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Pushed out of a tough crowd: centrosome aberrations promote invasiveness

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Centrosome defects are observed in a broad array of solid and liquid tumors and are associated with advanced disease and poor patient prognosis. Unexpectedly, centrosome aberrations are present in only a subset of cells within a tumor and are poorly tolerated in non-transformed cells, raising the conundrum of why centrosome aberrations are maintained during tumor evolution. New work by Ganier *et al* published in *The EMBO Journal* shows that centrosome defects can function in a non-cell-autonomous manner to force mitotic cells out of an epithelium, providing a plausible mechanism to promote dissemination of metastatic cells.

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See also: **O Ganier *et al***

Centrosomes are the major microtubule organizing centers of mammalian cells and play a key role in most microtubule-related processes, including organizing cell shape and polarity, and guiding formation of a bipolar spindle during mitosis. To coordinate these processes, centrosome number and function must be controlled within the cell division cycle. Cells begin the cycle with a single centrosome that duplicates exactly once, to ensure cells have two copies of this organelle when they divide (Nigg & Holland, 2018). The two centrosomes then instruct the formation of a bipolar mitotic spindle, upon which the chromosomes are segregated. Centrosome aberrations are frequent in human tumors and can be subdivided into either numerical or structural alterations. Numerical alterations reflect increases in the number of centrosomes and are able to promote mitotic chromosome segregation errors that are sufficient to drive spontaneous tumor development in mice (Levine *et al*, 2017). On the other hand, structural defects encompass alterations in centrosomes shape and size. Although conceptually distinct, numerical and structural centrosome defects often coexist in many tumors. While the effect of numerical centrosome alterations has been intensively studied (Chan, 2011), the consequence of structural aberrations on cancer initiation and progression has received little attention.

To investigate the impact of centrosome aberrations in an intact epithelium, Ganier *et al* (2018) employed overexpression of Ninein-like protein (NLP) to model structural centrosome aberrations in 3D epithelial cultures. NLP is a centrosome protein that interacts with the γ-tubulin ring complex to promote microtubule nucleation (Casenghi *et al*, 2003). Increased expression of NLP is commonly observed in human tumors (Li & Zhan, 2011), and overexpression of NLP leads to a change in centrosome size and structure that is highly reminiscent of that observed in cancer cells (Schnerch & Nigg, 2016). Interestingly, the authors observed extrusion, or "budding", of individual cells from acini harboring NLP-induced centrosome aberrations. Importantly, all of the extruded cells were mitotic. Moreover, most of the budding cells were viable and some were observed to continue dividing after departing the epithelium. Budding was rarely observed in cells containing supernumerary centrosomes, suggesting that this phenotype is primarily a consequence of structural, rather than numerical, centrosome aberrations.

To study how structural aberrations in centrosomes promote budding of mitotic cells, the authors examined the arrangement of E-cadherin junctions in NLP-overexpressing epithelial cells. Increased levels of NLP interfered with the establishment of adherens junctions in proliferating cells, leading to the weakening of contacts between dividing cells and their neighbors. NLP overexpression also disrupted spindle orientation, consistent with the established role of adherens junctions in orienting the cell division axis in an epithelium. Previous work has shown that increased microtubule polymerization leads to activation of the small GTPase Rac1, which in turn promotes increased Arp2/3-dependent actin polymerization that destabilizes E-cadherin junctions (Godinho *et al*, 2014). Since the enlarged centrosomes of NLP-overexpressing cells nucleated more microtubules with an enhanced stability, the authors tested whether suppressing Rac1 or Apr2/3 activity could rescue the phenotypes caused by NLP overexpression. Importantly, pharmacological inhibition of either Rac1 or Arp2/3 activity prevented the loss of E-cadherin junctions in NLP-overexpressing cells and suppressed both the spindle orientation defects and the budding phenotype. These data suggest that stabilization of microtubules in cells with elevated NLP expression leads to excessive activation of Rac1-Arp2/3, which in turn suppresses the formation of E-cadherin junctions between mitotic cells and their neighbors. Since centrosome amplification, which does not promote appreciable budding, also disrupted E-cadherin junctions, weakening of E-cadherin junctions appears to be necessary, but not sufficient, for the extrusion of mitotic cells from the epithelium.

To explore the involvement of additional mechanisms required for budding, the

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authors examined the frequency of cellular extrusion in acini containing different proportions of NLP-overexpressing cells. Surprisingly, the extent of budding within an acinus did not show a linear correlation with the percentage of NLP-overexpressing cells. Rather, extensive budding was only observed when the proportion of NLP-overexpressing cells exceeded ~50%, suggesting that budding requires cooperation between cells within the epithelium. Importantly, cells did not have to overexpress NLP to bud; in fact, the authors observed a preference for non-NLP-overexpressing cells to be excluded from the epithelium. Taken together, this suggests that budding is a non-cell-autonomous response to NLP-induced centrosome aberrations.

To investigate the mechanical properties of cells with centrosome aberrations, atomic force microscopy (AFM) was used to measure the stiffness of cells within a confluent monolayer. The authors observed that interphase cells within an epithelium were stiffer than mitotic cells. At first glance, these data appear to contradict previous work that showed cells increase stiffness during mitosis (Stewart *et al*, 2011). However, these previous measurements were made on isolated, single cells, rather than cells in an epithelium, indicating that stiffness is influenced by cell–cell contact and physical confinement within the epithelium. Importantly, NLP overexpression increased the stiffness of both interphase and mitotic cells. This led the authors to suggest that epithelia containing a critical fraction of NLP-overexpressing cells increase stiffness to a level that is sufficient to selectively squeeze out softer mitotic cells, resulting in budding. Since inhibiting actin polymerization reduced cellular stiffness and blocked budding, the authors propose that budding caused by NLP overexpression requires both E-cadherin loss and increased cellular stiffness (Fig 1). By contrast, centrosome amplification resulted in the nucleation of more dynamic microtubules that did not at the same time increase cell stiffness, explaining why such alteration is insufficient

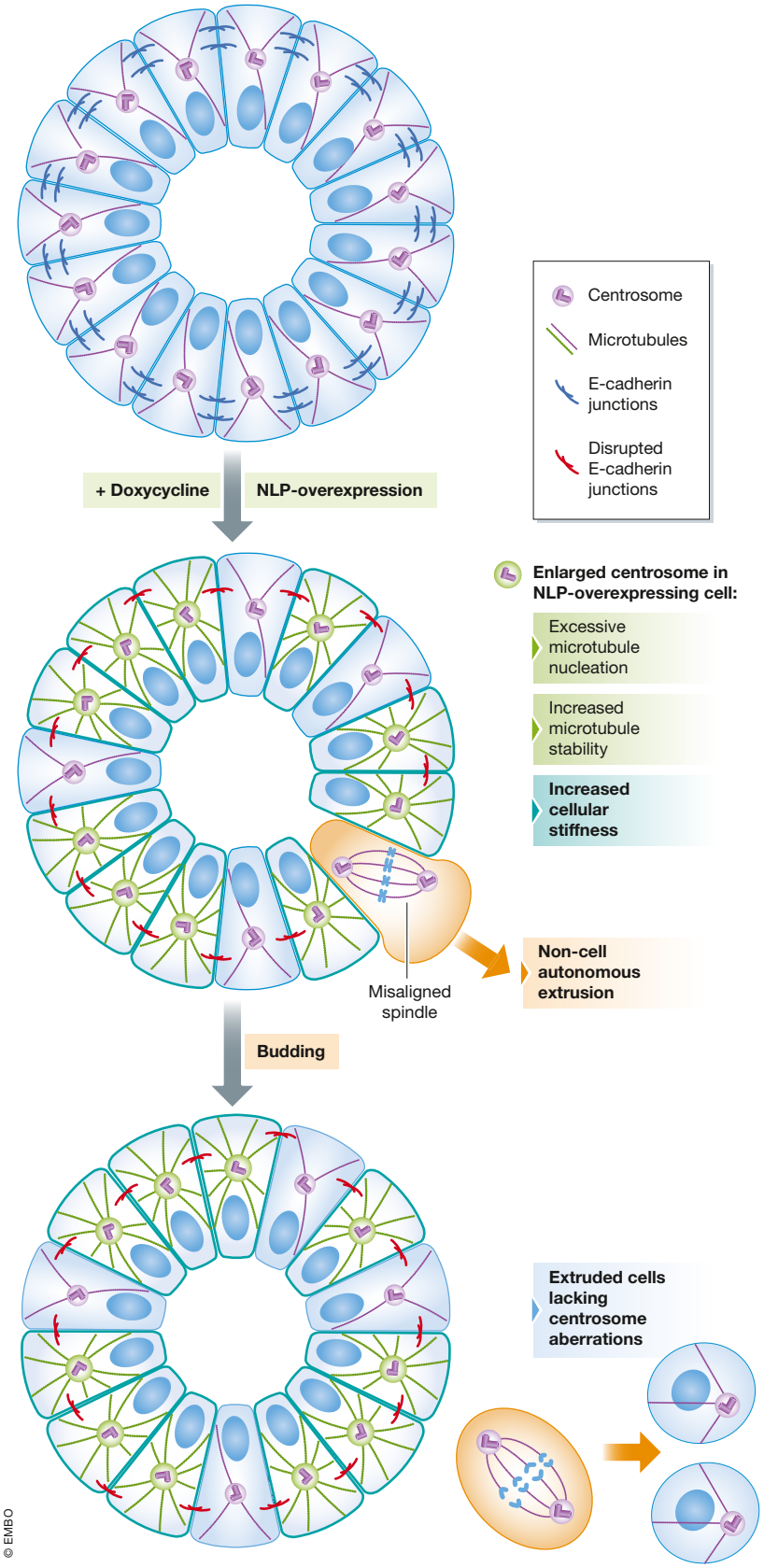

Figure 1. Structural centrosome aberrations promote cellular extrusion.
Schematic illustration of non-cell-autonomous extrusion and budding of mitotic cells from epithelial layers containing cells with structural centrosome aberrations. See text for detail.

to promote the extrusion of mitotic cells from the epithelium.

Studies in cells and animal models have shown that supernumerary centrosomes contribute to tumorigenesis, at least in part, by promoting mitotic chromosome segregation errors that facilitate the evolution of malignant karyotypes (Ganem *et al*, 2009; Silkworth *et al*, 2009). However, additional mechanisms for how centrosome aberrations impact tumor behavior have also been put forth, including recent work that showed centrosome amplification promotes invasive phenotypes in a 3D culture system by increasing Rac1 activity and disrupting cell–cell adhesion junctions (Godinho *et al*, 2014). The study by Ganier *et al* (2018) now expands our understanding of the impact of centrosome aberrations in tumors by offering additional insight into a pathway through which centrosome aberrations can induce an invasive phenotype. Perhaps most importantly, the budding phenotype the authors observe is non-cell-autonomous, offering an explanation for how centrosome abnormities could contribute to metastasis, by disseminating cells that themselves do not harbor potentially deleterious centrosome alterations. More broadly, this work suggests metastatic lesions may not have to possess all the genetic alterations required for dissemination, raising the possibility that centrosome aberrations could have a larger clinical impact than previously thought.

As with all discoveries, many new questions are raised. It remains technically challenging to continuously visualize the escape of cells from the primary tumor all the way to a distant metastatic site. Nevertheless, accessing the long-term survival of the cells extruded from the epithelium, and whether they can contribute to seeding of new tumors *in vivo*, will be required to solidify a role of budding in promoting metastasis. It would also be interesting to evaluate whether the spontaneous tumors formed in NLP-overexpressing mice show increased metastatic potential (Shao *et al*, 2010). Finally, we would benefit from a more detailed understanding of the cause of structural centrosomes aberrations in tumorigenesis and how to faithfully model such alterations

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