CINcere Modelling: *What Have Mouse Models for Chromosome Instability Taught Us?*

Judith E. Simon, Bjorn Bakker and Floris Foijer

Abstract

Chromosomal instability (CIN) is a process leading to errors in chromosome segregation and results in aneuploidy, a state in which cells have an abnormal number of chromosomes. CIN is a hallmark of cancer, and furthermore linked to ageing and age-related diseases such as Alzheimer's. Various mouse models have been developed to explore the role of CIN in ageing and cancer. While these models reveal only a modest contribution of CIN to the initiation of cancer, they also clearly show that CIN is a powerful accelerator of cancer in a predisposed background. Other than cancer, CIN also appears to provoke premature ageing in some of the CIN models. In this review, we discuss the phenotypes of the various available mouse models, what we have learnt so far, and importantly, also which questions still need to be addressed.

Keywords

Aneuploidy · Chromosomal instability · Cancer · Ageing · Mouse models

Contents

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1 Chromosomal Instability and Aneuploidy

During each cell division our genetic code is replicated, followed by symmetrical segregation of all chromosomes into the emerging daughter cells. Cancer cells occasionally exhibit errors segregating their chromosomes, a process known as chromosomal instability (CIN), leading to cells with abnormal numbers of chromosomes, a state defined as aneuploid. In addition to whole chromosome abnormalities, CIN can also lead to structural abnormalities such as amplifications, deletions or translocations, either through defects in the DNA damage machinery or as a direct result of chromosome missegregation events (Janssen et al. 2011). Although numerical and structural abnormalities frequently coincide, in this review we will focus on how mouse models have contributed to our understanding of the consequences of whole chromosome instability.

David von Hansemann was the first to report abnormal chromosome numbers in carcinoma samples in 1890, long before the relationship between chromosomes and the genetic code had been established (Hardy and Zacharias 2005; Bignold et al. 2006; Siegel and Amon 2012). Early in the 20th century, Theodor Boveri showed that aneuploidy leads to abnormal development or even death by injecting two sperms instead of one into sea urchin embryos. These observations led to the hypothesis that aneuploidy can lead to cancer or developmental defects (Boveri and Manchester 1995; Bignold et al. 2006; Boveri 2008, Ried 2009). Since then, many studies confirmed that CIN is a hallmark of human malignancies, affecting 2 out of 3 cancers (Duijf et al. 2013). More recently, aneuploidy has also been associated with ageing and age-related diseases (Faggioli et al. 2012). For instance, trisomy for chromosome 21 is frequently found in plaques in Alzheimer patients' brains (Iourov et al. 2009). Conversely, people with Down syndrome develop early onset Alzheimer's disease (Lai and Williams 1989), further emphasizing the relationship between trisomy 21 and neurodegenerative disease.

Although CIN has been associated with cancer for more than a century, we are only beginning to understand the consequences of CIN and aneuploidy at the cellular and molecular level. CIN is believed to accelerate the evolution of cancer cells by facilitating gain of oncogenes and loss of tumour suppressor genes. Paradoxically, when modelled in yeast strains (Torres et al. 2007) or mouse embryonic fibroblasts (MEFs) (Williams et al. 2008), aneuploidy appears to decrease rather than increase cell proliferation, suggesting that cancer cells find ways to cope with the adverse effects of aneuploidy. However, as transformation of aneuploid cells into aneuploid cancer cells can only occur in vivo by definition, animal models for CIN are essential to solve this paradox.

2 Provoking CIN In Vivo

Several processes that safeguard correct chromosome segregation have been targeted to engineer mouse models for CIN. Figure 1 shows a schematic overview of a large number of genes that have been targeted for this purpose. One of the first models specifically designed to study the in vivo consequences of CIN is the Mad2 knockout mouse, targeting the spindle assembly checkpoint (SAC) (Dobles et al. 2000). The SAC prevents missegregation of chromosomes by inhibiting metaphase to anaphase progression until all chromosomes are properly attached to kinetochores in a bi-oriented fashion. Defects of the SAC therefore result in flawed chromosome segregation, which makes the SAC an attractive target to model CIN in vivo. A second means to induce CIN in vivo is by interfering with kinetochore integrity, a protein structure that connects the centromeric DNA to the mitotic spindle. This has been done by removing structural components of the kinetochore (e.g., CenpB, CenpC) or alternatively, by stabilizing kinetochore-microtubule attachments through e.g., overexpressing Mad2 or Hec1 (Diaz-Rodríguez et al. 2008; Kabeche and Compton 2012). Centrosomes are the microtubule-organizing centres in the cell from which the mitotic spindle emanates (Ganem et al. 2007; Gordon et al. 2012). Abnormal centrosome numbers can either result in multipolar divisions or, when supranumeral centrosomes cluster, predispose for lagging chromosomes in mitosis (Ganem et al. 2009). Therefore, a third way to provoke CIN in vivo is by inducing centrosome amplification, e.g., through overexpression of Plk4 (Ko et al. 2005; Marthiens et al. 2013a). A fourth approach to induce CIN in vivo is by disrupting the cohesion complex, a ring like structure that holds the sister chromatids together during interphase. Cohesion defects have been modelled by abrogating components of the cohesion complex (e.g., SA1), but also by deregulating upstream players such as pRb (Coschi et al. 2010; Manning et al. 2010; Van Harn et al. 2010). Similarly, various other genes have been knocked out in the mouse which indirectly affect chromosome segregation.

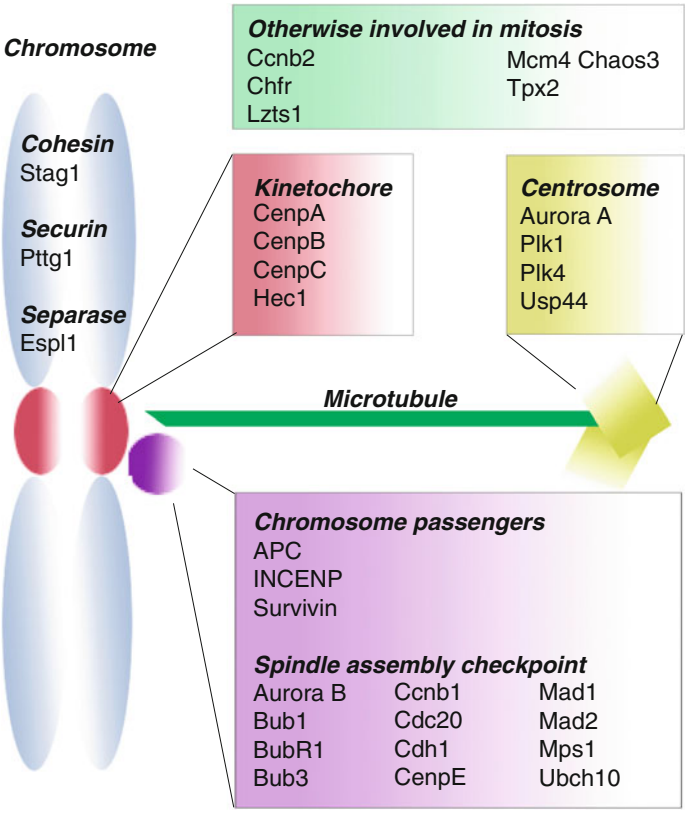

Fig. 1 Schematic overview of various genes targeted to provoke CIN in vivo

3 In Vivo Consequences of CIN

In the last two decades, a large number of mouse models for chromosome instability have been engineered. Hereunder, we summarize the findings from these models asking the following questions:

1. Is CIN a bona fide instigator of cancer?
2. Which genes collaborate with CIN in vivo converting aneuploid cells into aneuploid cancer cells?
3. What are other consequences of CIN in vivo?

4 Can CIN Initiate Cancer?

CIN has detrimental consequences for cells grown in vitro (Kops et al. 2004; Williams et al. 2008; Torres et al. 2008), yet, two out of three human tumours are aneuploid (Weaver and Cleveland 2006; Duijf et al. 2013). This raises the question whether CIN is an initiating factor in cancer, a facilitator or merely a side effect of tumorigenesis. In the vast majority of all models (see Fig. 1), full inactivation of the targeted genes resulted in early embryonic lethality. Although the time of embryonic death varied between genotypes (Table 1), embryos were typically lost before embryonic day 10, which presumably was the result of aneuploidy in the inner cell mass of the developing embryos (Dobles et al. 2000; Weaver and Cleveland 2006; Foijer et al. 2008; Holland and Cleveland 2009; Schvartzman et al. 2010). To circumvent embryonic lethality, phenotypes of heterozygous mice were monitored, or in some cases, conditional alleles were engineered. Even though tumour phenotypes have been reported for many of these models (Table 1) tumour incidence is relatively low, with in most cases fewer than 50 % of the mice developing cancer. Moreover, tumours only arise late in the life of the mice, with latencies typically ranging from 12 to 24 months (Table 1). The most frequent pathologies observed include lymphoma, lung and liver tumours. Furthermore, not all models develop spontaneous tumours, for instance in case of the Bub family members [Bub1, Bub3, Rae1 (Kalitsis 2000; Babu et al. 2003; Wang et al. 2004; Baker et al. 2006a; Jeganathan et al. 2007; Baker et al. 2009)]. There is no clear correlation between the severity of the tumour phenotypes and the mechanism that drove CIN in the mice (i.e. SAC mutation, cohesion defects, centrosome abnormalities etc.). Expression levels of the CIN-provoking genes on the other hand appear to be a better predictor of tumour incidence: phenotypes were most severe in cases where CIN-driving proteins were overexpressed to high levels [e.g. Mad2, Cyclin B1, Cyclin B2, Hec1, Plk4 (Ko et al. 2005; Sotillo et al. 2007; Baker et al. 2008; Diaz-Rodríguez et al. 2008)] possibly because the relative effect on protein expression (several folds overexpression) was more dramatic than in heterozygous mice, where protein levels were typically reduced $\sim$50 %. However, as tumour latency is high in these models as well, additional hits must be required for aneuploid cells to become malignant.

5 Does CIN Predispose to Cancer?

Exposure to carcinogens is a powerful tool to assess tumour predisposition in vivo. Given the relative weak tumour phenotypes of CIN mice, various CIN models were exposed to carcinogens (Table 1) to assess whether CIN is a powerful collaborator in transforming cells. Indeed, carcinogens aggravated the tumour phenotypes of some of the CIN mice, more than their control counterparts. For instance, when Mad1 heterozygous mice were treated with Vincristine (a microtubule-depolymerizing agent), 40 % of the mice developed mostly lung tumours, while no tumours were detected in control-treated mice (Iwanaga et al. 2007). Likewise, carcinogens

Table 1 List of various mouse models engineered to provoke CIN in vivo, with phenotypes and observed aneuploidy levels in vivo and in vitro where quantified

Group	Gene	−/−	±	Cancer predisposed (chemical or genetic collaboration)	Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer (Schvartzman et al. 2010)	References
Spindle assembly checkpoint	AuroraB	EL	>60 %; 24 mo	Tumour suppression upon DMBA + TPA-induced (not sig.)	ND	ND		Fernández-Miranda et al. (2011)
	Bub1	EL (E6.5)	VNODD	DMBA-induced-57 %, $p53^{+/-}$; 16.6 mo	ND	ND		Jeganathan et al. (2007), Baker et al. (2009)
	Bub1 hypomorph	n/a	50 %; 12 mo	78 %, $p53^{+/-}$; 12 mo	ND	15 % (seg. defects)		Jeganathan et al. (2007), Baker et al. (2009)
	Bub3	EL (E6.5)	VNODD	DMBA-induced	10 % (splenocytes)	20 %		Kalitsis (2000), Babu et al. (2003), Kalitsis et al. (2005), Baker et al. (2006b)
	Bub3; Rae1	ND	VNODD	DMBA-induced	40 % (splenocytes)	40 %		Babu et al. (2003), Baker et al. (2006b)
	BubR1	EL (E6.5)	VNODD	DMBA-induced	Polyploidy in megakaryocytes	15 %	Yes	Wang et al. (2004), Baker et al. (2004)
	BubR1 hypomorph	n/a	Premature ageing	DMBA- and azoxymethane-induced	30 % (splenocytes)	35 %	Yes (MVA)	Baker et al. (2004)
	BubR1 overexpression	n/a	Delayed ageing	DMBA-induced, but decreased susceptibility than WT	1 % (splenocytes)	9 % (WT comparable)		Baker et al. (2013a)
	Ccnb1 (Cyclin B1) overexpression	n/a	>75 %; (lung, lymphoma, liver, lipoma)	~80 %, APC+/min; 40 % (WT comparable; skin), DMBA-treatment	hi 20 %, lo 12 % (splenocytes)	31 % (ctrl. 15 %)		Nam and van Deursen (2014)

(continued)

Table 1 (continued)

Group	Gene	−/−	±	Cancer predisposed (chemical or genetic collaboration)	Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer (Schvartzman et al. 2010)	References
	Cdc20 AAA mutant (does not bind to Mad2)	EL (E12.5)	50 %; 24 mo	ND	35 % ($Cdc20^{AAA/+}$, splenocytes)	28 % ($Cdc20^{AAA/+}$ and 52 % of		Li et al. (2009)
	Cdh1	EL (E10.5)	17 % females – (mammary); mild brain abnormalities and altered behaviour	Tumour suppression upon TPA/DMBA treatment	ND	Increased (not quantified)		García-Higuera et al. (2008)
	CENPE	EL (<E7.5)	20 % (lung, spleen); 19–21 mo	Tumour suppression upon DMBA treatment or $p19^{Arf}$ loss	40 % (splenocytes)	20 % (up to 70 % at high passage)		Weaver et al. (2003, 2007)
	Mad1	EL	20 % (lung); 18–20 mo	Vinicristine-induced	ND	10 %		Iwanaga et al. (2007)
	Mad2	EL	30 % (lung); 18 mo	ND	ND	55 %		Dobles et al. (2000), Michel et al. (2001)
	Mad2 overexpression	n/a	50 % (lymphomas, lung and liver); 20 mo	DMBA-induced	Aneuploid tumours (not quantified)	50 %	Yes	Sotillo et al. (2007)
	Mps1 (T-cell restricted)	VVNOD	~50 % (lymphoma) 17 mo	100 %, $p53^{+/-}$; 5 mo	>90 % of cells aneuploid	ND		Foijer et al. (2014)
	Rae1	EL (E6.5)	No spont. tumorigenesis	DMBA-induced	10 % (splenocytes)	20 %		Babu et al. (2003), Baker et al. (2006b)
	Ubch10 overexpression	n/a	Expression level dependent: 40–80 % (lymphoma, lung adenoma, lipoma and liver and skin)	Yes, but not significantly different compared to wild type	4–19 % hi-lo, 5 mo (splenocytes); 52–64 % (lymphoma)	28–33 % (WT 13 %)	Yes	van Ree et al. (2010)

(continued)

Table 1 (continued)

Group	Gene	−/−	±	Cancer predisposed (chemical or genetic collaboration)	Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer (Schvartzman et al. 2010)	References
Kinetochore	CENPA	EL (E6.5)	VNODD	ND	Chromosome missegregation in E6.5 CENPA$^{-/-}$ embryos	n/a		Howman et al. (2000)
	CENPB	VNODD	VNODD	ND	ND	ND		Hudson et al. (1998), Perez-Castro et al. (1998), Kapoor et al. (1998)
	CENPC	EL (E3.5)	VNODD	ND	Aberrant mitosis and micronuclei in early embryos	n/a		Kalitsis et al. (1998)
	Hec1 overexpression	n/a	13 % (lung), 26 % (liver); 67 wk, 60 wk	ND	ND	25 %	Yes	Diaz-Rodríguez et al. (2008)
Cohesion	Espl1 (separase)	EL (E6.5)	Espl1$^{+/H}$; VNODD	86 % (lymphomas), p53$^{-/-}$; 4 mo—50 % (carcinoma), p53$^{+/-}$	57 % (splenocytes); 84 % (bone marrow)	ND	Yes	Mukherjee et al. (2011)
	Espl1 overexpression (mammary restricted)	n/a	80 % (mammary), 11 mo	100 % (mammary), p53$^{+/-}$; 14 mo	>80 % (mammary tumours)	ND		Mukherjee et al. (2013)
	Stag1 (exon 3 and 4, encoding SA1-cohesin subunit)	EL (between E12.5 to E18.5)	40–50 % (haematoma, lung, fibrosarcoma, liver, vascular, pancreas); 24 mo	Resistance to 3MC and DEN induced fibrosarcomas and liver tumours	40 % (fetal liver)	>70 %		Remeseiro et al. (2012)
	Pttg (securin)	Reduced testis, spleen and thymus weight.	n/a	Tumour protective, pRb$^{+/-}$	ND	15 % (WT 1 %)		Wang et al. (2001), Chesnokova et al. (2005)

(continued)

Table 1 (continued)

Group	Gene	−/−	±	Cancer predisposed (chemical or genetic collaboration)	Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer (Schvartzman et al. 2010)	References
	Pttg (securin) overexpression	n/a	Enlarged pituitary; altered nuclear morphology	>80 % (pituitary), $pRb^{+/-}$; 10 mo	ND	ND		Abbud et al. (2005), Donangelo et al. (2006)
Chromosome passengers	APC/MIN	EL (<E8.5)	Intestinal tumours; 3 mo	ND	Aneuploidy and abnormal mitosis in crypt cells	Increased, not quantified		Su et al. (1992), Oshima et al. (1995), Rao et al. (2005), Caldwell et al. (2007)
	Incenp	EL (3.5–8.5)	VNODD	ND	Abnormal nuclear morphology hyperdiploid content in E3.5 embryos	n/a		Uren et al. (2000)
	Survivin	EL (6.5)	VNODD	ND	Giant nuclei in early embryos	n/a		Uren et al. (2000)
Centrosome	Aurora A overexpression (mammary restricted)	n/a	Increased p16 expression	45 %, $p53^{-/-}$ (mammary gland); 4.5 mo	ND	13.6 %		Zhang et al. (2004, 2008)
	Plk1	EL (E10.5)	27.5 % (lymphoma, lung); 12.5–17.5 mo	100 % (lymphoma, lung), $p53^{-/-}$	12 % (splenocytes)	ND		Lu et al. (2008)
	Plk4 overexpression (CNS restricted)	n/a	Microcephaly, 100 % post-natal lethality; <1 wk	100 % lethality, $p53^{-/}$; 5 mo	31.7 % centrosome amplification (neural stem cells); >60 % aneuploidy of chr. 18 in $p53^{-/-}$	ND		Marthiens et al. (2013b)

(continued)

Table 1 (continued)

Group	Gene	−/−	±	Cancer predisposed (chemical or genetic collaboration)	Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer (Schvartzman et al. 2010)	References
	Usp44	VNODD	$Usp44^{+/-}$ 20 %, $Usp44^{-/-}$ 50 %; 15 mo (lung, liver, lymphoma, sarcoma)	n/a	8 %, 5 mo; 16 %, 15 %, 15 mo (splenocytes)	18 % (WT 13 %)	Yes	Zhang et al. (2012)
Otherwise involved in mitosis	Ccnb2 (Cyclin B2) overexpression	n/a	>70 % (lung, lymphoma, liver, lipoma); 14 mo	>80 %, $APC^{+/min}$; >80 % (lung), DMBA-treatment	18 % (splenocytes)	36 % (ctrl. 16 %)		Nam and van Deursen (2014)
	Chfr	VNODD	$Chfr^{-/-}$ 50 %; 20 mo	DMBA-induced	ND	25 %		Yu et al. (2005)
	Mcm4 Chaos3	$Chaos^{3/-}$ EL (E14.5)	$Mcm4Chaos^{3/+}$ (mammary); 12 mo	ND	ND	ND		Shima et al. (2007)
	Tpx2	EL (E8.5)	53 % (lymphoma, lung)	no	18.3 %, 16 wk; 27 %, 90 wk (splenocytes) 48.9 %, 90 wk (lymphomas)			Aguirre-Portolés et al. (2012)

Genes that have been used to generate CIN mouse models

EL embryonic lethal; *VNODD* viable, no overt developmental defects; *ND* not determined; *n/a* not applicable

(NMBA or DMBA) accelerated tumorigenesis in Lzts1-deficient and Chfr-deficient mice (Yu et al. 2005; Vecchione et al. 2007). Furthermore, even in CIN mice without a tumour phenotype (e.g., $Bub1^{+/-}$, $Bub3^{+/-}$, $Rae1^{+/-}$ and $Bub3^{+/-}$ $Rae1^{+/-}$), DMBA treatment had a stronger tumour promoting effect than on wild type mice (Baker et al. 2006b; Jeganathan et al. 2007). As carcinogens reduce tumour latency and increase tumour incidence in a CIN background, also these experiments indicate that additional mutations are required for a CIN cell to transform into a malignant cell.

6 Which Genes Collaborate with CIN in Cancer?

To test which genetic alterations collaborate with CIN in tumorigenesis, various CIN models were crossed into cancer-predisposed backgrounds. For instance, when CIN was combined with p53 heterozygosity, [Bub1, Espl1, Mps1 (Baker et al. 2009; Mukherjee et al. 2011; Baker et al. 2013b)] tumour incidence dramatically increased while tumour latencies decreased. In all reported cases, tumours had lost the remaining p53 wild type allele, indicating that full p53 loss and CIN synergize in tumorigenesis (Baker et al. 2009; Foijer et al. 2014). However, as CIN further increased tumour incidence of $p53^{null}$ mice, CIN must have facilitated cancer formation through additional genomic alterations as well. Furthermore, CIN provoked by Bub1 hypomorphic alleles or Cyclin B1 overexpression accelerates tumours in a Apc^{min} background (Baker et al. 2008, 2009). However, in other tumour predisposed backgrounds (e.g., pRb or Pten heterozygousity) CIN has no effect on tumour incidence (Baker et al. 2009).

7 CIN as a Tumour Suppressor

In some cases CIN can also act in a tumour suppressive manner. For instance, CIN driven by SA1 heterozygosity delays 3-methyl-colanthrene (3-MC)-induced fibrosarcoma and diethyl-nitrosamine (DEN)-induced liver tumours (Remeseiro et al. 2012). Similarly, even though $Cdh1^{+/-}$ mice and $CenpE^{+/-}$ mice are more susceptible to spontaneous tumours, they are more resistant to carcinogenic insults than their wild type counterparts (Weaver et al. 2007; García-Higuera et al. 2008). Furthermore, CIN can also delay tumorigenesis in some genetically predisposed models, for instance by delaying $p19^{Arf}$ or Pten loss-driven tumours (Weaver et al. 2007; Baker et al. 2009). Why then is CIN tumour promoting in one setting, but tumour suppressive in another? The answer might lie in the levels of CIN. CIN is quite toxic and provokes an 'aneuploidy stress' response in untransformed cells (Kops et al. 2004; Torres et al. 2007; Williams et al. 2008; Foijer et al. 2013). However, aneuploid cancer cells also exhibit this stress response (Dürrbaum et al. 2014; Foijer et al. 2014), suggesting that aneuploid cancer cells still suffer from the disadvantageous effects of CIN. Therefore, the levels of CIN occurring in premalignant cells could be a determining factor for the outcome. The fact that $p19^{Arf}$ loss provokes aneuploidy itself fits with this hypothesis, as CenpE heterozygosity would

exacerbate CIN to a level that is toxic for cancer cells (Silk et al. 2013). However, further experiments are required to determine at what level CIN is beneficial for cancer cells and at what level the balance is tipped.

8 What Other Phenotypes Are Provoked by CIN?

There is increasing evidence that aneuploidy also occurs in untransformed tissues, with liver being the most well-known example. Up to half of both human and murine hepatocytes are aneuploid (Duncan et al. 2012a, b), but it is unclear why hepatocytes evolved to become aneuploid. One suggestion is that particular karyotypes are selected for during hepatotoxic insults, making the hepatocytes more resistant to injury (Duncan et al. 2012b). Other studies quantified over 30 % of normal human neuroblasts to be aneuploid (Rehen et al. 2001, 2005), which has been suggested to contribute to the plasticity of neurons (Kingsbury et al. 2005). However, when provoked in a random fashion, CIN appears to mostly have disadvantageous effects on brain functioning, as mice heterozygous for Cdh1 exhibit defects in neuromuscular coordination and learning (García-Higuera et al. 2008). The interfollicular epidermal cells in mouse skin on the other hand appear to cope surprisingly well with CIN as they tolerate full abrogation of the SAC provoked by Mad2 loss, which results in dramatic aneuploidy (Foijer et al. 2013). The hair follicle stem cells that reside in the same compartment do not cope at all and disappear, resulting in mice with functional skin, but without hair (Foijer et al. 2013). Together these data clearly indicate that CIN is tolerated by some cell lineages, but not others, underscoring the importance of in vivo modelling.

9 Linking Ageing and CIN In Vivo

Ageing is the time-dependent functional decline in the fitness of cells, organs and organisms. A common hallmark of ageing is genomic instability, as exemplified by genetic alterations in old blood cells (Forsberg et al. 2012; López-Otín et al. 2013). Some of the CIN mouse models also suggest a role for aneuploidy in ageing. For instance, BubR1 hypomorphic mice are not only prone to severe aneuploidization, but also display progeroid pathologies. Similar to BubR1, combined Bub3/Rae1 haploinsufficiency also results in a premature ageing phenotype, albeit less severe than the BubR1 hypomorphic mice (Baker et al. 2006b), MEFs isolated from BubR1 hypomorphic mice express various ageing-associated markers such as p53, p21, $p19^{Arf}$ and $p16^{Ink4a}$. Interestingly, when $p16^{Ink4a}$ positive cells are killed in vivo using a $p16^{Ink4a}$-promotor regulated suicide construct, ageing pathologies induced by a reduction of BubR1 protein levels are dramatically delayed (Baker et al. 2011). The pathologies observed in BubR1 hypomorphic mice mimic those of patients with multi-variegated aneuploidy (MVA), a disease that frequently coincides with mutations in *BUB1B*, the gene encoding BUBR1 (Hanks et al. 2004,

2006; Matsuura et al. 2006). Furthermore, BubR1 expression levels decline with age providing further evidence for a role of BubR1 in ageing (Baker et al. 2011) in mice. Even more striking, when BubR1 is overexpressed, a dose-dependent delay in the onset of ageing is observed, as well as protection against developing chemically-induced tumours (Baker et al. 2013a). As discussed above, in most tested cases overexpression of CIN-controlling proteins increases CIN and predisposes for cancer (Sotillo et al. 2007; Diaz-Rodríguez et al. 2008; Fernández--Miranda et al. 2011). Apparently, BubR1 is the exception that forms the rule, but future work should reveal whether BubR1 has a unique role in the SAC or whether it has additional roles that can explain the beneficial effects of an overdose of BubR1.

10 What Have We Learnt from Modelling CIN in the Mouse so Far?

As most tumours are aneuploid to some extent, CIN makes an attractive target for therapy. For this, understanding how CIN is signalled is crucial. A large number of mouse models have been engineered over the last 15 years specifically for this purpose, with a wide variety of phenotypes summarized in Table 1. Even though many of the targeted genes will have other roles than safeguarding faithful chromosome segregation, some common conclusions can be drawn from the cumulative data. The first important conclusion is that CIN alone is not sufficient for efficient tumourigenesis and that CIN alone mostly has disadvantageous effects on cell proliferation. This has important implications for therapy targeting aneuploid cancer, as discussed below. A second conclusion is that CIN facilitates tumourigenesis efficiently in some tumour-predisposed backgrounds, chemical or genetic. However, when CIN is aggravated and becomes too severe, it can actually suppress tumour formation in the mouse, which can also be exploited in cancer therapy. A third and perhaps the most important conclusion is that several unaddressed questions remain before we can develop therapeutic strategies targeting aneuploid cell progeny, some of which are discussed below. Although all models discussed here were designed to study the consequences of CIN in vivo, the majority mimic a situation that is not typically found in human cancers, as loss of genes that regulate chromosome segregations are rarely lost in human cancer (Schvartzman et al. 2010; Foijer 2012). Even though those models mimic chromosome missegregation and its consequences, overexpression of CIN-modulating genes is more common [e.g., Mad2 overexpression, which is seen in many tumours (Hernando et al. 2004; Sotillo et al. 2007)]. Possibly, mimicking the CIN-provoking mutations that are found in human cancers would result in a physiologically more relevant CIN level, thus adding to our understanding of CIN and its role in tumorigenesis. A lot can be learned about affected pathways from in vitro studies as well. For instance, Donnelly and colleagues have shown that increased HSF1 activity can play a facilitating

role in coping with aneuploidy-induced proteotoxic stress by regulating the gene expression of various heat shock proteins (Donnelly et al. 2014)

11 Questions that Need to Be Addressed

11.1 Which Mutations Make an Aneuploid Cell an Aneuploid Cancer Cell?

Some tumour suppressor genes, (e.g., p53) were found to accelerate the malignant transformation of aneuploid cells, but the mechanism behind this collaboration remains unclear. As CIN alone is a poor initiator of cancer, pathways that convert aneuploid cells in aneuploid cancer cells make up important therapeutic targets. So far, CIN-collaborating genes were picked in an 'educated guess' approach. However, to identify in an unbiased fashion the pathways that convert CIN cells into CIN cancer cells, (in vivo) genetic screens are required.

11.2 At What Rate Is CIN Tumorigenic and at What Levels Tumour Suppressive?

The effects of CIN across the various mouse models are diverse, but it is unclear why. It is inevitable that the levels of CIN are different among the various CIN models, but there is no clear correlation between the levels of aneuploidy and the resulting phenotype based on the available data. However, as the level of CIN might determine whether tumours are promoted or are suppressed (Silk et al. 2013), high resolution quantification of CIN will be crucial when targeted in therapy. Furthermore, even though aneuploidy is a hallmark of cancer, the actual rates of chromosome missegregation (i.e., the CIN rates) in human cancer are unknown. To quantify these, primary (tumour) cells need to be fully karyotyped at the single cell level at various stages. So far, most studies have relied on metaphase-spread based (spectral) karyotyping using dividing cell populations, such as primary MEFs or tumour cell lines. However, this technique cannot be applied to most primary tumour cells as they do not divide frequently (Mitchison 2012; McGranahan et al. 2012). A new, but costly approach to quantify karyotypes of single cells is next-generation sequencing (NGS) (Knouse et al. 2014; Bakker et al. 2015). However, to quantify aneuploidy, full coverage (or even multiple coverage) per cell is not a requirement. 1–2 % coverage per cell will be sufficient to quantify chromosome numbers for an individual cell, allowing sequencing libraries of many cells to be pooled in each sequencing lane. Single cell karyotyping will allow us to faithfully measure in vivo missegregation rates (i.e. CIN) by assessing subtle karyotype differences between cells within one tumour (karyotype heterogeneity) (Bakker et al. 2015). Such technology will allow us to determine at which rate CIN is tumorigenic or tumour suppressive in mouse models and what the CIN rates are in human primary tumours.

11.3 What Determines the Tissue Specific Response to CIN?

There is a marked difference as to how cell lineages respond to CIN. For instance, CIN is highly toxic to embryonic stem cells (Burds et al. 2005), but quite well tolerated by interfollicular epidermal cells (Foijer et al. 2013), hepatocytes and possibly neurons (Rehen et al. 2001, 2005; Kingsbury et al. 2005; Duncan et al. 2012a). As of yet, it remains unclear what determines this differential response. Possibly, some cell lineages such as stem cells, induce a stronger stress response upon aneuploidy, resulting in apoptosis or differentiation. Alternatively, aneuploidy-tolerating cells spend more time in pro-metaphase and therefore have more time to correct improper kinetochore-microtubule attachments, thus reducing the missegregation rates and therefore reducing aneuploidy to tolerable levels. Indeed some cell types tolerate at least some aneuploidy including neurons and hepatocytes. However, further in vivo experiments are required to assess which molecular pathways make up the response to aneuploidy at the tissue level and how the differential wiring of these pathways in different cell lineages determines the fate of aneuploid cells.

11.4 What Are the Molecular Mechanisms that Explain the Link Between CIN and Ageing?

Some of the CIN mouse models exhibit a premature ageing phenotype, most clearly knockout models of Bub family proteins (BubR1, Bub3/Rae1) (Baker et al. 2004, 2006b). Conversely, BubR1 transgenic mice show increased lifespan, clearly implicating BubR1 with ageing (Baker et al. 2013a). This data, together with the observation that BubR1 expression decreases with ageing in wild type animals, (Baker et al. 2004, 2006b), suggest that CIN may play a role in natural ageing. Why were phenotypes only described for Bub protein members? Possibly, (subtle) signs of premature ageing were overlooked in other CIN models, as these models were developed specifically to study the relationship between CIN and cancer and not ageing, (Ricke and van Deursen 2013). Indeed, a more detailed analysis of transcriptomes of $Mad2^{null}$ epidermal cells suggests an ageing-like response in murine skin following SAC abrogation (Foijer et al. 2013), suggesting that CIN indeed provokes a premature ageing response in untransformed tissue. However, more detailed and high resolution mapping of CIN in ageing human tissues is required to confirm physiological relevance for a potential link between CIN and ageing. When this link is confirmed, the underlying molecular mechanisms that link CIN and ageing should be elucidated, employing exciting and possibly new, more human relevant CIN mouse models.

11.5 What Is the Potential of CIN-Targeting Therapy?

Aneuploidy is a hallmark of cancer and selectively killing aneuploid cells would therefore be a powerful means to treat cancer. The various mouse models for CIN have revealed that there are three possible outcomes for aneuploid cell progeny depending on the tissue affected: (1) cell death (e.g., in case of hair follicle stem cells), (2) cellular senescence (evidenced by premature ageing and upregulation of the senescence marker $p16^{Ink4a}$) and (3) tolerance of aneuploidy (Fig. 2). The latter outcome is the most dangerous, as proliferating aneuploid cells can further evolve into aneuploid cancer cells. Therefore, to target aneuploid cancer, those cells that tolerate aneuploidy will need to be forced to either commit suicide or senesce. There are multiple ways as to how such therapy could work, ranging from very broad spectrum to highly 'personalized' therapies. As discussed above, too much CIN is detrimental to cells (Silk et al. 2013). Therefore, further increasing CIN in aneuploid tumours could be a broad-spectrum way to target aneuploid cancer cells. Indeed, mild CIN renders cells more sensitive to therapeutics that exacerbate CIN such as low doses taxol (Janssen et al. 2009). However, the inherent risk to this therapy is

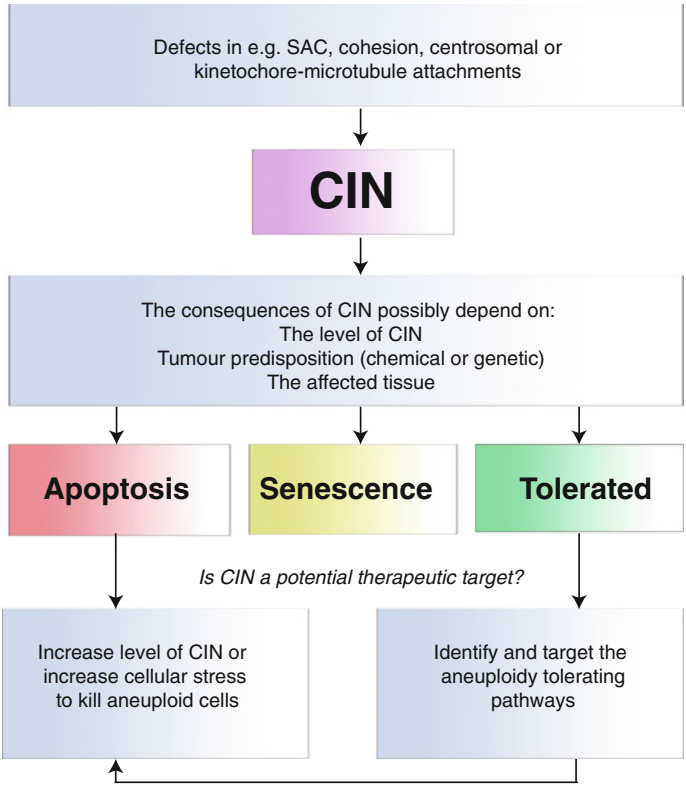

Fig. 2 Flowchart summarizing the in vivo consequences of CIN and therapeutic promise

that untransformed (non-CIN) cells will also be exposed to CIN and might convert into a new CIN tumour over time. A second approach of targeting CIN cells is by modulating the pathways that regulate cell fate following aneuploidization. In this approach, the pathways that result in cell death of (embryonic) stem cells following CIN are artificially activated in aneuploid cancer cells, resulting in cancer cell death. However, before feasibility of such therapy can be assessed, CIN-responsive pathways need to be mapped first. Instead of targeting aneuploidy-signalling pathways, therapy can also target the downstream consequences of CIN. For instance, one common response to aneuploidy is a deregulation of cellular metabolism, which affects untransformed cells as well as cancer cells (Williams et al. 2008; Torres et al. 2008; Foijer et al. 2014). The first proof of principle evidence for such therapy is just emerging. Recent studies are showing that energy stress inducer AICAR and the Hsp190 inhibitor 17-AAG selectively can kill aneuploid (cancer) cells by enhancing aneuploidy-induced stress (Tang et al. 2011; Ly et al. 2013; García Martínez et al. 2014). The next step to this will be to test whether this is also effective in vivo. A fourth 'personalised medicine' approach to tackle aneuploid cancer is by targeting the mutation that is driving CIN. One candidate for such therapy is Hec1, as it is frequently overexpressed in a variety of cancers. Indeed, inhibition of the Hec1/Nek2 pathway results in reduced tumour growth in a xenograft mouse model (Wu et al. 2008), providing proof of principle evidence for this approach. Similarly, gene products that collaborate with CIN in transformation can be targeted using molecular therapy. For the latter, we first need to identify candidate targets, for instance in in vivo genetic screens. However, for molecular therapy full sequencing of the tumour is a requirement. However, as sequencing costs are rapidly decreasing and the number of specific pathway inhibitors are rapidly increasing, this approach might become feasible within the near future.

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