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The kinetochore and cancer: what's the connection?

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The molecular mechanisms ensuring accurate chromosome segregation during meiosis and mitosis are critical to the conservation of euploidy (normal chromosome number) in eukaryotic cells. A dysfunctional kinetochore represents one possible source for chromosome instability (CIN) and the generation of aneuploidy. The kinetochore is a large complex of proteins and associated centromeric DNA that is responsible for mediating the segregation of sister chromatids to daughter cells via its interactions with the mitotic spindle. Continued identification of conserved kinetochore components in model systems such as yeast has provided a rich resource of candidate genes that may be mutated or misregulated in human cancers. Systematic mutational testing and transcriptional profiling of CIN candidate kinetochore genes should shed light on the kinetochore's role in tumorigenesis, and on the general role CIN plays in cancer development.

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Introduction: chromosome instability and cancer

The molecular mechanisms ensuring accurate chromosome segregation during mitosis are critical to the conservation of euploidy (normal chromosome number) in eukaryotic cells. Errors in this process (chromosome nondisjunction and chromosome loss) result in aneuploidy and the phenotypic consequences of these imbalances in chromosome number are usually profound [1]. In humans, errors in mitotic chromosome segregation may play a role in the onset of neoplasia by reducing tumor suppressor gene dosage or by amplifying oncogenes. Mutations that cause genomic instability are now recognized as being important predisposing conditions for cancer [2,3,4••]. For instance, genomic instability in colon cancers arises by one of two mechanisms: microsatellite instability (MIN), where defects in mismatch repair lead to increased mutation rates; or chromosome instability (CIN), where improper sister chromatid segregation (or some related mechanism) leads to aneuploidy [5]. The MIN phenotype was first described in 1993 and led to the rapid identification of DNA mismatch repair gene mutations as predisposers to colon cancer [6–8]. The CIN phenotype, which is observed in the large majority of colon tumors was first described in 1997; colon tumors that had been characterized as exhibiting *states* of aneuploidy were shown to exhibit 10- to 100-fold higher *rates* of CIN relative to normal cells or to diploid (MIN) cancers [9].

The underlying CIN phenotype that predominates in colon cancer is not an exception to the rule; nearly all solid tumors exhibit genomic instability at the chromosomal level [10]. Experimental evidence strongly supports the hypothesis that the CIN phenotype occurs early in the development of cancer, and represents an important step in the initiation and/or progression of the disease [3,4••,11]; therefore a major goal has been to determine the genetic basis of CIN in tumors. One approach to identifying mutations responsible for CIN in cancer cells is to test for mutations in genes known to be important for chromosome segregation in human cells, or in human homologs of CIN genes discovered in model organisms, which serve as cross-species CIN candidate genes. Vogelstein and colleagues initially showed that the hBUB1 (identified originally in yeast as a mitotic spindle checkpoint mutant) is mutated in ~5% of colorectal tumors [12]. The recent report that germline biallelic mutations in another spindle checkpoint gene, hBUB1B, is associated with inherited predispositions to cancer strongly supports a causal link between CIN and cancer development [13••]. To date, there is evidence for somatic mutations in eight candidate CIN genes in colon cancers (APC, CDC4, hBUB1, hRod, hZW10, hZwilch, MRE11, Ding) [4••,10,14•]; however, many other candidate CIN genes remain untested. Even as the genetic basis for CIN in colon cancers is beginning to be understood, mutations leading to CIN in other types of cancer remain unknown. The daunting task of screening hundreds of CIN candidate genes in colon and other cancers lies ahead.

Candidate CIN genes encode proteins that function in all aspects of chromosome segregation, including proteins that function at kinetochores, telomeres and origins of replication, and in MT dynamics, sister chromatid cohesion, DNA replication, DNA repair, DNA condensation and cell cycle checkpoints. Of these, the kinetochore offers a logical choice for mutational testing since four

out of eight candidate CIN genes known to be mutated in CIN colon cancers encode kinetochore proteins. Furthermore, the ~100 predicted human genes that encode kinetochore components comprise a large mutational target that could be mutable to a CIN phenotype [15]. For example, kinetochore proteins constitute a significant portion of the collection of chromosome transmission fidelity (*ctf*) mutants identified in a classical genetic screen in yeast (9 out of the 24 CTF genes cloned and characterized to date) [16]. In this review, we will summarize recent discoveries of novel eukaryotic kinetochore proteins, and will highlight key findings pertaining to the connection between cancer and kinetochore dysfunction.

The kinetochore

The kinetochore (which consists of CEN DNA and associated proteins) is a macromolecular complex that is critical to the process of chromosome segregation. The kinetochore is responsible for mediating attachment of sister chromatids to the spindle microtubules (MTs) and for directing chromosome movement during mitosis and meiosis [17,18]. Remarkably, the kinetochore also monitors MT attachment and is critical in sensing the completion of metaphase (bi-polar attachment of all chromosomes) before allowing anaphase to begin [19,20]. To fulfill these critical tasks, the kinetochore acts as a central hub where kinetochore proteins, centromeric chromatin, cohesions and spindle checkpoint and MT-associated proteins gather to coordinate chromosome segregation. In budding yeast, often considered the simplest kinetochore with CEN DNA of only 125bp, >60 kinetochore proteins have been identified [21]. The number of proteins functioning at the mammalian kinetochore, which can span megabases of repetitive DNA, is predicted to be >100 [15]. Kinetochore proteins are classified as structural or regulatory, and ~40 of the yeast kinetochore proteins are considered to be structural components necessary for physically bridging CEN DNA to the spindle MTs [21]. Kinetochore components are further classified as inner, central, and outer kinetochore proteins on the basis of their proximity with the CEN DNA. The other proteins — including spindle checkpoint proteins, motor proteins, MT-associated proteins, and regulatory proteins such as the Ipl1 kinase — function to regulate kinetochore–MT attachment and to coordinate events within the cell cycle [21,22].

Work on identifying the protein components necessary to build a functional kinetochore has been undertaken in a number of model organisms, as reviewed in [15,21,23–25]. However, the list of kinetochore associated proteins continues to grow; the most recent additions within the last two years are listed in Table 1. The identification of novel kinetochore proteins has been aided greatly by the joint application of affinity purification using epitope tags and mass spectrometry [26–29]. However, other methodologies remain critical to the advancement of our knowledge, including the use of genetic screens to identify mutants that enhance (or suppress) chromosome loss rates [30,31]. Candidate protein selection based on homology to known kinetochore counterparts in other organisms has also provided recent success [32,33]. Of particular note is the continued discovery of conservation between kinetochore proteins of higher eukaryotes and yeast [29,31,33]. The conservation of individual kinetochore proteins and the overall organization of protein complexes, as is the case for the human and yeast Ndc80 complex, suggests that the basic building blocks of kinetochores in these organisms may not be as different as first suspected based on the differences in underlying DNA sequence and size [34–36]. Continued identification of conserved novel kinetochore components in yeast and other model organisms provides an important source of CIN candidate genes that may be mutated in cancers.

The cancer connection

Mutations in structural kinetochore proteins have not yet been identified in cancer cells, but most have not been tested. However, spindle checkpoint components, which monitor kinetochore–MT attachment, have been found mutated in cancer (Table 2). Spindle checkpoint proteins alert the cell to potential chromosome segregation errors by specifically binding to kinetochores that have not attached to MTs. Two spindle checkpoint proteins, hBUB1 and hBUBR1, are mutated in several cancer types at a low frequency [12,37–40]. Another spindle checkpoint component, hMAD2, is mutated in gastric cancers [41], and downregulated in cancer cell lines [42–44]. Heterozygous MAD2 mice also develop lung tumors at high rates after long latencies, suggesting that biallelic expression of MAD2 is important for its function [45]. Further support for the association between kinetochore dysfunction and cancer comes from Wang *et al.*, who analyzed 100 human homologs of CIN genes identified in yeast and flies, including six kinetochore/spindle checkpoint proteins, and identified mutations in hRod, hZw10 and hZwilch (Table 2), which together account for ~2% of colorectal cancers [14•]. These proteins function together as the RZZ complex to recruit the dynein–dynactin complex and MAD1–MAD2 to the kinetochore. The RZZ complex is thought to have a role in spindle checkpoint activation and inactivation (reviewed in [46]). The infrequent mutation rate in spindle checkpoint proteins poses the possibility that CIN in cancer cells can be caused by mutation of any one of many genes involved in chromosome segregation, including other kinetochore proteins. Because of the large number of candidate genes that could be mutated to give a CIN phenotype, the frequency of a particular mutation may be low as is observed for the spindle checkpoint proteins. Alternatively, mutations in a small subset of CIN genes may account for the majority of CIN in a given tumor type (e.g. colon). Systematic mutational testing of kinetochore

Table 1

Kinetochore proteins identified in the past 2 years.

Protein	Organism	Description	Reference
Hsk2 Hsk3	*S. cerevisiae*	DASH complex members, localize to microtubules and the outer kinetochore	[27,30]
Sgo1	*S. cerevisiae* *S. pombe* *H. sapiens*	Protects centromeric cohesion; required for spindle checkpoint function in *S. cerevisiae*	[33,66,67]
Spc105 YDR532c	*S. cerevisiae*	Spc105 and Ydr532c co-purify, interact with the Ndc80 complex, and Mtw1	[68]
Sim4	*S. pombe*	Coiled-coil protein associated with the centromere central core region, requires Mis6 to localize to the kinetochore	[69]
Mis13	*S. pombe*	Associated with the centromere central core region, interacts with Mis12 and Spc7	[29]
Mis14-18	*S. pombe*	Required for the formation or maintenance of specialized chromatin of centromeres. Mis15-18 are part of the CENP-A recruitment pathway, Mis16 function is conserved in humans	[31]
Spc7	*S. pombe*	Associated with the centromere central core region, interacts with Mal3, Mis12 and Mis13	[29,70]
Sgo2	*S. pombe*	SGO1 paralog required for mitotic chromosome segregation, Bub1 is required for localization of Sgo2 to the kinetochore	[33]
Knl-3 Kbp2-5 Mis12	*C. elegans*	Involved in generating and maintaining the kinetochore–microtubule interface, co-purifies with KNL-1, KNL-3, NDC-80, and Nuf2. Localize to kinetochores from prophase through completion of mitosis	[28]
Cenp-A	*X. laevis*	Centromere-specific histone H3 variant	[71]
Spc24 Spc25	*X. laevis* *H. sapiens*	Members of the Ndc80 complex, required to establish and maintain kinetochore microtubule attachments. RNAi knockdown of hSpc25 results in loss of Hec1/hNdc80 and Mad1 from the kinetochore	[34,72]
Borealin	*H. sapiens*	Member of the Aurora B kinase/INCENP/Survivin complex required for stability of the mitotic spindle	[73]
Zwilch	*Drosophila* *H. sapiens*	Forms a complex with ZW10 and ROD	[74]
c20orf1720 DC8 PMF1 KIAA157	*H. sapiens*	Form a complex with hMis12 that is similar to complexes found in both *S. pombe* and *S. cerevisiae*. c20orf1720 and DC8 shown to localize to kinetochores	[29]
p30	*H. sapiens*	Component of the inner centromere may have a role in formation of centromeric chromatin	[26]
Nup107-160 complex	*H. sapiens*	Sub-complex of nuclear pores, localizes to kinetochores from prophase to anaphase	[75]

proteins in cancers should shed light on their role in tumorigenesis and the frequency of specific mutations.

While systematic mutational testing is just beginning to be undertaken [14•], expression studies have suggested a correlation between overexpression of several kinetochore proteins and cancer (X). CENP-A, a histone H3-like protein that is unique to CEN DNA and crucial to CEN chromatin maintenance, is overexpressed and mistargeted in colorectal cancer tissues [47]. Overexpressed CENP-A localizes to the entire chromosome and dissociates from native centromeres. This causes a subset of kinetochore proteins to be recruited to non-centromeric chromatin, leading to ectopic formation of pre-kinetochore complexes, which might deplete some kinetochore components, disrupt the native centromere–kinetochore complex and cause CIN [48]. Another inner kinetochore protein, CENP-H, which is important for kinetochore organization, is also upregulated in colorectal cancer tissues [49•]. Transfection of a CENP-H expression plasmid into diploid cell lines induces aneuploidy and increases the incidence of aberrant micronuclei, suggesting that upregulation of CENP-H can lead to a CIN phenotype. In addition, Aurora-B (AIM-1) and INCENP, two chromosome passenger proteins that localize to the kinetochore from prophase to metaphase and to the mitotic spindle in cytokinesis, are upregulated in tumor cell lines [50–52]. Aurora-B phosphorylation is required for chromosome condensation, controlling MT dynamics including destabilizing syntelic MT attachments to kinetochore, and regulation of cytokinesis (reviewed in [53]). Aurora-B-overexpressing cells exhibit CIN and contain multinuclei, and injection of these cells into nude mice induces tumor growth [51,54]. In reverse, block of Aurora-B expression increases the latency period and reduces the growth of thyroid anaplastic carcinoma cells [51], supporting a link between Aurora-B expression and cancer initiation or progression. Similarly, overexpression of CENP-F (mitosin) correlates with tumor proliferation and metastasis; hence, CENP-F is suggested to be a potentially valuable proliferation marker for diagnosis and prognosis [55–60]. CENP-F is a cell-cycle-regulated protein that associates

Table 2

Association of kinetochore gene mutation or misregulation with cancer.

Kinetochore/spindle checkpoint gene	Mutation/misregulation	Frequency*	Tumor type	Reference
hBUB1	Dominant negative heterozygous deletion and missense mutation	2/19	Colorectal cancer	[12]
	Heterozygous missense mutation	1/30	Lung tumor	[37]
	Heterozygous missense mutation	1/10	Acute T-cell lymphoblastic leukemia	[38]
	Dominant negative heterozygous deletion in kinetochore localization domain	1/2	Acute lymphoblastic leukemia	[39]
	Deletion in kinetochore localization domain	2/2	Hodgkin's lymphoma	[39]
	Overexpressed	30/36	Gastric cancer	[76]
hBUBR1	One heterozygous and one homozygous missense mutation, one homozygous deletion	3/10	Acute T-cell lymphoblastic leukemia	[38]
	Downregulated (10 fold)	3/109	Colorectal cancer and others	[40]
	Overexpressed	19/28	Gastric cancer	[76]
hBUB3	Overexpressed	26/34	Gastric cancer	[76]
MAD2	Missense mutation	22/49	Gastric cancer	[41]
	Downregulated	1/1	Breast cancer cell line	[42]
	Downregulated	2/5	Nasopharyngeal cancer cell lines	[43]
	Downregulated	3/7	Ovarian cancer cell lines	[44]
hRod	Homozygous missense mutation	1/192	Colorectal cancer	[14•]
hZw10	Heterozygous missense mutation	2/192	Colorectal cancer	[14•]
hZwilch	Heterozygous premature truncation	1/192	Colorectal cancer	[14•]
CENP-A	Overexpressed (1.5–32.5 fold)	11/11	Colorectal cancer	[47]
CENP-H	Overexpressed (1.7–9.6 fold)	15/15	Colorectal cancer	[49•]
CENP-F (mitosin)	Amplified (1.6–2.5 fold)	7/72	Head and neck squamous cell carcinomas	[57]
	Overexpressed (2.1–4.2 fold)	25/72		
	Overexpressed	25/26	Salivary gland tumor	[54]
HEC1 (highly expressed in cancer; hNDC80)	Overexpressed	9/9	Cervical, acute lymphocytic leukemia, breast and colorectal cancer lines	[77]
Aurora-B (AIM1)	Overexpressed	12/12	Thyroid cancer lines	[51]
	Overexpressed	7/7	Colorectal cancer	[50]
INCENP	Overexpressed (2.4–4.7 fold)	4/4	Colorectal cancer cell lines	[52]

* shown as number of positive patients or cells lines as a fraction of the total number tested.

with the outer kinetochore in M phase and is rapidly degraded upon completion of mitosis. It associates preferentially with kinetochores of unaligned chromosomes, and may play a role in the spindle checkpoint [61–63].

The evidence above suggests that overexpression of kinetochore components may contribute to tumor progression by driving CIN. Stoichiometric expression of kinetochore components may be important for normal kinetochore assembly and the dosage may be crucial for spindle checkpoint signaling. However, it is possible that overexpression is a consequence rather than a cause of dysfunctional cell cycle regulation in carcinogenesis. To delineate the causal relationship between kinetochore protein mutation/misregulation and cancer development, further functional studies must be performed in diploid cell lines or mouse models to investigate whether kinetochore mutation/misregulation leads to CIN or cellular transformation.

Therapeutic applications

Knowledge of the mutational spectrum of CIN genes in cancer could have several important practical applications. First, it would allow sub-classification of tumors based on the specific CIN gene mutation, which could have implications for improved diagnostics, prognostication, or predictions of response to therapy. Second, if a defined subset of CIN genes represents the major CIN mutational targets in cancer, they may provide a rationale for therapeutic design. That is, although CIN may be important in the development of a tumor, the specific CIN mutation may define an 'Achilles heel' (relative to adjacent normal tissue) allowing selective killing of tumor cells [64,65]. In this regard, an on-going effort in model organisms such as yeast has been to construct a comprehensive synthetic lethal genetic interaction map, identifying pairs of non-allelic gene mutations 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 suggest candidate drug targets for killing specific cancers. By definition, these second-site loss-of-function mutations (which are otherwise non-lethal in the CIN-gene wild-type cells) define proteins that, when reduced in activity, cause lethality in the reference CIN mutant. These second-site genes therefore may suggest cross-species candidate proteins in humans that when inhibited (e.g. by a drug) would

specifically kill tumor cells relative to normal cells. If kinetochore proteins turn out to represent a significant fraction of the CIN mutational spectrum in cancer, it is conceivable that second-site genes will exist that are synthetically lethal in combination with various kinetochore gene mutations, and therefore provide common drug targets for killing a broad spectrum of CIN cancers.

Concluding remarks

Designing effective therapeutics for cancer will rely on us first understanding the genetic basis of cancer, including the cause of CIN and its contribution to human cancers. This will involve systematic mutational testing and transcriptional profiling of candidate CIN genes, of which the kinetochore represents a logical choice due to the large number of protein constituents and the established connections between kinetochore dysfunction and cancer.

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