Centrosome amplification and cancer: a question of sufficiency

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Centrosome amplification is a common feature of many types of cancer, but whether it is a cause or consequence is hotly debated. In this issue of *Developmental Cell*, Levine et al. (2017) provide strong evidence that centrosome amplification is sufficient to initiate tumorigenesis in a mouse model.

Centrosomes consist of a pair of centrioles surrounded by a matrix of pericentriolar material, and they are the major microtubule organising centers of animal cells. They play an important part in many cell processes, including organising the poles of the mitotic spindle, which precisely partitions the duplicated chromosomes into two daughter cells during mitosis (Conduit et al., 2015). Centrosome numbers are normally tightly regulated so that, during division, each daughter cell inherits only one centrosome, which is then duplicated once to produce the two centrosomes that, in turn, help to form the next spindle. It has long been recognised that having too many centrosomes (centrosome amplification) can lead to the formation of multipolar spindles that mis-segregate chromosomes during mitosis, thereby leading to aneuploidy. Such chromosomal instability is a hallmark of cancer, and Theodor Boveri proposed more than a hundred years ago that centrosome amplification might generate the aneuploidy that he believed was the root cause of cancer.

In the last decade or so, it has become clear that aneuploidy can play an important part in both the initiation and evolution of cancer, and that centrosome amplification can lead to an increase in aneuploidy. In many dividing somatic cells, centrosome amplification leads to the transient formation of multipolar spindles, but the extra centrosomes eventually cluster together to form a spindle with just two poles, resulting in a bipolar division. Nevertheless, the transient formation of a multipolar spindle is sufficient to increase the rate of chromosome mis-segregation, so that centrosome amplification usually leads to a significant, but modest, increase in aneuploidy. In theory, therefore, centrosome amplification should promote cancer by promoting aneuploidy, but it has remained unclear how important a part centrosome amplification really plays in driving tumourigenesis. After all,

cancer cells are highly abnormal, so it is perhaps not surprising that the normal tight regulation of centrosome numbers is perturbed; perhaps centrosome amplification is a relatively benign consequence of cancer, rather than an important driver of cancer. In their study in this issue of *Developmental Cell*, Levine et al. (2017) set out to tackle these issues in a mouse model, in particular focusing on the key question of whether centrosome amplification alone is sufficient to induce tumours.

The recent elucidation of a conserved pathway of centrosome duplication has provided the means to directly address this question. Polo-like-kinase 4 (Plk4) is a universal initiator of centriole (and so centrosome) duplication, and cells that overexpress Plk4 form too many centrosomes. Thus, overexpression of Plk4 potentially provides a way to amplify centrosomes in otherwise normal cells. The key question then is whether Plk4 overexpression pre-disposes such cells to form tumors and, if so, why?

This experiment was first attempted in the fruitfly *Drosophila* (Basto et al., 2008). Transgenic flies constitutively overexpressing Plk4 exhibited high levels of centrosome amplification and a modest increase in aneuploidy. Although significantly delayed in development, these flies were viable and fertile, indicating that centrosome amplification was surprisingly well tolerated. No spontaneous tumors were observed in these flies but, in a classical tissue-transplantation model, developing brain tissue from these flies could spontaneously form tumors, while normal brain tissue could not. Interestingly, centrosome amplification perturbs the asymmetric divisions of the neural progenitors in the developing fly brain, leading to their mis-specification and over-proliferation, a defect that could contribute to tumour initiation. A different study using the same brain transplantation assay concluded that centrosome defects were tumorigenic, but other non-centrosomal defects that induced aneuploidy were not (Castellanos et al., 2008). Moreover, transplanted wing tissue from flies that overexpress Plk4 could also form tumors, even though this tissue does not contain asymmetrically dividing progenitors (Sabino et al., 2015). Thus, centrosome amplification (and centrosome defects more generally) in the developing fly brain appear to promote tumorigenesis

independently of aneuploidy, while in the developing wing they seem to do so through the induction of aneuploidy.

The first attempt to address these issues in a mouse model gave a very surprising result (Marthiens et al., 2013). Conditional Plk4 overexpression specifically in the developing mouse brain produced centrosomal amplification but not tumors, and the brain was actually too small (a condition termed microcephaly). In these mice, centrosome amplification generated aneuploidy, which appeared to trigger cell death leading to microcephaly, which could be partially rescued by ablating p53 (which can induce cell death or cell senescence in response to various cell stresses, including aneuploidy and centrosome amplification). Intriguingly, centrosome defects in humans have also been strongly linked to microcephaly (Conduit et al., 2015).

Several studies then examined the effects of Plk4 overexpression in other mouse tissues after birth. In one study, the general overexpression of Plk4 led to significant centrosome amplification in the liver and skin but not in the lung or kidney (Vitre et al., 2015). These mice, however, showed no increase in spontaneous tumor formation or decreased survival compared to controls, even when p53 levels were reduced (p53+/-) or eliminated (p53-/-). As expected, perturbing p53 alone induced many tumors, but this was not exacerbated by Plk4 overexpression. Two similar studies also concluded that the widespread (Coelho et al., 2015) or skin-specific (Serçin et al., 2016) overexpression of Plk4 did not lead to an increase in spontaneous tumor formation, but in these cases Plk4 overexpression in a p53-/- background led to a dramatic increase in the speed of tumor initiation. Thus, while there was general agreement that Plk4 overexpression does not induce spontaneous tumors, it seems it can enhance tumor initiation in a p53-/- background, at least in certain tissues and under certain conditions.

In this issue of *Developmental Cell*, Levine et al. (2017) use Plk4 overexpression to drive widespread, but low level, chronic centrosome amplification in mice, and they followed these mice for a longer period than in previous studies. Unexpectedly, starting at 35 weeks of age, the mice

exhibited a dramatic increase in the spontaneous formation of lymphomas, squamous cell carcinomas, and sarcomas, and these tumours exhibited the aneuploidy and chromosomal instability typical of many human tumours. Although centrosome amplification promoted the initiation of tumours, it did not seem to dramatically affect their progression. Importantly, the authors used several methods to assess the status of p53 in these tumors. Although there was some variation in p53 levels, there was a consistent reduction in the expression of p53-target-genes, suggesting that the p53 pathway was at least partly inactivated. So, although this study is the first to demonstrate that centrosome amplification can initiate cancer in an intact mouse model, it may be that a similar underlying mechanism is at least partly responsible; if given enough time, the aneuploidy induced by centrosome amplification can lead to p53-pathway down-regulation, and so, eventually, to tumorigenesis.

Taken together, these experiments in flies and mice support Boveri's original hypothesis that centrosome amplification promotes tumorigenesis, but it remains unclear how it does so. In all the experiments discussed above centrosome amplification was correlated with an increase in aneuploidy, which seems the most likely culprit, at least in mice and the developing fly wing. These experiments, however, also illustrate the complexity and heterogeneity of the response to Plk4 overexpression in different tissues. Therefore, it seems premature to conclude that centrosome amplification *only* promotes cancer formation by increasing chromosome mis-segregation and aneuploidy. Indeed, other mechanisms have been proposed, such as a centrosome amplification leading to cell invasiveness (Godinho et al., 2014) or defects in spindle orientation, which could lead either to hyperplasia or cell fate-specification defects (Basto et al., 2008; Serçin et al., 2016). Moreover, these experiments rely almost exclusively on Plk4 overexpression to drive centrosome amplification; Plk4 is a protein kinase whose overexpression could influence many cellular processes that depend on the microtubule cytoskeleton or even have unsuspected roles in other processes. Despite this caveat, the findings of Levine et al. (2017), together with the preceding work in both fly and mouse, provide strong support for the idea that centrosome amplification can play an important part in cancer initiation and progression.

The resolution of this debate will help focus attention on the key questions of the frequency and genesis of centrosome amplification, the relative contribution of centrosome amplification to cancers of different origins, and whether centrosome amplification offers a potential therapeutic target.

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