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The Roles of E2F5 in Tumor Cell Cycle: The Gatekeeper or Destroyer?

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Abstract: E2F5 is a member of E2F transcription factor superfamily, controlling many molecular activities, such as cell proliferation, cell differentiation, DNA repair and cell death. Therefore, it is closely related to the occurrence, development and prognosis of a variety of cancers. In recent years, with the rapid development of bioinformatics, genomics and epigenetics, this study has further elucidated of E2F5 in the tumor cell cycle. Based on the latest research reports, this study reviewed the structural composition, dynamic activity regulation of E2F5, and how its transcription program driven by carcinogenic activity changed the progress of various tumor cell cycles, especially how it converted from a "gatekeeper" to a "destroyer", thus affecting abnormal biological behaviors of tumor cells. Our aim is to provide a new direction for the development of E2F5 targeted therapy strategies and drug resistance in the future.

Keywords: E2F5; Retinoblastoma protein; Tumor cell cycle

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1. Introduction

In the early 1990s, Joe Nevins et al. found an active factor that induced the transcriptional activation of adenovirus E2 promoter at the early stage of replication, named as adenovirus early region 2 binding factor (E2F). They also found that adenovirus early region 1A (E1A) transforming protein could cause the reverse transcriptional activation of adenovirus E2 promoter, and stimulate the entry of cell cycle by inhibiting E1 related protein pRB and inducing the release of E2F agonist [1]. Subsequently, in 1995 Victoria buck screened another new member

of E2Fs family in a yeast two hybrid test. The homology of DNA binding domain with E2F4 was 87%, and the homology of tag box and pocket protein binding domain with the same region of E2F4 was respectively 75% and 72%, and the protein sequence and molecular tissue also had the same amino acid residues as E2F4. Based on this similarity, he named the new member E2F5, which represents a subfamily of E2F protein family with E2F4, and defined its molecular structure and physiological function for the first time [2].

Retinoblastoma protein (RB) plays the important role in regulating gene transcription and chromatin remodeling, affecting cell cycle progression, cell senescence and tumorigenesis. As the first tumor suppressor discovered, RB is absent or mutated in at least one third of human tumors. Rb-E2F pathway integrates proliferation signals with cell cycle checkpoints to ensure proper cell division and genomic integrity [3]. The precise regulation of E2F activity makes RB plays a central role in determining cell fate.

Cancer is a polygenic disease in the eukaryotic cell cycle, involving the genetic and epigenetic changes of key genes in the cell cycle. The cell cycle is a series of highly organized events. Multiple checkpoints have been set up to monitor the growth signal and DNA integrity during cell proliferation. Among them, G_1/S phase transition is crucial to maintain DNA conservation. The CDK-RB-E2F axis is the core transcription mechanism driving the cell cycle process, determining the spatiotemporal nature of genome replication and the accuracy of genetic material transmission. As the final effector, E2F transcription factor family is encoded by eight genes into transcriptional activators and transcriptional inhibitors, which are involved in many important cellular processes, such as cell proliferation, differentiation and apoptosis. As one of the classic E2F family inhibitors, the expression of E2F5 is cell cycle dependent and mainly regulates gene expression in G_1 and S phases, just like the gatekeeper standing in front of the checkpoint. Abnormal expression and activity of E2F5 may lead to malignant cell proliferation, which is a common phenomenon in various cancers.

Therefore, this article describes the structural composition of E2F5 and its dynamic activity regulation in the cell cycle, focusing on how its transcription program driven by carcinogenic activity changes the progress of tumor cell cycle, converted from the gatekeeper to the destroyer, then affects the abnormal biological behavior of tumor cells such as proliferation, migration, invasion and apoptosis.

2. E2F5 molecular structure

In cytogenetics, E2F5 gene is located on human chromosome 8q21.2 and contains 10 exons. It is encoded by 346 amino acids and has a predicted molecular weight of 38 kDa. E2F5 contains three highly conserved active regions: the wing helix DNA binding domain, the dimer domain, and the reverse transcription activation domain. The E2F family recognizes TTT (C/G) (C/G) (C/G) CGC, a consistent DNA sequence in the promoter of target genes. The E2F5 dimer domain consists of a heterodimer helix Helicon domain and a heterodimer β - sandwich domain connected by two small helixes and two small chains. Before binding to DNA, E2F5 needs to interact with transcription factor dimers (TFDP1, TFDP2 and TFDP3) to form a dimer through protein-protein interaction through oligomerization sites on the dimer domain, containing leucine zipper (LZ) and marker frame (MB) domains [1,4]. The amino acid sequence at the oligomerization site has strong conservation, and connecting with the DNA binding domain and forms a certain spatial conformation. It is reported that TFDP1 and TFDP2 can be identified in humans, mice, dogs and other species, while TFDP3 is not found in mouse cells, but can be captured in humans and highly expressed in many malignant tumors [5]. Although TFDP3 has high sequence homology with TFDP1 and TFDP2, and combining with E2F family members to enhance E2F DNA binding activity. However,

TFDP3 can down regulate E2F family mediated transcriptional activation. Most E2Fs and TFDP1/2 are mainly nuclear localization, While the subcellular localization of TFDP3 is cell cycle specific. It is expressed in the nucleus of G_1 phase and the end of mitosis, and in the cytoplasm of S phase and G_2 phase, which is similar to the cell cycle specific localization of E2F4 and E2F5.In quiescent cells (G0), the inhibited E2F5 mainly interacts with TFDP1/2 to block the transcription of cell cycle related genes. In circulating cells, E2F5 and TFDP1/2/3 are shuttled into the cytoplasm after the pocket protein in the middle and late stages of G_1 is phosphorylated and inactivated. At the same time, E2F1-3 combines with TFDP1/2 to activate target gene transcription, promote G_1–S phase transition, or form a complex with TFDP3 to inhibit its activity level, resulting in E2F activity threshold not reaching the limit point of G_1–S phase transition, and cell cycle is blocked in G_1 phase [6].

When E2F and DP protein family members interact as E2F/DP heterodimers, E2F has physiological functions and its transcriptional activity is regulated by the physical association of different pocket protein family members. The members of pocket protein family are composed of N-terminal domain, pocket domain and disordered sequence, including C-terminal domain, inter domain linker and pocket ring. The pocket domain is divided into two non-covalently interacting subdomains composed of folded cyclins and three additional helixes. It is combined with E2F reverse transcription activation domain through LxCxEcleet. The C-terminal domain not only forms another binding plane with the marker frame domain of E2F/DP heterodimer, but also contains the docking sites of a variety of kinases and phosphatases. It is reported that the deficiency of LXCXE binding function of RB will lead to the significant increase of E2F3 level and promote tumorigenesis, and the deletion of LXCXE binding domain will also lead to the instability of mouse genome, emphasizing that RB plays an important role in maintaining the stability of chromosome structure [7,8].

Different pocket protein subtypes (RB, p107 and p130) are different from each other. For example, p107 and p130 have 54% sequence homology, 30% homology with RB and similar domain structure [9]. Both pocket proteins can bind to E2F5, but p107 and p130 almost only bind specifically to E2F4 and E2F5. Tyler J. Liban et al. discovered that the C-terminal domain of p107 has a higher affinity for E2F4/5 by using structural and biochemical analysis, which is necessary to mediate the growth inhibition function, and p107 is only related to E2F4/5 [3].

Unlike E2F1-3, E2F4 and E2F5 do not have inherent nuclear localization signals (NLS), but contain nuclear export signals (NES). The nuclear localization signal activity is provided by DP heterodimer chaperone or pocket protein trans. The nuclear cytoplasmic localization of E2F5 in the cell cycle is consistent with the expression fluctuation of pocket protein in regulating nuclear accumulation, indicating that the nuclear cytoplasmic shuttle activity mediated by E2F nuclear accumulation mechanism plays a significant role in the cell cycle [10].

3. Role of E2F5 in cell cycle

Cell cycle refers to the process from the end of parental cell division to the end of offspring cell division. It is composed of G_1, S, G_2 and M phases, among which G_1, S and G_2 phases are collectively referred to as interphase. Mammalian cells are highly controlled by both positive and negative growth regulatory signals in the G_1/S phase. By regulating the transcriptional activation of E2F, the cells make irreversible DNA replication. Accordingly, E2F is crucial in the proliferation control of normal cells and tumor cells.

A single subtype of E2F will show different expression levels and activity patterns throughout the cell cycle. The nature of cell cycle dependent dynamic gene expression is driven by the sequential combination of E2F activators and inhibitors with target promoters, requiring the coordination of gene expression and cell cycle

regulatory proteins. One of the key periods is the transition from G_1 phase to S phase, and E2F5, RB and other afferent regulatory factors play a pivotal role in this transition process. They are strictly regulated in transcription, mRNA stability, post-translational modification, protein-protein interaction and protein stability. Here this study summarizes the main regulatory mechanisms of E2F5 in the cell cycle.

3.1. Pocket protein regulates E2F5 by phosphorylation

Pocket protein family is a critical negative regulator of cell cycle, regulating transcription by directly inhibiting E2F and recruiting transcriptional co regulators that modifying histone and chromatin structures. In G_0 phase, dephosphorylated pocket protein combines with E2F5 reverse transcription activation domain to form inhibition complex with a variety of cofactors on the target gene promoter, directly inhibiting the expression of target gene. In G_1 phase, when mitogen activated protein kinase signaling pathway transmits extracellular signals through kinase cascade reaction, it triggers cell proliferation, forms a complex between cyclin and cyclin dependent kinase (CDK), phosphorylates serine 807/811 (S807/811) and threonine 821 (T821) sites of RB, and turns RB into pRB. After RB inactivation, E2F5 is released from the complex and shuttles to the cytoplasm, increasing E2F activator activity and inducing transcriptional activation of G_1–S phase target genes, promoting the process of cell cycle. RB plays a major role in protecting genomic integrity and preventing uncontrolled cell proliferation, and is a key barrier to prevent tumorigenesis.

RB is an important barrier to prevent tumorigenesis, plays the crucial role in protecting genome integrity and preventing uncontrolled cell proliferation. RB knockout is fatal in mouse embryos, and knockout of p107 or p130 will lead to a viable phenotype [11,12]. In addition, the tumor suppressive characteristics of RB gene are considered to be stronger than p107 and p130, and mutations are often found in human tumor cells [13,14]. In contrast, p107 and p130 gene mutations are not common in cancer. Heterozygous mutant RB mice spontaneously develop tumors, while p107 and p130 mutant mice do not. Many tumorigenesis and pathological states are related to the loss of control caused by cell cycle checkpoint interruption. For example, overexpression of RB inhibits early events in G_1 phase and prevents cells from entering S phase. Then RB gradually phosphorylates from G_1 phase to G_2 phase, completing the complete closed loop of positive and negative regulatory signaling pathways in cell cycle. The accumulation of RB in the cytoplasm is significantly related to the phosphorylation of serine 795 (S795) site, resulting in the weakening of its tumor inhibitory activity in the nucleus [15].

On the one hand, RB combined with E2F5 to block the transcription of target genes, showing the ability of “inhibit activation”. On the other hand, RB-E2F5 complex retains the ability to bind to the promoter of the target gene, and recruits’ factors, such as histone deacetylases (HDACs) and methyltransferases, that affect the gene and chromatin structure, resulting in transcriptional inhibition, showing the ability of “active inhibition”. Loss of RB function or dysfunction of related pathways can lead to excessive cell proliferation, genomic instability and accumulation of gene mutations. Furthermore, RB signaling pathway is intertwined with other signaling cascades involved in cell cycle regulation and tumor inhibition, such as MARK, p53, PI3K-Akt-mTOR, increasing the complexity of cell cycle regulation.

In summary, the imbalance of RB-E2F5 signal will destroy the intracellular homeostasis. Whether it is gene mutation, functional defect, epigenetic change or environmental induction, it can release the carcinogenic potential of cells, leading to the occurrence, development and treatment resistance of tumors. On the contrary, changes in the expression or activity of Rb and E2F5 may lead to sensitivity or resistance to specific anti-tumor therapy, making them potential predictive markers of tumor therapy.

3.2. Regulation of subcellular localization on E2F5

Most of E2Fs were mainly located in nucleus, but E2F5 showed cell cycle specific localization. The nuclear export signal of E2F5 is mediated by a transporter protein (XPO1, also known as CRM1), which is located in the nucleus of quiescent cells and in the cytoplasm of circulating cells. Allen et al. found that the nuclear localization signal accumulation of E2F5 is produced by DP or supplied by physically related pocket protein trans. On the one hand, the cytoplasmic localization of E2F5 may be passive, and E2F5 combined with DP can overcome the interaction with nuclear localization signals. DP plays a dominant role in regulating nuclear localization signal in DP/E2F5. On the other hand, when E2F5 was co-expressed with D*P*-1 and p107, p107 significantly changed the nuclear localization ratio of DP/E2F5 (from ≤ 5% to > 90%), indicating that pocket protein trans provided nuclear localization to control the nuclear accumulation of DP/E2F5. It is worth noting that the nuclear accumulation of E2F5/DP is regulated by cell cycle. When E2F5 and D*P*-3 coexist, the proportion of cells in G_2/M phase will increase significantly. In contrast, under the same conditions, the co-expression of E2F5 and p107 lead to the accumulation of G_1 phase, and E2F5 is co-expressed with D*P*-3/p107 in the nucleus. Different E2F5 nuclear accumulation mechanisms have different biological consequences on cell cycle progression. When mediated by DP chaperone, cells receive stimulation to promote growth. While mediated by pocket protein, cell cycle progression is blocked, thus maintaining the transcriptional activity of E2F target gene [16].

3.3. Self-transcription and post transcriptional regulation of E2F5

In terms of self-transcription, E2F5 completes a cell cycle through self-regulation. in the early stage of G_1, E2F5 was released from the inhibition of pocket protein, to combine with pocket protein and return to the nucleus in the late stage of mitosis, formed a closed loop. At the post transcriptional level, E2F5 mRNA stability and translation are strictly regulated by a variety of miRNAs. For example, miR-154 is reported to be abnormally expressed in prostate cancer, hepatocellular carcinoma and renal cell carcinoma [17–19]. The former two are used as tumor suppressors to inhibit growth and proliferation, while in renal cell carcinoma, mir-154 is used as a carcinogen to participate in cancer cell proliferation and anti-apoptosis. Moreover, targeted miRNA has a profound impact on tumor drug resistance. Such as miRNA-34a targeted down regulated E2F5 expression, significantly enhancing the sensitivity of gastric cancer cells to paclitaxel treatment and overexpression of mir-544 in esophageal squamous cell carcinoma targets E2F5 to enhance cisplatin sensitivity [20,21]. Multiple miRNAs can adjust the expression and activity of E2F5, thereby regulating a variety of E2F5 target genes and affecting biological processes related to cell proliferation. On the contrary, E2F5 can regulate the expression of multiple miRNAs to build an interactive regulatory network.

4. Role of E2F5 in tumor cells

Malignant tumors produce drug resistance by changing the cell cycle process which also known as cell cycle specific mechanism or upstream signaling pathway which also known as cell cycle nonspecific mechanism and the imbalance of cell proliferation and apoptosis signal regulation is related to tumorigenesis. As a member of the core cell cycle mechanism, E2F5 is involved in the occurrence and development of a variety of cancers.

4.1 Renal cell carcinoma (RCC)

The expression level of E2F5 in clear cell renal cell carcinoma (ccRCC) decreased, and its low expression was significantly correlated with longer overall survival, disease-specific survival and progression free survival. It

was also an independent factor related to overall survival and progression free survival in patients with ccRCC, and its expression was significantly correlated with DNAmethylation and copy number [51]. E2F5 also showed a low expression level in chromophobe renal cell carcinoma (chRCC), and the expression level was correlated with the histological grade of chRCC, including T and N stages [52].

4.2. Ovarian cancer

E2F5 is overexpressed in the early stage of ovarian cancer, especially in serous and endometrioid ovarian cancer. The expression of E2F5 can be up-regulated to 5 times in the process of early progression to advanced stage, so it is considered to be a carcinogen. When E2F5 and CA125 are combined, the sensitivity and specificity of detection of epithelial ovarian cancer (EOC) can be improved to 97.9% and 72.5% [22,23]. Daniel et al. found that the response of different E2F to INF-γ intervention is heterogeneous, and the increased expression of E2F5 is the key factor for INF-γ to play an anti-proliferative role in ovarian cancer cells [24]. In recent years, E2F5 has been found to be highly expressed in cisplatin resistant ovarian cancer cells and promotes proliferation as a positive regulator, indicating that E2F5 may be a new target for the treatment of drug-resistant ovarian cancer. Knockdown of E2F5 can reduce the expression levels of cyclin D1, CDK4 and pRB through *Hippo* and *Wnt-β-Catenin* pathways, make cells accumulate in G_0/G_1 phase, and significantly block the cell cycle from entering G_1/S phase [25–27]. FAT4 can reduce the expression of E2F5, promote the transition of G_1/S phase of cell cycle, and accelerate cell growth and epithelial-mesenchymal transition [26].

4.3. Breast cancer

E2F5 specific signal is located between MOS (8q12) and MYC (8q24), which is also the place where 50–60% of breast cancer genes are amplified, indicating that E2F5 may cooperate with other oncogenes to promote cell transformation and thus promote the occurrence and development of tumors [28]. It is reported that the abnormal methylation degree of E2F5 in breast cancer tissues is low, and its promoter methylation frequency is also low, and the methylation degree of well differentiated tumors is often lower than that of poorly differentiated tumors [29]. Carson broeker et al. detected that E2F5 deficient mice retained the integrity of lactation function, but also spontaneously developed into highly metastatic breast cancer after a long incubation period [29].

In triple negative breast cancer (TNBC), overexpression of E2F5 was more common, and increased E2F5 was also observed in tissues with high Ki-67. In addition, the positive expression of E2F5 in patients with negative lymph nodes will show worse clinical prognosis and shorter disease-free survival. The overexpression of E2F5 is associated with invasive histopathology and worse clinical prognosis [30]. Down regulation of E2F5 could reduce the proliferation rate of tumor cells and induce the death of ER $^{(+)}$ breast cancer cells with wild-type TP53, but had little effect on TNBC cells with TP53 mutation and Her-2 $^{(+)}$ breast cancer cells. Although there was a slight increase in G_2/M phase cells in E2F5 deleted TNBC cells, the fluctuation of p21WAF1 expression was not found after E2F5 deletion in TNBC cells, and there was no increase in G_2/M phase cells in HER-2$^{(+)}$ breast cancer cells [31].

4.4. Hepatocellular carcinoma

The increased expression of E2F5 in hepatocellular carcinoma (HCC) tissues is significantly correlated with tumor stage and poor overall survival [32]. Ji et al. described that FOXN3 inhibits E2F5 expression by directly combining with its promoter, but this inhibition can be reversed by up regulating E2F5 [33]. Contrary to the above conclusion, Zou et al. found that the expression of E2F5 was down regulated in HBV infection related HCC cells, and the level of HBV infection was negatively correlated with the expression of E2F5, revealing that the cell growth promoting

mechanism of HBV may play an important role in the progress of HBV related HCC [34]. Jiang et al. identified that the 8q21.2 locus, also the E2F5 locus gene of primary HCC was repeatedly amplified through genome-wide, microarray, and comparative genomic hybridization analysis. Down regulation of E2F5 could significantly inhibit the growth of HCC cells and block the cell cycle at G_0/G_1 phase. E2F5 may be involved in the regulation of early G_1 events, including G_0/G_1 phase transition, lifting the restriction of checkpoints, inducing uncontrollable cell cycle progression in hepatocytes, and ultimately promoting cancer transformation through joint action with other carcinogens [35].

4.5. Prostatic cancer

E2F5 is a carcinogen gene in prostatic cancer (PCa). Overexpression of E2F5 was significantly correlated with high Gleason score, high transcription level, high biochemical recurrence risk, advanced tumor stage, metastasis and low survival rate after metastasis. E2F5 low expression tissues are often accompanied by abundant plasma cells and NK cells, driving antibody-dependent cell-mediated cytotoxicity (ADCC) to promote anti-tumor immunity. E2F5 may potentially affect the progress of PCA by interfering with anti-tumor immunity [36]. Increased copy numbers of E2F5 and MYC were also observed in the chromosome 8q21-24 region of PCa cells, and their synergistic effect may be related to invasive clinicopathological features [37]. Qi et al. discovered that endogenous CDK13 can promote the formation of circ-CDK13 and significantly promote the expression of E2F5 by reducing the expression of miR-212-5p/449. As a transcription activator of CDK13, E2F5 also stimulates the transcription of CDK13. This positive feedback regulation promotes the growth and proliferation of PCa cells, and this circ-RNA produced with the transcription of primary genes regulates the expression of primary genes and downstream target genes through positive feedback, which may be one of the important reasons for the drug resistance of tumor cells to molecular targeted drugs [38].

It is reported that E2F5 may be involved in the regulation of G_0/G_1 phase of PCa cells. Majumder s et al. found that the transcription level of E2F5 in tumor tissues with Gleason score of 6 was significantly higher than that in tumor tissues and benign prostatic hyperplasia (BPH) tissues with Gleason score greater than 6. They further verified that E2F5 and SMAD3 were co-located in PCa cells, and their distribution in the nucleus was increased. Down regulation of E2F5 would reduce the phosphorylation level of p38 and SMAD3, leading to the arrest of PCa cells in G_1 phase [39]. Li et al. reported that overexpression of E2F5 and (or) PFTK1 was significantly associated with the highly invasive PCa phenotype. Silencing E2F5 and PFTK1 not only significantly increased the proportion of G_0/G_1 phase cells, but also inhibited the expression of CDK2 and CDK4 [40]. Tariq a. bhat et al. clarified that decursin downregulated ERK1/2 phosphorylation by targeting EGFR-ERK1/2 pathway, increased the expression of p27, p107 and p130, but significantly reduced the expression levels of E2F5, CDK2 and CDK4, thereby inducing strong G_1 arrest and cell death of PCa cell [41]. Karmakar deepmala et al. showed that E2F5 as a bifunctional transcription factor, on the one hand inhibited the expression of TFPI2 (TFPI2 negatively regulated the level and activity of MM*P*-2 and MM*P*-9), on the other hand activated the transcriptional expression of MM*P*-2 and MM*P*-9 genes. They also found that artemisinin can reduce the expression of E2F5 and reverse the dysfunctional interaction between TFPI2 and MMPs in PC3 cells [42]. It has been reported that artemisinin can trigger G_1 phase arrest and improve the proliferation rate and invasiveness of PCa cells [43]. However, the relevant mechanism of artemisinin mediated E2F5 down-regulation remains to be explored. Similarly, Chapla Agarwal

et al. observed that IP6 could significantly reduce E2F5 protein levels by 70% with the increase of dose and the extension of treatment time, and reduce the molecular levels of CDK4, cyclin D1, pRB and other molecules involved in the control of G_1/S phase transition. Besides, IP6 also induced the expression of Cip1/p21 and Kip1/p27, resulting in the decrease of Bcl-2 and the increase of Bax/Bcl-2 ratio, and the strong accumulation of cell cycle in G_1 phase accompanied by the decrease of S phase and G_2/M phase [44]. According to the report, cell apoptosis often occurs in G_1 phase, and G_1/S phase arrest can accelerate cell apoptosis, suggesting that IP6 may simultaneously activate cell proliferation arrest and apoptosis to induce the growth inhibition of PCa cells [45].

4.6. Other malignant tumors

In head and neck squamous cell carcinoma (HNSCC), gastric cancer, colon cancer and esophageal squamous cell carcinoma (ESCC), E2F5 overexpression was found, and was significantly correlated with pathological malignancy, poor prognosis and low overall survival. It was also negatively correlated with the infiltration of activated dendritic cells, macrophages, neutrophils and NK cells [46–50]. At present, the "protective" characteristics of E2F5 in renal cell carcinoma are still unexplained, which may be related to cell proliferation, differentiation, DNA repair, cell cycle control and cell death, and the related mechanism remains to be further studied.

The stable expression of E2F5 can specifically induce quiescent cells to enter the cell cycle, and its abnormal expression behavior may be determined by the specific cell environment, the existence of overlapping binding sites and the interaction between promoter and target genes. In a study on cervical cancer associated with high-risk human papillomavirus (HPV) infection, HPV 18 converts E2F5 into an activator by directly transcribing and activating the major carcinogenic protein E7. E2F5 directly activates E6/E7 transcription, overcomes G_0/G_1 checkpoint blocking by degrading p53 and pRB, and indirectly and positively regulates the entry of cells into S phase. The transition of E2F5 from inhibition to activation may enhance the carcinogenic potential of HPV18 and promote the progression of cervical cancer [53]. Down regulation of E2F5 in pancreatic cancer and neuroblastoma can trigger G_0/G_1 phase block. Knockout of E2F5 in gastric cancer can increase the number of cells in G_1 phase and reduce the number of cells in S phase, leading to the stagnation of tumor cells in G_1/S phase [54–56]. In general, E2F5 seems to act as a gatekeeper to control the transcription and expression of target genes at immune checkpoints during early cell cycle events, especially the transition from quiescent cells to circulating cells. Its precise regulation of cell cycle is an important guarantee to ensure growth, proliferation and differentiation. The abnormal expression of E2F5 is common in various tumors, and it seems that the transition from the " gatekeeper" to the "destroyer" has made it the latest breakthrough point in current cancer treatment.

5. Conclusion and perspective

The abnormal activity of core cell cycle mechanism basically exists in all tumor types, and it is also the driving force of tumor occurrence and progression. E2F5, as one of the core gatekeepers of cell cycle, mainly regulates the gene expression in G_1/S phase, showing the characteristics of cell cycle dependence. Its precise expression and activity are crucial for protecting cells from abnormal proliferation, ensuring the spatiotemporal nature of genome replication and the accuracy of genetic material transfer. However, the abnormal expression and activity of E2F5 are also important mechanisms for the occurrence and development of various cancers. Cancer cells with uncontrolled proliferation use the characteristics of E2F5 in different ways, strengthening the uniqueness and dependence of E2F5 program driven by oncogenes. The abnormal expression of E2F5 in tumor cells may be a manifestation of tumor specificity. More and more evidences show that the change of E2F5 expression is the key

mechanism of chemotherapy resistance of cancer cells, especially in the presence of CDK4/6 inhibitors. Therefore, E2F5 is a potential biomarker for molecular diagnosis.

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Disclosure statement

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