Investigating aneuploidy's role in cancer cell fitness under various conditions of stress

Samuel Drew Rutledge

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
Department of Biological Sciences

Daniela Cimini, Chair
Silke Hauf
Richard Walker

June 30th, 2015
Blacksburg, VA

Keywords: Cancer, Aneuploidy

Investigating aneuploidy's role in cancer cell fitness under various conditions of stress

Samuel Drew Rutledge

ABSTRACT

The gain or loss of whole chromosomes, known as aneuploidy, is a distinguishing feature of cancer cells. The rapid gain or loss of hundreds of genes dramatically alters a cell's genomic landscape and is typically detrimental to cell survival under normal conditions. However, cancer cells display enhanced proliferation and overcome multiple conditions of stress, suggesting aneuploidy may increase cellular fitness. Furthermore, distinct patterns of aneuploidy are found in cancers from different anatomical sites. Despite these observations, scant research has sought to examine the role of aneuploidy in cancer, or determine whether aneuploidy is a driver or passenger mutation, or why certain aneuploidies appear to be selected for and others against. To investigate the role of aneuploidy in cancer cell fitness, we utilized the diploid colorectal cancer (CRC) cell line DLD1 and two trisomic variants carrying an extra copy of either chromosome 7 or chromosome 13, two trisomies frequently seen in colorectal cancer. To assess fitness, we compared proliferation, anchorage-independence, and invasiveness in aneuploid CRC cells versus their diploid counterpart when grown under various culture conditions, including regular media, serum-free media, cytotoxic drug-containing media, and hypoxia. We found that aneuploid cells proliferated better than diploid cells under all but standard culture conditions. Moreover, regardless of growth condition, we found that aneuploid CRC cells formed larger and more numerous colonies in soft agar (anchorage-independent growth), and displayed greater invasiveness (assessed by matrigel invasion assay). Taken together, these results indicate that aneuploidy enhances the fitness of CRC cells under stressful conditions that are likely to occur in the tumor microenvironment.

Acknowledgements

I would like to acknowledge the support of my family throughout my life, but especially during my final years at Virginia Tech. These words can never payback the debt owed to them for their relentless encouragement, unwavering devotion and dedication that helped see me through the most difficult times.

I would also like to thank my mentor and adviser, Daniela Cimini, as well as the entire Cimini lab for forging me into a better scientist and stronger person. I learned and grew through every experience and challenge through these interactions; this work would not have been possible without you. Thank you.

Table of Contents

List of Figures and Tables

Chapter 1: Introduction

1.1. Brief History of Cancer and Cancer Therapy

Over a century ago, Theodor Boveri, observed multipolar mitotic cell divisions lead to the formation of cell with an abnormal number of chromosomes, termed aneuploidy (Boveri, 1902). By the time of Boveri's observations, aneuploidy had already been associated with cancer [reviewed in (Heim and Mitelman, 2011)], leading Boveri to hypothesize that aneuploidy underlined malignant progression (Boveri, 1902; Boveri, 1914). Despite a tremendous collective effort to identify effective cancer therapies (Abbott, 2002; Marx, 2002), whether aneuploidy is a cause or a consequence of malignant transformation remains the subject of great debate.

The first effective chemotherapeutic agent was aminopterin, a methotrexate analog that was discovered in 1947 (Farber et al., 1948). Methotrexate acts during the synthesis (S-phase) phase of the cell cycle, targeting cells through inhibition of thymidylate synthesis (Hoffbrand and Tripp, 1972). However, following exposure to hypoxic stress, Chinese hamster ovary cells display increased resistance to methotrexate (Rice et al., 1986), overduplication of their chromosomes (Rice et al., 1986; Schimke et al., 1986), and higher ploidy compared to untreated cells (Baroja et al., 1998). Despite these associations, the cellular and molecular mechanisms directly responsible for generating cells with higher chromosome content remain unclear (Marx, 2002).

Although methotrexate remains the mainstay therapy for common cancers such as colorectal cancer, half a century after its discovery, the focus of cancer research and drug design have shifted from S-phase to mitosis. Mitosis became a successful antitumor target following the identification of several drugs, such as taxol, docetaxel, and vincristine. Although all these drugs are highly effective against cancer, they target all dividing cells without specificity [reviewed in (Weaver and Cleveland, 2005)]. Despite these advances, the therapeutic value of cancer drugs remains limited by the fact that tumor cells develop resistance (Lee et al., 2011) via complex biological mechanisms.

Recently, it has become clear that tumors are characterized by genetically heterogeneous landscapes (Gerlinger et al., 2012), referred to as intratumor heterogeneity. Consequently, eliminating most but not all cancer cells within a tumor cell population effectively selects for the most aggressive cancer cell sub-population. These cells, in turn, possess inherent resistance to the therapy used, promoting recurrence and, if untreated, metastasis. The clinical ineffectiveness forced another paradigm shift in cancer research and launched the search for specific gene mutations in the hope of designing personalized cancer therapies.

This paradigm shift produced cancer research greatest success, imatinib (Gleevec) (Horne et al., 2013). In patients with chronic myelogenous leukemia (CML) scientists observed a specific and recurrent chromosomal abnormality, known as the Philadelphia chromosome (Rowley, 2004). The Philadelphia chromosome was the result of a balanced translocation between chromosomes 9 and 22 that created a chimeric fusion protein, *BCR-ABL*, which Gleevec inhibits specifically (Capdeville et al., 2002). However, Gleevec's success is limited by the accumulation of additional chromosome aberrations and development of drug resistance (Hochhaus et al., 2002).

Despite the discovery of the Philadelphia chromosome in CML and Gleevec's effectiveness, similar clonal translocations and chimeric gene products are uncommon and ineffective therapeutic targets. To catalog the chromosome aberrations of specific cancers, researchers established the Mitelman database, a cancer chromosome database, which indicates that most cancer cells are aneuploid, containing both structural and numerical chromosome abnormalities (Table 1.1) (Cimini, 2008; Heim and Mitelman, 2011; Mitelman et al., 2014). Despite this widely acknowledged fact, research into aneuploidy's role in cancer biology remains in its early stages.

1.2. Overview of the Cell Cycle

Fundamental to life is the process of growth and replication. At the cellular level, this process is tightly regulated and proceeds through what is known as the cell cycle, a process of cell growth and division whose misregulation has been asserted to be at the core of cancer (Malumbres and Barbacid,

2001; Massague, 2004). The cell cycle can be broken down into four stages, G1, S, G2, and M (Figure 1.1), characterized by specific events. Cells in the G1 phase undergo growth and prepare to replicate their DNA by assuring, through the G1/S checkpoint, that their DNA is damage-free. Chromosomes are duplicated in S phase and further DNA repair occurs in the ensuing G2 phase as the cell prepares to undergo division during M phase (Blow and Tanaka, 2005; Wittmann et al., 2001), which is further divided into mitosis and cytokinesis. The process of mitosis is divided into five distinct stages: prophase, prometaphase, metaphase, anaphase, and telophase (Figure 1.2) (Blow and Tanaka, 2005; Rieder and Khodjakov, 2003), and will be discussed in the next section.

1.3. Mitosis

The start of mitosis, prophase, is marked by condensation of the genetic material into individual chromosomes. The two sister chromatids of each chromosome, products of DNA replication at the preceding S phase, are held together by cohesin protein complexes, whereas condensin protein complexes are responsible for chromosome condensation. An outer kinetochore (a large protein complex important for interaction with microtubules) assembles on each chromatid. At the same time, the interphase microtubule cytoskeleton disassembles and the two centrosomes (which duplicated in the preceding S phase) start nucleating short and dynamic microtubules and begin moving to opposite sides of the nucleus forming a microtubule-based bipolar mitotic spindle (Kapoor et al., 2000; Kashina et al., 1996). Nuclear envelope breakdown marks progression from prophase into prometaphase.

The breakdown of the nuclear envelope gives chromosomes the chance to interact with microtubules growing from the separated centrosomes. The fully assembled kinetochores form attachments with the microtubules, and typically one of the sister kinetochores establishes attachment first (monopolar attachment). This results in the rapid poleward movement of the chromosome (Rieder and Alexander, 1990), which only later may establish attachment at the other sister kinetochore (bipolar attachment). Once a chromosome becomes bipolarly attached, it moves to the spindle equator in a process named chromosome congression. Monopolar chromosomes can also move to the spindle equator with one

kinetochore still unattached (Kapoor et al., 2006; Cai et al., 2009). Regardless of how congression occurs, all chromosomes eventually become bipolarly attached and line up at the spindle equator.

Metaphase begins after all the chromosomes have congressed to the spindle equator forming what is known as the metaphase plate. Chromosomes aligned at the metaphase plate are not stationary but instead exhibit oscillatory movement (Skibbens et al., 1993). These oscillations result from microtubule-dependent forces acting on the chromosomes at the spindle equator (Civelekoglu-Scholey et al., 2013) and continue until the cohesin holding the sister chromatids together is broken down at anaphase onset (Haering and Nasmyth, 2003).

The sudden elimination of cohesin marks anaphase onset and enables the movement of separated sister chromatids to opposite poles (Blow and Tanaka, 2005). Chromosome poleward movement is usually defined anaphase A, whereas the spindle elongation that starts soon after sister chromatid separation is referred to as anaphase B.

In telophase, chromosome segregation to the spindle poles is completed, and the chromosomes decondense while the nuclear envelope reassembles around the decondensing chromosomes, packing the two sets of segregated chromosomes into two new daughter nuclei. Simultaneously, an actin-myosin contractile ring assembles in the region of the cortex corresponding to the spindle equator/midzone and pinches the cytoplasm in two in a process known as cytokinesis.

Cell division must be finely regulated as errors in this process can lead to chromosome mis-segregation and aneuploidy, as discussed in the next section.

1.4. Pathways to Aneuploidy

Aneuploidy, the condition of a cell possessing an incorrect number of chromosomes, arises as a consequence of chromosome missegregation during cell division. Aneuploidy arising in the germ line, and specifically occurring during meiosis, is a major cause of miscarriage and genetic diseases in humans (Brown, 2008; Duelli et al., 2005). Similarly, aneuploidy arising in mitotic cells is generally considered disadvantageous, as shown by a decrease in proliferative potential in mouse embryonic fibroblasts and

yeast (Cimini, 2008; Torres et al., 2008; Williams et al., 2008). While rates of missegregation are likely to vary based on cell type and environmental context, experiments in cell culture suggest chromosome missegregation occurs as frequently as once every 100 cell divisions (Yuen and Desai, 2008). Common pathways to aneuploidy include defects in the spindle assembly checkpoint (SAC), cohesion defects, and kinetochore mis-attachments.

1.4a. Defects in the Spindle Assembly Checkpoint

The mitosis-specific surveillance mechanism responsible for upholding chromosomal stability is known as the spindle assembly checkpoint (SAC). The SAC functions by monitoring the attachment of kinetochores to spindle microtubules (Cleveland et al., 2003), and prevents anaphase onset until all kinetochores have attached spindle microtubules. Once all chromosomes achieve bi-orientation, the SAC is satisfied, which then triggers degradation of the cohesin complexes holding sister chromatids and, hence, sister chromatid separation and anaphase onset. Unattached kinetochores produce a "wait anaphase" signal (Rieder et al., 1995), which prevents the metaphase-to-anaphase transition. Even a single unattached kinetochore is enough to maintain an active SAC and to prevent anaphase onset for some time (Rieder et al., 1995; Rieder et al., 1994), thereby allowing kinetochore attachment defects to be corrected (Wang et al., 2013; Wells, 1996).

The molecular components of the SAC were originally identified by isolating budding yeast strains that failed to arrest cell cycle progression in the presence of spindle poisons (from which the name "spindle assembly checkpoint") (Hoyt et al., 1991). Studying homologs of the genes discovered in budding yeast many eukaryotic equivalents were identified, including: MAD2, BUB1, BUB3, BUBR1 and MPS1 (Wassmann and Benezra, 2001). These SAC components were found to have multiple functions and interactions during mitosis, and elimination of any single component is enough to compromise SAC function. SAC proteins are recruited to unattached kinetochores and produce a soluble inhibitory complex that prevents the activation of the anaphase-promoting complex/cyclosome (APC/C). APC/C activation leads to the degradation of securin, which exists in a complex with the protein separase

prior to APC/C activation. Degradation of securin, thus, leads to activation of separase, which in turn cleaves the cohesin complexes, triggering anaphase onset (Holland and Cleveland, 2009).

Inactivation of the SAC results in massive chromosome missegregation and cell death (Kops et al., 2004; Michel et al., 2001). On the other hand, mild checkpoint defects may lead to single chromosome mis-segregation events and aneuploidy (Figure 1.3a) (Holland and Cleveland, 2009). Thus, significant efforts have been invested toward understanding whether mutations in SAC genes may be associated with cancer development and/or progression.

Mutations in the SAC gene BubR1 were found in some patients affected by the cancer predisposition syndrome mosaic variegated aneuploidy (Hanks et al., 2004; Matsuura et al., 2006). Animal models with haploinsufficiency or heterozygous mutations in SAC genes display an increase in aneuploidy, as well as an increased incidence of tumor development (Babu et al., 2003; Baker et al., 2005; Dai et al., 2004; Iwanaga et al., 2007; Jeganathan et al., 2007; Michel et al., 2001; Weaver et al., 2007). Moreover, mouse cells or HCT116 cells with a mutation in the SAC protein MAD2 were shown to display premature sister chromatid separation and increased rates of chromosome missegregation (Michel et al., 2001). Interestingly, although mutations in SAC genes have been reported in cancer (Cahill et al., 1998), only rarely are they seen in coding regions (Bharadwaj and Yu, 2004), and in general, very few mutations have been identified despite extensive analysis (Barber et al., 2008; Greenman et al., 2007; Schvartzman et al., 2010; Weaver and Cleveland, 2006; Wood et al., 2007). However, SAC genes are found to be frequently mis-expressed in cancer (Schvartzman et al., 2010). In mouse models, overexpression of MAD2 causes structural and numerical chromosomal alterations and tumorigenesis (Sotillo et al., 2007). Importantly, overexpression of MAD2 and the kinetochore component HEC1 are frequently observed in cancer and are associated with poor prognosis (Hayama et al., 2006; Hernando et al., 2004; Li et al., 2003; Tanaka et al., 2001).

1.4b. Cohesin Defects

Mutations in cohesin or cohesion related genes (separase or securin) lead to premature or delayed sister chromatid separation, promoting chromosome mis-segregation and aneuploidy (Figure 1.3b) (Solomon et al., 2011; Zhang et al., 2008). Accordingly, in experiments with budding yeast, mutations in cohesion related genes increase the rates of chromosome missegregation by allowing cells to complete mitosis in the presence of unaligned chromosomes (Elledge, 1996; Murray, 1995; Nasmyth, 1996; Paulovich et al., 1997). Interestingly, mutations in the human homologs of the aforementioned yeast genes were also found in colorectal cancer (Barber et al., 2008). Moreover, the down-regulation or genetic disruption of these human homologs in the human CRC cell line HCT116 produced defects in sister chromatid cohesion leading to chromosomal instability, and aneuploidy (Barber et al., 2008). Additionally, the cohesion-cleaving enzyme separase has been reported to be over-expressed in breast cancer (Zhang et al., 2008), further supporting the importance of cohesion integrity to normal cell division. However, it remains unclear to which extent cohesion defects contribute to human cancers.

1.4c. Kinetochore Attachment Defects

Accurate chromosome segregation is ensured by formation of amphitelic kinetochore attachment (Figure 1.4A), in which the two kinetochores of an individual mitotic chromosome bind to microtubules from opposite poles of the mitotic spindle. However, other attachments (monotelic, syntelic, and merotelic; see Figure 1.4B-D) may form early in mitosis and may lead to chromosome missegregation and aneuploidy under certain circumstances.

Monotelic attachments, in which only one kinetochore is attached and its sister is unattached, give rise to aneuploid daughter cells if left uncorrected (Figure 1.4B) (Cimini, 2008). However, this type of attachment is sensed by the SAC, and therefore can give rise to aneuploidy only when SAC signaling is impaired.

A syntelic attachment is formed when microtubules emanating from the same spindle pole attach to both sister kinetochores (Figure 1.4C). If allowed to persist into anaphase, syntelic attachments will produce aneuploid daughter cells (Cimini, 2008); however, it is believed that syntelic attachments are

corrected before anaphase onset, although the mechanism ensuring their correction remains unclear (Pinsky and Biggins, 2005).

Merotelic attachments, in which one kinetochore is bound to microtubules from both spindle poles (Figure 1.4D), occur frequently in early mitosis due to the stochastic interaction between kinetochores and microtubules (Cimini et al., 2004; Cimini et al., 2002; Cimini et al., 2003). Although usually corrected before anaphase onset (Cimini et al., 2003) merotelic attachments are not detected by the SAC (Cimini et al., 2004; Cimini et al., 2002; Rieder et al., 1997; Wise and Brinkley, 1997; Yu and Dawe, 2000) and can therefore persist into anaphase, producing lagging chromosomes (chromosomes lagging behind at the spindle equator) (Cimini et al., 2001) and potentially leading to aneuploidy (Cimini et al., 2002; Cimini et al., 2001) (Figure 1.4D).

1.5. *Chromosome Segregation Defects and CIN*

As described in earlier sections, cancer cells are generally aneuploid (Mitelman F, 2013). In addition to being aneuploid, cancer cells display high rates of chromosome missegregation (up to 100-fold above the rates found in normal cells), a phenomenon termed chromosomal instability, or CIN (Lengauer et al., 1997). Multiple mechanisms have been proposed to underlie CIN, including: defects in the mitotic checkpoint, impaired microtubule dynamics, presence of abnormal or supernumerary centrosomes, aneuploidy, and transient defects in spindle geometry [as reviewed in (Nicholson and Cimini, 2011)].

An early report characterizing colorectal cancer cells suggested that CIN cells treated with spindle poisons failed to arrest in mitosis, leading some researchers to propose that defects in the SAC may underline the high chromosome missegregation rates observed in CIN cancer cells (Cahill et al., 1998; Kops et al., 2005; Lengauer et al., 1998). However, later studies showed that CIN cancer cells arrest in mitosis when treated with spindle poisons, indicating that the SAC is fully functional in these cells (Tighe et al., 2001) and that SAC defects are unlikely to be the underlying cause of CIN.

Recent evidence suggests that merotelically attached anaphase lagging chromosomes are the most common chromosome segregation defect observed in CIN cancer cells (Bakhoum et al., 2014; Thompson and Compton, 2008). Two major mechanisms have been proposed to be responsible for the increased rates of merotelically-attached lagging chromosomes in CIN cancer cells, namely supernumerary centrosome and transient multipolarity (Ganem et al., 2009; Silkworth et al., 2009), and impaired microtubule dynamics.

Supernumerary centrosomes are seen in both solid mass and hematological cancers (Krämer et al., 2005; Lingle et al., 1998; Nigg, 2002), and may arise through numerous mechanisms, including cell fusion, centriole overduplication, mitotic slippage, and cytokinesis failure (Duelli and Lazebnik, 2007; Ganem et al., 2007; Nigg, 2002). Cancer cells with supernumerary centrosomes typically assemble multipolar mitotic spindles. Cells with multipolar spindles can undergo multipolar cell division, producing three or more daughter cells (Storchova and Pellman, 2004), which typically are not viable (Ganem et al., 2009). In most cases, however, CIN cancer cells cluster their centrosomes into two poles prior to anaphase onset and undergo bipolar cell division with high rates of anaphase lagging chromosomes (Ganem et al., 2009; Silkworth et al., 2009). The increased rates of anaphase lagging chromosomes are caused by an increase in the formation of merotelic kinetochore attachments during the transient multipolar state (Ganem et al., 2009; Silkworth and Cimini, 2012; Silkworth et al., 2009). Thus, the high rates of anaphase lagging chromosomes in CIN cancer cells are, at least in part, the result of increased rates of formation of merotelic kinetochore attachments (Ganem et al., 2009; Silkworth and Cimini, 2012; Silkworth et al., 2009).

The frequency of merotelic attachments is determined by the balance between the rate of kinetochore mis-attachment formation and the rate of correction. Mis-attachment correction requires turnover of kinetochore-bound microtubules, by which the plus-ends of microtubules detach from the kinetochore (followed by microtubule depolymerization) to allow for attachment to new microtubules. For correction or merotelic attachments, microtubules from one pole (the incorrect pole) must be replaced with microtubules from the correct pole (Cimini, 2007). In support of this, increased stability of

kinetochore-microtubules decreases correction of merotelic kinetochore attachments, thus increasing the frequency of anaphase lagging chromosomes (Bakhoum et al., 2008) and CIN cancer cells display increased kinetochore-microtubule stability (Bakhoum et al., 2009). Thus, CIN cancer cells are both prone to establishing erroneous kinetochore-microtubule attachments (Ganem et al., 2009; Silkworth et al., 2009) and less likely to correct such erroneous attachments (Bakhoum et al., 2009).

1.6. *Consequences of Aneuploidy*

Aneuploidy is a distinguishing feature of cancer cells. The rapid gain or loss of hundreds of genes dramatically alters a cell's genomic landscape and is typically detrimental to cell survival under normal conditions (Gao et al., 2007; Pollack et al., 2002; Thayer, 1996; Upender et al., 2004). In mammalian cancer cells and budding yeast, for instance, it has been shown that changes in mRNA expression correlate with chromosome copy number (Gao et al., 2007; Pavelka et al., 2010). More precisely, the gain of a specific chromosome leads to up-regulation of genes on that chromosome whereas the loss of a specific chromosome leads to down-regulation of the gene products encoded on that specific chromosome (Gao et al., 2007; Pavelka et al., 2010; Ried et al., 2012). Although this mechanism of aneuploidy-related gene mis-regulation is not always straightforward, it is typically detrimental, supporting Boveri's original idea that aneuploidy may have detrimental effects on cell proliferation and cellular physiology (Boveri, 1902).

Aneuploidy remains the leading cause of miscarriage and still birth in humans (Hassold and Hunt, 2001), as well as the cause of Down syndrome, the most common human genetic disease (Lejeune et al., 1959). Trisomy 21, the cause of Down syndrome, is the only human trisomy known to be compatible with survival to adulthood. Indeed, while trisomy 13 and trisomy 18 can survive birth, both lead to death within the first few months of life (Pai et al., 2003; Rasmussen et al., 2003). Interestingly, transcript levels of the genes carried on chromosome 21 are increased in Down syndrome patients (Mao et al., 2003), and chromosomes 13, 18, and 21 represent the smallest chromosomes with regard to the number of transcripts they encode (Torres et al., 2008). These observations support the notion that the gene content of a

chromosome plays a role in a cell's ability to tolerate the aneuploidy and the corresponding protein imbalances it causes (Duijf et al., 2013).

At the cellular level, aneuploidy has been shown to produce similar deleterious physiological effects, termed aneuploidy-associated stress, in both mammalian and yeast cells (Tang et al., 2011; Torres et al., 2007; Williams et al., 2008). Such effects are similar to those observed in cells derived from Down syndrome individuals (Korenberg et al., 1994; Williams et al., 2008), and reduce cellular fitness (Torres et al., 2007) by impairing proliferation and growth under normal conditions (Pavelka et al., 2010; Torres et al., 2007). These deleterious effects are thought to arise as a consequence of proteotoxic stress (Oromendia et al., 2012; Stingele et al., 2012), which refers to an excess of proteins aggregating in folded and misfolded conformations as a result of the aneuploidy-induced protein imbalance (Donnelly et al., 2014; Oromendia et al., 2012).

However, despite the generalized deleterious effects of aneuploidy, there are circumstances in which aneuploidy can confer a selective advantage by increasing stress tolerance. For example, the gain of chromosome VI (which carries the β-tubulin gene) in *Saccharomyces cerevisiae* is lethal (Anders et al., 2009; Katz et al., 1990; Torres et al., 2007), but the addition of chromosome XIII (encoding α-tubulin) restores viability (Anders et al., 2009). Furthermore, aneuploidy has been shown to confer drug resistance in pathogenic yeast (Selmecki et al., 2006) and increase their virulence (Poláková et al., 2009). Finally, aneuploidy improves budding yeast's survival under conditions of limited nutrients (Dunham et al., 2002; Gresham et al., 2008; Pavelka et al., 2010), or in the face of genomic damage or alteration (Hughes et al., 2000; Rancati et al., 2008; Vernon et al., 2008).

Aneuploidy appears to affect higher eukaryotic cells similarly to how it affects yeast. For instance, colon epithelial cells trisomic for chromosome 7 out-compete their diploid counterparts for growth under serum-free conditions (Ly et al., 2011). Similarly, tyrosinemia-induced stress in the mouse liver is overcome by emergence of aneuploid hepatocytes lacking chromosome 16 that carries the homogentisic acid dioxygenase gene (Duncan et al., 2012). However, it also appears that the same

aneuploidy may produce different effects in different cell types, as shown by a study in which the gain of identical trisomies in different cell types produced different patterns of gene expression changes (Upender et al., 2004). This is likely a consequence of the fact that cells from different tissues/organs rely on the expression of different sets of genes for survival and proliferation (Liu et al., 2008). Indeed, microarray analysis of cells from different tissues demonstrates that normal diploid cells display tissue-specific patterns of gene expression (Hsiao et al., 2001; Liu et al., 2008). These observations may explain the multi-fold increased risk and incidence of hematological cancers (particularly leukemias) and resistance to developing solid mass tumors of Down syndrome patients compared to diploid individuals (Hasle et al., 2000; Rabin and Whitlock, 2009). Interestingly, analysis of the cancer karyotype database, known as the Mitelman Database (Mitelman F, 2013), indicates that aneuploid karyotypes are specific to the cancer's tissue and anatomical site-of-origin (Gebhart and Liehr, 2000; Mitelman F, 2013). A number of studies have also found recurrent patterns of chromosome gains and losses during cancer progression and metastasis to be specific to the cancer's site-of-origin (Bardi et al., 1995; Ried et al., 2012). This phenomenon has been termed karyotype spatiotemporal progression because the karyotypic changes are found to vary with both space and time (Upender et al., 2004).

Table 1.1. Aneuploidy in cancer cells from ten of the most commonly affected sites in humans

Lung	413/435 (94.9%)
Colon	301/340 (88.5%)
Stomach	167/180 (92.8%)
Liver	110/155 (71.0%)
Breast	598/800 (74.8%)
Cervix uteri	75/84 (89.3%)
Corpus uteri	116/165 (70.3%)
Ovary	386/422 (91.5%)
Prostate	151/186 (81.2%)
Bladder	157/192 (81.8%)

Adapted from Cimini, 2008.

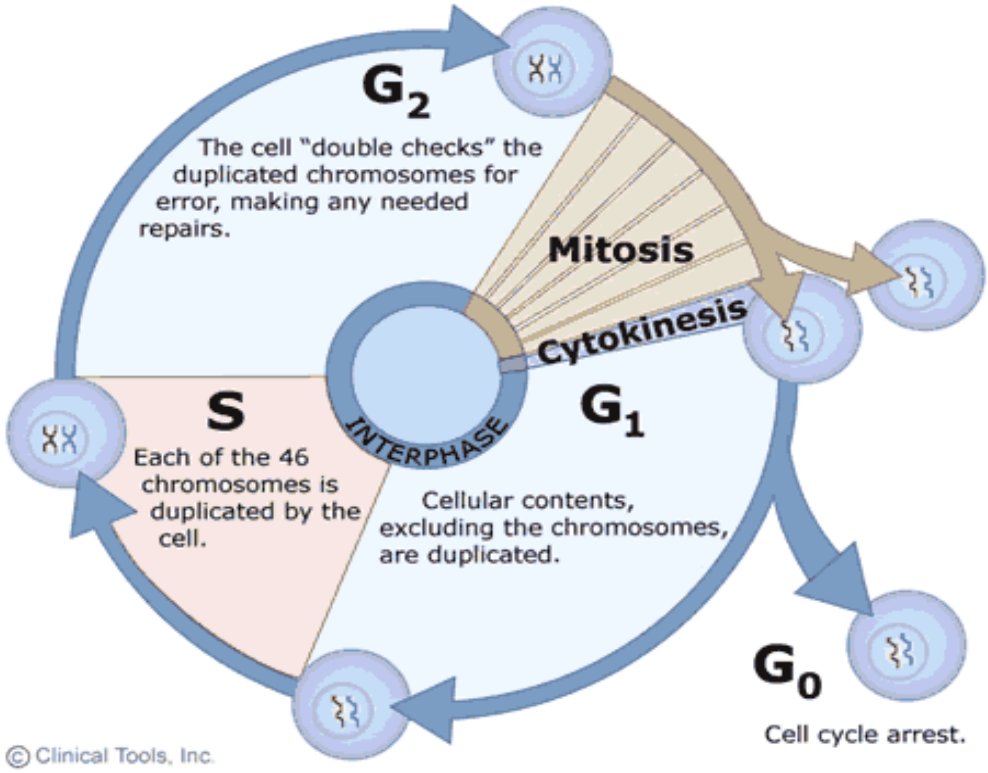

Figure 1.1: Cell Cycle.
A graphical depiction of the cell cycle, outlining each stage

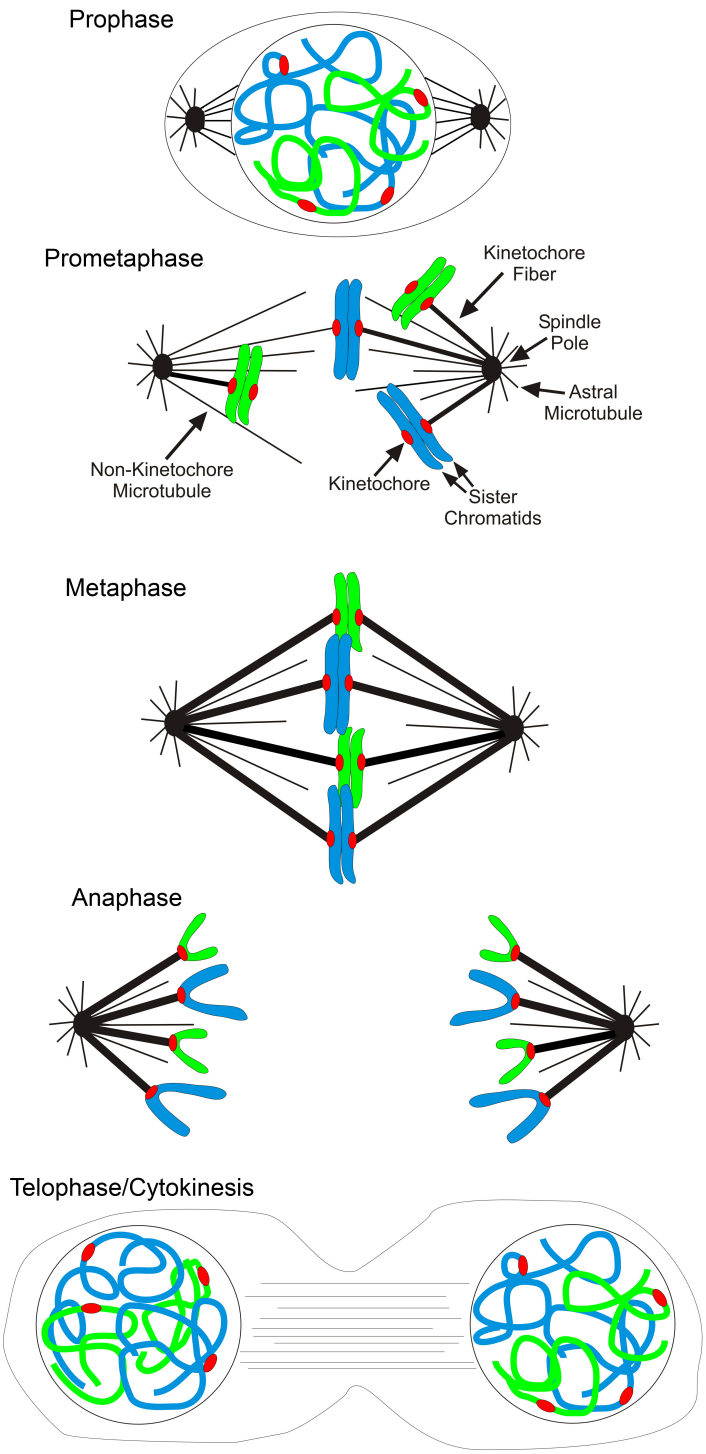

Figure 1.2: Stages of Mitosis: prophase, prometaphase, metaphase, anaphase, and telophase/cytokinesis. [Adapted from (Cimini and Degrassi, 2005)]

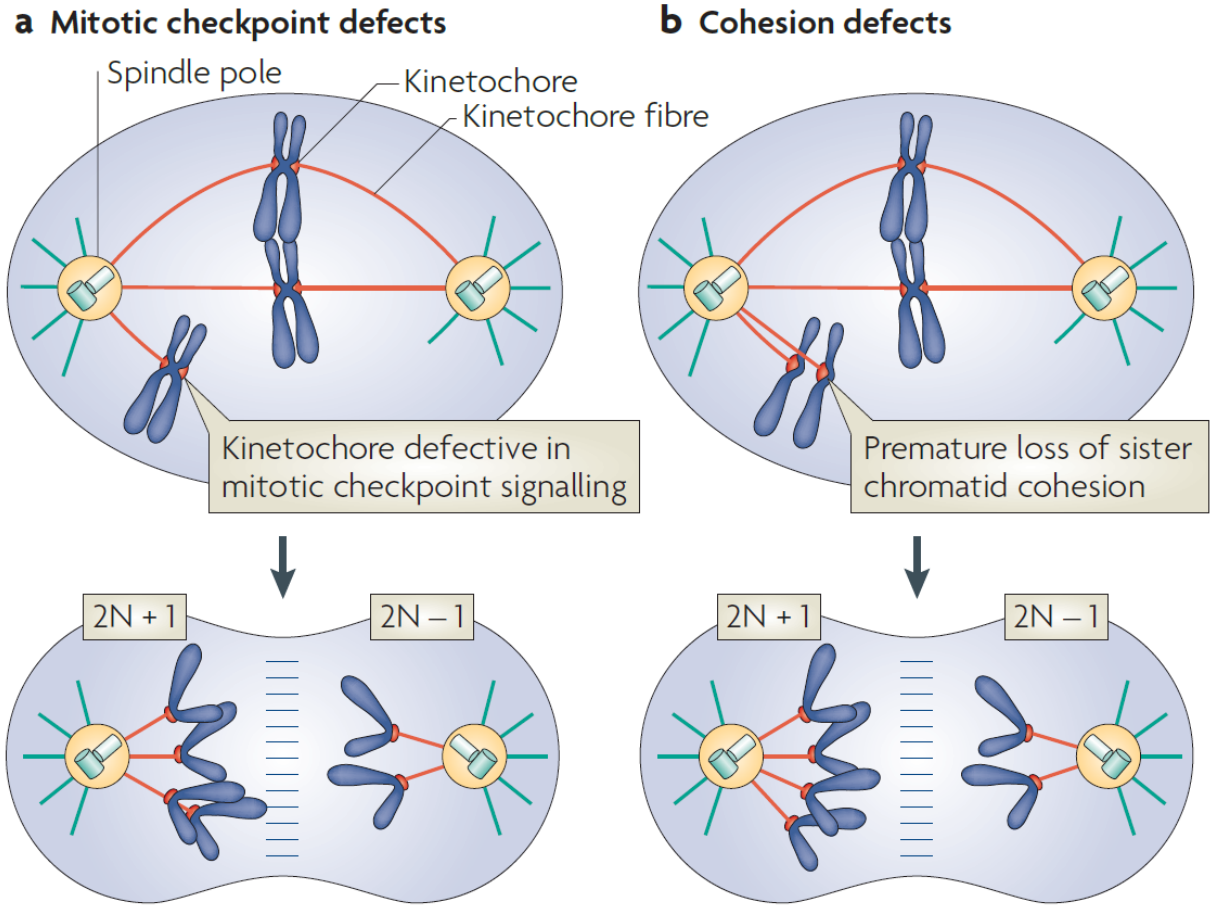

Figure 1.3: Pathways to Aneuploidy. A) Mitotic checkpoint defects allow undetected kinetochore misattachments to persist and chromosomes to mis-segregate. B) Cohesion defects lead to premature loss of sister chromatid cohesion and chromosome mis-segregation. [Adapted from (Holland and Cleveland, 2009)]

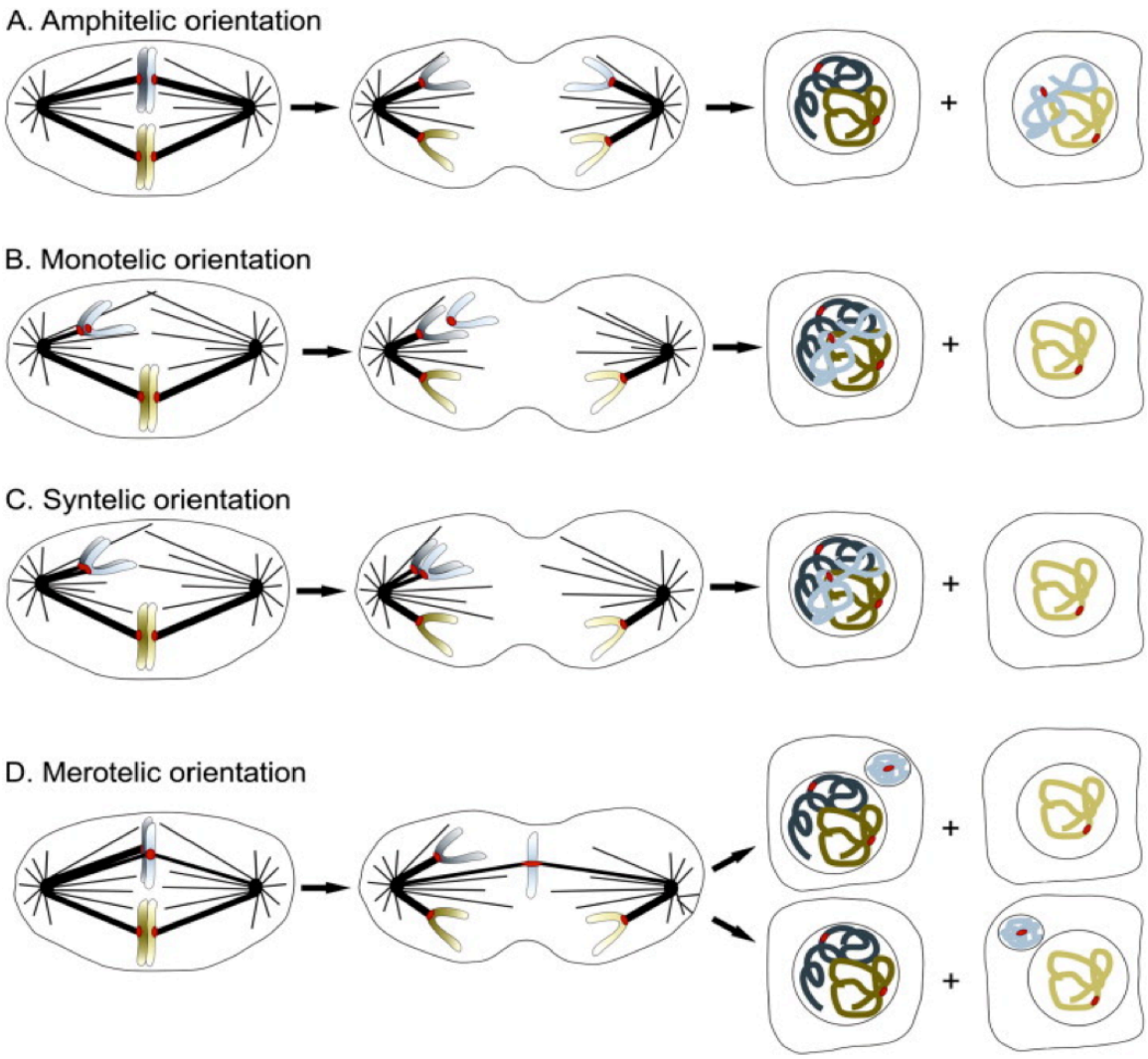

Figure 1.4: Chromosome-Kinetochore attachments during mitosis A) Amphitelic attachment formed when both kinetochores of each mitotic chromosome attach to microtubules emanating from opposite spindle poles; B) Monotelic attachments arise when only one kinetochore attaches and its sister is unattached; C) Syntelic attachments result from microtubules of the same spindle pole attaching to both sister kinetochores; D) Merotelic attachments are formed when one kinetochore is attached to microtubules emanating from both spindle poles and are capable of eluding detection by the SAC which may lead to aneuploidy progeny [Adapted from (Cimini, 2008)].

Chapter 2

Aneuploidy enhances the hallmarks of cancer cells.

Samuel D. Rutledge[1,2], Courtney Kantzler[2], Darawalee Wangsa[3], Shiv D. Kale[2], Daniela Cimini[1,2]*

[1]Department of Biological Sciences and [2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061 – USA

[3]Genetics Branch, National Cancer Institute, NIH, Bethesda, MD, 20892 – USA

*Corresponding author

Manuscript submitted for publication to *Scientific Reports*

Keywords: aneuploidy, karyotype, cancer

Author Contributions

Conceived and designed the experiments: SDR, DC. Performed the experiments: SDR, CK, DW, SDK. Analyzed the data: SDR, CK. Contributed reagents/materials/analysis tools: DW, SDK, DC. Wrote the paper: SDR, DC. Final approval of the manuscript: SDR, CK, DW, SDK, DC.

Abstract

An abnormal chromosome number, a condition known as aneuploidy, is a ubiquitous feature of cancer cells. A number of studies have shown that aneuploidy impairs cellular fitness. However, there is also evidence that aneuploidy can arise in response to specific challenges and can confer a selective advantage under certain environmental stress. Cancer cells are likely exposed to a number of challenging conditions arising within the tumor microenvironment. To investigate whether aneuploidy may confer a selective advantage to cancer cells, we employed a controlled experimental system. We used the diploid, colorectal cancer (CRC) cell line DLD1 and two DLD1-derived CRC cell lines carrying single-chromosome aneuploidies, namely trisomy 7 (DLD1+7) and trisomy 13 (DLD1+13), to assess a number of cancer cell properties. Such properties, which included rates of proliferation and apoptosis, anchorage-independent growth, and invasiveness, were assessed both under normal culture conditions and under conditions of stress (i.e., serum starvation, drug treatment, hypoxia). Overall, our data show that aneuploidy can confer selective advantage to cancer cells under environmental stress conditions. These findings indicate that aneuploidy can increase the adaptability of cells, even those, such as cancer cells, that are already characterized by increased proliferative capacity and aggressive tumorigenic phenotypes.

Introduction

Fundamental to the survival of any organism is the balance between cell proliferation and cell death, which is required to ensure organismal development and to maintain healthy tissues and organs. The death and proliferation of normal, healthy cells is ensured by their ability to respond and modulate growth and death signals. As opposed to healthy cells, cancer cells are characterized by the ability to escape such signals, thus becoming capable to evade apoptosis and proliferate independent of growth signals (Hanahan and Weinberg, 2011). Several other features, typically referred to as "hallmarks of cancer" (Hanahan and Weinberg, 2011), are shared by many cancer cells independent of their origin. One such feature ubiquitous in cancer cells is aneuploidy (Cimini, 2008; Mitelman et al., 2014; Nicholson and Cimini, 2011). Inspired by his studies in sea urchin embryos, Theodor Boveri proposed, over a century ago, that the abnormal chromosome numbers (aneuploidy) found in cancer cells were responsible for cancer cells' abnormal behavior (Boveri, 1914; Boveri, 2008). Nevertheless, the effect of aneuploidy on cancer cell behavior is still unclear and abnormal chromosome numbers are generally acknowledged to negatively affect cell function (Torres et al., 2008). Indeed, aneuploidy is the leading cause of miscarriage in humans (Hassold and Hunt, 2001), and mosaic aneuploidy is typically associated with inherited disorders (Biesecker and Spinner, 2013). Moreover, recent studies aimed at investigating the effect of aneuploidy on cell physiology have revealed that aneuploidy negatively affects cellular fitness (Torres et al., 2008) in a number of experimental systems, including mouse embryonic fibroblasts (Williams et al., 2008) and budding yeast (Torres et al., 2007). Nevertheless, there is also evidence that aneuploidy can confer a selective advantage in certain contexts. For instance, aneuploidy was shown to be an acquired trait in strains of *Candida albicans* that developed resistance to antifungal drugs (Selmecki et al., 2006; Selmecki et al., 2008). Similarly, budding yeast was shown to adapt to the lack of a key molecular motor through the acquisition of aneuploid karyotypes (Rancati et al., 2008). Moreover, aneuploid budding yeast strains were shown to display a growth advantage under a number of environmental stresses, despite their reduced fitness when grown under optimal conditions (Pavelka et al., 2010). Finally, aneuploidy was proposed to contribute to adaptation of liver cells in response to hepatic injury (Duncan et al., 2012;

Duncan et al., 2010) and is required for normal development of the Drosophila rectum (Fox et al., 2010; Schoenfelder et al., 2014). These findings suggest that aneuploidy may confer a similar selective advantage to cancer cells. Moreover, the observation that certain aneuploidies can be either recurrent in cancers of different origin or specifically recurring in cancers from individual anatomical sites (Nicholson and Cimini, 2013) suggests that, as observed in fungi (Pavelka et al., 2010; Selmecki et al., 2006; Selmecki et al., 2009) or in mouse hepatocytes (Duncan et al., 2012), specific aneuploidies may confer selective advantage in a given environment, but not in others.

Addressing the question as to whether aneuploidy can confer a selective advantage to cancer cells can be very challenging, given that cancer cell karyotypes are very complex (Cimini, 2008; Nicholson and Cimini, 2013; Weaver and Cleveland, 2006), and typically characterized by high degrees of aneuploidy, as well as numerous chromosome rearrangements. Moreover, many cancer cells also display chromosome numerical instability (CIN), which generates chromosome numerical heterogeneity within cancer cell populations (Lengauer et al., 1997; Nicholson and Cimini, 2011; Thompson and Compton, 2008). To avoid such complexity, we chose to address the effect that aneuploidy may have on cancer cell properties in a simplified experimental system. Specifically, we performed a series of assays in the diploid, chromosomally stable (non-CIN) colorectal cancer cell line DLD1 (Lengauer et al., 1997) and two DLD1-derived cell lines that were previously generated via microcell-mediated chromosome transfer (Upender et al., 2004) and carry an extra copy of either chromosome 7 or chromosome 13, two chromosomes frequently gained in colorectal cancer (Bardi et al., 1995; Ried et al., 2012). This experimental set-up is advantageous for several reasons. First, it allows investigation of the specific effects of individual aneuploidies on the properties of cells that already display a transformed phenotype, thus providing insight on how aneuploidy may contribute to tumorigenesis. Second, it includes trisomies that may play different roles in cancer. Indeed, gain of chromosome 7 and chromosome 13 are both frequently found in colorectal cancer (Bardi et al., 1995; Ried et al., 2012). But gain of chromosome 7 is also seen in many other types of cancer (Nicholson and Cimini, 2013), whereas gain of chromosome 13 appears to be exclusive to colorectal cancer (Nicholson and Cimini, 2013; Ried et al., 2012).

Results

To explore whether aneuploidy confers a selective advantage to cancer cells, we made use of two trisomic cell lines derived from the diploid (2N = 46), chromosomally stable colorectal cancer cell line DLD1(Lengauer et al., 1997). The DLD1-derived trisomic cell lines used in this study carried an extra copy of either chromosome 7 (DLD1+7) or chromosome 13 (DLD1+13) (Nicholson et al., 2015; Upender et al., 2004). We assessed a number of properties, including proliferative capacity, apoptosis evasion, anchorage-independent growth, and invasiveness, which are known to correlate with tumorigenic capacity (Hanahan and Weinberg, 2011; Shin et al., 1975). Such properties were assessed under both standard culture conditions (normal) and under a number of culture conditions that are representative of possible conditions occurring in the tumor microenvironment (Hanahan and Weinberg, 2000) concomitantly with the aneuploidies under study. We reasoned that this strategy would allow us to identify a potential selective advantage conferred by the extra chromosome. The selective conditions included nutrient starvation (serum-free culture medium), exposure to 10 μM of the chemotherapeutic drug 5-fluorouracil (5-FU), and hypoxia. The performance of the trisomic cells under such selective conditions was compared to that of the diploid parental cell line.

Aneuploidy suppresses cell proliferation under normal culture conditions, but favors cell proliferation under selective conditions.

We first examined the proliferative capacity of the three cell lines grown under various conditions by determining growth curves over a period of 6 days (Figure 1). We found that under normal conditions, the DLD1 cells displayed faster growth rates compared to each aneuploid variant (Figure 1A). Under both serum-free and 5-FU conditions (Figure 1B-C), the proliferation rates were lower than those observed under normal culture conditions for all cell lines. However, the trisomic cell lines displayed higher proliferation rates than the parental diploid cell line, with the DLD1+13 cells proliferating better than both DLD1+7 and DLD1 cells (Figure 1B-C). Moreover, the DLD1+13 cells displayed faster

proliferation rates than either of the other two cell lines also under hypoxic conditions, whereas the DLD1+7 cells did not display any growth advantage when cultured under hypoxic conditions (Figure 1D).

Cell division vs. suppression of apoptosis as a mechanism to increase proliferation rates.

To assess whether the higher proliferation rates were linked to increased cell division rates, we quantified the mitotic index for the three cell lines under all culture conditions (Figure 2A-B). We found that the diploid cells displayed a higher mitotic index under normal culture conditions (Figure 2B), whereas both trisomic cell lines displayed higher mitotic indices compared to DLD1 cells grown under serum free or 5-FU conditions (Figure 2B). Under hypoxic conditions, the DLD1+7 cells displayed the lowest mitotic index, whereas DLD1 and DLD1+13 cells displayed similar mitotic indices (Figure 2B). However, it is worth noting that the mitotic indices displayed by DLD1 and DLD1+7 cells under hypoxic conditions were higher than those observed under either of the other two selective conditions and similar to those observed under normal culture conditions (Figure 2B). Similarly, the mitotic index for DLD1+13 cells subjected to hypoxia was higher than the mitotic index measured for DLD1+13 cells under any other culture condition (Figure 2B).

Because overall proliferation rates are determined by the combined rates of cell division and cell death, we also quantified the rates of apoptosis by TUNEL assay in all cell lines under all culture conditions (Figure 2C-D). Under normal culture conditions, DLD1 cells showed the lowest incidence of apoptosis compared to DLD1+7 or DLD1+13, whereas their rate of apoptosis was the highest among the three cell lines both under serum-free and 5-FU conditions (Figure 2D). Under hypoxic conditions, the DLD1+7 cells displayed the highest rate of apoptosis among the three cell lines (Figure 2D), which is consistent with the observation that DLD1+7 cells displayed the lowest proliferation rates among the three cell lines under hypoxic conditions (Figure 1D). The TUNEL assay data also showed that the rates of apoptosis did not vary much for DLD1+13 cells grown under different conditions, whereas the DLD1 cells showed higher rates of apoptosis under all selective conditions compared to normal culture

conditions, and DLD1+7 cells displayed lower rates of apoptosis under normal and serum-free conditions compared to their rates of apoptosis in 5-FU and hypoxia (Figure 2D).

Aneuploidy can increase anchorage-independent growth of cancer cells cultured under selective conditions.

We next performed a soft agar assay to examine the ability of the three cell lines to grow independent of anchorage *in vitro*, a phenotype that correlates with tumorigenicity (Shin et al., 1975). As a negative control we used the non-transformed, immortalized, hTERT-RPE1 cell line. As expected, these cells were unable to form colonies in soft agar (Figure 3A). On the other hand, all three DLD1 cell lines formed colonies under all culture conditions (Figure 3B-H), consistent with anchorage-independent growth being the phenotype most consistently associated with tumorigenicity (Shin et al., 1975). Although the three cell lines formed similar numbers of colonies under most culture conditions (Figure 3E-H), some differences were noted. Under hypoxic conditions, DLD1+7 cells formed more colonies compared to the diploid DLD1 cells (Figure 3H). Moreover, although DLD1+7 and DLD1+13 formed similar numbers of colonies compared to the diploid DLD1 cells under both serum-free (Figure 3F) and 5-FU (Figure 3G) conditions, the colonies formed by the aneuploid cells were significantly larger for both these selective culture conditions (Figure 3J-K). There was no significant difference in the size of colonies formed by the three cell lines under normal (Figure 3I) or hypoxic (Figure 3L) culture conditions.

Aneuploidy increases the invasive capacity of colorectal cancer cells.

To assess the invasive ability of aneuploid cells compared to the diploid parental cell line, we performed a matrigel invasion assay (Figure 4). We found that under all culture conditions, the number of cells migrating through the matrigel was higher for the aneuploid cell lines compared to the diploid parental cell line (t-test, $p<10^{-4}$ for each aneuploid cell line compared to diploid cells under all culture conditions; Figure 4E-H).

Discussion

Taken together, our data show that aneuploidy can confer a selective advantage under conditions of environmental stress. In some of our assays, there was no difference in behavior between diploid and aneuploid colorectal cancer cells and in general, the diploid cancer cells performed better under normal culture conditions. This last observation is not surprising, given that such culture conditions were optimized for DLD1 cells. A similar proliferative advantage of euploid vs. aneuploid cells under standard culture conditions was previously shown in both yeast and mouse embryonic fibroblasts (Chen et al., 2015; Pavelka et al., 2010; Torres et al., 2007; Williams et al., 2008). On the other hand, whenever we found the aneuploid cells to display an advantage, with the exception of the matrigel invasion assay, this occurred under selective culture conditions, indicating that aneuploidy can confer a selective advantage to cancer cells, as previously shown in yeast (Chen et al., 2015; Pavelka et al., 2010; Rancati et al., 2008). Our results are also consistent with the previous finding that trisomy 7 was associated with an acquired resistance of epithelial colon cells to growth in serum-free conditions (Ly et al., 2011). However, our findings are even more striking in that they show that aneuploidy increases the tolerance of cancer cells to environmental stresses beyond that seen in cells that are already transformed and tumorigenic, and presumably already adapted to proliferate under certain environmental stresses.

Aneuploidy has been shown to cause transcriptomic changes that, for the most part, correlate with the specific aneuploid chromosome(s) (Gao et al., 2007; Habermann et al., 2007; Stingele et al., 2012; Torres et al., 2007; Upender et al., 2004). However, when examining the proteomic changes linked to aneuploidy, the situation is more complex. Whereas some studies have reported proteomic changes to scale up with changes in chromosome copy number in yeast (Pavelka et al., 2010), other studies in yeast and human cells reported that protein abundance does not vary significantly as a result of aneuploidy and/or that the proteomic changes do not necessarily correlate with the specific aneuploidy (Gemoll et al., 2013; Habermann et al., 2007; Stingele et al., 2012; Torres et al., 2007). Nonetheless, a general link between aneuploidy and phenotypic traits is strongly supported by experimental data (Pavelka et al., 2010; Sheltzer et al., 2011; Tan et al., 2013; Zhu et al., 2012). Moreover, aneuploidy-specific phenotypes,

resulting from the altered levels of proteins encoded by genes on the aneuploid chromosomes, have been reported in a number of different organisms and contexts. For instance, the chromosome 5 aneuploidy emerging in *Candida albicans* exposed to fluconazole (Selmecki et al., 2006; Selmecki et al., 2009) was shown to confer drug resistance by inducing overexpression of two genes encoding, respectively, for the drug target and for a trascriptional regulator of efflux pumps (Selmecki et al., 2008). Similarly, the acquisition of trisomy 7 in colon epithelial cells cultured in serum-free conditions was associated with overexpression of the epidermal growth factor receptor, encoded on chromosome 7 (Ly et al., 2011). Finally, a specific cytokinesis failure phenotype was recently shown to be associated with trisomy 13 and caused by overexpression of Spartin, a protein encoded by a gene (SPG20) on chromosome 13 (Nicholson et al., 2015). However, the selective advantage we observed in our study seems unlikely to depend on overexpression of specific genes on the aneuploid chromosomes. If that were the case, we would expect to find consistently significant differences between trisomy 7 and trisomy 13, whereas we only found minor differences between the two trisomic cell lines. Thus, although the specificity of chromosome 13 gain for colorectal cancer (Nicholson and Cimini, 2013) may suggest a specific advantage conferred by this chromosome to colorectal cancer cells, such advantage may be linked to conditions other than those tested in our study. Instead, for the selective conditions assessed here, we found a general selective advantage conferred by aneuploidy. This observation suggests that the advantage conferred by these two chromosomes may depend on a common phenotypic effect observed for both trisomies. We propose that such an effect may be the increased rate of chromosome mis-segregation recently reported for DLD1+7 and DLD1+13 cells compared to diploid DLD1 cells (Nicholson and Cimini, 2013). In DLD1+7 and DLD1+13 cells, high rates of chromosome mis-segregation are associated with high rates of karyotypic heterogeneity (Nicholson and Cimini, 2013), similarly to findings from other studies showing a link between aneuploidy and chromosome instability (CIN) in both yeast and human cells (Biron-Shental et al., 2015; Duesberg et al., 1998; Reish et al., 2006; Reish et al., 2011; Sheltzer et al., 2011; Zhu et al., 2012). Cells with high degrees of CIN are expected to display high degrees of phenotypic heterogeneity (Chen et al., 2015; Nicholson and Cimini, 2015) and this

karyotypic/phenotypic heterogeneity will allow aneuploid cells to be more "adaptable" (Chen et al., 2015; Nicholson and Cimini, 2015). Indeed, high karyotypic/phenotypic heterogeneity increases the chance that some cells within the population may possess the phenotype required for increased fitness in a certain environment.

Finally, our matrigel invasion assay provided an interesting observation in that aneuploid colorectal cancer cells are significantly more invasive than diploid cells not only under selective conditions, but also under normal culture conditions. One could envision migration through an extra-cellular matrix as a challenge of its own. Therefore, the observation that aneuploid cells migrate better through the matrigel regardless of the culture conditions, reinforces the idea that aneuploidy increases the adaptability of cells, even those, such as cancer cells, that are already characterized by increased proliferative capacity and aggressive tumorigenic phenotypes.

In conclusion, our work shows that addition of one extra chromosome is enough to enhance certain hallmarks of cancer cells. This can be explained by the recently reported increase in chromosome mis-segregation and CIN as a result of the addition of an extra chromosome (7 or 13) to DLD1 colorectal cancer cells (Nicholson et al., 2015). The increased CIN, in turn, can generate phenotypic variability and increased adaptability, thus allowing aneuploid cancer cells to perform better than diploid cancer cells under a number of environmental challenges. This paradigm is consistent with the widespread aneuploidy observed in solid tumors (Mitelman et al., 2014), with the association between CIN and poor patient prognosis (Carter et al., 2006; Walther et al., 2008), and with the link between CIN and drug resistance (Lee et al., 2011; Swanton et al., 2009).

Materials and Methods

Cell lines and culture conditions. The DLD1 cell line was obtained from American Type Culture Collection (ATCC, VA, USA), DLD1+7 and DLD1+13 cell lines were generated previously by microcell-mediated chromosome transfer (Upender et al., 2004), and DLD1+13 cells were sub-cloned as previously described (Nicholson et al., 2015). All the DLD1 cell lines were maintained in RPMI 1640 medium (ATCC, VA, USA) supplemented with 10% FBS (Gibco, NY, USA) and antibiotic/antimycotic mixture (Gibco, NY, USA). The hTERT-RPE1 cells (ATCC, VA, USA) were maintained in DMEM/F12 medium (Gibco, NY, USA) supplemented with 10% FBS (Gibco, NY, USA) and antibiotic/antimycotic mixture (Gibco, NY, USA). All cells were kept in a humidified incubator at 37°C with 5% CO_2.

For exposure to selective conditions, culture medium was prepared as follows: normal culture medium without FBS (Serum-free); normal culture medium with 10 μM 5-fluorouracil (5-FU) (Sigma-Aldrich, MO, USA); normal medium pre-conditioned in hypoxia chamber for 48 hrs (Hypoxia). For all experiments, except those in hypoxic conditions, cells were incubated with the appropriate culture media in a humidified incubator at 37°C with 5% CO_2 for the duration of the experiment. For experiments in hypoxic conditions, cells were maintained inside a modular incubator chamber (Hillups-Rothenberg, CA, USA) in which humidity was maintained by addition of a 60 mm Petri dish containing 5 ml of water. Prior to each use, the chamber was flushed with 5% CO_2, 1% O_2, and N_2 before being sealed and stored at 37°C in an incubator. Cells were removed from the chamber for not more than one hour to conduct experiments, after which time the chamber was again flushed with 5% CO_2, 1% O_2, and N_2 before being sealed and stored at 37°C. Hypoxic conditions were confirmed using a Cyto-ID Hypoxia Detection Kit (Enzo Life Sciences, NY, USA) and fluorescence microscopy detection according to the manufacturer's instructions.

All experiments were repeated three times and the data are reported as mean and S.D. Additional details are provided in the figure legends.

Growth curves. To determine the proliferative capacity over time, we plated each DLD1 cell line in a 6-well plate (6 wells total) at a density of $1.5x10^5$ cells per well. Following 24 hrs of growth in normal media, the cells were washed twice in PBS and re-incubated under selective culture conditions. For the 4-6-day samples, the media was replenished half-way through the experiment. At 24-hour intervals for six days, the media from one well of each DLD1 cell line was removed, the cells were washed twice with PBS and trypsinized. Cells were then vigorously re-suspended in media to a final total volume of 3 ml. From this, a 100 μl sample was thoroughly pipetted and mixed with an equal amount of 0.4% trypan blue solution (Gibco, NY, USA), and subjected to cell counting. Cell counts were performed by hemocytometer (Bright-Line, PA, USA) and averaged from four samples.

Mitotic index analysis. $1.5x10^5$ cells suspended in normal media were plated on 22x22 mm acid-washed sterile glass coverslips inside 35 mm Petri dishes. After 24 hours, cells were incubated under the appropriate culture conditions for additional 24 hrs. Cells were then washed twice with PBS, fixed with freshly prepared 4% paraformaldehyde (Fischer Scientific, NJ, USA), washed twice more with PBS, and DAPI stained. For each sample, the number of mitotic cells was counted over a total of at least 1,000 cells.

TUNEL assay. $0.5x10^5$ cells suspended in normal media were plated on 22x22 mm acid-washed sterile glass coverslips inside 35 mm Petri dishes. After 24 hours, cells were incubated under the appropriate culture conditions for additional 24 hrs. Cells were then washed twice with PBS, fixed with freshly prepared 4% paraformaldehyde (Fischer Scientific, NJ, USA), washed twice more with PBS + 0.01% sodium citrate, and finally stained using an *in situ* cell detection kit (Roche, Basel, Switzerland). Cells were then washed three times with PBS before DAPI staining. For each sample, the number of TUNEL positive cells was recorded over a total of at least 1,000 cells. Positive and negative controls were used according to the manufacturer's guidelines.

Fluorescence microscopy. For both mitotic index and TUNEL assay quantification, samples were viewed on a Nikon Eclipse TE2000-U inverted microscope (Nikon Instruments Inc., NY, USA) equipped with a swept field confocal system (Prairie Technologies, WI, USA), a 100x/1.4 NA Plan-Apochromatic objective, and an automated ProScan stage (Prior Scientific, Cambridge, UK). The confocal head was accessorized with a multiband pass filter set for illumination at 405, 488, 561, and 640 nm and illumination was obtained through an Agilent MLC400 monolithic laser combiner (Agilent Technologies, CA, USA) controlled by a four channel acousto-optic tunable filter. Digital images were acquired with a HQ2 CCD camera (Photometrics, AZ, USA). Stacks of images were acquired through the Z axis at 0.6 μm steps. Exposure time, Z-axis position, laser line power, and confocal system were all controlled by NIS Elements AR software (Nikon Instruments Inc., NY, USA) on a PC computer (Dell, TX, USA).

Soft agar assay. 1.5 ml of a 1:1 mixture of 2% agar (Fischer Scientific, NJ, USA) and 2x DMEM (Gibco, NY, USA) media (prepared according to the specific selective condition for each sample) was poured evenly into a 35 mm Petri dish. After cooling, 1.5 ml of a 1:1 mixture of 1.4% agar (Fischer Scientific, NJ, USA) and 2X DMEM (prepared according to the specific culture condition for each sample) media was used to re-suspend $1x10^5$ cells and then poured on the bottom solidified agar layer. After solidification of the top agar layer, 2 ml of RPMI 1640 media (prepared according to the specific selective condition for each sample) were added and the samples incubated in a humidified incubator at 37°C with 5% CO_2, or in the hypoxia chamber. The colonies were grown for 22 days and media changed every 4-5 days. Ten randomly selected fields of view were imaged on a Nikon Eclipse Ti inverted microscope (Nikon Instruments Inc., NY, USA) equipped with a 20x/0.4 NA ADL phase contrast objective, phase-contrast transillumination, transmitted light shutter, ProScan automated stage (Prior Scientific, MA, USA), and a HQ2 CCD camera (Photometrics, AZ, USA). For each field of view, Z-stacks were acquired by imaging 10 focal planes at 100 μm steps and colony number and size were

measured for colonies present on different focal planes. The total number of colonies was quantified for all ten fields of view and the size of each colony was quantified by measuring the length of the longest axis. hTERT-RPE1 cells were used as a negative control and as a baseline for colony size measurements. Individual dot-like structures, corresponding to individual non-proliferating cells, were observed at low density in hTERT-RPE1 samples. The average size of these structures was 15 μm, and this was considered as the lower size limit for colony size measurements in DLD1 cell lines.

Matrigel invasion assay. The matrigel invasion assay was performed using an 8 μm pore transwell PET membrane (BD Biosciences Inc., MA, USA). The matrigel mixture (Corning Inc., MA, USA) was reconstituted with the appropriate culture media to a concentration of 200 μg/ml and poured evenly over the transwell PET membrane; 10^5 cells were re-suspended in RPMI 1640 media and plated onto the matrigel; 500 μl of media were also added to the bottom well and the chambers were incubated. After 24 hrs, both the media in the bottom well and that in the top chamber were replaced with media prepared according to the specific culture condition for each sample. After incubating for additional 24 hrs, non-invasive cells were scraped off the chamber side of the transwell membrane and invasive cells were fixed with 100% methanol, washed twice with PBS, and stained with a 1% Giemsa solution. Quantification was performed by light microscopy on a Nikon Eclipse TS100, using a 20x/0.4 NA ADL phase contrast objective and counting the total number of cells from 6 random fields of view.

Acknowledgements

We would like to acknowledge Thomas Ried (National Cancer Institute, NIH) for providing the aneuploid cell lines, Maria Vila Casadesús for assistance with statistical analysis of the growth curve data, and Josh Nicholson for helpful suggestions during the initial stages of this project. We also thank Silke Hauf and Rich Walker for providing important feedback throughout the execution of this project. Finally, we thank all the members of the Hauf and Cimini labs for helpful comments and discussions. SDR was partly supported through Graduate Teaching Assistantships provided by the Dept. of Biological Sciences (Virginia Tech). Work in the Cimini lab partly funded by NSF grant MCB-0842551 and HFSP grant RGY0069/2010.

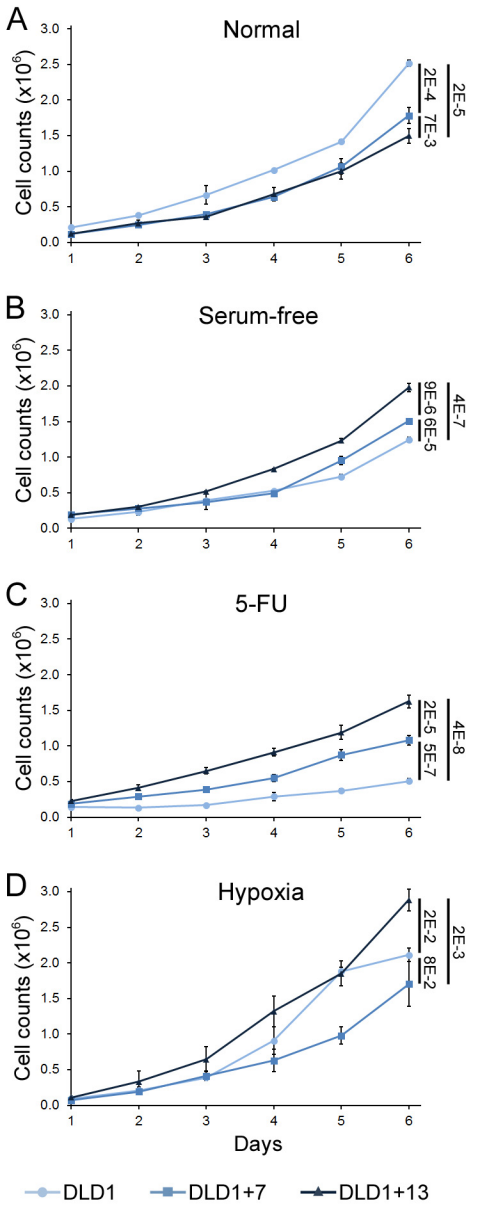

Figure 2.1: Aneuploidy suppresses cell proliferation under normal culture conditions, but favors cell proliferation under selective conditions. The graphs show growth curves for DLD1, DLD1+7, and DLD1+13 cells cultured for six days under different conditions. Data for cells cultured in normal media (**A**), serum-free media (**B**), media containing 10 μM 5-FU (**C**), or incubated under hypoxic conditions (**D**) are reported as mean and standard deviations from three biological replicates. Similar results were found

by automated cell counting (data not shown). For statistical analysis, the growth curves in logarithmic scale were fit to linear functions and analyzed using R software package. The numbers to the right of the graphs indicate the p values for comparison of end-point data. Statistical analysis was also performed on the trends in cell growth using the geepack R package for longitudinal data analysis (Hojsgaard et al., 2006) and showed that all pairwise comparisons were significantly different ($p<4.5E-7$), except for DLD1+7 vs. DLD1+13 under normal culture conditions.

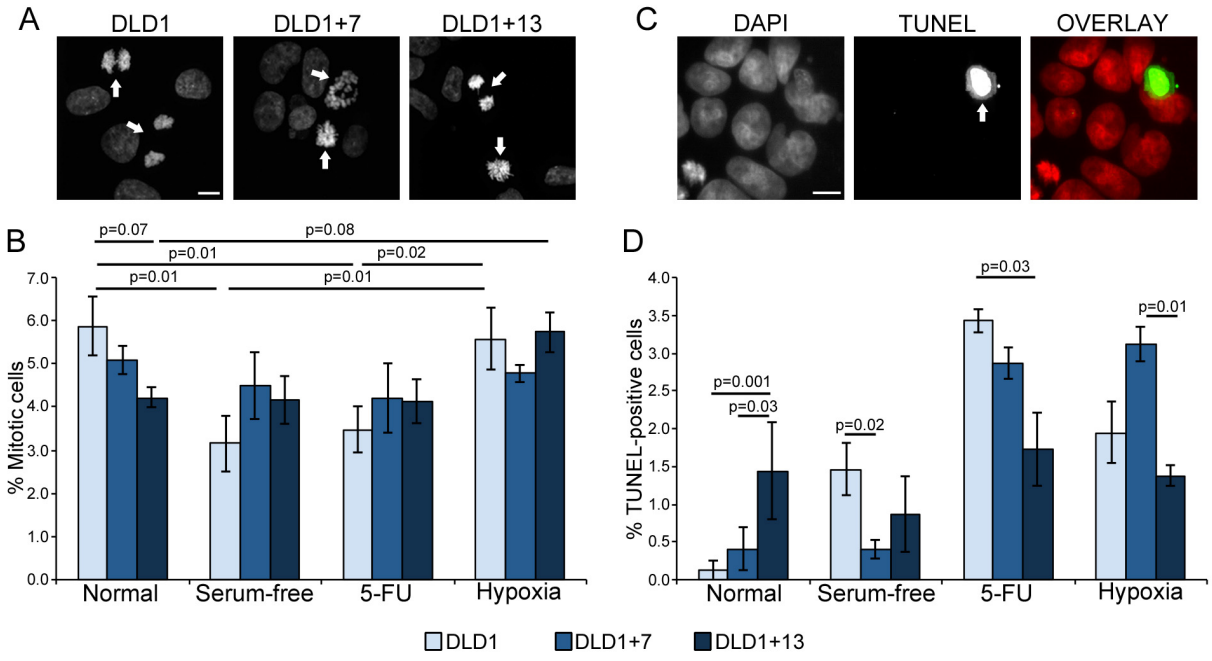

Figure 2.2: Mitotic index and apoptosis in diploid vs. aneuploid colorectal cancer cells.

(**A**) Representative images of DLD1, DLD1+7, and DLD1+13 cells grown under normal culture conditions and stained with DAPI. Arrows point at mitotic cells. Images are maximum intensity projections of Z-stacks acquired at 0.6 μm steps. Scale bar, 10 μm. (**B**) Mitotic indices for the three cell lines cultured under different conditions. The data are reported as mean and standard deviations from three biological replicates. Statistical analysis was performed using the χ^2 test and only p values that were significant or close to significance are reported in the figure. (**C**) Representative TUNEL assay image of DLD1+13 cells cultured under normal conditions. Arrow points at TUNEL-positive cell. Images are maximum intensity projections of Z-stacks acquired at 0.6 μm steps. Scale bar, 10 μm. (**D**) Quantification of TUNEL-positive cells in the three cell lines cultured under different conditions. The data are reported as mean and standard deviations from three biological replicates. Statistical analysis was performed using the χ^2 test and only p values that were significant or close to significance are reported in the figure.

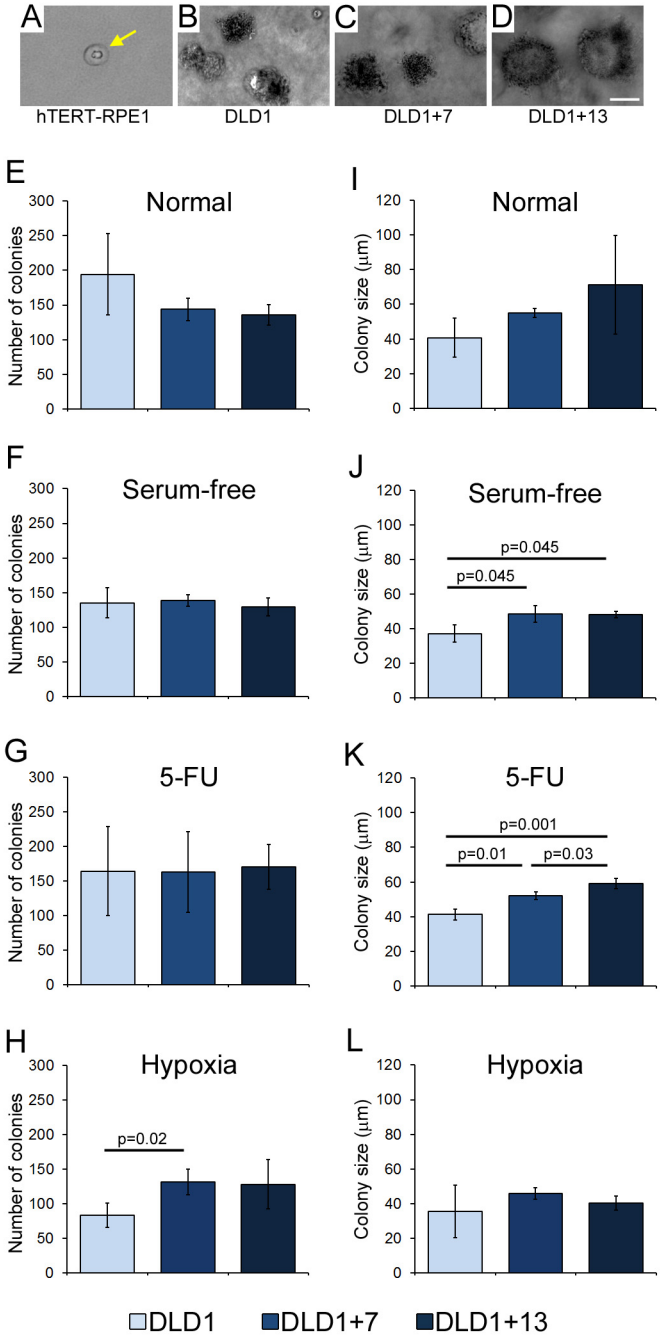

Figure 2.3: Aneuploidy can increase anchorage-independent growth. Anchorage-independent growth was assessed by testing the ability of DLD1, DLD1+7, and DLD1+13 cells to form colonies on soft agar when cultured under different conditions. (**A**) Representative image of hTERT-RPE1 cells on soft agar. These non-transformed cells were used as a negative control and as a baseline for colony size

measurements. Individual dot-like structures (yellow arrow), corresponding to individual non-proliferating cells, were observed at low density. The average size of these structures was 15 μm, and this was considered as the lower size limit for colony size measurements in DLD1 cell lines, although most of the cancer cell line colonies were well above 15 μm in size. (**B-D**) Representative colonies formed by DLD1, DLD1+7, and DLD1+13 on soft agar under normal culture conditions. Scale bar, 50 μm. (**E-H**) Total number of colonies from ten randomly selected fields of view of soft agar plates with the various cell lines under different culture conditions. (**I-L**) Average size of colonies formed by the various cell lines under different culture conditions. For (**E-L**), the data are reported as mean and standard deviations from three biological replicates. Statistical analysis was performed using the t-test and only p values that were significant or close to significance are reported in the figure.

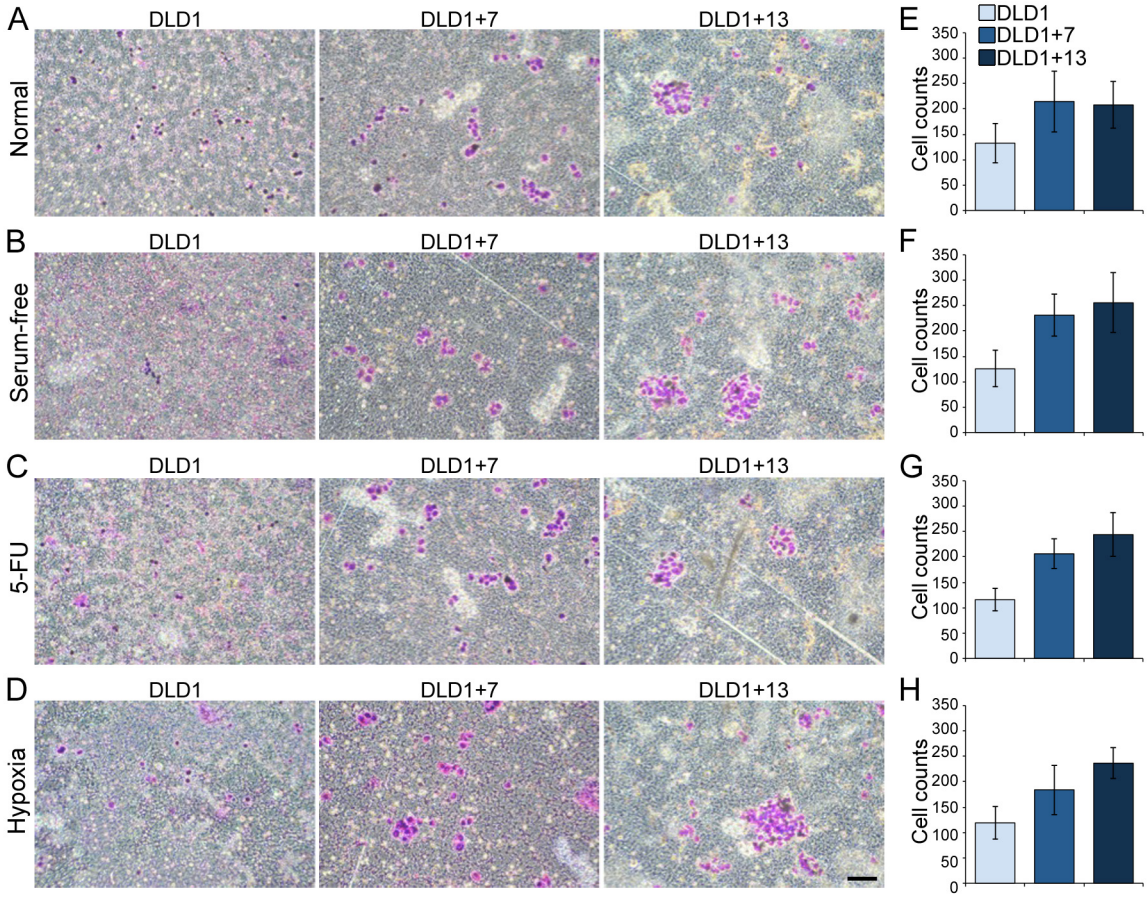

Figure 2.4: Aneuploidy increases invasiveness of cancer cells. The invasive capacity of the three different cell lines was assessed using a matrigel invasion assay. (**A-D**) Examples of Giemsa-stained invasive DLD1, DLD1+7, and DLD1+13 cells cultured under different conditions. Scale bar, 100 μm. (**E-H**) Quantification of invasive DLD1, DLD1+7, and DLD1+13 cells cultured under different conditions. The data are reported as mean and standard deviations from three biological replicates. Statistical analysis showed that significantly larger numbers of aneuploid compared to diploid cells migrated through the matrigel layer (t-test, $p<10^{-4}$ for each aneuploid cell line compared to diploid cells under all culture conditions).

Chapter 3

Conclusions and perspectives

Our results demonstrate that aneuploidy confers a selective advantage to and heightens the tumor-related properties of cancer cells cultured under conditions of environmental stress. Although aneuploidy is a well-established feature of cancer cells (Mitelman et al., 2014), the selective benefit of aneuploidy, generally thought to be detrimental to cellular fitness (Torres et al., 2007), remains unclear. Our findings support other recent work in yeast (Pavelka et al., 2010) and in immortalized human colon epithelial cells (Ly et al., 2011) demonstrating aneuploidy improves cancer cell fitness and enhances its tumorigenic properties.

Many recurrent patterns of chromosome gains and losses have been recognized in cancer. For instance, chromosome 7 gain is detected in 40% of early CRC (Bomme et al., 1994; Habermann et al., 2007), but is also prevalent in many other cancers (Bean et al., 2007; Beroukhim et al., 2010). On the other hand, gain of chromosome 13 appears specific to CRC and is rarely observed in other cancers (Nicholson and Cimini, 2013; Ried et al., 2012). Finally, gain of 3q is observed in a number of different cancers, but rarely seen in CRC (Nicholson and Cimini, 2013; Ried et al., 2012). Using previously generated CRC cells containing an additional individual copy of either chromosome 7 or 13 (Upender et al., 2004), we were able to compare how specific aneuploidies affected the fitness of cancer cells (see chapter 2). Upender and colleagues, in a previous study, performed microarray analysis on both these aneuploid variants (DLD1+7 and DLD1+13), as well as their parental cell line (DLD1) and an aneuploid CRC cell line carrying an extra copy of chromosome 3 (DLD1+3) (Upender et al., 2004). The microarray analysis reported a 2-fold gene mis-expression threshold. Some common cancer aneuploidies may be favorably selected due to their ability to promote overexpression of genes that are found to be beneficial to most cancer cells. For instance, human colon epithelial cells were found to spontaneously gain chromosome 7 and maintain it clonally when passaged under low serum conditions (Ly et al., 2011). In these cells, gain of chromosome 7 was associated with over-expression of the epidermal growth factor

receptor (EGFR) gene located on chromosome 7, band p11 (Ly et al., 2011). However, this EGFR mis-expression (Briand et al., 1996; Garewal et al., 1990; Sareen et al., 2009), as well as gain of chromosome 7 (Nicholson and Cimini, 2013) are not CRC-specific. To start exploring whether certain cancer-specific aneuploidies are selected in favor or against as a result of the associated overexpression of genes that confer a selective advantage at a specific anatomical site, I compared the microarray data from Upender et al. to the TiGER database (Liu et al., 2008), which reports the expression profiles for healthy tissue cells from a number of different anatomical sites. I reasoned that genes of potential interest for CRC would be those specifically mis-expressed in the trisomic DLD1 cell lines and that are typically expressed in colon cells (Table 3.1). Genes of interest would be those that are down-regulated in DLD1+3 cells, but typically expressed in normal colon cells. This may suggest that such genes are essential to proliferation/survival of colon cancer cells and may be why trisomy of chromosome 3 is rarely observed in CRC (Ried et al., 2012). Other genes of potential interest are those that are typically expressed in healthy colon cells and overexpressed in DLD1+13 cells. This may suggest that such genes are similarly important for proliferation/survival of colon cells and that their overexpression may enhance colon cell proliferation/survival, which would explain why trisomy 13 is commonly found in CRC (Nicholson and Cimini, 2013; Ried et al., 2012). To test the above hypotheses, one could experimentally induce down-regulation or overexpression of the selected genes in colon epithelial cells and assess whether such mis-expression confers tumor-related properties, such as those assessed in our study (Chapter 2). Moreover, if my hypothesis is correct, knocking down, in DLD1+13 cells, the expression of genes specifically overexpressed as a result of trisomy 13, should reduce some of the tumor-related properties of these cells.

Additionally, it would be important to determine whether gene expression changes based on a cell's particular context, and thus gene expression analysis could be repeated under stress conditions to compare with expression data under normal conditions. However, gene expression profiles could change under stress as a result of many factors. A simple idea is that certain cellular pathways are activated in response to the specific stress inflicted to the cells and this would result in specific changes in gene expression profiles. However, because certain aneuploidies are known to confer selective advantage under

certain conditions (Chen et al., 2015; Ly et al., 2011; Rancati et al., 2008; Selmecki et al., 2006; Selmecki et al., 2009) changes in gene expression profiles observed in cells growing under selective conditions may be the result of activation of specific pathways in combination with stress-induced karyotypic changes. Finally, one should not disregard the fact that aneuploidy itself can increase the rates of chromosome mis-segregation (Nicholson and Cimini, 2015; Nicholson et al., 2015; Sheltzer et al., 2011; Thompson and Compton, 2010) and that environmental stresses themselves can generate aneuploidy (Chen et al., 2015; Ly et al., 2011; Rancati et al., 2008; Selmecki et al., 2006; Selmecki et al., 2009; Tang et al., 2011). Thus, analysis of stress-induced changes in gene expression profiles should be accompanied by karyotypic analysis (e.g., by aCGH or SKY).

Clearly, the relationship between aneuploidy, environmental stresses, and evolution of cancer karyotypes is very complex. However, the propensity of cancer cells to genomic instability has recently been suggested as a therapeutic target by leveraging therapeutic and environmental pressures to select for a specific karyotype so as to channel cancer toward a specific path (Chen et al., 2015). Essential to launch such an investigation is identifying the conditions and/or therapeutics that could select for a specific karyotype. This has previously been accomplished in yeast (Chen et al., 2012; Chen et al., 2015) and it stands to reason is feasible in human cancer cells (Chen et al., 2015). Further encouragement comes from those experiments that have demonstrated selection for specific karyotypes by long term passaging or extended exposure to high doses of therapeutics meant to induce resistance (Chen et al., 2015; Ly et al., 2011; Rancati et al., 2008; Selmecki et al., 2006; Selmecki et al., 2009; Tang et al., 2011). Similarly, it would be interesting investigating whether any drug or condition associated with selection of either chromosome 7 or chromosome 13 could increase the percentage of cells carrying extra copies of these chromosomes, or select against their acquisition. This could be achieved by passaging our DLD1 aneuploid variants over the long term under specific selective conditions.

Table 3.1: Gene expression profiles of normal colon epithelial cells mis-regulated in infrequently and frequently seen aneuploidies of colon cancer

#	Gene	DLD1+3	Ratio (+3/DLD1)	DLD1+7	Ratio (+7/DLD1)	DLD1+13	Ratio (+13/DLD1)
1	KRT20	Up	13.33	Up	2.42		
2	GPA33					Up	2.53
3	STEAP2			Up	2.10		
4	CFTR	Down	0.16				
5	HNF4A	Down	0.43	Up	2.00		
6	HEPH			Up	3.39	Up	2.79
7	SIM2					Up	2.11
8	VIL1	Down	0.40				
9	TFF3			Up	2.46	Up	2.24
10	FUT3					Up	3.48
11	FXYD3	Down	0.38	Up	3.35		
12	SLC12A2	Down	0.44				

The genes listed in the second column are genes that are expressed in normal colon epithelium and exhibit a 2-fold or more under- (Down) or over- (Up) expression level in the trisomic cell lines when compared to the expression levels in DLD1.

References

Abbott, A. 2002. Cancer research: On the offensive. *Nature*. 416:470-474.

Anders, K.R., J.R. Kudrna, K.E. Keller, B. Kinghorn, E.M. Miller, D. Pauw, A.T. Peck, C.E. Shellooe, and I.J. Strong. 2009. A strategy for constructing aneuploid yeast strains by transient nondisjunction of a target chromosome. *BMC genetics*. 10:36.

Babu, J.R., K.B. Jeganathan, D.J. Baker, X. Wu, N. Kang-Decker, and J.M. van Deursen. 2003. Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. *The Journal of cell biology*. 160:341-353.

Baker, D.J., J. Chen, and J.M.A. van Deursen. 2005. The mitotic checkpoint in cancer and aging: what have mice taught us? *Current Opinion in Cell Biology*. 17:583-589.

Bakhoum, S.F., G. Genovese, and D.A. Compton. 2009. Deviant kinetochore microtubule dynamics underlie chromosomal instability. *Curr Biol*. 19:1937-1942.

Bakhoum, S.F., W.T. Silkworth, I.K. Nardi, J.M. Nicholson, D.A. Compton, and D. Cimini. 2014. The mitotic origin of chromosomal instability. *Current Biology*. 24:R148-R149.

Bakhoum, S.F., S.L. Thompson, A.L. Manning, and D.A. Compton. 2008. Genome stability is ensured by temporal control of kinetochore–microtubule dynamics. *Nature cell biology*. 11:27-35.

Barber, T.D., K. McManus, K.W. Yuen, M. Reis, G. Parmigiani, D. Shen, I. Barrett, Y. Nouhi, F. Spencer, S. Markowitz, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, C. Lengauer, and P. Hieter. 2008. Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. *Proc Natl Acad Sci U S A*. 105:3443-3448.

Bardi, G., T. Sukhikh, N. Pandis, C. Fenger, O. Kronborg, and S. Heim. 1995. Karyotypic characterization of colorectal adenocarcinomas. *Genes Chromosomes Cancer*. 12:97-109.

Baroja, A., C. de la Hoz, A. Alvarez, R. Vielba, R. Sarrat, J. Aréchaga, and J.M. de Gandarias. 1998. Polyploidization and exit from cell cycle as mechanisms of cultured melanoma cell resistance to methotrexate. *Life sciences*. 62:2275-2282.

Bean, J., C. Brennan, J.Y. Shih, G. Riely, A. Viale, L. Wang, D. Chitale, N. Motoi, J. Szoke, S. Broderick, M. Balak, W.C. Chang, C.J. Yu, A. Gazdar, H. Pass, V. Rusch, W. Gerald, S.F. Huang, P.C. Yang, V. Miller, M. Ladanyi, C.H. Yang, and W. Pao. 2007. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci U S A*. 104:20932-20937.

Beroukhim, R., C.H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J.S. Boehm, J. Dobson, M. Urashima, K.T. Mc Henry, R.M. Pinchback, A.H. Ligon, Y.J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M.S. Lawrence, B.A. Weir, K.E. Tanaka, D.Y. Chiang, A.J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F.J. Kaye, H. Sasaki, J.E. Tepper, J.A. Fletcher, J. Tabernero, J. Baselga, M.S. Tsao, F. Demichelis, M.A. Rubin, P.A. Janne, M.J. Daly, C. Nucera, R.L. Levine, B.L. Ebert, S. Gabriel, A.K. Rustgi, C.R. Antonescu, M. Ladanyi, A. Letai, L.A. Garraway, M. Loda, D.G. Beer, L.D. True, A. Okamoto, S.L. Pomeroy, S. Singer, T.R. Golub, E.S. Lander, G. Getz, W.R. Sellers, and M. Meyerson. 2010. The landscape of somatic copy-number alteration across human cancers. *Nature*. 463:899-905.

Bharadwaj, R., and H. Yu. 2004. The spindle checkpoint, aneuploidy, and cancer. *Oncogene*. 23:2016-2027.

Biesecker, L.G., and N.B. Spinner. 2013. A genomic view of mosaicism and human disease. *Nat Rev Genet*. 14:307-320.

Biron-Shental, T., M. Liberman, M. Sharvit, R. Sukenik-Halevy, and A. Amiel. 2015. Amniocytes from aneuploidy embryos have enhanced random aneuploidy and signs of senescence - Can these findings be related to medical problems? *Gene*.

Blow, J.J., and T.U. Tanaka. 2005. The chromosome cycle: coordinating replication and segregation. *EMBO reports*. 6:1028-1034.

Bomme, L., G. Bardi, N. Pandis, C. Fenger, O. Kronborg, and S. Heim. 1994. Clonal karyotypic abnormalities in colorectal adenomas: clues to the early genetic events in the adenoma-carcinoma sequence. *Genes Chromosomes Cancer*. 10:190-196.
Boveri, T. 1902. Über mehrpolige mitosen als mittel zur analyse des zellkerns.
Boveri, T. 1914. Zur frage der entstehung maligner tumoren. Gustav Fischer.
Boveri, T. 2008. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci*. 121, Supplement 1:1-84.
Briand, P., K.V. Nielsen, M.W. Madsen, and O.W. Petersen. 1996. Trisomy 7p and malignant transformation of human breast epithelial cells following epidermal growth factor withdrawal. *Cancer Res*. 56:2039-2044.
Brown, S. 2008. Miscarriage and its associations. *In* Seminars in reproductive medicine. Vol. 26. © Thieme Medical Publishers. 391-400.
Cahill, D.P., C. Lengauer, J. Yu, G.J. Riggins, J.K. Willson, S.D. Markowitz, K.W. Kinzler, and B. Vogelstein. 1998. Mutations of mitotic checkpoint genes in human cancers. *Nature*. 392:300-303.
Capdeville, R., E. Buchdunger, J. Zimmermann, and A. Matter. 2002. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nature Reviews Drug Discovery*. 1:493-502.
Carter, S.L., A.C. Eklund, I.S. Kohane, L.N. Harris, and Z. Szallasi. 2006. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet*. 38:1043-1048.
Chen, G., W.D. Bradford, C.W. Seidel, and R. Li. 2012. Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy. *Nature*. 482:246-250.
Chen, G., W.A. Mulla, A. Kucharavy, H.J. Tsai, B. Rubinstein, J. Conkright, S. McCroskey, W.D. Bradford, L. Weems, J.S. Haug, C.W. Seidel, J. Berman, and R. Li. 2015. Targeting the adaptability of heterogeneous aneuploids. *Cell*. 160:771-784.
Cimini, D. 2007. Detection and correction of merotelic kinetochore orientation by Aurora B and its partners. *Cell Cycle*. 6:1558-1564.
Cimini, D. 2008. Merotelic kinetochore orientation, aneuploidy, and cancer. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 1786:32-40.
Cimini, D., L.A. Cameron, and E. Salmon. 2004. Anaphase spindle mechanics prevent mis-segregation of merotelically oriented chromosomes. *Current biology*. 14:2149-2155.
Cimini, D., and F. Degrassi. 2005. Aneuploidy: a matter of bad connections. *Trends in cell biology*. 15:442-451.
Cimini, D., D. Fioravanti, E. Salmon, and F. Degrassi. 2002. Merotelic kinetochore orientation versus chromosome mono-orientation in the origin of lagging chromosomes in human primary cells. *Journal of cell science*. 115:507-515.
Cimini, D., B. Howell, P. Maddox, A. Khodjakov, F. Degrassi, and E. Salmon. 2001. Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *The Journal of cell biology*. 153:517-528.
Cimini, D., B. Moree, J.C. Canman, and E. Salmon. 2003. Merotelic kinetochore orientation occurs frequently during early mitosis in mammalian tissue cells and error correction is achieved by two different mechanisms. *Journal of cell science*. 116:4213-4225.
Cleveland, D.W., Y. Mao, and K.F. Sullivan. 2003. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell*. 112:407-421.
Dai, W., Q. Wang, T. Liu, M. Swamy, Y. Fang, S. Xie, R. Mahmood, Y.-M. Yang, M. Xu, and C.V. Rao. 2004. Slippage of mitotic arrest and enhanced tumor development in mice with BubR1 haploinsufficiency. *Cancer research*. 64:440-445.
Donnelly, N., V. Passerini, M. Dürrbaum, S. Stingele, and Z. Storchová. 2014. HSF1 deficiency and impaired HSP90-dependent protein folding are hallmarks of aneuploid human cells. *The EMBO Journal*. 33:2374-2387.
Duelli, D., and Y. Lazebnik. 2007. Cell-to-cell fusion as a link between viruses and cancer. *Nat Rev Cancer*. 7:968-976.

Duelli, D.M., S. Hearn, M.P. Myers, and Y. Lazebnik. 2005. A primate virus generates transformed human cells by fusion. *The Journal of cell biology*. 171:493-503.

Duesberg, P., C. Rausch, D. Rasnick, and R. Hehlmann. 1998. Genetic instability of cancer cells is proportional to their degree of aneuploidy. *Proc Natl Acad Sci U S A*. 95:13692-13697.

Duijf, P.H., N. Schultz, and R. Benezra. 2013. Cancer cells preferentially lose small chromosomes. *International journal of cancer. Journal international du cancer*. 132:2316-2326.

Duncan, A.W., A.E. Hanlon Newell, W. Bi, M.J. Finegold, S.B. Olson, A.L. Beaudet, and M. Grompe. 2012. Aneuploidy as a mechanism for stress-induced liver adaptation. *The Journal of clinical investigation*. 122:3307-3315.

Duncan, A.W., M.H. Taylor, R.D. Hickey, A.E. Hanlon Newell, M.L. Lenzi, S.B. Olson, M.J. Finegold, and M. Grompe. 2010. The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature*. 467:707-710.

Dunham, M.J., H. Badrane, T. Ferea, J. Adams, P.O. Brown, F. Rosenzweig, and D. Botstein. 2002. Characteristic genome rearrangements in experimental evolution of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A*. 99:16144-16149.

Elledge, S.J. 1996. Cell Cycle Checkpoints: Preventing an Identity Crisis. *Science*. 274:1664-1672.

Farber, S., L.K. Diamond, R.D. Mercer, R.F. Sylvester Jr, and J.A. Wolff. 1948. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (aminopterin). *New England Journal of Medicine*. 238:787-793.

Fox, D.T., J.G. Gall, and A.C. Spradling. 2010. Error-prone polyploid mitosis during normal Drosophila development. *Genes Dev*. 24:2294-2302.

Ganem, N.J., S.A. Godinho, and D. Pellman. 2009. A mechanism linking extra centrosomes to chromosomal instability. *Nature*. 460:278-282.

Ganem, N.J., Z. Storchova, and D. Pellman. 2007. Tetraploidy, aneuploidy and cancer. *Current opinion in genetics & development*. 17:157-162.

Gao, C., K. Furge, J. Koeman, K. Dykema, Y. Su, M.L. Cutler, A. Werts, P. Haak, and G.F. Vande Woude. 2007. Chromosome instability, chromosome transcriptome, and clonal evolution of tumor cell populations. *Proc Natl Acad Sci U S A*. 104:8995-9000.

Garewal, H., P. Meltzer, J. Trent, R. Prabhala, R. Sampliner, and M. Korc. 1990. Epidermal growth factor receptor overexpression and trisomy 7 in a case of Barrett's esophagus. *Dig Dis Sci*. 35:1115-1120.

Gebhart, E., and T. Liehr. 2000. Patterns of genomic imbalances in human solid tumors (Review). *International journal of oncology*. 16:383-482.

Gemoll, T., J.K. Habermann, S. Becker, S. Szymczak, M.B. Upender, H.P. Bruch, U. Hellman, T. Ried, G. Auer, H. Jornvall, and U.J. Roblick. 2013. Chromosomal aneuploidy affects the global proteome equilibrium of colorectal cancer cells. *Anal Cell Pathol (Amst)*. 36:149-161.

Gerlinger, M., A.J. Rowan, S. Horswell, J. Larkin, D. Endesfelder, E. Gronroos, P. Martinez, N. Matthews, A. Stewart, P. Tarpey, I. Varela, B. Phillimore, S. Begum, N.Q. McDonald, A. Butler, D. Jones, K. Raine, C. Latimer, C.R. Santos, M. Nohadani, A.C. Eklund, B. Spencer-Dene, G. Clark, L. Pickering, G. Stamp, M. Gore, Z. Szallasi, J. Downward, P.A. Futreal, and C. Swanton. 2012. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *New England Journal of Medicine*. 366:883-892.

Greenman, C., P. Stephens, R. Smith, G.L. Dalgliesh, C. Hunter, G. Bignell, H. Davies, J. Teague, A. Butler, C. Stevens, S. Edkins, S. O'Meara, I. Vastrik, E.E. Schmidt, T. Avis, S. Barthorpe, G. Bhamra, G. Buck, B. Choudhury, J. Clements, J. Cole, E. Dicks, S. Forbes, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, A. Jenkinson, D. Jones, A. Menzies, T. Mironenko, J. Perry, K. Raine, D. Richardson, R. Shepherd, A. Small, C. Tofts, J. Varian, T. Webb, S. West, S. Widaa, A. Yates, D.P. Cahill, D.N. Louis, P. Goldstraw, A.G. Nicholson, F. Brasseur, L. Looijenga, B.L. Weber, Y.E. Chiew, A. DeFazio, M.F. Greaves, A.R. Green, P. Campbell, E. Birney, D.F. Easton, G. Chenevix-Trench, M.H. Tan, S.K. Khoo, B.T. Teh, S.T. Yuen, S.Y. Leung, R.

Wooster, P.A. Futreal, and M.R. Stratton. 2007. Patterns of somatic mutation in human cancer genomes. *Nature*. 446:153-158.
Gresham, D., M.M. Desai, C.M. Tucker, H.T. Jenq, D.A. Pai, A. Ward, C.G. DeSevo, D. Botstein, and M.J. Dunham. 2008. The repertoire and dynamics of evolutionary adaptations to controlled nutrient-limited environments in yeast. *PLoS Genet*. 4:e1000303.
Habermann, J.K., U. Paulsen, U.J. Roblick, M.B. Upender, L.M. McShane, E.L. Korn, D. Wangsa, S. Kruger, M. Duchrow, H.P. Bruch, G. Auer, and T. Ried. 2007. Stage-specific alterations of the genome, transcriptome, and proteome during colorectal carcinogenesis. *Genes Chromosomes Cancer*. 46:10-26.
Haering, C.H., and K. Nasmyth. 2003. Building and breaking bridges between sister chromatids. *Bioessays*. 25:1178-1191.
Hanahan, D., and R.A. Weinberg. 2000. The hallmarks of cancer. *Cell*. 100:57-70.
Hanahan, D., and R.A. Weinberg. 2011. Hallmarks of cancer: the next generation. *Cell*. 144:646-674.
Hanks, S., K. Coleman, S. Reid, A. Plaja, H. Firth, D. FitzPatrick, A. Kidd, K. Méhes, R. Nash, and N. Robin. 2004. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. *Nature genetics*. 36:1159-1161.
Hasle, H., I.H. Clemmensen, and M. Mikkelsen. 2000. Risks of leukaemia and solid tumours in individuals with Down's syndrome. *The Lancet*. 355:165-169.
Hassold, T., and P. Hunt. 2001. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet*. 2:280-291.
Hayama, S., Y. Daigo, T. Kato, N. Ishikawa, T. Yamabuki, M. Miyamoto, T. Ito, E. Tsuchiya, S. Kondo, and Y. Nakamura. 2006. Activation of CDCA1-KNTC2, Members of Centromere Protein Complex, Involved in Pulmonary Carcinogenesis. *Cancer Research*. 66:10339-10348.
Heim, S., and F. Mitelman. 2011. Cancer Cytogenetics: Chromosomal and Molecular Genetic Abberations of Tumor Cells. Wiley. com.
Hernando, E., Z. Nahle, G. Juan, E. Diaz-Rodriguez, M. Alaminos, M. Hemann, L. Michel, V. Mittal, W. Gerald, R. Benezra, S.W. Lowe, and C. Cordon-Cardo. 2004. Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. *Nature*. 430:797-802.
Hochhaus, A., S. Kreil, A.S. Corbin, P. La Rosee, M.C. Muller, T. Lahaye, B. Hanfstein, C. Schoch, N.C. Cross, U. Berger, H. Gschaidmeier, B.J. Druker, and R. Hehlmann. 2002. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia*. 16:2190-2196.
Hoffbrand, A., and E. Tripp. 1972. Unbalanced deoxyribonucleotide synthesis caused by methotrexate. *British medical journal*. 2:140.
Hojsgaard, S., U. Halekoh, and Y. J. 2006. The R package geepack for generalized estimating equations. *J Stat Softw*. 15:1-11.
Holland, A.J., and D.W. Cleveland. 2009. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nature reviews Molecular cell biology*. 10:478-487.
Horne, S.D., J.B. Stevens, B.Y. Abdallah, G. Liu, S.W. Bremer, C.J. Ye, and H.H. Heng. 2013. Why imatinib remains an exception of cancer research. *Journal of Cellular Physiology*. 228:665-670.
Hoyt, M.A., L. Totis, and B.T. Roberts. 1991. S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function. *Cell*. 66:507-517.
Hsiao, L.-L., F. Dangond, T. Yoshida, R. Hong, R.V. Jensen, J. Misra, W. Dillon, K.F. Lee, K.E. Clark, and P. Haverty. 2001. A compendium of gene expression in normal human tissues. *Physiological genomics*. 7:97-104.
Hughes, T.R., C.J. Roberts, H. Dai, A.R. Jones, M.R. Meyer, D. Slade, J. Burchard, S. Dow, T.R. Ward, M.J. Kidd, S.H. Friend, and M.J. Marton. 2000. Widespread aneuploidy revealed by DNA microarray expression profiling. *Nat Genet*. 25:333-337.
Iwanaga, Y., Y.-H. Chi, A. Miyazato, S. Sheleg, K. Haller, J.-M. Peloponese, Y. Li, J.M. Ward, R. Benezra, and K.-T. Jeang. 2007. Heterozygous Deletion of Mitotic Arrest–Deficient Protein 1 (MAD1) Increases the Incidence of Tumors in Mice. *Cancer research*. 67:160-166.

Jeganathan, K., L. Malureanu, D.J. Baker, S.C. Abraham, and J.M. van Deursen. 2007. Bub1 mediates cell death in response to chromosome missegregation and acts to suppress spontaneous tumorigenesis. *The Journal of cell biology*. 179:255-267.

Kapoor, T.M., T.U. Mayer, M.L. Coughlin, and T.J. Mitchison. 2000. Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. *The Journal of cell biology*. 150:975-988.

Kashina, A., R. Baskin, D. Cole, K. Wedaman, W. Saxton, and J. Scholey. 1996. A bipolar kinesin. *Nature*. 379:270.

Katz, W., B. Weinstein, and F. Solomon. 1990. Regulation of tubulin levels and microtubule assembly in Saccharomyces cerevisiae: consequences of altered tubulin gene copy number. *Mol Cell Biol*. 10:5286-5294.

Kops, G.J., D.R. Foltz, and D.W. Cleveland. 2004. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proceedings of the National Academy of Sciences of the United States of America*. 101:8699-8704.

Kops, G.J., B.A. Weaver, and D.W. Cleveland. 2005. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nature Reviews Cancer*. 5:773-785.

Korenberg, J.R., X.N. Chen, R. Schipper, Z. Sun, R. Gonsky, S. Gerwehr, N. Carpenter, C. Daumer, P. Dignan, C. Disteche, and et al. 1994. Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc Natl Acad Sci U S A*. 91:4997-5001.

Krämer, A., K. Neben, and A.D. Ho. 2005. Centrosome aberrations in hematological malignancies. *Cell biology international*. 29:375-383.

Lee, A.J., D. Endesfelder, A.J. Rowan, A. Walther, N.J. Birkbak, P.A. Futreal, J. Downward, Z. Szallasi, I.P. Tomlinson, M. Howell, M. Kschischo, and C. Swanton. 2011. Chromosomal instability confers intrinsic multidrug resistance. *Cancer Res*. 71:1858-1870.

Lejeune, J., M. Gautier, and R. Turpin. 1959. Study of somatic chromosomes from 9 mongoloid children]. *Comptes rendus hebdomadaires des séances de l'Académie des sciences*. 248:1721.

Lengauer, C., K.W. Kinzler, and B. Vogelstein. 1997. Genetic instability in colorectal cancers. *Nature*. 386:623-627.

Lengauer, C., K.W. Kinzler, and B. Vogelstein. 1998. Genetic instabilities in human cancers. *Nature*. 396:643-649.

Li, G.-Q., H. Li, and H.-F. Zhang. 2003. Mad2 and p53 expression profiles in colorectal cancer and its clinical significance. *WORLD JOURNAL OF GASTROENTEROLOGY*. 9:1972-1975.

Lingle, W.L., W.H. Lutz, J.N. Ingle, N.J. Maihle, and J.L. Salisbury. 1998. Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. *Proceedings of the National Academy of Sciences*. 95:2950-2955.

Liu, X., X. Yu, D.J. Zack, H. Zhu, and J. Qian. 2008. TiGER: a database for tissue-specific gene expression and regulation. *BMC bioinformatics*. 9:271.

Ly, P., U. Eskiocak, S.B. Kim, A.I. Roig, S.K. Hight, D.R. Lulla, Y.S. Zou, K. Batten, W.E. Wright, and J.W. Shay. 2011. Characterization of aneuploid populations with trisomy 7 and 20 derived from diploid human colonic epithelial cells. *Neoplasia*. 13:348-357.

Malumbres, M., and M. Barbacid. 2001. Milestones in cell division : To cycle or not to cycle: a critical decision in cancer. *Nat Rev Cancer*. 1:222-231.

Mao, R., C.L. Zielke, H. Ronald Zielke, and J. Pevsner. 2003. Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics*. 81:457-467.

Marx, J. 2002. Debate surges over the origins of genomic defects in cancer. *Science*. 297:544-546.

Massague, J. 2004. G1 cell-cycle control and cancer. *Nature*. 432:298-306.

Matsuura, S., Y. Matsumoto, K.i. Morishima, H. Izumi, H. Matsumoto, E. Ito, K. Tsutsui, J. Kobayashi, H. Tauchi, and Y. Kajiwara. 2006. Monoallelic BUB1B mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome. *American Journal of Medical Genetics Part A*. 140:358-367.

Michel, L.S., V. Liberal, A. Chatterjee, R. Kirchwegger, B. Pasche, W. Gerald, M. Dobles, P.K. Sorger, V.V. Murty, and R. Benezra. 2001. MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature*. 409:355-359.
Mitelman F, J.B.a.M.F.E. 2013. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer.
Mitelman, F., B. Johansson, and F. Mertens. 2014. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer http://cgap.nci.nih.gov/Chromosomes/Mitelman.
Murray, A.W. 1995. The genetics of cell cycle checkpoints. *Current Opinion in Genetics & Development*. 5:5-11.
Nasmyth, K. 1996. At the heart of the budding yeast cell cycle. *Trends in Genetics*. 12:405-412.
Nicholson, J.M., and D. Cimini. 2011. How mitotic errors contribute to karyotypic diversity in cancer. *Adv Cancer Res*. 112:43-75.
Nicholson, J.M., and D. Cimini. 2013. Cancer karyotypes: survival of the fittest. *Frontiers in oncology*. 3:148.
Nicholson, J.M., and D. Cimini. 2015. Link between Aneuploidy and Chromosome Instability. *International review of cell and molecular biology*. 315:299-317.
Nicholson, J.M., J.C. Macedo, A.J. Mattingly, D. Wangsa, J. Camps, V. Lima, A.M. Gomes, S. Doria, T. Ried, E. Logarinho, and D. Cimini. 2015. Chromosome mis-segregation and cytokinesis failure in trisomic human cells. *eLife*. 4.
Nigg, E.A. 2002. Centrosome aberrations: cause or consequence of cancer progression? *Nature Reviews Cancer*. 2:815-825.
Oromendia, A.B., S.E. Dodgson, and A. Amon. 2012. Aneuploidy causes proteotoxic stress in yeast. *Genes & development*. 26:2696-2708.
Pai, G.S., R.C. Lewandowski, and D.S. Borgaonkar. 2003. Handbook of chromosomal syndromes. J. Wiley.
Paulovich, A.G., D.P. Toczyski, and L.H. Hartwell. 1997. When Checkpoints Fail. *Cell*. 88:315-321.
Pavelka, N., G. Rancati, J. Zhu, W.D. Bradford, A. Saraf, L. Florens, B.W. Sanderson, G.L. Hattem, and R. Li. 2010. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature*. 468:321-325.
Pinsky, B.A., and S. Biggins. 2005. The spindle checkpoint: tension versus attachment. *Trends in cell biology*. 15:486-493.
Poláková, S., C. Blume, J.Á. Zárate, M. Mentel, D. Jørck-Ramberg, J. Stenderup, and J. Piškur. 2009. Formation of new chromosomes as a virulence mechanism in yeast Candida glabrata. *Proceedings of the National Academy of Sciences*. 106:2688-2693.
Pollack, J.R., T. Sørlie, C.M. Perou, C.A. Rees, S.S. Jeffrey, P.E. Lonning, R. Tibshirani, D. Botstein, A.-L. Børresen-Dale, and P.O. Brown. 2002. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proceedings of the National Academy of Sciences*. 99:12963-12968.
Rabin, K.R., and J.A. Whitlock. 2009. Malignancy in children with trisomy 21. *The oncologist*. 14:164-173.
Rancati, G., N. Pavelka, B. Fleharty, A. Noll, R. Trimble, K. Walton, A. Perera, K. Staehling-Hampton, C.W. Seidel, and R. Li. 2008. Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. *Cell*. 135:879-893.
Rasmussen, S.A., L.-Y.C. Wong, Q. Yang, K.M. May, and J. Friedman. 2003. Population-based analyses of mortality in trisomy 13 and trisomy 18. *Pediatrics*. 111:777-784.
Reish, O., N. Brosh, R. Gobazov, M. Rosenblat, V. Libman, and M. Mashevich. 2006. Sporadic aneuploidy in PHA-stimulated lymphocytes of Turner's syndrome patients. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology*. 14:527-534.

Reish, O., M. Regev, A. Kanesky, S. Girafi, and M. Mashevich. 2011. Sporadic aneuploidy in PHA-stimulated lymphocytes of trisomies 21, 18, and 13. *Cytogenetic and genome research*. 133:184-189.

Rice, G.C., C. Hoy, and R.T. Schimke. 1986. Transient hypoxia enhances the frequency of dihydrofolate reductase gene amplification in Chinese hamster ovary cells. *Proceedings of the National Academy of Sciences*. 83:5978-5982.

Ried, T., Y. Hu, M.J. Difilippantonio, B.M. Ghadimi, M. Grade, and J. Camps. 2012. The consequences of chromosomal aneuploidy on the transcriptome of cancer cells. *Biochimica et biophysica acta*. 1819:784-793.

Rieder, C.L., and S.P. Alexander. 1990. Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. *The Journal of cell biology*. 110:81-95.

Rieder, C.L., R.W. Cole, A. Khodjakov, and G. Sluder. 1995. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *The Journal of cell biology*. 130:941-948.

Rieder, C.L., and A. Khodjakov. 2003. Mitosis through the microscope: advances in seeing inside live dividing cells. *Science*. 300:91-96.

Rieder, C.L., A. Khodjakov, L.V. Paliulis, T.M. Fortier, R.W. Cole, and G. Sluder. 1997. Mitosis in vertebrate somatic cells with two spindles: implications for the metaphase/anaphase transition checkpoint and cleavage. *Proceedings of the National Academy of Sciences*. 94:5107-5112.

Rieder, C.L., A. Schultz, R. Cole, and G. Sluder. 1994. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *The Journal of cell biology*. 127:1301-1310.

Rowley, J.D. 2004. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Landmarks in Medical Genetics: Classic Papers with Commentaries*. 51:104.

Sareen, D., E. McMillan, A.D. Ebert, B.C. Shelley, J.A. Johnson, L.F. Meisner, and C.N. Svendsen. 2009. Chromosome 7 and 19 trisomy in cultured human neural progenitor cells. *PLoS One*. 4:e7630.

Schimke, R.T., S.W. Sherwood, A.B. Hill, and R.N. Johnston. 1986. Overreplication and recombination of DNA in higher eukaryotes: potential consequences and biological implications. *Proceedings of the National Academy of Sciences*. 83:2157-2161.

Schoenfelder, K.P., R.A. Montague, S.V. Paramore, A.L. Lennox, A.P. Mahowald, and D.T. Fox. 2014. Indispensable pre-mitotic endocycles promote aneuploidy in the Drosophila rectum. *Development*. 141:3551-3560.

Schvartzman, J.-M., R. Sotillo, and R. Benezra. 2010. Mitotic chromosomal instability and cancer: mouse modelling of the human disease. *Nature reviews Cancer*. 10:102-115.

Selmecki, A., A. Forche, and J. Berman. 2006. Aneuploidy and isochromosome formation in drug-resistant Candida albicans. *Science*. 313:367-370.

Selmecki, A., M. Gerami-Nejad, C. Paulson, A. Forche, and J. Berman. 2008. An isochromosome confers drug resistance in vivo by amplification of two genes, ERG11 and TAC1. *Molecular microbiology*. 68:624-641.

Selmecki, A.M., K. Dulmage, L.E. Cowen, J.B. Anderson, and J. Berman. 2009. Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. *PLoS Genet*. 5:e1000705.

Sheltzer, J.M., H.M. Blank, S.J. Pfau, Y. Tange, B.M. George, T.J. Humpton, I.L. Brito, Y. Hiraoka, O. Niwa, and A. Amon. 2011. Aneuploidy drives genomic instability in yeast. *Science*. 333:1026-1030.

Shin, S.I., V.H. Freedman, R. Risser, and R. Pollack. 1975. Tumorigenicity of virus-transformed cells in nude mice is correlated specifically with anchorage independent growth in vitro. *Proc Natl Acad Sci U S A*. 72:4435-4439.

Silkworth, W.T., and D. Cimini. 2012. Transient defects of mitotic spindle geometry and chromosome segregation errors. *Cell Div*. 7:19.

Silkworth, W.T., I.K. Nardi, L.M. Scholl, and D. Cimini. 2009. Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells. *PLoS One*. 4:e6564.

Skibbens, R.V., V.P. Skeen, and E. Salmon. 1993. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *The Journal of cell biology*. 122:859-875.

Solomon, D.A., T. Kim, L.A. Diaz-Martinez, J. Fair, A.G. Elkahloun, B.T. Harris, J.A. Toretsky, S.A. Rosenberg, N. Shukla, and M. Ladanyi. 2011. Mutational inactivation of STAG2 causes aneuploidy in human cancer. *Science*. 333:1039-1043.

Sotillo, R., E. Hernando, E. Díaz-Rodríguez, J. Teruya-Feldstein, C. Cordón-Cardo, S.W. Lowe, and R. Benezra. 2007. Mad2 overexpression promotes aneuploidy and tumorigenesis in mice. *Cancer cell*. 11:9-23.

Stingele, S., G. Stoehr, K. Peplowska, J. Cox, M. Mann, and Z. Storchova. 2012. Global analysis of genome, transcriptome and proteome reveals the response to aneuploidy in human cells. *Mol Syst Biol*. 8:608.

Storchova, Z., and D. Pellman. 2004. From polyploidy to aneuploidy, genome instability and cancer. *Nature reviews Molecular cell biology*. 5:45-54.

Swanton, C., B. Nicke, M. Schuett, A.C. Eklund, C. Ng, Q. Li, T. Hardcastle, A. Lee, R. Roy, P. East, M. Kschischo, D. Endesfelder, P. Wylie, S.N. Kim, J.G. Chen, M. Howell, T. Ried, J.K. Habermann, G. Auer, J.D. Brenton, Z. Szallasi, and J. Downward. 2009. Chromosomal instability determines taxane response. *Proc Natl Acad Sci U S A*. 106:8671-8676.

Tan, Z., M. Hays, G.A. Cromie, E.W. Jeffery, A.C. Scott, V. Ahyong, A. Sirr, A. Skupin, and A.M. Dudley. 2013. Aneuploidy underlies a multicellular phenotypic switch. *Proc Natl Acad Sci U S A*. 110:12367-12372.

Tanaka, K., J. Nishioka, K. Kato, A. Nakamura, T. Mouri, C. Miki, M. Kusunoki, and T. Nobori. 2001. Mitotic checkpoint protein hsMAD2 as a marker predicting liver metastasis of human gastric cancers. *Japanese journal of cancer research : Gann*. 92:952-958.

Tang, Y.C., B.R. Williams, J.J. Siegel, and A. Amon. 2011. Identification of aneuploidy-selective antiproliferation compounds. *Cell*. 144:499-512.

Thayer, M.J. 1996. Regulation of tissue-specific gene expression in microcell hybrids. *Methods*. 9:30-37.

Thompson, S.L., and D.A. Compton. 2008. Examining the link between chromosomal instability and aneuploidy in human cells. *The Journal of cell biology*. 180:665-672.

Thompson, S.L., and D.A. Compton. 2010. Proliferation of aneuploid human cells is limited by a p53-dependent mechanism. *J Cell Biol*. 188:369-381.

Tighe, A., V.L. Johnson, M. Albertella, and S.S. Taylor. 2001. Aneuploid colon cancer cells have a robust spindle checkpoint. *EMBO reports*. 2:609-614.

Torres, E.M., T. Sokolsky, C.M. Tucker, L.Y. Chan, M. Boselli, M.J. Dunham, and A. Amon. 2007. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science*. 317:916-924.

Torres, E.M., B.R. Williams, and A. Amon. 2008. Aneuploidy: cells losing their balance. *Genetics*. 179:737-746.

Upender, M.B., J.K. Habermann, L.M. McShane, E.L. Korn, J.C. Barrett, M.J. Difilippantonio, and T. Ried. 2004. Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res*. 64:6941-6949.

Vernon, M., K. Lobachev, and T.D. Petes. 2008. High rates of "unselected" aneuploidy and chromosome rearrangements in tel1 mec1 haploid yeast strains. *Genetics*. 179:237-247.

Walther, A., R. Houlston, and I. Tomlinson. 2008. Association between chromosomal instability and prognosis in colorectal cancer: a meta-analysis. *Gut*. 57:941-950.

Wang, C.-Y., L.-N. Liu, and Z.-B. Zhao. 2013. The role of ROS toxicity in spontaneous aneuploidy in cultured cells. *Tissue and Cell*. 45:47-53.

Wassmann, K., and R. Benezra. 2001. Mitotic checkpoints: from yeast to cancer. *Current opinion in genetics & development*. 11:83-90.

Weaver, B.A., and D.W. Cleveland. 2006. Does aneuploidy cause cancer? *Curr Opin Cell Biol*. 18:658-667.

Weaver, B.A., A.D. Silk, C. Montagna, P. Verdier-Pinard, and D.W. Cleveland. 2007. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer cell*. 11:25-36.

Weaver, B.A.A., and D.W. Cleveland. 2005. Decoding the links between mitosis, cancer, and chemotherapy: The mitotic checkpoint, adaptation, and cell death. *Cancer Cell*. 8:7-12.

Wells, W.A. 1996. The spindle-assembly checkpoint: aiming for a perfect mitosis, every time. *Trends in cell biology*. 6:228-234.

Williams, B.R., V.R. Prabhu, K.E. Hunter, C.M. Glazier, C.A. Whittaker, D.E. Housman, and A. Amon. 2008. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science*. 322:703-709.

Wise, D.A., and B. Brinkley. 1997. Mitosis in cells with unreplicated genomes (MUGs): spindle assembly and behavior of centromere fragments. *Cell motility and the cytoskeleton*. 36:291-302.

Wittmann, T., A. Hyman, and A. Desai. 2001. The spindle: a dynamic assembly of microtubules and motors. *Nature cell biology*. 3:E28-E34.

Wood, L.D., D.W. Parsons, S. Jones, J. Lin, T. Sjoblom, R.J. Leary, D. Shen, S.M. Boca, T. Barber, J. Ptak, N. Silliman, S. Szabo, Z. Dezso, V. Ustyanksky, T. Nikolskaya, Y. Nikolsky, R. Karchin, P.A. Wilson, J.S. Kaminker, Z. Zhang, R. Croshaw, J. Willis, D. Dawson, M. Shipitsin, J.K. Willson, S. Sukumar, K. Polyak, B.H. Park, C.L. Pethiyagoda, P.V. Pant, D.G. Ballinger, A.B. Sparks, J. Hartigan, D.R. Smith, E. Suh, N. Papadopoulos, P. Buckhaults, S.D. Markowitz, G. Parmigiani, K.W. Kinzler, V.E. Velculescu, and B. Vogelstein. 2007. The genomic landscapes of human breast and colorectal cancers. *Science*. 318:1108-1113.

Yu, H.-G., and R.K. Dawe. 2000. Functional redundancy in the maize meiotic kinetochore. *The Journal of cell biology*. 151:131-142.

Yuen, K.W., and A. Desai. 2008. The wages of CIN. *J Cell Biol*. 180:661-663.

Zhang, N., G. Ge, R. Meyer, S. Sethi, D. Basu, S. Pradhan, Y.-J. Zhao, X.-N. Li, W.-W. Cai, A.K. El-Naggar, V. Baladandayuthapani, F.S. Kittrell, P.H. Rao, D. Medina, and D. Pati. 2008. Overexpression of Separase induces aneuploidy and mammary tumorigenesis. *Proceedings of the National Academy of Sciences*. 105:13033-13038.

Zhu, J., N. Pavelka, W.D. Bradford, G. Rancati, and R. Li. 2012. Karyotypic determinants of chromosome instability in aneuploid budding yeast. *PLoS Genet*. 8:e1002719.