

The Emerging Link between Centrosome Aberrations and Metastasis

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Centrosome aberrations are commonly observed in human tumors and correlate with tumor aggressiveness and poor prognosis. Extra centrosomes drive mitotic errors that have been implicated in promoting tumorigenesis in mice. However, centrosome aberrations can also disrupt tissue architecture and confer invasive properties that may facilitate the dissemination of metastatic cells. Recent work has shown that centrosome defects facilitate invasion through cell-autonomous and non-cell-autonomous mechanisms, suggesting cancer cells can benefit from centrosome aberrations present in a subset of the tumor cell population. Here, we discuss how centrosome defects promote invasive behaviors that may contribute to initial steps in the metastatic cascade.

Centrosome Abnormalities Occur Frequently in Human Tumors

The centrosome is the major microtubule organizing center in animal cells and functions in controlling cell polarity, motility, proliferation, and division (Nigg and Holland, 2018; Conduit et al., 2015; Fu et al., 2015; Bornens, 2012; Nigg and Raff, 2009). Centrosomes are generally comprised of a pair of microtubule-based structures called centrioles that are embedded in a pericentriolar matrix (PCM) (Woodruff et al., 2014). Centrosomes form the poles of the bipolar microtubule spindle during mitosis and organize the microtubule cytoskeleton of many cell types during interphase. In quiescent cells, the older of the two centrioles functions as a basal body that assembles a primary cilium, which plays an important role in cell signaling (Sánchez and Dynlacht, 2016).

Centrosome number is tightly controlled in healthy proliferating cells. In G1 phase, cells contain a single centrosome that duplicates once producing two centrosomes, which form the bipolar spindle in mitosis (Nigg and Holland, 2018; Firat-Karalar and Stearns, 2014). The two centrosomes are equally partitioned during mitosis so that each daughter receives a single copy of the organelle. This duplication and segregation cycle maintains the correct number of centrosomes from one generation to the next. Proper control of centrosome number is critical for the appropriate functioning of the organelle in signaling and cell division (Nigg and Raff, 2009).

While centrosome homeostasis is strictly maintained in healthy cells, centrosome aberrations are commonly observed in human tumors. Centrosome defects in tumors can be broadly classified into numerical or structural alterations. Numerical alterations are an increase in centrosome copy number and may arise from defects in centrosome duplication, or perhaps more often as a consequence of failed cell divisions. In contrast to centrosome amplification, structural centrosome abnormalities in human tumors are less well characterized, with the most straightforward defects being an increase in centrosome size as result of an expansion of the PCM (Schnerch and Nigg, 2016).

Structural and numerical centrosome aberrations are frequently observed in solid and hematological malignancies

(Godinho and Pellman, 2014; Guo et al., 2007; Krämer et al., 2005; Pihan et al., 2003; Sato et al., 1999; Pihan et al., 1998). Centrosome aberrations are observed at all stages of tumor development, and the severity of the defects correlates with increased tumor aggressiveness and poor prognosis in some cancer types (Hsu et al., 2005; Skyldberg et al., 2001; Sato et al., 1999). In addition, karyotype analysis of tumors has drawn strong links between centrosome amplification and aneuploidy (Krämer et al., 2005; Miki et al., 2004; Krämer et al., 2003). Structural abnormalities such as altered centrosome size or shape and increased centriole length have also been observed in tumors (Martel et al., 2018; Lingle and Salisbury, 1999; Lingle et al., 1998). Although structural and numerical alterations often co-occur in tumors and induce some common effects, they can also promote mechanistically distinct cell behaviors as described further below (Arnandis et al., 2018; Ganier et al., 2018a; Ganier et al., 2018b; Godinho et al., 2014).

Studying sea urchin embryos more than 100 years ago, Theodor Boveri proposed that centrosome amplification promotes chromosome segregation errors, which drive tumorigenesis (Boveri, 1914). Today, it is widely accepted that increases in centrosome number disrupt the fidelity of cell division, leading to both numerical and structural alterations in the tumor cell karyotype (Crasta et al., 2012; Ganem and Pellman, 2012; Janssen et al., 2011; Ganem et al., 2009; Silkworth et al., 2009). A strong connection between centrosome amplification and tumorigenesis was first made in flies, where transplanted tissue containing extra centrosomes formed tumors that were capable of metastasizing (Basto et al., 2008). Moreover, experimentally inducing centrosome amplification in mouse models by increasing the levels of Polo-like kinase 4 (Plk4), the master regulator of centrosome biogenesis, has been shown to promote tumorigenesis (Levine et al., 2017; Serçin et al., 2016; Coelho et al., 2015). The tumors that form in mice with extra centrosomes show recurrent chromosomal copy number changes, suggesting that mitotic errors are likely to be a major contributor to tumorigenesis in this model (Levine et al., 2017). However, the effect of extra centrosomes *in vivo* is context dependent (Nigg and Holland, 2018). In the mouse brain, centrosome amplification promoted



cell death leading to microcephaly (Marthiens et al., 2013). Moreover, in the skin epidermis, centrosome amplification did not initiate spontaneous tumorigenesis or enhance the development of carcinogen-induced skin tumors (Vitre et al., 2015) but was able to accelerate tumors that develop in the absence of the tumor suppressor TP53 (Serçin et al., 2016).

In addition to playing important roles in cell division, centrosomes also organize the interphase microtubule network that controls cell shape, polarity, and motility. Structural and numerical centrosome alterations can, therefore, reorganize the microtubule cytoskeleton and disrupt tissue architecture, potentially providing a platform for metastatic cell dissemination (Nigg et al., 2017; Schnerch and Nigg, 2016). Indeed, recent work shows that centrosome aberrations may facilitate the dissemination of potentially metastatic cells through multiple distinct mechanisms (Armandis et al., 2018; Ganier et al., 2018a; Ganier et al., 2018b; Godinho et al., 2014). In this review, we first outline the key steps required for metastasis and then discuss work suggesting that centrosome aberrations can contribute to the initial steps in the metastatic cascade.

Fundamentals of Metastasis

The overwhelming majority of cancer mortality is caused by metastasis, a process in which cancer cells disseminate from the primary tumor and seed new colonies at distant sites (Siegel et al., 2018). The dissemination and metastatic outgrowth of cancer cells is a complex multi-step process. It involves the local invasion of primary-tumor cells into surrounding tissue, intravasation of these cells into the circulatory system, and subsequent extravasation back through the vascular walls. In this way, cancer cells travel to the parenchyma of a distant tissue and seed microscopic colonies that proliferate to form metastatic lesions (Lambert et al., 2017).

Invasion is the first step of metastasis and refers to the ability of cancer cells to escape the site of the primary tumor and enter into surrounding normal tissue. A central process in invasion is the activation of a cell biological program termed the epithelial to mesenchymal transition (EMT). The EMT program causes epithelial cells to adopt mesenchymal cell traits, such as increased motility, loss of cell-cell adhesions, and the ability to degrade components of the surrounding extracellular matrix (ECM). Induction of this developmental program is considered a key initiating step in metastasis that allows single cells to exit the primary-tumor site and invade surrounding parenchyma (Mittal, 2018; Kalluri and Weinberg, 2009).

The traditional view of metastasis is that activation of the EMT program allows single cells to disseminate from the primary tumor and seed the formation of clonal secondary tumors. However, more recent work has established that invasion by primary tumors often involves the collective migration of cohorts of cells that subsequently seed polyclonal metastatic outgrowths (Cheung and Ewald, 2016; Cheung et al., 2016; Gudem et al., 2015; Maddipati and Stanger, 2015; Aceto et al., 2014). The organization of these invasive tumor cell clusters contradicts the expected behavior of cells that have undergone complete EMT in that they retain cell-cell adhesion junctions (Chung et al., 2016; Veracini et al., 2015). However, the EMT program is not a binary switch, as carcinoma cells can adopt a mixture of epithelial and mesenchymal traits (Lambert et al., 2017). More-

over, in collectively migrating tumor cells, mesenchymal traits are occasionally observed in cells at the invasive front, possibly facilitating ECM degradation and the migration of the tumor cell cluster into the surrounding stroma (Westcott et al., 2015; Ye et al., 2015). Therefore, it is likely that the EMT program works together with additional pathways to promote metastatic dissemination.

After invasion into neighboring stromal tissue, cancer cells must then intravasate into the vasculature and travel to a distant site to colonize a secondary tumor. This process depends on multiple signaling pathways, proteases, and interactions with nearby cells to allow invasive cells to adhere to, and pass through, the endothelial membrane (Chiang et al., 2016). Once in the circulation, tumor cells travel as single cells or clusters until they become lodged in microvessels of distant tissues (Au et al., 2016; Aceto et al., 2014). The transit of circulating tumor cells (CTCs) can be aided by interactions with platelets, macrophages, and neutrophils, which coat CTCs to protect them from an immune attack and to facilitate docking at a distant site (Lambert et al., 2017).

CTCs are present for short periods in the circulation before becoming lodged in microvessels, where they may breach vessel walls and extravasate into the parenchyma. The mechanisms that control the subsequent proliferation of cancer cells in foreign tissue environments remain unclear. Successful colonization requires adaptive behaviors and the development of a metastatic niche through the expression of supportive growth factors and signaling molecules (Massagué and Obenauf, 2016). Furthermore, in some sites, disseminated tumor cells are able to remain dormant for many years before reinitiating tumor growth and colonizing a metastatic lesion (Braun et al., 2005).

Centrosome Aberrations Promote Invasive Phenotypes

The molecules and pathways required for tumor cell dissemination and subsequent metastatic outgrowth are beginning to come into focus. Nevertheless, we lack a comprehensive understanding of which features of the primary tumor are the major contributors to metastatic disease. As discussed earlier, centrosome aberrations are common in human tumors. New evidence suggests that these alterations can contribute to tumor cell dissemination through at least four different mechanisms, each of which is discussed in more detail below.

Centrosome Aberrations Induce Formation of Invadopodia

Centrosome amplification triggered by overexpression of PIK4 has been shown to promote the formation of invasive protrusions (invadopodia) in mammary epithelial acini grown in a three-dimensional culture (3D) system (Figure 1A) (Godinho et al., 2014). Invasive protrusions were accompanied by the degradation of ECM components, and in some instances resulted in the collective invasion of cells into the surrounding matrix. The increase in centrosomal microtubule nucleation in cells with extra centrosomes promoted activation of the small GTPase Rac1. Rac1 activity, in turn, initiated actin polymerization that disrupted cell-cell adhesion and promoted cell migration. Rac1 signaling is frequently upregulated in tumors, where it has been shown to promote invasion and metastasis (Figure 1A) (Bid et al., 2013). A similar invasive phenotype can be triggered by overexpression

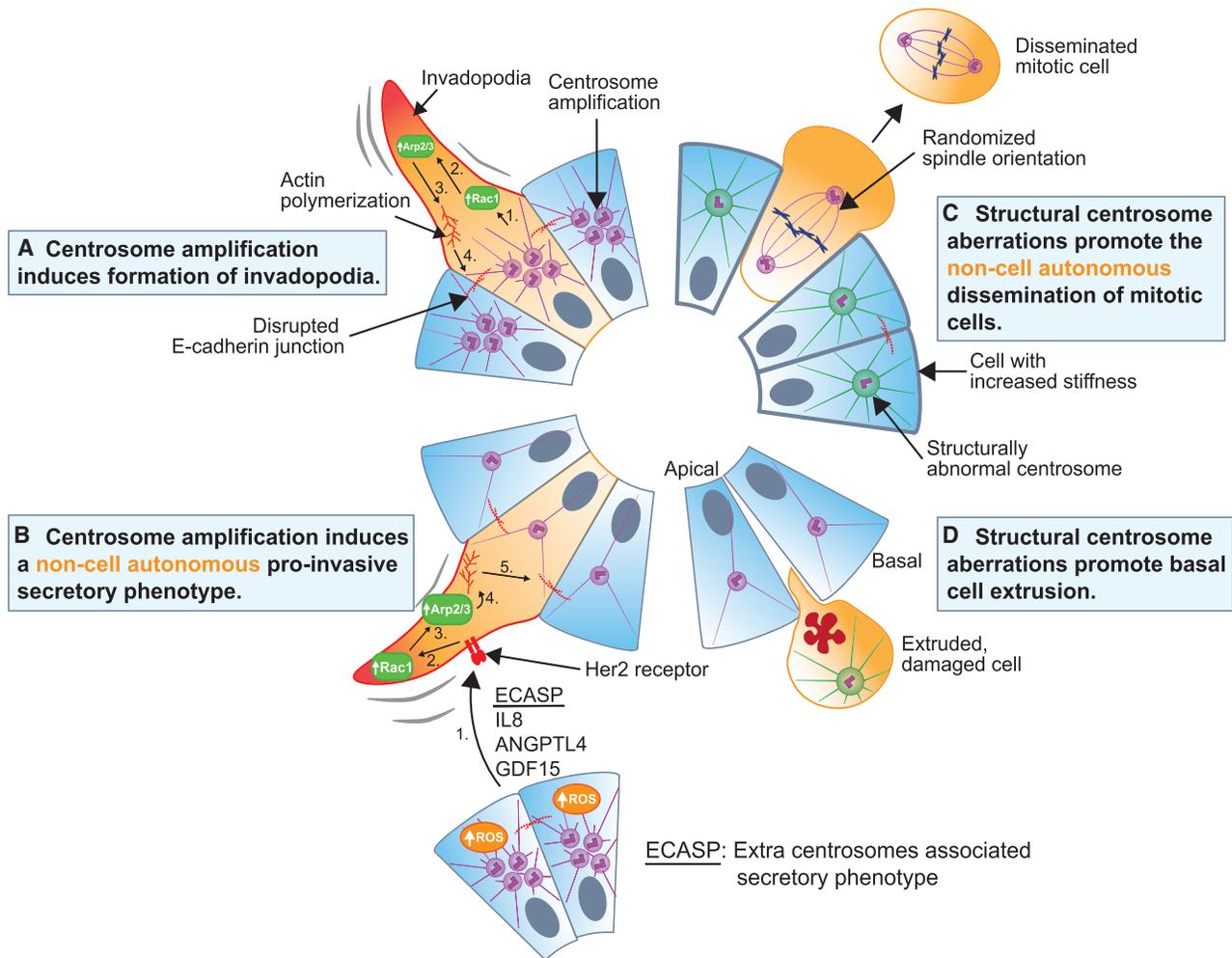


Figure 1. Mechanisms by which Centrosome Aberrations Can Promote Invasive Phenotypes

(A) Centrosome amplification induced by Plk4 overexpression leads to increased microtubule nucleation and dynamics. This increases Rac1 signaling (1), resulting in the activation of the Arp2/3 complex (2), increased actin polymerization (3), and weakening of E-cadherin junctions (4) to promote the formation of invasive protrusions (invadopodia) in cells with extra centrosomes.

(B) Pro-invasive factors secreted from cells with extra centrosomes promote the formation of invadopodia in cells with normal centrosome numbers. This ECASP is dependent on elevated reactive oxygen species in cells with amplified centrosomes. The secreted pro-invasive factors activate receptors (1), leading to increased Rac1 signaling (2), activation of the Arp2/3 complex (3), increased actin polymerization (4), and weakening of E-cadherin junctions (5) to promote invadopodia formation in cells with normal centrosome numbers.

(C) Structural centrosome aberrations induced by overexpression of Nlp lead to increased microtubule nucleation and stability. This increases interphase cell stiffness and disrupts E-cadherin junctions, promoting the selective extrusion of softer mitotic cells from the epithelium.

(D) Structural centrosome aberrations induced by overexpression of Nlp and CEP131 promote basal extrusion of damaged cells.

of Ninein-like protein (Nlp), a centrosome protein that interacts with the γ -tubulin ring complex to promote microtubule nucleation (Ganier et al., 2018b; Casenghi et al., 2003). Nlp overexpression leads to an increase in centrosome size that resembles structural centrosome alterations in cancer cells (Schnerch and Nigg, 2016). These findings suggest that centrosome aberrations may constitute a general mechanism for promoting Rac1 activation, the disruption of cell-cell junctions, and cell motility during tumorigenesis.

In addition to a primary role as a microtubule organizing center, the centrosome has been shown to directly nucleate actin filament assembly in an Arp2/3-dependent manner *in vitro* (Farina et al., 2016). In lymphocytes, Arp2/3-mediated actin nucleation at the centrosome is required to link this organelle

to the nucleus (Obino et al., 2016). The removal of centrosomal Arp2/3 following lymphocyte activation promotes centrosome detachment from the nucleus and recruitment to the immune synapse. Although centrosome-mediated actin assembly has not yet been implicated in promoting lamellipodium formation or cell movement, this represents another mechanism by which centrosomes could contribute to the cytoskeletal reorganization that precedes invasion.

Centrosome Amplification Induces a Pro-invasive Secretory Phenotype

In addition to promoting invadopodia formation through increased Rac1 signaling, centrosome amplification has been shown to promote the secretion of pro-invasive factors that induce non-cell autonomous invasion (Figure 1B) (Arnandis

et al., 2018). Conditioned media from cells with extra centrosomes induced the formation of invasive protrusions in 3D organoid cultures of cells with normal centrosome numbers. Cells with extra centrosomes were shown to secrete multiple pro-invasive factors that have been previously linked to cancer, invasion, and migration (such as IL-8, ANGPTL4, and GDF-15). Rac1 signaling was not required for the secretion of pro-invasive factors from cells with extra centrosomes, but it was required for the formation of invadopodia in cells with normal centrosomes responding to the secreted factors (Figure 1B). This suggests that the non-cell autonomous extra centrosomes-associated secretory pathway (ECASP) is distinct from the previously reported pathway that promotes the cell-autonomous formation of invasive protrusions in cells with supernumerary centrosomes (Godinho et al., 2014). Exactly how extra centrosomes promote the ECASP remains unclear, but the response relies partly on elevated levels of reactive oxygen species in cells with centrosome amplification. While much remains to be understood about the role of the ECASP in tumor development, the discovery of this pathway provides a mechanism explaining how extra centrosomes could induce paracrine invasion in nearby cells with a normal centrosome content (Arnandis et al., 2018). In the future, it will be interesting to test whether structural centrosome alterations can induce a pro-invasive secretory phenotype similar to that observed in cells with extra centrosomes.

Structural Centrosome Alterations Promote the Non-Cell-Autonomous Dissemination of Mitotic Cells

Although significant effort has focused on how centrosome amplification can contribute to tumorigenesis, the impact of structural centrosome aberrations has received much less attention. This is partly because defining centrosome structural defects is subjective, and it is less clear how to model these alterations experimentally. Recently, Nlp overexpression was used to experimentally produce structural centrosome alterations that are similar to those observed in cancer. Indeed, elevated expression of Nlp is commonly found in human tumors (Yu et al., 2009; Qu et al., 2008) and promotes tumorigenesis in mice (Shao et al., 2010).

Nlp-induced structural centrosome aberrations promoted the extrusion of individual cells from acini in a 3D culture model (Figure 1C) (Ganier et al., 2018b). This “budding” phenotype occurred specifically in mitotic cells without structural centrosome aberrations and could be prevented by blocking cells from entering into mitosis. Most of the extruded cells were viable, and some continued to proliferate after escaping from the epithelium, indicating that epithelial budding may promote metastatic dissemination of nearby cells with normal centrosomes. Interestingly, budding was not observed in cells with extra centrosomes, demonstrating that structural and numerical centrosome abnormalities can promote distinct types of invasive behaviors.

The mechanisms by which structural centrosome aberrations promote mitotic cell budding are two-fold. Nlp-induced structural centrosome aberrations triggered increased Rac1 signaling and actin polymerization, leading to weakening of E-cadherin-mediated cell-cell junctions and randomized mitotic-spindle orientation. However, centrosome amplification also increased Rac1 signaling and disrupted E-cadherin junctions but did not induce a budding phenotype, suggesting that additional alter-

ations are necessary for Nlp-induced dissemination. In contrast to cells with extra centrosomes, Nlp-induced structural centrosome aberrations markedly increased the stiffness of interphase cells in the epithelial sheet through increased microtubule nucleation and stability. This suggests that epithelia with a high density of Nlp-overexpressing cells may selectively squeeze out softer mitotic cells with destabilized E-cadherin junctions (Figure 1C). Importantly, the extruded mitotic cells did not often carry the centrosome alterations themselves, indicating that budding is a non-cell autonomous process that relies on cooperation between cells in an epithelium.

Structural Centrosome Alterations Promote Basal-Cell Extrusion

In addition to mitotic cell budding, structural centrosome aberrations have also been shown to promote the preferential extrusion of damaged cells toward the basal surface of epithelial monolayers (Figure 1D). Epithelia typically dispose of damaged cells by extruding them apically into the luminal cavity (Slattum and Rosenblatt, 2014). However, a switch in the directionality of cell extrusion has been observed in epithelia harboring oncogenic mutations (Ohsawa et al., 2018; Gu et al., 2015). If cell death is circumvented, basal extrusion may promote the accumulation of cells outside of the epithelial sheet, providing a first step toward metastatic dissemination. Similar to oncogenic mutations, centrosome structural aberrations induced by overexpression of Nlp have been shown to sensitize damaged epithelial cells to basal extrusion (Figure 1D). In addition, overexpression of the centrosome protein CEP131 creates distinct alterations in the centrosome structure that promote the basal extrusion of dying cells with CEP131-induced structural centrosome aberrations in the absence of any external damaging agent (Ganier et al., 2018a). If extruded cells harbor additional oncogenic alterations that promote survival, it is plausible that a reversal in the directionality of cell extrusion caused by centrosome aberrations could contribute to the dissemination of metastatic cells.

Implications for Metastasis

In summary, recent work has uncovered multiple mechanisms by which centrosome alterations may contribute to metastasis by facilitating invasion. This suggests that centrosome aberrations can influence cancer progression beyond simply promoting mitotic defects. Centrosome defects cause invadopodia formation and basal cell extrusion, which could act as first steps in the dissemination of genetically unstable cells with metastatic potential. Intriguingly, centrosome aberrations may also facilitate invasion in a non-cell autonomous manner by inducing a pro-invasive secretory phenotype or promoting mitotic cell budding. These findings could explain why centrosome aberrations are often associated with advanced tumor stage and metastasis (Hsu et al., 2005; Neben et al., 2003; Skyldberg et al., 2001; Sato et al., 1999). In addition, the non-cell autonomous effects of centrosome alterations suggest that cells with centrosome aberrations could promote invasive behavior in surrounding cells that lack these defects. In this manner, tumor cells can broadly benefit from the centrosome aberrations present in only a subset of cells in the tumor population.

Many of the experiments examining the impact of centrosome aberrations on invasion have focused on 3D culture models of mammary epithelial cells. However, metastasis is a highly inefficient process, with the vast majority of disseminating cells

destined to be eliminated or enter into a state of dormancy (Mas-sagué and Obenauf, 2016; Chambers et al., 2002). Therefore, from current experimental approaches, it remains unclear whether centrosome aberrations can give rise to invasive cells that survive long-term and seed metastatic lesions *in vivo*. Moreover, it is unknown whether the phenotypes observed in cell culture will translate to tissues *in vivo* considering the responses to centrosome alterations may vary in different tissue types. Therefore, although *in vitro* studies will continue to be important to define molecular mechanisms, *in vivo* animal models and intravital tumor imaging will be increasingly required to define to what extent, and by which mechanisms, centrosome aberrations contribute to metastasis.

Perspective

Experimental work in mouse models has demonstrated that structural and numerical centrosome aberrations can contribute to tumorigenesis (Levine et al., 2017; Serçin et al., 2016; Coelho et al., 2015; Shao et al., 2010). What remains to be clarified are the main mechanisms by which centrosome defects contribute to tumor formation and/or progression. It is now clear that structural and numerical alterations in centrosomes can promote distinct changes in cell physiology and behavior. This emphasizes the need to carefully define the properties of the centrosome aberrations present in various human tumors. However, while alterations in centrosome number are relatively simple to evaluate, determining what constitutes a structural defect in the centrosome is at present, relatively subjective. This issue is compounded by the fact that most studies analyzing primary tumors only use markers for the PCM and not the centriole. Consequently, centrosome defects such as altered centrosome size or shape, increased centriole length, and an expanded PCM cannot be easily distinguished. For example, centrosome amplification and PCM fragmentation arise through different mechanisms, but both lead to the formation of supernumerary PCM foci (Maiato and Logarinho, 2014). There is, therefore, a need to develop better methods to analyze and classify centrosome aberrations in human tumors to understand both the prevalence and consequences of these defects. Doing so could allow different centrosome alterations to be used as diagnostic or prognostic markers in human tumors.

An additional area of focus is to discern the origin of centrosome aberrations in human tumors. Genes encoding centrosome proteins are rarely found to be mutated in tumors, but the misregulated expression of centrosome components is more common (Gönczy, 2015; Chan, 2011; Nigg and Raff, 2009). Given that centrosome aberrations can promote invasive phenotypes through non-cell-autonomous mechanisms, it is plausible that only a portion of cells in a primary tumor harbor the genetic alterations responsible for causing the centrosome defects (Arandis et al., 2018; Ganier et al., 2018b). In addition, changes in cell cycle progression, DNA damage, and failed cell divisions are common deficits in tumors that can indirectly impact centrosome number (Douthwright and Sluder, 2014; Inanç et al., 2010; Ganem et al., 2009). Relevant to understanding the origin of centrosome defects in cancer is the question of how faithfully the current experimental models recapitulate the centrosome alterations observed in human tumors. For example, Plk4 has been implicated in centrosome-independent functions,

and these may contribute to the phenotypes observed following overexpression of the kinase (Kazazian et al., 2017; Rosario et al., 2015). Moreover, it is unclear how closely Nlp overexpression mirrors centrosome structural alterations observed in tumors. Ultimately, improved understanding of the origins of centrosome alterations in cancer will lead to the creation of better experimental models that will more closely phenocopy the defects observed in tumors. The development of these models will allow a more in-depth analysis of the contribution of structural and numerical centrosome alterations to invasion and metastatic disease *in vivo*.

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