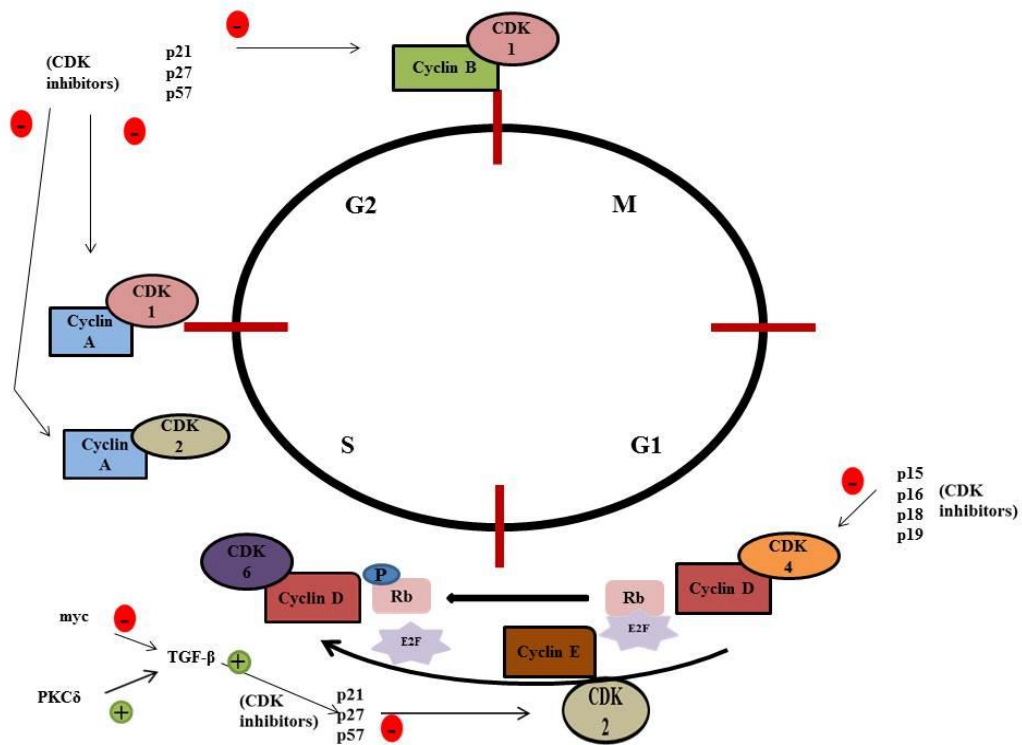


The control of the cell cycle with particular emphasis on the G1 / S transition

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Reglering av cellcykeln med tyngdpunkt på G1 / S transitionen

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SAMMANFATTNING

En viktig orsak till tumörers uppkomst står att finna i en defekt hos cellcykelns reglering. En felaktig reglering av cellcykelns G1-fas tros vara en betydande orsak. Anledningen till att G1-fasen är extra känslig beror på att den styrs av många olika signaler på olika nivåer (såväl yttre faktorer som cykliner och cdk's). I denna del av cellcykeln fattas viktiga beslut. G1-fasen kan delas upp i två delar, G1_{pm} och G1_{ps}. De innehåller varsin restriktionspunkt som måste passeras för att cellerna ska kunna gå in i S-fasen. Den första restriktionspunkten, R1, är beroende av tillväxtfaktorer och där bestäms *om* en cell ska fortsätta till S-fasen. Den kontrollerar således den cellcykel som innefattar DNA-replikation och mitos. Den andra restriktionspunkten, R2, som ligger i G1_{ps} är bl a beroende av mängden näring och bestämmer *när* en cell ska gå vidare in i S-fasen. Genom denna reglering justeras cellernas storlek så att dottercellerna är lika stora när de ska gå in i S-fasen. Tumörutveckling kan ske först när de transformerande cellerna har kunnat ta sig förbi både R1 och R2.

G1-S gener kodar för flertalet proteiner som reglerar kaskadsignaler nedströms ner till kärnan där de slutligen kan verka som t.ex. transkriptionsfaktorer. Ras är ett av de viktigaste proteinerna som inte bara reglerar en utan flera intracellulära signalvägar som innehåller överlappande signaler. Dessa överlappande signaler är ett av de viktigaste kvarvarande vita fälten inom cancerforskningen.

Men de andra faserna i cellcykeln kan också påverka tumörutvecklingen. Ett exempel som uppmärksammas på senare år är oupplösta och ofullständigt replikerade delar av arvsmassan i S-fasen som fortsätter vidare till M-fasen utan att cellen stoppas.

Det finns ännu många frågetecken som måste rätas ut. Sålunda arbetas det intensivt på att se hur onkogener kan tystas och därmed få defekta celler att gå i apoptos eller åldras. Dessa forskningslinjer kan innebära revolutionerande framsteg på vägen mot lösningen av cancers gåta.

SUMMARY

Tumour development occurs to a large extent because of dysregulation of the cell cycle. Of particular importance are defects occurring in the G1 phase. The reason why G1 is critical is because of the influence of several signals (external signals as well as cyclins and cdks) on this stage. The G1 phase can be divided into two parts, G1_{pm} and G1_{ps}. Each part has its “own” restriction point which needs to be passed in order to progress to S phase. The first restriction point, R1, is growth-factor dependent and decides *if* the cell progresses to S phase and hence also controls the chromosomal cell cycle i.e. DNA-replication and mitosis. The second restriction point, R2, located in G1_{ps} is nutrient dependent and decides *when* the cell will progress to S phase. By doing so it controls that the daughter cells are the same size before they enter S phase. Transformed cells need to overcome both R1 and R2 in order to induce tumour development. Transition from G1 to S is driven by genes that encode several proteins that regulate downstream signals. Ras is one of the most important proteins that not only regulate one downstream pathway but several overlapping pathways. Even though the main emphasis is on the G1 phase other phases can also affect the transformation process. For instance unsolved and incompletely replicated DNA in the S phase without a cell cycle stop before M phase is an area that has aroused recent interest. There are several issues that need further investigation. The findings of novel suppressor genes that inhibit oncogene expression and consequently induce apoptosis and cell senescence may contribute to the solving of the cancer riddle.

INTRODUCTION

When a cell is preparing to undergo division, it has to pass several distinct phases in a tightly controlled fashion. Apart from what can be described as a “household control” procedure, namely the doubling of all its components, the key issue is whether the cell decides to progress towards mitosis or simply stop in the process (Mitchison, 1971).

This stop/go control has aroused a great deal of interest and from a cell cycle point of view, one specific phase, G1 phase, holds the key to a range of unresolved issues. In this phase the cell can be influenced by different signals and stimuli that decide whether the cell should progress to the S phase and mitosis or leave the cell cycle and enter a reversible state of quiescence (G0) (Larsson et al., 1985a, 1985b).

One of the hallmarks of transformation is that cells that should respond to suboptimal conditions by entering G0, instead progress towards S and mitosis. There are many explanations for this phenomenon including aberrant growth signals, the absence of inhibitors and negative control mechanisms. But the key issue remains how the stop/go switch in G1 is controlled and how it can be circumvented during transformation (Hanahan and Weinberg, 2011). This paper aims to clarify hitherto unresolved issues as regards how the control mechanisms operate in G1 on a molecular level.

MATERIAL AND METHODS

This review is based on articles from the webpages “Web of science” and “PubMed”. The search terms used for the literature search were: cell cycle, tumour, retinoblastoma, checkpoints, cyclin-dependent kinase, CDK, cyclin D, cyclin E, G1 and G1/S. The search limitations that were used were language and sometimes also searching only on the authors.

LITERARY REVIEW

Overview of the cell cycle

The cell cycle consists of five different phases which all have to be completed in order to make the cell ready for division (Zetterberg and Killander, 1965) (fig. 1). Firstly a doubling of all subcomponents (e.g. RNA, protein and membrane lipids) needs to be completed and it takes place through the whole cell cycle. During the S phase the DNA replication as well as histone synthesis takes place, i.e. the doubling of the genome, and in the M phase first the nucleus and then the whole cell undergoes division. In between these phases there are two gaps called G1 and G2 (Howard and Pelc, 1951). Here, DNA damages and replication errors are repaired (Massagué, 2004). G2 is dedicated to the control of the replication machinery, the repair of eventual errors and to ensure that the cell is ready for mitosis. The G1 phase is particularly sensitive to signals that control cell cycle progression and ultimately the decision to divide. These signals can be positive (e.g. growth stimulatory signals) or negative (e.g. apoptotic or differentiation signals) and ultimately control the activity of different cyclin-dependent kinases (CDKs) that are the key operators that traverse stage specific cell cycle check points. Even if the cell cycle is defined as one cycle it can be divided in two parts, one chromosomal cycle where DNA replication and mitosis takes place, i.e. the doubling of the cellular components, and one growth cycle that involves cellular enlargement. In the G1 phase one critical point called “restriction point” (R) was discovered forty years ago. When the cell has passed through this checkpoint is it programmed to continue to the next division (Pardee, 1974).

Moreover, G1 is the phase where normal cells under suboptimal conditions can decide to leave the cell cycle and pause in a reversible state of quiescence called G0. Tumour cells lack the capacity to enter G0 which enables them to undergo sustained proliferation and oncogenic transformation (Larsson et al., 1986, 1985a, 1985b; Massagué, 2004). In addition there are several substances and factors that can interfere with and eliminate functional cell cycle controls.

Recently, several gene mutations that encode proteins that affect the G1 phase have been found to facilitate transformation and therefore point at this phase as especially important to investigate. Another interesting general theory is that cell damage induces both tumour development and cell aging (Hanahan and Weinberg, 2011). The difference is that normal cells enter senescence while tumour cells can overcome this barrier and continue to growth. In other words, protection against cell damage also protects the cell from aging and tumour development.

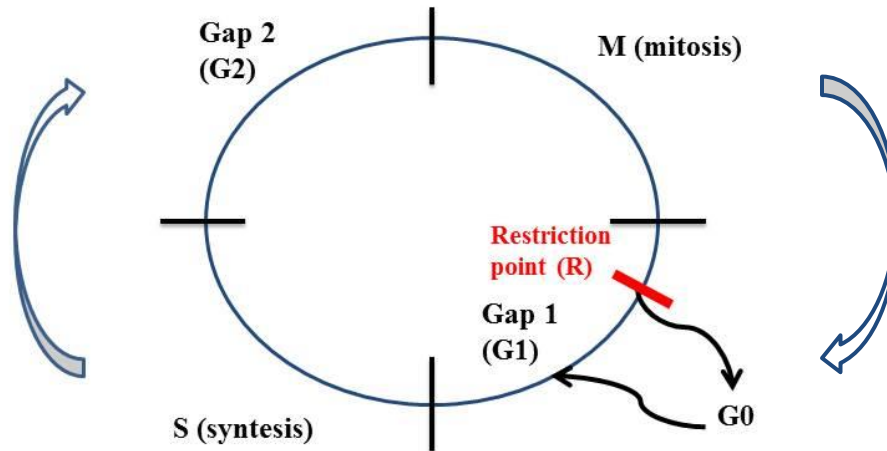


Figure 1. Overview of the cell cycle

Critical checkpoints

$G1_{pm}$ and $G1_{ps}$

The G1 phase can be divided into two parts, a constant post mitotic phase ($G1_{pm}$) where the cell spends approximately 4 hours, and a more variable pre synthetic phase ($G1_{ps}$) (Larsson et al., 1985a) (fig. 2). Only when the cell is located in the $G1_{pm}$ it has the opportunity to leave the cell cycle and enter G0 in response to the absence or presence of different growth factors. Hence $G1_{pm}$ decides if a cell should continue through the cell cycle or not.

Cells in the $G1_{ps}$ are already committed to DNA synthesis and are resistant to external growth factors. In contrast to $G1_{pm}$, during $G1_{ps}$ the cell decides when it is going to enter the S phase and the duration in the $G1_{ps}$ varies between different cells (Larsson et al., 1985a). The rationale for this variability is that a small cell stays longer in the $G1_{ps}$ than a large cell, probably because the differences in size between cells must be adjusted before entering the S phase (Larsson et al., 1985b).

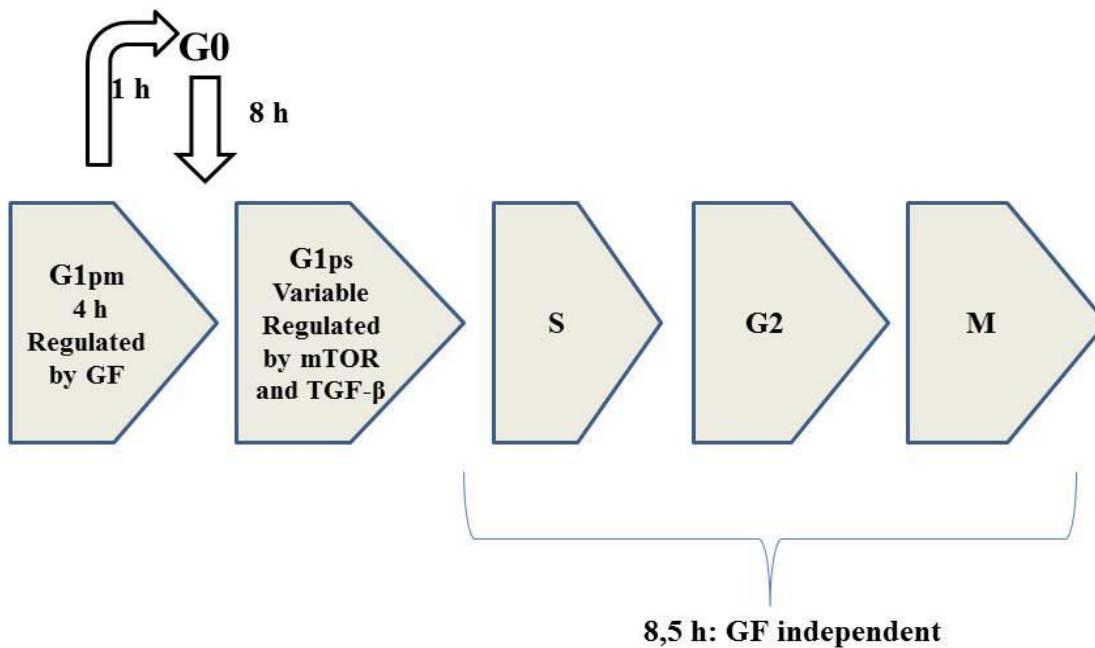


Figure 2. Regulation and duration of $G1_{pm}$ and $G1_{ps}$

Gulliver (2013) has launched an interesting theory which aims to explain the decision whether a cell leaves $G1_{pm}$ to arrest or continues to $G1_{ps}$. The decision requires a machinery that is influenced by several signals, each of them depending of each other like the layers of an onion. Inmost we have the transcribed and translated proteins encoded by the S genes. This depends on Rb phosphorylation status and E2F binding. That in turn depends on CDKs and other inhibitors in different phases of the cell cycle. Furthermore, this depends on growth factors and growth inhibitors also in the different phases that then depend on the presence of tumour suppressor and proto-oncogenes. Outhermost we have the influence of hormones.

However, there is another theory that contradicts this “indecision theory” that predicts that the cell already has the machinery to promote cell cycle progression or arrest and just has to be driven by endogenous and exogenous factors. These factors (e.g. hormones or their imitators) regulate the machinery at different phases in the cell cycle. When the G1 and S phase are regulated they impact on the proliferation rate and cell enlargement. These factors can also modify the response to inhibition signals and facilitate sustained cell proliferation. Factors that regulate mitosis, e.g. oestrogen, are dependent on the endogenous hormone level in the body and that in turn depends on several things: the cells capacity for hormone production, ultimate microenvironment for hormone production, existence of receptors for specific hormones, higher expression of pro-mitogenic receptors than anti-mitogenic receptors and the age (Engström, 2013).

G1 to S transition

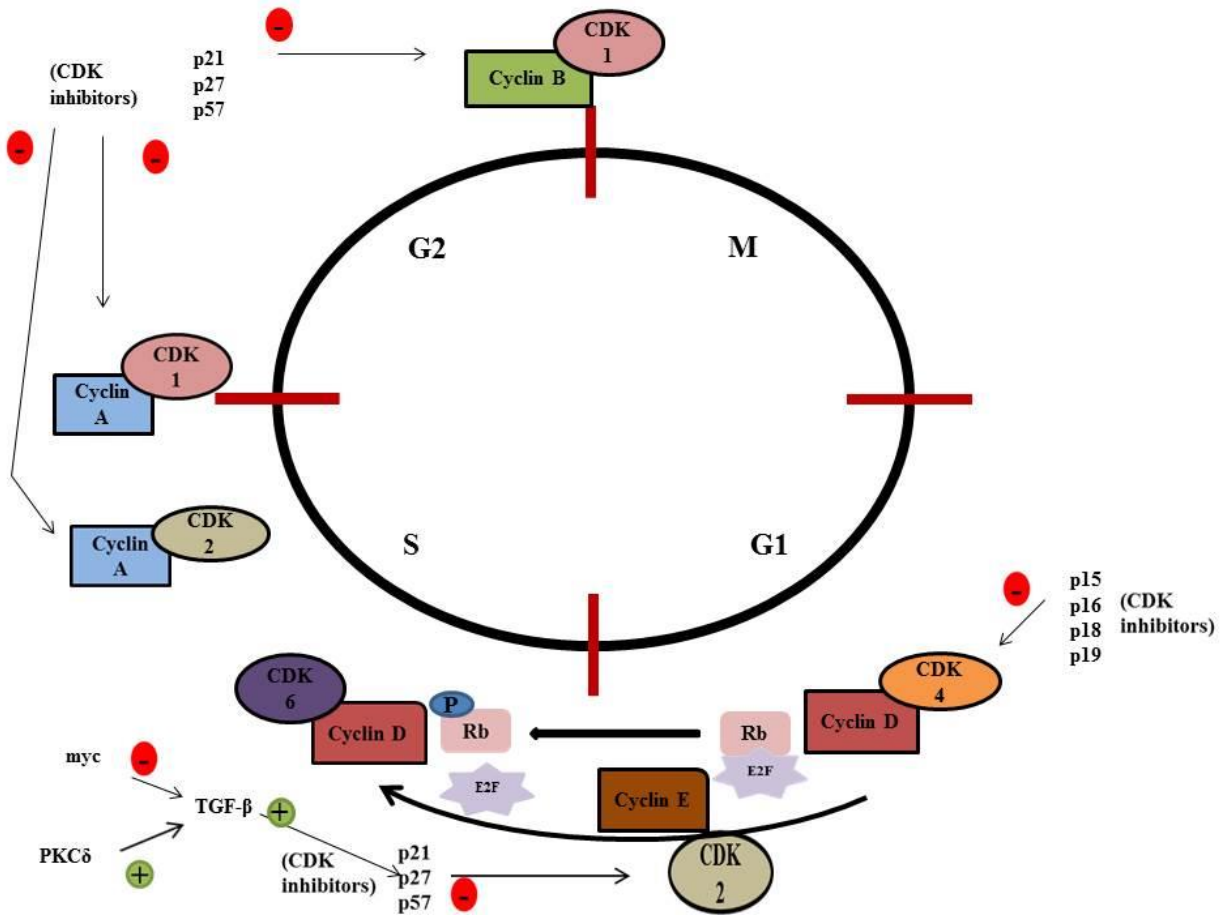
To progress through the G1 phase the cell has to pass two restriction points (Foster et al., 2010). The first one called R1, sites at the end of the G1_{pm}, controls the chromosomal cell cycle and is growth factor dependent. When the cell passes this it is programmed to progress the cell cycle to the next mitosis. R1 corresponds to the original restriction point (R) (Pardee, 1974). Up to this point the cell could be exposed to a variety of stimuli, factors and components that affect the outcome and the cell can decide if it would like to arrest in the G0 phase. The second restriction point, R2, is located in the G1_{ps} interval and is referred to as a “cell growth” checkpoint because it is dependent of nutritional supply. This “cell growth” checkpoint controls the cellular enlargement (growth cell cycle) and is regulated by mTOR (the mammalian target of rapamycin) and TGF- β (transforming growth factor β) and it is distinguished from R1 both in a temporal and genetic fashion (Foster et al., 2010). Moreover, tumour cells have lost their ability of adequately responding to the control mechanisms of both restriction points and progress through the cell cycle.

According to classic findings, two growth factors with dual roles were used for an experimental approach to dissect different control points in the cell cycle. When a cell is arrested in G0 it is dependent on platelet-derived growth factor (PDGF) to re-enter the cell cycle, but for the following progression through G1_{ps} it is dependent on insulin-like growth factor-1 (IGF1) (Zetterberg and Killander, 1965). Hence, it is reasonable to assume that PDGF increases levels of cyclin D whereas IGF1 increases PI3K and mTOR, thereby passing both of the checkpoints required for G1 to S transition (Glass, 2010).

Cyclin-dependent kinases (CDKs) and cyclins are required for progression from G1 to S phase. Cyclin-dependent kinases form complexes with diverse cyclins that are specific for each phase of the cell cycle (fig. 3). It is above all the CDK1 (also called CDC28) that is regulated by cyclins and affect processes necessary for the cell cycle progression (Koltovaya, 2013).

Although different cell types progress through the cell cycle in response to different signals, they all have one thing in common, they still have to activate CDKs (Massagué, 2004).

Cyclin D and E are important for the progression through the G1 phase. Cyclin D peaks in the G1_{pm} phase and cyclin E in the G1_{ps}. Mitogenic stimuli increase the level of cyclin D which form a complex with CDK4/6. This complex hypophosphorylates Rb and after this cyclin D-dependant phase the cyclin E/CDK2 complex is activated. Cyclin E/CDK2 hyperphosphorylates and inactivates retinoblastoma (Rb) which makes it possible for the transcription factor E2F to be activated. E2F activates the transcription of cyclin E and other compounds (e.g. cyclin D, c-myc and DNA polymerase) that are needed for S phase progression and subsequent DNA replication (Massagué, 2004; Mayank et al., 2014).



Figur 3. Cyclin, CDKs and its inhibitors

The Cdk activity is low in the G0 and early G1 phase, thus Rb stays hypophosphorylated and binds E2F and transcription cannot be initiated (Mayank et al., 2014).

Cyclin D levels peaks at R1(Harbour et al., 1999).

Cyclin E forms a complex with CDK2. There are two types of cyclin E, Cyclin E1 and E2. In normal cells cyclin E1 peaks in the G1/S phase and is thereafter deregulated by the SCFFbw7 ligase complex during the S and G2/M phase. In transformed cells this pulsatile expression is deregulated which results in high levels of cyclin E1 in the S phase that in turn leads to abnormal proliferation and genomic instability. Cyclin E2 is also uncontrolled in transformed cells and displays an enhanced and continuous expression during the S phase.

Until recently, the knowledge was limited to the influence of cyclin E1 on tumourigenesis and thereby potential therapeutic routes were directed against cyclin E1 as well. Recent studies have shown that also cyclin E2 has a great impact because of the differences in regulation during the S phase between cyclins E1 and E2. The quantity of cyclin E1 mRNA and cyclin E2 mRNA differs in various cancer cells and are thought to be independently expressed (Caldon et al., 2013).

Cyclin E is degraded through ubiquitin mediated proteolysis by an ubiquitin ligase called SCFFbw7 that binds to cyclin E. This binding depends on the phosphorylation of the site where degradation starts who is located at the C- and N-terminal of cyclin E. If the phosphorylation

doesn't begin at these sites cyclin E cannot be degraded, This has been observed in tumour cells and promotes sustained proliferation (Minella et al., 2008).

In normal cells there are CDK inhibitors that inhibit cyclin-cdk complexes when they are overexpressed (fig. 3). The CDK inhibitors p15, p16, p18 and p19 regulate cyclin D/CDK4/6 and p21, p27 and p57 inhibit cyclin E/CDK2 (Sherr and McCormick, 2002). Sherr and McCormick, (2002) also discuss that the cyclin E/CDK2 inhibitor p27 even affect cyclin D/CDK4/6. When cyclin D increases, p27 is sequestered which activate cyclin E/CDK2 and influences it to release p27 that in turn inhibit cyclin D/CDK4/6. However, there is one example of a theory where cyclin D does not need to sequester p27 to activate cyclin E. Instead a hypothetical feedback loop where cyclin E/CDK 2 phosphorylates p27 to make it more sensitive to ubiquitination and degradation by the proteasome at the same time as the complex also inactivates Rb and activates E2F which in turn increases cyclin E, has been proposed. In conclusion, the signals have reached full circle and progression towards the S phase has been enable (Martinsson et al., 2005). Another inhibitor of cyclin E/CDK 2 in G1_{ps} is the transforming growth factor- β (TGF- β) which acts by elevating p27 (Massagué, 2004) (fig. 3). Hence TGF- β induces cell cycle arrest but only in the absence of myc since the upstream signals of myc, mTOR and PLD, inhibit TGF- β . Tumour promoters (e.g. phorbol esters) that induce progression through G1 and are activated by Ras require the inhibition of protein kinase C δ (PKC δ) in order to progress to S phase. Thus, when PKC δ is activated the TGF- β mediated cell cycle arrest is operational (Lu et al., 1997) (fig. 3).

The tumour suppressor gene LIMD1 binds to Rb and prevents phosphorylation and inhibits E2F release. Therefore, cell cycle arrest and downregulation of S phase gene expression are induced, indicating the important role of LIMD1 as a regulator of the cell cycle.

In the nucleus Rb is hypophosphorylated when it is stimulated by growth suppressing signals. LIMD1 can bind to Rb, but LIMD1 also has the capability to move between the nucleus and cytoplasm, which points at an interesting theory that it is possible for cytoplasmic cell cycle specific signals to enter the nucleus and affect Rb (Mayank et al., 2014). However, further studies are required to understand the specific mechanisms concerning LIMD1s roll as an Rb- and cell cycle regulator especially if LIMD1 directly competes with cdk for the binding site. Only 50 % of the E2F suppression can be explained by LIMD1 binding to Rb. Thus further insights independent of Rb are needed to explain the other aspect of the E2F suppression.

Protein phosphate 2A (PP2A) is a tumour suppressor that regulates the G1/S transition and is inhibited by Simian Virus 40 (SV40) small T antigen. The complex role of PP2A in tumourigenesis motivate further research about the requirement for transformation of cells, thus it is interesting to investigate mechanisms which compensate the lack of PP2A (Boehm et al., 2005).

M phase

There is upcoming evidence that unsolved and incompletely replicated chromosomes in S phase remain unfinished when the cell progresses to the M phase. In the M phase these uncompleted DNA structures constitute a threat to the chromatid segregation and are thought to have an impact on human disease and aging. Moreover, it has been shown that the yeast *Saccharomyces Cerevisiae* can enter M phase before DNA-replication is completed (Mankouri et al., 2013).

There are four potential theories why cells enter M phase even though the S phase is not absolutely completed. Firstly, the S phase checkpoint cannot detect small regions of unreplicated DNA. Secondly, some regions of the genome with important structural functions (e.g. centromere and telomerase) are inherently difficult to replicate and segregate together with the rest of the DNA. Thus, cells have learned to deal with this inevitable situation and may also take advantage of it. Thirdly, it might be a fundamental aspect of a normal cell cycle progression and accomplish important biological functions.

Fourthly, the M phase is considered more hazardous than the S phase and the inability of a total DNA replication may be a minor concern in the overall context. When the cell commits to mitosis in the G1 phase it will continue as quickly as possible to the M phase in order to minimise the risk for chromosomal defects. Moreover, cells are able to correct unresolved problems in the subsequent G1 phase.

This theory arouses an interesting possibility that suppressing mitotic defects by promoting mitotic deviation may result in healthy aging (Mankouri et al., 2013).

Intracellular regulatory pathways

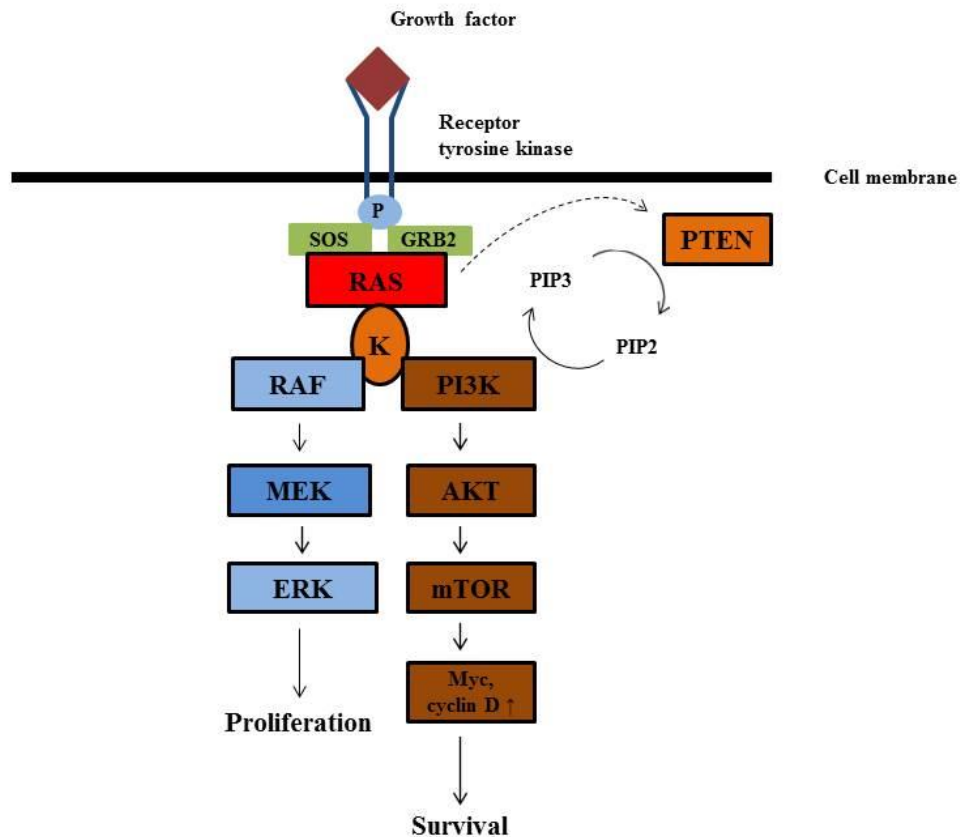
To influence the cell with different stimuli, e.g growth factors, signals have to be transmitted by intracellular pathways from the extracellular area to the nucleus. Receptors are connecting the outside and inside of the cell. A major group of receptors are the type 1 growth factor receptors (ERBB/HER) that correspond to epidermal growth factor (EGF) stimuli and promote cell proliferation. Every EGF binds to a specific receptor. ERBB consists of an extracellular ligand-binding domain that is connected with an intracellular tyrosine kinase domain (TK) that in turn affects a cascade of proteins in the intracellular pathway. To activate the TK and thus also getting the cascade effect, a ligand containing an EGF-like domain has to bind to the extracellular part of ERBB inducing a conformation change that facilitates dimerization between the receptors that in turn phosphorylate the downstreaming proteins. ERBBs affect the following downstreaming pathways: PI3K/Akt, Ras/MAPK, PLC γ 1/PKC, STAT and Par6-atypical PKC, all of them influencing the cell by e.g inhibition of apoptosis, promoting proliferation, differentiation and angiogenesis. Mutations and oncogenetic presence in these pathways leads to dysregulation of these functions, hence tumour development is a common result (Wieduwilt and Moasser, 2008).

Ras

Ras transduces intracellular signals and is activated by a variety of growth factors (Westermarck and Hahn, 2008). Moreover, the *Ras* oncogene could activate mutations that are considered to be the first proliferation signal upon binding of a growth factor to its receptor. Transformed Ras elicitate intracellular signals in the absence of growth factor receptor activation and increase the cyclin D levels (Harbour et al., 1999). In conclusion, tumour transformation induced by Ras mutation is dependent on cyclin D.

In normal cells when growth factors activate the Ras/Raf pathway a protein kinase cascade is induced that in turn increases cyclin D, myc and PTEN (phosphatase and tensin homolog) expression (Gille and Downward, 1999) (fig. 4). In addition, the Ras/Raf pathway includes the protein kinase mTOR whose activity increases myc expression and cyclin D activity.

PTEN is a tumour suppressor that catalyses the reaction from phosphatidylinositol-3,4,5-tri-phosphate (PIP3) to phosphatidylinositol-4,5-bis-phosphate (PIP2) and the reverse reaction is catalysed by PI-3-kinase (PI3K) (Yuan and Cantley, 2008, p. 2) (fig. 4). PI3K activity is common in human cancer and probably plays an important role in cell cycle progression since it increases PIP3 in the absence of PTEN (Manning and Cantley, 2007). Furthermore, PIP3 recruits pleckstrin homology (PH) domains e.g. phosphoinositide-dependent kinase 1 (PDK1) that in turn phosphorylates Akt which activates mTOR and induces G1 progression (Huang and Manning, 2008).



Figur 4. Intracellular signaling by receptor activation of Ras

Amongst unresolved issues are the overlapping signals that alleviate $G1_{pm}$ to $G1_{ps}$ transition. Ras is the most important factor since it activates multiple downstreaming proteins and pathways e.g. Raf/MEK/MAP kinase pathway, PI3K, mTOR, Ral-GDS, PLD, cyclin D, c-myc. Therefore Ras should be able to dysregulate R1 and R2.

Both Ras and myc are required for cell transformation. Ras activates myc expression and could not transform primary cells without myc.

Cyclin D- null mice are resistant to transformation by Ras but not by myc. This indicates at the importance for Ras to activate downstreaming pathways.

In addition, the most important target of Ras is the Raf/MEK/MAP pathway to stimulate cyclin D and $G1_{pm}$ to $G1_{ps}$ passage.

Myc

C-myc plays an interesting and important role in cell cycle control because of its involvement in several different pathways that are influenced by external and internal signals. C-myc acts in concert with other factors on cell metabolism, proliferation, apoptosis and differentiation. For instance, c-myc increases the level of CDK 4, cyclin D, cyclin E and inhibits p27.

Growing cells show a higher expression of c-myc than arrested cells. Tumour cells that are growing faster than normal cells express higher c-myc activity. In transformed cells c-myc promotes a circuit, it induces chromosomal translocation or gene amplification which in turn activates more c-myc expression (Dang, 1999).

Src

C-Src is a non-receptor tyrosine kinase which phosphorylates other proteins in order to induce proliferation, differentiation, angiogenesis and/or cell to cell adhesion. These multifaceted functions facilitate the promotion of metastatic changes (Yeatman, 2004).

To activate different signals (e.g. proliferation and differentiation) Src has to interact with different factors e.g. cell surface receptors and steroid hormone receptors (Ishizawa and Parsons, 2004). Src is activated by phosphorylation on the Tyr-416 residue and inhibited by phosphorylation on Tyr-527 which induces a binding to the SH2 domain making the kinase inactive (fig. 5). Moreover elevated levels of c-Src expression are shown in many different tumour forms.

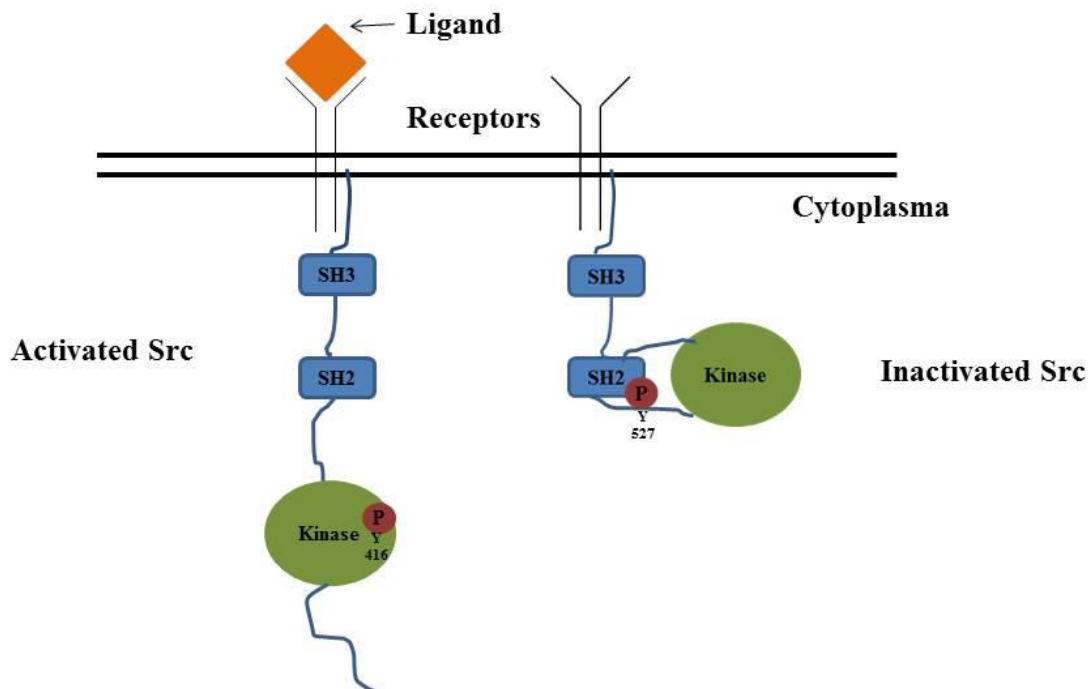


Figure 5. Activation of Src

The mammalian target of rapamycin (mTOR)

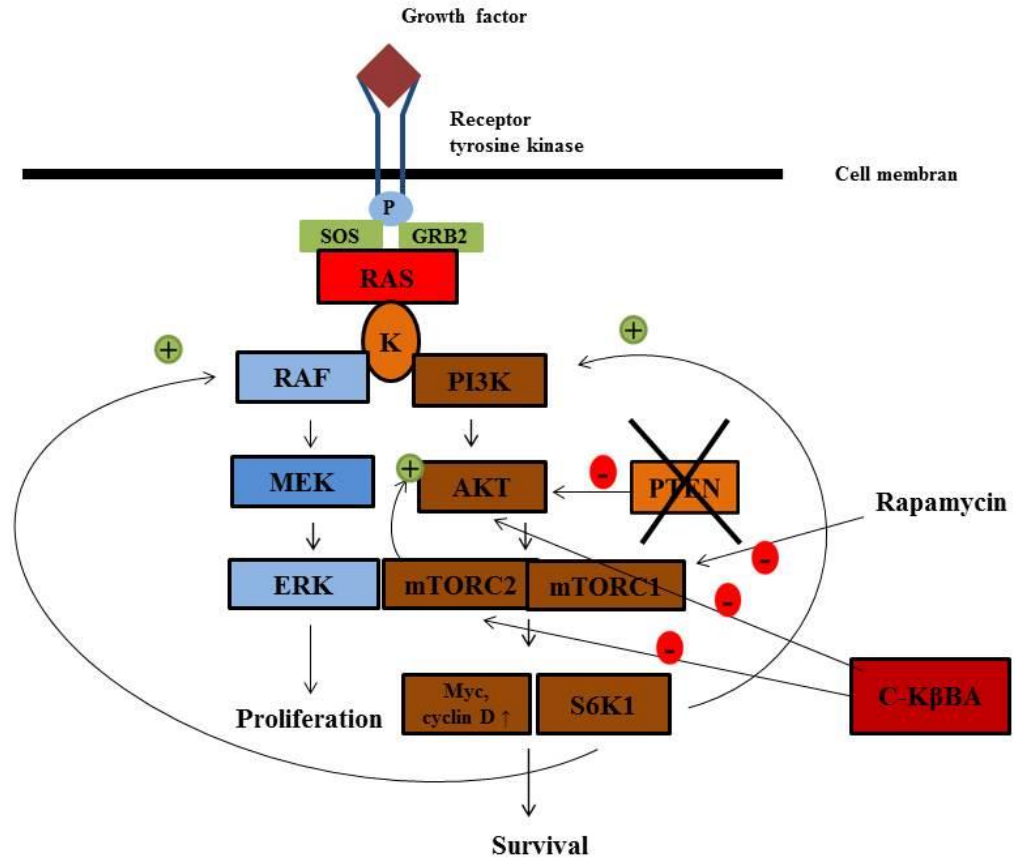
mTOR is a serine/threonine protein kinase which regulates important cell functions e.g. growth, survival, proliferation and transcription (Hay and Sonenberg, 2004). Hence, mTOR plays a major role in G1 phase. Moreover dysregulated mTOR is a common phenomenon observed in tumours. The mTOR is an important regulator of R2, thus also the transition through G1_{ps} (Foster et al., 2010) (cf above). mTOR is activated by amino acids and cell cycle arrest in G1_{ps} is induced when the level of amino acids is low. Re-entry to the cell cycle at R2 when amino acids levels increase takes about two hours compared with 12 hours at the restriction point affected by growth factors (Zetterberg et al., 1995).

One factor that changes the normal mTOR expression is Rheb – a GTPase. Rheb activates mTOR and originates from the Ras pathway, indicating that Ras and mTOR act in concert in transformation of normal cells (Sun et al., 2008).

The mTOR inhibitor C-K β BA (3-cinnamoyl-11-keto- β -boswellic acid) is a new derivate indicating proapoptotic and antiproliferative activity on prostate cancer cell lines (PC-3). C-K β BA acts cytotoxic and triggers apoptosis in PC-3. PC-3 lacks PTEN which is a negative regulator of Akt and that, in turn, leads to activation of Akt (fig. 6). When PTEN is overexpressed in PC-3 it's notable that PC-3 becomes less sensitive to C-K β BA proposing that the aim target of C-K β BA might be Akt or downstream factors e.g. mTOR. Moreover, C-K β BA reduces cyclin D1 and thus makes the cell arresting in G1 phase (Morad et al., 2013).

Next, the mTORs activity is regulated by ligands e.g. phosphatidic acid and rapamycin who bind to the part at mTOR called the FRB domain. It is showed that the C-K β BA also binds to the FRB domain and even harder than the ligands which allow competition between these (Morad et al., 2013).

Morad et al., (2013) also discuss the importance of finding a better mTOR inhibitory-therapy against cancer that can overcome the limitations of rapamycin. Rapamycin cannot prevent the upregulation of the MEK-ERK and PI3K/Akt signalling pathway as a result of the S6K1-mediated feedback loop and the mTORC2 activation of Akt that in turn result in prosurvival and proliferative signals that repress the effect of rapamycin (fig. 6). However, C-K β BA differs here in a positive way by lacking this activation of the Akt feedback loop. This probably depends on a partial inhibition of the mTORC2, a subunit of mTOR, or directly inhibition of Akt (fig. 6).



Figur 6. Effects of C-K β BA in PC-3 cells

DISCUSSION

The cell cycle concept has been around since mitosis in a growing cell population was discovered. The discovery of the S-phase by Howard and Pelc (1951) was paramount for the understanding of the interphase, ie the time spent between two cell divisions. During the 1980:s a lot of interest was focussed on the variability in intermitotic time between individual cells (Brooks et al., 1980) as well as the regulatory steps in G1 (Larsson et al., 1985a, 1985b; Pardee, 1974). Later research pointed at cyclins and cdk:s as key operators in the regulation of the cell cycle (Evans et al., 1983; Hartwell, 1967; Nurse et al., 1976).

The cell cycle can be regulated by a variety of external factors, e.g. growth factors and cytokines normally operating through membrane bound receptors that transduces a signal to the cell nucleus (Massagué, 2004). The G1 phase is the most important phase where external signals play different roles in different subsections. Two restriction points R1 and R2 are cyclin regulated checkpoints that need to be passed in order to initiate DNA-synthesis in the S-phase. Transformation of normal cells to tumour cells therefore needs to dysregulate both R1 and R2, that in turn are regulated by cyclin D and cyclin E (Foster et al., 2010).

Once the signal has reached the interior of the cell membrane, Ras is activated. Ras is an example of a protein that induces overlapping signals and activates multiple downstream targets (Gille and Downward, 1999). Several of them result in activation of the mTOR pathway, thereby regulating R2 and consequently affect enlargement, survival, proliferation. Ras can also act through its most important target, the Raf/MEK/MAP kinase pathway and elevate cyclin D levels and in doing so, it also regulates R1.

The normal defence against unwarranted progression through G1 is either differentiation, apoptosis (programmed cell death) or cell senescence (ageing) (Hanahan and Weinberg, 2011). When a cell is transformed, all these defence mechanisms are incapacitated. It is important to consider also antiapoptotic mechanisms as well as immortalisation as important hallmarks of transformation.

A more fundamental issue is why mammalian cells are imperfect in the first place. It has been known for a long time that the time cells spend in interphase varies considerably. In this respect they differ from lower organisms, e.g. the slime mould *Physarum polycephalum* where nuclear division is more or less perfectly synchronised (Loidl and Sachsenmaier, 1982). However there are some interesting observations. When two daughter cells, i.e. siblings, were studied with respect to intermitotic time, it was found that their differences were smaller than when cells were compared at random (Brooks et al., 1980). Moreover, we now know that the cell possesses delicate machineries to repair defects in DNA-sequence and can correct mutations quite swiftly. We also know that the cell cycle machinery will be used at great length to ensure that the cell size homeostasis is upheld in a cell population (Zetterberg and Killander, 1965).

Although G1 is considered to be the main regulatory phase and very much the target responsible for tumour transformation, dysregulation in other phases may also have an impact on tumour development. For instance, unresolved and incompletely replicated chromosomes in the S phase that progress to the M phase (Mankouri et al., 2013).

In conclusion, there are still remaining issues including the enigmatic overlapping signals in G1 but also black holes in our understanding of the other phases. An improved understanding of these key regulatory issues will facilitate the development of improved diagnostic features as well as novel therapies against cancer.

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