Cell Cycle and Cancer

4

Manzoor Ahmad Mir and Shazia Sofi

4.1 Introduction

Cancer is the most lethal disease leading to a low life expectancy worldwide (Mir and Mir (2022); Mir et al. 2022a–e; Sung et al. 2021). According to World Health Organization (WHO) estimations for 2019, cancer is the third or fourth top cause of death before the age of 70 in 23 countries and the first or second leading cause in 112 of 183 nations (Bray et al. 2021). Hence, cancer is a major life-threatening disease that poses a great challenge to the present biomedical knowledge and treatments. Unfortunately, the complexity of the disease at the tissue level makes it difficult to accurately diagnose it and ensure that treatment is effective (Meacham and Morrison 2013; Fisher et al. 2013). Prostate, lung and bronchi, colon and rectum, and urine bladder are the main organs in men that are most severely impacted by cancer. Breast, lung, bronchus, colon, rectum, uterine corpus, and thyroid cancer prevalence in women have been found to be highest, correspondingly. This data estimates that prostate and breast cancer as the most prevalent type of cancer seen in men and women, respectively (Mir and Gul 2022; Siegel et al. 2020). While as blood cancer, and cancers related to the brain and lymph nodes, are the most common cancers found in children that account for about 28% of all cancers in children (Schottenfeld and Fraumeni 2006; Mehraj et al. 2022).

M. A. Mir (✉) · S. Sofi
Department of Bioresources, School of Biological Sciences, University of Kashmir, Srinagar, J&K, India
e-mail: drmanzoor@kashmiruniversity.ac.in

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4.2 Cancer Development

A condition known as cancer causes some body cells to grow out of control and propagate to other parts of body to form new tumours (a process called metastasis). The progression of cancer, termed as carcinogenesis, can be best described by enlisting all the features of cancer cells that stir up the process and hence make them distinct characteristics of cancer cells. Cancer progression depends upon the procurement of several abnormal properties like: self-supporting proliferation, insensitivity to anti-proliferative signals, failure of cancer cells to undergo apoptosis, lower requirement of growth factors, angiogenesis and, for malignancy, tissue invasion and metastasis (Mir and Haq 2022; Hanahan and Weinberg 2000). The transition from normal cell to cancer cell that is termed as transformation is a multistep process and can be divided into three distinct stages: initiation, promotion, and progression (Kinzler and Vogelstein 1996). Initiation is a process in which genomic changes get accumulated in cells and they are able to form tumours. Promotion is associated with increased proliferation of initiated cells (Mir et al. 2022d). Progression is marked by acquiring additional genetic changes that lead to malignancy and metastasis. Progression encompasses a substantial growth in tumour size and either growth-related or mutually exclusive metastasis (Sherr 2000) Fig. 4.1.

4.3 Cancer: Cell Cycle Dysregulation

Cancer is being increasingly viewed as a malfunctioning cell cycle. It indicates that the most exhibiting cause of the tumorigenesis is the defective cell cycle machinery leading to unregulated cell proliferation. The main targets of the disease are either the components of the cell cycle itself or the upstream signalling events that ultimately trigger cell cycle events. Although the cancer development process suggests that every tumour is defective in one or more aspect of cell cycle control, but carcinogenesis implies that apart from inducing defects in cell cycle machinery. Cancer can be viewed as a stepwise process that eventually leads to a dysregulated cell cycle (Sherr and Roberts 2004). Human cancers have been linked to cell cycle dysregulation in the past two decades, supported by a vast body of literature (Malumbres and Barbacid 2001). Tumour cells acquire mutations that induce mitosis and create obstructions in responding to anti-mitogenic signalling that leads to abnormal proliferation (Malumbres and Barbacid 2001; Massagué 2004).

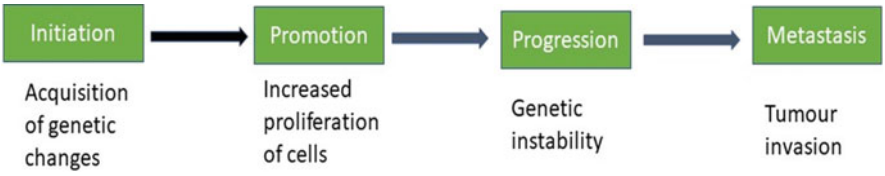

Fig. 4.1 Different stages of cancer development

Additionally, most tumours develop chromosomal instability (CIN), a malfunction that results in alterations to the number of chromosomes, and genomic instability (GIN), which causes additional mutations (Mir et al. 2022d; Kastan and Bartek 2004). Together, these changes lead to proliferative benefits as well as greater vulnerability to the accumulation of further genetic changes that aid in tumour development and the acquisition of more aggressive phenotypes. The three main cell cycle disorders that are either directly or indirectly brought on by insufficient cyclin-dependent kinase (CDK) regulation are unscheduled proliferation, GIN, and CIN (Kops et al. 2005).

4.4 Cell Cycle

The sequence of activities known as the cell cycle occurs when cellular components are duplicated and then properly divided into daughter cells. DNA replication in eukaryotes is restricted to a specific S-phase, also known as synthesis, and chromosomal segregation takes place during the M-phase of mitosis. S-phase and mitosis are separated by the two Gap phases, G1 and G2 (Malumbres and Barbacid 2005). Instead of being inactive, cells acquire mass during these times, as well as integrate growth signals, organize a replicated genome, and get ready for chromosome segregation. The cyclin-dependent kinases (CDKs) are the main enzymes that control how the cell cycle develops. These serine/threonine protein kinases phosphorylate important substrates to advance mitosis and boost DNA synthesis (Weinert and Hartwell 1988; Mehraj et al. 2021).

4.5 Cell Cycle Entry and Progression

The cells choose whether to start DNA replication and go through the cell cycle or to stay in the G1 phase, which is the pre-replicative phase, before going through the S-phase. During the G1 phase, cells can also enter the quiescent phase, also known as the G0 phase, which is a non-proliferative phase. Many of the cells in the adult body must enter the G1 phase in order to start DNA replication and the cell cycle (Pennycook and Barr 2020). Once DNA replication in S-phase is complete, cells can decide to enter M-phase by starting chromosomal condensation and central chromosome alliance. M-phase precisely separates the DNA which is duplicated (mitosis) and divides the whole cellular material into two new daughter cells. In M-phase, which also restarts the cell cycle so that interphase returns, cells commit to segregating the genetic material (Qayoom and Bhat 2020; Rubin et al. 2020).

4.6 Cell Cycle Checkpoints

Cell cycle checkpoints operate the cell cycle's integrity and appropriate advancement. Before moving on to the following phase of the cell cycle, these checkpoints ensure that the operations at each phase have been correctly completed. Cell cycle checkpoints are biochemical signalling pathways that can monitor and detect various kinds of structural DNA flaws or changes in how the DNA functions. They then trigger a cellular response that initiates DNA repair and slows the course of the cell cycle. Because the checkpoint pathways have not changed throughout time, checkpoint failure results in cancer cells continuing to develop (Nasmyth 1996). Checkpoint responses are an important factor in determining whether cells will survive or die. Seven checkpoints have been identified so far in the eukaryotic cell cycle: quiescent, G1/S, replicative S, and G2 checkpoints, the mitotic checkpoint, cytokinesis checkpoint, and the DNA damage checkpoints. Checkpoints remove those cells by causing permanent cell cycle arrest or cell death when DNA damage is irreparable (Mir et al. 2022d). Similar to this, cells fight off genotoxic stressors till the very end using a variety of strategies, including complex survival pathways between DNA synthesis and the divisional phase of the cell cycle, there are two gap periods (Pardee 1989). Eukaryotic cell cycle progression requires the coordinated activity of proteolytic enzymes and a number of kinase cascades (King et al. 1994, Malumbres and Barbacid 2005). Throughout the cell cycle, cyclins go through a continual cycle of synthesis and degradation, timely controlling kinase activity (Malumbres and Barbacid 2005). Three interphase CDKs (CDK2, CDK4, and CDK6), a mitotic CDK (CDK1, also known as cell division control protein 2 (CDC2), and ten cyclins from four different classes make up the CDK-cyclin that directly drives the cell cycle (the A-, B-, D-, and E-type cyclins) (Peng et al. 1998). The mammalian cyclins are broadly classified into A, B1, B2, C, D, E, H, T (Table 4.1). The cyclin box, a domain used to bind and activate Cdks, is a region of homology shared by all cyclins. However, not all cyclins and Cdks are involved in controlling the cell cycle. Apoptosis, DNA repair, differentiation, and transcription regulation are some of the additional roles that have been discovered (Roy et al. 1994; Rickert et al. 1996).

Table 4.1 Cyclins and cell cycle checkpoints

Cyclins	Associated CDKs	Function
Cyclin A	Cdk1, Cdk2	S-phase entry and G2/M transition
Cyclin B	Cdk1	Entry into mitosis and G2 exit
Cyclin C	Cdk8	Transcriptional regulation
Cyclin D (D1, D2, D3)	Cdk4, Cdk6	G0 to S-phase transition G1/S-phase transition, G2 to M-phase transition
Cyclin E	Cdk2	Entry into S-phase
Cyclin H	Cdk7	Transcriptional regulation, Cdk activation
Cyclin T	Cdk9	Transcriptional regulation

Checkpoints in the cell cycle let important cellular processes like DNA replication to stop. When complete cellular division might be harmful, such as in the presence of DNA damage, these checkpoints are used (Kim et al. 2005). Most DNA damage checkpoint signalling pathways culminate on the inactivation of either CDK1/cyclin or CDK2/cyclin complexes as the primary regulators of mammalian cellular progression (Richardson and Jasin 2000). The intra-S checkpoint in mammalian cells is crucial for stopping the advancement of the S-phase in the presence of DNA damage (Hartwell and Weinert 1989; Qayoom et al. 2021). The serine-threonine checkpoint kinases CHK1 and CHK2 are phosphorylated and activated upon the detection of a DSB by a variety of kinases, such as PI3 K's, ATM (Ataxia-Telangiectasia Mutated), and ATR (ATM and Rad3-related). CHK1 and CHK2 subsequently phosphorylate and stabilize TP53 (p53) (Sørensen and Syljuåsen 2012). Following p53 stabilization, the CDK inhibitory protein p21WAF1/CIP1 is transactivated by p53. Here, CDK2/cyclin E activity is efficiently suppressed by p21WAF1/CIP1, blocking the G1/S transition and the start of DNA synthesis (Nyberg et al. 2002; Shechter et al. 2004; Iyer and Rhind 2017; Chehab et al. 1999). One crucial step in maintaining the G1/S DNA damage checkpoint is the activation of p53 and p21WAF1/CIP1 through checkpoint-mediated activation, which inhibits CDK2. Loss of p53 or p21WAF1/CIP1 impairs the cellular response to DNA damage, and mice lacking these proteins are more prone to developing cancer (Chehab et al. 2000; Shieh et al. 2000; Bartek and Lukas 2003; Mehraj et al. 2022). By encouraging the degradation of CDC25 phosphatases, CHK1 and CHK2 can also have a secondary effect on CDK activity (Kastan et al. 1992; Gu et al. 1993; Harper et al. 1993; Mitra et al. 1999). The CDC25 phosphatases are strong CDK/cyclin complex activators that work in direct opposition to the WEE1/MYT1 phosphorylation-induced inhibition of the glycine-rich CDK inhibitory loop domain. These residues are threonine 14 (T14) and tyrosine 15 (Y15) in CDK1 and CDK2. Both CDK2/cyclin E and CDK1/cyclin B must have these residues dephosphorylated by the CDC25 dual-specificity phosphatases in order to fully activate their respective kinases (Donehower et al. 1992; Brugarolas et al. 1995; Mir and Mehraj 2019). Thus, DNA damage is a strong initiator of CDK inhibition that can be brought on by the stimulation of CDK inhibitory proteins as well as the destruction of CDK activators.

4.7 Regulation of Cyclin-CDK Complexes

Beyond the cell cycle, cyclins, Cdks, and CKIs can influence these cellular and developmental processes. Particular focus is placed on the possibility that kinase-dependent or -independent pathways may be used to carry out each of these procedures. Most cyclins enhance Cdk activity, but CKIs decrease it. CKIs are divided into two groups based on the structure and Cdk specificity of each group. The Ink4 family includes the genes p16INK4a, p15INK4b, p18INK4c, and p19INK4d. However, the Cip/Kip family members are more adaptable and characteristically prevent the actions of cyclin A-, B-, D-, and E-dependent kinase

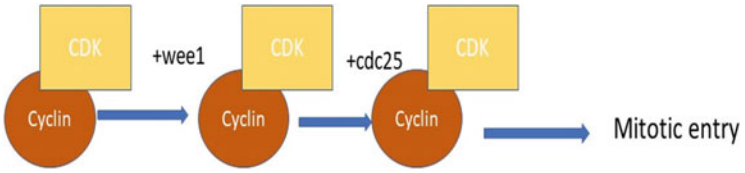

Fig. 4.2 Cell cycle regulation

complexes (Martín-Caballero et al. 2001). Based on sequence homology, more members have been added to the Cdk, cyclin, and CKI families., it has become evident that the original criteria used to classify the founding members are no longer applicable. For instance, it was originally believed that cyclins are solely Cdks' regulatory components, that Cdk/cyclin complexes are the only ones that CKIs can inhibit, and that Cdks and cyclins must interact for Cdks to become active. Despite this deviation from the usual cooperative behaviour, recent studies have amply shown the functions of separate subunits without complex formation, and as a result cyclins, cdks, and CKIs are now believed to have a diversity of cell cycle-independent functions in mammals. Cdk4 and Cdk6 are the primary targets of Cdkn2d (Mailand et al. 2000). Recent research has abundantly demonstrated the functions of individual subunits without complex formation. The Rb/E2F pathway, which is intimately tied to cell cycle control, is one of the most well-studied instances of how cell cycle regulators affect transcription (Mailand et al. 2002). Members of the E2F family of transcription factors are bound and sequestered by the retinoblastoma protein (Rb), p107 (Rbl1), and p130 (Rbl2) in the hypophosphorylated state (Busino et al. 2003). Cdk4/6 and Cdk2 are in charge of sequentially phosphorylating Rb, reducing its inhibition of E2F and enabling the activation of genes required for boosting S-phase entry and DNA synthesis. They do this in collaboration with their respective catalytic partners, D- and E-type cyclins (Fig. 4.2).

4.8 Activation by Phosphorylation

The protein kinase activity of Cyclin-cdk complexes depends on the phosphorylation state of CDK subunit. The activation is completed in two steps and involves binding of cyclins and subsequent phosphorylation by the CDK activating kinases (CAK). For efficient CAK phosphorylation, association of CDK with its cyclin subunit is required in human Cdc2 residue at 161 positions. This type of phosphorylation is activating in nature (Hoffmann et al. 1994; Sørensen et al. 2003; Jinno et al. 1994). Phosphorylation is enhanced by the binding of cyclins as it affects cyclin binding sites (Molinari et al. 2000). CDK activation is completed in two steps, first the binding of CDK2 with cyclin A brings a substantial conformational variation in the kinase activity and modulates the binding ability of ATP constituent of the substrate; second, the activation segment's threonine residue (Thr160 in the human CDK2

sequence) is phosphorylated by CAK to enhance protein substrate binding and align substrates for phosphoryl transfer (Malumbres et al. 2004; Malumbres and Barbacid 2009). In CDK7 phosphorylation occurs at activation site (threonine 170 in human sequence). But also has a second site of phosphorylation in the activation (Atherton-Fessler et al. 1993) segment (Ser 164) (Malumbres et al. 2009). When compared to the rest of CDKs, phosphorylation is not important for the CAK activity. CAKs actively phosphorylate CDKs that are bound to their relevant cyclins. They do not phosphorylate CDKs in monomeric form even if they do so they are phosphorylated very poorly. in monomeric state activation segment cannot be accessed by CAKs (Dyson 1998; Sherr and Roberts 1999).

4.9 CDK Inhibition by Phosphorylation

In contrast to the activation of CDK complexes by phosphorylation, cyclin-CDK complexes can also be inactivated by phosphorylation at the sites of inhibitory phosphorylation. In higher vertebrates the adjacent threonine residues at 14th position and Tyr at 15th position in CDC2 and CDK2 are the sites of inhibitory phosphorylation. The actual mechanism of inhibition is still not clear. Phosphorylation of CDK1 by wee1 at Thr 15 and Thr 14 is also inhibitory in nature that keeps kinase activity of CDk1 low and prevents cells from initiating mitosis until their size is adequate. During entry into M-phase the activity of wee1 is decreased by various regulators and hence activity of CDK1 is increased (Matsushime et al. 1992; Harbour and Dean 2000; Mir and Mehraj 2019).

4.9.1 CDK Inhibitors (CKI's)

Regulation of cyclin-CDK complexes is also contributed by CDK inhibitor proteins. These inhibitor proteins inhibit the kinase activity of CDKs by interfering with their binding with cyclins that is necessary for the activation of cyclin-CDK complexes. There are two types of CDK inhibitor proteins.

4.9.2 CDK Interacting Protein/Kinase Inhibitory Protein (CIP/KIP)

Family of CKIs are the negative regulators of G1 phase cell cycle progression [70]. CIP/KIP family includes P21, P27, P57 that inhibit a wide array of cyclin-CDK complexes. CIP/KIP proteins play many other important roles outside the nucleus. P27kip1 regulates actin dynamics and cell migration (Won et al. 1992). Another member of the family P21cip1 has an ability of inhibiting Rho-kinase (ROCK). P57kip2 regulates subcellular localization (Sherr and Roberts 1999). A cell cycle arrest occurs in G-1 phase in variety of cell types by forming complexes of cyclins D1-D3, CDK4 or CDK6 and cyclin E or cyclin A CDK2 (El-Deiry et al. 1993).

4.9.3 Inhibitors of Kinase (INK4)

INK-4 Family is another type of CKI's and include that contribute to cell cycle control in mammals. INK4 members include P15, P16, P18, and p19. These proteins inhibit the activity of CDK4 and CDK6 with D-type cyclins (Harper et al. 1993) (Fig. 4.3).

P16 has an important role in regulating the Rb. P16 is a tumour suppressor protein that plays a major role in slowing down the pace of Rb and hence deregulates the cell cycle. In human tumours P16 gene is mutated in a high proportion. Cells in which P16 is deleted, P15 also gets affected simultaneously. In such cells the levels of Rb do not influence P15 but in turn get incited by growth-inhibitory cytokine TGF-β (Polyak et al. 1994; Toyoshima and Hunter 1994; Tanaka et al. 2002) that binds to CDK4 and CDK6 and carries on the phosphorylation.

P18 and P19—regulate the activities of cyclin/CDK4 and cyclin/CDK6 complexes but exert no effect over cyclin E/CDK2. Cyclin A/CDK2 or cyclin B/CDK2. The net effect of the inhibition applied by P18 and P19 coordinates with inhibition of G1 phase progression in mammalian cells (Okamoto et al. 1994; Otterson et al. 1994; Koh et al. 1995). The inactivation of INK4 inhibitors or the overexpression of D-type cyclins, cdk4 and cdk6, are thought to be the causes of Rb′ s functional inactivation. Rb that has been hyperphosphorylated cannot bind to or inhibit E2F transcription factors, as was previously mentioned. The discovery that ectopic production of D-type cyclins in dormant cells increases the expression of at least some E2F-regulated genes supports this concept (Ouelle et al. 1995; Pomerantz et al. 1998; Zhang et al. 1998). Although E2F gene mutations in human malignancies have not yet been discovered, there is compelling circumstantial evidence that dysregulation of E2F transcriptional control is a critical step in carcinogenesis. In cell culture-based transformation tests, some E2F genes have been demonstrated to serve as oncogenes (Hunter and Pines 1994; Sherr 1996). Furthermore, it has recently been demonstrated that uncontrolled expression of E2F1 in a transgenic

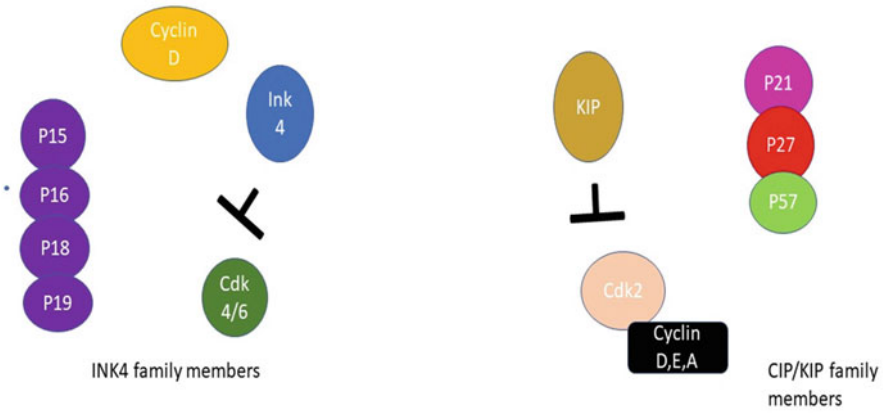

Fig. 4.3 Classes of CDK inhibitors

Table 4.2 Cyclin-dependent kinase inhibitors

CKI's	Different Types of CKI's	Role played
CIP/KIP	P21	Inhibition of Cdk2
	P27	Inhibition of CyclinE-Cdk2
	P57	Cdk4 and Cdk6
INK4	P15	
	P16	
	P18	
	P19	

mice model works in conjunction with either an active Ras gene or a p53 deficit to promote the growth of skin cancers (Reynisdóttir and Massagué 1997; Sangfelt et al. 1997) (Table 4.2).

4.10 Role of M-C

M-CDK commonly called as mitosis promoting factor or maturation promoting factor is the cyclin-CDK complex that is synthesized during the S and G-2 phase. M-CDK promotes the entry into mitosis (M-phase) and meiosis by causing phosphorylation of a wide variety of proteins. M-CDK activity is inhibited by wee1 protein kinase which phosphorylates a tyrosine residue at 15th position in the CDK subunit therefore inhibiting the premature entry of cells into mitosis. The inhibitory role played by wee1 is opposed by a protein phosphatase cdc25 that removes the inhibitory phosphate group and results in the activation of M-CDK and drives the G2/M transition. Yoshio Masui, a researcher in Toronto, identified MPF as a component that promotes egg maturation that involves the meiotic phase. After purifying MPF from the Xenopus frog, Jim Maller and Fred Lokha in Denver further refined Yoshio's cell free assays for monitoring MPF.

4.11 Role of APC/C Activators During Mitotic Division

APC/C is an E3 ubiquitin ligase that facilitates the metaphase to anaphase transition and exit from mitosis by targeting a set of regulatory proteins. APC/C activation requires association with two homologous activators cdc20 and cdh1 (cdc-homologue1). APC/C initiates metaphase-anaphase transition by mediating the degradation of anaphase inhibitor Pds1/securing ensuing separation of cohesion complex which holds the sister chromatids together. After anaphase, APC/CCdh1 mediates the final degradation of mitotic B-type cyclins and several other proteins (Motokura et al. 1991; Bodrug et al. 1994; Lovec et al. 1994; Wang et al. 1994; Morse et al. 1997) as the cell exits mitosis and enters G1. In S-phase and G2, the APC/C is inactive to allow accumulation of proteins required for building the mitotic spindle. APC/C mediated proteolysis of key regulatory proteins drives the cell from

G2 through M-phase into G1 (m. Accordingly, the APC/C is under a strict temporal control so these targets are destroyed in the correct order. APC/CCdc20 is controlled by at least four ways to achieve this. First, transient transcription from the S-phase through the G2 phase and proteolysis in the G1 phase both affect Cdc20 levels (Mir et al. 2022d). Once linked, Mad2p, a part of the spindle assembly checkpoint (SAC) pathway, inhibits APC/CCdc20 in G2 (Leach et al. 1993; Wölfel et al. 1995; Easton et al. 1998). Additionally, the Protein Kinase A (PKA) enzyme directly phosphorylates Cdc20 to block its function when the DNA damage checkpoint pathway is activated (Kamb 1998). The spindle checkpoint signal is silenced when bi-polar attachment of the chromosomes on the metaphase plate allowing securin (Pds1) ubiquitylation/destruction and anaphase to occur.

4.12 Spindle Assembly Checkpoint

Spindle assembly checkpoint ensures correct chromosomal alignment and microtubule attachment at the metaphasic plate. The spindle assembly checkpoint keeps track of the mitotic spindle's flaws and delays sister chromatid segregation until all flaws have been fixed (Mir et al. 2022d) (Fig. 4.4). APC/C is blocked by these spindle microtubules' improper kinetochore attachment, which sends out a negative signal: thereby Inhibiting the metaphase to anaphase transition as a result. The mitotic checkpoint pathway's best-studied components are Mad1, Mad2, Mad3, Bubr1, Bub3, and Mps1, which were first discovered in budding yeast (Lapointe

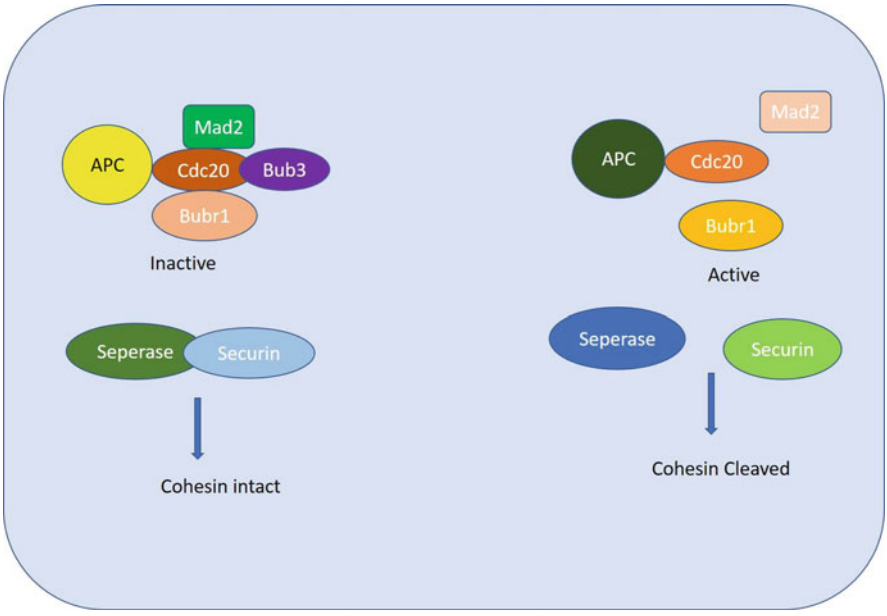

Fig. 4.4 Spindle assembly checkpoint signalling

Table 4.3 Spindle assembly checkpoint proteins and their functions

SAC proteins	Functions
Mad1	Inhibits the activity of APC/C and prevents anaphase onset before the spindle is built
Mad2	APC/C inhibitor
Bubr1	Inhibits Cdc-20-Apc activity
Bub3	Prevents early anaphase entry and mitotic exit

et al. 1996; Johnson 1995). The downstream target of the multi subunit machinery is APC/C complex that results in destruction of several proteins and mitotic cyclins (Shao and Robbins 1995). Mad2 is an essential APC/C inhibitor and prevents anaphase onset. Bubr1 works in harmony with Mad2 and inhibits cdc20-APC activity (Table 4.3). Only after the proper alignment of all the chromosomes at kinetochore correctly at the metaphasic plate spindle assembly checkpoint is finally turned off the localization of the Mad2 and Bubr1 to the kinetochore may be dependent on one or many proteins like Aurora B kinase (Mir et al. 2022b) (Fig. 4.4).

4.13 DNA Damage Checkpoints

Prevent the daughter cells from acquiring mutant DNA. A signal transduction mechanism set off by the damaged DNA prevents cell cycle advancement until the DNA is fully repaired. DNA double-strand breaks (DSBs) during interphase result in an immediate signalling response that is reliant on the checkpoint protein kinase mutant ataxia telangiectasia (ATM). The resultant alteration of ongoing transcription levels and patterns, activation of DNA repair machinery, and interaction with cell cycle regulators all result in a slowing or cessation of the cell cycle (Mir et al. 2022d). The primary mechanism for limiting the accumulation and spread of genetic errors during cell division is this biological response to DNA damage. Once activated by the DNA damage sensor complex MRN (MRE1, RAD50, and NBS1), ATM phosphorylates a wide range of substrates. The transcription factor p53 and the protein kinase CHK2 are significant targets for cell cycle regulation. The mutant checkpoint protein kinase ataxia telangiectasia is required for the fast-signalling response that DNA double-strand breaks (DSBs) during interphase cause (ATM). The response modifies ongoing transcription levels and patterns, activates DNA repair machinery, and interacts with cell cycle regulators, slowing or halting the advancement of the cell cycle (Hartwell and Weinert 1989). This biological response to DNA damage substantially prevents the accumulation and spread of genetic mistakes during cell division. Despite the fact that the protein kinase CHK2 and the transcription factor p53 are essential for cell cycle regulation, a variety of substrates are phosphorylated by ATM when the DNA damage sensor complex MRN (MRE1, RAD50, and NBS1) activates it. To prevent the commencement of the S-phase, P53 activates the CDK inhibitor p21, which significantly

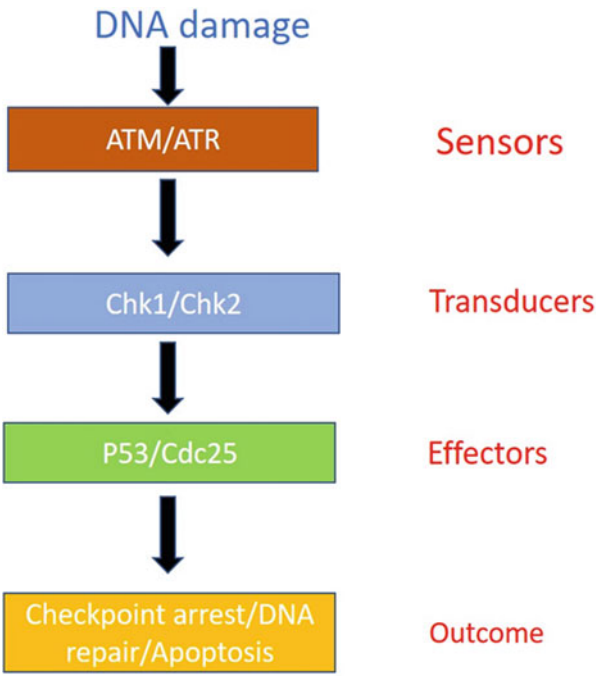

Fig. 4.5 DNA damage signalling cascade

inhibits cyclin-CDK complexes in G1 (Fig. 4.5). When CDC2551 is degraded during the S and G2 phases, CDK1 is phosphorylated under the direction of WEE1 to delay the onset of mitosis. P53 and ATM are not as crucial for slowing or stopping cell cycle progression during tumour growth because of some protein redundancy with other proteins. Despite the fact that the protein kinase CHK2 and the transcription factor p53 are essential for cell cycle regulation, a variety of substrates are phosphorylated by ATM when the DNA damage sensor complex MRN (MRE1, RAD50, and NBS1) activates it. To prevent the commencement of the S-phase, P53 activates the CDK inhibitor p21, which significantly inhibits cyclin-CDK complexes in G1. When CDC2551 is degraded during the S and G2 phases, CDK1 is phosphorylated under the direction of WEE1 to delay the onset of mitosis. P53 and ATM are not as crucial for slowing or stopping cell cycle progression during S and G2 stages because of some redundancy with other proteins. DNA end resection at DSBs is regulated by the cell cycle, which has an impact on the repair method of choice (Mir et al. 2022d). Because the concept of "severe" varies depending on the environment and the type of cell, judgments about a cell's fate are not uniform or always easy to predict. Apoptosis, permanent cell cycle stoppage, and senescence are the three events that cells can experience. The cell cycle arrest is either reversible (quiescence) or irreversible (apoptosis) if the cell does not go through this process during the pre-replicative G1 phase (senescence) (Hayles et al. 1994). Long-term arrest during the S or G2 phases, however, primarily results in cells permanently terminating the cell cycle through senescence or death. The

inability to re-enter the cell cycle is largely caused by P53-controlled mechanisms (Nurse and Bissett 1981; Lohka et al. 1988; Peters 1998). P53 activates the CDK inhibitor p21, which largely inhibits cyclin-CDK complexes in G1, to stop the onset of the S-phase. In the S and G2 phases, CHK2 degrades CDC2551, which promotes CDK1 phosphorylation under the control of WEE1 to prevent mitotic entry. P53 and ATM are less crucial for slowing or stopping cell cycle progression during the S and G2 phases due to some DNA replication checkpoint redundancy. DNA end resection at DSBs is regulated by the cell cycle, which has an impact on both the repair procedure and the DNA damage signalling cascade. The majority of DSB repair techniques employed during G1 focus on non-homologous end joining because DNA end resection is not occurring. However, repair through homologous recombination is made easier by the resection of DNA ends following DSBs during the S and G2 stages. The degree of the DNA damage determines how a cell will turn out because the concept of "severe" varies depending on the environment and the type of cell, judgments about a cell's fate are not uniform or always easy to predict. Processes for DSB repair are activated during G1 Phase of cell cycle. Apoptosis, permanent cell cycle stoppage, and senescence are the three events that cells can experience. The cell cycle arrest is either reversible (quiescence) or irreversible (senescence) if the cell does not undergo apoptosis during the pre-replicative G1 phase. In contrast, a cell is more likely to irreversibly exit the cell cycle through senescence or apoptosis when it is arrested for an extended period of time in the S or G2 phases. The inability to re-enter the cell cycle is largely caused by P53-controlled mechanisms (Crasta et al. 2006).

4.14 Therapeutic Agents

Targeting checkpoint controls to create novel therapeutic approaches for this disease offer a number of opportunities given that the breakdown of regular cell cycle regulation is a characteristic of cancer (Mir et al. 2022d). These techniques involve targeting medicines, arresting proliferating cells at specific stages of the cell cycle that may make them more susceptible to treatment with other therapeutic agents like radiation, and inducing checkpoint arrest that results in cytostasis and ultimately apoptosis towards particular cell cycle regulatory components. The process of causing DNA damage and thereafter apoptosis is one of the most well-known chemotherapy strategies. Cell cycle arrest can occur at both the G1/S and G2/M checkpoints in response to substances like cisplatin and nitrogen mustard, which cause DNA cross-links and chromosome breakage. Cyclin/cdk2 and cyclin/cdk4 complexes are inhibited in p21, and as a result Rb is hypo phosphorylated (Zhan et al. 1993; Guillot et al. 1997). Up-regulation of p21 also causes PCNA to be sequestered, which aids in G1/S arrest. DNA damage can trigger the G2/M checkpoint either through p53-dependent or independent mechanisms (Agarwal et al. 1995; Guillot et al. 1997). Phosphorylation of both cdk1 and p21 are necessary for entrance into M and can take part in the G2/M checkpoint for DNA damage because they are unable to stop and fix their damaged DNA, tumour cells with inactive p53

can circumvent the G1/S checkpoint and show increased sensitivity to DNA-damaging substances like cisplatin (Fan et al. 1997; Mir and Agrewala 2008). Taxol and vinca alkaloids, two microtubule inhibitors, interfere with normal tubulin polymerization/depolymerization and mitotic spindle formation (Schiff and Horwitz 1980; Gorbsky 1997). As a result, cells either start a p53-dependent arrest at the radiosensitive mitotic spindle assembly checkpoint (Schiff and Horwitz 1980) or proceed through M and become aneuploid and arrest in G1 (Andreassen et al. 1996; Cahill et al. 1998; Mir 2015). These medications cause G2/M arrest, which is accompanied by stability of the cyclin B/cdc2 complexes. Treatment of tumour cells with microtubule inhibitors may experience apoptosis after G1 or G2 arrest (Woods et al. 1995). Radiosensitizers made from microtubule inhibitors have also demonstrated efficacy in clinical settings. Combining chemotherapy and radiation therapy with taxol, a drug that prevents cells from completing the mitotic spindle assembly checkpoint can increase a tumour's sensitivity to radiation treatment (Liebmann et al. 1994; Chen et al. 1997). Radiosensitizers made from microtubule inhibitors have also demonstrated efficacy in clinical settings (Mir et al. 2022d). Combined Taxol chemotherapy/radiation therapy, prevents cells from dividing at the mitotic spindle assembly checkpoint, which can increase the sensitivity of cancers that are resistant to radiation treatment (Linke et al. 1996; Sofi et al. 2022).

4.15 Summary

The cell cycle represents a sequence of coordinated events that allow the cells to grow and divide. The cell cycle machinery is driven by the systemized action of cyclins and CDKs. The combined activity of these proteins drives the cell cycle progression. The fidelity of cell cycle is maintained by cell cycle checkpoints that operate as a surveillance mechanism and ensure the faithful replication and repair of genome. These checkpoints delay the cell cycle progression in response to irreparable DNA damage. The fidelity of this process is destroyed by mutations that prevent apoptosis and compromise cell cycle exit. These mutations disrupt the signalling pathways and their downstream counterparts CDKs and cyclins. CDK activity is the most targeted activity due to their major role in cell cycle progression, they are antiproliferative and arrest cells in G1 or G2/M phase and also trigger apoptosis. Cell cycle checkpoints which play a pivotal role in driving cell cycle need to be defective for a cell to become cancerous. Cancer cells continue to divide, despite the accumulation of genetic errors as DNA damage checkpoints are compromised in the cell cycle.

4.16 Further Readings

The readers can further read about the role of CDKs in breast cancer by going through the following papers

- https://doi.org/10.1080/13543784.2022.2097067
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8167670/

For more insights about the topic, we would suggest detailed findings from the books of (Mir MA, 2022) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2014-0-02898-5 (Mir MA, 2021) https://doi.org/10.52305/WXJL6770, from cancer.net website, https://www.cancer.net/cancer-types/breast-cancer/types-treatment. Also, the readers can have a look upon the following visual presentations for the better conceptual understanding of CDKs and their role in breast cancer

https://youtu.be/0Sj3rbJPeXQ
https://youtu.be/RXsWAvdWG0s
https://youtu.be/YA67P2k2d6A

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