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Mouse Models for Chromosomal Instability

Floris Foijer
European Institute for the Biology of Aging (ERIBA)
University Medical Center Groningen, Groningen
The Netherlands

1. Introduction

Chromosomal instability

Cancer is the result of several genetic alterations that overrule a cell's protection mechanisms against unscheduled proliferation (Hanahan & Weinberg, 2000). As the vast majority of human tumours show chromosomal instability (CIN), CIN is believed to be an important driver and facilitator of oncogenic transformation. CIN can result in structural abnormalities such as focal deletions, amplifications or translocations (structural CIN). Alternatively, CIN causes numerical abnormalities (*i.e.* aneuploidy) with cells showing high rates of losses and gains of whole chromosomes leading to dramatic karyotypic variability between cells. Whereas alterations to single or small groups of oncogenes/ tumour suppressor genes can explain the malignant effect of structural CIN, the cancerous effect of numerical CIN is mostly attributed to the loss of heterozygosity (LOH) of tumour suppressor genes (Jallepalli & Lengauer, 2001, Kops et al., 2005). Structural and numerical CIN often coincide in tumours and numerical CIN can in fact provoke structural CIN as well (Janssen et al., 2011). Although CIN appears to be a potent driver of genomic reorganization, it comes at cost for cells. For instance, numerical CIN in tissue culture cells kills cells within six generations (Kops et al., 2004). Furthermore, in depth analysis of aneuploid yeast strains and mouse embryonic fibroblasts (MEFs) carrying an extra chromosome revealed that aneuploidy slows cell proliferation down and deregulates the metabolic homeostasis (Torres et al., 2007, Williams et al., 2008). However, as the majority of human tumours are aneuploid, cancer cells must have found a way to circumvent the detrimental consequences of CIN. This chapter reviews several mechanisms that can drive numerical CIN and discusses several of the mouse models that were engineered to mimic these conditions with the aim to study the *in vivo* consequences of numerical CIN.

2. Origins of whole chromosome instability

Kinetochores and centromeric proteins

Correct chromosome distribution critically depends on correct attachment of chromosomes to a bipolar mitotic spindle. Defects in the machinery regulating the formation of a proper bipolar spindle have been associated with human cancer and aneuploidy (Fukasawa, 2005, Kops et al., 2005, Yuen et al., 2005). Kinetochores are macromolecular protein structures that

link the centromeric DNA to the mitotic spindle. The inner kinetochore plate is the DNA interacting domain and is comprised of several structural proteins such as the centromeric proteins (CENP). Some of these CENP genes (*e.g. CENPA, CENPF* and *CENPH*) frequently show altered expression in human cancer, underscoring the importance of a precisely balanced protein composition of the kinetochore (Yuen et al., 2005).

The mitotic spindle checkpoint

To ensure the accurate segregation of chromosomes, cells initiate segregation only after the correct attachment of all chromosomes to microtubules of the mitotic spindle. The spindle checkpoint (SAC) operates at the outer kinetochore plate by monitoring the attachment of the kinetochore to the microtubules. Improperly attached kinetochores recruit a family of SAC proteins (Mad1, Mad2, Bub1, BubR1, Bub3, Mps1, Rod and ZW10) (Musacchio & Salmon, 2007). Additionally, CENPE, a motor protein, is recruited to unattached kinetochores, where it is implicated in regulating microtubule attachment (Kops et al., 2005). Even a single unattached kinetochore is sufficient to trigger a robust spindle checkpoint response that prevents premature anaphase onset and thus missegregation of chromosomes (Rieder et al., 1995, Rieder et al., 1994). This robust response is achieved by CDC20 sequestering by the SAC proteins, the specificity factor required for anaphase promoting complex/cyclosome (APC/C) activity. Activation of the APC/C, an E3 ubiquitin ligase, is essential for anaphase progression, as it is responsible for the degradation of several mitotic proteins including Securin and Cyclin B1. Securin degradation leads to activation of Separase, which cleaves Cohesin that glues sister chromatids together. Cyclin B1 degradation results in the inactivation of CDK1 and thereby allows mitotic exit (Karess, 2005, Kops et al., 2005, Musacchio & Salmon, 2007). Thus, precise regulation of APC/C activity by the SAC allows for timely and accurate progression of mitosis preventing numerical CIN.

Tension versus attachment

Kinetochore-microtubule attachment by itself is not sufficient to satisfy the spindle checkpoint. To achieve proper segregation, it is also essential that one kinetochore of a pair is attached to microtubules from one spindle pole and the other kinetochore to the opposite pole (Biggins & Walczak, 2003). This correct geometry of attachment generates tension between the sister chromatids, which is another prerequisite for mitotic progression. Aurora B is implicated in sensing this tension and correcting erroneous attachments (Andrews et al., 2003). In the absence of tension, for instance when both sister chromatid kinetochores are connected to the same centrosome, Aurora B kinase activity remains uninhibited. This results in the release of improper microtubule attachments and allows reattachment of the kinetochores with microtubules emanating from the opposite pole (Andrews et al., 2003, Liu et al., 2009, Musacchio & Salmon, 2007). While details of error correction mechanisms are just emerging, drugs targeting this pathway (*e.g.* Aurora inhibitors) are already in clinical trials for cancer (Jackson et al., 2007).

Centrosomes

Centrosomes are essential for bipolar spindle formation during mitosis, as they are the major microtubule organizing centres of the cell. The presence of two centrosomes is therefore critical for accurate chromosome segregation into two daughter cells. Centrosome number is tightly controlled: centrosomes are duplicated during S-phase and segregated

into the two daughter cells during mitosis (see for detailed review: (Doxsey et al., 2005, Fukasawa, 2005, Nigg, 2007, Tsou & Stearns, 2006)). Defects in these control mechanisms can lead to abnormal centrosome numbers and are thus another cause of numerical CIN. In agreement with this, most tumours exhibit abnormal centrosome numbers (Nigg, 2006, Pihan et al., 1998). It was originally hypothesized that abnormal centrosome numbers would provoke CIN through multipolar mitoses. Although careful tracking revealed that such divisions occasionally do take place, the resulting daughter cells all died in the next cell cycle. It's therefore unlikely that cells undergoing multipolar divisions contribute to a developing tumour. Instead, in a large proportion of the cells supranumeral centrosomes clustered to form one 'supercentrosome' allowing 'normal' bivalent divisions to take place. Such divisions frequently coincided with lagging chromosomes in the next mitosis, presumably the result of merotelic attachments caused by abnormal centrosomal structure (Ganem et al., 2009). Therefore, lagging chromosomes might very well be the driving force behind numerical CIN associated with supranumeral centrosomes.

3. Mouse models for whole chromosome instability

To test whether numerical CIN is an early event in tumourigenesis and thereby infer whether it can be causative of cancer, several proteins playing a role in accurate segregation were disrupted in mice. The results of these studies are discussed below and collectively underscore the importance of the spindle checkpoint in the different aspects of tumourigenesis.

Loss of centromeric (CENP) genes frequently causes embryonic lethality

CENPA, B and C reside at the inner plate of the kinetochore and bridge between the centromeric DNA and the kinetochore-docking microtubules emanating from the centrosomes. Several of these genes have been knocked out in mice to determine their role in the prevention of aneuploidy. Whereas homozygous CENPB loss resulted in no overt phenotypes other than slightly lower testis and body weights (Hudson et al., 1998, Kapoor et al., 1998, Perez-Castro et al., 1998), disruption of CENPA or CENPC led to embryonic lethality in early stages of embryogenesis (E3.5-E6.5). The early embryos show micronuclei, a lowered mitotic index (indicative of mitotic delay), and enlarged nuclei (suggestive of tetraploid cells). Heterozygous mice develop normally and are fertile, although animals were not tested for cancer predisposition using carcinogens or in combination with other predisposing mutations (Howman et al., 2000, Kalitsis et al., 1998). Animal longevity and spontaneous cancer predisposition are currently being tested [Andy Choo, personal communication].

Spindle checkpoint inactivation interferes with mammalian development

The first mouse model for germline SAC inactivation was the Mad2 knockout. Homozygous Mad2 loss results in embryonic lethality at approximately day E6.5 (Dobles et al., 2000). Similar to the Mad2 knockout, BubR1, Bub1, Mad1, Bub3, Rae1 and CENPE inactivation are also incompatible with mammalian development beyond day E6.5-E8.5. In all cases, massive apoptosis of the inner cell mass (ICM) was observed, presumably a consequence of numerous missegregation events in these highly proliferative cells (Baker et al., 2004, Baker et al., 2006, Iwanaga et al., 2007, Jeganathan et al., 2007, Kalitsis et al., 2000, Putkey et al., 2002, Q. Wang et al., 2004, Weaver et al., 2007). Inactivation of the APC/C activating factors

Cdc20 or Cdh1 provoke embryonic lethality as well, but later in gestation with embryos developing up to E12.5 (Garcia-Higuera et al., 2008, Li et al., 2009).

The chromosomal passenger proteins Incenp, Survivin and Aurora B are part of the chromosomal passenger complex. This complex is essential for forming a bipolar spindle by measuring tension during metaphase and is involved in later events in cytokinesis as well (Lens et al., 2006). Knockouts for Survivin or Incenp revealed that ablation of either protein causes embryonic lethality before day E8.5, similar as observed for CENP proteins and SAC protein knockouts (Cutts et al., 1999, Uren et al., 2000).

Does reduced expression of CIN protective genes result in spontaneous tumour formation?

Several cancers show decreased expression of spindle checkpoint proteins, indicating that a partially compromised checkpoint can be the underlying cause for tumour aneuploidy (Weaver & Cleveland, 2006). As homozygous inactivation of SAC components aborts embryonic development well before mid-gestation, heterozygous mice (Mad1, Mad2, BubR1, Bub1, Bub3, Rae1, Cdc20, Cdh1 and Cenp-E) were used to study the *in vivo* consequences of a partially defective spindle checkpoint. In all cases, heterozygotes are born at a Mendelian ratio and none show clear developmental defects, except for a mild hematopoietic defect in BubR1 heterozygotes (Babu et al., 2003, Baker et al., 2006, Garcia-Higuera et al., 2008, Iwanaga et al., 2007, Jeganathan et al., 2007, Kalitsis et al., 2005, Li et al., 2009, Michel et al., 2001, Q. Wang et al., 2004, Weaver et al., 2007). However, despite normal development, Mad2, Mad1 and CENPE heterozygotes develop cancers in a substantial number of the animals (20-30%), albeit relatively late (at 18-20 months of age). Lung tumours are predominant, and CENPE heterozygotes develop hematopoietic malignancies as well (Iwanaga et al., 2007, Michel et al., 2001, Weaver et al., 2007), indicating that Mad1, Mad2 and CENPE function as haplo-insufficient tumour suppressors in the mouse by suppressing CIN. Heterozygousity for the SAC-downstream-targets Cdh1 or Cdc20 predisposes for late tumours as well: up to 50% of the Cdc20 hypomorphic mice develop tumours, mostly in the hematopoietic compartment, whereas Chd1 heterozygotes mainly develop solid tumours in the mammary gland, lungs, liver and testes (Garcia-Higuera et al., 2008, Li et al., 2009). Heterozygous BubR1, Bub1, Bub3 and Rae1, and even Bub3; Rae1 double heterozygotes do not develop any malignancies, suggesting that in those models aneuploidy is not sufficient for tumours to arise (Baker et al., 2004, Baker et al., 2006, Jeganathan et al., 2007, Q. Wang et al., 2004). Finally, similar to SAC knockout heterozygotes, Survivin$^{+/-}$ and Incenp$^{+/-}$ mice are indistinguishable from their wildtype littermates. Whether these animals are cancer-predisposed is currently not known [Andy Choo, personal communication].

In another approach, a hypomorphic allele for Bub1 was created, reducing protein levels down to 20% of wildtype levels. These mice develop several malignancies within 18-20 months of age, mainly lymphomas, lung and liver tumours (Jeganathan et al., 2007). Using the same approach, a hypomorphic allele for BubR1 was engineered (reducing BubR1 protein levels by 90%). Surprisingly, although these mice develop massive aneuploidy in several tissues, they are not cancer-predisposed, suggesting that aneuploidy does not cause cancer *per se* (Baker et al., 2004).

BubR1 hypomorphs develop another phenotype though: they age prematurely, as evidenced by decreased subcutaneous fat and spinal kyphosis (spinal deformation or hump)

and muscle atrophy. The median lifespan of these animals is six months and none of them age over 15 months (Baker et al., 2004), which might obscure a late-in–life cancer phenotype. Bub3; Rae1 double heterozygotes develop a similar phenotype, albeit less severe as these animals survive up to 27 months (Jeganathan et al., 2007). Interestingly, BubR1 levels seem to decline in aging wildtype tissue, which suggests a role for this gene and possibly aneuploidy in natural aging as well (Baker et al., 2004). However, why the premature aging phenotype affects these two knockouts and not other aneuploidy-related mouse models is currently unknown.

Phenotypes of the various mouse models are summarized in Table 1. Taken together, the results show that SAC defects have a profound effect on development and cancer predisposition. Additionally, mouse models targeting the SAC have revealed a potential role for aneuploidy in aging.

Aneuploidy as a collaborating factor in tumorigenesis

Even though mouse models clearly indicate that numerical CIN can contribute to *tumour initiation*, the actual tumour phenotypes of these mouse models are relatively weak, with tumours arising sporadically and relatively late in life. Why is this effect so weak despite the high incidence of aneuploidy in human cancer? One explanation is that the current mouse models rely on partial inactivation of the spindle checkpoint, since complete inactivation is incompatible with embryonic development. Another possible explanation for the weak phenotypes is that aneuploidy requires predisposing mutations in order to be tumorigenic and therefore plays a more important role in *tumour progression*. To test the latter hypothesis, several of the described models were exposed to carcinogenic drugs or combined with other mouse models carrying predisposing mutations in established tumour suppressor genes.

Indeed, tumour phenotypes are exacerbated by carcinogenic insults. For instance, whereas Bub1, Bub3, Rae1 and Bub3; Rae1 double heterozygous mice are not clearly cancer predisposed, DMBA treatment (a known carcinogen) provokes more tumours in all four knockouts than in wildtype mice (Baker et al., 2006, Jeganathan et al., 2007). This is also true for DMBA-treated BubR1 hypomorphs (Baker et al., 2004). Furthermore, BubR1 heterozygotes are more susceptible to the formation of intestinal tumours in response to treatment with the colon carcinogen azoxymethane (Dai et al., 2004). Finally, 40% of Mad1 heterozygous mice treated with Vinicristine (a microtubule-depolymerizing agent) develop tumours whereas control-treated mice do not develop any (Iwanaga et al., 2007). Similarly, numerical CIN synergizes with predisposing mutations. For instance, decreased levels of Bub1 accelerate tumorigenesis in a $p53^{+/-}$ or $Apc^{+/Min}$ background. In this setting, numerical CIN acts by facilitating LOH of the remaining wildtype allele of the tumour suppressor gene (Baker et al., 2009).

Aneuploidy as a tumour suppressor?

Although decreased levels of Bub1 have a profound effect on tumour initiation in p53- of Apc-mutated backgrounds, this synergy is not observed in Rb or Pten heterozygous mice. Paradoxically, Bub1 hypomorphism acts tumour suppressive in $Pten^{+/-}$ mice, with $Pten^{+/-}$; Bub1 hypomorphs developing fewer hyperplastic prostate lesions than $Pten^{+/-}$ mice expressing wildtype Bub1 levels (Baker et al., 2009). Similarly CENPE heterozygousity delays tumourigenesis in a $p19^{Arf}$-deficient background. Whereas normally $p19^{Arf-/-}$ animals develop lymphomas and sarcomas with an average latency of 6-7 months (Kamijo et al.,

1997), tumour latency is delayed to 12 months in CENPE$^{+/-}$ mice. Furthermore, despite a predisposition for hematopoietic malignancies, the number of spontaneous liver tumours in CENPE$^{+/-}$ mice is reduced 3-fold and liver tumour sizes are remarkably smaller. Finally, CENPE heterozygousity also delays DMBA-induced tumours (Weaver et al., 2007). These observations suggest that aneuploidy can delay tumourigenesis as well and that the response to aneuploidy might very well be tissue specific.

Overexpression of genes with a role in chromosome segregation

Diminished Mad2 expression, mutations and even gene loss do occur in cancer, but are infrequent (Percy et al., 2000, X. Wang et al., 2002, X. Wang et al., 2000). More often Mad2 expression is elevated (Alizadeh et al., 2000, Hernando et al., 2004, van 't Veer et al., 2002). This upregulation is likely the result of increased E2F activity in cancers, as the RB (retinoblastoma) – E2F pathway is altered in more than 80% of human tumours (Malumbres & Barbacid, 2001) and the E2F transcription factor family is an important modulator of Mad2 transcription (Hernando et al., 2004). Furthermore, deregulation of the RB pathway has been associated with CIN (Lentini et al., 2006, Lentini et al., 2002, Schaeffer et al., 2004), and elevated Mad2 levels can provide an explanation for this. Indeed, reducing Mad2 protein levels to wildtype levels in Rb deficient cells reduces the number of aneuploid cells. Conversely, overexpression of either E2F or Mad2 in wildtype cells increases aneuploidy (Hernando et al., 2004). These results strongly suggest that unscheduled activation of E2F contributes to aneuploidy through elevated Mad2 expression. To test if this is true *in vivo*, an inducible Mad2 overexpression mouse model was engineered. These mice display a wide spectrum of tumours (lung adenomas, lymphomas, hepatocellular carcinomas and fibrosarcomas) with a similar latency (~20 months), but higher frequency (~50% at 20 months) than Mad2 heterozygous mice (Sotillo et al., 2007). These results seem somewhat counterintuitive: why would increasing levels of checkpoint protein lead to cancer? Perhaps, delayed degradation of both Securin and Cyclin B1 during mitosis causes defects in mitotic exit and thus explains the observed chromosomal aberrations and subsequent tumourigenesis. Even though this data indicates a clear role for Mad2 overexpression in tumorigenesis, several other mechanisms might contribute to the CIN phenotype of cells with aberrant E2F activity. For instance, Rb inactivation in mouse embryonic fibroblasts also reduces cohesion between sister chromatids, leading to premature chromosome segregation and CIN (Coschi et al., 2010, Manning et al., 2010, van Harn et al., 2010). Furthermore, inducible overexpression of Hec1, another Rb-interacting protein residing at the kinetochore, provokes aneuploid cancers 15-18 months following induction (Diaz-Rodriguez et al., 2008) similar to Mad2 overexpressing mice. Therefore, even though overexpression of Mad2 has a clear cancer phenotype, it is unlikely that Mad2 is the only downstream target of the RB pathway involved in the maintenance of a euploid genome.

Other mouse models for numerical CIN

Not all aneuploidy is the result of spindle checkpoint failure; other mitotic defects can also perturb chromosome segregation and thus cause aneuploidy. Lzts1 was discovered as a gene that is frequently lost in breast, lung, gastric, esophageal, prostate, and bladder cancers. Lzts1 impairs Cyclin B1 activation late in mitosis, resulting in lowered Cyclin B1-Cdk1 activity in mitosis and premature mitotic exit, similar to Mad2 overexpression. Indeed, Lzts1-deficient mice develop a wide spectrum of tumours within 19 months of age with a

high penetrance. Lzts1 loss also facilitates carcinogen-induced tumourigenesis, as NMBA treatment resulted in a tumour incidence of 100% in both Lzts1-deficient and heterozygous animals, whereas only 15% of the treated control animals develop tumours. Therefore, premature mitotic exit through decreased Cyclin B1 levels might be an important driver of (numerical) CIN and cancer (Vecchione et al., 2007).

Entry into mitosis is guarded by a prophase checkpoint that can be activated by chromosomal damage or disruption of microtubules (Mikhailov et al., 2002, Pines & Rieder, 2001). Chfr is a potential E3 ubiquitin ligase that functions early in prophase (Matsusaka & Pines, 2004), presumably by controlling expression levels of mitotic proteins such as Aurora A. Unlike SAC components, homozygous deletion of Chfr in the mouse is dispensable for normal development. However, half of the Chfr-deficient animals develop tumours (lymphomas, lung, liver and intestinal tumours) within 20 months of age and furthermore the knockouts show accelerated tumour development upon carcinogenic insults (DMBA). (Yu et al., 2005). Both phenotypes (Lzts1, Chfr) are very similar to those of the cancer-predisposed SAC-compromised heterozygotes, emphasizing the importance of accurately regulated mitotic kinase activity (Cyclin B1-Cdk1, Aurora A) in preserving normal chromosomal numbers and suppression of cancer.

Micronuclei formation is another indication of chromosomal instability. An elegant genetic screen for genes that facilitate micronucleus formation in erythrocytes identified a point mutation in Mcm4 (Chaos3). Mcm4 is part of the Mcm2-7 replication licensing complex and therefore a crucial part of the DNA replication machinery. Mcm4 mutant mice showed decreased expression of several Mcm proteins and primary cells from this mouse also showed increased sensitivity for the replication inhibitor aphidicolin in line with a defect in DNA replication (Shima et al., 2007). However, as this mutation was identified as a mutation that provokes micronuclei formation, this model suggests that defects in DNA replication can cause numerical chromosomal abnormalities as well.

The Adenomatous polyposis coli (APC) gene is a tumour suppressor gene that is often mutated in human colorectal cancers (note that this gene is unrelated to the anaphase promoting complex APC/C). In addition to APC's well-known role in regulating/dampening β-catenin's transcriptional activity and cell proliferation, multiple lines of evidence indicate that mutated APC contributes to chromosome missegregation and numerical CIN. For instance, cell culture studies have shown that APC localizes to kinetochores and microtubule ends. Perturbing APC function by mutation, overexpression or depletion causes aneuploidy (Draviam et al., 2006, Green et al., 2005, Tighe et al., 2004). In addition, mice expressing truncated APC mutant protein display multiple intestinal tumours with aberrant mitosis and polyploidy, indicating a strong link between the loss of APC's mitotic function and tumourigenesis (Caldwell et al., 2007, Dikovskaya et al., 2007, Oshima et al., 1995, Su et al., 1992). Interestingly, this phenotype can be substantially aggravated by concomitant heterozygous BubR1 loss (Rao et al., 2005). Finally, transcriptome analysis of APC-mutated cells revealed that the mutation resulted in the increase of BubR1 and Mad2 expression, which is also observed in adenomas and colorectal carcinomas (Dikovskaya et al., 2007). Together, these findings illustrate an important link between deregulation of APC and the proteins that control mitosis in the progression from early adenomas to aggressive carcinomas.

Lessons learnt from Mouse Embryonic Fibroblasts

MEFs isolated from several of these mouse models have been invaluable in estimating the levels of aneuploidy occurring *in vivo*. However, as complete ablation of spindle checkpoint genes resulted in embryonic lethality before day E8.5 and MEFs are typically isolated at E15.5-14.5, it has been difficult to generate MEFs from the homozygous knockouts. Therefore heterozygous MEFs were analyzed for the extent of aneuploidy (Table I). All heterozygous MEFs show significantly elevated numbers of aneuploid cells compared to wildtype control MEFs, ranging from 10% aneuploidy in the Mad1$^{+/-}$ MEFs to 50% in the Mad2 heterozygotes and Mad2 overexpressing cells. Note that wildtype MEFs typically show less than 10% aneuploid cells (Iwanaga et al., 2007, Michel et al., 2001, Sotillo et al., 2007). In addition, BubR1 hypomorphic and Bub3; Rae1 heterozygous MEFs exhibit premature senescence and increased protein levels of senescence-associated p19^{Arf}, p21^{Cip1} and p16^{Ink4a} (Baker et al., 2004, Baker et al., 2006) in agreement with the observed premature aging phenotype.

Even though all SAC-compromised MEFs show aneuploidy to a greater or lesser extent, the level of aneuploidy in MEFs does not fully correlate with cancer incidence (Table I). However, aneuploidy analyses might lead to different results depending on the passage number of the MEFs. For instance, detailed analysis of Cenp-E heterozygous MEFs revealed that up to 70% of the cells are aneuploid at high passage numbers whereas only 20% are aneuploid in early passages (Weaver et al., 2007). Furthermore, culturing MEFs *in vitro* might not be the best representation for every cell type *in vivo*. Therefore, even though MEF studies are useful to estimate CIN rates *in vitro*, *in vivo* assessment of aneuploidy will be crucial to link the extent of aneuploidy to cell fate.

4. Conclusions

Aneuploidy as a primary cause for cancer?

Since the first notion of a possible link between chromosomal abnormalities and malignant transformation by Theodore Boveri (Boveri, 1902, Boveri, 1914), we have learned a great deal about the causes and consequences of chromosome missegregation. Mouse models have provided crucial insight into how the SAC and mitotic machinery protect against CIN en cancer. The mouse models discussed in this chapter are far from complete especially as structural CIN and numerical CIN appear to be intertwined (Janssen et al., 2011). Therefore, mutations in genes protecting against structural CIN (Brca2, Mcm4, p53bp1, etc) might very well lead to aneuploidy too, even though these models were not the focus of the current chapter.

Complete inactivation of genes involved in chromosome segregation appears to be uncommon in human cancer. Why then is the vast majority of human cancers aneuploid? Transcriptome analysis of numerous cancers has revealed that overexpression of spindle checkpoint genes is more frequent than reduced expression (Oncomine database (Rhodes et al., 2004)). However, in many cases SAC gene products show mutations or truncations (Cahill et al., 1998, Kim et al., 2005, Olesen et al., 2001, Percy et al., 2000, Scintu et al., 2007, Tsukasaki et al., 2001). Possibly, mutated SAC proteins can act in a dominant-negative fashion, and therefore attenuate spindle checkpoint function. Careful biochemical analysis of such mutant gene products for their effect on the SAC status in combination with new mouse models for these cancer-associated mutations should clarify this issue.

Mouse models for systemic SAC inactivation unequivocally show that removing the spindle checkpoint is not tolerated during embryonic development, when cells are dividing at maximum speed, and results in massive aneuploidy and apoptosis. This agrees fully with the observation that SAC inactivation is not tolerated in cancer cell lines (Kops et al., 2004). However, p53 inactivation in Mad2 deficient MEFs (derived from Mad2$^{-/-}$; p53$^{-/-}$embryonic stem cell lines) partly rescues this lethality, as these cell lines show better survival than Mad2$^{-/-}$ MEFs, even though both cell lines become highly aneuploid (Burds et al., 2005). Therefore, p53 inactivation might synergize with SAC inactivation in malignant transformation by (partly) rescuing SAC-deficiency-induced cell death. Indeed, p53 heterozygosity appears to collaborate with Bub1 hypomorphism in transformation.

Partial inactivation of spindle checkpoint genes does not interfere with normal development (Table 1) and the average life span of these mice is unaltered. While some heterozygotes succumb to tumours at 18-20 months, others age without any cancer phenotype. The Bub3; Rae1 double heterozygotes show signs of premature aging (but not cancer), but do not succumb to the consequences of this phenotype earlier than their wildtype littermates (Baker et al., 2006). All together, the current mouse models for CIN argue that aneuploidy can provoke cancer. It remains unclear why some models are more prone to cancers than other, despite the fact that heterozygous MEFs from all mouse models show substantial aneuploidy, where tested. The only effect on life span is observed in BubR1 hypomorphic mice, which die a few months earlier due to premature aging (Baker et al., 2004). Premature aging coincides with increased numbers of senescent cells *in vivo* and furthermore, MEFs showed increased levels of the senescence-associated p16^{Ink4a}, p19^{Arf} and p21^{Cip1} proteins. Remarkably, *in vivo* clearance of p16^{Ink4A} positive (*i.e.* senescent) cells can delay several of the premature aging sings in BubR1 hypomorphic mice (Baker et al., 2011), suggesting that senescence and aging , in addition to cancer, are important consequences of (numerical) CIN *in vivo*.

Premature senescence might also explain why Cenp-E$^{+/-}$ mice are less susceptible to tumours in a p19^{Arf} negative background or when tumours were induced chemically (Weaver et al., 2007). For instance, loss of p19^{Arf}, an activator of p53 activity, might lead to aberrant DNA damage signalling resulting in genomic instability. Similarly, challenging animals with carcinogenic compounds will induce CIN. Together with Cenp-E heterozygosity, the cumulative aneuploidy might rise to levels that ultimately result in senescence, instead of increased proliferation and cancer. These findings emphasize once more the importance of studying the relationship between senescence, aneuploidy and cancer.

Future directions

What are the next steps that will bring us closer to a therapy that specifically targets aneuploid cancers? We have now learnt that dramatic numerical CIN (e.g. by SAC inactivation) kills cells *in vitro* and interferes with development *in vivo*. We also now know that aneuploidy deregulates cell metabolism and restricts cell proliferation at the cellular level. Partial SAC inactivation appears to predispose for cancer in some models and to provoke a progeria/senescence phenotype in others. However, even though the vast majority of human tumours are aneuploid, few mutations in the SAC cascade have been identified, even though many human cancer cell lines display

an abnormal response to anti-cancer drugs that normally activate the SAC, such as Paclitaxel.

Now that several conventional knockout mouse models have been analyzed in depth, we need to address more specific questions on the role that aneuploidy plays in cancer, for instance:

- Is full SAC inactivation tolerated in adult tissues and if so, does it require additional mutations such as p53 inactivation?
- What are the consequences of aneuploidy *in vivo* and do these resemble the consequences as found in aneuploid MEFs or yeast strains (Torres et al., 2007, Williams et al., 2008)?
- Which genes collaborate with expansion of aneuploid cell progeny?
- What determines the cellular response to aneuploidy (proliferation/senescence)?
- Can we specifically kill aneuploid cells, leaving euploid cells untouched?

To answer these questions more sophisticated mouse models are required. For instance, to answer the first question, conditional mouse models are needed. In such models the knockout allele can be deleted in tissues of choice leaving other tissues unaffected. Using this approach embryonic lethality can be circumvented in many cases. In addition, such models allow for comparing the differential responses to aneuploidy from tissue to tissue for each conditional knockout.

In addition, highly aneuploid tumour panels are required to extract the molecular consequences to aneuploidy in transformed cells. For instance, by comparing the molecular responses in different aneuploid tumour types (different knockouts, different tissues), common responses to aneuploidy can be extracted. Understanding this response is of vital importance before even starting to develop drugs that specifically kill aneuploid cells. These tumour cohorts can also be used to screen for additional mutations that occurred during tumour development. By extracting common mutations occurring in aneuploid tumours arising in different tissues and models, recurring pathways can be identified that aneuploid cells rely on to survive. With the recent advances in high throughput sequencing technology such endeavours are becoming more and more feasible.

Does this mean that we are still far away from the first therapies that target aneuploid cells? Maybe not. A recent study has identified a few promising compounds that specifically target aneuploid cell lines making use of the deregulated metabolism of these cells (Tang et al., 2011). Such drugs can be tested in an *in vivo* setting using conditional mouse models for CIN.

Finally, we need a nifty model to visualize aneuploid cells and their response in the *in vivo* setting. One possible approach is to engineer a mouse model in which a single chromosome can be tracked, for instance a transgenic mouse strain expressing a fluorescent artificial transcription factor. By tracking aneuploidy as it arises in early tumour lesions or in aging tissues the direct consequences, but also the more long term effects of aneuploidy can be monitored in an *in vivo* setting. Such a model will provide unique insight into a developing tumour or otherwise affected tissue, but, even more important, can also be used to visualize the clearance of aneuploid cell progeny in response to newly developed drugs.

Spindle checkpoint genes		Phenotypes +/- animals						
Genes	*-/-*	*+/-*	*Cancer prone?*	*Chemical induced cancer?*	*Other Phenotypes*	*Aneuploidy in tissue?*	*% Aneuploid MEFs*	*Reference*
Bub1	Embryonic lethal (E6.5)	Viable, no overt developmental defects	No	DMBA-induced	No	ND	ND	(Jeganathan et al., 2007)
Bub1 hypo-morph	NA	NA	50% have developed tumours by 20 months (lymphomas, lung and liver tumours)	ND	No	ND	15% (segregation defects)	(Jeganathan et al., 2007)
Bub3	Embryonic lethal (E6.5)	Viable, no overt developmental defects	No	DMBA-induced	No	10% (splenocytes)	20%	(Babu et al., 2003, Baker et al., 2006, Kalitsis et al., 2000, Kalitsis et al., 2005)
Bub3; Rae1	ND	Viable, no overt developmental defects	No	DMBA-induced	Premature aging	40% (splenocytes)	40%	(Babu et al., 2003, Baker et al., 2006)
BubR1	Embryonic lethal (E6.5)	Viable, no overt developmental defects	No	DMBA-induced	Hematopoietic defect	Polyploidy in megakaryocytes	15%	(Baker et al., 2004, Q. Wang et al., 2004)
BubR1 hypo-morph	NA	NA	No	DMBA-induced and azoxymethane induced	Premature aging	30% (splenocytes)	35%	(Baker et al., 2004)
$Cdc20^{AAA}$ mutant (does not bind to Mad2)	Embryonic lethal (E12.5)	Viable, no overt developmental defects	50% of the mice developed tumours by 24 months	ND	No	35% ($Cdc20^{AAA/+}$, splenocytes)	28% of $Cdc20^{AAA/+}$ and 52% of $Cdc20^{AAA/AAA}$	(Li et al., 2009)
Cdh1	Embryonic lethal (E10.5)	Viable, no overt developmental defects	17% of the females develop mammary tumours	Tumour suppression upon TPA/DMBA treatment	No	ND	Increased, not further quantified	(Garcia-Higuera et al., 2008)
CENPE	Embryonic lethal (< E7.5)	Viable, no overt developmental defects	20% develop tumours by 19-21 months of age (both lung and spleen)	Tumour suppression upon DMBA treatment	Tumour suppression in a $p19^{Arf-/-}$ background	40% (splenocytes)	20% (up to 70% at high passage)	(Weaver et al., 2003, Weaver et al., 2007)
Mad1	Embryonic lethal	Viable, no overt developmental defects	20% develop tumours within 18-20 months, (lung)	Vinicristine-induced	No	ND	10%	(Iwanaga et al., 2007)

Mad2	Embryonic lethal (E6.5)	Viable, no overt developmental defects	30% develop tumours at 18 months (lung)	ND	No	ND	55%	(Dobles et al., 2000, Michel et al., 2001)
Mad2 over-expression	NA	NA	50% develop tumours by 20 months (lymphomas, lung and liver)	DMBA-induced	No	Aneuploid tumours (not quantified)	50%	(Sotillo et al., 2007)
Rae1	Embryonic lethal (E6.5)	Viable, without developmental defects	No	DMBA-induced	No	10% (splenocytes)	20%	(Babu et al., 2003, Baker et al., 2006)
Structural centromeric proteins	**Phenotypes +/- animals**							
	-/-	*+/-*	*Cancer prone?*	*Chemical induced cancer?*	*Other Phenotypes*	*Aneuploidy in embryos or tissues?*	*% Aneuploid MEFs*	*Reference*
CENPA	Embryonic lethal (E6.5)	Viable, without developmental defects	ND	ND	NA	Chromosome missegregation in E6.5-/- embryos	NA	(Howman et al., 2000)
CENPB	Viable, no phenotype	Viable, no phenotype	ND	ND	Lower body and testis weight	ND	ND	(Hudson et al., 1998, Kapoor et al., 1998, Perez-Castro et al., 1998)
CENPC	Embryonic lethal (E3.5)	Viable, without developmental defects	ND	ND	NA	Aberrant mitosis and micronuclei in early embryos	NA	(Kalitsis et al., 1998)
Hec1 overexpression	NA	NA	13% develop lung tumours and 25% develop liver tumours 15-18 months after induction	ND	No	ND	25%	(Diaz-Rodriguez et al., 2008)
Chromosomal passenger genes/ mitotic spindle binding proteins	**Phenotypes +/- animals**							
	-/-	*+/-*	*Cancer prone?*	*Chemical induced cancer?*	*Other Phenotypes*	*Aneuploidy in embryos/ tissues?*	*% Aneuploid MEFs*	*Reference*
APC/MIN	Embryonic lethal (<E8.5)	Viable	Develop tumours within 3 months (intestine)	ND	Anaemia, presumably due to intestinal bleeding	Aneuploidy and abnormal mitosis in crypt cells	Increased, not quantified	(Caldwell et al., 2007, Oshima et al., 1995, Rao et al., 2005, Su et al., 1992)

Incenp	Embryonic lethal (E3.5-8E.5)	Viable, no overt developmental defects	ND	ND	NA	Abnormal nuclear morphology hyperdiploid content in E3.5 embryos	NA	(Uren et al., 2000)
Survivin	Embryonic lethal (E6.5)	Viable, no overt developmental defects	ND	ND	NA	Giant nuclei in early embryos	NA	(Uren et al., 2000)
Genes otherwise involved in mitosis		**Phenotypes -/- animals***						
	-/-	*+/-*	*Cancer prone?*	*Chemical induced cancer?*	*Other Phenotypes*	*Aneuploidy in tissue?*	*Aneuploidy in MEFs?*	*Reference*
Ltzs1	Viable, no developmental defects	Viable, no developmental defects	All the -/- and 60% of the +/- mice develop tumours at 8-24 months (lymphomas, mammary, liver and liver)	NMBA-induced	No	ND	25% (lagging chromosomes)	(Vecchione et al., 2007)
$Mcm4^{Chaos3}$	$Mcm4^{Chaos3/-}$ embryonic lethal E14.5	Viable, no developmental defects	$Mcm4^{Chaos3/+}$ develop mammary tumours within a year	ND	Classic minichromosome loss phenotype in blood	ND	ND	(Shima et al., 2007)
Chfr	Viable, no developmental defects	Viable, no developmental defects	50% of the -/- animals develop tumours within 20 months	DMBA-induced	No	ND	25%	(Yu et al., 2005)

*in case of Mcm4 mice -/- refers to $Mcm4^{Chaos3/+}$ mice

Table 1.

5. References

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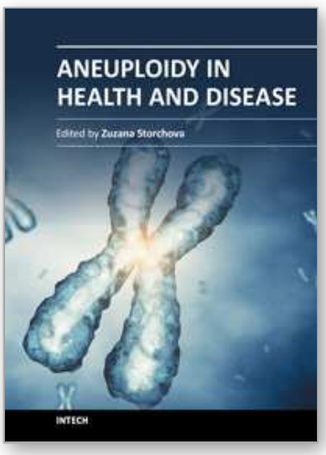

Aneuploidy in Health and Disease

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Aneuploidy means any karyotype that is not euploid, anything that stands outside the norm. Two particular characteristics make the research of aneuploidy challenging. First, it is often hard to distinguish what is a cause and what is a consequence. Secondly, aneuploidy is often associated with a persistent defect in maintenance of genome stability. Thus, working with aneuploid, unstable cells means analyzing an ever changing creature and capturing the features that persist. In the book Aneuploidy in Health and Disease we summarize the recent advances in understanding the causes and consequences of aneuploidy and its link to human pathologies.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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