Short Communication

Many Ways to the Cell Cycle Exit after Inhibition of CDK4/6

(cell cycle / cell growth / cyclin-dependent kinase / senescence / CDK4/6 inhibitor)

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Abstract. Cyclin-dependent kinases (CDKs) are master regulators of proliferation, and therefore they represent attractive targets for cancer therapy. Development of selective CDK4/6 inhibitors including palbociclib revolutionized the treatment of advanced $HR^+/HER2^-$ breast cancer. Inhibition of CDK4/6 leads to cell cycle arrest in G0/G1 phase and eventually to a permanent cell cycle exit called senescence. One of the main features of the senescence is an increased cell size. For many years, it was believed that the non-dividing cells simply continue to grow and as a result, they become excessively large. There is now emerging evidence that the increased cell size is a cause rather than consequence of the cell cycle arrest. This review aims to summarize recent advances in our understanding of senescence induction, in particular that resulting from treatment with CDK4/6 inhibitors.

Progression through the cell cycle is governed by various cyclin-dependent kinases (CDKs), including CDK4/6, which promote progression through the G1 phase, CDK2, which triggers replication, and CDK1, which is essential for entry to mitosis. Given the importance of CDK activity for proliferation, CDKs are attractive pharmacological targets for cancer therapy. CDK4/6 inhibitors including palbociclib have recently entered clinics and are now being used for treatment of advanced hormone receptor-positive (HR^+), human epidermal growth factor receptor 2-negative ($HER2^-$) breast cancers (Fassl et al., 2022; Morrison et al., 2023). It is well established that extended CDK4/6 inhibition leads to a permanent cell cycle exit called senescence, and the underlying molecular mechanisms represent an active field of research (Wagner and Gil, 2020; Schmitt et al., 2022; Wang et al., 2022a, b) (Fig. 1).

Under normal circumstances, the cell size progressively increases during the cell cycle and culminates by cellular division that gives rise to two daughter cells of comparable size. Thus, the cell growth and proliferation are usually closely associated (Björklund, 2019; Zatulovskiy and Skotheim, 2020). The homeostatic cell size is maintained by activation of mitogen-activated protein kinase p38 (p38MAPK aka p38) in small cells, which extends the duration of G1 to allow cell growth (Liu et al., 2018; Tan et al., 2021). As the cell grows, the cytoplasmic concentration of Rb protein (or another cell cycle inhibitor Whi5 in yeast) drops and eventually results in progression to S phase (Schmoller et al., 2015; Zatulovskiy et al., 2020). Blocking the cell cycle progression may disconnect the tight regulation of cell growth, allowing the arrested cell to acquire cell mass. Indeed, in the presence of palbociclib, cells approximately double their size within two days and triple within four days (Foy et al., 2023). This cellular outgrowth depends on the mammalian target of rapamycin (mTOR) pathway and can be completely blocked by mTOR inhibitors (Crozier et al., 2023; Foy et al., 2023). Interestingly, the increased cell size is recognized as cellular stress. Several recent studies reported a causal connection between the abnormal cell size and activation of tumour suppressor p53, which triggers expression of p21 and subsequently the cell cycle arrest (Crozier et al., 2023; Foy et al., 2023; Manohar et al., 2023). These enlarged G1 cells are negative for markers of DNA damage (including γ-H2AX and 53BP1 nuclear foci), indicating that p53 activation is not primarily caused by genotoxic stress (Crozier et al., 2022; Wang et al., 2022a). Instead, enlarged G1 cells show increased activity of the p38 kinase, which acts upstream of p53 and

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Abbreviations: 53BP1 – TP53-binding protein 1, APC/C – anaphase-promoting complex/cyclosome, CDKs – cyclin-dependent kinases, g-H2AX – histone H2AX phosphorylated at Ser139, $HER2^-$ – human epidermal growth factor receptor 2-negative, HR^+ – hormone receptor-positive, MCM4/5/6 – DNA replication licensing factor MCM4/5/6, mTOR – mammalian target of rapamycin, p38MAPK – mitogen-activated protein kinase p38, RB1 – retinoblastoma-associated protein.

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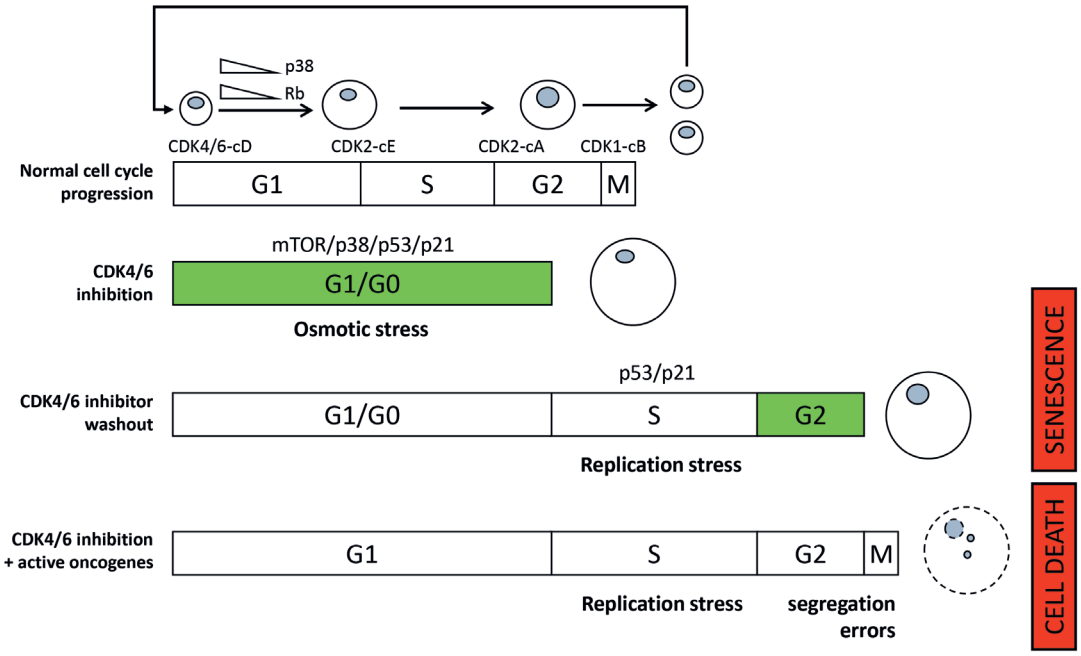

Fig. 1. Cell cycle progression depends on sequential activation of CDKs. The activity of p38 and mTOR promotes cell growth in G1 phase. Cells transit to S phase upon inactivation of Rb by CDK2-dependent phosphorylation and by a decrease of its concentration in growing cells. Upon inhibition of CDK4/6, progression through G1 is blocked, but cells continue to grow. Osmotic stress in enlarged cells leads to activation of p53/p21 and promotes permanent cell cycle exit. Cells that escape this block (after removal of CDK4/6 inhibitor or cells with increased oncogenic signalling) experience replication stress during S phase, activate p53/p21, and subsequently exit the cell cycle in G2. Cells that fail to arrest in the checkpoint accumulate massive DNA damage and segregation errors and are eliminated by cell death.

triggers the arrest (Crozier et al., 2023). The primary reason for p38 activation seems to be the osmotic stress arising from the changed composition of the cytosol during excessive cellular outgrowth (Crozier et al., 2023).

In extremely enlarged cells, the nuclear DNA is no longer sufficient to maintain the level of transcription proportional to the cell size, which eventually leads to a phenomenon called cytoplasmic dilution (Neurohr et al., 2019). This global change in proteomic composition of the cytosol of enlarged cells is evolutionarily conserved from yeast to human and contributes to induction of senescence (Neurohr et al., 2019; Lanz et al., 2022). Large proteomic screens identified hundreds of proteins that become sub-scaled (including RB1, MCM proteins, HGB2 and ribosomal components) or super-scaled (including CDC27, CYB5R3, endoplasmic reticulum proteins, lysosomal proteins) during the cell growth; nevertheless, the precise mechanisms linking the global proteome changes with the cell cycle arrest still remain to be defined (Cheng et al., 2021; Zatulovskiy et al., 2022).

Upon removal of CDK4/6 inhibitors, a fraction of quiescent cells re-enter the cell cycle and renew the proliferation (Foy et al., 2023). However, cells that experienced an extended G0 arrest typically pass through the rest of the cell cycle with difficulties. For instance, cells recovering from G0 arrest show significantly slowed down replication, possibly due to decreased expression of proteins involved in pre-replicative complex assembly (such as MCM4/5/6) and nucleotide metabolism (such as RRM2) (Crozier et al., 2022, 2023). As a consequence, these cells activate p53 and p21 in the subsequent G2 phase and exit the cell cycle with 4N DNA content (Crozier et al., 2022, 2023). Similar replication defects were previously reported in cells re-entering the cell cycle from contact inhibition-induced quiescence, suggesting that impaired replication is not a consequence of enlarged cell nuclei but rather reflects incomplete licensing of replication origins (Matson et al., 2019). The cell cycle exit from G2 depends on activation of the p53/p21 pathway, which leads to premature activation of anaphase-promoting complex/cyclosome (APC/C) and degradation of cyclin B (Krenning et al., 2014; Müllers et al., 2014). In addition, CDK4/6 have recently been implicated in sustaining expression of *CCNA2*, which is a prerequisite for activation of CDK2, indicating that inhibition of CDK4/6 may also directly induce the cell cycle exit from G2 (Cornwell et al., 2023).

Several recent studies have demonstrated that increased oncogenic signalling (such as activation of Ras or c-Myc) allows cells to progress through the cell cycle in the absence of CDK4/6 activity (Foy et al., 2023; Zhang et al., 2023). Such override of the cell cycle arrest may increase cytotoxicity in cancer cells due to massive accumulation of DNA damage. Recent advances in un-

derstanding the molecular mechanisms underlying these events may facilitate development of rational combinations of CDK4/6 inhibitors in cancer therapy.

Conflict of interests

No conflict of interests exists.

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