Cell

Centrosome Amplification Can Initiate Tumorigenesis in Flies

Renata Basto,[1,6,*] Kathrin Brunk,[1,5] Tatiana Vinadogrova,[2,3,5] Nina Peel,[1,4,5] Anna Franz,[1] Alexey Khodjakov,[2,3] and Jordan W. Raff[1,*]
[1]The Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN, UK
[2]Wadsworth Center, New York State Department of Health, Albany, NY 12201, USA
[3]Department of Biomedical Sciences, State University of New York, Albany, NY 12222, USA
[4]Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[5]These authors contributed equally to this work
[6]Present address: UMR 144, CNRS, Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France
*Correspondence: renata.basto@curie.fr (R.B.), j.raff@gurdon.cam.ac.uk (J.W.R.)
DOI 10.1016/j.cell.2008.05.039

SUMMARY

Centrosome amplification is a common feature of many cancer cells, and it has been previously proposed that centrosome amplification can drive genetic instability and so tumorigenesis. To test this hypothesis, we generated *Drosophila* lines that have extra centrosomes in ~60% of their somatic cells. Many cells with extra centrosomes initially form multipolar spindles, but these spindles ultimately become bipolar. This requires a delay in mitosis that is mediated by the spindle assembly checkpoint (SAC). As a result of this delay, there is no dramatic increase in genetic instability in flies with extra centrosomes, and these flies maintain a stable diploid genome over many generations. The asymmetric division of the larval neural stem cells, however, is compromised in the presence of extra centrosomes, and larval brain cells with extra centrosomes can generate metastatic tumors when transplanted into the abdomens of wild-type hosts. Thus, centrosome amplification can initiate tumorigenesis in flies.

INTRODUCTION

Centrosomes are the main microtubule organizing centers in animal cells, and they comprise a pair of centrioles surrounded by an amorphous pericentriolar material (PCM) (Bornens, 2002; Kellogg et al., 1994). Centrosomes play an important part in organizing many cell processes, particularly during mitosis where they organize the poles of the mitotic spindle. The idea that centrosome amplification can contribute to tumorigenesis was first proposed by Theodor Boveri almost one hundred years ago (Boveri, 2008; Wunderlich, 2002). Boveri was aware that malignant cells often had an abnormal complement of chromosomes, and he had shown that the presence of extra centrosomes in sea urchin embryos invariably led to chromosome missegregation, as the chromosomes were randomly distributed among the spindle poles formed by the multiple centrosomes. This elegant hypothesis, however, was largely ignored as the discovery of oncogenes led to the idea that tumorigenesis is a multistep process involving the accumulation of several mutations or epigenetic changes that ultimately give rise to a cancer cell. Nevertheless, it remains a fact that genetic instability is a common feature of many different types of cancer.

Centrosome amplification is a common feature of many cancer cells (D'Assoro et al., 2002a, 2002b; Nigg, 2006; Pihan et al., 1998, 2001). Moreover, levels of centrosome amplification are often correlated with levels of genetic instability (Brinkley, 2001; Ghadimi et al., 2000; Lingle et al., 2002). Thus, it is now widely assumed that centrosome amplification inevitably leads to genetic instability, and that this can be a significant factor in the generation of fully transformed cancer cells. In support of this possibility, it has recently been shown that inducing genetic instability in mice can increase the rates of tumor formation in some, but not all, tissues (Weaver et al., 2007).

Centrosome amplification, however, does not necessarily lead to spindle multipolarity (Quintyne et al., 2005; Ring et al., 1982). In at least some cell types, extra centrosomes can "cluster" together during mitosis, and the cells often ultimately divide in a bipolar fashion. Indeed, it is thought that many cancer cells in culture have evolved mechanisms to cluster their centrosomes during mitosis so they avoid generating high (and potentially lethal) levels of aneuploidy during every round of cell division (Brinkley, 2001). Thus, the consequences of amplifying centrosomes within the context of a normal developing organism are far from clear.

In flies and humans, the protein kinase SAK/PLK4 plays a critical part in initiating centriole duplication, and the overexpression of this protein can drive centriole overduplication in cells (Bettencourt-Dias et al., 2005; Habedanck et al., 2005; Kleylein-Sohn et al., 2007). In this study, we have used stable *Drosophila* transgenic lines overexpressing SAK to drive centrosome amplification in ~60% of somatic cells. This has allowed us to assess the long-term consequences for an organism of having cells with too many centrosomes. Surprisingly, we find that cells with extra centrosomes invariably divide in a bipolar fashion in vivo, and the presence of extra centrosomes does not

generate large-scale genetic instability. The asymmetric division of the neural stem cells (neuroblasts), however, is perturbed, and ~10% of these cells ultimately divide symmetrically. Most importantly, we show that the transplantation of brain cells with too many centrosomes can induce the formation of metastatic tumors in normal hosts.

RESULTS

Flies Overexpressing GFP-SAK Have Too Many Centrosomes in ~60% of Their Somatic Cells but Are Viable and Fertile

It has previously been shown that the overexpression of the centriole replication protein SAK/PLK4 leads to the formation of extra centrosomes in cells (Bettencourt-Dias et al., 2005; Habedanck et al., 2005; Kleylein-Sohn et al., 2007; Peel et al., 2007). To analyze the consequences of centrosome amplification within the context of a developing multicellular organism, we analyzed stable transformed *Drosophila* lines that expressed a GFP-SAK fusion protein under the control of the Ubiquitin promoter. This promoter is expressed at moderately high levels in all cells (Lee et al., 1988), and it leads to a dramatic overexpression of centriole duplication proteins, as these proteins are normally expressed at very low levels in cells (Peel et al., 2007). We generated several independent transformed lines, all of which showed similar degrees of centrosome amplification; we analyzed two of these lines in detail. Both lines behaved in an essentially indistinguishable manner in all the experiments reported here, so we simply refer to them as *SAKOE* lines, unless otherwise stated.

Quantification of centrosome number in *SAKOE* third-instar larval brain cells revealed that ~60% of these cells contained more than two centrosomes (Figures 1A–1C), and we obtained similar results in larval imaginal disc cells (data not shown). Note that in this and all subsequent experiments dots were only counted as centrosomes if they were stained by both centriolar and PCM or microtubule (MT) markers. Usually, 3 to 6 centrosomes were present in the cells with extra centrosomes, but cells with higher numbers were occasionally observed. To confirm that these structures were centrosomes, we performed an electron microscopy (EM) analysis of fixed whole-mount brains. In wild-type (WT) brains, two centrioles were identified at each spindle pole (n = 4) (Figure 1D). In contrast, half of the spindle poles we examined in *SAKOE* cells contained multiple centrioles (n = 9/18) (Figure 1E). Thus, the extra centrosomes we observe in the *SAKOE* cells contain morphologically recognizable centrioles.

We have previously shown that *DSas-4* mutant flies (that lack centrioles and centrosomes) are morphologically normal and are only slightly delayed in development compared to WT flies (Basto et al., 2006). Surprisingly, we found that *SAKOE* flies were also morphologically normal (Figure S1 available online), but they exhibited a much longer delay in development compared to WT and *DSas-4* mutants (Figure 1F). To test if this developmental delay was caused by the presence of extra centrosomes, we overexpressed GFP-SAK in a *DSas-4* mutant background. These flies contained no detectable centrioles (data not shown) and, like *DSas-4* mutants alone, they were only slightly delayed in development (Figure 1F). We conclude that it is the presence of extra centrosomes in *SAKOE* flies that delays their development.

Despite the delay in development, adult *SAKOE* flies were viable and fertile, although a significant fraction of eggs laid by *SAKOE* females (~60%) died early in development due to an accumulation of mitotic errors (Peel et al., 2007). Nevertheless, transgenic fly lines containing extra centrosomes in ~60% of their somatic cells can be maintained in the laboratory as viable and fertile stocks for many generations (at present we have maintained these stocks for nearly 2 years).

Mitosis in Cells with Extra Centrosomes

These findings indicate that the presence of extra centrosomes in the majority of somatic cells in an organism is compatible with normal development and long-term survival. This suggests that extra centrosomes do not dramatically interfere with cell division and cell-cycle progression. To better understand how cells divide in the presence of extra centrosomes we examined fixed *SAKOE* third-instar larval brain cells.

It has previously been shown that centrosomes behave asymmetrically in WT neuroblasts, with one centrosome associated with more PCM and nucleating more MTs than the other throughout interphase and during the early stages of mitosis (Rebollo et al., 2007; Rusan and Peifer, 2007). This asymmetry was noticeable during early mitosis in WT neuroblasts (Figures 1A and 2A) but was often not apparent in *SAKOE* neuroblasts with extra centrosomes (Figures 1B and 2B). In these early mitotic cells, all centrosomes were associated with PCM and MTs and the centrosomes were often of different sizes, but it was usually not possible to identify a single "dominant" centrosome, either in terms of PCM recruitment or MT nucleation (Figure 2B). Thus, centrosome asymmetry appears to be disrupted in neuroblasts with extra centrosomes.

In WT brains, 98% of cells (n = 250 cells) formed a bipolar spindle by metaphase (Figure 2C, see also Figure S2). Surprisingly, 93% of cells with extra centrosomes, (n = 500 cells) had also formed a bipolar spindle by metaphase (Figures 2D and S2). Usually, several of the centrosomes were clustered at the poles of the spindle, but we also often observed centrosomes that were not associated with either pole (Figure 2D, see also Figure S3). In metaphase and anaphase cells, the non-pole-associated centrosomes usually contained less PCM than the centrosomes located at the poles, and they were usually not associated with robust asters of MTs (arrows, Figures 2D–2F, see also arrows in Figure 3E), suggesting that they were partially inactivated. Importantly, we made similar observations on the clustering and partial inactivation of extra centrosomes in living *SAKOE* brain cells (Figure S3 and Movies S1–S4).

Surprisingly, the frequency of multipolar and abnormal metaphase spindles was only slightly higher in *SAKOE* cells (2% and 5%, respectively, n = 500 cells) than in WT cells (0% and 2%, respectively, n = 250 cells) (Figure S2). And, by the time cells entered anaphase, the spindles were always bipolar in both WT and *SAKOE* brains (n = 200 cells and n = 400 cells, respectively), while the frequency of aneuploidy was only slightly higher in *SAKOE* brains (1.75%, n = 345 cells) compared to WT (0.7%, n = 150 cells) (Table 1). The mitotic index, however, was significantly higher in *SAKOE* brains (2.6% ± 0.5%, n = 7797 cells from 4

Download English Version:

https://daneshyari.com/en/article/2038141

Download Persian Version:

https://daneshyari.com/article/2038141

Daneshyari.com