Abstract. The centrosome is a small organelle located near the nucleus which acts as the microtubule organizing center for the cell. Abnormalities in centrosome replication resulting in centrosome amplification have been identified in most of the common human cancers and implicated in the development of genetic instability and cancer. Research in centrosomes is evolving rapidly with identification of key regulatory proteins. This knowledge could help to explain the mechanisms of action of existing cancer treatments and also lead to the development of new molecularly-targeted therapy.

The centrosome is a small organelle located near the nucleus. In eukaryotic cells it is composed of a pair of cylindrical centrioles positioned perpendicular to each other. The infrastructure of each centriole is made up of nine triplet microtubules. α and β tubulin protein comprise the subunits of the microtubule fiber (17). Additionally, the centrioles are surrounded by an electron dense amorphous pericentriolar material (PCM) which contains many proteins, some of which have been identified, including γ-tubulin and pericentrin (10, 39).

The main function of the centrosome is to act as the microtubule organizing center (MTOC) for the cell. In this role it establishes polarity and orientation of microtubules during interphase. Then, during cell division, the centrosome nucleates the growth of microtubules and directs the assembly of the mitotic spindle. The centrosome also seems to play a crucial role in cytokinesis. Microsurgical ablation of centrosomes in BSC-1 African green monkey kidney cells caused a significant rate of cytokinesis defects (31).

The number of centrosomes and their method of replication are critical issues. The exact mechanism of centrosome replication is not well understood. However, it is clear that normally the centrosome divides only once per cell cycle. If the centrosome does not replicate, this results in a monopolar spindle and the cell cannot divide. If the centrosome were to duplicate multiple times within a single cell cycle, this would result in centrosome amplification and possible formation of multipolar spindles. D’Assoro et al. defined centrosome amplification as when centrosomes appear significantly larger than normal, contain more than four centrioles, and/or when more than two centrosomes are present within a cell (9). The hypothesized mechanisms for cells to possess an excess number of centrosomes include: a) multiple duplication of centrosomes within a single cell cycle; b) failure to undergo cytokinesis; c) uncontrolled separation of centriole pairs; and d) overexpression of certain PCM components resulting in acentriolar centrosomes (16). Another possibility is fragmentation of the centrosome into smaller ectopic functional fragments (4).

Centrosomes have recently generated much interest with researchers linking it to cancer (3, 9, 11, 27, 29). However, this is certainly not a new concept having been first suggested by Theodor Boveri nearly a century ago (2). He had the foresight to hypothesize that the malfunction of centrosomes could result in chromosome missegregation, leading to malignant tumors. Here we review recent findings regarding centrosomes and cancer and discuss the potential implications for therapy.

Molecular biology of centrosomes

The recent interest in centrosomes has largely been driven by the development of molecular biology techniques and identification of key proteins involved in the regulation of
centrosome duplication. Although not completely understood, it is apparent that a complex balance of enzymes control centrosome assembly and function.

p53. It has long been known that p53, a tumor suppressor gene frequently mutated in human cancers, physically associates with centrosomes (5). However, Fukasawa et al. made a major breakthrough discovery in centrosome cancer research when they found that loss of p53 resulted in abnormal amplification of centrosomes. Immunostaining of p53-deficient mouse embryonic fibroblasts with anti-γ-tubulin revealed more than 30% of the cells contained in excess of two centrosomes during interphase, and more than 50% contained multipolar spindles during mitosis (14). The same group later corroborated their results in vivo by analyzing cells from organs of p53 deficient young mice. Again it was observed that 20-30% of interphase cells contained multiple centrosomes. Also frequent genetic instability in the form of aneuploidy and amplification of the c-myc proto-oncogene, DHFR, and CAD genes were seen (15). The next step was human analyses of p53 in advanced stage squamous cell carcinomas of the head and neck which showed a correlation between centrosome amplification and p53 mutations. Five of twelve tumors from patients were identified as having p53 mutations by testing for direct sequences or loss-of-heterozygosity, and all five of these tumors also showed centrosome amplification (7). Altogether this data implies that centrosome amplification is one of the mechanisms behind the chromosome instability caused by the p53 mutation. It is yet to be established exactly how centrosomes become amplified in p53-deficient cells. However, it is known that one of p53’s targets downstream is p21 which in turn interacts with Cdk2-E (40).

Cdk2-E. In order to study the individual components necessary for centrosome reproduction, an in vitro assay was developed in which S-phase-arrested frog egg extract supported repeated centrosome reproduction, free of the normal cell cycle blocks (19). p21 and p27 are known to be natural inhibitors of Cdk2 (13). After selective inhibition of Cdk2-E activity with a recombinant variant of p27, time-lapse video microscopy showed centrosome reproduction was significantly decreased. Addition of purified Cdk2-E reversed this blockage (19). During the same time period Lacey et al. independently investigated Cdk2-E and reported similar results. In addition they developed an in vitro assay in which paired centrioles could be observed to separate within the centrosome. However, when incubated with p21, centriole separation was inhibited (25). Both of these studies support the idea that Cdk2-E is required for centrosome duplication. Since Cdk2-E is also a key enzyme governing cell cycle progression, this probably provides a way for these two processes to be synchronized, and gives a suggestion of how cells ensure that centrosomes replicate only once during the cell cycle.

Aurora A. Aurora A is another important kinase involved in centrosome regulation. It has been the subject of several recent reviews (6, 41). The gene was originally identified in the fruit fly Drosophila melanogaster because its mutation in the fly cells caused disruption in mitosis by preventing centrosome separation (18). The human analogue’s name is now generally accepted as aurora A, but in the past the nomenclature was somewhat confusing because it was also called aurora-2, aik1, btak, stk15, or ark1. Aurora A is a member of a family of serine/threonine kinases, which are thought to be key regulators of centrosome duplication, chromosome segregation and cytokinesis. Several years ago it was postulated that aurora A kinase had oncogenic properties because it was found to be overexpressed in breast cancer cell lines, and mapped to chromosome 20q13, a region frequently amplified in cancers (38). In support of this concept, overexpression of aurora A in cell culture studies induced centrosome amplification, aneuploidy and transformation (43). Again the mechanism of exactly how aurora A overexpression causes centrosome amplification is unknown. However, a study by Meraldi et al. indicated that overexpression of aurora A was not directly related to excessive centrosome duplication; instead, it was due to a defect in cell division and consequent formation of tetraploidization. Also, absence of p53 exacerbated the generation of extra centrosomes, showing a possible link between these two proteins (30).

BRCA1. Another connection between centrosome abnormalities and tumorigenesis is the finding that breast cancer suppressor gene 1 (BRCA1) is involved in centrosome regulation. The BRCA1 mutation is found in a large percentage of familial breast and ovarian cancers (21). The function of BRCA1 has been difficult to determine because it interacts with many proteins that play important roles in multiple biological pathways. An important initial observation was that BRCA1 interacts with γ-tubulin and associates with the centrosome during mitosis (20). Then investigators deleted part of the BRCA1 gene in mouse embryonic fibroblast cells causing 25% of these cells to contain more than 2 centrosomes (42). These findings seem to echo the earlier p53 results.

It seems that several classes of centrosome regulators are developing. The tumor suppressor genes p53 and BRCA1 are negative regulators, since their absence results in centrosome amplification. Then there are basic enzymes such as Cdk2-E which must be present for centrosome duplication to occur. Their absence results in blockage of centrosome duplication. Finally there are positive regulators such as aurora A whose overexpression causes centrosome amplification.
Association with cancer

Aneuploidy, the gain or loss of one or more chromosomes, is a common feature of most human cancers and it is suspected to be an early event in tumorigenesis. With newfound appreciation for the importance of centrosomes, investigators using immunostaining techniques and electron microscopy have detected abnormal centrosomes in a large number of tumor types. Lingle et al. examined specimens from 35 high-grade human breast tumors and observed that the centrosomes displayed structural abnormalities including increase in centrosome number and volume, accumulation of excess pericentriolar material, supernumerary centrioles and inappropriate phosphorylation of centrosomal proteins. Quantitative analysis showed that normal cells contained a mean number of 1.5 centrioles per cell. In contrast tumor cells contained a mean number of 4.3 centrioles per cell. In addition the centrosomes in the breast tumor cells showed functional abnormalities characterized by increased microtubule nucleating capacity. Pihan et al. examined tumor-derived cell lines, as well as tissue obtained from biopsies of malignant tumors of the breast, prostate, lung, colon and brain. Using pericentrin antibody, centrosome defects were observed in 81 out of 87 tumors and 8 out of 8 tumor-derived cell lines. The percentage of cells with abnormal centrosomes ranged from 9% to 67% in the different tumor-derived cell lines. Centrosome abnormalities have also been identified in carcinomas of the head and neck, pancreas, gallbladder and bile ducts, bladder and adrenocortical tumors. In these reports there is a wide range in the observed percentage of cells with centrosomal abnormalities. Probably multifactorial reasons account for this difference including inherent heterogeneity of the tumor types and between patients, and also variation between investigator’s criteria for what is considered an abnormal centrosome. Some have more strict criteria reporting only the percentage of cells with greater than two centrosomes, while others are also including cells with alterations in centrosome size and shape.

Lingle et al. analyzed 20 invasive breast tumors by fluorescence in situ hybridization with centromeric probes in order to measure chromosome instability. They found a significant positive linear correlation between centrosome size and number with aneuploidy and chromosomal instability. A simple carcinogenesis model would be one in which inappropriate levels of enzyme activity causes centrosome defects, leading to the assembly of aberrant mitotic spindles and missegregation of chromosomes. This genetic instability could result in gains and losses of genes that confer tumorigenic potential. However, this theory is still unproven, and others have proposed that the order is actually reversed where aneuploidy comes first and causes centrosome abnormalities rather than vice versa. One argument for centrosome defects being the initial event is that they have been observed in a significant fraction of pre-invasive in situ carcinomas of the cervix, prostate and breast indicating that this phenomenon occurs during the earliest stages of cancer development.

Centrosomes and cancer therapy

For some cancer treatments that are already commonly in use, new evidence is revealing that their underlying mechanisms of action may involve centrosomes. For example, this is the case for radiotherapy, a mainstay of cancer treatment. Sato et al. examined the centrosomes in a human osteosarcoma cell line using immunofluorescence microscopy with an antibody to γ-tubulin before and after exposure to 10 Gy radiation. Only 1.3% of untreated cells contained more than two centrosomes but, 72 hours after irradiation, about 60% of the cells contained multiple centrosomes. These cells also frequently displayed multipolar spindles and nuclear fragmentation. The authors concluded that abnormal centrosome function could be one of the mechanisms involved in radiation-induced cell death. It should be noted, however, that 10 Gy radiation given in a single high dose is not typically used clinically in human patients. Therefore extrapolation from their results to doses used in therapeutic radiotherapy is uncertain.

Sato et al. later corroborated their results in 9 other tumor-derived cell lines. In untreated cells, a mean of 12.0% cells contained multiple centrosomes compared to a mean of 37.2% of cells postirradiation. Furthermore, infection of cells with a recombinant adenovirus causing overexpression of p21 inhibited postirradiation centrosome amplification, and these cells were protected from radiation-induced cell death. These results suggest that radiation does not act directly on the centrosomes but instead on the regulatory enzymes which control centrosome duplication.

Based on these findings we suggest a threshold level hypothesis in which centrosome abnormalities may lead to carcinogenesis or cell death, depending on the degree of severity. If centrosome damage is at a low level, daughter cells are still capable of sequestering their multiple centrosomes into two functional poles and remain viable. The presence of multiple centrosomes increases the rate of genetic instability promoting tumor formation. On the other hand, if centrosome damage is severe as is the case after irradiation with high doses, this leads to a catastrophic event and eventually to apoptosis or another form of cell death.

The antitumor drug Taxol is another cancer treatment in widespread use which seems to impair centrosome function. Abal et al. treated leukemia and ovarian cancer cell lines with a fluorescent derivative of Taxol to investigate its binding site. Instead of uniformly labeling the microtubule cytoskeleton, the specific binding sites were found to be the...
centrosome and the spindle pole microtubules. Abnormal mitotic features such as monopolar and multipolar spindles were frequently observed in cells treated with the fluorescent taxoid. These cells then developed apoptosis and cell death. The author concluded from the results that taxoid binding to centrosome caused impairment of its duplication or separation during interphase and was a primary cause for cell cycle disruption and taxoid-induced cell death.

As we develop further understanding of carcinogenesis and the regulatory enzymes involved, there is potential for the development of new, effective, molecule-targeted cancer therapy, for example an inhibitor against aurora A (41). Kimura et al. cloned the gene Aik which has a high homology with the aurora family of protein kinases, and examined its expression in various human tissues using Northern and Western blotting analysis (23). Aik was predominantly expressed in tissues with proliferating cells. Thus, its expression was very high in testis and weak in skeletal muscle, thymus and spleen. Also expression was high in the HeLa cancer cell line. This suggests that aurora A activity is, for the most part, elevated in tumor cells compared to normal cells and there is potential for its selective inhibition. Cheetham et al. studied the crystal structure of aurora A and discovered pockets in its ATP-binding site and a unique conformation of the activation loop, which could be exploited to design a selective inhibitor (8).

Conclusion

Advances in molecular biology have recently shed light on the key proteins regulating centrosome replication and function. This has revived interest in Boveri’s hypothesis that centrosome abnormalities may lead to the development of malignant tumors. An association has been established since investigators have discovered centrosome amplification to be a common finding in human cancers. Yet further work must be done before causality can be proven. Also research into centrosomes is unearthing tantalizing clues that this inconspicuous organelle may play an important role in the mechanism of action for cell death in existing cancer treatments such as radiotherapy and Taxol. Future investigation into centrosomes could prove to be beneficial in many ways. Measuring the degree of centrosome amplification could be used to predict prognosis or response to therapies such as radiation. There is also potential for the development of new cancer therapies which target the centrosome or its regulatory proteins.

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References


