Current Biology 18, 1209–1214, August 26, 2008 ©2008 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2008.07.029

Report

Centrosome Dysfunction in *Drosophila* Neural Stem Cells Causes Tumors that Are Not Due to Genome Instability

Elisabeth Castellanos,[1] Paloma Dominguez,[1,3] and Cayetano Gonzalez[1,2,*]
[1]Cell Division Group
IRB-Barcelona
PCB
c/ Baldiri Reixac 10-12
08028 Barcelona
Spain
[2]Institucio Catalana de Recerca i Estudis Avançats
Passeig Lluis Companys 23
08010 Barcelona
Spain

Summary

Genome instability (GI) and centrosomal alterations are common traits in human cancer [1, 2]. It is suspected that centrosome dysfunction may cause tumors by bringing about GI, but direct experimental proof is still lacking [3]. To explore the possible functional link between centrosome function and overgrowth, we have assayed the tumorigenic potential of a series of mutants that affect different centrosomal proteins in *Drosophila*. We have found that a significant number of such mutant conditions are tumorigenic in larval brain tissue, where self-renewing asymmetric division of neural stem cells is frequent, but not in symmetrically dividing epithelial cells. We have also found that mutations that increase GI without causing centrosome dysfunction are not tumorigenic in our assay. From these observations, we conclude that the tumors caused by centrosome dysfunction cannot be explained solely by the resulting genome instability. We propose that such tumors might be caused by impaired asymmetric division of neural stem cells [4]. These results show that centrosome loss, far from being innocuous, is a potentially dangerous condition in flies.

Results and Discussion

Centrosome dysfunction is frequent in cancer, but it is still unclear whether it contributes to, or results from, malignant transformation [3]. To asses whether centrosome dysfunction can cause tumors, we have carried out an unbiased test based on assaying the tumorigenic potential of well-characterised mutants that affect centrosome function. These include mutants in centriolar proteins that are required for PCM stabilization (Asl [5]) or for centriole duplication (DSas-4 and DSas-6 [6]), a kinase that regulates centriole duplication (Sak [6]), components of the PCM that are essential for the microtubule-nucleation activity of the centrosome (Cnn [7, 8] and gammaTUB23C [9]), a protein that localizes in both centrioles and PCM and is required for the efficient recruitment of several PCM components (Plp [10]), and the centrosome-regulatory protein kinases Polo [11] and AurA [12]. Besides their key roles in the centrosome cycle, these two kinases have recently been reported to regulate asymmetry in larval NBs, and their loss of function results in supernumerary larval NBs at the expense of neurons [13–15]. Altogether, such mutant collection brings about a range of phenotypes that provides a fair representation of the centrosome abnormalities that are characteristic of human tumors [2].

Our assay was based on the allograft-transplantation procedure, which has been extensively used to identify and characterize tumor suppressors in *Drosophila* [16] (see Supplemental Data, available online). Whereas wild-type larval brain tissue hardly grows after implantation into the abdomen of adult flies, tissue mutant for a number of tumor suppressors can grow to many-fold the size of the implant, in some cases invading different organs and killing the hosts [17]. We found that pieces of larval brain tissue mutant for $pPlp^{2172}$, cnn^{F04547}, or $gammaTUB^{PI}$ did not show significant growth at a frequency detectable in our assays (Table 1B, Figure 1A). However, the remaining mutant conditions resulted in the growth of tumors with frequencies that are similar to those of previously characterized brain-tumor suppressors [17]. Implants of larval brain-tissue mutant for asl^{1}, sak^{c06612}, or $dsas\text{-}6^{c02901}$ (henceforth *asl*, *sak*, and *dsas-6*) displayed very substantial growth, typically expanding over a quarter of the abdominal cavity, in 4%, 2%, and 1% of hosts, respectively (Table 1B, Figure 1B). However, these tumors never grew enough to fill the abdomen, nor did they significantly shorten the host's lifespan. In contrast, implants of $dsas\text{-}4^{l(3)s2214}$, $polo^{1}$, $aurA^{8839}$, and $aurA^{37}$ larval brain tissue grew unrestrained, filling the abdominal cavity and eventually killing the host, in 6%, 10%, 86%, and 10% of the cases, respectively (Table 1B; Figure 1C). Moreover, in addition to the major tumor mass, these implants originate small colonies scattered on different parts of the host's anatomy, like those previously observed in tumors caused by mutants in several brain-tumor-suppressor genes [17, 18]. Some such "fly micrometastases" can be observed in the ovary (Figures 1D–1G), revealing the capacity of the tumor cells to penetrate through peritoneal and muscle sheaths. We did not observe such fly micrometastases in *asl*, *sak*, and *dsas-6* tumors.

To further characterize the nature of these tumors, we assayed their growth potential by serial retransplantation. We found that *asl*, *sak*, and *dsas-6* tumors were unable to grow upon retransplantation into new healthy hosts (Figure 2A). In contrast, $dsas\text{-}4^{l(3)s2214}$, $polo^{1}$, and $aurA^{8839}$ (henceforth *dsas-4*, *polo*, and *aurA)*-derived tumors can be maintained for years after biweekly serial retransplantation, thus revealing an endless ability to generate more tumor mass. Interestingly, the number of implanted hosts that develop a tumor steadily increases to > 70% by the 4^{th} transfer generation (T4) and to nearly 100% by T10. This is a dramatic increase for *polo* and *dsas-4* tumors that grew in less than 10% of the hosts in T0 (Figure 2A). Host lethality also increases over time in *dsas-4*, *polo*, and *aurA* tumors. In T0, *dsas-4* and *polo* tumors take, on average, 38 and 20 days to kill the hosts, respectively, and host lethality during the first 10 days after implantation is essentially null (Figure 2B). Yet, by T4, host lethality during the same period is > 50%, and it reaches more than 70% by T10 (Figure 2B).

*Correspondence: gonzalez@irbbarcelona.org
[3]Present address: Microscopy Unit, CABIMER, Parque Cientifico y Tecnologico Cartuja 93, Avda, Americo Vespucio s/n, 41092 Sevilla, Spain

Table 1. Assaying Tumor Growth by Allograft Culture

Genotype of Implanted Brain Tissue[a]	Total Number of Implants	Host that Developed Tumors (%)
Control (w^{1118})	250	0
Centrosome function[b]		
dsas-$4^{l(3)s2214}$	120	8 (6,5%)
asl^{1}	140	6 (4%)
$pPlp^{2172}$	60	0
cnn^{f04547}	100	0
cnn^{f04547}/cnn^{hk21}	20	0
$polo^{1}$	60	6 (10%)
$aurA^{8839}$	30	26 (86,5%)
$aurA^{37}$ MARCM	40	4 (10%)
$\gamma tub23C^{Pl}$	60	0
sak^{c06612}(plk4)	130	3 (2%)
dsas-6^{c02901}	140	2 (1%)
Genome stability[c]		
$l(3)11m\text{-}254^{1}/l(3)11m\text{-}254^{5}$	95	0
$l(3)K43^{1}$	75	1 (1%)
asp^{L1}	60	0
asp^{L1}/asp^{E3}	70	0
atm^{3}/atm^{6}	110	0
flb^{1}/fbl^{3}	50	0
fbl^{2}/fbl^{3}	50	0
fbl^{1}/fbl^{2}	50	0
fbl^{3}	100	0
dia^{9}	70	0
$l(3)7m\text{-}62^{1}/l(3)7m\text{-}62^{5}$	100	0
$l(3)7m\text{-}62^{1}/l(3)7m\text{-}62^{4}$	50	0
$l(3)7m\text{-}62^{4}/l(3)7m\text{-}62^{5}$	50	0
$l(3)7m\text{-}62^{1}$	50	0
tsr^{2}	100	0
tsr^{1}/tsr^{2}	50	0
$bubR1^{1}$	105	0
bub3	100	0
rod^{AG1}	100	0
M-5 Birmingham	70	0
X-Rays	60	0
Genotype of Implanted Imaginal Discs[d]	**Total Number of Implants**	**Host that Developed Tumors (%)**
Control (w^{1118})	100	0
lgl^{4}	50	9 (18%)
$polo^{1}$	60	0
aur^{8839}	60	0
dsas-$4^{l(3)s2214}$	100	0
asl^{1}	100	0
sak^{c06612}(plk4)	100	0
dsas-6^{c02901}	100	0

Pieces of larval brain tissue or imaginal discs, wild-type or mutant for a series of genes required for centrosome function and genome stability, were implanted into the abdomen of adult hosts for determination of their growth potential.

[a] None of 250 control implants grew to any significant extent in this assay.

[b] Implants of larval brain tissue homozygous for mutants that affect centrosome function. Six out of nine genes assayed have a tumor-suppressor activity. In the case of $aurA^{37}$, the implant was brain tissue containing MARCM-induced mutant clones.

[c] Only one out of the twelve tested experimental conditions that cause GI gave rise to a tumor.

[d] None of the centrosome mutants that cause tumors in larval brain tissue cause tumors in imaginal discs.

We then determined the extent of chromosome instability (CIN) in *polo, aurA*, and *dsas-4* homozygous brains and tumor lines. Unfortunately, the relatively small mass of *dsas-6, asl,* and *sak* tumors does not afford this type of analyses. We found that in larval brains there are no significant differences in DNA content per cell, as determined by FACS, between *polo, aurA*, *dsas-4,* and a wild-type control, except for a minor increase in 4n cells in *aurA* (Figure 3A, top). Consistent with published data [12, 19], aneuploid or polyploid cells account for 20% of the cells arrested in mitosis in *polo* and *aurA* brains (Figure 3A, bottom). Also consistent with published results [32], only a small fraction of d*sas-4* cells have abnormal karyotypes even though *dsas-4* tumors occur as frequently, and are as malignant, as *polo* tumors. In addition, 20% of *dsas-4* cells show precocious sister-chromatid separation after colchicine treatment. CIN is dramatically increased in the tumor lines as compared to the mutant brains. By T10, the DNA-content profiles obtained by FACS are markedly shifted toward levels $\geq$ 4n (Figure 3B) and karyotypes are highly polyploidy in > 95% of tumor cells (Figure 3B). In addition to these quantitative changes, many cells from *dsas-4* tumor lines show aberrantly condensed chromatin, which was not observed at a significant level in *dsas-4* larval brains. Therefore, as in many tumor types in mammals [1], CIN is a major trait in *polo, aurA*, and *dsas-4* fly neoplasms.

Being immortal, lethal to the host, deadlier as they age, invasive, and genomically unstable, *polo, aurA*, and *dsas-4* tumors can be graded as malignant neoplasms, very similar to those caused by mutants that disrupt the asymmetric-cell-division machinery in larval neuroblasts [16]. Using the same criteria, *asl*, *sak*, and *dsas-6* tumors can be graded as benign hyperplasias. Thus, regardless of their malignant or benign nature, six out of the nine assayed mutant conditions that cause centrosome dysfunction result in tumors. It is formally possible that the genes affected by these mutants might have additional, non-centrosome-related functions that could be responsible for the observed tumor suppressor activity. More likely, these results strongly suggest that loss of centrosome function facilitates tumorigenesis in *Drosophila*. Notably, the mutants for any of the three components of the centriole-duplication pathway that we have tested, *dsas-4*, *sak*, and *dsas-6*, are tumorigenic, showing that centrosome loss, far from being innocuous, is a potentially dangerous condition in flies.

If centrosome dysfunction caused tumors through genome instability (GI), it would be expected that GI alone, without centrosome dysfunction, should cause tumors as well. To test this hypothesis, we assayed the tumorigenic activity of mutants (Table S1) in genes required for the chromosome-replication checkpoint: ataxia telengiesctasia mutated (*atm*) [20]; the spindle assembly checkpoint: *bub3* [21], bub related one (*bubR1*) [22], and rough deal (*rod*) [23]; spindle assembly: abnormal spindle (*asp*) [24]; chromatin condensation: l(3)11 m-254, and l(3)K43 [25]; and cytokinesis: l(3)7 m-62 [25], fumble (*fbl*) [26], twinstar (*tsr*) [27], and diaphanous (*dia*) [27]. We also assayed larval brain tissue in which GI was induced by exposure to X-rays or by somatic mobilization of the multiple P elements of the Müller-5 "Birmingham M" strain [28]. Such collection of experimental conditions brings about a fair recapitulation of the most frequent types of defects in chromosome number and integrity reported in human tumors [1], such as chromosomal rearrangements, aneuploidy, and different levels of polyploidy, including, notably, tetraploidy, which is suspected to be an unstable state that contributes to aneuploidy [3] (Figure S1).

We found that, except for $l(3)K43^{1}$, which, besides its effect on chromosome condensation and integrity, also causes amorphous microtubule-organizing centers [29], none of the experimental conditions tested gave rise to tumors in our assays (Table 1C). However, the implications of these results