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Spindle Multipolarity Is Prevented by Centrosomal Clustering

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Spindle Multipolarity Is Prevented by Centrosomal Clustering

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Most tumor cells are characterized by increased genomic instability and chromosome segregational defects, often associated with hyperamplification of the centrosome and the formation of multipolar spindles. However, extra centrosomes do not always lead to multipolarity. Here, we describe a process of centrosomal clustering that prevented the formation of multipolar spindles in noncancer cells. Noncancer cells needed to overcome this clustering mechanism to allow multipolar spindles to form at a high frequency. The microtubule motor cytoplasmic dynein was a critical part of this coalescing machinery, and in some tumor cells overexpression of the spindle protein NuMA interfered with dynein localization, promoting multipolarity.

Hyperamplification of the centrosome has been observed in many tumor tissues and cell lines and is linked with both aneuploidy and tumorigenesis (*1–4*). The extent of genomic instability is generally correlated with the degree of centrosomal abnormalities (*3–5*). Furthermore, centrosome abnormalities are more severe in high-grade and recurrent tumors and in cell lines that show aggressive malignant phenotypes (*1–4*). In mitosis, supernumerary centrosomes can lead to an increase in spindle poles, and multipolar spindles are found in many cancer cell types (*6*).

Although centrosome amplification is clearly important for multipolar spindles, the presence of extra centrosomes does not always lead to multipolar spindle formation. Certain cell types can apparently suppress multipolarity and form a bipolar spindle during mitosis even though the centrosomes are amplified (*7–9*). Furthermore, some centrosomal defects induce centrosomal amplification without multipolarity (*10*). To suppress multipolar spindles, the cell could functionally silence the extra centrosomes, preventing them from forming a spindle pole and leaving only two centrosomes active. Alternatively, a cell could coalesce the extra centrosomes into only two functional spindle poles (*7*, *11*, *12*).

The spindle protein NuMA has been shown to be critical for spindle assembly (*13–17*), and the *NUMA1* gene maps to one of the most frequently amplified chromosomal segments in cancer cells (*18*, *19*). We examined two cell lines that exhibited relatively high amounts of NuMA expression (Fig. 1A) and that had ~20% multipolar spindles (Fig. 1, B and D). If the overexpression of NuMA is driving multipolarity, then reduction of NuMA by small interfering RNA (siRNA) should lead to a return of bipolar spindles. Three days after a single siRNA transfection (*20*), NuMA amounts were reduced (Fig. 1C and fig. S1), whereas amounts of the associated proteins dynein and dynactin were unchanged (fig. S2) (*16*). To examine only cells that received siRNA (~50%), we labeled the siRNA duplex with a fluorescein marker. In both the UPCI:SCC103 and UPCI:SCC078 oral cancer cell lines, transfection with the NuMA siRNA nearly eliminated multipolar spindles (Fig. 1, B and D). Similar results were observed for the SK-HEP-1 liver adenocarcinoma cell line (Fig. 1D). When the NuMA level was allowed to recover 10 days after a single siRNA treatment, the frequency of multipolarity returned to that of untreated cells. Thus, overexpression of NuMA perturbs the ability of these cells to coalesce supernumerary centrosomes into a single pole. NuMA provides a cohesive force in maintaining spindle microtubules around a single centrosome (*13*, *16*), but these results suggest a previously unknown role for elevated amounts of NuMA as an inhibitor of centrosome coalescence in cells with supernumerary centrosomes.

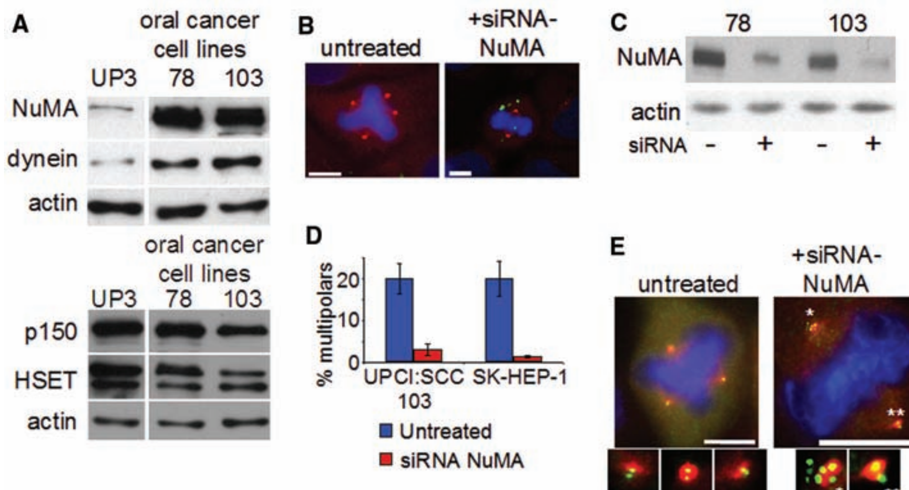

Fig. 1. Reduction of NuMA decreases spindle multipolarity. (**A**) Immunoblots of whole-cell extracts from the indicated cell lines were probed with antibodies to NuMA, dynein intermediate chain, the dynactin subunit p150^{Glued}, and HSET. Only NuMA and dynein showed an increase in expression in cancer cells when compared to normal oral keratinocytes (UP3) (*24*). (**B**) UPCI:SCC103 cells were stained with antibodies to centrosomal γ-tubulin (red), fluorescein-labeled siRNA (green), and the DNA dye 4′,6′-diamidino-2-phenylindole (DAPI) (blue) before and after siRNA transfection. (**C**) NuMA protein reduction after transfection. (**D**) Decrease in multipolar spindles in metaphase cells after siRNA to NuMA. In each case, the decrease in multipolar spindles was matched by an increase in bipolar spindles. (**E**) UPCI:SCC103 cells stained with antibodies to centrin-2 (green) and γ-tubulin (red) and with DAPI (blue). Magnified views of spindle poles are shown at bottom at 3× magnification. Asterisks match the magnified images with the larger image. Bars indicate 10 μm in all figures.

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Does NuMA act directly on the spindle or exert its effect on other spindle proteins? Dynein (*13*, *16*), its associated activator dynactin (*21*), and the kinesin motor HSET (*22*) all localized along the spindle microtubules in noncancer HEK293 cells (Fig. 2) (*22*, *23*). Whereas HSET and dynactin localized normally in the oral cancer cells, spindle dynein immunoreactivity was absent or sharply reduced (Fig. 2), although dynein intermediate chain expression was elevated in these cells (Fig. 1A) (*24*). Punctate dynein staining was still visible in the cancer cells, probably representing nonspindle vesicle-associated motor molecules. Reduction of NuMA did not interfere with dynein localization at the centrosomes of interphase cancer cells. A survey of various cancer cell types showed a similar loss of spindle dynein, but not dynactin, immunolabeling from nearly all cultured cancer cell lines tested (table S1).

Because NuMA overexpression led to multipolar spindle formation and dynein plays a critical role in spindle formation and maintenance, we tested whether the overexpression of NuMA was the cause of the change in spindle-associated dynein. When NuMA amounts were reduced in the UPCI:SCC103 oral cancer cell line (or UPCI:SCC078), dynein was visible on nearly all of the spindles (Fig. 3A, image e). More than 90% of the spindles in UPCI:SCC103 cells lacked dynein staining before treatment, whereas after siRNA treatment >80% of the transfected cells had visible dynein staining (Fig. 4A). Thus, overexpression of NuMA induced both a change in spindle dynein and multipolarity in these cancer cell lines. Similar results were observed for the SK-HEP-1 liver adenocarcinoma cell line (table S1).

However, spindle dynein delocalization in other cancer cell lines is not dependent on NuMA. In the UPCI:SCC070 cells, which do not have *NUMA1* amplification (*19*), reduction of NuMA had no effect on spindle multipolarity and did not restore dynein staining (Fig. 3A, image f). Similar observations were made in other tumor cell lines (table S1). Although a reduction of spindle dynein staining seems to be a common feature of many cancer cell lines, only some cell types achieve this change by overexpression of NuMA.

Could a change in spindle dynein account for the failure of centrosome clustering in cancer cells? We first checked for a correlation between the frequency of multipolar spindles and centrosomal amplification or dynein localization in various cancer cell lines. Of the tested lines, only those with centrosomal amplification and no dynein immunolabeling on the mitotic spindle were able to induce multipolar spindles in >10% of the metaphase cells (table S2). However, other unknown variables could also account for the differences between these different cell lines.

To confirm that these two factors were critical for spindle multipolarity, we determined whether inducing extra centrosomes and/or inhibiting dynein function would result in multipolarity in noncancer cells. We treated HEK293 cells with the microtubule inhibitor Colcemid (Irvine Scientific, Santa Ana, CA) for 28 to 36 hours, and the frequency of cells with extra centrosomes increased from <5% to ~80% (Fig. 3B, images a and a', and fig. S3). The increase in spindle multipolarity was limited to between ~8% and ~20% of the metaphase cells (Fig. 4, A and B; this would still be relatively high for the untreated cancer cells). Thus, centrosome amplification alone leads to only a limited increase in multipolarity in this nontumor cell line. To inhibit dynein, we transfected the HEK293 cells with either plasmids expressing NuMA (*14*) to mimic the overexpression seen in the oral cancer cells or a plasmid expressing the dynein-binding fragment of p150^{Glued}, CC1 (*25*). Overexpression of NuMA was able to displace dynein from the spindle of ~50% of the HEK293 cells, reproducing the change we saw in cancer cells with *NUMA1* amplification, but by itself did not increase the frequency of multipolar spindles (Figs. 3B, images b and b' and 4, B and C) (*21*). Similarly, expression of CC1 reduced dynein on the spindle as expected but only marginally elevated spindle multipolarity (Fig. 4, B and C). Thus, inhibition of dynein or amplification of centrosomes alone was sufficient to induce only a modest increase in multipolar spindles. However, when cells were treated with both Colcemid and overexpression of either NuMA or CC1, multipolar spindle frequency increased to ~60 to 70% of metaphase cells (Figs. 3B, images c and c' and 4, B and C).

We also tried two other methods of amplifying centrosomes in conjunction with inhibition of dynein. HEK293 cells were transfected with a plasmid expressing the centrosomal kinase hMps1 (*26*). Centrosomal amplification rose from 6.1% to 36% of the cells, but multipolarity only increased when cells were cotransfected with plasmids expressing NuMA or CC1 (fig. S4, A and B). Similarly, the preexisting centrosomal coalescence of N1E-115 cells (*8*, *9*) was eliminated by NuMA or CC1 overexpression, and spindle multipolarity jumped from ~5% to ~80%. Another human oral cancer cell line (UPCI:SCC114) was found to possess similar, but less pronounced, centrosomal

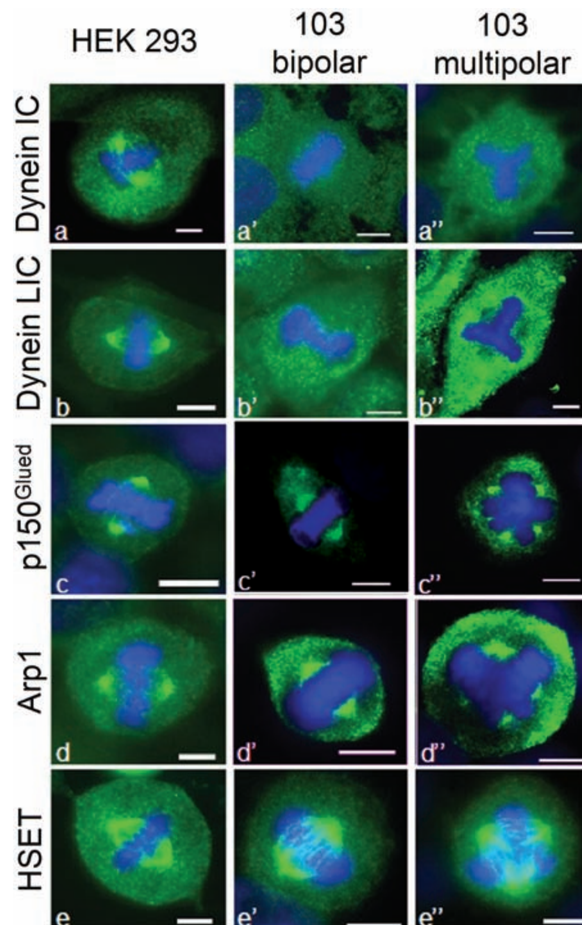

Fig. 2. Dynein is depleted from the spindle in oral cancer cells. HEK293 (images a to e) and UPCI:SCC103 (images a' to e' and a'' to e'') cells were stained with antibodies to dynein, the p150^{Glued} or Arp 1 subunits of dynactin, or HSET (green) and with DAPI (blue). Spindles in UPCI:SCC103 cells lacked visible dynein but were positive for dynactin and HSET. Antibodies to two different subunits of the dynein macromolecular complex gave similar results, indicating that the loss of immunoreactivity was not likely to be caused by epitope masking.

clustering like that of N1E-115. Unlike the other human cancer lines we tested that did not show centrosomal clustering, expression of either NuMA or CC1 alone in UPCI:SCC114 markedly elevated spindle multipolarity in the absence of Colcemid exposure or hMps1 overexpression (fig. S4, C to E).

It appears that spindle multipolarity arises via two distinct steps, an increase in centrosome number and an inhibition of centrosomal coalescence (*7*, *11*, *12*). Centrosome hyperamplification has been described in numerous tumor types (*1*–*4*). However, a second change leading to a loss of centrosome coalescence was required to manifest the multipolar phenotype. Thus, clustering may be an important mechanism for preserving genomic stability in noncancer cells. Overcoming centrosomal clustering appears to involve a change in spindle dynein, either a reduction of dynein amounts or a change to a more diffuse position within the spindle such that the strong fluorescent signal is not seen. It is unlikely that dynein is completely inhibited in the spindle of cancer cells, because we did not see the splaying of spindle poles observed after injection of antibodies to dynein (*21*) and we observed NuMA labeling on the spindle, which requires transport by dynein (*27*). Apparently, enough dynein activity remains to prevent these phenotypes. However, these results show that dynein plays an important role in maintaining the coalescing mechanism to prevent multipolar spindles.

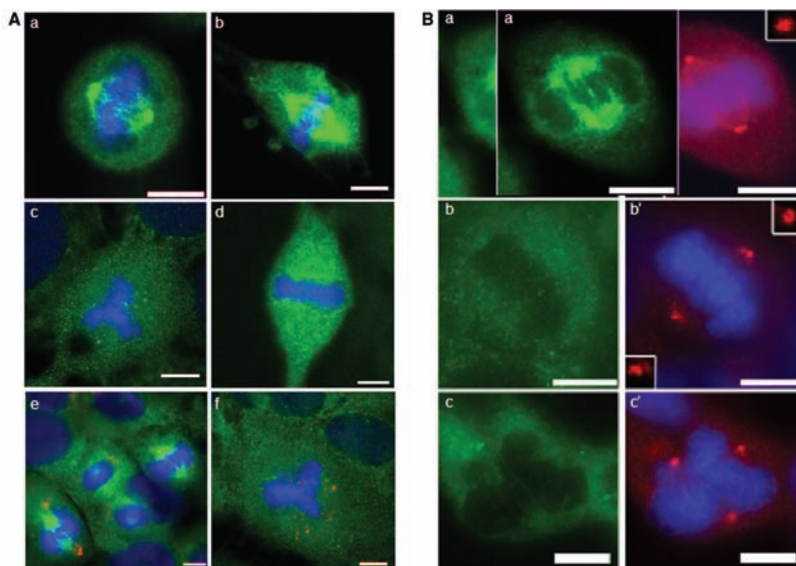

Fig. 3. Dynein is restored to spindles after siRNA-mediated knockdown of NuMA. (**A**) Cells stained with antibodies to dynein intermediate chain (green) and DAPI (blue). Normal human oral keratinocytes (UP3) (image a) and skin fibroblasts (image b) showed the expected spindle-associated dynein. Untransfected metaphase UPCI:SCC103 showed little or no detectable dynein on multipolar (image c) or bipolar spindles (image d). Dynein returned to spindles in UPCI:SCC103 cultures transfected with NuMA siRNA (red, image e) but not for UPCI:SCC070 (image f). (**B**) Inhibition of dynein or NuMA overexpression stimulated formation of multipolar spindles only in cells with amplified centrosomes. HEK293 cells were treated with Colcemid (a and a'), or overexpression of NuMA (images b and b'), or both (images c and c'). Antibodies used in (A) images and (B) images a to c were anti-dynein LIC (green) and in (B) images a' to c' were anti-γ-tubulin (red). DAPI, blue. Insets, 3× magnifications.

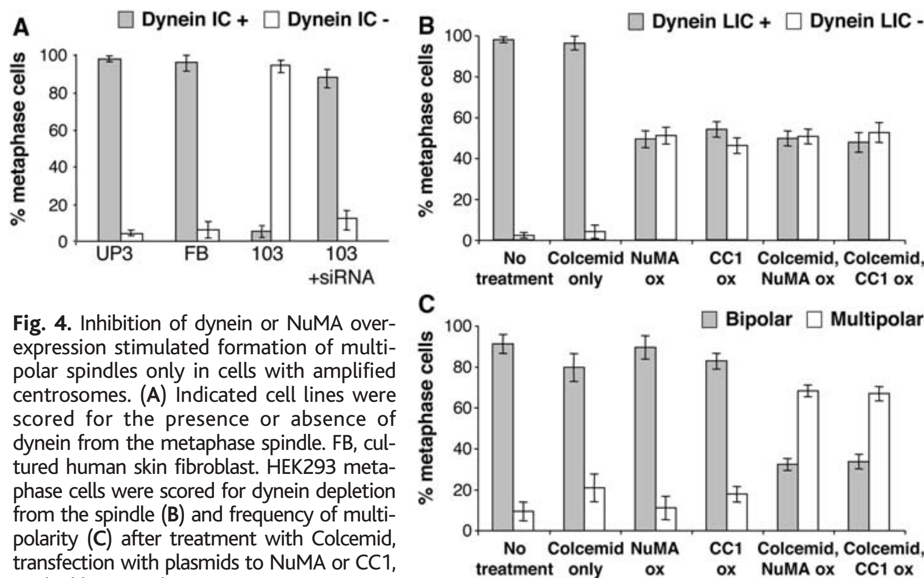

Fig. 4. Inhibition of dynein or NuMA overexpression stimulated formation of multipolar spindles only in cells with amplified centrosomes. (**A**) Indicated cell lines were scored for the presence or absence of dynein from the metaphase spindle. FB, cultured human skin fibroblast. HEK293 metaphase cells were scored for dynein depletion from the spindle (**B**) and frequency of multipolarity (**C**) after treatment with Colcemid, transfection with plasmids to NuMA or CC1, or double treated. ox, overexpression

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Figs. S1 to S4
Tables S1 and S2

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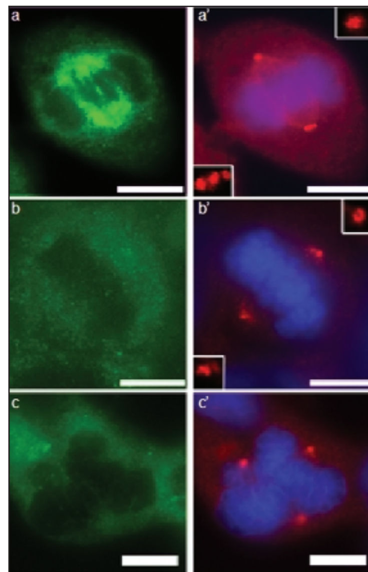

Reports: "Spindle multipolarity is prevented by centrosomsal clustering" by N. J. Quintyne *et al.* (7 Jan. 2005, p. 127). There was an error in Fig. 3. Panel B, a was mistakenly printed twice, with the second printing slightly overlapping panel B, a′. The corrected figure is shown here.