealed that incomplete cytokinesis precedes re-fusion, one might speculate that re-fusion might be based on an intrinsic mitotic failure. In concordance, mutations of the midbody protein KLHDC8B in HL were recently reported.[26,27] Furthermore, downregulation of this gene in HeLa cells induced increased frequencies of binucleated cells.[28] Therefore, upcoming studies will address the functional role of midbody-associated proteins in HRS cells to further illuminate the molecular basis of the described re-fusion phenomenon.

Understanding the mechanisms involved in fusion-based RS-cell formation could lead to new therapeutic intervention strategies and might have implications beyond HL, as RS-like cells are also regularly seen in other lymphoproliferative disorders, including infectious mononucleosis, B-cell chronic lymphocytic leukemia or T-cell lymphomas.[1,2,3]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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