Actin and Microtubules: Working Together to Control Spindle Polarity

Fengfeng L. Xu[1] and William S. Saunders[1,*]
[1]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA
*Correspondence: wsaund@pitt.edu
DOI 10.1016/j.ccr.2008.08.007

Centrosomes are frequently amplified in cancer cells, but centrosome clustering pathways act to minimize their detrimental impact on mitosis. Recent data published online in *Genes & Developments* by Kwon et al. (2008) suggest that these pathways involve microtubule motors, actin, and focal adhesions. Since centrosomal amplification is rarely seen in normal cells, could blocking clustering lead to the selective killing of tumor cells?

Cancer cells exhibit a variety of mitotic defects; among the best characterized is enhanced centrosome number and microtubule nucleation at the spindle poles. Centrosome amplification has been linked to increased tumorigenesis and decreased survival in numerous studies (Pihan et al., 2003). Microtubules are nucleated and the minus ends of the polar filaments are often anchored at the centrosome. From the free plus ends, dynamic microtubules perform two essential functions during mitotic chromosome division and segregation. First, they grow and shrink to capture the condensed chromosomes, also serving as a track for chromatid separation during anaphase. Second, they bind and crosslink the microtubules from the other centrosome to form a bilaterally symmetrical spindle that can separate the chromatids into two equal sets. The bilateral symmetry of the spindle is critical to ensure that duplicated chromatids are separated into only two half-sets and that genetic continuity between cellular generations is maintained.

The functional unit of the spindle is the microtubule, and since there are multiple chromosomes, there must be multiple microtubules to attach the different chromosomes to the centrosome. This presents an organizational challenge for the cell in that it must link multiple microtubules to a single centrosome but allow the other centrosome to separate and form the other half-spindle. In cancer cells, this organizational requirement becomes even more complicated with the presence of additional centrosomes and spindle poles. In cancer cells, but not normal cells, supernumerary centrosomes often separate away from each other to form an aberrant multipolar spindle. The result for mitosis can be catastrophic, with chromosomes dividing into seemingly irregular and incomplete sets that do not match the chromosome complement of the parent. It would seem the cell could not survive such a hyperreductional division, and indeed, cells with multipolar spindles do not give rise to survivable clones in culture (Stewénius et al., 2005). However, Basto et al. (2008) recently observed that flies with numerically enhanced centrosomes remarkably maintained a mostly stable genome through several generations and survived to adulthood with multicentromeric and diploid cells. However, these cells were tumorigenic in transplantation assays. This poses the questions of how cells can tolerate extra centrosomes and still divide with a stable genome and, if those mechanisms are inhibited, whether cancer cells with amplified centrosomes will be killed selectively.

One mechanism for minimizing the impact of extra centrosomes is to cluster them together to form a bipolar spindle. While the molecular mechanisms behind clustering are still being identified, some common themes are emerging. Clustering appears to require the spindle assembly checkpoint (SAC). This pathway monitors kinetochore attachment to the spindle and can delay anaphase until all of the chromosomes are attached. Centrosomal clustering cannot occur in human retinal pigmented epithelial cells immortalized with telomerase RPE1 cells treated to increase centrosome number or the near tetraploid *Drosophila* S2 cell line when SAC function is inhibited (Basto et al., 2008; Yang et al., 2008; Kwon et al., 2008). When the SAC is blocked, cells divide with multipolar spindles and chromosomes segregated inefficiently. It remains uncertain whether the SAC can detect the presence of an extra centrosome or whether extra centrosomes indirectly activate the SAC by interfering with chromosome attachment. A second mechanism for clustering centrosomes is the activity of minus-end-directed microtubule motors like dynein or Ncd/HSET (Quintyne et al., 2005; Basto et al., 2008, Kwon et al., 2008). These molecular motors could either crosslink the microtubules from different centrosomes together or transport factors to the centrosome that are required for clustering (Figure 1).

Kwon et al. (2008) suggest an additional clustering mechanism from an unexpected source, the actin cytoskeleton. The investigators screened a *Drosophila* RNAi knockdown library for functional inhibition of clustering. Strikingly, they found a number of inhibitory targets in proteins associated with the actin cytoskeleton and focal adhesions (FAs), the sites of cell attachment to the extracellular matrix. Actin is a major component of the spindle, but its function there is poorly defined. However, the functions of the proteins identified in the RNAi screen of Kwon et al. suggest that the actin connection with spindle poles may be more related to actin's role at the cell cortex and FAs. When the Ncd/HSET motor was inhibited in S2 cells, tracked centrosomes could be seen to migrate apart, a motility that was blocked by LatA depolymerization of actin filaments. In aneuploid breast cancer cells, the actin linkage to FAs during interphase correlates with

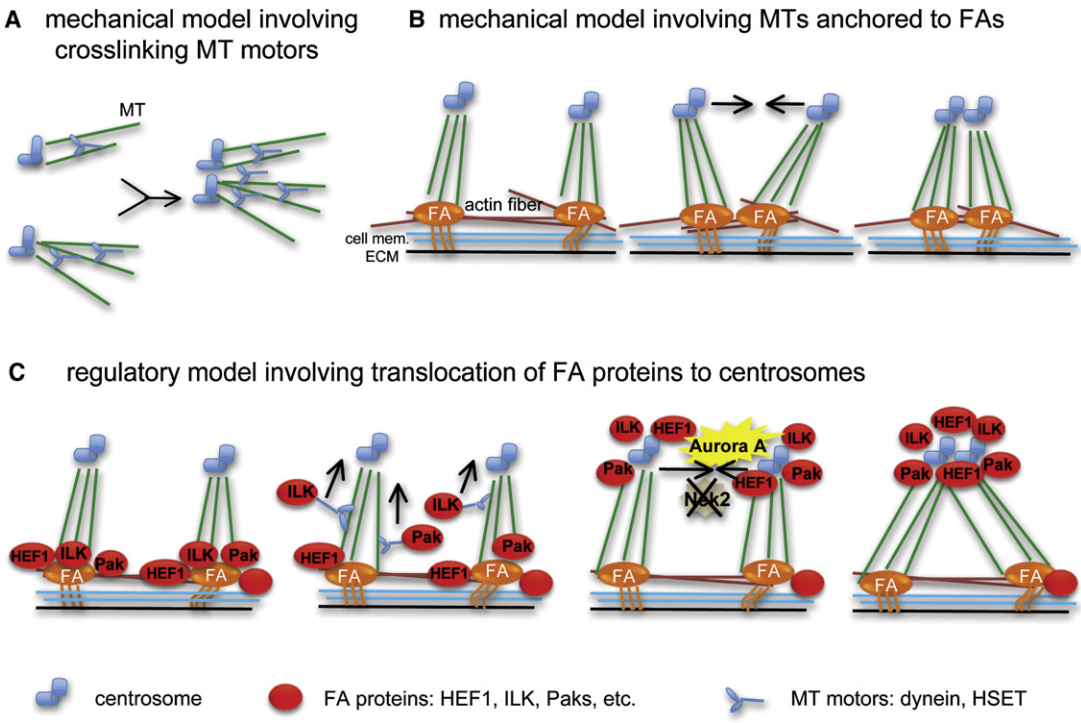

Figure 1. Three Models of Mechanisms that May Contribute to Centrosome Clustering
(A) Microtubule (MT) motors such as dynein or HSET may crosslink microtubules from adjacent centrosomes.
(B) Focal adhesions (FAs) and cortical contraction may influence centrosome position through microtubule attachments. ECM = extracellular matrix.
(C) FA proteins transported to centrosomes by molecular motors may affect clustering.

the residual adhesive retraction fibers (RFs) found in rounded mitotic cells. The positioning of RFs also correlates with the pattern of cell division during mitosis. For example, three fiber attachments to the substratum were seen in cells dividing with a tripolar spindle. Furthermore, the investigators tested the role of cell adhesion patterns and the effect of RF positioning in centrosome clustering and multipolar mitosis. They deposited the FA attachment protein fibronectin on glass coverslips in patterns that either enhanced ("Y" or "O") or inhibited ("H") the frequency of multipolar spindles. These results therefore demonstrate the influence of interphase cell shape on the faithfulness of mitosis in aneuploid cancer cells.

FAs are anchored to the actin cytoskeleton but have well-established interactions with microtubules that influence FA stability and actin polymerization (Palazzo and Gundersen, 2002). Kwon et al. (2008) demonstrated that actin inhibition from LatA treatment together with Ncd/HSET depletion has synergistic effects on spindle polarity, suggesting that actin and microtubules, or associated proteins, cooperate to inhibit multipolar mitosis. Drawing from these and previous findings, we propose the following models of mechanisms for actin, FAs, and microtubules to cluster extra centrosomes. One model suggests that microtubules serve as direct mechanical couplings to link spindle poles to FAs and actin filaments (Figure 1B). When actin fibers or FAs change their positions in the cell, centrosome positioning will also change, which may facilitate centrosome association. An alternative model follows observations that FAs are disassembled upon microtubule targeting (Palazzo and Gundersen, 2002) and that some FA proteins translocate to centrosomes via microtubule motors. We suggest that some of these transported centrosomal proteins may function to cluster centrosomes. Several FA proteins are relocated to centrosomes and may play a role in centrosome clustering, including HEF1, integrin-linked kinase (ILK), and p21-activated protein kinases (Paks). HEF1 depletion triggers centrosomal splitting, and HEF1 can inhibit the centrosomal protein Nek2 (Pugacheva and Golemis, 2005). HEF1 could promote centrosome clustering by inhibiting Nek2. Furthermore, HEF1 (Pugacheva and Golemis, 2005), ILK (Fielding et al., 2008), and Paks (Zhao et al., 2005) are required for Aurora A activity, which is required for spindle pole formation and may have an indirect effect on centrosome positioning (Figure 1C). A variety of other FA proteins have also been found in centrosomes, including zyxin, paxillin, Ajuba, and Rab11-FIP4 (Fielding et al., 2008), and may contribute to centrosome movement or linkage.

Can we use this new information to kill cancer cells while sparing normal cells? Kwon et al. (2008) tested this by examining a cadre of cancer cell lines with siRNA against the microtubule motor HSET. Diploid cells were tolerant of this knockdown, but up to 90% of total cells in populations with amplified centrosomes were inhibited by HSET knockdown, leading to senescence and apoptosis. Targeting microtubule motors involved in centrosome clustering with small-molecule inhibitors may therefore be a promising strategy for uniquely targeting dividing malignant cells.

REFERENCES

Basto, R., Brunk, K., Vinadogrova, T., Peel, N., Franz, A., Khodjakov, A., and Raff, J.W. (2008). Cell *133*, 1032–1042.

Fielding, A.B., Dobreva, I., and Dedhar, S. (2008). Cell Cycle *7*, 1899–1906.

Kwon, M., Godinho, S.A., Chandhok, N.S., Ganem, N.J., Azioune, A., Thery, M., and Pellman, D. (2008). Genes Dev. *22*, 2189–2203.

Palazzo, A.F., and Gundersen, G.G. (2002). Sci. STKE *2002*, PE31.

Pihan, G.A., Wallace, J., Zhou, Y., and Doxsey, S.J. (2003). Cancer Res. *63*, 1398–1404.

Pugacheva, E.N., and Golemis, E.A. (2005). Nat. Cell Biol. *7*, 937–946.

Quintyne, N.J., Reing, J.E., Hoffelder, D.R., Gollin, S.M., and Saunders, W.S. (2005). Science *307*, 127–129.

Stewénius, Y., Gorunova, L., Jonson, T., Larsson, N., Hoglund, M., Mandahl, N., Mertens, F., Mitelman, F., and Gisselsson, D. (2005). Proc. Natl. Acad. Sci. USA *102*, 5541–5546.

Yang, Z., Loncarek, J., Khodjakov, A., and Rieder, C.L. (2008). Nat. Cell Biol. *10*, 748–751.

Zhao, Z.S., Lim, J.P., Ng, Y.W., Lim, L., and Manser, E. (2005). Mol. Cell *20*, 237–249.