Role of chromosomal instability in cancer progression

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Abstract

Cancer cells often display *chromosomal instability* (CIN), a defect that involves loss or rearrangement of the cell's genetic material – chromosomes – during cell division. This process results in the generation of aneuploidy, a deviation from the haploid number of chromosomes, and structural alterations of chromosomes in over 90% of solid tumours and many haematological cancers. This trait is unique to cancer cells as normal cells in the body generally strictly maintain the correct number and structure of chromosomes. This key difference between cancer and normal cells has led to two important hypotheses: (i) cancer cells have had to overcome inherent barriers to changes in chromosomes that are not tolerated in non-cancer cells and (ii) CIN represents a cancer-specific target to allow the specific elimination of cancer cells from the body. To exploit these hypotheses and design novel approaches to treat cancer, a full understanding of the mechanisms driving CIN and how CIN contributes to cancer progression is required. Here, we will discuss the possible mechanisms driving chromosomal instability, how CIN may contribute to the progression at multiple stages of tumour evolution and possible future therapeutic directions based on targeting cancer chromosomal instability.

Key Words

- ▶ chromosomal instability
- ▶ cancer

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Mechanisms driving chromosomal instability

A defining feature of chromosomal instability is the unequal separation of newly replicated chromosomes to daughter cells during mitosis (Lengauer *et al.* 1998, McGranahan *et al.* 2012). These *chromosome segregation errors* (Fig. 1A) increase genetic heterogeneity between tumour cells at each cell cycle, allowing rapid evolution of tumour genomes and presenting a challenge to cancer patient treatment (see below). Chromosome segregation errors can be observed in tumour-derived cell lines (Cimini *et al.* 2001, Burrell *et al.* 2013) and also in tumour samples (Matsuda *et al.* 2015) and represent a tool for identifying chromosomally unstable tumours (Montgomery *et al.* 2003). Because chromosome segregation errors can be observed during mitosis, much work has focussed on elucidating mitotic defects that contribute to

chromosomal instability (CIN) in cancer. A brief summary of these defects is given below as this subject has been extensively covered elsewhere (Funk *et al.* 2016, Naylor & van Deursen 2016, Sansregret & Swanton 2017). Proper control of the dynamics of microtubules is essential for the correct attachment of chromosomes to the mitotic spindle. Defects in the dynamics of microtubule assembly and depolymerisation have been proposed as mechanisms promoting CIN in cancer. For example, hyperstable microtubules or increased microtubule assembly rates can promote incorrect chromosome-spindle attachments, lagging chromosomes and aneuploidy (Fig. 1B) (Bakhoun *et al.* 2009a, Ertych *et al.* 2014). Defects in the mitotic checkpoint, also known as the spindle assembly checkpoint (SAC), a cellular surveillance mechanism that

A Chromosome segregation errors

B Improper kinetochore-microtubule attachments

Figure 1

Chromosome segregation errors. (A) Cartoon illustrating the three major classes of chromosome segregation error. Lagging centric chromosomes, identified by antibodies targeting centromere-bound proteins, are likely to be whole chromosomes. Acentric fragments, lacking centromeric protein signals, and chromatin bridges, are likely to reflect structurally abnormal chromosomes. (B) Two types of incorrect kinetochore-microtubule attachment are shown, merotelic attachment where one or both sister kinetochores are attached to microtubules emanating from both centrosomes, and syntelic attachment where both sister kinetochores are attached only to a single centrosome.

ensures mitosis cannot complete until all chromosomes are correctly attached to the mitotic spindle, have also been proposed to contribute to CIN. For example, mutant or deregulated key checkpoint genes have been found in some cancers (Cahill *et al.* 1998, Hernando *et al.* 2004, Ryan *et al.* 2012), and certain cancer predisposition syndromes such as mosaic variegated aneuploidy (MVA) are caused by mutations in checkpoint proteins including BubR1 (Hanks *et al.* 2004), CEP57 (Snape *et al.* 2011) and recently TRIP13 (Yost *et al.* 2017). The latter study is particularly interesting as it demonstrated a direct link between TRIP13 downregulation and the development of Wilms tumour in children, and furthermore, suggested that the nature of the MVA-causing mutation dictates the likelihood of cancer developing in the patient (Yost *et al.* 2017). Functional experiments in cancer cell lines have demonstrated that the mitotic checkpoint retains activity in some cancer cell lines (Gascoigne & Taylor 2008, Burrell *et al.* 2013) but not others (Ryan *et al.* 2012); therefore, mitotic checkpoint dysfunction is unlikely to fully explain cancer CIN. A further mechanism that has been proposed to generate CIN is multipolar spindle assembly mediated by extra centrosomes, which leads to asymmetric chromosome segregation, which will result in increased aneuploidy (Nigg 2002). However, this mechanism alone cannot explain CIN, firstly the frequencies of multipolar division are not high enough, and secondly the progeny fail to keep proliferating, indicating that multipolar mitosis does not cause persistent chromosomal instability in cancer cells (Ganem *et al.* 2009, Silkworth *et al.* 2009).

In addition to defects in cellular processes orchestrating the mechanisms of mitosis, it has been shown that chromosome mis-segregation can be caused by defective processes earlier in the cell cycle that interfere with normal chromosome sequence or structure. For example, defects in cohesion or condensation of sister chromatids can predispose to aberrant kinetochore-microtubule attachments (Jallepalli *et al.* 2001, Barber *et al.* 2008, Manning *et al.* 2010, Tanno *et al.* 2015). Furthermore, mutations in cohesion gene STAG2 (Solomon *et al.* 2011) and other cohesion complex members (Solomon *et al.* 2014) have been associated with aneuploidy and tumourigenesis. Chromosomes can also be rendered incompatible with correct progression through mitosis following disruptions at the genetic sequence level. For example, it has been shown in human cells that telomere fusions can promote whole chromosome loss (Pampalona *et al.* 2010). DNA damage can also lead to mis-segregation of damaged chromosomes and chromosome fragments at mitosis (Kaye *et al.* 2004). A more subtle effect of an activated DNA damage response in mitosis was also recently shown to impair normal chromosome attachment error correction machinery, resulting in merotelic attachments and aneuploidy (Bakhoum *et al.* 2014). Defects in the prevention of genome re-replication have additionally been shown as a non-mitotic route to changes in centromere number, albeit only in a yeast system to date (Hanlon & Li 2015). Furthermore, it was shown that, in colorectal cancer, defects in DNA replication ('replication stress') are a major driver of CIN

(Burrell *et al.* 2013). Replication stress occurs at early stages of tumourigenesis in colorectal cancer and other cancer types (Bartkova *et al.* 2005) and can create structurally abnormal chromosomes that cannot be correctly segregated during mitosis (Burrell *et al.* 2013) through a variety of mechanisms that remain poorly characterised, particularly in mammalian systems (Mankouri *et al.* 2013, Mizuno *et al.* 2013, Magdalou *et al.* 2014, Ait Saada *et al.* 2017). A key question remains whether CIN in other cancer types is also driven by replication stress.

Adding an additional layer of complexity to the challenge of elucidating drivers of cancer chromosomal instability is the concept of aneuploidy-driven CIN. Gross changes in chromosome number including polyploidy have been shown to deregulate the genome in yeast (Storchova *et al.* 2006) and mammalian (Li *et al.* 1997) systems. In addition, studies in yeast (Sheltzer *et al.* 2011, Zhu *et al.* 2012) and more recently in human cells (Nicholson *et al.* 2015, Passerini *et al.* 2016) have shown that the presence of a single extra chromosome appears to be sufficient to initiate further chromosome mis-segregation events, with mechanisms underlying this beginning to be elucidated. In some cases, aneuploidy-driven CIN appears to depend upon deregulated gene expression of specific genes (Nicholson *et al.* 2015), and as such may depend upon the identity of the chromosome in aneuploidy. In another case, a global increase in DNA replication stress was observed, that precipitated further genomic instability (Passerini *et al.* 2016). In contrast, however, analysis of trisomic cells from human patients with congenital aneuploidy syndromes did not reveal any increased chromosome instability (Valind *et al.* 2013), sparking lively debate in the field (Duesberg 2014, Heng 2014, Valind & Gisselsson 2014a,b).

Given the multitude of distinct genetic lesions observed between tumour types and also between patients and even regions of the same tumour, it is likely that mechanisms driving CIN vary between, and within cancer types, and it will therefore be important to assess the mechanisms driving CIN in a cancer-specific manner. Moreover, since recent work has highlighted potential differences between some commonly used cancer cell lines and their intended tumour model, particularly in ovarian cancer (Anglesio *et al.* 2013, Domcke *et al.* 2013), it is critical to assess CIN mechanisms in cell line panels that represent the genetic features of their intended represented cancer type as closely as possible. In addition to carefully selecting cell lines to analyse, it may be advisable, where feasible, to move towards the classification of CIN mechanisms from tumour samples directly isolated from

patients, such as circulating tumour cells (CTCs) or other forms of liquid biopsy such as ascites-derived tumour cells (Penner-Goeke *et al.* 2017). Such efforts would not only alleviate concerns about applicability of tumour-derived cell lines but also allow the assessment of CIN in a temporal manner (Penner-Goeke *et al.* 2017). This would provide an important advancement of our understanding of the dynamics of CIN during tumour evolution. Current knowledge of how CIN varies over the lifetime of a tumour, and in response to treatment, is currently limited; however, elegant studies utilising tumour multiregion sequencing in patients before and after therapy have begun to elucidate the complex dynamics of CIN and tumour evolution over tumour lifetimes and how this may impact patient survival (Murugaesu *et al.* 2015, Jamal-Hanjani *et al.* 2017).

CIN: a clinical problem

Chromosomal instability predicts drug resistance and poor prognosis in multiple cancer types (Swanton *et al.* 2009, McGranahan *et al.* 2012) with several recent studies demonstrating the impact of chromosomal instability, tumour heterogeneity and clonal evolution on chemotherapy resistance and metastasis (Bakhoun *et al.* 2011, Gerlinger *et al.* 2012, McGranahan *et al.* 2012, Bashashati *et al.* 2013, Castellarin *et al.* 2013, Murugaesu *et al.* 2015, Jamal-Hanjani *et al.* 2017). Chromosomal instability thus renders tumours more difficult to treat, but precise reasons for this are currently poorly defined. Chromosomally unstable cell lines derived either by direct manipulation of Mad1 levels to induce CIN (Ryan *et al.* 2012), or from tumours (Lee *et al.* 2011), are intrinsically more resistant to chemotherapy, and CIN promotes massive genomic variation that provides an ideal substrate for *acquired resistance* (the outgrowth of a pre-existing clone under novel selection conditions – see Fig. 2). In addition, it is thought that chromosomal instability can act as an adaptive mechanism to allow cancer cells to widely sample a range of genotypes and phenotypes during periods of stress. This concept has been clearly demonstrated in single-celled organisms (Rancati *et al.* 2008, Selmecki *et al.* 2009, Pavelka *et al.* 2010, Yona *et al.* 2012, Chang *et al.* 2013), however, demonstrating this in human cells has been more challenging. There is evidence that aneuploid human cells, although generally appearing less fit than their euploid counterparts under ‘standard’ conditions (Williams *et al.* 2008, Thompson & Compton 2010) display advantages under selective conditions (Rutledge *et al.* 2016) and

Figure 2

Major pathways to chemotherapy resistance. Three different but not necessarily mutually exclusive pathways to resistance are depicted. *Innate resistance* allows tumour growth to continue unchecked by the application of chemotherapy, for example, in the case of patients with non-responsive tumours. *Adaptive resistance* can occur if a pre-existing tumour cell or clone is present in the primary tumour that is capable of survival in the presence of chemotherapy agent. Tumours will initially respond and regress, but relapse will occur, seeded from the resistant clones. *Acquired resistance* occurs when genetic or other mechanisms allowing the development of novel traits is present. Chemotherapy itself may also promote genome mutation and rearrangement to facilitate this process. Cells that chance upon a resistant phenotype are then able to seed tumour regrowth. A prediction of these distinct pathways to resistance is that the relapsed tumour may display different levels of heterogeneity or CIN depending on which pathway was most prevalent during tumour regrowth.

that aneuploidy of pluripotent stem cells promotes their efficient adaptation to culture conditions (Barbaric *et al.* 2014, Na *et al.* 2014). Further, aneuploidy in murine liver cells promotes adaptation to chronic liver injury (Duncan *et al.* 2012). Additionally, elegant work in mice models used Mad2 knockout to confer adaptive advantage by increasing chromosome mis-segregation, which promoted efficient tumour relapse following withdrawal of KRAS dependency (Sotillo *et al.* 2010). However, it remains challenging to test these principles in controlled human cellular systems directly as this requires the ability to manipulate rates of chromosomal instability.

CIN-mediated genetic and phenotypic diversity in tumours thus has important implications for chemotherapy resistance via three possible pathways (Fig. 2): (i) *innate resistance*, perhaps linked to the necessity of cancer cells to tolerate chromosomal instability and aneuploidy, may provide a higher threshold of tolerance to chemotherapy agents. It is also possible that aneuploidy-mediated growth rate reduction seen in some models provides a quiescent state and concomitant protection from DNA damaging or other chemotherapeutics; (ii) *acquired resistance*, due to high initial substrate (tumour cell population) diversity generated prior to chemotherapy treatment, provides a high probability that a sub-clone is present that intrinsically carries protection against the chemotherapeutic agent used. This sub-clone can then outgrow sensitive tumour cells that are eliminated during treatment (Greaves & Maley 2012); (iii) *adaptive resistance*, where a resistant sub-clone

may not exist prior to treatment, but may be generated during or following chemotherapy treatment. This might depend on a mutator phenotype that can generate genetic diversity via chromosomal instability or small-scale sequence level changes such as point mutations. This mutator capability could be either intrinsic to the initial tumour, for example tumour CIN, and might also be provided or augmented by the genome-destabilising effects of cytotoxic chemotherapeutic agents.

Targeting CIN therapeutically

Chromosomal instability: a balancing act

A key defining feature of chromosomally unstable cells is that they apparently tolerate a multitude and shifting spectrum of chromosomal defects that are strongly deleterious in normal cells (Williams *et al.* 2008, McClelland *et al.* 2009, Thompson & Compton 2010). Induction of aneuploidy in diploid systems leads to multiple cellular changes that result in cell death or cell cycle arrest, reviewed in (Santaguida & Amon 2015). Chromosomally unstable cells have overcome these arrest points by multiple mechanisms, some of which are beginning to be elucidated (Li *et al.* 2010, Lopez-Garcia *et al.* 2017). Moreover, tumours appear to have evolved a beneficial and tolerable level of CIN. Studies have shown that disrupting this balance can impact tumour progression. CENP-E is a key player during mitosis, essential for the correct alignment and

segregation of chromosomes (Yen *et al.* 1992). Fine-tuning levels of chromosome segregation errors using CENP-E deregulation in mouse models have shown that CIN can act either as a tumour suppressor or to promote tumour formation depending on the rate of chromosome segregation errors (Weaver *et al.* 2007, Silk *et al.* 2013, Zasadil *et al.* 2016). An advantage of manipulating this nexus of chromosome segregation is that it removes potential confounding effects from additional roles of other genes that have been experimentally manipulated to examine the effects of aneuploidy on tumour progression (reviewed in Giam & Rancati 2015). Analysis of chromosomal instability levels in patients echoed this work from model systems, demonstrating that patients with tumours exhibiting 'extreme' CIN showed better prognosis (Birkbak *et al.* 2011). This was supported by subsequent studies in breast cancer (Roylance *et al.* 2011, Jamal-Hanjani *et al.* 2015), and also more recently in a pan-cancer analysis (Andor *et al.* 2016).

Modulating CIN levels in cancer for therapeutic benefit

These findings suggest that there may be a window of opportunity to target CIN cancers by driving them over the edge of tolerable CIN (Janssen *et al.* 2009, Silk *et al.* 2013, Zasadil *et al.* 2016). However, there is a potential worry that such approaches might negatively affect portions of the tumour that may reside in a 'low CIN' fraction and that would be elevated to higher CIN levels with deleterious consequences for patients. An alternative strategy would be to *decrease* CIN, with the aim of preventing CIN-driven chemotherapy resistance (*adaptive resistance*, Fig. 2). Elegant genetic manipulation of CIN levels by modulating the expression of the mitotic checkpoint regulator Mad2 in an Hras^{v12} mouse fibrosarcoma model demonstrated a delay in tumour formation when CIN levels were decreased (Schvartzman *et al.* 2011). In addition, recent studies took advantage of the fact that aberrant microtubule dynamics driving CIN (discussed above) can be modulated to test the role of CIN in tumourigenesis. One study found that limiting CIN in a mouse model of CRC lead, seemingly counter-intuitively, to increased tumour growth (Ertych *et al.* 2014). However, in light of the knowledge that CIN and aneuploidy can carry a fitness cost, this is perhaps not surprising. Furthermore, this study was performed in the absence of chemotherapy treatment, and it would therefore be very interesting to extend these studies to examine the response to chemotherapy of such 'CIN-limited' tumours. A similar approach to limit CIN driven by aberrant microtubule dynamics

was recently taken by the use of a drug to potentiate MCAK, the molecular motor that depolymerises MTs and overexpression of which can limit CIN caused by hyperstable microtubules (Orr *et al.* 2016). However, in this interesting study, the hypothesis of whether limiting CIN might impact tumour progression was unable to be tested due to the unexpected adaptation of CIN cancer cells to the agent, resulting in the re-establishment of high rates of chromosome mis-segregation. Such adaptation did not occur following manipulation of protein-level of the microtubule depolymerase MCAK in earlier studies (Bakhoun *et al.* 2009b), suggesting a small molecule-specific effect promoting the adaptation to CIN reduction. This re-establishment of chromosomal instability in a cell culture-based model system is perhaps surprising in the absence of any obvious drive to maintain CIN, such as might be expected to occur in a tumour environment, and hints that there might be an innate drive to maintain genetic instability within chromosomally unstable cancer cells. More recently, it has been shown that lengthening mitosis by manipulating the function of the master regulator of mitotic protein degradation, the APC/C complex, can also reduce chromosome mis-segregation (Sansregret *et al.* 2017). In addition to limiting mitosis-led mechanisms of CIN, approaches to limit replication stress driven CIN also exist. A potential therapeutic avenue for reducing replication stress-mediated CIN has been provided by studies showing that chromosome segregation errors can be prevented by reducing replication stress by the addition of nucleosides (Bester *et al.* 2011, Burrell *et al.* 2013, Ruiz *et al.* 2015). Thus, tools are beginning to emerge to allow the role of CIN throughout multiple stages of tumourigenesis and relapse to be elucidated. A potential drawback of such approaches to decrease tumour CIN as a therapeutic approach is that CIN-limiting effects might be negated if chemotherapeutic agents themselves are able to promote chromosomal instability. Furthermore, studies testing this hypothesis have to date been limited to the use of relatively homogenous cell lines, whereas tumours are vastly heterogeneous, potentially encompassing multiple types or levels of chromosomal instability, and possibly rendering it difficult to elicit a uniform CIN-limited state.

Evolutionary traps

This concept was defined following elegant studies demonstrating the possibility of using genetic instability-driven adaptation to 'fix' a population in a particular evolutionary fitness peak, effectively reducing the heterogeneity of the population and reducing its ability to

effectively adapt to a second insult (Chen *et al.* 2015). This concept was also echoed in the study described previously using small-molecule inhibitors to reduce CIN caused by deviant microtubule dynamics where treated cell populations became specifically desensitised to Aurora B inhibitors (Orr *et al.* 2016).

Outstanding questions

In order to direct efforts to target chromosomal instability in cancer patients, we need to understand better the mechanisms and contributions of CIN throughout the history of a tumour. For example, is CIN operational at consistent rates throughout tumour development, or in short bursts (*punctuated evolution*)? Is CIN active during regrowth of tumour cells following chemotherapy treatment, or is the genetic heterogeneity of the remaining tumour cell population sufficient to house a pre-existing clone that can re-seed tumour growth? Lastly, is CIN elevated following treatment with cytotoxic or targeted therapies? It will be important to answer these questions to allow novel strategies targeting CIN to be taken forward to pre-clinical and clinical trials, coupled with the further advancement of assays for CIN from patients over time so we can discover whether chromosomal instability can be manipulated in ways that enhance the quality of life for cancer patients.

Conclusion

Current cancer therapy approaches including personalised targeted therapies and immunotherapies are often thwarted by the existence of chromosomal instability and tumour heterogeneity enabling rapid adaptation to treatment and the almost inevitable relapse of tumours. We are hopeful that future research to determine root cases of CIN, and methods to limit, or specifically elevate CIN, can be used against the disease in two key ways; by providing new information to drive future drug discovery efforts specifically targeting vulnerabilities of chromosomally unstable cells and by providing opportunities to test whether modulating CIN is a feasible strategy, a key hypothesis that may represent a paradigm shift in the approach to treating cancer.

Declaration of interest

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