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## COMMENTARY

# Is Centrosomal Protein 70, a Centrosomal Protein with New Roles in Breast Cancer Dissemination and Metastasis, a Facilitator of Epithelial-Mesenchymal Transition?



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Microtubules are driving mechanisms of chromosomes, intracellular organelle movement, and cell shape and motility. Microtubules are organized on centrosomes, which are assembled in microtubule-organizing centers (MTOCs). In mitosis, there is no nuclear envelope and centromeres associated with microtubules are mainly involved in chromosome redistribution into daughter cells. In differentiated cells, the MTOCs are also dispersed in the cytoplasm (noncentrosomal MTOCs) and interact with the minus end of microtubules through  $\gamma$ -tubulin.<sup>1</sup> However, it is not known if the microtubule contribution to tumor biology is only by facilitating tumor aneuploidy. During tumor dissemination, a process not linked to cell division, important changes take place in cell shape and motility. Microtubules are dynamic because of their inherent structural instability, and this plasticity facilitates their reorganization during the epithelial-mesenchymal transition (EMT) induced by transforming growth factor- $\beta$ . Therefore, it is feasible that proteins controlling microtubule dynamics can also modulate processes that require changes in cell shape and motility, such as EMT, which are important for tumor dissemination.

### Cep70 Contributes to Tumor Phenotype

A report by Shi et al in this issue of *The American Journal of Pathology* brings attention to an alternative mechanism by which the centrosomal protein (Cep), Cep70, can contribute to the tumor phenotype, particularly in a subset of luminal breast cancer cases, in which Cep70 is mostly

cytosolic.<sup>2</sup> Ceps in cancer have been mainly associated with their role in the generation of tumor aneuploidy. However, the Cep family of centrosomal proteins is composed of 32 members, and for most of them, the specific cellular functions are unknown.<sup>3</sup> These proteins have been implicated in the regulation of microtubule attachment to the centromere, and therefore play an important role in mitosis and its chromosomal aberrations, such as aneuploidy. Centrosomes are present in both proliferating and resting cells, and they are essential for microtubule organization and function; centrosome amplification contributes to cellular invasion by a yet uncharacterized oncogene-like mechanism.<sup>4</sup>

Approximately more than half of luminal breast cancer cases, particularly those with lower levels of estrogen and/or progesterone receptors, have significantly higher levels of Cep70 protein. Shi et al<sup>2</sup> report that this increased Cep70 expression is because of increased *CEP70* gene expression, without gene amplification, which correlated with a higher dissemination and metastatic potential. Consistent with this observation, the overexpression of *CEP70* induces centrosome abnormalities and microtubule disorganization in pancreatic cancer.<sup>5</sup> Moreover, in breast cancer cell lines,

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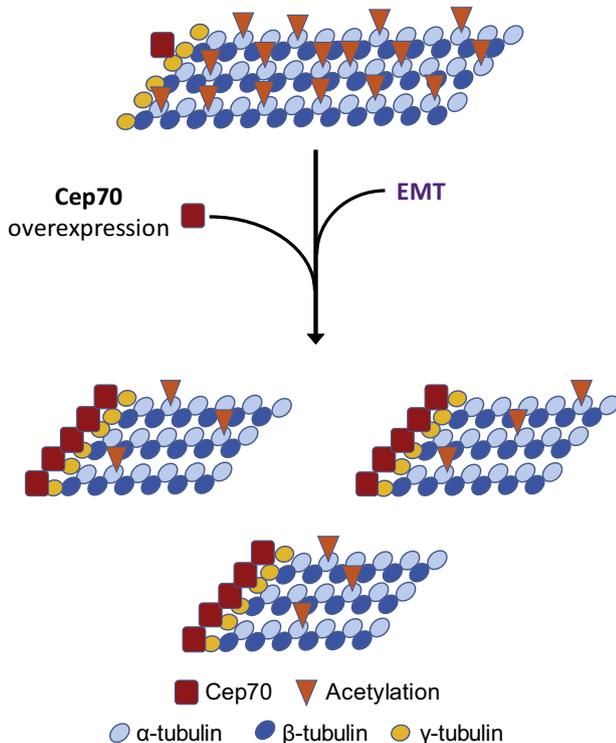
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*CEP70* overexpression also induced an increase in cell migration and metastasis in animal models.<sup>2</sup> Depletion of *Cep70* resulted in a reduction of tumor cell motility and breast cancer metastasis in lung.<sup>2</sup>

## Potential Underlying Mechanisms

The above-mentioned observations suggest a novel biological role for *Cep70* by a yet unknown mechanism. This effect of *Cep70* is likely to be a consequence of the interaction of *Cep70* with other centrosomal and microtubule proteins, which not only contribute to chromosome segregation, but also to changes in cell shape and motility, two components of the tumor phenotype. It is possible that these phenotypic effects are a consequence of facilitating the EMT by *Cep70* (Figure 1).

Microtubules are composed of tubulin subunits that are dynamically regulated, and control chromosome movement in mitosis, nuclear movement, and cell motility and shape.<sup>6–8</sup> Mechanistically, the effects of *Cep70* are a likely consequence of the role of *Cep70* on microtubule dynamics.<sup>5</sup> The alteration of microtubule assembly and disassembly can have an effect on cell shape and motility, and *Cep70* is a new component participating in EMT. This



**Figure 1** Effect of centrosomal protein (*Cep* 70) on microtubule organization. *Cep70* overexpression and epithelial-mesenchymal transition (EMT) induce a disorganization of microtubules by causing a variation in their number and size (increases and decreases), as well as an altered pattern of covalent modifications. These dynamic microtubule alterations contribute to generation of aneuploidy by irregular distribution of chromosomes in daughter cells and to increased cell migration and formation of microtentacles that facilitate tumor cell dissemination and metastasis.

remodeling will facilitate both dissemination and formation of metastasis. In this context, *Cep70* interacts with  $\gamma$ -tubulin and is critical for mitotic spindle assembly.<sup>9</sup> But the recent study of *Cep70* on other aspects of the tumor phenotype reveals its novel contributions in cancer.<sup>2</sup> A functional regulatory link is likely to be a consequence of the effect of *Cep70* on microtubule dynamics and covalent modifications. *Cep70* binds to the ring of  $\gamma$ -tubulin protein at the minus end of microtubules, which interacts with centrosomes that play a fundamental role in the structural organization of cell shape and motility. Tumor aneuploidy or abnormal increase in chromosome numbers is a characteristic of tumor cells whose underlying mechanisms are not well known. In this context, the role played by proteins involved in different steps of chromosome segregation is likely to be important in the pathogenic mechanism. Proteins involved in aneuploidy have, in general, received limited attention in oncology. In this context, proteins interacting with centrosomes and microtubules are likely candidates to play a major regulatory role, because centrosomal aberrations have been associated with aneuploidy and cancer progression.<sup>10</sup>

In centrosomes, *Cep70* binds to the minus end of microtubules and promotes their assembly by increasing elongation, but not nucleation, which occurs at the plus end.<sup>11</sup> *Cep70* has been shown to increase the number of microtubules.<sup>5</sup> Therefore, proteins interacting with microtubules can alter the balance between these two processes. The minus end of microtubules interacts with MTOCs and noncentrosomal MTOCs, which also bind to  $\gamma$ -tubulin rings and are regulated by interactions with additional proteins that stabilize microtubules, as in the case of calmodulin-regulated spectrin associated protein (CAMSAP).<sup>1</sup> Alternatively, other proteins, such as ninein, can contribute to anchor new microtubules.<sup>1</sup> Moreover, *Cep70* has a long-range effect on microtubule fibers affecting the  $\alpha/\beta$  tubulin dimer, and this may contribute to its effect on elongation at the plus end. However, the mechanisms behind dynamically complex processes remain largely unknown. The *Cep70* long-range effect is likely to be indirect on additional proteins or their covalent modifications, and might also require conformational changes in the microtubule fiber.

Microtubule fibers are regulated by several covalent modifications of tubulin. In microtubules,  $\alpha$ -tubulin acetylated in lysine 40<sup>12</sup> is deacetylated by two deacetylases, the NAD-dependent Sirt2 and the NAD-independent histone deacetylase 6 (HDAC6). These two deacetylases have overlapping and distinct roles on tubulin acetylation and its distribution on the fiber. The loss of  $\alpha$ -tubulin K40-acetylation is associated with the promotion of EMT induced by transforming growth factor- $\beta$ .<sup>13</sup> Inhibition of deacetylase activity can alter the acetylation pattern, but how this local effect at the minus end is transmitted to the fiber remains unknown. The inactivation of Sirt2 facilitates the K40 hyperacetylation of tubulin in a subset of

perinuclear microtubules, which are also not accessible to HDAC6,<sup>14</sup> and causes a local alteration in the acetylation pattern of the microtubule fiber. Therefore, these variations in the acetylation pattern are likely to affect microtubule interactions with other proteins that affect either microtubule structure and dynamics, their protein associations, or their functions. Microtubule post-translational modifications might control MTOC and noncentrosomal MTOC locations.<sup>1</sup> Cep70 can also regulate the stability of microtubules by its interaction with HDAC6 in the cytoplasm and increasing tubulin acetylation.<sup>15</sup> In support of this role for microtubule acetylation, variation in the level of tubulin acetylation modifies the migration properties of neural cells.<sup>16</sup> In this context, it is important to know how Cep70 levels alter the acetylation pattern of microtubules and of their associated proteins in tumor cells. Other proteins, such as coiled-coil protein associated with myosin II and DISC1 (CAMDI), also interact in microtubules, with HDAC6 inhibiting its activity and resulting in increased tubulin acetylation and neural cell motility.<sup>16</sup> Thus, an altered tubulin acetylation pattern might contribute to cell migration. Moreover, Cep70 might also contribute to cell migration by facilitating tubulin detyrosylation, which is a necessary step to facilitate EMT and promote endothelial engagement.<sup>17</sup>

Cep70 also contributes to angiogenesis that also requires cellular migration and reorganization of vascular endothelial cells.<sup>18</sup> Cep70 is important for cdc42 and Rac1 activation,<sup>18</sup> and both proteins are regulators of cell motility and angiogenesis<sup>19</sup>; regulation of small GTPases by acetylation of specific guanine nucleotide exchange factors contributes to cell motility.<sup>20</sup> Consistent with this role on motility, depletion of Cep70 in breast cancer cell lines resulted in a defective cell migration.<sup>2</sup> Moreover, the altered stability of microtubules also facilitates the formation of microtentacles in breast cancer circulating cells and enhances their metastatic potential.<sup>21</sup>

## Cep70 in Cancer Cell Biology

To understand the complex role of Cep70 in cancer cell biology, it will be necessary to understand its regulation and the signals that impinge on it, directly or indirectly, as well as the interplay among the different proteins that bind and form a complex at the minus end on the  $\gamma$ -tubulin ring. This may function by acting on microtubule ends by regulating their assembly and disassembly, as well as by their effect on the  $\alpha/\beta$  tubulin subunits in the fiber, which undergo covalent modifications and interactions with a large number of proteins. Cep76, another centrosomal protein, is regulated by phosphorylation and acetylation.<sup>22</sup> Therefore, it is likely that similar covalent modifications will be affected by Cep70, which might also be phosphorylated, and contribute to the coordination of its roles in different biological processes.

The findings in this report<sup>2</sup> open up several questions regarding the role of Cep70 in cancer. Is this effect of Cep70 on cell migration also common to erbB2 or triple-negative breast cancer cases, or to other types of carcinomas? How are mitogenic and cell adhesion signals connected to *CEP70* gene expression? Does Cep70, by modulating tubulin covalent modifications, alter microtubule functions? What is the functional link between Cep70 and EMT induced by transforming growth factor- $\beta$ ? Can this role of Cep70 on cellular motility be disrupted by pharmacological intervention, and thus be of potential use as a target with the aim of preventing tumor dissemination?

## Cep70 as a Therapeutic Target

Oncologic treatments targeting microtubules can likely function, not only by blocking chromosome segregation in mitotic cells, but also by interfering with cell movement and migration, and thus reducing the likelihood of dissemination and metastasis formation. The role played by Cep70 makes it a potential therapeutic target, with the advantage that it is located in a more restricted and localized position compared with the number and size of microtubules, which constitute a much more dispersed intracellular target.

## Conclusion

A better understanding of the minus end of microtubules and its pharmacological manipulation can open up new therapeutic opportunities to control or prevent tumor dissemination.

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