Research & Reviews: Medical and Clinical Oncology

Cell Cycle Dysregulation in Cancer: Molecular Mechanisms and Therapeutic Implications

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Review Article

Received: 09-Dec-2023, Manuscript No. MCO-23-122432; **Editor assigned:** 11-Dec-2023, Pre QC No. MCO-23-122432(PQ); **Reviewed:** 25-Dec-2023, QC No. MCO-23-122432; **Revised:** 01-Jan-2024, Manuscript No. MCO-23-122432(R); **Published:** 08-Jan-2024, DOI: 10.4172/medclinoncol.8.1.001.

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Citation: Inayat M, et al. Cell Cycle Dysregulation in Cancer: Molecular Mechanisms and Therapeutic Implications. 2024;08:001.

ABSTRACT

This review details the numerous regulatory mechanisms that govern cellular homeostasis and their significance for cancer development. The control focuses on proliferation, growth arrest, and apoptosis. These activities control cell destiny and avoid abnormalities.

This topic focuses on the cell cycle, a tightly synchronized sequence. Cancer is characterized by recurrent deregulation of this system. CDKs and cyclins coordinate the positive acceleration of cell cycle progression. Cyclin-Dependent Kinase Inhibitors (CKIs) impede growth in response to regulatory signals. Cancer originates from abnormal expressions of genes that promote cell development and repression of genes that inhibit cell growth in this carefully balanced system.

As we get further into the molecular landscape, we focus on cancer's modest cell cycle disruption. Lack of cell cycle checkpoint regulation causes genetic instability and uncontrolled cell proliferation in cancer. Hyperactivating mutations in growth signaling networks and the lack of tumor suppressor proteins accelerate cancer cell proliferation. The cell cycle machinery, which integrates upstream

signaling cascades, is a promising diagnostic and therapeutic target. Expanding on this scientific inquiry, DNA replication machinery and cell division proteins are examined in detail. This review finds novel cancer biomarkers that might be utilized for diagnosis and prediction. This lays the groundwork for cell cycle-targeted therapies.

Keywords: Cancer treatment; Cell cycle; Tumorigenesis; Biomarker; DNA replication; Mitosis

INTRODUCTION

Cancer, a diverse and challenging group of diseases, remains a substantial worldwide health obstacle. Cancer is characterized by the unregulated proliferation and division of cells, which disturbs the tightly controlled mechanisms of the cell cycle. Cancer is believed to occur when cellular proliferation surpasses the rate of cellular death, resulting in uncontrolled cell division [1]. Current consensus acknowledges that cancer is more precisely characterized as a consequence of dysfunctions in the control of the cell cycle. This leads to the survival and proliferation of damaged or mutant cells, which would normally be eliminated, resulting in the accumulation of mutations [2]. These characteristics encompass the loss of cellular specialization, independence from external growth impulses, unrestricted ability to replicate, heightened ability to invade surrounding tissues, and reduced susceptibility to drugs [3]. The cell cycle is a highly regulated series of events that controls cell division and growth and is crucial for maintaining the stability of multicellular organisms. Abnormalities in the cell cycle machinery primarily cause cancer start and development. Comprehending the complex connection between cancer and the cell cycle is crucial for deciphering the fundamental processes of cancer development, pinpointing possible targets for treatment, and progressing precision medicine strategies. This study article examines the complex relationship between cancer and the cell cycle, investigating the detailed molecular mechanisms that cause irregular cell proliferation. It also establishes the basis for novel approaches in cancer detection, therapy, and prevention.

The correlation between age and the occurrence of cancer has been widely understood to indicate that the development of tumors necessitates the accumulation of mutations and changes in gene expression, affecting several genes with varied roles. Mutations mostly arise in proto-oncogenes and tumor suppressor genes. Proto-oncogenes typically exert their influence at various stages of cell proliferation, but they can facilitate the formation of tumors when they undergo mutations. Furthermore, the mutation of tumor suppressor genes will impede the suppression of cell cycle progression, hence promoting aberrant growth [4].

Growth-regulating systems often preserve homeostasis under normal circumstances [1,5]. The regulation of homeostasis in a cell is determined by the equilibrium between cell proliferation, growth arrest, and apoptosis [6]. An imbalance in the equilibrium between cell proliferation and apoptosis can lead to hyperplasia or neoplasia [1]. Hyperplasia may be reversed when the stimulus is eliminated, whereas cancer cells undergo permanent changes. Cancer cells have a distinctive trait of being unaffected by growth stimuli as a result of mutations in intracellular signaling pathways.

This autonomy enables the cell to re-enter the cell cycle, regardless of whether the external stimulation is positive or negative [2]. Thus, it is clear that cells that are prone to genetic abnormalities might contribute to the development of cancer in the initial phases of carcinogenesis [6]. Nevertheless, effective regulatory systems identify

cellular harm [7]. The destruction of the injured cells serves to reduce the malignant development, which occurs through two separate mechanisms [8].

1. The initial stage is necrosis, which is distinguished by cellular enlargement and rapid deterioration.
2. The second kind, apoptosis, is distinguished by cellular contraction accompanied by the formation of blisters on the plasma membrane and fragmentation of DNA.

The control of apoptotic death, which occurs as a result of DNA damage, is a crucial process in stopping the proliferation of altered cells and so reducing the risk of pre-cancerous cell growth. Several proteins have a dual function in both the cell cycle and apoptosis. The magnitude of DNA damage and the concentration of these proteins dictate the fate of the cell, either its advancement or demise [9,10]. Environmental insults can cause DNA damage, which in turn disrupts cell cycle regulatory processes. Cell cycle checkpoints govern cell growth and development processes, overseeing the vast and complicated network of relationships [9]. The mutation of these checkpoints is observed in all forms of cancer [4].

The inhibition of tumor growth through the processes of growth arrest, DNA repair, and apoptosis is crucial in preventing the development of potential malignancies. The tumor suppressor gene, p53, is commonly referred to as the "guardian of the genome" due to its crucial function in safeguarding the genome against the growth of altered cells [11,12]. Tumor cells arise when mutations occur in tumor suppressor genes, which regulate the cell cycle checkpoints, enabling damaged cells to advance through the cell cycle [13].

LITERATURE REVIEW

The intricacies of cell cycle

The process of cell division and replication, known as the cell cycle, is a fundamental biological mechanism. It is a complex series of processes that allows cells to undergo growth and replication. The cell cycle and cancer formation are influenced by two categories of genes: Oncogenes (such as Her2/neu, Ras, c-Myc, etc.) and tumor suppressor genes (such as p53 and Rb). The cell cycle consists of four consecutive stages.

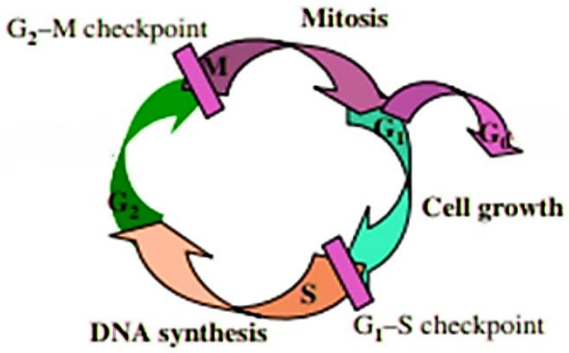
The cell cycle is a highly controlled process that controls cell division. It consists of several important stages, including the S phase, which is responsible for DNA replication, and the M phase, which is responsible for cell division. The phases are divided into gap phases, G1 and G2, with G1 being responsible for responding to growth signaling networks and G2 preparing for mitosis [14]. G0 is a condition in which cells briefly exit the cell division cycle as a result of variables such as excessive cell density or lack of mitogens [15]. Alternatively, cells can permanently leave the cell cycle and reach either a terminally differentiated or senescent state.

The cell cycle is controlled by the Cyclin-Dependent Kinase (CDK) family and its regulatory partners, cyclins [16]. Cyclin D-CDK4, cyclin D-CDK6, and cyclin E-CDK2 are responsible for propelling the cell through the G1 phase, reaching the restriction point, and ensuring the cell's commitment to completing the cell cycle. Cyclin A-CDK2 initiates the S phase, whereas G2 and mitosis are regulated by Cyclin B-CDK1[17,18]. Checkpoints are responsible for monitoring the transitions between each phase of the cell cycle, hence guaranteeing the proper sequence of events [19].

When abnormalities or incomplete events are identified, checkpoint pathways activate effectors that trigger cell cycle arrest until the problem is rectified [20,21]. Effector proteins, such as CDK inhibitors (CDKIs), are involved in the cessation of cell cycle advancement. G1 arrest can be triggered by the Ink4 family (INK4A(p16), INK4B (p15), INK4C (p18), and INK4D (p19)) of CDKIs, which inhibit the activity of CDK4 and CDK6. Alternatively, it can be

induced by the Cip/Kip family of inhibitors (p21, p27, p57), which decrease the activity of CDK2 as shown in Figure 1[22, 23].

Figure 1. Cells progress by sequential events influenced by external and internal cause.

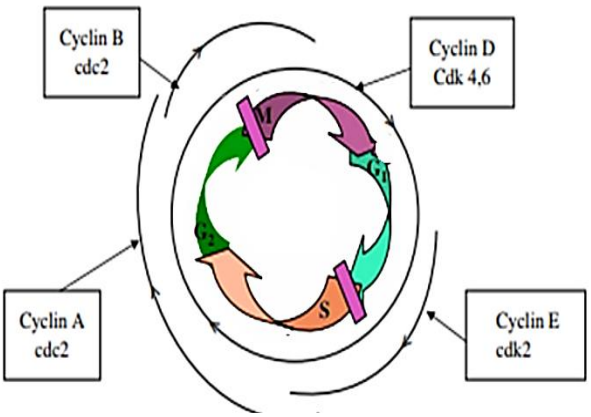


G1-s phase

After undergoing mitosis, daughter cells that reach the G1 phase have the potential to complete the whole cell cycle once more. During this phase, cellular stimulation and proliferation take place, or the cell may enter a temporary state of dormancy (G0 phase). Once the restriction point, known as the Retinoblastoma gene (Rb), is successfully crossed during the late G1 phase, the cell will proceed to the S phase, during which DNA synthesis takes place. It is crucial to complete this phase expeditiously as DNA is more susceptible to damage at this stage, when the bases are exposed and the DNA strands are split [7].

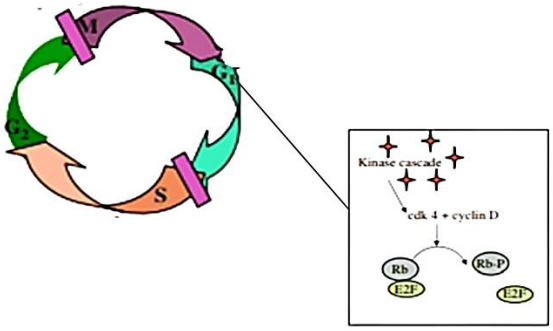
In the early stage of G1, the cell gets signals that stimulate growth by raising the levels of cyclin D. This is followed by an increase in the levels of cyclin E in the later stage [7,10,24]. The levels of cyclin D rise in reaction to the Ras-dependent kinase cascade [12]. The management of a negative growth rate, however, is governed by a restriction point in the G1 phase that dictates the advancement of the cell in the cycle. The restriction point is regulated by the growth inhibitory factor Rb, which functions as a decisive element in deciding whether the cell progresses into the S phase [10,24]. Currently, the cell is either determined to finish the cell cycle or halted [25]. Therefore, cells that have passed this threshold reach the late G1 phase and make an irreversible as shown in Figure 2.

Figure 2. Cyclin D increases in early G1 and stays stable throughout the cycle. These are followed by cyclin E in late G1-S, A in early S-G2, and B in G2-M.



The cell cycle is controlled by CDK-induced Rb protein phosphorylation [12]. Hypophosphorylated E2F is inhibited by Rb. Cyclin-CDK complexes phosphorylate Rb [10,25]. Phosphorylated Rb protein releases E2F, which stimulates S phase gene transcription as shown in Figure 3 [7, 12,13,26].

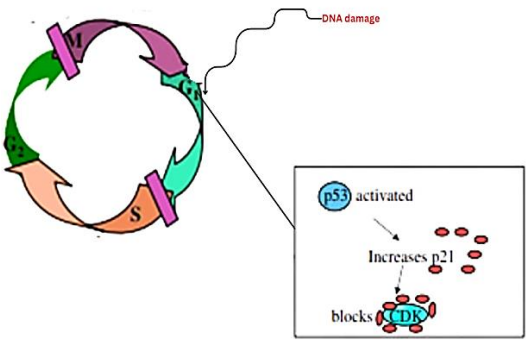
Figure 3. G1-S cell cycle progression.



The orderly processes outlined above are disturbed when cells sustain damage, for as after exposure to radiation [27]. An unorganized sequence of events occurs, resulting in a faulty cellular cycle. Modifications such as the removal of genes, increased expression, or changes in individual nucleotides impact the rate at which a cell divides. Many malignancies exhibit point mutations or omissions [5]. p53 is a widely distributed transcription factor that safeguards DNA integrity by recognizing DNA damage [7,13]. p53 levels rise in reaction to DNA damage by halting the cell in the G1 phase to facilitate DNA repair [4,28]. The method of detecting this harm is not evident. The factors that determine whether a damaged cell undergoes cell arrest or cell death are still unknown, however it is established that DNA damage can lead to either outcome, as well as DNA repair.

According to one explanation, cells have the ability to detect when the damage they have suffered is too severe to be fully repaired. This detection then activates a process called p53-dependent apoptosis, which leads to cell death [10]. However, the cellular response to DNA damage triggered by p53 appears to be dependent on the specific kind of cell [11,12]. Bellamy demonstrated that the process of growth arrest may be induced by exposing actively dividing fibroblasts to a radiation dose of 5 Gy of gamma-rays. In contrast, the identical radiation dose resulted in apoptosis for actively dividing intestinal crypt cells. p53 also activates the transcription of p21, which is a CDK inhibitor, in response to DNA damage [13,29]. Cyclin A is expressed specifically during the S phase of the cell cycle. It forms an association with CDK2 in the early phase and with cdc2 in the later phase. The cyclin-CDK complex is believed to be involved in the start of DNA synthesis [13]. Suppression of CDK expression would consequently result in cell arrest [24], as illustrated as shown in Figure 4.

Figure 4. G1–S cell cycle arrest.



p53 stimulates the suppression of cell growth to enhance DNA repair and induce apoptotic cell death [5]. p53 is activated by the ATM (Ataxia-Telangiectasia Mutated) kinase in order to coordinate DNA repair and apoptosis through DNA damage signaling pathways [30]. ATM is responsible for detecting and repairing double strand breaks, while ATR (ATM and Rad3-related) is responsible for responding to other forms of DNA damage [31]. The process that initiates discrimination is not clearly understood. The activation and regulation of the growth arrest and DNA damage gene (Gadd45), which hinders cell proliferation, may be influenced by p53.

It is established that p21Cip1/WAF1 has a crucial function in p53-induced arrest in G1. Additionally, it interacts with and deactivates the Proliferating Cell Nuclear Antigen (PCNA), a component of the DNA polymerase complex. This action prevents DNA synthesis but does not affect the repair pathway [7,26,32,33]. Furthermore, ICBP90, a newly discovered nuclear protein, is now proposed to be one of the targets for the p53/ p21Cip1/ WAF1 mediated pathway, and hence, it plays a crucial role in the arrest of the G1/S cell cycle [30].

Dang et al. conducted studies that identified human Rad9 and Rad17 proteins as essential for the activation of the early S phase checkpoint and the subsequent preservation of chromosomal stability. Additionally, they suggest that Rad9 facilitates the phosphorylation of a substantial protein complex by ATR kinase, transmitting checkpoint signals and enhancing subsequent cellular activity [31]. The loss of p53 activity and Rb protein can occur due to gene mutation, gene deletion, or interaction with other proteins. Conversely, the overexpression of CDK4 and CDK6 inhibitors can inhibit the function of p53. The evidence clearly demonstrates that the absence of p53 function leads to the buildup of cancer-causing mutations in the genome, as its ability to regulate cell death has been eliminated [7].

Genetic abnormalities in genes located downstream of p53, such as p15, p16, and p27, can also lead to disruption of the cell cycle. The inhibitors of Cyclin-Dependent Kinases (CDK), namely p21, p27, and p57, hinder the functions of CDKs and cyclin-CDK complexes [7,33]. They also inhibit Cyclin Activating Kinases (CAK), which in turn hinders the phosphorylation of Rb, so stopping the cell from progressing into the S phase [7,10,12,26]. The malfunctioning of CDKs, crucial regulators in the cell cycle, has been demonstrated to lead to malignant effects. The CDK4 gene has been discovered in a chromosomal area that is frequently amplified in human sarcomas. Additionally, the p15 and p16 genes, which act as inhibitors of CDK4 and CDK6, respectively, have been found to be altered in some cancers [30]. The reason for this could be the oncogenic properties of the mutant CDK4, which becomes unresponsive to the inhibitory effects of p16 [31].

Apoptosis mediated by p53: The genes TP53 and BCL-2 are crucial in the process of apoptosis [34,35]. Typically, functioning p53 controls an intricate system of connections that dictate the outcome of a cell. The nuclear phosphoprotein C-Myc promotes p53, which triggers apoptosis by transcriptionally stimulating genes that control this programmed cell death. Studies have demonstrated that C-Myc has a significant involvement in apoptosis, which includes both p53-dependent and p53-independent pathways [13,25]. C-Myc is not only linked to the production of Fas ligand and Fas receptor, but it is also believed to control the transcription of Bax, which triggers the release of Cytochrome c [25]. Nevertheless, when p53 is not functioning properly, injured cells have the potential to go into mitosis, resulting in the accumulation of unregulated mutations due to the absence of cell cycle checkpoints [9,32]. Mdm2, the negative regulator of p53, has been demonstrated as an alternative mechanism that can circumvent the regulatory function of p53. The overexpression of the MDM2 gene has been observed in leukemia, lymphoma, sarcoma, glioma, and breast cancer, suggesting a bypass of p53-mediated growth regulation [4]. Apoptosis is a regular and well-structured process that enables the elimination of impaired and abnormal cells that have the potential to cause injury [4,5,11,25,36,37]. Cancer formation is more likely to occur at two levels when

there is dysfunction in the systems within the apoptotic pathway [38]. At the initial stage, mutations in the genes responsible for regulating apoptosis enable mutant cells, which would typically be removed through apoptosis, to persist [36,38]. In the second stage, cells that are multiplying and have developed resistance to programmed cell death, known as apoptosis, due to harsh internal conditions, are permitted to continue existing by a process similar to Darwinian selection in cancer cells [38].

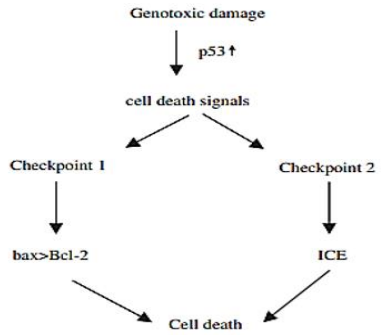
Anti-apoptotic mechanisms: These systems pertain to the cell's ability to survive, which becomes problematic when mutant cells are encouraged. The gene BCL-2 acts as a prominent inhibitor of cell death, therefore protecting against apoptosis [6,7,32]. Additional members of this family, such as Bcl-xL, MCL, and bag-1, function as inhibitors of cell death, whereas bad, bax, and bik promote apoptosis [6]. Tumour promoters function as factors that enhance cell survival by inhibiting apoptosis and increasing the likelihood of injured cells surviving [9]. The c-Myc protein enhances cell proliferation by inhibiting the expression of GADD45 and cki genes p15, p21, and p27 [10, 39]. In the absence of survival factors, the oncogene c-Myc, which also facilitates cell death, mostly induces apoptosis in cells [29,32]. Bcl-2 operates by redirecting c-Myc signals, resulting in cell growth instead of cell death [29,31,33].

The specific mechanism that influences the pro or anti-apoptotic process remains unclear. Nevertheless, certain evidence indicates that Bcl-2, situated on the external membrane of the mitochondria, impedes the liberation of Cytochrome-c from the mitochondria [38,40], hence triggering the activation of caspases (apoptotic proteases), which subsequently initiates apoptosis [6,12]. Consequently, the levels of Bcl-2 expression play a crucial role in this apoptotic pathway, and cells that have high amounts of Bcl-2 may avoid undergoing cell death [25]. Nevertheless, research has demonstrated that very elevated levels might induce cellular apoptosis [10]. The transcription factor NF-kB is recognized for its ability to increase the expression of the Bcl-2 family. Bcl-2 also controls the anti-apoptotic function of Ras, a G protein located in the cell membrane [4].

Another regulation mechanism of Bcl-2 involves preventing cell death by suppressing the activity of other growth regulators, such as bax [6,7]. The precise equilibrium between bax and Bcl-2 is crucial in determining the occurrence of apoptosis [7,27].

Apoptotic checkpoints: Apoptosis requires the levels of bax to surpass those of Bcl-2 [11,27]. Checkpoint 1 in apoptosis has been designated as such in previous research [7]. Furthermore, when wild-type p53 is present, insufficient levels of Bcl-2 expression will lead to apoptosis [9]. In the event of a reversal, the outcome would be an upregulation of Bcl-2 in the absence of normal p53 and with elevated rates of cell mutation, as a consequence of active cell proliferation [7,8]. Bcl-xL, the predominant protein of the Bcl-2 family, exhibits comparable behaviour to Bcl-2 [5,37]. By contrast, the smaller proteins Bcl-XS and Bak inhibit the activity of Bcl-2, making the cell vulnerable to apoptosis [32, 41]. The second stage in the process of programmed cell death (apoptosis), as shown in Figure 5.

Figure 5. Apoptotic checkpoints.



Is not clearly characterized although it involves the activity of an enzyme called Interleukin-Ib Converting Enzyme (ICE), which interacts with enzymes responsible for repairing DNA [7]. Poly Adenosine diphosphate-Ribose Polymerase (PARP) is responsible for detecting DNA breaks and plays a key role in DNA repair processes. When ICE deactivates PARP, it triggers apoptosis because the fragmentation of the DNA becomes irreversible [32].

G2-m phase

Multiple cellular reorganization activities take place during the G2 phase, which involve the control of unaddressed DNA damage. The G2 checkpoints oversee the monitoring of all these processes. The G2 phase checkpoints necessitate the involvement of the cyclin-CDK-cki system to remove cells that have sustained damage and progressed from the G1 phase [7]. This checkpoint functions as a mechanism to inhibit chromosomal segregation in the case that the chromosomes are not structurally sound [12]. Chromatid alignment and division into the two daughter cells take place during the M phase. The spindle assembly checkpoint oversees the precise positioning of chromosomes. If the centromeres are not connected to the microtubules, mitosis is postponed. Cyclin-Dependent Kinases (CDKs), specifically cdc2 and cyclin B, are essential for the occurrence of these events [7]. The Cdc2-cyclin B complex, sometimes referred to as MPF (Mitosis Promoting Factor), has a crucial function in facilitating the transition into the M phase. The early initiation of MPF is regulated by a nuclear protein called Wee 1, which, together with myt1, keeps cdc2 in a phosphorylated condition throughout interphase.

In order for the cell to advance through this phase, the cdc2 protein, which is inactive when phosphorylated, needs to be activated by the cdc25b and cdc25c protein phosphatases [42]. The level of cdc25b rises during the G2 phase, which helps to activate the cdc2-cyclin B complex. Excessive production of cdc25b can cause early cell division and increased vulnerability to harm, due to the rise in cdc25c levels triggered by the activation of cdc2-cyclin B complex. The protein cdc25c moves from the cytoplasm to the nucleus, where its levels build up and promote a further increase in MPF through a positive feedback mechanism [5,43]. Discovered that aurora-A kinase is activated as a result of cdc2-cyclin B activity. Aurora-A is believed to be triggered indirectly by phosphorylation mediated by cdc2-cyclin B. This activation process is thought to involve a positive feedback loop, similar to that of Polo-like kinase. In order to commence cell development, the presence of cdc2-cyclin B inside the nucleus is necessary [43]. Suggests that the activation of aurora-A could potentially result in the movement of the nucleus and complete activation of cdc2-cyclin B, which in turn triggers the initiation of mitosis. Moreover, the excessive expression of aurora-A has been detected in many types of cancer and is believed to disrupt the normal regulation of checkpoints, hence promoting the development of tumours [43]. The proteins p21 and p53 hinder the progression of injured cells in the G2-M phase from undergoing a second round of DNA replication by suppressing the activity of CDKs [12,29]. p53 hinders this transition by enhancing the transcription of the 14-3-3 δ gene, which obstructs entry into G2. In the cytoplasm, 14-3-3 δ binds with cdc25c phosphatase [12]. The 14-3-3 δ-cdc25c complex hinders the nuclear entry of cdc25c, resulting in DNA blockage and cellular arrest (Figures 6 and 7).

Figure 6. G2-M cell cycle progression.

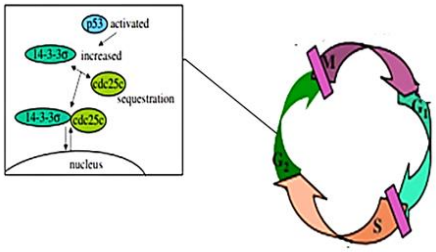
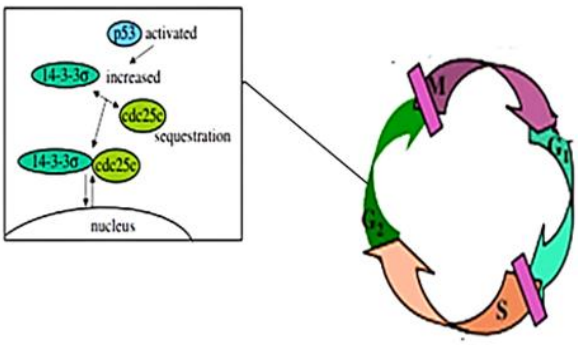


Figure 7. G2-M cell cycle progression.



The cell cycle as a point of convergence for oncogenic signalling pathways

Utilizing genome-wide analysis to examine the intricate and partially overlapping upstream signaling networks that regulate cell proliferation, differentiation, and invasion has yet to be established as a standard approach for clinicopathological evaluation. The initial investigations utilizing microarray-based gene expression profiling revealed highly promising prognostic and predictive patterns, indicating that this approach could potentially supplant conventional clinicopathological indicators in the near future [19-21]. However, further research has demonstrated that microarrays' ability to forecast and anticipate outcomes only provides supplementary information and cannot supplant conventional clinicopathological factors. Regrettably, the practical effectiveness of prediction methods utilizing gene expression has not lived up to expectations for numerous tumor types, and the roster of discovered genes can exhibit significant instability [22,23]. For example, the categorization of molecular subtype classes of breast cancer using dendrograms derived by hierarchical cluster analysis has been found to be subjective, with little agreement among different observers [24]. The impact of prognostic markers on reducing the utilization of hazardous chemotherapy in patients is still uncertain. For instance, while the Mamma Print 70-gene signature is anticipated to detect 10-15% of patients who might avoid chemotherapy, findings from a recently concluded feasibility study indicate that this expectation is excessively optimistic [25].

Transcriptomic profiling has been limited in its ability to predict treatment response accurately. The correlation between treatment response and predictive signatures is not consistent, and the predictive utility of these signatures has been severely diminished when tested on validation cohorts [26,27]. Furthermore, obtaining surgical material for microarray analysis, especially for tiny tumors, poses a significant challenge in the regular clinical environment and has financial consequences. The intricate architecture of these signaling networks also hinders the effectiveness of employing targeted medicines. The hypothesis of 'oncogene addiction' proposes that a tumor will exhibit a persistent reliance on a specific alteration of a single gene [28].

Cancer cells are inherently unstable and exhibit many changes [29]. Therefore, the cancer evades the targeted therapy and avoids the intended target [15,30]. This phenomenon is seen in the predominantly unsatisfactory outcomes of clinical trials for targeted therapy in cancer, where the most favorable outcome is a modification of the tumor's normal progression. Nevertheless, a remedy has not yet been discovered [31-38]. A different strategy involves directing attention toward the cell cycle machinery, which serves as a central hub for processing information received from upstream signaling networks [3,39,40]. It is worth mentioning that the clinic's most successful treatments before and after surgery are drugs that target the cell cycle (Table1). The DNA replication initiation pathway, which is a fundamental aspect of the cell division cycle, has gained significant attention as a specific target in recent years [3,41–43]. The DNA replication initiation mechanism is considered a crucial and decisive stage in growth regulation, located at the point where the intricate and divergent upstream signaling networks converge [40].

This particular element of the cell cycle mechanism functions as an intermediary hub, linking growth signaling networks with the onset of DNA synthesis. As a result, it has the potential to be a valuable target for both diagnostic and therapeutic purposes [3].

Regulation of cell cycle progression

Extensive research has been conducted in the past ten years to study the molecular mechanisms that regulate the different phases of the cell cycle, from G1 to M [44]. The fundamental component of the regulatory machinery governing cell cycle progression consists of a group of enzymes known as Cyclin-Dependent Kinases (CDKs). The active forms of CDKs consist of a kinase and a cyclin, which together form a complex of at least two proteins (Table 1). They frequently include additional proteins with activities that are not well understood. These complexes experience alterations in the kinase and cyclin components, which are thought to propel the cell from one phase of the cell cycle to another [44,45]. According to this paradigm, the progression of the cell cycle is regulated by the specific set of proteins that are either activated or deactivated by phosphorylation. This phosphorylation process is influenced by the activity of the CDKs during that particular stage, as seen in Figure 1. Mammalian cells express a series of kinase subunits (CDK4, CDK6, CDK2, and CDC2) and a series of cyclins (cyclin D, E, A, and B) when they transition from G1 to mitosis. The CDK4 and CDK6 proteins form a complex with one of multiple D-type cyclins, which has a role in the early stage of the G1 phase, most likely in response to growth stimuli. The presence of CDK2, in combination with either cyclin E, cyclin A, or both, is crucial for the transition from G1 to S phase and for DNA replication, respectively. The presence of CDC2 in conjunction with cyclin A and cyclin B is crucial for the process of mitosis. Further CDKs and cyclins will be included in this list (Table 1).

Table 1. Cyclin-dependent kinase with their respective regulatory cyclins.

S. No.	Cyclin-Dependent Kinases (CDKs)	Regulatory cyclins
1	Cdc2	Cyclin A and B
2	Cdk2	Cyclin A, E and D
3	Cdk3	Cyclin E
4	Cdk4	Cyclin D1, D2 and D3
5	Cdk5	P35
6	Cdk6	Cyclin D1, D2 and D3
7	Cdk7	Cyclin H
8	Cdk8	Cyclin C
9	Cdk9	Cyclin T

The transition of cells from one phase of the cell cycle to another is rigorously governed by numerous regulatory mechanisms that influence the transcription of cyclin genes, the degradation of cyclins, and the phosphorylation-mediated alteration of kinase subunits. Several positive or negative feedback loops also play a role in the course of the cell cycle. The activity of CDK is enhanced by its interaction with cyclins and by the phosphorylation of the threonine in the T-loop by the CDK Activating Kinase (CAK), which is a serine/threonine kinase that is also involved in transcription and DNA repair [46]. The process of inhibitory phosphorylation occurs when neighboring threonine and tyrosine residues (T14/Y15 in CDC2) are phosphorylated by dual specific kinases (Wee1 and MYT1). The relief of this inhibition occurs when the CDC25 phosphatases remove the phosphate groups from these residues, which then initiates the process of entering mitosis [47,48].

CDKs and cyclin partners serve as positive regulators that promote cell cycle progression. In contrast, Cyclin-Dependent Kinase inhibitors (CKIs) act as negative regulators that halt cell cycle progression in response to regulatory signals. CKIs can exert a negative regulatory effect on CDK activity through direct interaction with CDK. There exist two categories of CKIs. The inhibitory function of the INK family, consisting of INK4A (p16), INK4B (p15), INK4C (p18), and INK4D (p19), is achieved by binding to CDK4 and CDK6, thereby blocking their interaction with D-type cyclins. The CIP/KIP family consists of three members, namely CIP1 (p21), KIP1 (p27), and KIP2 (p57). These members combine with the G1/S CDKs to create heterotrimeric complexes. Cyclin-dependent kinase inhibitors (CKIs) are activated in response to several physiological events [49,50]. In cells that are not actively dividing, the levels of KIP1 are often elevated. CIP1 functions as a downstream mediator of p53, a crucial tumour suppressor involved in the regulation of the DNA damage checkpoint.

The important aspect in regulating the cell cycle is the restriction point. Upon reaching this stage, the cell becomes permanently dedicated to progressing to the subsequent phase of the cell cycle (Figure 6). The regulation of the restriction point is facilitated by the cyclin D and cyclin E-dependent kinases. The retinoblastoma protein family, consisting of pRB, p107, and p130, are the main targets of CDK4/6 and CDK2 during G1 development [47,51]. These compounds act as inhibitory regulators at the restriction point. The E2F family of transcription factors is a significant target of pRB in the regulation of early G1 cell cycle progression [52]. E2Fs control the activity of numerous genes involved in both the transition through the restriction point, such as cyclin E, and the progression through the S phase, such as dihydrofolate reductase and thymidylate synthase. Furthermore, pRb exhibits certain functions that are not dependent on CDK, including the suppression of certain promoters and the regulation of RNA pol III activity [53,54]. Hence, the reintroduction and manifestation of pRb in tumour cells can halt growth through mechanisms that are not reliant on the creation of the CDK complex.

Abnormalities in the cell cycle control proteins in cancer: Abnormalities in cyclins and CDKs

Recent evidence suggests that the development of tumours is often linked to genetic changes or irregularities in the expression of specific cyclins, CDKs, and CKIs in diverse forms of human cancers [55,56]. In 1991, researchers discovered a potential cancer-causing gene called an oncogene. This oncogene is made up of the Parathyroid hormone gene (PRAD1) fused with the gene responsible for producing cyclin D1. The discovery was made in a human parathyroid adenoma [57,58]. This result yielded the initial indication that cyclins could have a direct role in certain types of human malignancies. Later, evidence was provided to support the role of cyclins in different types of human cancer cells. The tumours studied encompassed B cell lymphoma, breast, stomach, colon, and oesophageal carcinomas, along with various other cancer types [59]. Undoubtedly, the upregulation of cyclin D1 is a common anomaly observed in human cancer, with a prevalence of around 60% in breast malignancies, 40% in colorectal cancers, 40% in squamous carcinoma of the head and neck, and 20% in prostate cancers [56,60-62]. In line with these findings, cyclin D1 can replace or partially replace specific oncogenes in the cellular transformation experiment [63,64]. The cyclin E gene, which functions during the late G1 phase of the cell cycle, is excessively expressed and improperly controlled in various types of human malignancies [65]. Instances of CDKs and its regulators being amplified and overexpressed in human malignancies are infrequent. Several publications have shown that the CDK4 gene is over expressed in specific tumor cell lines [59]. Although CDKs have been implicated in cancer, their overall role in the disease has not been conclusively established, similar to cyclins.

Deviation in cyclin-dependent kinase inhibitors

Two classes of Cyclin-Dependent Kinase Inhibitors (CDKIs) have been identified and classified according to their selectivity. The INK4 family consists of four members: p15 (INK4B), p16 (INK4A), p18 (INK4C), and p19 (INK4D) [66].

The INK4 proteins selectively inhibit the cyclin D-dependent kinases (CDK4 and CDK6) [66,67]. The INK4A (p16) protein, initially recognized as a CDK4-interacting protein that suppresses CDK4 kinase activity [68] , has been assigned to chromosome 9p21[69]. Intensive research has been conducted to investigate the role of INK4A in carcinogenesis, as it is associated with a cluster of genetic changes in malignancies. INK4A gene mutations and deletions are commonly observed in several types of human malignancies and altered cells [70,71]. The malignancies encompassed in this list are melanoma, acute lymphocytic leukemia, osteosarcoma, lung cancer, brain cancer, breast cancer, head and neck cancer, bladder cancer, and ovarian cancer. The frequent inactivation of INK4A in these tumours indicates that the absence of INK4A confers a specific advantage in cellular development as shown in Table 2.

Table 2. Alterations of INK4A reported with respect to the type of tumor.

S. No.	Type of tumor	Alteration reported
1.	Adult Lymphocyte Leukemia	Deletion
2.	Carcinoma of Bladders	Deletion
3.	Gliosarcoma	Deletion
4.	Head and Neck Tumours	Mutation
5.	Melanoma	Mutation
6.	Oral Squamous Cell Carcinoma	Mutation

INK4B is located directly next to INK4A at the INK4 gene on chromosome 9p21. Its expression is stimulated in response to treatment with Transforming-Growth Factor beta (TGF-β) [72]. The INK4B sequence exhibits a high degree of similarity to INK4A, around 70% at the amino acid level. The virtually comparable molecular behaviour of INK4B, acting as a CDK4/6 inhibitor, suggests that it may have a role in suppressing tumours. While there is ample evidence supporting the tumor suppressor function of INK4B, its specific role in tumor suppression remains uncertain. INK4B mutations in tumor cell lines have been either absent or infrequent. Most tumours are affected by homozygous deletions that primarily target either both the INK4A and INK4B loci, or INK4A alone [73]. INK4B deletions have been identified in a limited number of leukemia and lymphoma patients [73-75]. Conversely, the occurrence of hyper methylation in the INK4B gene appears to be common in multiple types of cancer [74,76-78]. These findings indicate that the methylation-induced silencing of the INK4B promoter plays a significant role in the progression of tumours.

The CIP/KIP family, which belongs to the second group of CKIs, exhibits homology in the N-terminal CDK inhibitory region. These include p21(CIP1/WAF1), p27 (KIP1), and p57 (KIP2). The p21CIP1 protein forms complexes with and blocks the activity of several CDK-cyclin combinations, such as CDK2-cyclin E and CDK4-cyclin D1. The expression of p21CIP1 is directly stimulated by p53 [79]. This highlights p21CIP1 as a possible agent involved in p53-dependent tumor suppression. Moreover, the excessive production of p21CIP1 can induce G1 cell cycle arrest. Thus far, there have been no documented instances of p21CIP1 modifications in tumours or cell lines. If p21CIP1 plays a crucial role in p53-dependent tumor suppression, it is reasonable to anticipate that mutations in p21CIP1 may be present in a portion of tumours and cell lines. Hence, the genetic data supporting p21CIP1 as a universal tumor suppressor remains inconclusive. The p27KIP1 protein was discovered to be a CDK-binding protein that is activated by TGF-β and contact suppression of cell growth [80, 81].

The protein sequence of p27KIP1 exhibits certain resemblances to p21 and also hinders many CDKs. There have been no documented mutations in the p27KIP1 gene in tumour and cell lines. Nevertheless, the diminished

presence of p27KIP1 is commonly observed in human malignancies. The cancers that are included are breast, prostate, stomach, lung, skin, colon, and ovarian cancers [82-88]. Remarkably, a comparatively elevated level of p27KIP1 expression is detected in a set of human oesophageal cancer cell lines [89]. In addition, it is worth noting that multiple human colon and breast cancer cell lines exhibit elevated amounts of p27KIP1. Still, three normal human mammary cell lines display low levels of this protein. Despite the high degree of malignancy in small-cell carcinomas of the lung, it is shown that this gene is also overexpressed [90]. The heightened upregulation of p27KIP1 in cancer cells appears contradictory, given no mutations of this gene have been detected or are exceedingly few in different types of tumours [91]. One potential explanation for the elevated levels of p27KIP1 in certain cancer cells is that these cells have developed resistance to the suppressive actions of this protein. Additional research will be necessary to determine the precise involvement of p21CIP1 and p27KIP1 in the progression of cancer.

Control of cell cycle as a potential for cancer treatment

The frequent disruption of cell cycle control in human cancer has identified potential targets for therapeutic intervention. Restoring adequate restriction point control in cancer cells may enable them to revert to a dormant state. Alternatively, it could exploit their uncontrolled proliferation to induce apoptotic death, or to specifically expose cancer cells to cytotoxic treatments [92]. CDKs are currently being specifically targeted due to their pivotal role in regulating the course of the cell cycle. The most straightforward and promising approach is to design inhibitors that can effectively block the action of CDK. Significant endeavors from multiple organizations have resulted in the identification, improvement, and description of powerful CDK inhibitors. CDK inhibitors has three features that render them highly appealing as anti-tumor medicines. Initially, they exhibit strong anti-proliferative properties by halting the progression of cells in the G1 or G2/M phases [93,94].

Additionally, they induce apoptosis, either independently or in conjunction with other therapies [95]. Furthermore, the suppression of Cyclin-Dependent Kinases (CDKs) can play a role in promoting cellular differentiation [96]. Currently, there are only accessible data on the clinical trials of flavopiridol and UCN01 (7-hydroxystaurosporine), but ongoing research is being conducted on numerous other CDK inhibitors. Gene therapy techniques are being developed using negative regulators of cell cycle progression, such as INK4A, p21CIP1, and p27KIP1, to prevent cell transformation and reduce the growth of cancer.

Gene therapy remains a promising but elusive option for cancer treatment. In order to achieve successful tumor regression, it is crucial to have a thorough understanding of the origins of cancer, which will enable the selection of the most effective therapeutic gene. The gene delivery device should be customized to provide optimal transmission of the therapeutic gene to the specific target tissue. In the foreseeable future, there will be an availability of novel pharmacological molecules and gene therapy techniques. They will contribute to the battle against cancer by expanding our comprehension of the cell cycle and its association with cancer.

CONCLUSION

The cell cycle engine is a potential target for diagnosis and treatment of cancer due to its position downstream in the complex oncogenic signalling networks and its crucial role in the abnormal cell proliferation that is a hallmark of all malignancies. Furthermore, both the structure and mechanism of many of its components are evolutionarily conserved, indicating that clinical applications are likely to be applicable to a wide range of tumor types. This stands in sharp opposition to the selective focus on mutations specific to cancer. The examination of potential cell cycle biomarkers in human tissues, together with associated clinical outcome measurements, is currently a vital means of translating fundamental findings in the field of cell cycle research into practical diagnostic and

therapeutic applications. The cellular mechanisms responsible for controlling and regulating the sequence of events in the cell cycle are abundant and intricate. Our knowledge and investigation of the role of tumor suppressors and apoptotic pathways have made significant advancements. Nevertheless, a multitude of enquiries still lack resolution. The cell cycle contains regulating mechanisms that are now poorly understood, or for whose counterparts in mammalian cells have not yet been identified. Cell cycle checkpoints have significant opportunities for targeting in chemotherapeutic and bio therapeutic approaches. The survival of cancer cells after radiotherapy or chemotherapy is determined by the absence of repair pathways. This might result in either increased vulnerability to these treatments in the presence of damage, or more resistance when the apoptotic response is more significant. However, a more comprehensive comprehension of the molecular constituents implicated in cell cycle checkpoints and DNA repair pathways is necessary in order to effectively influence and capitalize on the mechanisms.

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