Cell Cycle Arrest: An Impending Therapeutic Strategy to Curb Cancer

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Abstract

Eukaryotic cell division is divided into several phases and each of these phases has their own control mechanisms. Failure of any of these control mechanisms may lead to development of errors which may be propagated to up-coming generations leading to development of carcinogenic phenotype. Therefore, cell cycle has become an attractive target in anticancer research which is mainly focused on dealing with the regulators and checkpoints involved in the progression of cell cycle. The major components involved in controlling the cell cycle are cyclins, cyclin-dependent kinases (CDKs), and cyclin-dependent kinase inhibitors (CDKIs). Apart from these, an efficient DNA repair system and the proper assembly of spindle fibers also contribute to smooth progression of cell cycle. Therefore, in addition to the great dependency of anticancer research on cyclins, CDKs, and CDKIs, DNA repair system and assembly of spindle fiber

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also contribute to the foundation of anticancer research. In this chapter, we describe cell cycle and its importance in anticancer research, the clinical studies based on cell cycle to curb neoplastic development, and approaches used in anti-tumor research to counter cancer progression.

Keywords

Cell cycle · Cyclins · Checkpoints · Cancer · Anticancer therapy

3.1 Introduction

The cell cycle is a coordinated sequence of events that deals with duplication of genomic material and subsequent distribution of duplicated genetic material leading to the division of cells [1]. In the case of eukaryotes, the cell cycle has been categorized into several phases including Gap 1 (G1) phase, DNA synthesis (S) phase, Gap 2 (G2) phase, and Mitosis (M) phase. In first three phases, a cell prepares itself for division, and in M phase, segregation of chromosomes occurs followed by division of cells [2]. The M phase is progressed by initiation of prophase where nuclear envelop is disappeared and chromosomes become visible as chromatids. Prophase is followed by the alignment of chromosomes in metaphase, segregation of sister chromatids in anaphase, and subsequent movement of chromosomes at opposite poles in telophase followed by the division of genetic material leading to next interphase which is characterized by G1, S, and G2 phases as shown in Fig. 3.1 [3, 4]. The interphase is although a resting phase, but prepares a cell for the actual M phase, since a cell performs a normal metabolic role in interphase to duplicate its genetic material in S phase followed by DNA proof-reading, and preparation of M phase by the end of G2 phase. Additionally, G0 phase is a part of cell cycle in which cells are quiescent but have the potential of division under proper stimulus. Strict regulation of all the events in cell cycle is important for duplication of genetic material with high fidelity and its transfer in next generation with great accuracy since, even subtle errors in the cell cycle may lead to the fatal outcomes that may manifest in the development of complex diseases such as cancer. This chapter aims to provide a glimpse of the cell cycle and its crucial component with emphasis on the regulation of cell cycle in development as well as prevention of cancer.

3.2 Regulation of Cell Cycle by Interacting Partners

Several regulatory components are involved in the hassle-free progression of the cell cycle. These components work in a fashionable manner. Cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CDKIs) are the key components involved in

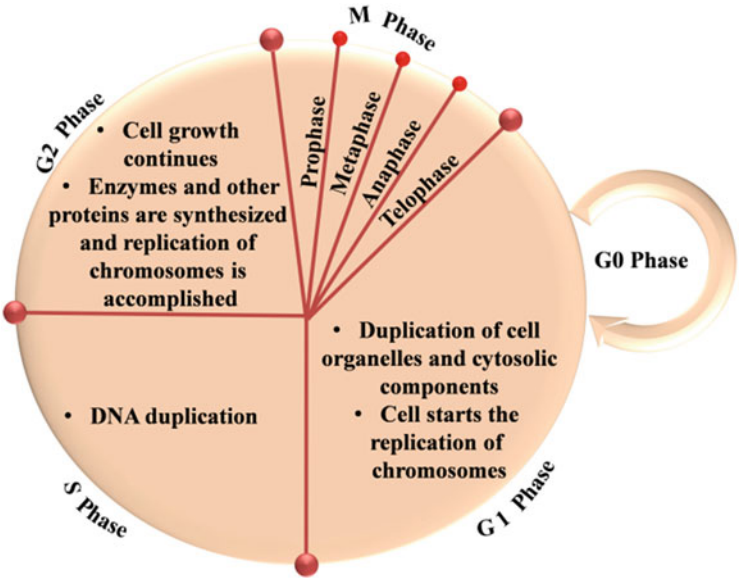

Fig. 3.1 Different phases of the cell cycle. Cell cycle comprises G1 phase, S phase, G2 phase, and M phase. Duplication of genetic material and cell organelles to assist in remaining cell cycle phases starts in G1 phase. S phase is represented by actual duplication of genetic material while in G2 phase, a cell continues to grow by completing its genetic content. M phase is demonstrated by actual segregation of chromosomes followed by division of cells. In G0 phase, cells undergo quiescence and may participate in division under the effect of proper signal

Table 3.1 The functional role of CDKs and cyclins in different phases of cell cycle (adapted and modified from Bai et al. [5])

CDKs	Cyclins	Cell cycle phase
CDK1	Cyclin A	G2/M transition
CDK1	Cyclin B	M
CDK2	Cyclin A	S
CDK2	Cyclin E	G1/S transition
CDK4	Cyclin D1, D2, and D3	G1
CDK6	Cyclin D1, D2, and D3	G1

regulating the cell cycle which perform in a coordinated manner to ensure proper progression of the cell cycle. The following few sections are briefly focused on the description of each of these regulatory components. Additionally, different interacting partners involved in progression of cell cycle are given in Table 3.1 below.

Cyclins

Cyclins are proteins known to regulate the progression of the cell cycle by their ability to complex with appropriate CDK partners. The expression of a particular

cyclin occurs in a particular phase of cell cycle, therefore, there is a sequential change in the expression pattern of cyclins which is dependent on specific cell progression phase.

Of the two types of cyclins, including cell-cycle related cyclins, viz. Cyclin A, B, D, and E, and non-cell cycle-related cyclins, viz. Cyclin C and H, cell-cycle related cyclins such as cyclin D and E play a pivotal role in G1 to S phase transition of the cell cycle [6]. Similarly, cyclin A forms the complex with CDK1 and CDK2 and plays a key role in S and M phase transition. The accumulation of cyclin A starts during the S phase and is down-regulated before commencement of M phase [7]. Similarly, cyclin B regulates the M phase and is required for a cell to enter and proceed through M phase. Therefore, cyclic change in the levels of cyclins is necessary in cell cycle progression.

Cyclin-Dependent Kinases (CDKs)

CDKs are about 300 amino acid proteins that contain binding motifs favoring the binding of appropriate cyclins. On binding to cyclins as their preferred binding partners, CDKs become catalytically active [8, 9]. Unlike cyclins, the expression of CDKs remains constant throughout the cell cycle, and several members of CDK family switch their association with cyclins, and their functional activities vary in accordance with a particular cell cycle phase. Notably, four different CDKs, namely, CDK 1, 2, 3, and 4 are responsible for governing the progression of the cell cycle [10]. In this way, at the G1/S phase transition, CDK4/6 and CDK 2 are required to make the cells to enter in S phase. CDK2 remains active throughout the S phase, and its activity declines after the cell exits S phase [9]. Similarly, CDK 1 is active during the G2 phase with persistent activity during mitosis [6]. CDK 1 associates with cyclin A and B, and acts on the interface of the G2/M phase. The accumulation of cyclin A and B and their degradation at the initiation of anaphase leads the cells to enter and exit mitosis, respectively. Therefore, periodic changes in the activities of CDKs are required for transition in phases of the cell cycle.

CDK Inhibitors (CDKIs)

CDKIs are up-regulated in response to a variety of anti-proliferative signals. CDKIs are known to regulate the activity and functions of CDK family members [11]. CDKIs are majorly categorized in two families, namely, CIP/KIP family of universal cyclin/CDK inhibitors, and INK4 family. The members of CIP/KIP family include p21 $^{Waf1/Cip1}$, p27 ^{Kip21}, and p57 ^{Kip2} proteins, and are known to bind and inhibit both cyclins, through their conserved LFG residues present in their cyclin box motif, and CDKs concurrently [12]. On the other hand, the members of INK4 family including p16 ^{INK4a}, p15 ^{INK4b}, p18 ^{INK4c}, and p19 ^{INK4d}, specifically bind and inhibit cyclin D, CDK4, and CDK6 (Fig. 3.2) [13].

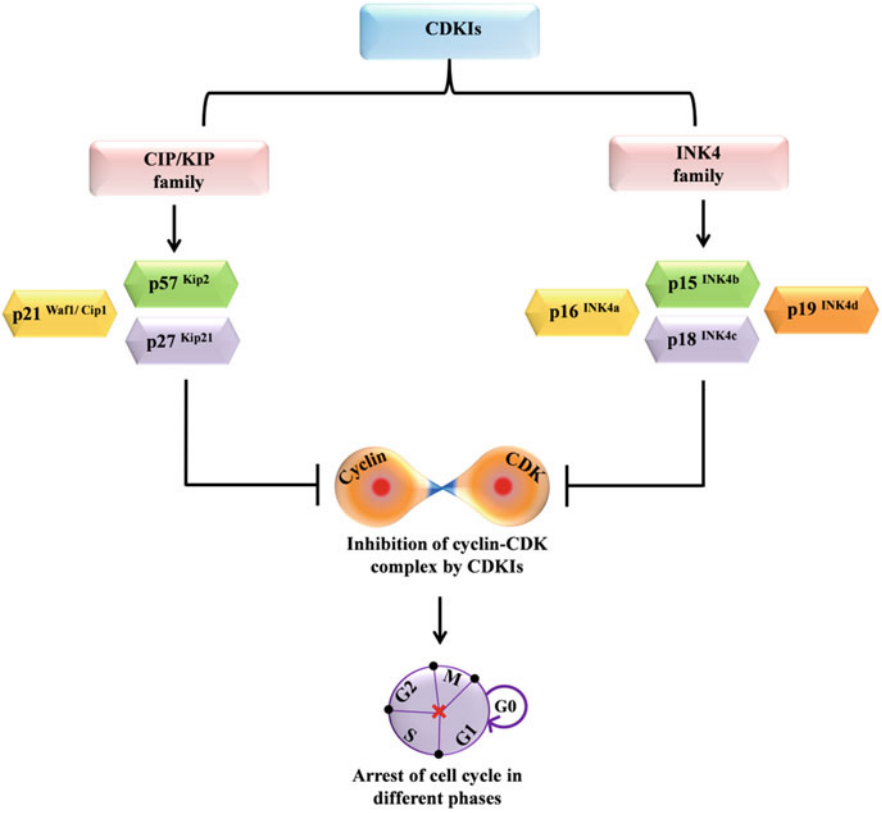

Fig. 3.2 Different families of CDKIs controlling the cell cycle. CDKIs of CIP/KIP family include p21 $^{Waf1/Cip1}$, p27 ^{Kip21}, and p57 ^{Kip2}, while CDKIs of INK4 family include p16 ^{INK4a}, p15 ^{INK4b}, p18 ^{INK4c}, and p19 ^{INK4d}. The members of both CDKI families work in a coordinated manner so as to inhibit the progression of cell cycle under certain circumstances

It is noteworthy that the relative concentration and distribution of the members of these two families determine the progression of the cell cycle. For instance, p21 plays a significant role in the inhibition of CDK kinase activity and inhibits the replication of DNA. Additionally, it is also known to arrest the cell cycle in G 1 phase so as to allow a cell to repair its DNA damage; which is seen when p53 is up-regulated (Fig. 3.3) [14]. Therefore, CDKIs act as a surveillance system to regulate the faithful progression of the cell cycle.

3.3 Cell Cycle Checkpoints

The status of the cell cycle progression from one phase to next is ensured by chronological activation as well as inactivation of a plethora of *regulatory gates* which are known as cell cycle checkpoints. These checkpoints monitor the status of

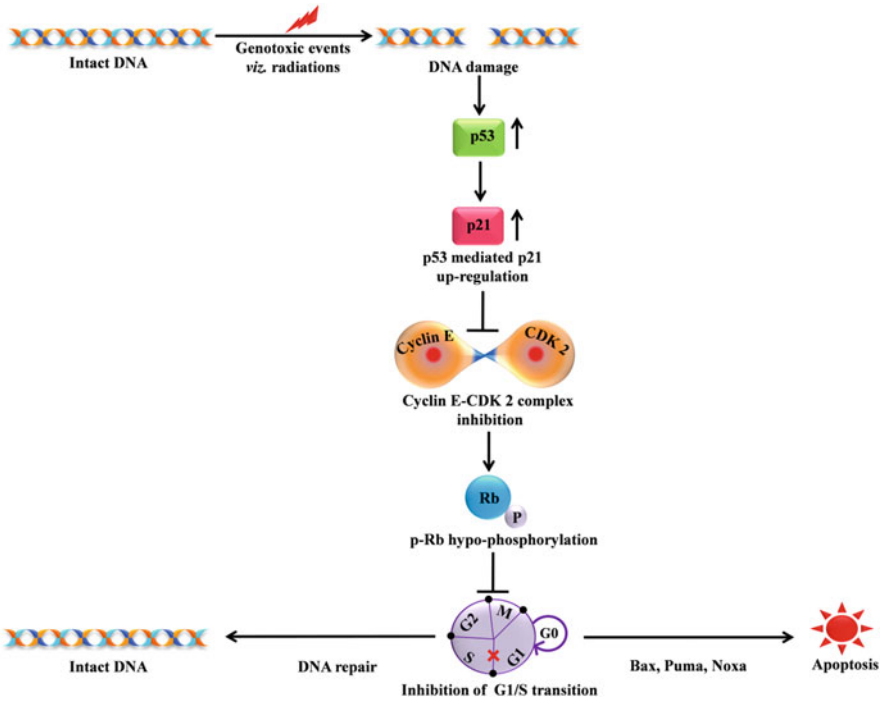

Fig. 3.3 Regulation of cell cycle under genotoxic stress. When DNA is damaged, p53 dependent up-regulation of p21 leads to inhibition of cyclin E-CDK2 complex resulting in hypophosphorylation of Rb protein which is accomplished by inhibition of cell cycle, DNA repair, and apoptosis

dividing and non-dividing cells [15]. Functionally, checkpoints are subsets of gene products that function in a sequential and controlled manner to ensure the fidelity in the cell cycle progression. If any of these checkpoints are mutated or altered, they confer independence in the cell cycle progression; which was otherwise dependent on successful completion of on-going cellular progression. Cells can arrest the progression of the cell cycle transiently so as to overcome the stress, viz. DNA damage. Otherwise, if the stress is irreversible, then checkpoints can direct a cell to programmed cell death. Alteration in the reliability of checkpoints can manifest with an expansion of DNA damage and permanent genetic lesions over several generations. It is noteworthy that cell cycle checkpoints are often hampered in cancerous cells resulting in the propagation of tumorigenic growth [16]. Hence, a cell has to pass through a huge number of internal checkpoints to ensure proper forwarding of genetic information to daughter generation [3, 17, 18]. The following few sections are focused on the type of cell cycle checkpoints and their importance in cancer.

G1/S Checkpoint

The inhibition of G1 phase cyclin and CDK complexes plays a significant role in maintaining the G1/S checkpoint [19]. As discussed earlier, CDKs can be negatively regulated by CDKIs. Among CDKIs, the members of the INK4 family are known to inhibit CDK4 and CDK6 during the G1 phase, while the members of CIP/KIP family can inhibit the activity of CDKs in all phases of the cell cycle (Fig. 3.2), thereby firmly maintaining the G1/S checkpoint. Furthermore, when a normal cell faces the genotoxic insult, transcription of p21, an important member of the CDKI family is up-regulated by p53 protein. Subsequently, p21 binds and inactivates cyclin E-CDK2 complex leading to hypophosphorylation of pRB followed by arresting the cell cycle from G1/S transition, allowing a cell to repair DNA damage, accumulate apoptotic factors such as Puma, Bax, Noxa, and up-regulate oxidative stress response as shown in Fig. 3.3. Additionally, p16 arrests the cell cycle in the G1 phase in p53 independent manner in response to DNA damage by abrogating cyclin D/CDK4 and cyclin D/CDK6 dependent pRB phosphorylation [20, 21]. Therefore, G1/S checkpoint acts by targeting two important tumor suppressor pathways which are often deregulated in a variety of human cancers.

S Phase Checkpoint

The S phase checkpoint, also known as intra-S phase checkpoint, operates to avoid the duplication of damaged DNA to transfer in mitosis further. This checkpoint is regulated by two different signaling pathways which include ATM/ATR-Chk1-Cdc25A and ATM-Nbs1-SMC1 [22]. DNA damage induced by ionizing radiations of UV radiations may provoke either of these pathways to arrest the cell cycle in the S phase. ATM or ATR results in phosphorylation of Chk1 that in turn phosphorylates Cdc25 A on serine residues maintaining the required concentration of Cdc25 A. The augmented functional activity of Chk1 and Chk2 leads to Cdc25 A down-regulation resulting in subsequent inhibition and inactivation of Cdk2-cyclin E complex in response to genotoxic insult [23]. ATM-mediated phosphorylation of Nbs1 on Ser 343 residue and some other residues results in activation of Nbs1-Mre11-Rad50 complex which is involved in S phase arrest [24, 25]. Similarly, cohesin protein SMC1 is also phosphorylated by ATM on Ser 957 and Ser 966 depending on the phosphorylation status of Nbs1, which is essential in S phase arrest of the cell cycle. Several other components including BRCA1, FANCD2, MDC1, and p53 BP1 are also involved in intra-S checkpoint [22, 26].

G2 Phase Checkpoint

If a cell feels genotoxic stress, then the cell can trigger a checkpoint mechanism arresting the cell cycle in G2 phase. For instance, ATM (ataxia-telangiectasia mutated)- and ATR (ATM and Rad3-related)-dependent signaling can arrest the

cell cycle in G2 phase by inhibiting CDK1 as a consequence of DNA damage. If a cell is exposed to ionizing radiations, ATM-dependent checkpoint kinase 2 (Chk2) activation can be seen. Whereas if a cell is exposed to ultraviolet radiation insult, ATR dependent Chk1 activation is prevalent [27]. Chk1 and Chk2 are known to phosphorylate Cdc25 C, thus generate a docking site for 14-3-3 proteins which leads to nuclear export and cytoplasmic sequestration of phosphatases followed by inhibition of CDK1 resulting in G2 phase arrest of the cell cycle [27].

Previously, studies have revealed that sustained G2 arrest can be mediated by p53 as a consequence of DNA damage in cancerous cells [28, 29]. p53 leads to transcriptional up-regulation of 14-3-3σ and p21 thereby inhibits G2 progression as a consequence of cytoplasmic sequestration and thus inactivating CDK1-cyclin B complex, respectively [29–32]. Additionally, once accumulated, p21 may cause the arrest of the cell cycle in G2 phase (Fig. 3.3) by disturbing the interaction of proliferating cell nuclear antigen and Cdc25 C [33].

Mitotic Spindle Checkpoint

The attachment of microtubules and chromosomes is under the strict control of mitotic spindle fiber checkpoint. This checkpoint monitors the accurate segregation of chromosomes during anaphase. Kinetochore associated proteins including MAD2, BUBR1, BUB1, BUB3 proteins are key components of mitotic spindle checkpoints [34]. Out of these, MAD2 and BUB are known to directly interact and inhibit APC machinery preventing the entry of cells in anaphase in case of mitotic spindle fiber dysfunction. Similarly, BUB1 and BUB3 also contribute to mitotic arrest in case of spindle dysfunction [34].

3.4 Dysregulation in Checkpoint Leading to Cancer

Cancer is the second leading cause of death in developed countries including United States [35, 36]. Abnormal cell proliferation due to the loss of cell cycle checkpoints is a key hallmark of cancer and also crucial for cancer progression [37–39]. Indeed, modulation in the machinery of cell cycle progression occurs in a variety of cancers. A healthy cell considers such modulations as a genetic insult which results in dysregulation of tumor suppressor genes which are considered as a suitable target for the implication of anticancer regimens [40]. For instance, regulation of cell cycle progression by tumor suppressor Rb protein plays a central role in curbing tumor development since oncogenic modulation in cyclins, CDKs, and other regulators of pRB is prevalent in a plethora of human cancers, viz. retinoblastoma, osteosarcoma, and many other cancers [41]. In cancers where pRB protein encoding is normal, even a subtle alteration in the alteration in signaling pathways regulating pRb can be frequently observed with augmented levels of cyclin D and cyclin E, deletion of p 16, and enhanced amplification of genes encoding CDK4 and CDK6 [41]. It is noteworthy that nearly half of the metastatic breast cancers are manifested with

increased expression of cyclin D as compared to normal breast epithelium in the vicinity [42]. In support of this, previously it has been speculated that transgenic mice overexpressing either human cyclin D1 or cyclin E in breast cells are more prone to develop breast adenocarcinomas [43, 44]. Likewise, sarcomas, melanomas, gliomas, and breast cancer have also shown amplification in CDK4/6 encoding genes [45]. Therefore, cell cycle dysregulation as a consequence of an alteration in cell cycle machinery is a major phenomenon detected in various cancer types.

Alteration In Cellular Checkpoint Proteins

The molecular events of checkpoint proteins play a crucial role in cell cycle regulation and these checkpoints altered during cancer progression [46]. Gene encoding cell cycle checkpoint proteins may undergo several genetic alterations leading to the development of cancer. For instance, mutations in p53 are one of the most often reported genetic alterations in human cancers [21]. Germline mutations in p53 are responsible for Li–Fraumeni syndrome which is manifested with provoked incidences for the development of breast cancer, brain tumors, and sarcomas [47]. The normal function of p53 may be altered by several cellular proteins such as Mdm2. This protein binds with p53 and leads to ubiquitin-mediated proteasomal degradation. Additionally, overexpression of Mdm2 may result in subsequent inactivation of p53 [48, 49]. Similarly, CDK1 modifications are also very often in human tumors. Apart from this, lower expression levels of p27 are found in aggressive breast cancers [50, 51], which may be more susceptible to oncogene-dependent transformation [52]. Similarly, lower expression levels of p27 are found in human bladder cancer [53]. Furthermore, either deletion or epigenetic modification, viz. methylation of p15 and p16 is related to human melanomas, lymphomas, and many other cancers [45]. Similarly, lower expression levels of p57 are associated with human bladder cancers [53] and epigenetic modification, viz. methylation of p15 and p16 or their deletion is linked with human mesotheliomas, melanomas, lymphomas, and pancreatic cancers [45].

Alteration in Spindle Fiber Checkpoint

The development of a plethora of human cancers is also linked to modulation in spindle checkpoints. For example, mutations in BUB1 have been identified and linked with the development of human colon cancer [54] which promotes the tumorigenic transformation of cells lacking BRCA2 breast cancer susceptibility gene [55]. Previously it has also been reported that MAD2 haploinsufficiency results in premature anaphase and chromosome instability in mammalian cells, resulting in increased incidences of lung cancer development [56]. Hence, alteration in either of the spindle fiber checkpoint components may manifest in the development of cancerous growth.

Alteration in DNA Repair System

Mutations in the components of the DNA repair pathway may also lead to the development of tumors due to sustained DNA damage. For instance, in ataxia-telangiectasia, a familial disease, ATM mutations are manifested with increased chances of lymphomas, breast cancers, and leukemias [57].

3.5 Therapeutic Approaches to Curb Cell Cycle in Cancer

It is clear that even subtle alterations in the cell cycle result in the development of a plethora of human cancers. Moreover, pieces of evidence have also supported the fact that cells with defective checkpoint functions are more prone to develop cancer. Fortunately, it also provides the opportunity to the scientific community to develop effective therapeutic regimens against carcinogenesis. Hence, the research is always focused on the development of alternative approaches to deal with cancer. The efforts against cancer are focused on the identification of novel, efficient, and potent drug molecules which have potential to target cell cycle checkpoints by considering (1) the use of high-throughput screening of anticancer lead molecules (2) the use of structure-based rational drug designing strategies for the development of small molecules against cancer, and (3) the use of genetics, proteomics, and metabolomics to identify potent anticancer therapeutics. The following few sections are focused on such approaches in a battle against cancer.

Screening of Novel Anticancer Molecules

Strategies involving the search for novel molecules have been employed to identify anticancer compounds against cancer. Previously, the National Cancer Institute (NCI) examined the inhibitory activity of about 70,000 small molecules against 60 different cells of human cancer origin [58]. Similarly, a group of authors also used NCI cell lines to examine the transcriptional levels of genes involved in cell cycle arrest and correlated the outcomes with standard anticancer chemotherapeutics [59]. Previously, it has been seen that the p53 status of cells is a crucial determinant of chemosensitivity since cells with mutant p53 are less responsive towards chemotherapeutic agents as compared to wild type cells [60]. Similarly, cDNA microarray studies have also been used earlier to examine the gene expression status of cell lines responding to the treatment with chemotherapeutic agents. Such evidence provide a valuable and definitive link between chemosensitivity and gene expression [61].

Apart from this, high-throughput screening has also been implemented in order to identify potent small molecules against cell cycle checkpoint components. For instance, breast cancer cells expressing mutant p53 were used in one of such studies where the G2 phase arrest of the cell cycle was induced by radiations. The cells were then co-treated with nocodazole, a microtubule inhibitor, and extracts from marine invertebrates. Consequently, isogranulatimide was identified as a novel inhibitor of

the G2 phase working in synergism with ionizing radiations [62]. Similarly, eight novel molecules with potent anti-mitotic efficacy were identified from 24,000 extracts from marine invertebrates and plants [63].

Genomic Approaches

Genetic approaches to counter cancer primarily depend on (1) conservation of cellular checkpoint pathways and (2) ease of manipulation in the genome of the organism under investigation. Therefore, *Saccharomyces cerevisiae* provides an excellent choice to be considered as a system to encounter against cancer [64]. Previously, anticancer drugs were screened on several strains of *S. cerevisiae* containing known mutations in cellular checkpoint pathways Notably, the toxicity profiles of ionizing radiations and chemopreventive therapeutic regimens were different from one another in several strains with defined mutations indicating the importance of particular mutation in cell cycle checkpoint and DNA repair pathways and thus giving a clue for deciding the therapeutic regimen [65]. Similarly, to identify selective peptide inhibitors and to identify novel cellular therapeutic candidates for anticancer drugs, *Schizosaccharomyces pombe* has also been used [66]. Additionally, the benefits can be taken from yeast genome which can be combined with cDNA microarrays to examine the changes in expression patterns of genes involved in cell cycle checkpoints after treatment with anticancer therapeutics [67]. Indeed, this approach has been used to generate a database of several cell cycle mutants of *S. cerevisiae* to screen novel anticancer molecules and ionizing radiations [68] and fortunately, the analysis of their profiles has demonstrated novel candidates in cell cycle regulatory pathways.

Chemical Approaches

Since the activity of cell cycle components such as CDKs is often deregulated in cancer, inhibitors of CDKs may be effective anticancer agents. For instance, Flavopiridol arrests the cell cycle in G1/S and G2/M phases by acting as CDKI and inhibiting CDK1, 2, and 4. Flavopiridol also acts synergistically with other anticancer drugs and has potent anticancer efficacy in human cancer cells and several in vivo xenograft tumor studies with mice [69]. Additionally, a number of phase 1 studies and phase 2 studies conducted on subjects with lung, renal, colorectal, and esophageal cancers have demonstrated the anticancer potential of Flavopiridol. Furthermore, several anticancer studies with breast and prostate cancer and non-Hodgkin's lymphoma are in process with Flavopiridol [45]. Furthermore, chemopreventive potency of several agents such as ionizing radiations can be enhanced by therapeutic agents such as caffeine or pentoxifylline which disturb G2 checkpoints [70, 71]. Similarly, UCN01 has also demonstrated anticancer activities against a variety of in vitro and in vivo cancer models by acting as a potent inhibitor of several kinases including Akt, protein kinase C, CDKs, and PDK 1. The

anticancer properties of UCN01 involve a variety of cellular pathways including prevention of nucleotide excision DNA repair, inhibition of G2 checkpoint kinase Chk1 thereby arresting the cells in G1/S phase followed by apoptosis [72–77]. Similarly, histone deacetylase inhibitors including FR901228 and MS27275 have shown promising anticancer activity in vitro [78], in vivo [79], and in clinical studies [80]. Therefore, a huge number of plant derived active pharmaceutical ingredients such as curcumin, quercetin, isothiocyanates, gambogic acid, carnosol, and many others are involved in cancer chemoprevention by targeting cell cycle as a preferable anticancer therapy [81–85].

3.6 Experiences from Clinical Studies

From the above discussion it is clear that arresting the cell cycle can be an impending strategy to curb the progression of cancer. Moreover, several clinical studies have also supported a positive correlation between cell cycle arrest and cancer prevention. Inhibition of CDK4/6, aurora kinase, Wee1 kinase, spindle proteins, viz. Kinesin, and microtubules have been seen as some of potent therapies against cancer in a variety of clinical studies [5, 86]. Recently, Mills et al. [87] have reviewed a number of clinical studies justifying the involvement of cell cycle arrest as a potent therapeutic anticancer strategy [87]. Furthermore, some of the completed clinical studies are enlisted below in Table 3.2.

3.7 Conclusion and Future Perspectives

For sustained development of novel and effective anticancer therapeutics, it is necessary that therapeutic agents must have the ability to identify the molecular differences between healthy and cancerous cells. Thereafter, therapeutic agents should selectively target tumor cells keeping the healthy cells intact and alive. Hence, the cytotoxic efficacy of such agents should be at par or well enough to affect cancer cells only. Unfortunately, with partial success in hand, the desired treatment of cancer is not possible. This is further aided by a poor prognosis of cancers in initial stages. However, mechanism-based approaches such as the use of proteomics and genomics have provided enormous opportunities to the scientific community and clinicians, to come up with effective treatment regimens against cancer. Although to fulfill the lacunae in existing treatment approaches, there is a consistent need to develop technologies with enough potential to identify the cell cycle checkpoint components with extreme precision. Additionally, advanced drug-delivery strategies, for instance, nano-encapsulation, may also aid up in present-day treatment approaches giving more effective therapeutic outcomes against cancer. The scientific community should also focus on exploiting the novel, in-depth, and mechanistic approaches to meet the need for early diagnosis and effective anticancer treatment.

Table 3.2 Different clinical trials considering cell cycle arrest as a potent anticancer therapy (Data obtained from: www.clinicaltrials.gov)

Sr. no.	Trial Id	Type of cancer/study involved	Drug molecule under test	Phase	Status	Sponsor name	No of subjects enrolled
1	NCT 00141297	Neoplasms, lymphoma, non-Hodgkin	PD-0332991	I	Completed	Pfizer	74
2	NCT 00840190	Solid tumors, hematologic malignancy	P1446A-05	I	Completed	Piramal Enterprises Limited	29
3	NCT 00407498	Neoplasm	P276-00	I	Completed	Piramal Enterprises Limited	50
4	NCT 00772876	Advanced refractory malignancies	P1446A-05	I	Completed	Piramal Enterprises Limited	39
5	NCT 00292864	Solid tumors	SNS-032 injection	I	Completed	Sunesis Pharmaceuticals	25
6	NCT 00446342	B-lymphoid malignancies, chronic lymphocytic leukemia, mantle cell lymphoma, multiple myeloma	SNS-032 injection	I	Completed	Sunesis Pharmaceuticals	21
7	NCT 01335256	Neoplasms	BAY1000394	I	Completed	Bayer	10
8	NCT 02540876	Metastatic malignant neoplasm, solid neoplasm, unresectable malignant neoplasm	Ilorasertib	I	Completed	University of Chicago, National Cancer Institute	12
9	NCT 00899054	Squamous cell carcinoma of head and neck	P276-00, radiation: External beam radiotherapy (EBRT)	I/II	Completed	Piramal Enterprises Limited	23
10	NCT 01291017	Non-small cell lung cancer	PD0332991	II	Completed	University of Florida	19
11	NCT 01096342	Refractory multiple myeloma	Dinaciclib	II	Completed	National Cancer Institute	16

(continued)

Table 3.2 (continued)

Sr. no.	Trial Id	Type of cancer/study involved	Drug molecule under test	Phase	Status	Sponsor name	No of subjects enrolled
12	NCT 01624441	Estrogen receptor negative HER2/Neu negative male breast carcinoma, progesterone receptor negative recurrent breast carcinoma, stage IV breast cancer AJCC v6 and v7, triple-negative breast carcinoma	Dinaciclib, Epirubicin hydrochloride	I	Completed	National Cancer Institute	40
13	NCT 01684215	Neoplasms, breast neoplasms	PD-0332991, Letrozole	II	Completed	Pfizer	61
14	NCT 02457351	Medical oncology	BAY 1000394, Itraconazole (Sporanox)	I	Completed	Bayer	14
15	NCT 01711528	Recurrent plasma cell myeloma	Bortezomib, Dexamethasone, Dinaciclib	I	Completed	National Cancer Institute	41
16	NCT 02047890	Neoplasms	BAY 1000394 BAY 1000394	I	Completed	Bayer	12
17	NCT 00824343	Squamous cell carcinoma of head and neck	P276-00	II	Completed	Piramal Enterprises Limited	86
18	NCT 00871910	Solid Tumors, lymphoma, non-Hodgkin, multiple myeloma	SCH 727965, Aprepitant, Ondansetron, Dexamethasone	I	Completed	Merck Sharp & Dohme Corp.	81
19	NCT 00871663	Solid tumors, lymphoma, non-Hodgkin, multiple myeloma, leukemia, lymphocytic chronic. B-cell	SCH 727965	I	Completed	Merck Sharp & Dohme Corp.	123
20	NCT 02441946	Breast cancer, hormone receptor positive tumor, early-stage breast carcinoma	Abemaciclib, Loperamide, Anastrozole	II	Completed	Eli Lilly and Company	224

21	NCT 01652144	Mantle cell lymphoma	AT7519M	II	Completed	NCIC Clinical Trials Group, Astex Pharmaceuticals, Inc., Canadian Cancer Trials Group	12
22	NCT 01627054	Chronic lymphocytic leukemia	AT7519M	II	Completed	NCIC Clinical Trials Group, Astex Pharmaceuticals, Inc., Canadian Cancer Trials Group	7
23	NCT 01188252	Neoplasms	BAY 1000394	I	Completed	Bayer	112
24	NCT 01515176	Chronic lymphocytic leukemia, prolymphocytic leukemia, recurrent small lymphocytic lymphoma, refractory chronic lymphocytic leukemia	Dinaciclib, biological: Ofatumumab	I/II	Completed	National Cancer Institute	36
25	NCT 01546038	Acute myeloid leukemia	PF-04449913, low dose ARA-C (LDAC), Decitabine, Daunorubicin, Cytarabine	II	Completed	Pfizer	255

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