Loss of the tumour-suppressor genes CHK2 and BRCA1 results in chromosomal instability

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Abstract
CHK2 (checkpoint kinase 2) and BRCA1 (breast cancer early-onset 1) are tumour-suppressor genes that have been implicated previously in the DNA damage response. Recently, we have identified CHK2 and BRCA1 as genes required for the maintenance of chromosomal stability and have shown that a Chk2-mediated phosphorylation of Brca1 is required for the proper and timely assembly of mitotic spindles. Loss of CHK2, BRCA1 or inhibition of its Chk2-mediated phosphorylation inevitably results in the transient formation of abnormal spindles that facilitate the establishment of faulty microtubule–kinetochore attachments associated with the generation of lagging chromosomes. Importantly, both CHK2 and BRCA1 are lost at very high frequency in aneuploid lung adenocarcinomas that are typically induced in knockout mice exhibiting chromosomal instability. Thus these results suggest novel roles for Chk2 and Brca1 in mitosis that might contribute to their tumour-suppressor functions.

Introduction
Aneuploidy is defined as a chromosome content that deviates from the euploid chromosome number and represents a major hallmark of human cancer. In cancer cells, aneuploidy is typically generated by the perpetual gain or loss of whole chromosomes during mitosis and this phenotype is referred to as CIN (chromosomal instability) [1,2]. It is not completely clear whether CIN is a cause or rather a consequence of tumorigenesis, but recent work has shown that CIN can directly contribute to tumour development [3]. In particular, several knockout mice modelling CIN have demonstrated that the experimental induction of aneuploidy can cause the formation of tumours. Intriguingly, lung adenocarcinomas are the predominant form of cancer observed in these mice, suggesting that lung epithelial cells might possess a particularly high propensity to develop tumours in response to CIN [3].

The molecular mechanisms generating CIN in human cancer and the mechanisms of CIN-induced tumorigenesis are not well understood, but are clearly a central subject of cancer research. Since chromosome missegregation takes place in mitosis, it is conceivable that the analysis of the abnormal progression of mitosis must be the key in understanding the molecular basis of CIN.

Molecular mechanisms of CIN
Possible defects that result in reduced mitotic fidelity include abnormal chromosome cohesion, a compromised SAC (spindle assembly checkpoint), faulty kinetochore–microtubule attachments, the formation of aberrant mitotic spindles and supernumerary centrosomes (reviewed in [4]) (Figure 1).

The premature resolution of sister chromatid cohesion is a mechanism that might lead to the untimely separation of sister chromatids, thereby enhancing the possibility of chromosome missegregation and aneuploidy. In fact, chromatid cohesion defects can be detected in human colon cancer cells, and loss of securin or overexpression of separase, both of which are key regulators of chromatid cohesion, can lead to CIN and tumorigenesis [5–7].

Premature separation of sister chromatids can also be mediated by a compromised SAC. The SAC represents a mitotic surveillance mechanism preventing the onset of anaphase until all chromosomes are properly attached to the mitotic spindle and tension is generated across the sister kinetochores [8]. Various SAC proteins, including Mad1 (mitotic arrest-deficient protein 1), Mad2, Bub1 (budding uninhibited by benzimidazoles 1), BubR1 (Bub1-related kinase 1) and Bub3, are specifically recruited to unattached or tensionless kinetochores, resulting in the formation of a mitotic checkpoint complex that inhibits the ubiquitin ligase activity of the APC/C (anaphase-promoting complex/cyclosome). The APC/C, in turn, is responsible for the mitotic ubiquitination of securin and cyclin B, the degradation of which is required for the onset of anaphase and the exit from mitosis [9]. Thus the SAC-mediated inhibition of the APC/C protects cells from untimely separation of sister chromatids and from premature exit from mitosis. Therefore the SAC is essential for the maintenance of chromosomal stability. Importantly, the analyses of several SAC-knockout mice have revealed a link between a weakened SAC, CIN and tumorigenesis [3]. However, mutations in SAC genes are rarely found in human tumours and most cancer cells exhibiting CIN have a robust

Key words: chromosomal instability, lung adenocarcinoma, mitosis, tumour-suppressor gene.

Abbreviations used: APC/C, anaphase-promoting complex/cyclosome; BRCA1, breast cancer early-onset 1; Bub, budding uninhibited by benzimidazoles; CHK2/Chk2, checkpoint kinase 2; CIN, chromosomal instability; MAD1/Mad, mitotic arrest-deficient protein; WAG, mitotic centrome-associated kinase; FRB, Polo-like kinase 1; SAC, spindle assembly checkpoint.

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Figure 1 | Mechanisms of CIN
Possible mitotic defects that can result in CIN include reduced chromatid cohesion (A), a compromised SAC (B), microtubule-kinetochore malattachments (C) and supernumerary centrosomes (D). All of these mechanisms result in reduced mitotic fidelity that is either caused by the premature entry into anaphase in the presence of not completely aligned chromosomes or by the generation of isolated lagging chromosomes during anaphase that are not recognized by the SAC.

Therefore loss of SAC function is unlikely to be a major mechanism for CIN in human cancer.

A more widespread mechanism of CIN is represented by the persistence of incorrect kinetochore–microtubule attachments associated with the generation of isolated lagging chromosomes during anaphase [12,13]. In particular, syntelic (both kinetochores are connected to spindle microtubules emanating from one pole) and merotelic (one kinetochore connected to spindle microtubules emanating from both poles) microtubule attachments, which are not recognized by the SAC, can significantly reduce the fidelity of mitosis [14]. Therefore correction mechanisms are necessary for resolving malattachments before the onset of anaphase to allow accurate chromosome segregation.

Importantly, cancer cells with CIN often exhibit hyperstable kinetochore–microtubule attachments that might be associated with a reduced correction capacity, and this can promote the appearance of lagging chromosomes and missegregation during anaphase [18].

In a normal cell, bipolar spindle formation favours correct bi-oriented chromosome attachments, but abnormal spindle morphologies in cancer cells, however, might be an important source for chromosome malattachments. For instance, it has been recently shown that supernumerary centrosomes cause the formation of transient multipolar spindles, which promote the malattachment of chromosomes to spindle microtubules followed by chromosome missegregation [19].

Interestingly, multipolar spindle intermediates are typically resolved by centrosome clustering at the poles, and this allows the assembly of bipolar anaphase spindles and chromosome segregation, albeit with reduced fidelity. Similar alterations in cancer cells resulting in transient spindle abnormalities might contribute significantly to CIN, but are obviously difficult to detect.

There are several possible mechanisms of CIN and it is conceivable that subtle defects in the normal mitotic progression, such as transient spindle abnormalities rather than severe mitotic abrogation, might be the major source of CIN in human cancer [4]. Thus it is of great importance to define those subtle mitotic alterations and to identify the underlying gene alterations in human cancer in order to understand the mechanisms of CIN and tumour development.

CHK2 (checkpoint kinase 2) is required for the maintenance of chromosomal stability by ensuring proper and timely mitotic spindle formation
CHK2 is a candidate tumour-suppressor gene that has been implicated previously in the DNA-damage response pathway [20]. It encodes a kinase that is activated in response to DNA strand breaks and it was suggested that Chk2 might positively regulate the tumour-suppressor protein p53 by direct phosphorylation. However, it is currently unclear whether this regulation indeed applies to human somatic cells and what the exact function of Chk2 after DNA damage is [21–23].

During our efforts to identify putative tumour-suppressor genes that are required for the maintenance of chromosomal stability we, unexpectedly, found that CHK2 represents such a gene. We found that loss of CHK2 or abrogation of its kinase activity induces aneuploidy in otherwise chromosomally-stable human colon carcinoma cells, indicating that the loss of CHK2 is directly linked to CIN [24]. Moreover, even a partial repression of CHK2 is sufficient to induce aneuploidy in non-transformed human fibroblasts. Importantly, similar analyses with isogenic TP53 (tumour protein 53)-deficient cells showed no alterations of the karyotype and loss of CHK2 was not associated with DNA damage, suggesting a novel, p53- and DNA-damage-independent function of Chk2 in mitosis and for the maintenance of chromosomal stability.

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Indeed, detailed analyses of the progression of mitosis revealed a requirement of Chk2 for accurate spindle assembly and for timely progression of mitosis. In fact, aberrant spindle structures, which are associated with chromosome alignment defects and a mitotic delay, are apparent in CHK2-deficient cells. Intriguingly, live-cell analyses revealed that spindle formation defects appear only transiently and are resolved before anaphase onset. These transient spindle abnormalities most probably facilitate merotelic chromosome attachments responsible for the elevated number of lagging chromosomes that are observed in the absence of CHK2. Indeed, the generation of lagging chromosomes and CIN can be suppressed by expression of the microtubule-destabilizing kinesin MCAK, suggesting that chromosome malattachments are the source of lagging chromosomes and CIN after loss of CHK2 [24]. Thus, together with previous work (reviewed in [4]), these studies strongly suggest that chromosome malattachments facilitated by transient abnormal mitotic spindle formation induced by the loss of CHK2 might be an important source of CIN in human cancer.

**CHK2 is frequently lost in human lung adenocarcinomas**

The experimental induction of CIN in various mouse knockout models has demonstrated a direct link between CIN and tumorigenesis [3]. Intriguingly, the generation of aneuploidy, for example after heterozygous knockout of the SAC gene MAD2, results predominantly in the induction of lung adenocarcinomas, the most frequent form of lung cancer in humans [3,25]. Thus lung epithelial cells might be especially prone to transformation in response to CIN and therefore it is of particular interest to identify gene alterations in lung adenocarcinomas that are associated with CIN. Given the novel role of CHK2 in the maintenance of chromosomal stability, we analysed CHK2 in human lung cancer [24]. Mutations in the CHK2 gene have been identified previously in various cancers, including colon, breast and lung cancer, but at rather low frequency [26]. We investigated the expression level of CHK2 in human lung cancer specimens by immunohistochemical analyses and found a loss of CHK2 expression in approximately half of the lung cancer tissues analysed. Most importantly, the Chk2 protein was found to be lost preferentially in lung adenocarcinomas [24]. Thus we suggest that CHK2 might represent an important tumour-suppressor gene in lung adenocarcinomas that is required for the maintenance of chromosomal stability.

**The tumour-suppressor protein BRCA1 (breast cancer early-onset 1) is a relevant mitotic target of Chk2 and is required for chromosomal stability**

It has been shown previously that the Chk2 kinase is activated in response to DNA damage and can phosphorylate the tumour-suppressor protein BRCA1, which might be important for subsequent DNA repair [27]. Intriguingly, we found that Chk2 kinase activity is clearly required for a normal mitotic spindle assembly and for ensuring chromosomal stability. Accordingly, Chk2 kinase activity is elevated in mitotic cells, even in the absence of DNA damage. Moreover, during mitosis Chk2 seems to phosphorylate BRCA1 on the same residue following DNA damage (on Ser538) and this phosphorylation is essential for the normal progression of mitosis and for accurate chromosome segregation. Loss of BRCA1 completely mimics the mitotic defects seen after loss of CHK2, indicating that Chk2 might positively regulate BRCA1 for its mitotic function [24]. These observations fit well with previous studies that showed a possible involvement of BRCA1 in mitotic spindle assembly. Interestingly, BRCA1 is localized at mitotic centrosomes where it might regulate spindle assembly factors, including NuMa (nuclear mitotic apparatus protein), RHAMM (receptor for hyaluronic acid-mediated motility), TPX2 (target protein for *Xenopus* kinesin-like protein 2) and Nlp [28,29]. In addition, the ubiquitin ligase activity of BRCA1 appears to be required to control centrosome dynamics and function by targeting γ-tubulin, suggesting an even more complex role of BRCA1 in the function of mitotic centrosomes [30]. However, it remains to be seen whether the Chk2-mediated phosphorylation of BRCA1 controls its ubiquitin ligase activity at centrosomes and whether BRCA1 is the only target for Chk2 in mitosis. Nevertheless, the Chk2–BRCA1 axis clearly fulfils an important role for mitotic spindle assembly and for the maintenance of chromosomal stability (Figure 2).

**Open questions**

The novel and unexpected role of CHK2 and BRCA1 for the proper mitotic progression and chromosomal stability raises several interesting points that are eagerly awaited to be addressed. It is currently not clear what the exact function of the Chk2–BRCA1 axis in mitosis and for mitotic spindle assembly is. Since BRCA1 has been implicated in the regulation of centrosomal proteins that actively participate in spindle assembly [28–30], it is obviously important to ask whether Chk2-mediated phosphorylation contributes to this regulation. Moreover, Chk2 might directly regulate the intrinsic E3 ligase activity of BRCA1 that has been shown to be involved in proper microtubule nucleation in mitosis [31]. In this regard, it is important to note that another mitotic kinase, Aurora-A, can phosphorylate BRCA1 on Ser538 [32], and this modification might play an important role in the regulation of its mitotic ubiquitin ligase activity of BRCA1 [33]. Thus it will be interesting to dissect the roles of the two mitosis-associated phosphorylations on BRCA1 mediated by Aurora-A and Chk2.

Another important issue is the evaluation of the network responsible for the activation of Chk2 in mitosis. After DNA damage, Chk2 is phosphorylated and activated by the ATM (ataxia telangiectasia mutated) kinase on Thr68 [20], and we found the same residue to be phosphorylated.
Chk2 and BRCA1 are required for the maintenance of chromosomal stability

In normal mitotic cells (left-hand panel), active Chk2 (as indicated by its activation-associated phosphorylation on Thr\(^{68}\) and Thr\(^{383/387}\)) phosphorylates BRCA1 on Ser\(^{988}\). This phosphorylation is essential for normal spindle assembly and for accurate chromosome segregation, possibly mediated by the regulation of centrosomal proteins. After the loss of CHK2 (right-hand panel), BRCA1 cannot be phosphorylated on Ser\(^{988}\) anymore, leading to aberrant spindle structures, which facilitate chromosome malattachments resulting in the generation of lagging chromosomes during anaphase. Thus loss of CHK2 or BRCA1 promotes chromosome missegregation, leading to aneuploidy. Examples of immunofluorescence microscopy experiments showing mitotic spindles and chromosome segregation in CHK2-proficient and -deficient cells are given [mitotic spindles, α-tubulin (green); kinetochores, CREST (red); chromosomes, 4′,6-diamidino-2-phenylindole (blue); scale bar, 10 μm]. The persistence of lagging chromosomes after loss of CHK2 (indicated by the arrow) inevitably results in aneuploidy, as depicted by the broad distribution of individual chromosome number in HCT116 and isogenic CHK2-deficient cells. Data are reprinted from [24], with permission.

In mitosis [24]. Is there a possible cross-talk of the DNA damage signalling pathway and mitotic regulation? To answer this question, it will be essential to identify the kinase that phosphorylates Chk2 during mitosis. Interestingly, it has been found that the SAC kinase Mps1 as well as the mitotic Plk1 (Polo-like kinase 1) can directly phosphorylate Chk2 [34,35], suggesting that the SAC and Plk1 might functionally interact with Chk2. Clearly, more work is required to investigate these potential branches of Chk2 regulation in mitosis.
Since loss of the Chk2–BRCA1 pathway induces chromosome malattaches that obviously cannot be resolved, it will be also an important issue to clarify whether these malattaches that result in lagging chromosomes simply ‘overload’ the correction capacity or if the Chk2–BRCA1 axis is actively involved in the chromosome attachment correction machinery. The latter would imply a possible functional interplay of Chk2–BRCA1 and the Aurora-B kinase or MCAK at kinetochores.

Answering these key questions will help to understand the role of the Chk2–BRCA1 pathway in the maintenance of chromosomal stability.

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References

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