Review

Cell-cycle Checkpoints and Aneuploidy on the Path to Cancer

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Abstract. *The cell cycle is a complex sequence of events through which a cell duplicates its contents and divides, and involves many regulatory proteins for proper cellular reproduction, including cyclin proteins and cyclin-dependent kinases, oncogenes and tumor-suppressor genes, and mitotic checkpoint proteins. Mutations of any of these regulatory mechanisms can lead to reproduction of cells carrying genetic mutations or abnormal numbers of chromosomes, resulting in genomic instability. Chromosomal instability, contributing to genomic instability, refers to abnormalities in the number of chromosomes, and leads to aneuploidy. The role of aneuploidy in cancer cell development is often disputed, as conflicting hypotheses and research make it unclear as to whether aneuploidy is a cause or consequence of cancer. Here, we present an overview of the importance of cell-cycle checkpoint regulation and chromosomal instability in the development of cancer, and discuss evidence for conflicting arguments for the role of aneuploidy in cancer, leading us to conclude that further investigation of this role would benefit our understanding of cancer development.*

The cell cycle is a complex sequence of events through which a cell duplicates its contents and divides, resulting in two genetically identical daughter cells. This cycle, and its regulation, is essential to cell growth and reproduction, and involves many regulatory proteins such as cyclin proteins and cyclin-dependent kinases (CDKs), oncogenes and tumor-suppressor genes in interphase, and mitotic checkpoint proteins that allow stages of the cell cycle to proceed, or

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inhibit this procession. Mutations and deficiencies in regulation throughout the cell cycle, however, can lead to serious diseases such as cancer. This review compares the cell cycle in normal cells to that in cancer cells, with a focus on the regulatory proteins involved, and the role of deficiencies in these proteins in the development of cancer.

The Cell Cycle in Normal Cells

The cell cycle comprises of interphase, which consists of the G_1, S, and G_2 phases, and the mitotic (M) phase (1). During interphase, a cell prepares for division by means of growth and DNA replication. The G_1 phase is the gap between the end of cytokinesis of a previous division and the beginning of S phase, and is the phase in which the cell grows in preparation for DNA replication, as well as the phase in which it is decided whether a cell will divide again or enter G_0, a resting phase. A removal of growth factors in early G_1 will send the cell into G_0, but their removal later on in G_1, after the restriction checkpoint, will allow the cell to continue into S phase (2). During S phase, DNA replication occurs and each chromosome is duplicated, becoming two sister chromatids. G2 marks the gap between the end of S phase and the start of mitosis. Here, the cell synthesizes materials needed for mitosis, such as RNA and proteins (3).

The M phase begins with mitosis, which is subdivided into five phases, namely prophase, prometaphase, metaphase, anaphase and telophase, and ends in cytokinesis (1). Reproduction through the cell cycle results in genetically identical daughter cells.

Regulation of the Cell Cycle

Throughout these phases of the cell cycle, regulation is essential in the proper production of daughter cells. The fundamental aspect of regulation lies in cyclin proteins and cyclin-dependent kinases (CDKs), which form complexes and catalyze progression through the cell cycle when activated (2). As a result of the systematic synthesis and destruction of cyclin throughout the cycle, CDKs are only

activated at certain times within the cell cycle, a key factor in cell-cycle regulation (4). Once activated by cyclins, CDKs phosphorylate specific substrates that drive events of the cell cycle and cell division.

These regulatory roles of cyclin–CDK complexes allow for 'checkpoints' during the cell cycle. Cell-cycle checkpoints are responsible for ensuring that each earlier process has been completed before the cell moves on to the next phase of the cycle. Activation of a checkpoint, meaning a possible error has been detected, arrests the cell cycle in its current phase through changes in CDK levels and activation, preventing improper cellular reproduction. Regulatory checkpoints include the G_1/S or restriction checkpoint, the G_2/M or DNA replication checkpoint, and the metaphase/anaphase or spindle apparatus checkpoint. The restriction checkpoint is influenced primarily by growth factors, cell size, cell nutrition, and DNA damage (2, 5). The DNA replication checkpoint is primarily influenced by improper DNA replication and damage, and the metaphase/ anaphase checkpoint is influenced by chromosome attachment to the mitotic spindle (1). Should any processes be found incomplete, or damage be evident at these checkpoints, cyclin–CDK regulatory activity is blocked, preventing the cell from progressing through the cycle until these issues are resolved and the cell is prepared for the next phase (1, 6).

Specifically, three CDKs are involved in regulation during interphase (CDK2, CDK4, and CDK6), regulating exit from and entrance into sub-phases (7). Detection of DNA damage signals the inhibition of these CDKs, inducing cell-cycle arrest. CDK4 and CDK6 activation influences the progression of G_1 early on, binding with cyclin-D to phosphorylate pRb, the retinoblastoma protein, preventing it from binding and inhibiting the E2F transcription factor, which transcribes the necessary proteins for the G_1/S transition and promotes the cell to the next phases of the cycle. pRb is responsible for inactivating E2F during phases such as G_0 and M. When CDK4 and CDK6 are inhibited at the detection of DNA damage, therefore, they do not inactivate pRb, allowing it to bind to and inhibit E2F and preventing the transcription of necessary proteins, a process that resumes when the checkpoint deems the cell to be properly prepared (7). CDK2 is also involved in the inactivation of pRb, and also plays a role in DNA repair and replication, phosphorylating substrates necessary for DNA replication (8, 9).

Cell-cycle Regulation and Cancer
Oncogenes and Tumor Suppressors

Oncogenes and tumor-suppressor genes play a large role in regulation of the cell cycle, particularly those of the p53 and pRb pathways, which are involved in the restriction checkpoint.

p53, a tumor-suppressor protein, responds to DNA damage during G_1 such as mismatches and single-stranded DNA, initiating transcription of *p21*, a CDK-interacting protein that inhibits activation of the necessary CDKs of G1 to phosphorylate pRb, and therefore preventing progression to DNA synthesis (11). p53 is also highly involved in apoptosis in cases of irreparable damage (12). Mutations in *p53* are extremely common in human cancer, and result in lack of *p21* transcription and therefore dysfunctional or lack of arrest of G1 in the presence of DNA damage, allowing this damage to continue on in the cycle without repair (13). Evidence of this p53 dependence during G_1 has been shown through studies of *p53*-null transgenic mice, and failure of their cells to arrest during G_1 when faced with DNA damage (14). As a result, the mutation or damage becomes permanent in the genome (2). Studies of mutations in *p53*, which are often accompanied by conformational changes of the protein that allow for detection of these mutations (11), in mice have shown mutant p53 to result in susceptibility to tumors such as lung adenocarcinomas, osteosarcomas, and lymphomas (15).

Related to the p53 tumor suppressor, the murine double minute-2 (*MDM2*) oncogene is also involved in the DNA damage checkpoint. MDM2 proteins abrogate the checkpoint by binding to mutated, wild-type p53 and inhibiting its function through these interactions (16, 17).

pRb, discussed previously, is another tumor suppressor, and a target of CDKs (CDK2, CDK 4, and CDK6) involved in G1 and the restriction checkpoint (5, 18). Defective pRb function results in a lack of binding to E2F, which then is allowed to transcribe such proteins without regulation and promote the cell through the cycle (19).

Cyclin D1 has been shown to be amplified in cancer such as breast cancer (20). Cyclin D1 affects the function of pRb through bonding with CDK 4 and CDK6, which phosphorylate pRb.

Aneuploidy and Cancer

Another source of development of cancer and tumorigenesis lies in defective regulation during mitosis. Aneuploidy involves the presence of an abnormal number of chromosomes in a cell, and is an extremely common characteristic of tumor cells involving chromosomal instability (21, 22). Chromosomal instability refers to an increased rate in chromosomal abnormalities, such as deletions or duplications, leading to an unequal distribution of DNA in daughter cells. High rates of chromosomal instability can lead to aneuploidy, which is often observed in cancer. For example, whole chromosome gains, such as the gain of chromosome 8, have been found as a common error in karyotype in acute myeloid leukemia (23).

One hypothesis as to why this abnormal segregation of chromosomes may lead to tumor development is that should

a cell be missing parts of or whole chromosomes, they also lose regulatory genes included in those parts, such as tumor-suppressor genes (24). These abnormal numbers may also result in aberrant gene expression and varying levels of genomic instability (25).

Studies suggest that a normal cell, such as one from the RPE-1 or HCT116 cell line, missegregates a chromosome once in every one hundred rounds of division (26). These missegregations are thought to be caused by errors surrounding the spindle checkpoint, such as abnormal centrosome numbers and incorrect kinetochore-microtubule attachments. For example, merotelic attachments, in which one single kinetochore of a sister chromatid pair is attached to microtubules from both poles of the cell, may arise from an excess number of centrosomes (27). Ganem *et al*. suggested through generation and imaging of cells that differed in centrosome numbers that extra chromosomes result in high frequencies of merotelic attachments and missegregation leading up to anaphase (27). It is especially important to look into the mechanisms surrounding the spindle checkpoint, as the cell cycle is reliant upon this checkpoint to ensure proper segregation of chromosomes prior to division.

Before anaphase and chromosomal segregation begins, sister chromatids are held together by cohesin, a protein complex (28). The proteolysis of sister chromatid cohesion 1 (SCC1), a cohesin subunit, by separase, a protease, triggers anaphase and chromosomal segregation. Once proper attachment of chromosomes to the spindle fibers is confirmed, a separase inhibitor called securin is ubiquitinated by the anaphase-promoting complex or cyclosome APC/C. Without securin to inhibit separase, it is activated, proteolysis of SCC1 occurs, and anaphase begins (28). Upon detection of improper connection of chromosomes to the spindle apparatus at kinetochores, it is thought that these kinetochores send out a checkpoint signal to arrest the cell cycle, signaling the inhibition of APC/C, and therefore inhibiting segregation (29, 30). Studies involving laser ablation of one unattached kinetochore showed that a cell could proceed on through mitosis when ablation occurred, suggesting that unattachment is the source of the inhibitory signal (29).

While complete failure at this spindle checkpoint leads to cell death (31), impairments in, but not failure of, the checkpoint resulting from defects surrounding the proteins involved in this process have been found in tumor cells. These proteins, after detection of incorrect attachment of chromosomes to the spindle apparatus, inhibit the procession of steps surrounding APC/C, therefore preventing the initiation and promotion of segregation and anaphase. The ubiquination abilities of APC/C are dependent on the binding of the cell division cycle protein 20 (CDC20), which recruits substrates to APC/C and activates the process (32). Other proteins involved in a functioning spindle checkpoint include the mitotic arrest deficient protein 2 (MAD2) and the budding uninhibited by benzimidazole-related protein 1 (BUBR1). MAD2 binds to CDC20, inhibiting the protein and preventing the ubiquination of securin by APC/C (33). BUBR1 also inhibits the APC/C and CDC20 complex, independently of MAD2 (33). Alterations in the expression of these checkpoint proteins can result in incorrect segregation of chromosomes due to failure to arrest before corrections can be made, leading to aneuploidy. These aberrant chromosome numbers are often seen in cancer, evidenced by the commonality of spindle checkpoint deficiency in tumor cells (22).

Mutations in checkpoint proteins have often been found in association with colorectal cancer, tumor cells of which have commonly been found to show evidence of aneuploidy due to chromosomal instability (22, 31, 34). Additionally, Li and Benezra reported that a breast cancer cell line with reduced MAD2 level (T47D) failed to arrest at the spindle checkpoint in response to inhibitory effects on the spindle apparatus (35). Mutations or dysregulation of other checkpoint proteins such as budding uninhibited by benzimidazole protein 1 (BUB1) and BUBR1 have also been observed in colorectal and lung cancer, as well as in leukemia and lymphoma (36-38). Gemma *et al*. screened the DNA sequences of human lung cancer cell lines for mutations in the *BUB1* gene, and found various alterations to this gene in some of these cell lines (36). Ohshima *et al*. found mutations or deletions in the BUB1 and BUBR1 genes in adult T-cell leukemia/lymphoma (37). Shichiri *et al*. found irregularities in the expression of BUB1 and BUBR1 in colorectal cancer, and concluded that the abnormal expression of these checkpoint genes may lead to aneuploidy and tumor metastasis (38).

The Role of Aneuploidy in Cancer Development

While more and more is being discovered regarding these protein irregularities resulting in improper spindle checkpoint function, the question still persists of whether aneuploidy is a cause or a consequence of cancer and tumor development. An argument for aneuploidy as a consequence, rather than a cause, of cancer lies in the knowledge that the commonly found mutations in regulatory pathways such as the p53 and pRb pathways lead to genomic and chromosomal instability, and that the frequent aneuploidy in cancer cells is a result of this chromosomal instability (39). Manning *et al*. showed that depletion of pRb, which as discussed above can cause errors at the restriction checkpoint, also results in errors in the structure of centromeres, leading to higher frequencies of merotelic attachment and therefore higher rates of improper chromosomal segregation. This ultimately can lead to abnormalities involving whole-chromosome losses/gains

(40). In these cases, aneuploidy results from alternative forms of instability, and, although it affects the development of these cells, it is a consequence of other causes.

On the other hand, studies involving the observation of chromosome numbers in cancer cell lines, such as trisomy of certain chromosomes, show a resultant duplication of mutated alleles, connecting aneuploidy to tumorigenesis (41, 42). Beghini *et al*. suggested that trisomy of chromosome 4 leads to the duplication of the mutant tyrosine kinase receptor (KIT) allele observed in acute myeloid leukemia (41), and Zhuang *et al*. observed duplication of another mutated tyrosine kinase receptor (MET) allele resulting from trisomy of chromosome 7 in papillary renal carcinoma (42).

In general, the particular role that aneuploidy plays in cancer development is still unclear, and as is whether it contributes to the causes of cancer or is a result of other causes. Future investigations of these phenomena should improve our understanding of cell-cycle regulation in cancer cells, and may point to newer approaches to treating cancer.

Conclusions and Future Directions

There are varying underlying mechanisms by which cell division and reproduction is regulated. Dysregulation of these mechanisms can have detrimental and even lethal effects on a cell and on the body. Uncorrected mistakes in the cell cycle, including DNA damage and mutations or improper chromosomal segregation and aneuploidy, result in genomic instability, a distinct characteristic of cancer. Knowledge of the means by which tumorigenesis occurs is important in exploring treatments of, and eventually cure of, cancer. Discussion of the role of aneuploidy in cancer development points to an area for which more research is encouraged, and also to an area which may offer an important piece in completing the puzzle to explain cancer development.

Conflicts of Interest

The Authors declare no competing financial interests.

Acknowledgements

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