to whether any correlation exists between the spectrum of germline mutations and spectrum of phenotypic (somatic) mutations in patients with the tumors.

When and How Should Requests Be Made to Perform Genetic Testing and How and When Should Testing Be Performed?

It is not easy to obtain genomic information, especially for practicing physicians, when there is no or little family history of cancer or when the patient's relatives are not very familiar with recent genetic knowledge, including the significance of DNA. Even after overcoming the hurdles of the difficulty of obtaining DNA and conducting a genomic analysis, the odds of obtaining a result that can completely explain the patient's condition are seldom high. Several studies, including the study by Paunu et al. (Paunu et al., 2001), did not reveal any germline *TP53* mutations in familial glial tumors. Portwine also reported negative findings in a search for germline *TP53* mutations in children with sporadic brain tumors (Portwine et al., 2001). The prevalence of germline *TP53* mutations that have biological significance in asymptomatic populations appears to be extremely low, perhaps only 0.3% (Palmero et al., 2008), and it is difficult to persuade the healthy relatives to undergo genetic testing. We consider the likelihood of detection of a germline *TP53* mutation in the adolescent cancer patient in Case 2 to have been very small. Most brain tumors are not thought of as genetic or hereditary diseases, and brain tumors are still basically classified according to their morphology and somatic genetic information (Burger and Scheithauer, 2007). Despite anecdotal reports of brain tumors as genetic manifestations, the overall attempt to identify constitutional genetic changes in brain tumor patients has been tedious until recently. Li et al. (1995) investigated TP53 germline mutations in 80 unselected glioma patients and found a *TP53* germline mutation in only 1 of the 65 cases, that did not have a peculiar family history, as opposed in 3 of the 15 cases with a familial or "unusual" personal history. As stated in the introduction, taking a complete family history is often difficult, and cases in family members who would have developed cancer but died at an early age of some other cause would be missed. When the parents have few siblings, it is very difficult to conclude that the occurrence of a disease in a particular case is sporadic. Then, how about "unusual" cases? In many of the cases in which we persuaded young patients and family members to undergo DNA testing and obtained their consent the results were negative, and the reason the patient suffered this particular disease, which was unusual for his or her age remained a mystery to the parents or relatives. There are still no valid scientific criteria for performing or recommending genetic tests in clinical settings. The criteria are often subjective and depend on the feeling of the attending physician or his or her enthusiasm regarding how far to pursue the investigation of the cause of the disease in cases that arouse suspicion in attending clinicians. Unusual clusterings of malignant tumors in a family attract general practitioners attention, but the absence of a family history of cancer or difficulty in obtaining it often impede further investigation by genetic analysis. The recent worldwide trend toward small families further reduces the possibility of identifying a peculiar pedigree to analyze for genetic disease. Then, can luck alone be expected to result in the identification of significant mutations?

In compensation the era of small family sizes and the rigorous procedures required to obtain patient consent, a wide variety of genetic test, technologies are now available and are easier and friendlier than ever before. A genome-wide, personal whole genome sequence strategy is now available, and it will become less costly in the near future. The introduction of cytogenetic arrays in recent years has facilitated the discovery of unexpected changes in copy numbers, small deletion/insertion polymorphisms, amplifications, and rather rare (0.1–5%) single nucleotide polymorphisms (SNPs) when we attempt to determine the etiology of a common disease in an unusual situation (Rieber et al., 2009; Schwarzbraun et al., 2009). The era of personal sequencing is coming, and we, as one of the part of medical systems these days, i.e., as a patient, a family member of the patient, counselor, physician, insurer, or scientist, may have to prepare for the time when germline tests will be routinely performed in every patient with an "unusual" brain tumor.

Syndromes Expanding?

Mismatch deficiencies are one of the well known genetic causes of brain tumors. Paraf proposed the

existence of two types of brain tumor-polyposis (BTP) syndrome (Paraf et al., 1997). The BTP syndrome type 1 is characterized by non-polypotic (thus, the name is paradoxical) colorectal tumors and microsatellite instability, the same as found in hereditary non-polypotic colorectal cancer (HNPCC). BTP1 syndrome corresponds to Turcot syndrome (MIM accession 270630) and/or Muir-Torre syndrome (MIM accession 158320), which can also have glioblastoma multiforme as a component of the syndrome (Park et al., 2009). BTP syndrome type 2 is characterized by brain tumors associated with adenomatous polyposis coli. We are not sure whether our Case 2 is a case of BTP syndrome type 1, because of the absence of microsatellite instability in the colorectal cancer in Case 2. It would be inappropriate to call cases like Case 2 "brain tumor-*polyposis* syndrome". In conclusion, we are compelled to expand the concept of brain tumor-colorectal tumor syndrome to include what we refer to here as "*TP53* disorders".

Problems in Screenings and Preventions

The unusual occurrence of common cancers in subjects harboring a germline mutation of *TP53* prompted us to consider the feasibility of detecting or mass screening for such carriers in the general population. An R337H mutation of *TP53* was found in two of 750 healthy subjects in a study conducted in southern Brazil (Palmero et al., 2008). A thorough characterization of this germline mutation (variant) in terms of its penetrance and the cancer spectrum of the subjects' families is under way, and justification of neonatal mass screening for this variant has been debated (Achatz et al., 2009). To justify this kind of attempts of neonatal mass screening, we must be ready and feasible for detection of the early occurrence of tumors in carriers, and we also have to have the tools to treat the tumors or to reduce the hazard caused by tumors in the carriers. Endoscopic surveillance of the gastrointestinal (GI) tract for cancer and surveillance for breast cancer by mammography would be an acceptable choice for many subjects instead of more invasive measures. Some GI tumors can be treated endoscopically, and some breast tumors can be cured by minimally invasive surgery. The situation in regard to brain tumors is different. CT, MRI, and positron emission tomography (PET) are powerful diagnostic imaging methods for detecting small tumors, but is it acceptable for the carriers of germline *TP53* mutations to undergo craniotomy or stereotactic radioneurosurgery? Since carriers of germline *TP53* mutations are thought to be more sensitive to radiation it is not certain that the gamma knife is the best choice of treatment.

Many investigators are attempting to find agents that will reduce the risk associated with a germline heterogeneous *TP53* mutation. One bold, promising strategy would be to use p53-activating drugs to treat patients with germline heterozygosity of *p53*. Deacetylase inhibitors are promising candidates for such drugs that would reduce the risk associated with germline heterogeneous *TP53* mutation, because acetylation of the C-terminal region of TP53 protein up-regulates its transcriptional and transactivational activity. Treatment with a deacetylase inhibitor is expected to compensate for the depleted function of mutant p53 in the *p53*-heterozygous cells by increasing the acetylation level of wild-type p53. One of the deacetylase inhibitors, the sirtuin (histone deacetylase type [HDAC] III) -targeting inhibitor, is particularly noteworthy, because the sirtuin (HDAC III) is catalytically different from the other histone deacetylases. The members of the sirtuin deacetylase family require NAD to exert their deacetylase activity, and these characteristics may lead to novel efficacy against cancer cells and cancer-predisposing cells. There is a mouse model with haploinsufficiency of p53 that is prone to develop various tumors (Jacks et al., 1994), and *tailless*, a nuclear receptor gene has recently been shown to contribute to brain tumor initiation in the p53 haploinsufficient mouse (Liu et al., 2010). Such models will provide monitoring systems to test the preventive efficacy of drugs against tumorigenesis in persons with greater genetic susceptibility to cancers. Various sirtuin inhibitors, both natural and synthetic, have been evaluated in vitro (Kahyo et al., 2008), and application of these drugs to an animal model and then clinically must be assessed in the near future.

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7

Centrosome Abnormality and Human Lung Cancer

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1. Introduction

The centrosome, which functions as a major microtubule-organizing center (MTOC), is composed of a pair of centrioles and surrounding protein aggregates called pericentriolar material (PCM); at any given time during the cell cycle, each cell contains one or two centrosomes (Fukasawa, 2007). Centrosomes play a crucial role in the formation of bipolar mitotic spindles, which are essential for accurate chromosome segregation (Zyss & Gergely, 2009). Numerical and functional abnormalities of centrosomes result in an increase in aberrant mitotic spindle formation, merotelic kinetochore-microtubule attachment errors, lagging chromosome formation, and chromosome segregation errors, all of which are thought to be possible causes of chromosome instability (Ganem et al., 2009; Nigg & Raff, 2009). Centrosome abnormalities and chromosome instability are characteristics of human lung cancer (Masuda and Takahashi, 2002; Koutsami et al., 2006; Jung et al., 2007; Shinmura et al., 2008), and abnormalities in genes responsible for centrosome regulation have been reported in lung cancer (Fukasawa, 2007; Lee et al., 2010). In this Review, the status of centrosome abnormalities in lung cancer, the mechanisms responsible for inducing centrosome abnormalities, and the relationship between centrosome abnormalities and chromosome instability are summarized.

2. Centrosome abnormalities in human lung cancer: Mechanisms causing centrosome abnormalities and chromosome instability

The presence of two centrosomes at mitosis is an important factor in the formation of bipolar mitotic spindles. Therefore, the numerical integrity of centrosomes is carefully controlled in human cells, and abrogation of this control results in centrosome amplification. First, we describe the normal centrosome duplication cycle, followed by three reports on centrosome abnormalities in lung cancer. Next, we describe investigations of the mechanism responsible for inducing centrosome amplification. Finally, we summarize the possible reasons why centrosome abnormalities cause chromosome instability.

2.1 Centrosome duplication cycle in human cells

Centrioles are cylindrical structures (-0.2 μm in diameter and 0.2-0.5 μm in length) and are composed of nine triplet microtubule arrays organized around a central cartwheel. Centrioles contain several tubulin isoforms and non-tubulin proteins such as CETN2, CP110,

SAS-6, and SAS-4 (Bettencourt-Dias & Glover, 2009). In animal cells, a pair of centrioles is embedded in a cloud of electron dense material known as PCM, and both structures constitute a larger structure named the centrosome, which serves as the main MTOC during both interphase and mitotic phase (Vorobjev & Nadezhdina, 1987). Centrosome duplication occurs once per cell cycle and is subject to strict control within cells. To organize a bipolar mitotic spindle, a centrosome is duplicated in S phase, additional PCM proteins are recruited during centrosome maturation in G2, and the two centrosomes separate at mitotic entry (Figure 1). The primary function of PCM is microtubule nucleation. The assembly of microtubules is initiated on a γ-tubulin ring complex (γTuRC), composed by γ-tubulin and additional subunits known as γ-tubulin complex proteins (Teixidó-Travesa et al., 2010).

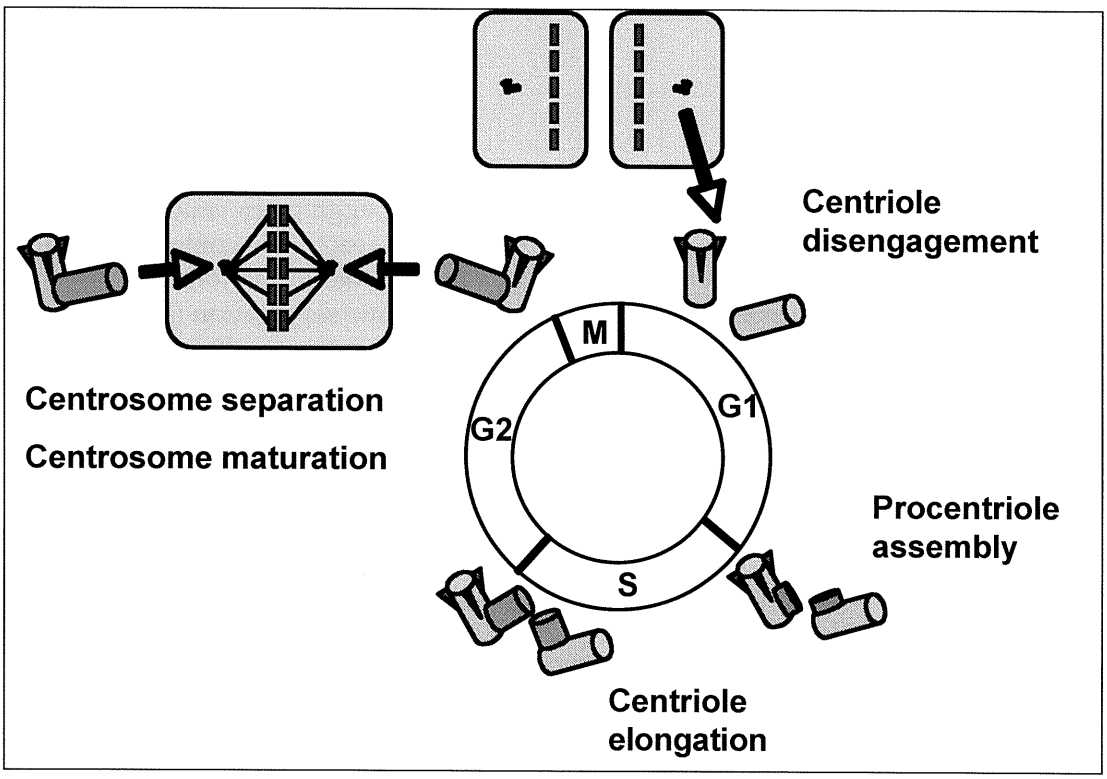

Fig. 1. Centrosome duplication cycle.

The centrioles duplicate once per cell cycle. The formation of the daughter centriole on each mother centriole occurs during the late G1 and S phases of the cell cycle. The daughter and mother centrioles are tightly associated in an orthogonal manner until the end of mitosis, and centriole disengagement occurs during mitotic exit. The initiation of centriole duplication requires the activity of several proteins, such as Cdk2-cyclin E and PLK4 kinases. The procentriole starts to assemble, and elongation depends on several proteins including centrin, CEP135, and γ-tubulin. During G2 phase, additional PCM proteins are recruited, and centrosome maturation requires the activity of Aurora A and PLK1 kinases. During late G2, the daughter centriole of the parental pair acquires subdistal appendages. Then, the two duplicated centrosomes separate and move to opposite end of the cell (centrosome separation). Finally, the two centrosomes form the poles of the bipolar mitotic spindle.

2.2 Centrosome abnormalities in lung cancer

Centrosome amplification has been reported in a variety of human primary cancers (e.g., breast cancer, lung cancer, bladder cancer, pancreatic cancer, and prostatic cancer) (Pihan et al., 1998; Sato et al., 1999; Pihan et al., 2001; Kawamura et al., 2004; Zyss & Gergely, 2009). With regard to primary lung cancer, Koutsami et al. (2006) examined 68 primary non-small cell lung carcinomas (NSCLCs) for the presence or absence of centrosome amplification using an immunofluorescence analysis with a monoclonal antibody for γ-tubulin, a centrosome marker; they showed that 36 (53%) of the 68 NSCLCs exhibited centrosome amplification. Centrosome amplification was not associated with clinicopathological markers such as stage, tumor grade, and histological subtype, but was associated with aneuploidy. Jung et al. (2007) examined 175 NSCLCs for centrosome abnormalities using an immunofluorescence analysis with an anti-γ-tubulin antibody; they showed that 50 (29%) of the 175 NSCLCs exhibited a centrosome abnormality. Aneuploidy, p16 expression, and the loss of pRB expression were significantly associated with centrosome abnormalities. Shinmura et al. (2008) examined 182 primary lung carcinomas for the presence or absence of centrosome amplification using an immunohistochemical analysis with an anti-γ-tubulin antibody and showed that 67 (37%) of the 182 cancers exhibited centrosome amplification. Thus, centrosome amplification is a common abnormality seen in human primary lung cancers.

2.3 Mechanisms inducing centrosome abnormalities

An immunofluorescence analysis using an antibody for centrosome or centriole markers in cultured cell lines can be used to determine the status of the centrosome number in the cells. The involvement of many kinds of agents and genes in centrosome regulation has been examined using such analyses. Here, these analyses are divided into those using lung cells and those using cells derived from other organs.

2.3.1 Mechanisms identified by using the lung cells

Holmes et al. (2006) showed that chronic exposure to lead chromate causes centrosome abnormalities and aneuploidy using WTHBF-6 cells, a cell line derived from normal human bronchial fibroblasts. Hexavalent chromium compounds [Cr(VI)] are human lung carcinogens (Le´onard & Lauwerys, 1980), and "particulate" Cr(VI) compounds are one of the most potent forms. They reported centrosome amplification in interphase and mitotic cells in response to treatment with lead chromate as a model particulate Cr(VI) compound. They suggested that one possible mechanism for lead chromate-induced carcinogenesis is through centrosome dysfunction, leading to the induction of aneuploidy. The same group (Holmes et al., 2010) also showed that chronic exposure to zinc chromate, another particulate Cr(VI) compound, induces centrosome amplification and spindle checkpoint bypass using human lung fibroblasts.

Arsenic is another environmental toxicant, and the biological effects of arsenic have been studied. Liao et al. (2007) showed that arsenic promotes centrosome abnormalities and cell colony formation in p53 compromised human lung cells. They used H1355 (a lung adenocarcinoma cell line with a p53 mutation), BEAS-2B (immortalized lung epithelial cells with functional p53) and pifithrin-α-treated BEAS-2B (p53-inhibited cells) and reported an increase in centrosome abnormalities in both arsenite-treated p53 compromised cell lines, compared with that in arsenite-treated BEAS-2B cells. Their findings provided evidence of

the carcinogenic promotional role of arsenic, especially in the presence of p53 abnormalities. The group also showed that arsenite promoted centrosome abnormalities in the presence of a p53-compromised status induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (nicotine-derived nitrosamine ketone, NNK) using BEAS-2B cells (Liao et al., 2010). Their findings provided evidence of an interaction between arsenite and cigarette smoking.

Benzo[*a*]pyrene diol epoxide (B[*a*]PDE), the ultimate carcinogenic metabolite of benzo[*a*]pyrene, has been implicated in the mutagenesis of the *p53* gene, which is involved in smoking-associated lung cancer. Shinmura et al. (2008) showed that the exposure of *p53*-deficient H1299 lung cancer cells to B[*a*]PDE resulted in S-phase arrest, leading to abnormal centrosome amplification. They also revealed that the centrosome amplification could be primarily attributed to excessive centrosome duplication, rather than to centriole splitting, and the forced expression of POLK DNA polymerase, which has the ability to bypass B[*a*]PDE-guanine lesions in an error-free manner, suppressing B[*a*]PDE-induced centrosome amplification. The B[*a*]PDE exposure also led to chromosome instability, which was likely to have resulted from centrosome amplification. Thus, they concluded that B[*a*]PDE contributes to neoplasia by inducing centrosome amplification and consequent chromosome destabilization in addition to its mutagenic activity.

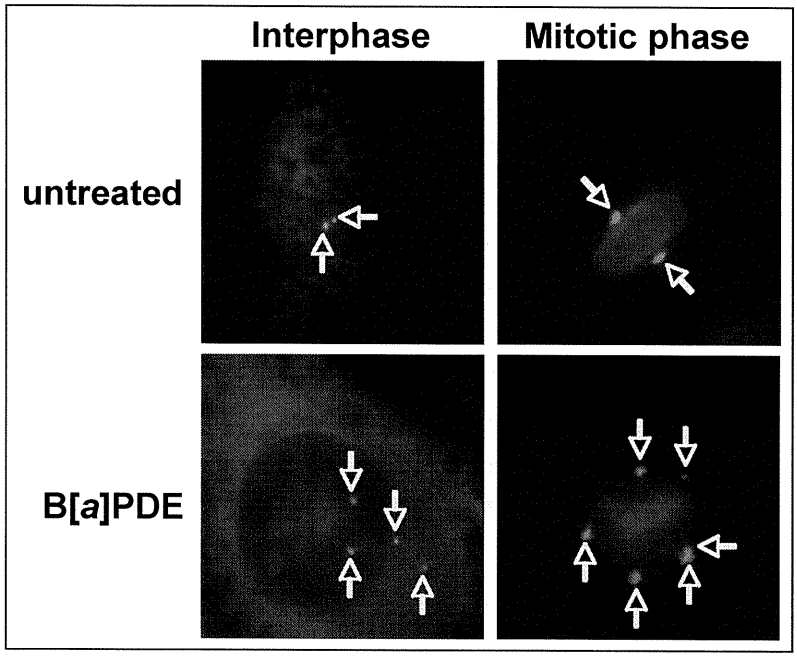

Fig. 2. Induction of centrosome amplification in *p53*-deficient H1299 lung cancer cells by exposure to benzo[*a*]pyrene diol epoxide (B[*a*]PDE).

H1299 cells were exposed to 0.6 μM B[*a*]PDE for 72 hr and then immunostained with mouse anti-γ-tubulin monoclonal antibody (GTU-88; Sigma-Aldrich, St. Louis, MO, USA). Alexa Fluor 546 (red)-conjugated anti-IgG antibody (Molecular Probes, Eugene, OR, USA) was used to detect the antibody-antigen complexes. The nuclei were stained with 4',6-diamidino-2-phenylindol (DAPI, blue). An increase in the number of centrosomes, i.e., centrosome amplification, was observed in both interphase cells and mitotic phase cells. The arrows indicate the positions of centrosomes.

The lung is easily subjected to many kinds of environmental agents, some of which may be derived from cigarette smoking or occupational exposure. As described in the above three paragraphs, some environmental carcinogens induce centrosome amplification. Other environmental carcinogens attacking DNA may also induce centrosome amplification, since cell cycle arrest has been shown to occur during centrosome amplification. Further precise analyses of environmental agent-related centrosome amplification are needed to understand the relationship between environmental carcinogens and lung cancer more clearly.

The S-phase kinase-interacting protein-2 (SKP2) plays a key role in the progression of cells from a quiescent to proliferative state, and the SKP2 protein is overexpressed in lung cancer. Jiang et al. (2005) showed that the RNA silencing of SKP2 inhibits proliferation and centrosome amplification using the lung cancer cell lines A549 and H1792. Their results suggest that SKP2 plays an oncogenic role in lung cancer and has a centrosome regulating function.

NORE1 (RASSF5) is a member of the *RASSF* gene family, and NORE1A is the longest and major splice isoform of the *NORE1* gene (Nakamura et al., 2005). Its product, NORE1A, is a nucleocytoplasmic shuttling protein and has a growth-suppressive function (Moshnikova et al., 2006). Shinmura et al. (2011) showed that NORE1A suppresses the centrosome amplification induced by hydroxyurea using a *p53*-deficient H1299 lung cancer cell line, and NORE1A expression was down-regulated in NSCLC. Both of these findings imply that NORE1A has a key preventative role against the carcinogenesis of NSCLC.

2.3.2 Mechanisms identified using cells derived from other organs

CDK2-cyclin E, a known inducer of S-phase entry (Heichman, 1994), has an important role in the regulation of centrosome duplication (Hinchcliffe et al., 1999; Matsumoto et al., 1999). The activation of CDK2-cyclin E during late-G1 phase coordinates the initiation of centrosome and DNA duplication. Several CDK2-cyclin E targets, including nucleophosmin (NPM) (Okuda et al., 2000), have been identified. NPM binds and modulates the activities of multiple proteins including tumor suppressor proteins (e.g., p53) and some oncogenic proteins (e.g., ROCK2) (Colombo et al., 2002; Ma et al., 2006b). The reduced as well as increased expression of NPM can lead to the oncogenic transformation of cells. Actually, NPM is frequently mutated, lost or overexpressed in cancers (Grisendi et al., 2006), and both the overexpression and the depletion of NPM in cultured cells can lead to neoplastic transformation (Kondo et al., 1997; Grisendi et al., 2005). NPM localizes between the paired centrioles of the unduplicated centrosome, probably functioning in centriole pairing (Shinmura et al., 2005). When NPM is phosphorylated by CDK2-cyclin E, most of the NPM dissociates from the centrosomes, leading to the centrosome duplication. In this context, NPM negatively controls centrosome duplication; indeed, the depletion of NPM leads to centrosome amplification (Grisendi et al., 2005; Wang et al., 2005). NPM was reported to have the ability to control centrosome duplication in association with ROCK2 (Ma et al., 2006b), a member of the Rho-associated, coiled-coil containing protein kinase family that is frequently overexpressed in cancer (Nishimura et al., 2003). After NPM phosphorylation by CDK2-cyclin E, the binding between NPM and ROCK2 increases and ROCK2 is activated at centrosomes, leading to centrosome duplication (Ma et al., 2006b). In ROCK2 activation, the binding of Rho small GTPase to the auto-inhibitory region is also required (Kanai et al., 2010). Among three isoforms of Rho, both RhoA and RhoC, but not RhoB, promoted centrosome duplication and centrosome amplification.

Another target of CDK2-cyclin E in centrosome regulation is MPS1, a spindle checkpoint kinase that is localized at the centrosome (Fisk et al., 2003). MPS1 is stabilized and activated by CDK2-cyclin E phosphorylation and involved in centrosome duplication. Mortalin, a member of the heat-shock protein 70 molecular chaperone family, is localized at the centrosome and physically interacts with and is phosphorylated by MPS1. The phosphorylation of mortalin activates MPS1 in a positive-feedback manner, and this phenomenon is important for MPS1-related centrosome duplication (Kanai et al., 2007). Mortalin is frequently upregulated in cancers (Wadhwa et al., 2006).

CDK2 forms a complex with cyclin A in addition to cyclin E, and CDK2-cyclin A has been implicated in the regulation of centrosome duplication (Meraldi et al., 1999). CDK2-cyclin A and CDK2-cyclin E share some substrates (Tokuyama et al., 2001). The CDK2-cyclin A complex is active in S and G2 phases during the cell cycle, and CDK2-cyclin A may have a crucial role in centrosome over-duplication and/or amplification (Hanashiro et al., 2008). As another type of CDK-cyclin complex, the overactivation of CDK4/6-cyclin D has been shown to induce centrosome amplification (Nelsen et al., 2005). The major target of CDK4/6-cyclin D is the RB tumor-suppressor protein (Duensing et al., 2000). The conditional loss of *Rb* in mice results in centrosome amplification (Balsitis et al., 2003; Iovino et al., 2006).

CDK2 activity is also negatively controlled by the CDK inhibitor p21, one of the major transactivation targets of the p53 tumor-suppressor protein (Bálint & Vousden, 2001). p53 is involved in the regulation of centrosome duplication, which was first demonstrated in cells and tissues from *p53*-deficient mice (Fukasawa et al., 1996; Fukasawa et al., 1997). When cells are exposed to DNA-synthesis inhibitors such as hydroxyurea, centrosomes undergo reduplication without DNA synthesis, resulting in centrosome amplification (Balczon et al., 1995). Centrosome reduplication occurs efficiently when *p53* is mutated or lost (Tarapore et al., 2001a). In normal cells, p53 is stabilized under cellular stresses by the inhibition of MDM2, leading to the upregulation of p21, which blocks the initiation of centrosome reduplication through the inhibition of cyclin-CDK2 complexes (Bálint & Vousden, 2001). On the other hand, p21 is not upregulated in cells lacking *p53*, allowing the activation of CDK2, which in turn triggers centrosome reduplication.

Besides the p53-p21 pathway, p53 has the ability to control centrosome duplication. p53 is localized at centrosomes (Blair Zajdel & Blair, 1988; Brown et al., 1994; Tarapore et al., 2001b; Tritarelli et al., 2004; Ma et al., 2006a; Shinmura et al., 2007) and appears to control centrosome duplication independently of its transactivation function. Even if p53 is a mutant without transactivation function, p53 retains the ability to localize to centrosomes and partially suppresses centrosome duplication (Shinmura et al., 2007). However, the mechanism underlying this role of p53 is currently unknown.

The proteins that control p53 stability are also involved in the regulation of centrosome duplication. The ectopic expression of human papilloma virus (HPV) E6 protein, which promotes the degradation of p53, induces centrosome amplification (Duensing et al., 2000). MDM2 is an E3 ubiquitin ligase that promotes the degradation of p53 and is often overexpressed in cancers (Manfredi, 2010). The forced expression of MDM2 in cells containing wild-type p53 efficiently leads to centrosome amplification (Carroll et al., 1999). Aurora A kinase (AURKA) phosphorylates p53 at Ser315, resulting in MDM2-mediated p53 destabilization (Katayama et al., 2004), and the forced expression of Aurora A induces centrosome amplification (Zhou et al., 1998).

Polo-like kinase 1 (PLK1) is a key regulator of centrosome maturation (Barr et al., 2004; Bettencourt-Dias and Glover, 2007). Its deregulation is linked to centrosome abnormalities and oncogenesis (Zyss and Gergely, 2009). PLK1 belongs to the mammalian PLK family, which is comprised of five members (PLK1 - PLK4 and PLK5P) (Lens et al., 2010). PLK1 is involved in a variety of mitotic events, including centrosome maturation and separation, G2/M transition, mitotic spindle formation, chromosome segregation, and cytokinesis, and several kinds of PLK1 substrates are known (Barr et al., 2004; Petronczki et al., 2008). PLK1 targets multiple centrosomal proteins (e.g., γ-tubulin) to fulfill the mitotic function of centrosomes. Ninein-like protein (NLP) interacts with γTuRC during interphase, and participates in the establishment of the cytoplasmic microtubule network (Casenghi et al., 2003; Rapley et al., 2005). At the onset of mitosis, the cooperation of PLK1 and NLP promotes the centrosomal localization of γ-tubulin and other mitosis specific PCM components, resulting in a higher microtubule nucleation capacity of the mitotic centrosome (Casenghi et al., 2003; Rapley et al., 2005). The phosphorylation of NEDD1 by PLK1 is required for the targeting of γTuRC to the centrosome (Zhang et al., 2009). In mitosis, centrosomes must withstand the pulling forces exerted by chromosome-attached microtubules. To withstand such forces, PLK1 also plays a role in maintaining the structural integrity of the centrosome during mitosis (Oshimori et al., 2006). Kizuna is localized at the centrosomes and is phosphorylated by PLK1 during mitosis. The reduced expression of kizuna results in centrosome fragmentation and the dispersion of PCM, leading to the formation of aberrant mitotic spindles and chromosome segregation errors.

Another PLK, PLK4, is involved in recruiting the structural components required for the formation of procentrioles at the proximal side of the older centriole, in cooperation with CDK2-cyclin E (Habedanck et al., 2004). The upregulation of PLK4 expression is a strong stimulus for centriole multiplication (Kleylein-Sohn et al., 2007). The timely degradation of PLK4 by the SCF slimb ubiquitin ligase is important for the restriction of procentriole formation (Cunha-Ferreira et al., 2009). The SCF component CUL1 also functions as a centrosomal suppressor of centriole multiplication by regulating the PLK4 protein level (Korzeniewski et al., 2009). PLK4 kinase activity also regulates its own stability (Holland et al., 2010; Guderian et al., 2010). CEP152 interacts with PLK4 and CPAP and controls centrosome duplication in human cells (Dzhindzhev et al., 2010). PLK4 is transcriptionally regulated by p53 (Li et al., 2005). Clinically, the expression of PLK4 is upregulated in colon cancer (Macmillan et al., 2001), while the expression of PLK4 is downregulated in hepatocellular carcinoma because of promoter hypermethylation and the loss of heterozygosity (LOH) (Pellegrino et al., 2010; Rosario et al., 2010).

The role of the morgana/chp-1 in centrosome regulation has been reported by Ferretti et al. (2010). Mutations in morgana result in centrosome amplification. Morgana forms a complex with Hsp90, ROCK1 and ROCK2, and directly binds to ROCK2. Morgana downregulation promotes the interaction between ROCK2 and NPM, leading to an increase in ROCK2 activity, which in turn results in centrosome amplification. Morgana is downregulated in a large fraction of lung and breast cancers. They suggested that morgana plays a role in preventing centrosome amplification and tumorigenesis.

NLP, a previously described substrate of PLK1 (Casenghi et al., 2003), is a BRCA1-associated centrosomal protein that is involved in microtubule nucleation and spindle formation (Jin et al., 2009). NLP is overexpressed as a result of *NLP* gene amplification in lung cancer, and NLP overexpression causes centrosome amplification (Shao et al., 2010).

The *BRCA1* gene is responsible for susceptibility to familial breast/ovarian cancer and participates in diverse cellular functions (Venkitaraman, 2002). The BRCA1 is localized at the centrosomes (Hsu & White, 1998; Okada & Ouchi, 2003) and is involved in the regulation of centrosome duplication (Xu et al., 1999). BRCA1 is associated with BARD1, and this association mediates the ubiquitylation of γ-tubulin, which is important for maintaining the numeral integrity of centrosomes. The *BRCA2* gene is another causative gene of familial breast/ovarian cancer and its protein product functions in homologous recombination (HR) repair (Venkitaraman, 2002). The loss of *BRCA2* results in centrosome amplification (Tutt et al., 1999), implying a relationship between a defect in DNA repair and the abnormal amplification of the centrosomes. HR repair is mediated by several proteins including RAD51, and the downregulation of RAD51 leads to centrosome amplification (Bertrand et al., 2003). The reduced expression or loss of XRCC2, XRCC3, and RAD51B-D, which are other HR components, induces centrosome amplification and chromosome instability (Griffin et al., 2000; Smiraldo et al., 2005; Date et al., 2006; Renglin Lindh et al., 2007; Cappelli et al., 2011).

Centrosome amplification induced by DNA damage occurs during a prolonged G2 phase (Dodson et al., 2004). A centrosome-autonomous signal that involves centriole disengagement causes centrosome amplification in G2 phase after DNA damage (Inanç et al., 2010), suggesting that genotoxic stress can decouple the centrosome cycle and chromosome cycle.

The active nucleocytoplasmic transport of proteins is mediated by the nuclear localization signal (NLS) and nuclear export signal (NES) (Turner & Sullivan, 2008). NLS-containing proteins are transported from the cytoplasm to the nucleus, whereas NES-containing proteins are exported from the nucleus to the cytoplasm by XPO1, the human homolog of yeast Crm1. The inhibition of XPO1 causes centrosome amplification via the disruption of the nucleocytoplasmic transport of NPM (Forgues et al., 2003; Shinmura et al., 2005; Wang et al., 2005). XPO1 is involved in the centrosomal localization of various proteins (Han et al., 2008). Importin β and RANBP1 are other proteins involved in nucleocytoplasmic transport, and these proteins also have the ability to regulate centrosomes (Di Fiore et al., 2003; Ciciarello et al., 2004).

SGOL1 interacts with protein phosphatase 2A, is localized in the centromere, and prevents the cohesin complex from precocious cleavage at the centromere via the dephosphorylation of SA2, one of the cohesin subunits (Kitajima et al., 2006; Riedel et al., 2006). Clinically, SGOL1 expression is downregulated in colorectal cancer, and SGOL1-knockdown leads to centrosome amplification and chromosome instability in a colon cancer cell line (Iwaizumi et al., 2009; Dai et al., 2009). A SGOL1-P1 transcript containing an exon-skip of exon 3, resulting in the formation of a premature stop codon, is expressed in colorectal cancer, and the overexpression of SGOL1-P1 in a colon cancer cell line resulted in an increased number of cells with aberrant chromosome alignment, precociously separated chromatids, delayed mitotic progression, and centrosome amplification (Kahyo et al., 2011). Furthermore, the overexpression of SGOL1-P1 inhibited the localization of endogenous SGOL1 and cohesin subunit RAD21/SCC1 to the centromere, suggesting that SGOL1-P1 may function as a negative factor to native SGOL1 (Kahyo et al., 2011).

2.4 Relationship between centrosome abnormalities and chromosome instability

Chromosome instability is defined as a persistently high rate of the gain and loss of whole chromosomes (Thompson et al., 2010). Chromosome instability is a major source of

aneuploidy (Lengauer et al., 1997; Rajagopalan and Lengauer, 2004), and chromosome instability is thought to be involved not only in cancer initiation, where aneuploidy may have a causal role, but also in cancer development, where increased rates of chromosome missegregation may enable the clonal expansion of cells with a greater malignant potential (Rajagopalan & Lengauer, 2004; Weaver et al., 2007; Gao et al., 2007; Ganem et al., 2009). Defects in chromosome cohesion, weakened spindle assembly checkpoint (SAC) signalling, impaired microtubule-kinetochore attachment, defects in cell cycle regulation, and centrosome abnormalities can cause chromosome instability (Lingle et al., 1998; Draviam et al., 2004; Thompson & Compton, 2008; Weaver & Cleveland, 2008; Thompson et al., 2010). Regarding centrosome abnormalities, two mechanisms underlying chromosome instability have been proposed. The first mechanism is that centrosome amplification generates chromosome instability by promoting multipolar anaphase, which is an abnormal division that produces more than three aneuploid daughter cells (Nigg, 2002). The other mechanism is that centrosome amplification generates chromosome instability by promoting merotelic kinetochore–microtubule attachments (Ganem et al., 2009; Silkworth et al., 2009). Merotely is a type of error in which single kinetochores attach to microtubules emanating from different poles (Salmon et al., 2005; Cimini, 2008) and is common in cells showing chromosome instability (Thompson & Compton, 2008). Cells with centrosome amplification often coalesce the extra centrosomes during mitosis to ensure that anaphase occurs with a bipolar spindle (Quintyne et al., 2005). The extra centrosomes induce transient multipolar spindle intermediates prior to the coalescence of the centrosomes into bipolar spindles; this event increases the incidence of merotelic kinetochore–microtubule attachments and elevates the chromosome missegregation rates (Ganem et al., 2009; Silkworth et al., 2009). Ganem et al. (2009) showed that the presence of extra centrosomes is correlated with an increase in lagging chromosomes (Figure 3), promoting chromosome missegregation through excessive merotely induced by transient multipolar spindle intermediates. Since merotelic attachments are poorly sensed by the SAC (Salmon et al., 2005; Cimini, 2008), the merotelic attachments arising from centrosome amplification are not fully repaired and give rise to lagging chromosomes during anaphase, possibly leading to missegregation events.

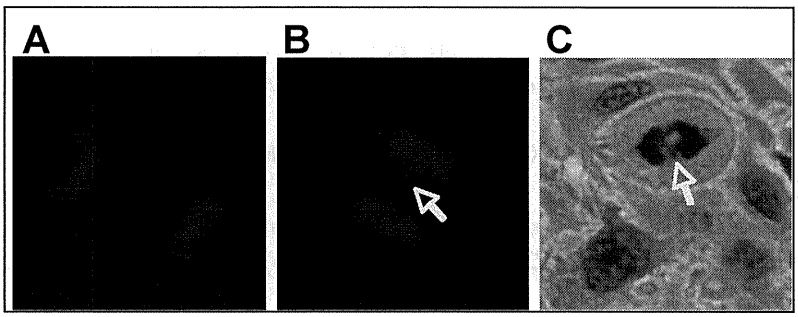

Fig. 3. Lagging chromosomes in human cancer cells.

(A, B) Lagging chromosome formation detected in a B[*a*]PDE-treated H1299 lung cancer cell line. (A) Normal segregation; (B) an anaphase cell showing lagging chromosome formation. The nuclei were stained with DAPI (blue). (C) Lagging chromosomes are shown in a hematoxylin-and-eosin-stained section of a squamous cell carcinoma of the lung. In (B) and (C), the arrows indicate lagging chromosomes.

3. Conclusion

The progress in our understanding of the relationship between centrosome abnormalities and cancer during the past 15 years has been enormous. We have learned that centrosome abnormalities are common among diverse human cancers including lung cancer. Many molecules are involved in the control of the numeral and/or functional integrity of centrosomes, and the abrogation of these mechanisms results in centrosome abnormalities, which promote chromosome instability. From a therapeutic standpoint, anti-cancer drugs targeting the centrosome have now been developed (Mazzorana et al., 2011). Future studies using a genome-wide approach and new scientific technologies will further increase our knowledge of the role of the centrosome in human cells, and such knowledge will likely help to establish effective cancer therapies.

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