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Spotlight

When E-cadherin is away, centrosomes can play

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Centrosome clustering is a process frequently used by cancer cells with extra centrosomes to avoid multipolar divisions. How cell-intrinsic properties influence clustering is not entirely known. In this issue, Rhys et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201704102) report an unexpected link between clustering capacity and cortical contractility through E-cadherin and DDR1 proteins.

During mitosis, a microtubule-based machinery, the mitotic spindle, is responsible for equal segregation of chromosomes in two daughter cells. The centrosome, the major organizer of the microtubule cytoskeleton in animal cells, nucleates microtubules and organizes the mitotic spindle poles, influencing not only efficiency of spindle assembly but also its bipolar status. Thus, strict control of centrosome duplication during interphase is required for cells to enter mitosis with only two centrosomes, which ensures the assembly of a bipolar spindle.

Defects in centrosome duplication, cytokinesis, or cell fusion can result in centrosome amplification, defined as the presence of more than two centrosomes in a cell. Centrosome amplification is a common feature of human cancers and it has been associated with tumor progression, poor prognosis, and metastasis (Godinho et al., 2009). When induced through overexpression of Plk4 (the master centriole duplication kinase), centrosome amplification is a tumor-initiating event in vivo (Basto et al., 2008; Coelho et al., 2015; Serçin et al., 2016; Levine et al., 2017) and causes developmental defects affecting the brain, such as microcephaly (Marthiens et al., 2013). Centrosome amplification correlates with numerical aneuploidy (the gain or loss of whole chromosomes) as the presence of extra centrosomes can lead to the formation of multipolar spindles, which leads to the generation of aneuploid daughter cells. The random distribution of chromosomes in more than two daughter cells, however, might generate a hurdle to tumor progression because imbalanced chromosome sets are normally associated with poor cell survival (Godinho et al., 2009). To escape this problem, cells with supernumerary centrosomes use mechanisms to suppress multipolar spindle assembly and ensure viability of daughter cells. Centrosome clustering is one of these mechanisms whereby the extra centrosomes coalesce together in two mitotic spindle poles, promoting the assembly of a pseudo-bipolar spindle. Different studies in various model systems have identified key factors essential for clustering (Basto et al., 2008; Kwon et al., 2008). These include mitotic timing, regulators of cell adhesion, and actin-dependent generators of cortical cues, which organize astral microtubules to generate the forces that guide clustering. One of the most important factors is HSET, a minus end–directed kinesin that has been implicated in spindle pole focusing. Interestingly, while HSET is dispensable in most cells that contain two centrosomes, it becomes absolutely essential in cells that contain extra centrosomes (Basto et al., 2008; Kwon et al., 2008).

Although our understanding of centrosome clustering mechanisms has increased in the past few years, an outstanding question remains unanswered: why do certain cell types show high clustering efficiency, whereas others fail to cluster extra centrosomes? In this issue, Rhys et al. investigated centrosome clustering in different nontransformed cell lines that contain similar degrees of centrosome amplification to study what determines cell type–specific centrosome clustering capacity. Interestingly, two distinct cell line groups with high (>80% of the cells cluster their centrosomes) and low (only <40% of the cells cluster their centrosomes) clustering efficiency were identified. The differences observed between these two groups were not explained by defects in centrosome inactivation, another mechanism that prevents multipolarity through the loss of pericentriolar material and hence microtubule nucleation. Furthermore, they were not caused by differences in mitotic timing or HSET levels and localization. Instead, Rhys et al. (2018) found a correlation between low clustering capacity and the presence of E-cadherin.

E-cadherin localizes at adherens junctions and is one of the most important cell–cell adhesion molecules in epithelial cells. E-cadherin junctions are active mechanical structures that regulate cortical contractility by coupling E-cadherin adhesion receptors to the actomyosin cortex. To test whether E-cadherin had a direct effect on centrosome clustering, Rhys et al. (2018) abolished E-cadherin expression from epithelial cell lines and found that this was sufficient to increase cortical contractility and, importantly, to improve centrosome clustering and cell viability.

A previous study demonstrated that cortical contractility is locally reduced in adherens junctions in an E-cadherin–dependent manner (Hidalgo-Carcedo et al., 2011). It has been shown that during interphase, E-cadherin recruits the discoidin domain receptor 1 (DDR1) triggering a signaling cascade that, through p190RhoGap, culminates in the local inhibition of RhoA and thus decreased cortical contractility (Hidalgo-Carcedo et al., 2011). Importantly, Rhys et al. (2018) observed that DDR1 also localized at the cell cortex during epithelial cell mitosis and that it was enriched at sites of cell–cell contact. Moreover, inhibition of DDR1 was sufficient to increase the efficiency of

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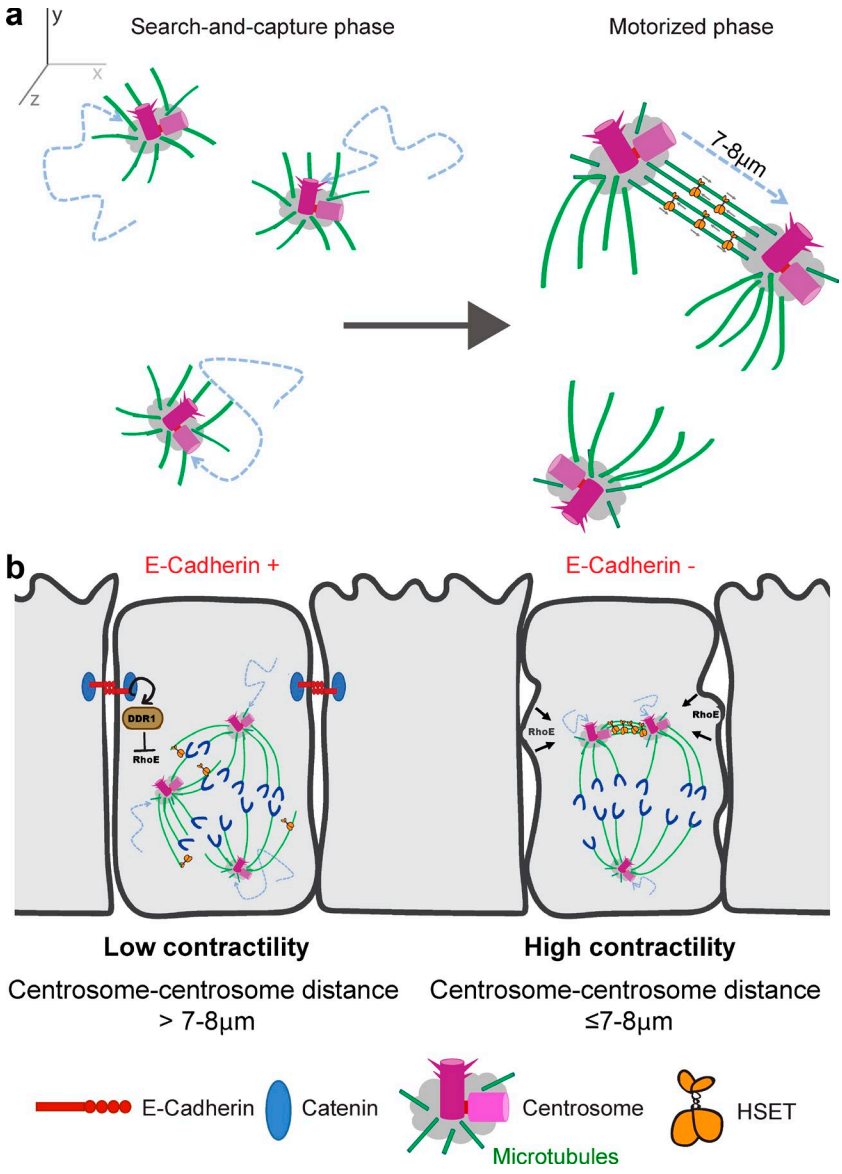

Figure 1. **Mechanisms of centrosome clustering in epithelial cells. (A)** Centrosome clustering is a two-step process: search-and-capture and motorized phases. **(B)** Influence of E-cadherin in centrosome clustering. E-cadherin presence (E-cadherin +) inhibits RhoE cortical contractility, which increases centrosome-oscillation frequencies and inter-centrosome distance. In the absence of E-cadherin (E-cadherin –), there is increased cortical contractility, which restricts centrosome movement and inter-centrosome distance (≤7–8 µm) and the HSET motor cross-links antiparallel MTs and promotes centrosome clustering.

centrosome clustering, even in epithelial cells that expressed high levels of E-cadherin. However, in this case, the authors rule out a role for p190RhoGap and RhoA. Instead, they identified RhoE as a DDR1 target, which was recruited to adherens junctions in a DDR1-dependent manner. These results suggest a model where the expression of E-cadherin in epithelial cells prevents cortical contractility through DDR1-induced RhoE inhibition, and ultimately influences the clustering capacity of extra centrosomes (Fig. 1).

These findings raise another question: is cortical contractility sufficient to induce centrosome clustering? And if this is the case, through which mechanism? The answer to this question came by analyzing the smallest angle between centrosomes in metaphase-like cells. Rhys et al. (2018) found that the smallest angle values were found in the population of cells that lacked E-cadherin. In other words, loss of E-cadherin resulted in centrosomes being positioned closer to each other. Furthermore, inhibition of cortical contractility was sufficient to revert this behavior, suggesting that inhibition of cortical contractility (favored by the presence of E-cadherin) directly impacts inter-centrosome distance and influences their ability to cluster. Importantly, the authors also showed that HSET does not influence the distance between centrosomes. A limiting distance between centrosomes as a requirement for clustering can indeed explain why, even in the presence of active HSET, cell lines of epithelial origin display low centrosome clustering capacity compared with the other cell lines.

Fueled by this attractive hypothesis, Rhys et al. (2018) characterized centrosome behavior using live-cell imaging. They found that centrosome clustering takes place in a biphasic manner. In the first phase of "search-and-capture," centrosomes moved in a stochastic manner, allowing them to be positioned in close proximity to each other. This was then followed by a second "motorized" phase, where closely positioned centrosomes (7–8 µm apart) were engaged in fast and directional movements toward each other to coalesce in one single pole just before anaphase onset (Fig. 1 a). Rhys et al. (2018) also provide evidence that, although HSET was not required for the first phase of search-and-capture, it was absolutely essential for the motorized phase where it promoted microtubule cross-linking and the subsequent clustering of centrosomes. Interestingly, Rhys et al. (2018) found that knockout of E-cadherin did not impair the

biphasic clustering of centrosomes in epithelial cells. Moreover, they found that the range of stochastic movements typical of the search-and-capture phase was significantly higher in epithelial cells expressing E-cadherin, suggesting a direct contribution of cell contractility in restricting centrosome movements during this phase. Rhys et al. (2018) not only describe the biphasic mode of centrosome clustering for the first time but they also provide a new paradigm to explain intrinsic differences in the efficiency of centrosome clustering across cell types. In this model, lack of E-cadherin at adherens junctions increases cortical contractility, restricting centrosome movement and increasing the probability of extra centrosomes being positioned at the minimal distance required for HSET to do its final job: bringing extra centrosomes together (Fig. 1 b).

Supernumerary centrosomes are a common feature of cancer cells (Godinho et al., 2009). Survival of cancer cells with centrosome amplification depends on their clustering capacity and their ability to assemble a pseudo-bipolar mitotic spindle that minimizes chromosome number deviations. One prediction of the results described by Rhys et al. (2018) is that tumor cells of epithelial origin should display poor cell survival because of low centrosome clustering efficiency. Yet, most human tumors are of epithelial origin and are known to contain extra centrosomes (Godinho et al., 2009). To solve this apparent paradox, Rhys et al. (2018) characterized a panel of 15 breast cancer cell lines, including luminal and basal subtypes, taking into account parameters related with centrosome amplification and E-cadherin/DDR1 levels. Importantly, they found that in the six cancer cell lines that displayed high frequency of centrosome amplification, neither the E-cadherin nor DDR1 proteins were detected, even though these were present in the other cell lines. In agreement with the proposed model, in these six cell lines, centrosome clustering was very efficient.

Overall, the study by Rhys et al. (2018) puts forward a model in which, upon centrosome amplification, the efficiency of centrosome clustering will depend on cell-intrinsic properties. The presence of E-cadherin at adherens junctions in epithelial cells negatively impacts centrosome clustering capacity. Unfortunately, it appears that transformed epithelial cells with extra centrosomes can escape multipolar divisions by down-regulating E-cadherin in order to increase their clustering efficiency and ensure cell survival. Of particular relevance to cell biology, this work supports the idea that the mechanisms promoting centrosome clustering rely on both intrinsic and extrinsic cues, which synergize to assemble a bipolar spindle and avoid the generation of high levels of aneuploidy. It will be interesting to investigate whether other factors that are influenced by cortical contractility, like mitotic rounding, also facilitate the positioning of extra centrosomes in close proximity to favor clustering. From a developmental biology point of view, it will be important to analyze centrosome clustering efficiency in different tissues of various origins. This type of analysis might help us understand how several tissue-specific responses to centrosome amplification arise (Basto et al., 2008; Marthiens et al., 2013; Coelho et al., 2015; Sabino et al., 2015; Serçin et al., 2016; Levine et al., 2017). Interestingly, this study also provides an explanation for the different outcomes observed when centrosome amplification was induced in the *Drosophila* wing disc epithelium and in *Drosophila* neural stem cells of the developing brain (Basto et al., 2008; Sabino et al., 2015). Centrosome amplification driven by Plk4 overexpression leads to efficient clustering in nonepithelial neural stem cells, but multipolar mitosis events were detected in the wing disc epithelium. Therefore, it will be important to determine whether the negative correlation between centrosome amplification and E-cadherin levels described by Rhys et al. (2018) extends beyond breast cancer cell lines. Intracellular heterogeneity represents an enormous obstacle in the treatment of human cancer. It has been proposed that inhibition of centrosome clustering, through the use of HSET inhibitors, for example, might specifically challenge the survival of cancer cells with extra centrosomes. In light of the results presented by Rhys et al. (2018), it now seems essential to take into consideration E-cadherin and DDR1 levels before considering HSET inhibition to prevent centrosome clustering.

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References

Basto, R., K. Brunk, T. Vinadogrova, N. Peel, A. Franz, A. Khodjakov, and J.W. Raff. 2008. Centrosome amplification can initiate tumorigenesis in flies. *Cell.* 133:1032–1042. https://doi.org/10.1016/j.cell.2008.05.039

Coelho, P.A., L. Bury, M.N. Shahbazi, K. Liakath-Ali, P.H. Tate, S. Wormald, C.J. Hindley, M. Huch, J. Archer, W.C. Skarnes, et al. 2015. Over-expression of Plk4 induces centrosome amplification, loss of primary cilia and associated tissue hyperplasia in the mouse. *Open Biol.* 5:150209. https://doi.org/10.1098/rsob.150209

Godinho, S.A., M. Kwon, and D. Pellman. 2009. Centrosomes and cancer: How cancer cells divide with too many centrosomes. *Cancer Metastasis Rev.* 28:85–98. https://doi.org/10.1007/s10555-008-9163-6

Hidalgo-Carcedo, C., S. Hooper, S.I. Chaudhry, P. Williamson, K. Harrington, B. Leitinger, and E. Sahai. 2011. Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. *Nat. Cell Biol.* 13:49–58. https://doi.org/10.1038/ncb2133

Kwon, M., S.A. Godinho, N.S. Chandhok, N.J. Ganem, A. Azioune, M. Thery, and D. Pellman. 2008. Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. *Genes Dev.* 22:2189–2203. https://doi.org/10.1101/gad.1700908

Levine, M.S., B. Bakker, B. Boeckx, J. Moyett, J. Lu, B. Vitre, D.C. Spierings, P.M. Lansdorp, D.W. Cleveland, D. Lambrechts, et al. 2017. Centrosome amplification is sufficient to promote spontaneous tumorigenesis in mammals. *Dev. Cell.* 40:313–322.e5. https://doi.org/10.1016/j.devcel.2016.12.022

Marthiens, V., M.A. Rujano, C. Pennetier, S. Tessier, P. Paul-Gilloteaux, and R. Basto. 2013. Centrosome amplification causes microcephaly. *Nat. Cell Biol.* 15:731–740. https://doi.org/10.1038/ncb2746

Rhys, A.D., P. Monteiro, C. Smith, M. Vaghela, T. Arnandis, T. Kato, B. Leitinger, E. Sahai, A. McAinsh, G. Charras, and S.A. Godinho. 2018. Loss of E-cadherin provides tolerance to centrosome amplification in epithelial cancer cells. *J. Cell Biol.* https://doi.org/10.1083/jcb.201704102

Sabino, D., D. Gogendeau, D. Gambarotto, M. Nano, C. Pennetier, F. Dingli, G. Arras, D. Loew, and R. Basto. 2015. Moesin is a major regulator of centrosome behavior in epithelial cells with extra centrosomes. *Curr. Biol.* 25:879–889. https://doi.org/10.1016/j.cub.2015.01.066

Serçin, Ö., J.C. Larsimont, A.E. Karambelas, V. Marthiens, V. Moers, B. Boeckx, M. Le Mercier, D. Lambrechts, R. Basto, and C. Blanpain. 2016. Transient PLK4 overexpression accelerates tumorigenesis in p53-deficient epidermis. *Nat. Cell Biol.* 18:100–110. https://doi.org/10.1038/ncb3270