Review

Chromosome instability and kinetochore dysfunction

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Summary. Chromosomal instability (CIN) has been recognized as a hallmark of human cancer and is caused by continuous chromosome missegregation during mitosis. Proper chromosome segregation requires a physical connection between spindle microtubules and centromeric DNA and this attachment occurs at proteinaceous structures called kinetochore. Thus, defect in kinetochore function is a candidate source for CIN and the generation of aneuploidy. Recently, a number of kinetochore components have been shown to be mutated and/or aberrantly expressed in human cancers, which suggests an important role of kinetochore for CIN and carcinogenesis. In this article, we will discuss about how kinetochore dysfunction causes CIN and might lead to the development of cancer.

Key words: Chromosonal instability, Aneuploidy, Kinetochore, Cancer

Introduction

Aneuploidy in tumour cells was first described almost a century ago and, since then, mitotic defects have been thought to be a direct cause of cancer (Hausemann, 1890). This aneuploidy was shown to occur due to the accelerated rate of gains or losses of whole or large portions of chromosomes, termed CIN, as the result of chromosome missegregation (Lengauer et al., 1998). Recently, the mechanism of proper mitotic processes has been unraveled and the aberrant function of factors involved in the equal chromosome segregation has been reported in various cancers, which suggests the important role of the factors for carcinogenesis. Among them, the centromere and kinetochore have a pivotal role in accurate chromosome segregation. Kinetochores are

formed on the centromeres in mitosis, when spindle microtubules attach to them to generate the physical forces necessary for chromosome movements, and they are also required for spindle checkpoint regulation. In this review, the recent progresses for understanding the involvement of mitotic defect, especially the aberrant kintochore function, for carcinogenesis will be discussed.

Chromosome instability and cancer

Mitotic defects in tumor cells were first described by David Hansemann more than a century ago (Hausemann, 1890). Subsequently, Theodor Boveri postulated that aneuploidy is a direct cause of cancer (Boveri, 1914). This hypothesis, however, was unappreciated because all current thinking about the cause of cancer is dependent on the somatic gene mutation hypothesis, which argued that tumors arise by activation of oncogenes and/or inactivation of tumor suppressor genes (Bishop, 1987). In recent years, several observations, described below, breathed new life into the old hypothesis of CIN and aneuploidy. First, 85% of colorectal cancers, and an even larger proportion of solid tumors, contain an abnormal chromosomal content (Lengauer et al., 1997, 1998; Rajagopalan et al., 2003; Rajagopalan and Lengauer, 2004). Second, experimentally transformed Chinese hamster cells showed 100% aneuploidy (Li et al., 1997). Third, aneuploidy occurs early in the development of cancer, which indicates that aneuploidy is an important step in the initiation and/or progression of cancer (Shih et al., 2001). One advantage that CIN contribute to tumorigenesis is that it could accelerate the rate of loss of heterozygosity (LOH) of a tumour suppressor gene and/or effectively amplify an oncogene by duplicating the chromosome. Inactivation of both alleles of a tumour suppressor gene must occur for a cell to acquire a growth advantage, thus, accelerated LOH is an apparent mechanism by which CIN can contribute significantly to the inactivation of a tumour suppressor gene. Furthermore, alteration of gene expression caused by CIN can also increase cell proliferation or decrease cell

death, essential processes for tumor development.

Mitotic defects and chromosomal instability

Chromosome missegregation during mitosis has been recognized as a main cause of CIN in cancers. There are many potential mitotic targets, such as chromosome condensation, sister-chromatid cohesion, kinetochore structure and function, centrosome/microtubule formation and dynamics, as well as "checkpoint" genes that monitor the proper progression of the cell cycle, all of which are indispensable for the proper segregation of chromosomes. Among them, a centrosome-associated kinase STK15/BTAK/auroraA is the first candidate gene responsible for CIN. It was amplified in multiple human tumor cell lines or primary cancers, and exogenous expression of the kinase in rodent and human cells induced unequal partitioning of chromosomes during mitosis and tumorigenic transformation of cells (Bischoff et al., 1998; Zhou et al., 1998). Human securin that prevents premature chromosome separation is also overexpressed in some tumors and exhibits transforming activity in NIH3T3 cells (Zou et al., 1999). Amplification of cyclin E and mutation of *hCDC4*, both previously implicated in G1-S phase transitions, have been identified in aneuploid cancers, and inactivation of hCDC4 in karyotypically stable colorectal cancer cells caused CIN (Rajagopalan

et al., 2004). Whether the defects in CIN genes are the direct cause for carcinogenesis still remains to be elucidated.

Kinetochore dysfunction and chromosomal instability

The centromeric DNA and kinetochore, essential for microtubule spindle attachments and the function of the mitotic checkpoint, are the most important elements required for equal sister-chromatids separation. The normal function of the spindle checkpoint is to ensure that all chromosomes are correctly aligned in metaphase cells and properly attached to the mitotic spindle before chromosome separation can proceed. Several types of cancer revealed mutations in either *hBuB1* or *hBuBR1* checkpoint genes (Cahill et al., 1998; Gemma et al., 2000; Ohshima et al., 2000; Ru et al., 2002; Shichiri et al., 2002) and germ line biallelic mutations in *hBuBR1* gene are associated with inherited predispositions to cancer (Hanks et al., 2004). Another spindle checkpoint component, *hMAD2*, is mutated in gastric cancers (Kim et al., 2005), and down regulated in cancer cell lines (Li and Benezra, 1996; Wang et al., 2000, 2002). Heterozygous *MAD2* mice develop lung tumors at high rates, suggesting that biallelic expression of *Mad2* is important for its function (Michel et al., 2001). In contrast, *hMad2* is overexpressed in retinoblastoma,
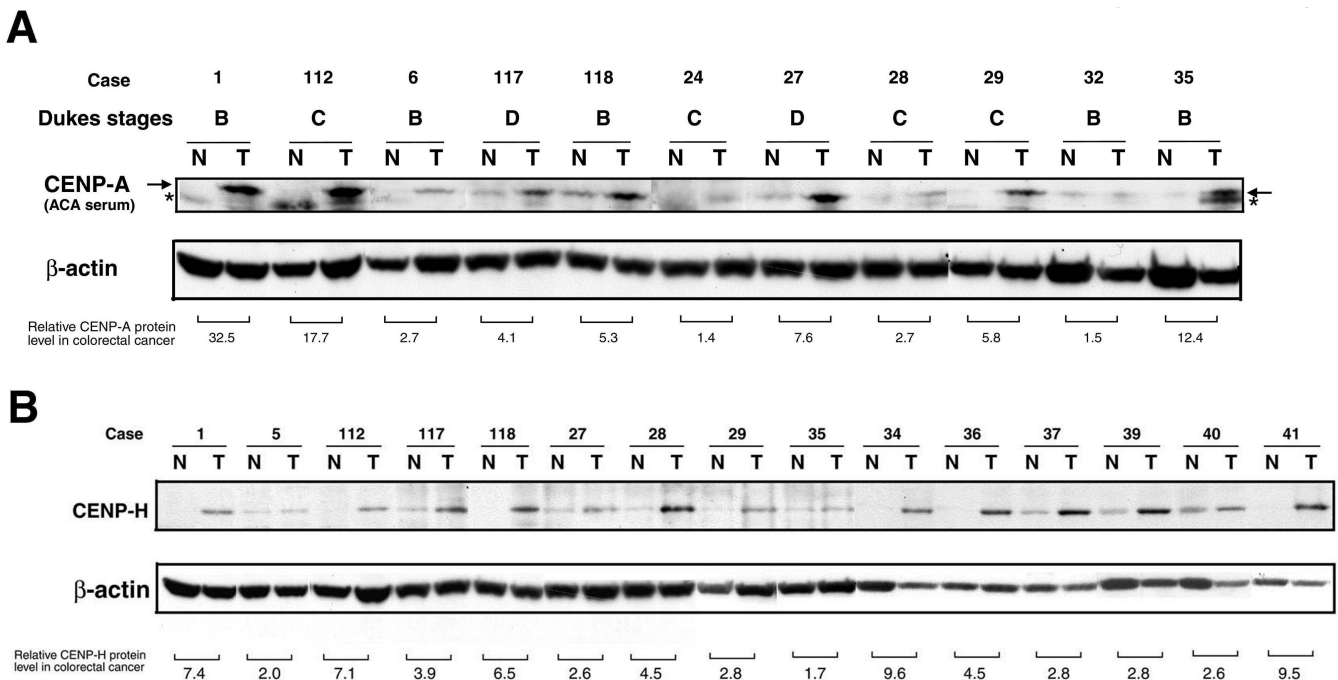

Fig. 1. CENP-A and CENP-H proteins were overexpressed in primary colorectal cancer tissues. Total protein lysate was prepared from matched samples of tumor (T) and adjacent normal tissue (N). Equal amounts of protein from each pair were resolved on 10-20% gradient polyacrylamide gel and immunoblotted with anti centromere (ACA) serum **(A)**, anti-CENP-H **(B)** antibodies. Immunoblot with anti-ß-actin is the loading control. The intensity of each band was measured with NIH Image and the relative CENP-A or CENP-H protein levels between tumor and normal tissue normalized with ß-actin were calculated.

A

	FISH	Immunostaining CENP-H
CEP8	a	b
CEP12	c	d

B
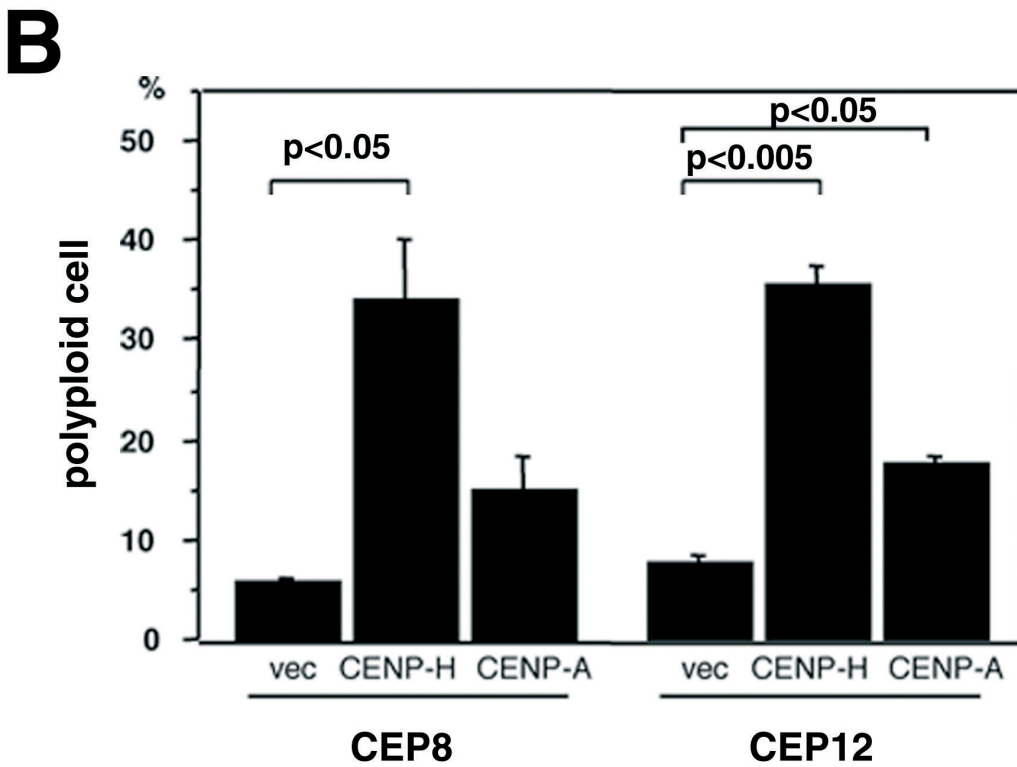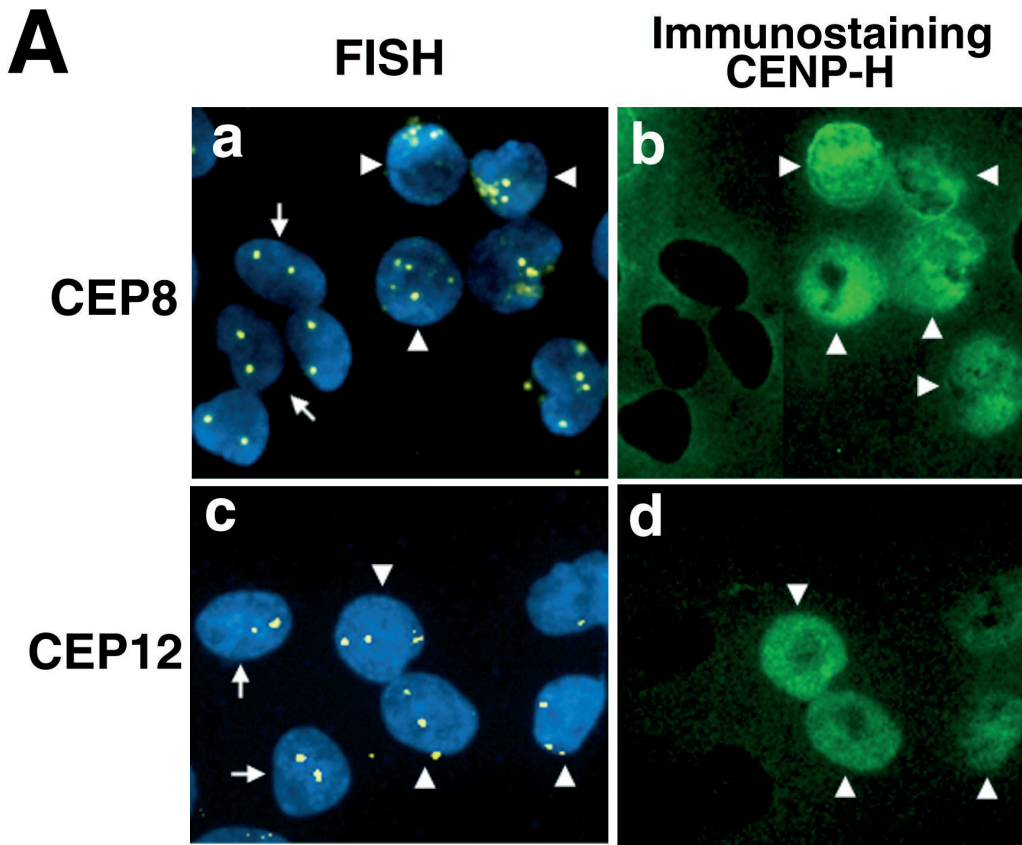
neuroblastoma, bladder carcinomas and the aberrant expression of hMad2 as a result of Rb pathway inactivation produces mitotic defects leading to aneuploidy (Hernando et al., 2004). Recently, the gene product of *adenomatous polyposis coli* (*APC*), the most frequently mutated gene in colorectal tumors, has been observed at the plus ends of kinetochore microtubules. Mutations in *APC*, similar to the mutations found in tumor cells, interfere with microtubule plus-end attachments and result in mitotic abnormalities (Kaplan et al., 2001; Fodde et al., 2001; Green and Kaplan, 2003). Furthermore, analysis of human homologs of CIN genes identified in yeast and flies found mutations in kinetochore/spindle checkpoint proteins, hRod, hZw10 and hZwilch, which account for ~2% of colorectal cancers (Wang et al., 2004). These observations implicate the mitotic spindle checkpoint as the point of failure in CIN.

The mounting evidence above suggested that mutations in mitotic checkpoint proteins might induce CIN, although such mutations have only rarely been found in human cancers. Mutations in structural kinetochore proteins have not yet been identified in cancer, whereas aberrant expression of the proteins has
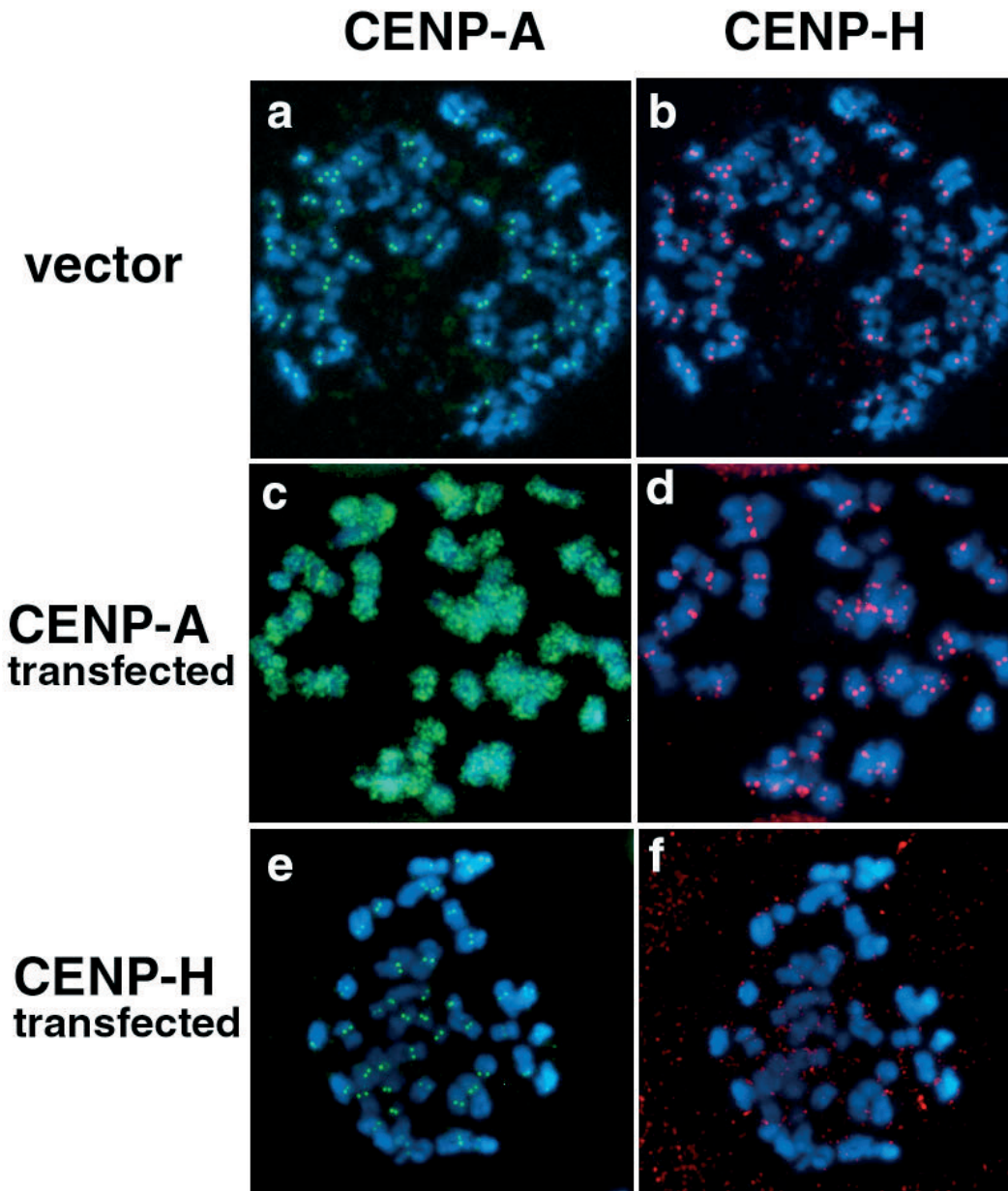

Fig. 3. Aberrant localization of centromere proteins in mitotic chromosomes of HCT116 cells overexpressing CENP-A and CENP-H. Mitotic chromosome spreads were prepared 48hrs after transfection of the CENP-A or CENP-H expression plasmid. Both CENP-A and CENP-H localized to the centromeres in the control cells (a,b). In cells overexpressing CENP-A, it localized to the entire chromosome, whereas CENP-H localized to the centromeres (c,d). In contrast, CENP-H disappeared from the centromeres of metaphase chromosomes prepared from cells overexpressing CENP-H, while CENP-A remained at the centromeres in those metaphase chromosomes (e,f).

been reported in various cancers. CENP-A, a histone H3-like protein that serves as both structural and functional foundations for the kinetochore, is overexpressed and mistargeted in colorectal cancer tissues (Fig. 1A) (Tomonaga et al., 2003). Overexpressed CENP-A localized to the entire chromosome and recruited a subset of kinetochore proteins to non-centromeric chromatin, suggesting a potential link between CENP-A mislocalization and CIN in cancer (Fig. 3) (Van Hooser et al., 2001; Tomonaga et al., 2003; Heun et al., 2006). Overexpression of Drosophila CENP-A also mislocalizes to non-centromeric chromatin and promotes formation of ectopic centromeres and multicentric chromosomes, which causes chromosome missegregation, aneuploidy, and growth defects (Heun et al., 2006). Thus, CENP-A mislocalization is one possible mechanism for CIN during cancer progression, as well as centromere plasticity during evolution. Another inner kinetochore protein, CENP-H, which is important for kinetochore organization, is also upregulated in colorectal cancer tissue (Fig. 1B) (Tomonaga et al., 2005). Transfection of a CENP-H expression plasmid into either diploid colorectal cancer cells or normal mouse fibroblasts induces aberrant mitosis, suggesting that upregulation of CENP-H can lead to a CIN phenotype (Fig. 2) (Tomonaga et al., 2005). In the CENP-H overexpressed cells, in great contrast to CENP-A overexpressed cells, CENP-H completely disappeared from the centromere of mitotic chromosomes, possibly by the disturbance of the stoichiometry of the kinetochore components (Fig. 3). Aurora-B (AIM-1) and INCENP, two chromosome passenger proteins that localize to the kinetochore from prophase to metaphase, are upregulated in tumor cell lines (Tatsuka et al., 1998; Adams et al., 2001; Sorrentino et al., 2005). Aurora-B-overexpressing cells exhibit CIN and injection of these cells into nude mice induces tumor formation (Ota et al., 2002). Conversely, inhibition of Aurora-B expression reduces the proliferation rate of thyroid cancer cells, suggesting the association between Aurora-B expression and cancer initiation and/or progression (Sorrentino et al., 2005). Overexpression of another kinetochore protein CENP-F has been reported to correlate with tumor proliferation and metastasis, although the precise function of CENP-F has not been understood (Clark et al., 1997; Liu et al., 1998; Erlanson et al., 1999; de la Guardia et al., 2001; Esguerra et al., 2004; Shigeishi et al., 2005). The evidence above suggests that overexpression of kinetochore proteins may induce CIN by disrupting the stoichiometry of the multi-protein kinetochore complex and contribute to tumor development. Further investigations are needed to clarify whether aberrant expression of kinetochore proteins is directly involved in carcinogenesis.

Clinical applications

Although the relative contribution of gene mutations vs. CIN in human cancers is still a matter of debate, CIN could be useful for clinical application. Aneuploid and/or chromosomally unstable cancers are likely to have a poorer prognosis than diploid cancers and the degree of aneuploidy correlates with the severity of the disease (Watanabe et al., 2001; Zhou et al., 2002). On the basis of the study of 104 breast carcinomas, aneuploid tumors showed higher malignancy potential than diploid tumors and subtype of aneuploidy improved grading of breast cancer (Kronenwett et al., 2004). The difference in rate of tumor progression between aneuploid and diploid tumors most likely reflects differences in rate of mutation due to chromosomal instability. Therefore, examining the ploidy of tumors may enable novel and powerful prognostic indicators for cancers. Recent reports demonstrated that CIN could also contribute to a cancer's ability to acquire chemoresistance (Sawyers, 2001; Wang et al., 2004). CIN might increase the rate of Darwinian adaptation to changing intracellular and extracellular environments. In this way, CIN is thought to contribute to cellular resistance to chemotherapeutic drugs such as imatinib or 5-FU. Finally, mutation or upregulation of CIN genes, such as kinetochore/spindle checkpoint genes, might allow selective killing of tumor cells by applying genetic approaches to the discovery of new anticancer drugs (Hartwell et al., 1997). Yeast genetics has been used to identify pairs of non-allelic gene mutants that are each individually viable, but lethal in combination. If the synthetic lethal interactions are conserved in humans, then the synthetic lethal interactors that are common to CIN mutants may be potential targets for anticancer drugs targets (Yuen et al., 2005).

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