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# Cyclin E in normal physiology and disease states

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## Abstract

E-type cyclins, collectively called cyclin E, represent key components of the core cell-cycle machinery. In mammalian cells two E-type cyclins, E1 and E2, activate the cyclin-dependent kinase CDK2 and drive cell-cycle progression by phosphorylating several cellular proteins. Abnormally elevated activity of cyclin E-CDK2 has been documented in many human tumor types. Moreover, cyclin E-overexpression mediates resistance of tumor cells to various therapeutic agents. Recent work reveals that the role of cyclin E extends well beyond cell proliferation and tumorigenesis, and it may regulate a diverse array of physiological and pathological processes. In this review we discuss these various cyclin E functions and the potential for therapeutic targeting cyclin E and cyclin E-CDK2 kinase.

### Keywords

cyclin E; cyclin-dependent kinases; CDK2; cell-cycle; cancer

## Regulation of the Cell-Cycle by E-cyclins: Mechanisms and Functions

**Cyclin** E functions during the **G1 and S-phases** of the **cell-cycle**. It was cloned as a cDNA that can rescue the proliferative block in *Saccharomyces cerevisiae* lacking cyclins CLN1–3, and later renamed cyclin E1. Cyclin E2 was discovered based on amino-acid sequence similarity to cyclin E1 [1]. Cyclins E1 and E2 (collectively called 'cyclin E' or 'E-cyclins') are expressed in all proliferating cell types, and share significant amino-acid sequence similarity (47% within the whole protein, 75% within the most conserved cyclin-box domain) [2]. The two E-type cyclins are thought to play largely overlapping functions, while in a few instances a specific role has been ascribed to a particular E-cyclin (see below). It is unclear whether these differences reflect distinct molecular functions of cyclin E1 versus E2, or different, cell type-specific regulation of their expression.

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E-cyclins bind and activate the **cyclin-dependent kinase** 2 (CDK2). Crystal structure of truncated cyclin E1 in complex with CDK2 revealed that cyclin E1 binds CDK2 mainly through the C-(PSTAIRE) helix and activation segment of CDK2. There are multiple contact residues on cyclin E protein, including the C-terminal part of the cyclin box, which makes additional contacts with CDK2 thereby strengthening the cyclin E-CDK2 interface [3]. Cyclin E can also activate CDK1 and CDK3 [1, 4]. Catalytically active E-CDK1 kinase was detected in cells of CDK2-knockout mice, and it was proposed that CDK1 compensates for the lack CDK2 in CDK2-null setting [5]. The biological significance of cyclin E-CDK1 and E-CDK3 in normal cells remains unclear.

During G1 phase progression of the somatic cell cycle, cyclin D-CDK4/6-mediated phosphorylation of the retinoblastoma protein (RB1) partially inactivates RB1's repressive function, leading to de-repression or activation of E2F family transcription factors. E2Fs, in turn, promote transcription of cyclin E genes. Once induced, cyclin E binds and activates CDK2. E-CDK2 kinase drives additional phosphorylation (hyperphosphorylation) of RB1, thereby increasing E2F activity and further transcriptionally upregulating cyclin E expression. This sequence of events forms an autoregulatory, positive-feedback loop that inactivates RB1 and sets in motion the E2F-dependent transcriptional program which is required for entry of cells into DNA synthesis (S-phase) (reviewed in [1, 4]). In addition to inactivating RB1, cyclin E-CDK2 plays several other important functions during late G1 and in S-phase, summarized in Box 1. Cyclin E-CDK2 kinase is regulated by the KIP/CIP family of cell cycle inhibitors ($p21^{CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$), which directly bind the complex and inhibit its catalytic activity. Cyclin E-CDK2 can phosphorylate and target for degradation its inhibitor, $p27^{KIP1}$, thereby amplifying CDK2-kinase activity.

As cells proceed through the S-phase, E-cyclins become degraded by the proteasome. The major degradation pathway involves Skp1-Cul1-F-box-protein (SCF) ubiquitin ligase. Degradation of cyclin E by this pathway is triggered by phosphorylation of cyclin E by CDK2 or GSK3. This creates a phosphodegron which is recognized by an F-box protein FBW7, a substrate recognition subunit of SCF (reviewed in [1]). In a complementary degradation pathway, the BTB-Cul3-Rbx1 ubiquitin ligase preferentially binds free cyclin E [6]. Cul3 recognizes cyclin E via the degron that is located within the N-terminal part of cyclin E [7].

Growing evidence indicates that in addition to their well-established cell-cycle roles, E-cyclins control a much wider array of physiological processes (Figure 1). In this review, we discuss various functions of E-cyclins, involvement of cyclin E-aberrations in disease states, and the utility of targeting cyclin E-CDK2 in treatment of cancer and possibly of other diseases.

## Cyclin E in Physiological Processes

### Cyclin E in Development

Cyclin E plays an essential role in development of several different organisms, as revealed by analyses of cyclin E loss-of-function mutants. In *C. elegans*, mutations of *cye-1* gene (encoding cyclin E) lead to cell-division defects in vulva and in somatic and germline

gonad tissues. *cye-1* mutants display underproliferation of many postembryonic blast lineages, severely diminished gut-cell **endoreplication**, and defects in fertility [8]. Also in *Drosophila*, cyclin E (DmcycE) is required for progression through the S-phase during embryogenesis. DmcycE loss-of-function mutants undergo first 16 divisions (likely due to the presence of maternally-encoded cyclin E), but fail to enter the S-phase thereafter. At later stages, no DNA-synthesis was detected, and endoreplication was abolished [9]. Proper downregulation of cyclin E was shown to be critical for cell-cycle exit; ectopic expression of DmcycE after the final mitosis resulted in progression through a complete additional cell-cycle [9]. In **eye imaginal discs**, ectopic expression of DmcycE triggered premature entry into S-phase and disrupted normal development [10].

In mice, the two E-cyclins play redundant roles in development. Mice lacking cyclin E1 or E2 are viable and develop essentially normally. Combined ablation of both E-cyclins resulted in lethality around day 10.5 of embryonic development due to placental defects [11, 12]. When provided with wild-type placentas, cyclin E-null embryos survived until day E18.5, and died due to heart abnormalities. Cyclin E-deficient embryos also displayed severely reduced endoreplication of **megakaryocytes** and **trophoblast-giant cells** [11, 12]. As mentioned above, *Drosophila* cyclin E was shown to play a key role in endoreplication [9], revealing that this function is evolutionarily conserved. In postnatal mice, an acute and global ablation of cyclin E had no adverse consequences for development and viability, indicating that cyclin E is not essential in an adult organism [13].

## Cyclin E in Male Meiosis

E-cyclins play an important role in male meiosis. Cyclin E2-deficient mice displayed a defect in **spermatogenesis** resulting in decreased male fertility. This phenotype was exacerbated in mice with reduced dosage of cyclin E1 ($E1^{+/-}E2^{-/-}$), and was most pronounced upon ablation of both E-cyclins in male germline ($E1^{\Delta/\Delta}E2^{-/-}$) [14]. These observations indicated that two E-cyclins perform redundant functions in spermatogenesis, with cyclin E2 playing the major role. Cyclin E-deficient spermatocytes displayed severe meiotic defects, such as abnormal pairing and synapsis of homologous chromosomes, associations of heterologous chromosomes, unrepaired double-strand DNA-breaks, disruptions in telomeric structure, and defects in Cdk2-localization [14]. The testicular phenotype of cyclin E-deficient mice resembles abnormalities seen in mice lacking Cdk2 [15, 16], suggesting an important function for cyclin E-Cdk2 during spermatogenesis. One possible mechanism was provided by the observation that cyclin E-Cdk2 phosphorylates Mybl1 and Dmrtc2, two meiotic transcription factors representing essential regulators of spermatogenesis [17]. It was also found that during meiotic prophase I, Cdk2 binds to promoters of many genes through an interaction with nuclear respiratory factor 1 (Nrf1). Several of Cdk2-bound genes are essential for spermatogenesis, in particular for meiotic prophase I [18]. The authors also showed that Cdk2 phosphorylates Nrf1 and negatively regulates its activity, and that ablation of Cdk2 resulted in an increased expression of Nrf1-target genes [18]. These observations suggest that cyclin E-Cdk2 might regulate spermatogenesis by directly controlling expression of key meiotic genes.

Other studies demonstrated that mutations within the human *CDK2* gene could also lead to defective spermatogenesis. A single-nucleotide polymorphism (rs3087335) in the human *CDK2* gene was implicated in male infertility [19]. Moreover, mice homozygous for Cdk2-Y15S alteration, mimicking human rs3087335, displayed disrupted spermatogenesis [19]. The Y15S substitution led to increased CDK2-activity, causing abnormal proliferation of spermatogonia and disrupting the differentiation process [20]. These findings suggest that fine-tuned cyclin E-CDK2 activity is essential for spermatogenesis. It is likely that further studies will uncover additional infertility cases displaying mutations within cyclin E/CDK2 genes.

## Cyclin E and Synaptic Function

Cyclin E is highly expressed in brains of adult animals, which are composed mostly of non-proliferating cells [2, 21]. In postmitotic, terminally-differentiated neurons cyclin E regulates the catalytic activity of a key neuronal kinase, **Cdk5**. Neuronal cyclin E physically interacts with Cdk5 and forms catalytically-inactive cyclin E-Cdk5 complexes. This prevents Cdk5 from binding to its activating partners p35 and p39, thereby inhibiting Cdk5-kinase activity [22]. Conditional ablation of cyclin E in mouse brains resulted in hyperactivation of Cdk5, leading to diminished number of **synapses** and reduced number and volume of **dendritic spines**. Consequently, mice with knockout of cyclin E in the nervous system displayed impaired **synaptic plasticity** and memory deficits [22]. Another study identified cyclin E1 as a key regulator of potassium channel in cortical neurons. Specifically, cyclin E1, by inhibiting Cdk5, blocks Cdk5-mediated phosphorylation of potassium Kv2.1-channel and promotes Kv2.1-channel dispersal. The authors demonstrated that through this mechanism, cyclin E1 offers neuroprotection against **excitotoxic cell death** [23].

These findings have potential implications for human neurological and neurodegenerative diseases. First, abnormal cyclin E levels might underlie learning disabilities and cognitive disorders. Second, hyperactivation of CDK5 has been implicated in human neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases [24]. Inhibition of Cdk5 activity or reduction of Cdk5 levels decreased pathological changes and reduced neuronal loss in an Alzheimer's disease mouse model and conferred neuroprotection in an animal model of Parkinson's disease [24]. Given the ability of cyclin E to inhibit Cdk5, these observations raise a possibility that manipulating cyclin E levels in neurons may have a protective effect against neuronal loss in patients suffering from Alzheimer's or Parkinson's disease, and may protect against excitotoxic cell death.

## Cyclin E Function in Stem Cells

Embryonic stem cells (ESC) divide rapidly and display a very short G1-phase. The activity of cyclin E-CDK2 in ESC is much higher than that in somatic cells, and it is constitutively active through the entire cell-cycle [25, 26]. The molecular role of such high levels of cyclin E-CDK2 in ESC is unclear. It seems likely that cell-cycle wide CDK2-activity and the resulting constitutive hyperphosphorylation of RB1 is responsible for very short G1-phase in ESC. Since ESC initiate differentiation during G1, short G1-phase may help to maintain ESC in an undifferentiated state [25]. Consistent with this, during **dissolution**

**of pluripotency** the activity of cyclin E-CDK2 decreases and becomes cell-cycle regulated. This is accompanied by a significant extension of G1-phase [26].

Cyclin E-CDK2 (along with D-cyclins) also maintains the undifferentiated, pluripotent state of ESC by phosphorylating key pluripotency-regulators Nanog, Oct4 and Sox2. This protects them from proteasomal degradation by promoting their interaction with Pin1 peptidyl-prolyl *cis-trans*-isomerase [27]. Consistent with this, ablation of D- and E-cyclins or reducing CDK2 levels in ESC resulted in the loss of the pluripotent state and triggered differentiation [27, 28].

Cdk2 (and Cdk1) phosphorylate in ESC a large number of epigenetic regulators representing writers and erasers of all major histone marks [29]. Hence, high activity of Cdk1/2 in ESC may serve to globally regulate the epigenetic landscape, thereby helping to maintain the undifferentiated and pluripotent state. Cyclin E-CDK2 may play a similar role in enforcing the undifferentiated state of **induced pluripotent stem cells** (iPSC). Indeed, downregulation of cyclin E or ectopic expression of $p21^{CIP1}$ in somatic cells decreased the efficiency of generating iPSC through somatic reprogramming [30].

Cyclin E also promotes stemness and inhibits differentiation of neural stem cells. Overexpression of cyclin E1 in embryonic mouse brains shortened G1-phase, increased self-renewal of neural stem cells and inhibited neurogenic differentiation [31]. Conversely, treatment of mouse embryos with a pan-CDK-inhibitor stimulated neurogenesis [32]. In addition, down-regulation of cyclin E and cyclin E-Cdk2 activity, caused by a mutation of *Aspm* gene, leads to a premature exhaustion of neural progenitor cells in mice [33].

In murine **hematopoietic stem cells** (HSC), cyclin E1 controls exit from quiescence, and aged cyclin $E1^{-/-}$ mice displayed increased fraction of quiescent HSC. Upon myeloablative stress induced by 5-fluorouracil, cyclin E1-null HSC were deficient in entering the cell-cycle, resulting in decreased hematopoiesis and reduced survival. On the other hand, increased quiescence provided cyclin E1-null cells with a competitive advantage in bone marrow serial-transplantation assays [34]. See also Box 2.

## Cyclin E in Disease States

### Cyclin E in Liver Pathology

Cyclin E was shown to play an important role during **liver fibrosis**. This pathology is characterized by deposition of extracellular matrix proteins, such as collagen. Collagen-producing cells, myofibroblasts, are derived from hepatic stellate cells. Cyclin E1 expression is crucial for hepatic stellate cells survival and transdifferentiation into myofibroblasts [35]. Cyclin E1 is upregulated in human fibrotic and **cirrhotic livers**, and in mouse livers undergoing fibrosis induced by administration of $CCl_4$. Cyclin E1-null mice were refractory to development of liver fibrosis following $CCl_4$ administration, revealing that loss of cyclin E1 protects against liver fibrosis *in vivo* [35]. Ablation of cyclin E1 also attenuated liver inflammation (induced by deletion of *NEMO*), a state which precedes liver fibrosis [36]. These observations raise a possibility that interfering with cyclin E1 function might protect against liver fibrosis. Indeed, liposome-mediated delivery of anti-cyclin E1 siRNA to mice

attenuated the inflammatory response to $CCl_4$ and protected mice against $CCl_4$-induced liver cirrhosis [37].

Cyclin E1 was also shown to play a rate-limiting role in cell-cycle entry of hepatocytes during liver regeneration. Ablation of cyclin E1 and Cdk2 inhibited S-phase entry and impeded liver-mass reconstitution after partial hepatectomy [38]. In contrast, ablation of cyclin E2 led to elevated cyclin E1 levels and resulted in excessive liver regeneration [39]. Hence, inhibition of cyclin E2 may augment liver regenerative process.

## Deregulation of Cyclin E in Cancer

High levels of E-cyclins have been documented in many human cancer types. In serous ovarian cancers, cyclin E-overexpression represents a very early event, occurring even before development of early-stage noninvasive 'serous tubal-intraepithelial carcinoma' [40]. These observations suggest that cyclin E-overexpression may represent an initiating event in tumor development. Mouse cancer models established the causative role of cyclin E-overexpression in tumorigenesis (Box 3).

Amplification of cyclin E1 and E2 genes (*CCNE1* and *CCNE2*), which is seen in about 8% of all cancers and most frequently in ovarian (~25%), endometrial (~14%), esophagogastric (~14%) and breast (~12%) cancers (data from TCGA), represents one of the major mechanisms underlying cyclin E deregulation. In addition, disruption of physiological cyclin E protein-degradation can also contribute to increased cyclin E levels. The most common mechanism involves inactivation of the F-box protein FBW7, a substrate-recognition subunit of Skp1-Cul1-F-box-protein (SCF) ubiquitin-ligase that normally mediates cyclin E-degradation [1, 4]. Moreover, reduced levels of Cul3 were found in human liver cancers and correlated with elevated cyclin E-expression [41]. Other mechanisms reported to deregulate cyclin E levels include overexpression of Id4, mesothelin, chromatin-modifying enzyme GCN5, RNA-binding protein HuR (which increases cyclin E1 mRNA stability) or activation of PKCiota (Figure 2) [1, 42–46]. It is not clear which of these mechanisms truly reflect the driving force of cyclin E overexpression, rather than correlating with high cyclin E levels.

Cyclin E-CDK2 activity can also be upregulated by oncogenic signaling pathways through downregulation, nuclear export or inactivation of KIP/CIP-inhibitors [4]. Moreover, several cancer types (such as breast carcinoma) express hyperactive low-molecular-weight cyclin E (LMW-E), generated from full-length cyclin E through proteolytic cleavage. LMW-E binds CDK2 with higher affinity than full-length cyclin E and is resistant to inhibition by KIP/CIP-inhibitors. Unlike full-length cyclin E, which is localized in the nucleus, LMW-cyclin E is mostly cytoplasmic, due to the lack of N-terminal nuclear-localization signal [47]. High levels of cyclin E, as well as the presence of cytoplasmic LMW-E, have been shown to correlate with cancer aggressiveness and unfavorable clinical outcome, and may serve as a prognostic marker of poor survival [1, 47].

## Deregulated Cyclin E Functions in Cancer

Given the well-documented role of cyclin E in promoting the cell-cycle, it seems logical that overexpressed cyclin E drives uncontrolled tumor cell division. In addition, deregulated cyclin E in cancer cells induces genome instability [48], which has been attributed to

the S-phase replication stress and mitotic defects. In contrast to the physiological role of fine-tuned E-CDK2 activity in loading of MCMs onto DNA pre-replication complexes and in DNA replication initiation (Box 1), deregulated and constitutively expressed cyclin E in tumor cells impairs loading of MCM proteins onto chromatin [49]. Cyclin E-overexpression is also associated with abnormally increased firing of DNA-replication origins [50], and results in interference between replication and transcription [50]. Overexpression of cyclin E causes decrease or exhaustion of substrates required for DNA replication, such as nucleotides and RPA [51, 52]. All these events lead to impaired DNA-replication fork progression and under-replication of DNA in S-phase. Cells with such S-phase defects enter mitosis with unreplicated genomic segments, which, in turn, leads to anaphase anomalies and results in chromosomal deletions [53].

Moreover, cyclin E-overexpressing cells proceed to anaphase without complete alignment of chromosomes, resulting in chromosome mis-segregation, polyploidy and mitotic failure. This phenotype has been attributed to abnormal inhibition of $APC^{Cdh1}$ by elevated cyclin E levels. Consequently, this leads to the aberrant accumulation of $APC^{Cdh1}$ substrates, such as cyclin B1 and securin, which are responsible for impairment of mitotic progression [54]. Compromised centrosome functions caused by the hyperactive E-CDK2 could also contribute to abnormal mitoses. Indeed, overexpression of cyclin E induces centrosome hyperamplification when combined with p53-loss [55]. Overactive cyclin E-CDK2 kinase also hyperphosphorylates centromere-associated protein CENP-A, an essential protein in chromosome segregation, and reduces its centromeric localization [56]. The notion that overexpressed cyclin E compromises the integrity of the genome has been verified *in vivo* by demonstrating that tumors arising in mice expressing degradation-resistant cyclin E1 ($E^{T393A}$) and mutant K-$Ras^{G12D}$ displayed chromosome-instability [57].

Other oncogenic mechanisms of cyclin E have been reported, and linked to phosphorylation of different cyclin E-CDK2 substrates. For instance, cyclin E-CDK2 was shown to suppresses cellular senescence induced by oncogenes, which was partly attributed to the ability of cyclin E-CDK2 to phosphorylate Myc [58, 59]. Phosphorylation of Smad3 by cyclin E-CDK2 inhibits its transcriptional activity, which might contribute to the aggressiveness of cyclin E-overexpressing breast cancers [60]. Cyclin E-CDK2 phosphorylates and stabilizes an anti-apoptotic protein Mcl-1, thereby inhibiting tumor cell death and promoting resistance to pro-apoptotic treatments [61]. Phosphorylation of a chromatin-modifying enzyme EZH2 by cyclin E-CDK2 was postulated to be critical for maintenance of triple-negative breast cancer (TNBC) identity, as inhibition of CDK2 converted TNBC cells to luminal estrogen-receptor α (ERα)-positive cells [62]. Lastly, cyclin E physically binds the androgen receptor and increases its transactivation activity in the presence of dihydrotestosterone, a mechanism that may be relevant in prostate cancer [63]. It should be noted that the *in vivo* significance of these various mechanisms and different outcomes of CDK2 inhibition remains unclear, and needs to be validated in the context of human tumors.

The tumor-specific cytoplasmic LMW-E may promote tumorigenicity due to its ability to phosphorylate or regulate cytoplasmic proteins, as well as its altered substrate preference [47]. For example, LMW-E physically interacts with the lipogenic enzyme ATP-citrate

lyase (ACLY) and enhances its activity. Depletion of ACLY inhibited LMW-E–induced anchorage-independent growth, migration, invasion and tumor growth *in vivo* [64].

## Cyclin E and Drug Resistance

Elevated cyclin E levels contribute to intrinsic and acquired resistance of cancer cells to a variety of therapeutic agents. Genome-wide analyses of ovarian cancers, where *CCNE*1 is frequently amplified, revealed that such amplification is associated with intrinsic resistance to standard platinum-**taxane** chemotherapy [65]. Since *CCNE1*-amplification is mutually exclusive with *BRCA1/2*-gene mutations, such insensitivity may be attributed to an intact BRCA1/2-pathway in these tumors [66]. Amplification of *CCNE1* and overexpression of cyclin E1 underlie the intrinsic and acquired resistance of HER2-positive breast cancers to treatment with **trastuzumab** [67]. In hormone receptor-positive breast cancers, the presence of cytoplasmic LMW-E correlates with resistance to treatment with aromatase-inhibitors [68]. Also high levels of cyclin E2 correlate with poor response to endocrine therapy [69]. Moreover, high levels of cyclin E or activation of cyclin E-CDK2, or the presence of cytoplasmic LMW-E were shown to underlie the intrinsic and acquired resistance to CDK4/6-inhibitors in this breast cancer type [70]. Consistent with these findings, analysis of PALOMA-3 trial for patients with hormone receptor-positive breast cancers revealed that high expression of cyclin E1 mRNA in metastatic lesions was associated with relative resistance to treatment with a CDK4/6 inhibitor palbociclib [71]. It is likely that hyperactivation of cyclin E-CDK2 by-passes the requirement for cyclin D-CDK4/6 in cell-cycle progression, consistent with genetic results [72]. Increased cyclin E1 levels also contribute to resistance of tumor cells to **BH3-mimetics**, through phosphorylation and stabilization of Mcl-1 by cyclin E-CDK2 [61].

## Targeting Cyclin E and Cyclin E-CDK2 axis for Cancer Treatment

As described above, activation of CDK2, the major catalytic partner of cyclin E, is thought to account for cyclin E's oncogenic functions. Surprisingly, CDK2 is not required for proliferation of several cancer cell lines [13, 73, 74]. However, CDK2 may play a rate-limiting function in specific cancer types (Figure 3). The requirement for CDK2 function has been particularly well documented in high-grade serous ovarian cancer (HGSC). It was reported that cyclin E-amplified HGSC cell lines critically depend on CDK2 for clonogenic survival and inhibition of CDK2 resulted in tumor cell apoptosis [75]. Also in melanoma cells, where CDK2 transcription is driven by the melanoma-specific transcription factor, MITF, CDK2 was shown to be essential to maintain cell proliferation [76]. Analyses of colon cancer cells engineered to express **analog-sensitive** CDK2 revealed that CDK2-kinase is required for proliferation and anchorage-independent growth [77]. In acute myeloid leukemia (AML) cells, CDK2 was found to suppresses cell differentiation, and depletion of CDK2 in human AML-xenograft models arrested tumor growth and induced differentiation [78].

In addition, inhibition of CDK2 was documented to have synergistic effects or synthetic lethality in specific contexts. For example, CDK2 inhibition does not significantly inhibit proliferation TNBC cells. However, blocking CDK2 activity was reported to convert TNBC cells to ERα-positive 'luminal-like' type, which become susceptible to treatment with

an anti-estrogen tamoxifen [62]. This suggests that a combination of CDK2 inhibitors and antiestrogens may represent a promising approach for TNBC treatment. Depletion or inhibition of CDK2 also reduces DNA-damage repair. It was proposed that CDK2-inhibition may enhance the response of cancer cells to DNA-damaging agents, and may be particularly efficacious in DNA-damage repair-deficient cancers, such as BRCA1- or ATM- deficient ones [79]. Lung cancer cells with activating *KRAS* mutations were found to be prone to CDK2 inhibitor-mediated anaphase catastrophe, possibly due to decreased levels of cyclin E-CDK2 centrosomal substrate CP110 in *KRAS*-mutant cells [80]. A synthetic lethal interaction between CDK2 inhibition and N-Myc overexpression was reported in neuroblastoma; silencing of CDK2 induced apoptosis of *MYCN*-amplified, but not non-amplified tumor cells [81]. Moreover, since the increased activity of cyclin E underlies resistance to several therapeutic compounds, cyclin E-CDK2 inhibition may represent a strategy to overcome this resistance. For instance, cyclin E-overexpressing, trastuzumab-resistant HER2-positive breast cancer cells are particularly sensitive to CDK2-inhibition [67]. Also palbociclib-resistant breast cancer cells undergo cell-cycle arrest upon combined inhibition of CDK2 and CDK4/6 [82, 83].

The reasons for different outcomes of cyclin E-CDK2 inhibition remain unclear and require further studies. It is possible that they truly reflect distinct functions played by cyclin E-CDK2 in different tumor types. However, many of them may reflect off-target effects of various non-specific inhibitors, or represent cell line-specific phenomena.

Cyclin E may have oncogenic functions that are CDK2- and kinase-independent. For example, CDK2 was shown to be dispensable for proliferation of several human liver cancer and TNBC cell lines, while cyclin E was essential [13, 74]. Consistent with these findings, analyses of mouse liver cancer models revealed that Cdk2 is required for tumor-initiation, but not for tumor-progression, while cyclin E was required at both stages [13, 84]. These findings are in line with an earlier observation that cyclin E-mutants defective in activating Cdk2 can transform rat embryo fibroblasts in cooperation with Ha-Ras [85]. While further studies are required to elucidate the underlying mechanisms, these observations suggest that some oncogenic functions of cyclin E may be independent of CDK2 kinase. Hence, targeting cyclin E, rather than inhibiting cyclin E-CDK2, might represent an attractive therapeutic strategy for certain cancer types.

## Concluding Remarks

Since the discovery of mammalian E-cyclins, great effort has been made to understand their functions in normal and pathological conditions. While cyclin E was cloned as a cell-cycle protein, subsequent work revealed that it plays cell-cycle-independent roles. As described here, cyclin E is involved in regulating neuronal physiology, stem cell-maintenance, spermatogenesis, development of heart, placenta and megakaryocytes. The molecular roles of cyclin E in these processes are only starting to be elucidated and require further studies (see Outstanding Questions).

In cancer cells, cyclin E was postulated to regulate many aspects of tumorigenesis, in addition to cell proliferation. Several molecular mechanisms were proposed to underlie these

various functions, but their *in vivo* relevance remains to be determined. It will be important to address these issues using mouse cancer models carrying defined genetic lesions, as well as by studying human tumors. Also, the role of CDK2 in proliferation (and possibly in regulating other cancer-relevant functions) remains unclear and requires further studies. Another unexplored issue is the impact of cyclin E-CDK2-inhibition on physiology of tumor-bearing individuals. Of note, CDK2 was found to promote production of cytokines by $CD4^+$ T-cells and to restrict the suppressive capacity of regulatory T-cells (Treg) [86, 87]. While involvement of cyclin E in these functions is not known – and hence this topic was not covered by the current review – it is likely that inhibition of CDK2 may affect the anti-tumor immune response of the hosts.

One of the major limitations in the field is the absence of CDK2-specific inhibitors. Much of the work was performed using non-specific pan-CDK inhibitors, which target other kinases. Also analyses using an acute depletion of CDK2 need to be treated with caution, as the interpretation may be confounded by the compensatory mechanisms [5]. Further studies using cancer cells and mice expressing analog-sensitive CDK2 in place of its wild-type counterpart will allow to decipher the range of CDK2 functions.

Another unresolved issue is the presence of specific roles for cyclins E1 and E2 in normal development and in cancer. *In vivo* proteomic approaches, such as identification of cyclin E1 versus E2 binding partners and substrates in different organs, and at different stages of cancer progression, will help to address this issue.

Lastly, cyclin E may be involved in non-proliferative pathological conditions, such as cognitive disorders or neurodegeneration. Further studies are needed to address the potential roles of cyclin E in these disease states.

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## Glossary

**Cyclins**
a class of proteins that control cell-cycle progression and cell-division. Cyclins bind, activate and provide substrate specificity to their catalytic partners, cyclin-dependent kinases (CDKs).

**Cell-cycle**
a tightly controlled sequence of events that take place during cell-division. Cell-cycle can be divided into Gap 1 (**G1**), DNA-synthesis (**S**), Gap 2 (G2) and mitosis (M) **phases**.

**Cyclin-dependent kinases (CDKs)**

a class of proline-directed serine-threonine kinases which are activated by cyclin proteins. Many CDKs are involved in driving cell-cycle progression through phosphorylation of cellular proteins.

**Endoreplication**
an unusual cell-cycle consisting of G1, S and G2 phases (no mitosis); leads to formation of polyploid cells with high DNA-content.

**Eye imaginal discs**
epithelial structures found during insect embryonic development.

**Megakaryocytes**
bone marrow cells that give rise to platelets.

**Trophoblast-giant cells**
large, polyploid cells of the placenta.

**Spermatogenesis**
the process of generating male germ cells.

**Cdk5**
cyclin-dependent kinase 5. Cdk5 is normally activated in neurons by its regulatory non-cyclin partners, p35 and p39; p35-Cdk5 and p39-Cdk5 complexes regulate neuronal development and physiology by phosphorylating several proteins [24].

**Synapses**
neuron-to-neuron contacts, often located in **dendritic spines** (protrusions emanating from dendrites of neurons).

**Synaptic plasticity**
activity-dependent modification of the strength of synaptic transmission, thought to underlie learning and memory.

**Excitotoxic cell death**
a type neuronal cell death induced by high levels of neurotransmitters.

**Dissolution of pluripotency**
a stage when pluripotent stem cells undergo cell-fate specification and start differentiating into different lineages.

**Induced pluripotent stem cells**
stem cells generated from somatic cells; pluripotent cells can give rise to all cells of a body.

**Hematopoietic stem cells**
stem cells which can generate all blood lineages. In contrast to embryonic stem cells, hematopoietic stem cells are largely quiescent.

**Liver fibrosis**

a pathological condition triggered by chronic liver injury caused by a persistent viral hepatitis infection, excessive alcohol abuse, or fatty liver disease.

**Liver cirrhosis**
late-stage liver disease in which liver tissue has been replaced with scar tissue.

**Taxanes**
a class of chemotherapeutic compounds which inhibit cell-division by preventing depolymerization of microtubules.

**Trastuzumab**
an anti-HER2 monoclonal antibody used to treat patients with HER2-positive cancers; also known as Herceptin.

**BH3-mimetics**
pro-apoptotic compounds which mimic BH3-proteins by binding and antagonizing anti-apoptotic BCL-2 family proteins.

**Analog-sensitive kinase**
genetically modified kinase of interest that can be selectively inhibited using a synthetic inhibitor compound.

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**Box 1.**

### Molecular Functions of Cyclin E in Driving The Cell-cycle

The best-documented cell-cycle substrate of cyclin E-CDK2 is the retinoblastoma protein, RB1. RB1 inhibits the activity of E2F transcription factors. Phosphorylation of RB1 de-represses E2Fs, which then induce genes required for S-phase entry and progression. Cyclin E-CDK2 also phosphorylates a CDK-inhibitor $p27^{KIP1}$, thereby targeting it for proteasomal degradation. This removes the barrier that restrains the activity of cyclin E-CDK2 and A-CDK2. E2F5, a member of E2F-family, represents another cyclin E-CDK2 substrate. Phosphorylation of E2F5 stimulates E2F5-dependent transcription. Cyclin E-CDK2 phosphorylates CBP/p300 and activates its histone acetyltransferase activity. Cyclin E-CDK2 also couples production of histones with entry of cells into S-phase, by phosphorylating NPAT, a protein that activates histone gene transcription. An important role for cyclin E-CDK2 in centrosome duplication was provided by the observations that this kinase phosphorylates centrosomal substrates such as NPM/B23 and CP110 (these findings were reviewed in [1] and [4]). Cyclin E-CDK2 phosphorylates and stabilizes Cdc6, a key component of DNA-pre-replication complexes [88]. The firing of DNA-replication origins and entry of cells into S-phase is driven by sequential action of Dbf4-dependent CDC7-kinase (DDK) and cyclin E-CDK2. These kinases collaborate to recruit DNA-replication proteins, such Cdc45 and GINS, to DNA replication origins and promote formation of the replicative helicase, the Cdc45-MCM2-7-GINS (CMG) complex [89]. Another function of cyclin E-CDK2 is phosphorylation and inactivation of Cdh1, a substrate-adaptor protein of the anaphase-promoting complex/cyclosome (APC/C). This allows accumulation of S-phase cyclins that are degraded in G1-phase by $APC^{Cdh1}$ [90]. Several members of mammalian SWI-SNF (BAF) complex, such as BRG1, BAF155 [91] and possibly SMARCA5 [92], represent substrates of cyclin E-CDK2. It has been proposed that activation of cyclin E-CDK2 in late G1-phase abolishes the growth-inhibitory effect of SWI-SNF, thereby contributing to entry of cells into S-phase [91]. Lastly, cyclin E-CDK2-mediated phosphorylation of SF3B1 (also known as SAP155), a subunit of the essential splicing factor SF3, may contribute to its dynamic interactions with nucleosome and chromatin during cell-cycle progression [93], although the specific splicing programs regulated by such phosphorylation remain to be discovered.

Some authors postulated that cyclin E may contribute to cell proliferation via a kinase-independent mechanism. A fraction of cyclin E was shown to localize to centrosomes in a CDK2-independent fashion, and to regulate centrosome duplication by facilitating the localization of centrosome-bound DNA-replication factor MCM5 [94]. Cyclin E was also implicated in loading of MCM proteins onto chromatin in cells exiting from quiescence [95].

**Box 2.**

### Cyclin E in Stem Cells of Invertebrates

The role of cyclin E in stem cells was also studied in invertebrates. *C. elegans* germline stem cells behave similarly to mammalian ESC, by having a rapid cell-cycle, short G1-phase and high cyclin E (CYE-1) levels and activity throughout the cell-cycle. CYE-1/CDK-2 controls the proliferative and meiotic cell-fate, with high CYE-1/CDK-2 levels promoting cell proliferation [96]. Conversely, RNAi-mediated depletion of CYE-1 or CDK-2 in *C. elegans* cells led to a switch from mitotic to meiotic cell-cycles [97].

In *Drosophila*, cyclin E-CDK2 is required for maintenance of ovarian follicle stem cells and germline stem cells. This function seems to be at least partially cell-cycle independent, as hypomorphic DmcycE mutations reduce stem cell maintenance despite normal proliferation rates of these cells [98]. Cyclin E also represents one of the major determinants of cell-fate in *Drosophila* central nervous system, where it is required for the maintenance of stem cell identity. At the molecular level, cyclin E inhibits the function of a transcription factor Prospero by facilitating its cortical localization [99]. Prospero is known to repress cyclin E expression, indicating the presence of a negative feedback loop in which the balance between cyclin E and Prospero determines whether stem cells continue to divide or activate the differentiation program [99].

**Box 3.**

### Mouse Models of Cyclin E-dependent Tumorigenesis

Analyses of mouse cancer models established the causative role of cyclin E-overexpression in tumorigenesis (Figure I). Transgenic mice engineered to overexpress cyclin E1 in mammary glands developed mammary hyperplasia, with 10% of animals displaying mammary carcinomas (reviewed in [1]). Overexpression of LMW-E triggered breast tumorigenesis with higher incidence than that induced by wild-type cyclin E, and tumors displayed increased metastatic potential (reviewed in [47]). LMW-E-driven tumorigenesis was strictly dependent on Cdk2, as crossing LMW-E mice with Cdk2-null animals abrogated breast cancer formation (reviewed in [47]). Also transgenic mice engineered to express degradation-resistant cyclin E1 mutant (T380A) in mammary tissue displayed breast tumorigenesis, especially when crossed into $p53^{+/-}$ background [100].

Transgenic expression of degradation-resistant human cyclin E1 (T62A/T380A) in lung alveolar type II and bronchioalveolar cells resulted in dysplasia and lung pulmonary adenocarcinomas [101]. Cyclin E1 knock-in mice ubiquitously expressing cyclin E1 (T393A) that is resistant to Fbw7-mediated degradation did not spontaneously develop tumors, but upon crossing with cancer-prone animals (p53-null mice or mice expressing oncogenically activated $K\text{-}Ras^{G12D}$), accelerated tumorigenesis in these strains. Cyclin E1 (T393A) mouse embryonic fibroblasts were hypersensitive to oncogenic transformation with activated H-Ras combined with p53-loss [57]. Transgenic mice engineered to overexpress cyclin E1 in T-lymphocytes displayed increased incidence of T-cell lymphomas upon treatment with a carcinogen N-methylnitrosourea. Tumors arising in these animals frequently displayed activating mutations in the *K-Ras* gene, indicating that cyclin E cooperates with oncogenic Ras in driving malignant transformation of the T-cell lineage [102].

While overexpression of cyclin E promoted tumorigenesis, ablation of cyclin E protected against the oncogenic transformation. Cyclin $E1^{-/-}E2^{-/-}$ mouse embryonic fibroblasts were refractory to *in vitro* transformation by different combinations of oncogenes [11]. Ablation of cyclin E1 protected mice against development of liver cancers induced by a mutagen diethylnitrosamine (DEN), by hepatocyte-specific overexpression of c-Myc, or by hepatocyte-specific deletion of NF-kappa-B essential modulator NEMO [36, 84]. Moreover, an acute ablation of E-cyclins in mice which developed DEN-induced liver cancers halted tumor progression, indicating that the continued presence of cyclin E is required for tumor growth [13].

## Outstanding Questions

Why is cyclin E required for development of some tissues, but not others? Does cyclin E regulate some cell type-specific proteins and controls unique cellular processes in these cyclin E-dependent compartments?

Do cyclins E1 and E2 perform identical functions under normal conditions, when both proteins are expressed?

How does overexpressed cyclin E-CDK2 promote tumorigenesis, beyond driving deregulated cell proliferation? What other functions and processes in tumor cells are affected by cyclin E-overexpression? What are phosphorylation substrates of cyclin E-CDK2 in various tumor types and at different stages of tumorigenesis such as tumor initiation, tumor progression and the metastatic spread?

Which cancer types depend on cyclin E-CDK2 for their proliferation (or for other cancer-related functions), and which molecular lesions confer dependency on cyclin E-CDK2?

Does cyclin E perform CDK2-independent and kinase-independent functions in tumorigenesis and what would be the molecular mechanism of such a role?

What is the impact of a global CDK2-inhibition on the physiology of tumor-bearing individuals, for example on the anti-tumor immune response?

Does deregulated cyclin E expression play role in pathogenesis of other human diseases, in addition to cancer?

## Highlights

- Cyclin E, a cell-cycle protein, plays a variety of non-canonical, cell-cycle-independent physiological roles, such as regulating liver function, neurophysiology, spermatogenesis, and stem cell maintenance.
- The diversity of cyclin E's physiological functions is achieved through activation or inhibition of cyclin-dependent kinases, and possibly also via kinase-independent mechanisms.
- Cyclin E is frequently overexpressed in cancer. Deregulated cyclin E expression causes uncontrolled proliferation, replication stress and genome instability of cancer cells, and is responsible for resistance of tumor cells to various therapeutic compounds.
- Targeting cyclin E may represent a very effective anti-cancer strategy.

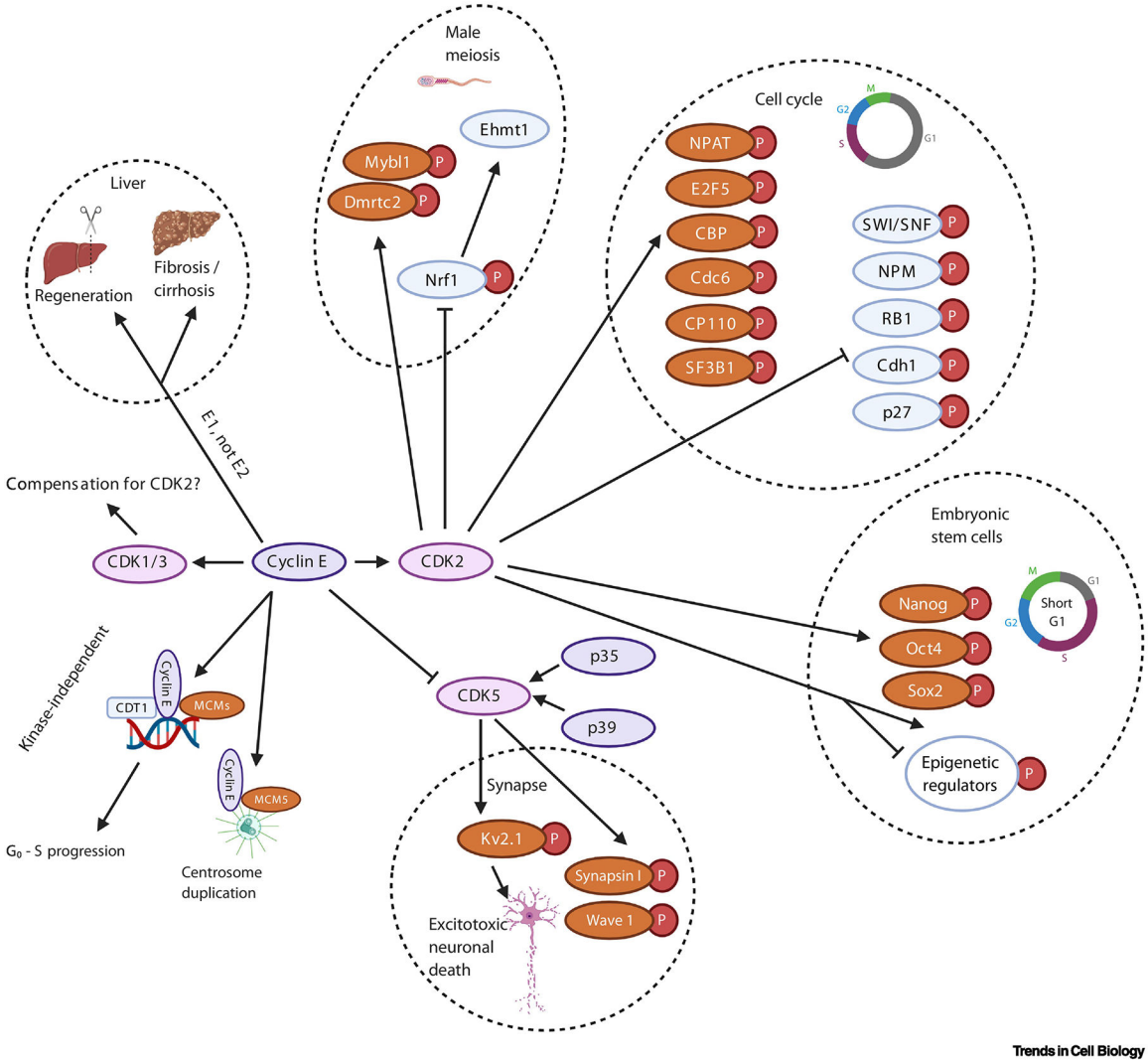


**Figure 1. Overview of Cyclin E Physiological Functions.**

Cyclin E exerts its physiological functions through cell cycle kinases (mainly CDK2). Cyclin E binds and activates CDK2, which then phosphorylates a range of substrates involved in different physiological functions such as cell cycle progression, male meiosis, and stem cell maintenance. Cyclin E also binds to CDK5 and sequesters it from its activating partners p35 and p39, and therefore negatively regulates its activity. This mechanism was shown to affect the synaptic functions in the brain. Cyclin E can also bind to CDK1 or CDK3, but the physiological significance and molecular functions of these complexes remain unknown. Lastly, cyclin E also plays kinase-independent functions, such as to facilitate loading of MCMs onto chromatin during G0 – S phase progression, and loading of MCM5 to the centrosomes during centrosome duplication. Abbreviations: E1, cyclin E1; E2, cyclin E2. Wave1, Wiskott-Aldrich syndrome protein family verprolin homologous protein 1, which together with Synapsin I represents synaptic CDK5 substrates. CP110, centriolar coiled-coil protein 110. Histone-lysine N-methyltransferase Ehmt1 represents a Nrf1 transcriptional target.

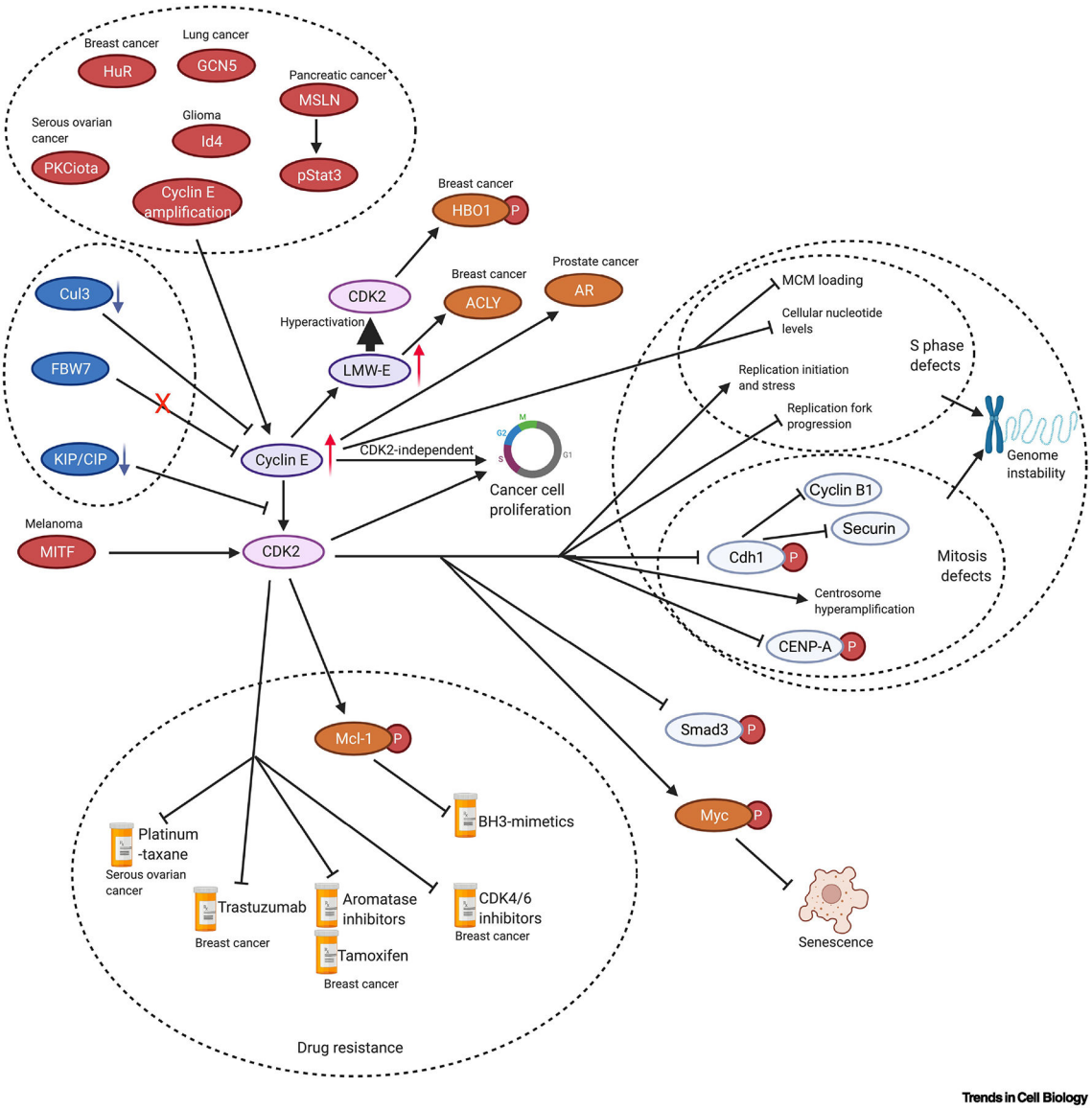


**Figure 2. Overview of Cyclin E Deregulation Mechanisms and Its Functions in Cancer.**

Cyclin E is frequently deregulated in cancer. Different mechanisms include amplification of cyclin E genes, abnormal upregulation of its expression by different factors, the loss or decreased activity of its turnover machinery, or downregulation of CDK2 inhibitors. Deregulated cyclin E hyperactivates CDK2, thereby affecting a variety of its downstream substrates. Cyclin E overexpression causes S-phase and mitotic defects which lead to genome instability in cancer cells. It also mediates resistance to a number of therapeutic reagents. The cancer-specific low-molecular-weight cyclin E can regulate cytoplasmic proteins such as ACLY, and can phosphorylate nuclear substrates, such as histone-acetyltransferase HBO1. Abbreviations: LMW-E, low-molecular-weight cyclin E; AR, androgen receptor; MCM, minichromosome maintenance complex; MSLN, mesothelin, which upregulates cyclin E1 expression via phosphorylated Stat3 (pStat3); ACLY, ATP-citrate lysase.

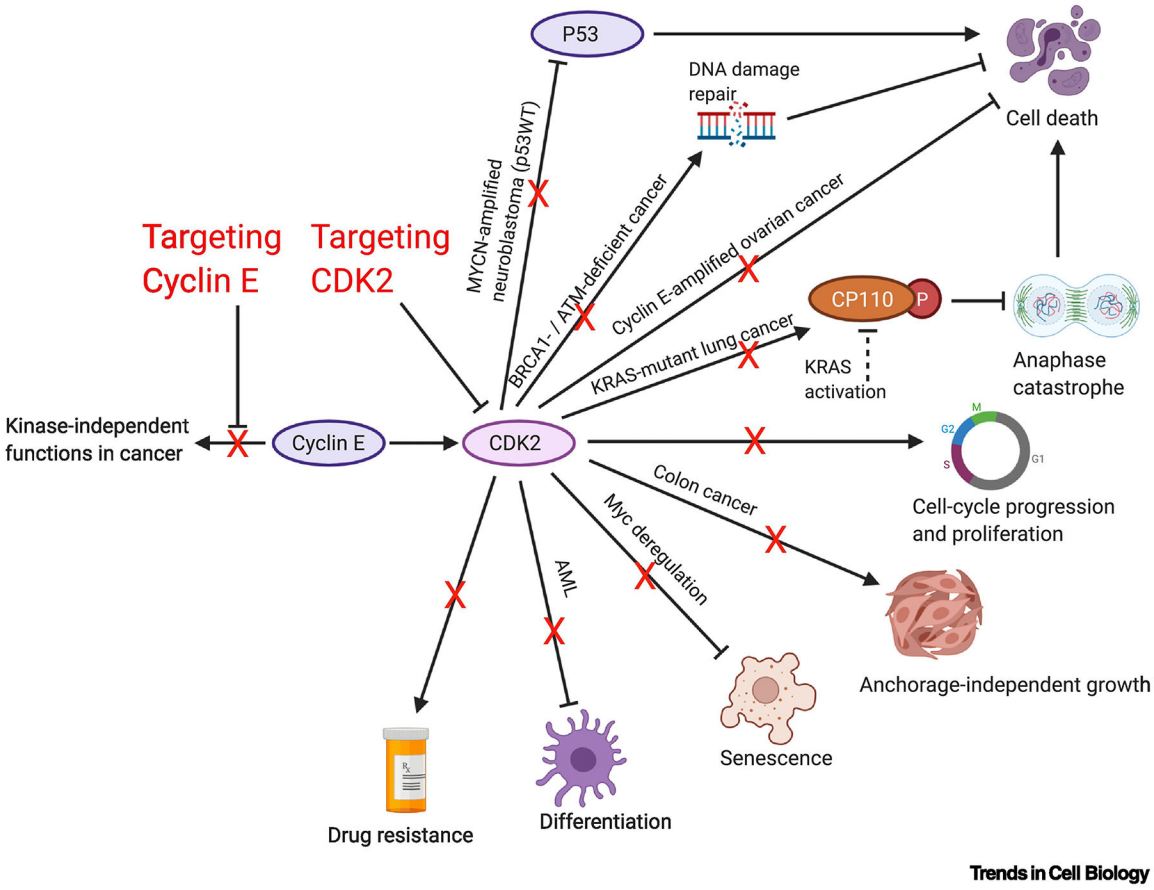


**Figure 3. Targeting Cyclin E-CDK2 in Cancer.**

Inhibition of CDK2, the major catalytic partner of cyclin E, can lead to a variety of therapeutically beneficial outcomes, such as halting cancer cell proliferation, causing cell death, and re-sensitizing resistant cells to therapeutic reagents. Dashed lines indicate that in KRAS-mutant lung cancer cells, activated KRAS was shown to decrease CP110 protein level. Abbreviations: CP110, centriolar coiled-coil protein 110; BRCA1, breast cancer type 1 susceptibility protein; ATM, ATM serine/threonine kinase; KRAS, KRAS proto-oncogene, GTPase; AML, acute myeloid leukemia.

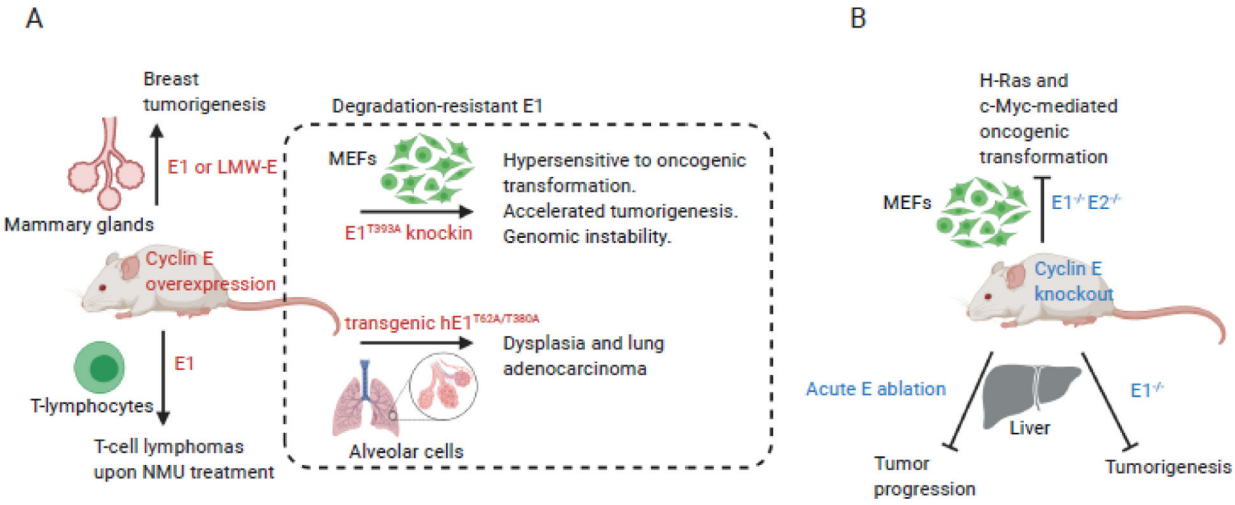


**Figure I. Mouse Models for Studies of Cyclin E in Cancer.**

Several cyclin E 'gain-of-function' (A) and 'loss-of-function' (B) models have been developed to study the function of cyclin E in cancer. Abbreviations: LMW-E, low-molecular-weight cyclin E; E1, cyclin E1; E2, cyclin E2; hE1, human cyclin E1; NMU, carcinogen N-methylnitrosourea; MEF, mouse embryonic fibroblasts.