CIN a cause, a consequence or something in between in tumour development

Abstract

Chromosome instability (CIN) is the process that leads to missegregation of chromosomes during cell division. CIN increases the likelihood that upon each cell division daughter cells end up with an aberrant number of chromosomes a status defined as aneuploidy. Two out of three solid tumours exhibit aneuploidy. While CIN is observed in the majority of tumours, mutations in genes that are involved in faithful chromosome segregation are rare. Furthermore, in vitro studies show that CIN decreases cellular fitness and is often detrimental. In vivo studies indicate a role for CIN in the development of tumours. However, if CIN is an initiator or facilitator in tumour development remains to be defined. CIN renders an increased rate of acquiring mutations due to chromosomal missegregation. Thus, the proposed role of CIN might be as the mechanism that promotes the transformation of non-malignant cells to malignant cells.

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Introduction

The process of cell division is a highly regulated process, for each cell division chromosomes must be duplicated and correctly segregated over the emerging daughter cells. When this process fails, the daughter cells might end up with too few or too many chromosomes. Cells that contain an aberrant number of chromosomes are termed aneuploid. Von Hanseman was the first to report on aberrant numbers of chromosomes in carcinoma samples in 1890. Additionally, Boveri showed that aneuploidy has detrimental effects on cell development and survival (reviewed by[1]). Together, these two observations led in the early 20th century to the hypothesis that aneuploidy may induce cancer and developmental defects.

Aneuploidy is a common form of genetic abnormality in cancer and affects two out of three solid tumours[2]. Cells acquire an aneuploid karyotype through a process defined as chromosomal instability (CIN). However, aneuploidy can occur without CIN. Systemic aneuploidy are the leading cause of miscarriages in humans[3]. There are few systemic aneuploid karyotypes which are compatible with life, e.g. down syndrome (trisomy 21) and klinefelter (XXY) syndrome.

In this essay I will discuss the role of aneuploidy and CIN in tumourigenesis and tumour progression. I describe the mechanisms which affect faithful chromosome segregation and lead to aneuploidy. I discuss what the effects of CIN are on cellular proliferation and growth. In addition, I will discuss different mice models created to study CIN. I will concentrate on mice models only, as this model is closely related to humans and it is proven to be a good model to study tumourigenesis. The main question of this essay is whether CIN initiates tumourigenesis, provides beneficial environment or is a side effect of tumour growth.

The role of CIN in tumourigenesis and tumour progression is not completely defined and still strongly debated. The discovery of oncogenes and tumour suppressor genes seemed to offer an explanation for the mechanism by which aneuploidy causes cancer. Namely, aneuploidy induces gain of oncogenes and loss of tumour suppressor genes and therefore induces tumourigenesis. This hypothesis did not result in a consensus on the molecular mechanism of tumourigenesis and is still investigated nowadays. It has been argued that aneuploidy is irrelevant to tumourigenesis[4], while others groups support the hypothesis that aneuploidy contributes to tumour progression but not tumour initiation[5]. Due to prevalence of aneuploidy in tumours, three different roles could be contributed CIN, it could initiate, facilitate or CIN could be a consequence of tumour growth. Clarification of CIN on tumour growth might elucidate if CIN is a *bona fide* target for cancer therapy.

Acquiring aneuploidy

The cell cycles is divided in five different phases: G0, G1, S, G2 and mitosis.

G0, G1, S, G2 can collectively be called interphase, and the cell spends most of its life in interphase. During the S phase, DNA is replicated in preparation for mitosis. Mitosis is the phase where the cell divides into two daughter cell and for correct cell division the cell must pass through certain mitotic phases. Mitosis consists of five morphologically distinct phases: prophase, prometaphase, metaphase, anaphase and telophase. The different phases serve a specific role in the process of cell division. During prophase, the replicated chromosomes are condensed, each duplicated chromosome consist of two identical sister chromatids which are joined by the centromere. DNA binding proteins bind to the chromosomes and regulate the process of condensation. Cohesin is a DNA binding protein complex that is involved in regulating sister chromatid cohesion by forming a ring around the two sister chromatids. Additionally, during prophase centrosomes start to move towards opposite poles to form the mitotic spindles. During prometaphase, the nuclear membrane is broken down and the microtubule spindles can now reach and pull on the sister chromatids. Microtubules pull the sister chromatids apart by binding to the kinetochores on opposite of the sister chromatids. Correct chromosome segregation requires the alignment of the sister chromatids on the metaphase plate or cell equator. Sister chromatids end up on the metaphase plate by a stochastic attachment and detachment of microtubule. This process can be described as a 'tug-of-war' between the opposite spindle poles. Followed by the prometaphase is the metaphase. During metaphase the "tug-of-war" continues until chromosomes align on the cell metaphase plate. Once chromosomes are aligned and tension on kinetochore is balanced the cell progresses into anaphase. In anaphase, chromosomes segregate to opposite poles of the cell to form new nuclei. In telophase the newly segregated chromosomes are enveloped by a nuclear membrane followed by cytokinesis. Figure 1 shows schematically an overview of the different phases of mitosis.

The process of chromosomal segregation is controlled by several independent mechanisms[6]. CIN disrupts faithful chromosomal segregation and thus results in an aneuploid karyotype. This might be due premature initiation of anaphase, defects in sister chromatid cohesion or supernumerary centrosomes. The next section will focus on the processes that lead to chromosomal instability and aneuploidy.

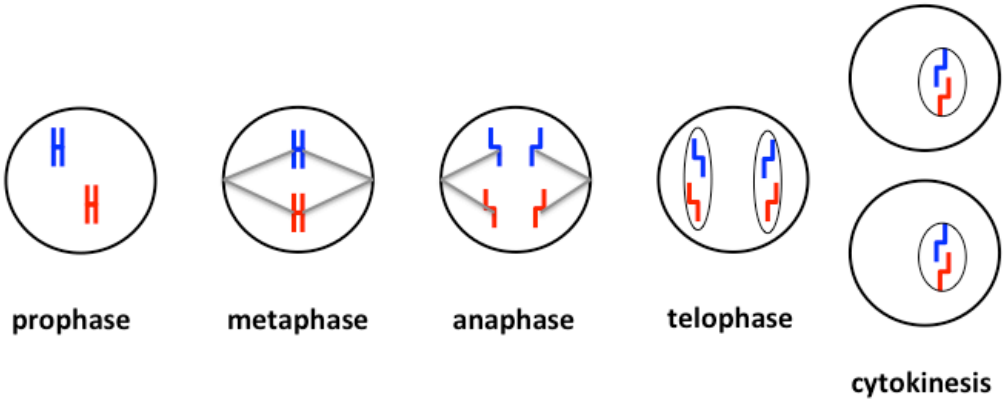

Figure1: schematic summary of in mitosis phases.
Prophase, nuclear membrane is broken down and mitotic spindles start to form. Prometaphase, (not depicted here) tug of war of chromosomes which result in alignment of chromosomes on cell equator. Metaphase, cells are aligned on metaphase plate and tension on chromosomes is balanced. Anaphase, cohesion between sister chromatids is lost and chromosomes move to opposite poles. Telophase, new nuclear membrane envelops the chromosomes followed by cytokinesis.

Mitotic checkpoint

The mitotic checkpoint, also known as spindle assembly checkpoint (SAC), regulates mitotic progression in response to attachment and tension on the kinetochore by the microtubules[7]. Mitotic checkpoint proteins are recruited to the kinetochores of unattached chromosomes. The SAC is actively preventing the initiation of anaphase when there is no tension or attachment on the kinetochore. In case of no tension SAC proteins are bound to the kinetochore. Proteins that are bound to the kinetochore are mitotic arrest deficient 1 (MAD1), mitotic arrest deficient 2 (MAD2), mitotic checkpoint protein BUB3, and BUB1-related 1 (BUBR1). Checkpoint kinase aurora B is concentrated in the proximity of the centromere and is involved in attachment and detachment of microtubule spindles to the kinetochores[8]. MAD1 is via protein-protein bindings associated to the kinetochore where it is required for MAD2 conformational change. MAD2 exist in two distinct conformations open (inactive) and closed (active) MAD2. Kinetochores actively recruit open MAD2 when not attached to mitotic spindles. In proximity to the kinetochores, open MAD2 is converted to closed MAD2 which then binds to cell division cycle protein 20 homolog (CDC20). MAD2 and CDC20 are subunits of the mitotic checkpoint complex(MCC)[9]. The MCC binds and thereby inactivates anaphase promoting complex (APC/C). APC/C is a multi-subunit E3 ligase that upon activation targets a number of proteins including securin and CDK1. The APC/C induces the degradation of securin which results in the release and activation of seperase[10]. Activated se parase hydrolyses cohesin which results in the loss of sister chromatid cohesion. Active APC/C, inactivates cyclin-dependent kinase-1 (CDK1) which releases cyclin B, released cyclin B

will be degraded by the proteasome (Figure **2**). The inactivation of CDK1 and the degradation of cyclin B initiate anaphase[10]. One single unattached kinetochore has such a dominant SAC signal that progression into anaphase is prevented[11]. A dysfunctional SAC due to mutations results in CIN. For example, depletion of MAD2 prevents the formation of the MCC. The MCC prevents premature initiation of anaphase. Premature initiation of anaphase increases the probability of missegregation[12]. Taken together, a functional SAC is essential for faithful chromosome segregation.

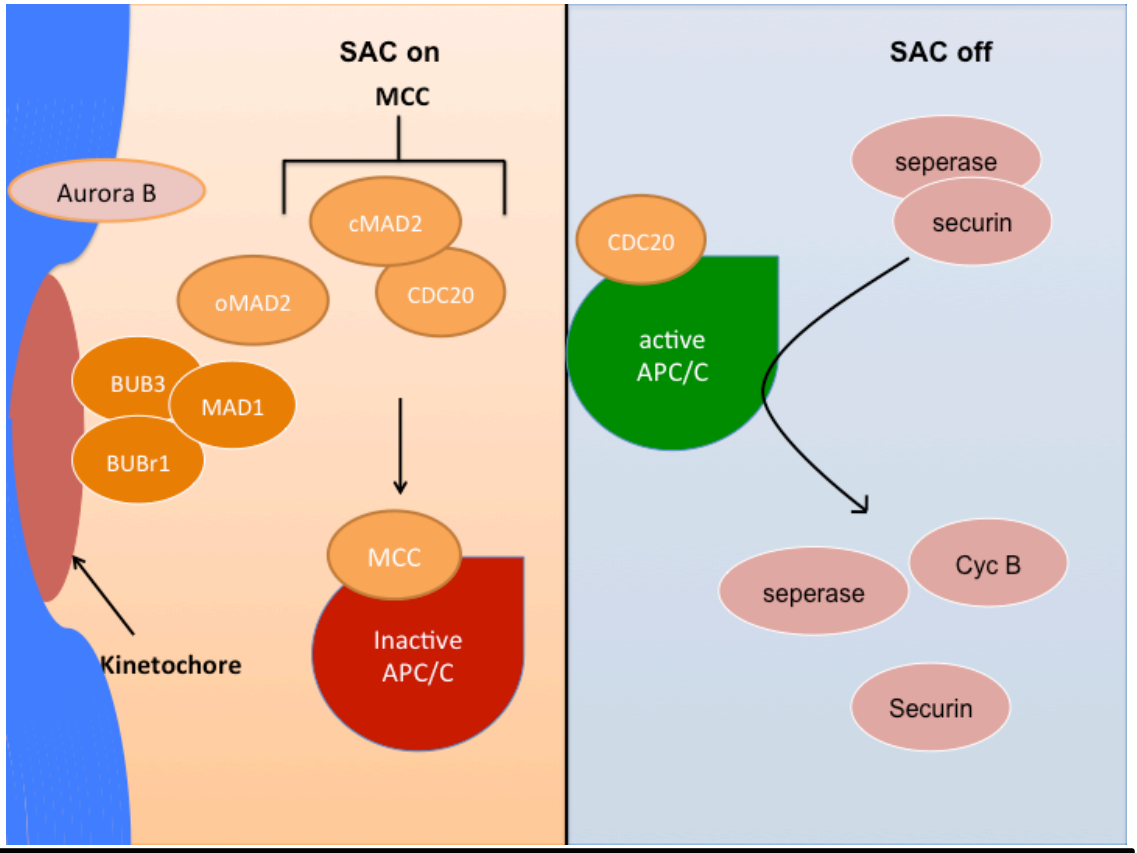

Figure 2: schematic overview spindle assembly checkpoint(SAC).
SAC is switched on when there is no attachment and tension on the kinetochore. BUB3, BUBr1 and MAD1 bind to the kinetochore which enables the conformational change of open MAD2 into closed MAD2 (cMAD2). cMAD2 and CDC20 bind and form mitotic checkpoint complex (MCC) which inhibits the APC/C. SAC is switched off when there is tension on the kinetochore and the APC/C is activated. Active APC/C ligates securin and seperase and frees cyclin B (Cyc B) which is degraded, the cell can progress to anaphase.

Merotelic attachment

Mitotic errors leading to aneuploidy can occur despite a functional SAC. For example when one kinetochore of a replicated chromosome is attached to microtubule from both centrosomes, resulting in the SAC requirements tension and attachment to be met. Such flawed attachments are called merotelic attachments. Since there is tension on the kinetochore SAC signalling is switched off[13] and there is no detachment of the microtubules. When there is no detachment of the microtubules from the kinetochore a

lagging chromosome is produced. Merotelic attachments are often resolved. However, in some cases the produced lagging chromosome will not align properly on the metaphase plate before the initiation of the anaphase. Therefore, the lagging chromosome can be excluded from both emerging daughter cells or is segregated into one daughter cell resulting in an aberrant number of chromosomes.

Supernumerary centrosome

A second mechanism that may cause aneuploidy with a functional SAC is the abnormal number of centrosomes. Centrosomes are organelles from where microtubules that interact with the chromosomes nucleate. Cells that contain a single centrosome create monopolar mitotic spindles. It is unlikely that all kinetochores are under tension with monopolar mitotic spindles. Since not all kinetochores are under tension SAC signalling is constantly switched on. A constant SAC signal prevents progression into anaphase and results in mitotic arrest. The majority of cells with monopolar mitotic spindles go into apoptosis during prolonged mitotic arrest[14], however a small amount of cells adapt. The adaptation process that enables cells to exit mitosis after prolonged mitotic arrest and produce tetraploid cells is not well understood.

In addition, multipolar mitotic spindles are frequently observed in tumours. Multipolar division occurs due to the over duplication of centrosomes. Multipolar division might give rise to aberrant number of daughter cells hence increased aneuploidy[15]. Multipolar division is often not compatible with life and results in cell death[16]. To avoid cell death, many cancer cells induce supernumerary centrosome clustering into two spindle poles thereby enabling bipolar division[17]. Bipolar division with supernumerary centrosomes have the tendency to produce lagging chromosomes which increases probability of aneuploidy progeny.

Sister chromatid cohesion

To identify a general mechanism that underlies the high incidence rate of aneuploidy observed in human cancers, a panel of aneuploid colorectal cancers was screened on genes involved in chromosome fidelity[18]. This led to the identification of a subset of genes that was mutated in these cancers. Ten out of eleven identified genes were directly linked to sister chromatid cohesion. Hence the hypothesis, defective proteins involved in sister chromatid cohesion renders increased missegregation rates thus aneuploidy. In line with this hypothesis the overexpression of proteins involved in sister chromatid cohesion induces aneuploidy[19]. Cohesin is a four-subunit protein complex that entraps DNA fibres within its ring-shaped structure. Cohesin plays a major role in interphase chromatin organization and sister chromatid cohesion (reviewed by[20]). Mutations in genes encoding for cohesin subunits and other regulators of the complex have been identified in several types of aneuploid tumours[21]. Kleyman and colleagues[22] showed that STAG2 (a

cohesion subunit) promotes the correction of kinetochore microtubules attachment and is involved in chromosome segregations. Taken together, defects in genes that are associated with sister chromatid cohesion might induce aneuploidy.

Microtubules kinetochore binding dynamics

The SAC controls correct chromosome segregation, by measuring tension and attachment on the kinetochore. Microtubules attach to kinetochores and provide tension on the kinetochore. In pro-metaphase the binding of microtubule to the kinetochore is an asynchronous and stochastic process[23]. Until bi-oriented attachment of microtubule and kinetochore is reached, incorrect attachments must be turned over. The detachment of microtubules from kinetochore is likely to be the rate-limiting step in correction of incorrect attachments[24]. As microtubule attachment is stochastic, the correction rate must be greater than the rate of new error formation to maintain mitotic fidelity. Recently, errors in kinetochore microtubule attachment that could cause chromosome missegregation gained more attention[25].

Detachment of incorrect orientated microtubule from the kinetochore creates the opportunity for a correctly orientated attachment. An unstable kinetochore microtubule attachment where microtubules detach frequently should improve the efficiency of error corrections[26]. Indeed, quantifying the stability of microtubules kinetochore attachment revealed that cells with CIN exhibited a hyper stable attachments[27]. The hyper stability leads to incorrect attachment which produces lagging chromosomes and increased missegregation rates. Primary tumours were found to exhibit lagging chromosomes similar to cancer cell lines that display hyper stable kinetochore microtubule attachment[28]. This suggest that CIN in primary tumours may be dependent on binding stability of microtubule to kinetochore attachment. Therefore, microtubule kinetochore stability might provide an answer on the prevalence of anueploidy in cancer cells. However, the exact molecular mechanism that regulates the stability of microtubule kinetochore attachment remains to be defined.

Oncogene-induced mitotic stress

Although it seems straightforward that aneuploid tumours harbour mutations in genes that are involved in faithful chromosomal segregation, surprisingly only a small amount of aneuploid tumours do exhibit mutations that are involved in SAC or sister chromatid cohesion[29]. Hence it is a puzzling observation that two out of three tumours are aneuploid with only a small percentage affected by mutations that render impaired chromosome segregations.

An explanation for why most tumours are aneuploid without mutations in genes that induce CIN is “oncogene-induced mitotic stress”[30]. For example, the inhibition of the tumour-suppressive pathway retinoblastoma-associated

protein (Rb) results in an up-regulation of MAD2[31]. Up-regulation of MAD2 has been shown in different independent studies to facilitates tumourigenesis and tumour progression[31,32]. Additionally, p53 another tumour suppressor, was found to repress MAD2, whereas a mutated p53 results in MAD2 up-regulation[31]. A p53 heterozygote knockout mice displayed CIN and generate aggressive lymphomas[33]. Taken together, Rb and p53 two major tumour suppressor pathways, can both directly be linked to a pathway that leads to CIN during mitosis.

The fact that mutations in oncogenes and CIN are frequently observed in human cancers might indicate a possible link[34]. The over activation of several oncogenes have been associated with CIN[35]. The over activation of B-raf is shown to lead to aberrant number of centrosomes and mitotic abnormalities. Hence, B-raf over activation causes chromosomal missegregation and lead to aneuploidy[36]. Upon amplification of CDK1 similar mitotic defects are observed. Short-term overexpression of CDK1 is suggested to induce CIN and aneuploidy[37].

The “oncogene-induced mitotic stress” model may provide an answer to the observation that mutations in genes that affect the chromosomal segregation machinery are rare whereas the majority of tumours are aneuploid. Hence, aneuploidy in most cases does not arise from mutations that directly effects the chromosomal segregation machinery but rather from mutations that indirectly affect the faithful chromosome segregation.

CIN in cancer

Tumour driving alteration mediated by CIN

CIN has detrimental effects on cellular survival in vitro[38], although CIN is prevalent in the majority of the tumours[29]. This raises the question whether CIN initiates tumourigenesis or is a mere side effect of tumour growth. In the absence or overexpression of MAD2, genomic instability increases. Sotillo and collegues[39] showed in 2007, that when MAD2 is transient overexpressed in mice, these mice develop tumours. Conversely Rowald and colleagues showed in 2016 that the overexpression of MAD2 together with KRAS induces a SAC over activation and delays the onset of tumours compared to the overexpression of KRAS alone. Taken together, MAD2 may induce tumourigenesis and may server as tumour suppressor in certain contexts.

Are there traits of CIN that can be termed as tumour promoting effects? Tumour development is described by stepwise acquisition of malignant features. Hanahan and Weinberg[40] defined eight hallmarks of cancer to better comprehend tumour development: Sustained proliferation signalling, evasion of growth suppressors, resistance to cell death, replicative immortality, adaptation of metabolism, induction of angiogenesis, invasion and metastasis and last evasion of immune destruction. CIN cells are prone to lose and or gain genetic information which might accommodate a sustained proliferation signal. Hence, cells that acquire mutations that sustain proliferation signalling

will outgrow cells that acquire detrimental mutations. Additionally, lagging chromosomes which are produced in the final stage of cell division of CIN cells often become trapped. The trapped chromosomes suffer from DNA double-stranded breaks[41], which are repaired by the non-homologous end joining pathway(NHEJ). NHEJ is known to be an error-prone repair mechanism and therefore results in increased mutations. CIN cells are likely to acquire mutations, these mutations might influence proliferation and growth signalling. Thus, there are traits of CIN that can be described as tumour promoting.

Furthermore, CIN might influence and accelerate tumour progression by remodelling the tumour microenvironment. Elevated chromosome missegregation rates increase the probability that tumour cells acquire traits that evade destruction by the immune-system[42]. Chang and colleagues[43] showed that a tumour with a defective antigen presentation cannot be recognized by the adaptive immune-system and evades destruction. In addition, CIN may lead to tumour cells secreting factors which induce an inflammatory response and promote tumour angiogenesis. Analysis of 20 diploid and 30 aneuploid human colon cancer samples showed a positive correlation between vascular endothelial growth factor (VEGF) expression levels and ploidy status[44]. This suggest that CIN may play a role in genetic alterations that induce angiogenesis. Taken together, CIN may promote tumour progression by inhibition of apoptosis, inducing proliferation and growth and adaptation of the microenvironment.

Tumour suppressive alteration mediated by CIN

Implied by the random nature in which chromosomes gain or lose content, CIN cells might acquire traits that inhibit tumourigenesis. In addition, CIN and aneuploidy are accompanied by a wide range of challenges the cell has to overcome to survive. Studies in cell lines showed that upon induction of CIN the effects are often detrimental for cellular survival (reviewed by[45]). This suggest that cells that exhibit CIN are more likely to acquire detrimental traits than traits that are tumour promoting. For example, in an attempt to accurately segregate chromosomes during mitosis, aneuploid cells prolong their metaphase. A prolonged mitotic arrest may result in the formation of cells with supernumerary nuclei, which proliferate at a slower rate or not at all[39]. Moreover, an unbalanced genome has been shown to lead to a distortion in protein stoichiometry[45].

Analysis of the transcriptome and proteome of aneuploid cells generated by chromosome transfer showed that most genes are expressed in proportion to their copy number[46]. Thus, aneuploid cells have to cope with dramatic changes in cellular protein levels. Many proteins that are subunits of protein complexes are unstable unless bound to their partners, and will often bind to cellular chaperones until they have formed the complex[47]. Since stoichiometry levels are affected in aneuploid cells the burden on the

chaperone system is increased. To maintain a tolerable cellular environment it is required to degrade misfolded and excess proteins. Chaperones together with proteasomal degradation and autophagy prevent cellular toxicity by reducing excess- and misfolded proteins. In aneuploid cells, these protein quality control systems can become overloaded which result in a state of proteo-toxicity (reviewed by[48]).

Aneuploid cells exhibit an up-regulation of cellular stress response including up-regulation of protein chaperons[49] and up-regulation of lysosome-mediated degradation[46]. The up-regulation of this stress response is required to maintain homeostatic protein levels with disrupted stoichiometry levels. However, an increasing amount of protein translation will eventually lead to the accumulation of miss folded proteins and ER-stress. Thus, aneuploid cells have to up-regulation the stress responses to reduce proteo-toxicity which results in a decreases cellular fitness.

Phenotypes of CIN models.

Since the discovery of aneuploidy in cancers cells in 1890 there is an increasing body of knowledge on aneuploidy and CIN. Studying CIN in cancer cell lines will not provide an insight on the role of CIN in tumour development. Cells from Cancer cell lines are always end-point cancer, these cells are already malignant. A different model is required to elucidate how malignant traits are acquired via CIN. To investigate the role of CIN in cancer development animal models were created.

To provoke CIN in vivo a wide range of models are designed to target proteins involved in faithful chromosomal segregation. The MAD2 knockout mouse was one of the first CIN models[50]. Mutations in genes coding for SAC proteins are attractive models to induce CIN in vivo since SAC has such a major role in mitosis[7]. However, the physiological relevance is debatable, as only a small amount of human tumour cells lose genes coding for SAC proteins[29]. A second option often used is interfering with kinetochore integrity. This is done by deletion of structural components of the kinetochore or by inducing hyper stabilization of microtubule kinetochore attachment[32]. Additionally, mutations in genes causing centrosome over duplication are used to induce lagging chromosomes. The overexpression of polo like kinase 4 (PLK4) causes centrosome over duplication and results in tumourigenesis[51]. The disruption of the cohesin complex is another approach to induce CIN in vivo. Modelling a defect cohesion complex is created through deregulating of upstream players for examples Rb or p53[33,52].

Homozygous knockout models

The challenge in modelling CIN in vivo is that the vast majority of all the models have proven to be embryonic lethal. Full inactivation of targeted genes resulted in embryonic lethality however dependent on the genotype, latency of death varied (reviewed by[53]). The first CIN model, the MAD2 knock out mice

was embryonic lethal[50]. These animal models are not suitable to study the effects of tumour development since the majority of these CIN models are embryonic lethal.

Heterozygote knockout models

To bypass homozygous knockout induced embryonic lethality, heterozygous knockout mice were designed. The heterozygote mice had full body knockout which results in an overall lower protein level. Heterozygotes MAD2 knockout mice developed in 30 % lung tumours with an average latency of 18 months[12]. The latency in which the mice acquired the tumour was relatively long ranging from 12 to 24 months. Additionally, in most cases only half or even less of the mice population acquired tumour phenotypes[53]. Thus, the knockout of genes inducing CIN provides a weak model to study tumourigenesis. Expression levels of CIN-provoking genes appear to be a better predictor of tumour incidence. In case of overexpression of CIN-driving proteins to high level such as MAD2 overexpression caused a stronger tumour phenotype than heterozygote knockout. An explanation for this is, when proteins are overexpressed proteins levels are more strongly affect compared to a heterozygous knock out where a reduction of 50% is often observed. These findings do not provide a clear answer whether CIN induces tumourigenesis. A tumour phenotype is observed in approximately half of the population with a latency over a year. Thus, CIN might induce tumourigenesis although additional mutations are required.

Chemically challenged CIN models

To asses whether CIN is a predisposition for cancer, CIN mice were challenged with various carcinogens. For example, MAD1 heterozygous knock out mice were treated with the carcinogenic vincristine. The challenged MAD1 knockout mice developed in 40% of the cases lung tumours, compared to the control group were no mice developed tumours[54]. Another carcinogenic challenge is the treatment with DMBA, an powerful immuno-suppressor. In CIN models without a tumour phenotype DMBA treatment had a stronger tumour promoting effect than observed in wildtype mice[55]. Surprisingly, there is an interesting observation regarding BUBR1. Baker and colleagues[56] showed that deletion of BUBR1 increases aneuploidy however the overexpression BUBR1 protects against aneuploidy. Interestingly, overexpression of BUBR1 also protected mice for tumourigenesis, even upon carcinogenic challenges mice. Hence, it might be that increased stability of the karyotype protects cells for tumourigenesis. In mice with CIN, carcinogens increase tumour incidence and decrease tumour latency. Thus, these studies with carcinogenic suggest that additional mutations are required for a CIN cells to transform into a tumour cell. These findings indicate, that CIN is predisposition for tumourigenesis.

CIN models in a predisposed background

In addition to chemically challenged CIN models, CIN models with a predisposed background. By silencing tumour suppressor genes such as p53, CIN mice develop tumours with shorter latency than traditional p53 knock out models. Donehower[57] was the first to report on Null(p53-/-) mice and heterozygote (p53+/-) mice. He showed that Null mice developed tumours within 5 months while in heterozygote only half of the mice developed tumours after 18 months. p53 heterozygote combined with Mps1 knock out mice developed tumours rapidly which were lethal in 3 to 4 months[33]. It was often observed in these mice that the one wild type allele of p53 was lost in the tumours. However, the loss of p53 wild type allele cannot solely account for the formation of these tumours. Since these animals showed a shorter latency of tumour development than the traditional p53 knock out mouse. Taken together, these observations suggest a synergetic role CIN and p53 loss in tumourigenesis.

Tumour-suppressive phenotypes of CIN

In some cases CIN models exhibit a tumour suppressive phenotype. For example in a phosphatase and tensin homolog(PTEN) loss driven tumour. Tumour progression of PTEN deficient tumours is delayed in mice that exhibit CIN compared to mice without CIN[59]. The proposed mechanism for this is that due to tumour progression levels of CIN increase which renders detrimental effects. Levels of CIN differ between cells whereas low levels are tolerated high levels may exert insuperable effects. CIN results in proteo-toxic stress and signals a state of stress in the cells[45]. The levels of CIN could be of influence on the level of toxicity, whereas toxic levels increase with the amount of CIN. The hypothesis of CIN tolerance levels was first proposed by Silk and colleagues[60]. To induce CIN a heterozygote knocked out CenpE mice was created. Subsequently the CenpE mice challenged mice with DMBA. It was observed that mice with heterozygote CenpE knockout developed fewer tumours than mice without CenpE knockout[60]. The CenpE knockout induced increasing levels of CIN which were detrimental for cellular survival. This suggest that tumour cells may not be able to cope with increasing CIN levels. Hence, this might provide therapy possibilities where CIN levels are therapeutic increased.

Discussion

A cause and effect relationship between CIN and cancer has been difficult to define. In vitro studies will not provide an answer on tumourigenesis effects of CIN. However, in vitro studies provided insight pathways that are deregulated by CIN. It has been shown that heat-shock-protein activity can play an important role in coping with proteo-toxic stress induced by aneuploidy[61]. Modelling CIN in vivo provided more insight on the development of tumours.

However, full knock out of genes that are involved in faithful chromosome segregation are embryonic lethal and therefore not informative. Heterozygote knock out models are difficult to study since tumour phenotype occur late in life. Therefore it is challenging to distinguish between spontaneous mutations or CIN induced mutations. Human tumours often exhibited over activation of genes involved in chromosomal segregation[39]. Hence, in vivo models overexpressing such genes are a more physiological relevant. A more physiological relevant animal model would contribute to our understanding of CIN in human tumours and the role in tumourigenesis. It is shown by independent studies that challenged CIN mice developed tumours at an increased rate compared to control mice (reviewed by[53]). Hence, the implication is that cells with CIN are predisposed to acquire tumours. CIN may contribute to tumour progression via numerous molecular mechanisms for example by immuno-editing or selection of beneficial mutations. Contribution of CIN to tumour progression is a balanced process where increasing levels of CIN may decreases cellular fitness.

Whether CIN initiates cancer or is a beneficial side effect is a question which remains unanswered. As proposed by Duijf and Benezra[2], the fate of cells that missegregated its chromosomes may be defined in an order of events. An oncogene or tumour suppressor gene is mutated which induces mitotic stress as a first event. This mutation renders CIN which at first decreases cellular fitness. Due to the nature of CIN daughter cells acquire mutations at an increased rate. Increasing the probability of a cell to emerge which can cope with the aneuploid induced stress. The transformation from non-malignant cell into a malignant cell comes with challenges the cell has to overcome. Additional mutations need to occur to overcome these challenges and drive tumour progression, CIN may provide the mechanism on how tumour cells accumulate mutations. To better understand the evolutionary process of transformation of an aneuploid cell to an aneuploid cancer cell, a high resolution genetic screening method is required. Single-cell sequencing provides high resolution data and may provide insights in the evolution of CIN cells to CIN cancer cells[62].

Future perspectives

Due to the dynamic nature of CIN and the ability to adapt the probability of tumour recurrence is high in CIN tumours. Therefore, CIN is often associated with poor clinical prognosis. As CIN is presented with numerous challenges a target of therapy might be to exploit those challenges and increase CIN up to a level where it is detrimental. Aneuploidy causes proteo-toxic stress, by blocking of the protein quality control machinery, proteo-toxicity will increase and may induce cell death. Heat shock protein 90 inhibitors are currently developed as frontline chemotherapeutics[63]. In this study the inhibitor which was given to the patients was well tolerated and showed some anti-tumour activity. According to the authors this served as a clinical proof of concept.

Additional studies of compounds that increase the burden on the protein quality control system may open up new avenues of aneuploid cancer treatment. To find these targets, studying the effect of aneuploidy on the protein quality control is required. The frontier is to elucidate how malignant cells cope with aneuploid induced stress.

Reference:

1. Holland, A. J. & Cleveland, D. W. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat. Rev. Mol. Cell Biol.* **10,** 478–87 (2009).
2. Duijf, P. H. G. & Benezra, R. The cancer biology of whole-chromosome instability. *Oncogene* **32,** 4727–36 (2013).
3. Brown, S. Miscarriage and Its Management. doi:10.1055/s-0028-1087105.
4. Hahn, W. C. *et al.* Creation of human tumour cells with defined genetic elements. *Nature* **400,** 464–8 (1999).
5. Zimonjic, D., Brooks, M. W., Popescu, N., Weinberg, R. A. & Hahn, W. C. Derivation of human tumor cells in vitro without widespread genomic instability. *Cancer Res* **61,** 8838–8844 (2001).
6. Kops, G. J. P. L., Weaver, B. a a & Cleveland, D. W. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer* **5,** 773–85 (2005).
7. Rieder, C. L., Cole, R. W., Khodjakov, A. & Sluder, G. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J. Cell Biol.* **130,** 941–948 (1995).
8. Krenn, V. & Musacchio, A. The Aurora B Kinase in Chromosome Bi-Orientation and Spindle Checkpoint Signaling. *Front. Oncol.* **5,** 225 (2015).
9. De Antoni, A. *et al.* The Mad1/Mad2 complex as a template for Mad2 activation in the spindle assembly checkpoint. *Curr. Biol.* **15,** 214–225 (2005).
10. Musacchio, A. & Salmon, E. D. The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* **8,** 379–93 (2007).
11. Rieder, C. L., Schultz, A., Cole, R. & Sluder, G. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J. Cell Biol.* **127,** 1301–1310 (1994).
12. Michel, L. S. *et al.* MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* **409,** 355–359 (2001).
13. Cimini, D. *et al.* Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *J. Cell Biol.* **152,** 517–527 (2001).
14. Orth, J. D., Loewer, a., Lahav, G. & Mitchison, T. J. Prolonged mitotic arrest triggers partial activation of apoptosis, resulting in DNA damage and p53 induction. *Mol. Biol. Cell* **23,** 567–576 (2012).
15. Brinkley, B. R. Managing the centrosome numbers game: From chaos to stability in cancer cell division. *Trends Cell Biol.* **11,** 18–21 (2001).
16. Ganem, N. J., Godinho, S. a & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460,** 278–82 (2009).
17. Lingle, W. L. & Salisbury, J. L. Altered centrosome structure is associated with abnormal mitoses in human breast tumors. *Am. J. Pathol.* **155,** 1941–51 (1999).

18. Barber, T. D. *et al.* Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 3443–8 (2008).
19. Zhang, N. *et al.* Overexpression of Separase induces aneuploidy and mammary tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 13033–8 (2008).
20. Losada, A. Cohesin in cancer: chromosome segregation and beyond. *Nat. Rev. Cancer* **14,** 389–93 (2014).
21. Guo, G. *et al.* Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. *Nat. Genet.* **45,** 1459–63 (2013).
22. Kleyman, M., Kabeche, L. & Compton, D. a. STAG2 promotes error correction in mitosis by regulating kinetochore-microtubule attachments. *J. Cell Sci.* 4225–4233 (2014). doi:10.1242/jcs.151613
23. Cimini, D., Moree, B., Canman, J. C. & Salmon, E. D. Merotelic kinetochore orientation occurs frequently during early mitosis in mammalian tissue cells and error correction is achieved by two different mechanisms. (2003). doi:10.1242/jcs.00716
24. Carolina, N. Elements of Error Correction in Mitosis : Microtubule Capture , Release , and Tension Capture / Release J /.\. **126,** 1241–1253 (1994).
25. Kabeche, L. & Compton, D. A. Cyclin A regulates kinetochore microtubules to promote faithful chromosome segregation. *Nature* **502,** 110–113 (2013).
26. Godek, K. M., Kabeche, L. & Compton, D. A. Regulation of kinetochore-microtubule attachments through homeostatic control during mitosis. *Nat. Rev. Mol. Cell Biol.* **16,** 57–64 (2015).
27. Bakhoum, S. F. & Genovese, G. Report Deviant Kinetochore Microtubule Dynamics Underlie Chromosomal Instability. *Curr. Biol.* **19,** 1937–1942 (2009).
28. Bakhoum, S. F., Danilova, O. V, Kaur, P., Levy, N. B. & Compton, D. A. Chromosomal Instability Substantiates Poor Prognosis in Patients with Diffuse Large B-cell Lymphoma. **17,** 7704–7711 (2011).
29. Rhodes, D. R. *et al.* Oncomine 3.0: Genes, Pathways, and Networks in a Collection of 18,000 Cancer Gene Expression Profiles 1. *Neoplasia* **9,** 166–180 (2007).
30. Malumbres, M. Oncogene-induced mitotic stress: P53 and pRb get mad too. *Cancer Cell* **19,** 691–692 (2011).
31. Sotillo, R., Schvartzman, J.-M., Socci, N. D. & Benezra, R. Mad2-induced chromosome instability leads to lung tumour relapse after oncogene withdrawal. *Nature* **464,** 436–40 (2010).
32. Kabeche, L. & Compton, D. A. Checkpoint-independent stabilization of kinetochore-microtubule attachments by Mad2 in human cells. *Curr. Biol.* **22,** 638–644 (2012).
33. Foijer, F. *et al.* Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *Proc. Natl. Acad. Sci. U. S. A.* **111,** 13427–32 (2014).
34. Fernández-Medarde, A. & Santos, E. Ras in cancer and developmental diseases. *Genes Cancer* **2,** 344–58 (2011).
35. Kamata, T. & Pritchard, C. Mechanisms of aneuploidy induction by RAS

and RAF oncogenes. *Am. J. Cancer Res.* **1,** 955–71 (2011).
36. Cui, Y., Borysova, M. K., Johnson, J. O. & Guadagno, T. M. Oncogenic B-RafV600E induces spindle abnormalities, supernumerary centrosomes, and aneuploidy in human melanocytic cells. *Cancer Res.* **70,** 675–684 (2010).
37. Nelsen, C. J. *et al.* Short term cyclin D1 overexpression induces centrosome amplification, mitotic spindle abnormalities, and aneuploidy. *J. Biol. Chem.* **280,** 768–776 (2005).
38. Kops, G. J. P. L., Foltz, D. R. & Cleveland, D. W. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 8699–704 (2004).
39. Sotillo, R. *et al.* Mad2 Overexpression Promotes Aneuploidy and Tumorigenesis in Mice. *Cancer Cell* **11,** 9–23 (2007).
40. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **144,** 646–674 (2011).
41. Janssen, A., van der Burg, M., Szuhai, K., Kops, G. J. P. L. & Medema, R. H. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science (80-.).* **333,** 1895–8 (2011).
42. Grivennikov, S. I., Greten, F. R. & Karin, M. Immunity, Inflammation, and Cancer. *Cell* **140,** 883–899 (2010).
43. Chang, C.-C. *et al.* Defective human leukocyte antigen class I-associated antigen presentation caused by a novel beta2-microglobulin loss-of-function in melanoma cells. *J. Biol. Chem.* **281,** 18763–18773 (2006).
44. Cristi, E. *et al.* Tumour proliferation, angiogenesis, and ploidy status in human colon cancer. *J. Clin. Pathol.* **58,** 1170–4 (2005).
45. Torres, E. M., Williams, B. R. & Amon, A. Aneuploidy: Cells losing their balance. *Genetics* **179,** 737–746 (2008).
46. Stingele, S. *et al.* Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol. Syst. Biol.* **8,** 608 (2012).
47. Boulon, S. *et al.* HSP90 and its R2TP/Prefoldin-like cochaperone are involved in the cytoplasmic assembly of RNA polymerase II. *Mol. Cell* **39,** 912–924 (2010).
48. Oromendia, A. B. & Amon, A. Aneuploidy: implications for protein homeostasis and disease. *Dis. Model. Mech.* **7,** 15–20 (2014).
49. Sheltzer, J. M., Torres, E. M., Dunham, M. J. & Amon, A. Transcriptional consequences of aneuploidy. TL - 109. *Proc. Natl. Acad. Sci. U. S. A.* **109 VN-,** 12644–12649 (2012).
50. Dobles, M., Liberal, V., Scott, M. L., Benezra, R. & Sorger, P. K. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell* **101,** 635–645 (2000).
51. Ko, M. a *et al.* Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis. *Nat. Genet.* **37,** 883–8 (2005).
52. Van Harn, T. *et al.* Loss of Rb proteins causes genomic instability in the absence of mitogenic signaling. *Genes Dev.* **24,** 1377–1388 (2010).
53. Simon, J. E., Bakker, B. & Foijer, F. in *Chromosomal Instability in Cancer Cells* 39–60 (2015). doi:10.1007/978-3-319-20291-4_2

54. Iwanaga, Y. *et al.* Heterozygous deletion of mitotic arrest-deficient protein 1 (MAD1) increases the incidence of tumors in mice. *Cancer Res.* **67,** 160–166 (2007).
55. Baker, D. J. *et al.* Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. *J. Cell Biol.* **172,** 529–540 (2006).
56. Baker, D. J. *et al.* Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. *Nat. Cell Biol.* **15,** 96–102 (2013).
57. Donehower, L. a. The p53-deficient mouse: a model for basic and applied cancer studies. *Semin. Cancer Biol.* **7,** 269–278 (1996).
58. Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. *Cell* **149,** 274–293 (2012).
59. Baker, D. J., Jin, F., Jeganathan, K. B. & van Deursen, J. M. Whole Chromosome Instability Caused by Bub1 Insufficiency Drives Tumorigenesis through Tumor Suppressor Gene Loss of Heterozygosity. *Cancer Cell* **16,** 475–486 (2009).
60. Silk, A. D. *et al.* Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumors. *Proc. Natl. Acad. Sci. U. S. A.* **110,** E4134-41 (2013).
61. Donnelly, N., Passerini, V., Dürrbaum, M., Stingele, S. & Storchová, Z. HSF 1 deficiency and impaired HSP 90 -dependent protein folding are hallmarks of aneuploid human cells. *EMBO J.* **33,** 2374–2387 (2014).
62. Bakker, B., van den Bos, H., Lansdorp, P. M. & Foijer, F. How to count chromosomes in a cell: An overview of current and novel technologies. *BioEssays* **37,** 570–577 (2015).
63. Wagner, A. J. *et al.* A phase i study of the HSP90 inhibitor retaspimycin hydrochloride (IPI-504) in patients with gastrointestinal stromal tumors or soft-tissue sarcomas. *Clin. Cancer Res.* **19,** 6020–6029 (2013).