Review

DOI: 10.5582/bst.2021.01072

The E2F transcription factor 2: What do we know?

Luwen Li[1,2,3], Shiguan Wang[4], Yihang Zhang[1,2,3], Jihong Pan[1,2,3,*]

[1] Biomedical Sciences College & Shandong Medicinal Biotechnology Centre, Shandong First Medical University, Ji'nan, China;
[2] Key Lab for Biotech-Drugs of National Health Commission, Ji'nan, China;
[3] Key Lab for Rare & Uncommon Diseases of Shandong Province, Shandong First Medical University, Ji'nan, China;
[4] Medical College, Shandong University, Ji'nan, China.

SUMMARY E2F transcription factor 2 (E2F2) is a member of the E2F family of transcription factors. The classical view is that some E2Fs act as "activators" and others "inhibitors" of cell cycle gene expression. However, the so-called "activator" E2F2 is particularly enigmatic, with seemingly contradictory roles in the cell cycle, proliferation, apoptosis, inflammation, and cell migration and invasion. How can we rationalize the apparently opposing functions of E2F2 in different situations? This is difficult because different methods of studying E2F2 have yielded conflicting results, so extrapolating mechanisms from an observed endpoint is challenging. This review will attempt to summarize and clarify these issues. This review focuses on genetic studies that have helped elucidate the biological functions of E2F2 and that have enhanced our understanding of how E2F2 is integrated into pathways controlling the cell cycle, proliferation, apoptosis, inflammation, and cell migration and invasion. This review will also discuss the function of E2F2 in cancer and other diseases. This review provides a strong basis for further research on the biological function and clinical potential of E2F2.

Keywords E2F2, biological effect, diseases

1. Introduction

The E2F transcription factor family, a cellular factor required for activation of the E2 adenoviral promoter (*1*), came to the forefront of cancer research when it was found to be associated with and regulated by the retinoblastoma protein RB. There are now eight known subclasses of E2F proteins (*2*); some are considered "activators" and others "inhibitors" of expression (*3,4*). This makes E2F2 enigmatic and unique, and despite being known for 35 years, its functions remain an active topic of research in diverse arenas such as biochemistry, cell and developmental biology, and oncology.

2. Structure and regulation

E2F2 contains a winged-helix DNA binding domain (DBD) (*5*) (Figure 1), a highly conserved domain that contributes to dimerization, expressed from eight chromosomal loci to regulate the transcriptional activity of other genes. The E2F2 protein also contains multiple protein-protein interaction domains, including a helix-loop-helix binding domain that mediates heterodimerization with Sp1, resulting in synergistic activation of transcription (*6,7*). Other domains include a cyclinA/cdk2 binding domain, hydrophobic heptad repeat dimerization domain, and Rb protein binding domain.

The activity of E2F2 is controlled by acetylation by P/CAF, p300/CBP, or a related acetyltransferase, which increases the protein stability and DNA binding and transactivation activity of E2F2. However, this acetylation can be reversed by "pocket proteins" (*i.e.*, the retinoblastoma protein (pRb) and Rb-related proteins p107 and p130) (*8,9*), which are regulated by cyclin dependent kinases (CDKs), form the CDK-E2F-pRb complex, and take part in transcriptional activities (*10*). pRb acts as a transcriptional repressor complex by recruiting histone deacetylase (HDAC) and remodeling chromatin. During transcriptional activation of cell cycle progression, pRb is phosphorylated by G1 cyclin-dependent kinase complexes (cyclinD/cdk4 and cyclinE/cdk2) that inhibit its ability to bind E2F2, which is released and becomes transcriptionally active (*11*).

The dimerization domain of E2F2 mediates heterodimerization with a DP protein such as DP-1, DP-2, or DP-3. This interaction is required for formation of functional transcription factors that can bind to DNA with high affinity. DP proteins were originally identified as binding to differentiation to regulate transcription

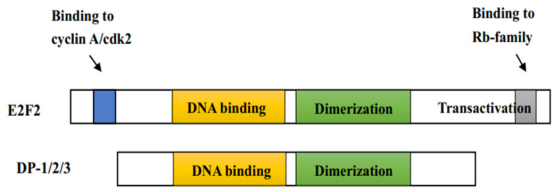

Figure 1. The structure of E2F2 as well as the similar structure of DP proteins. The highly conserved winged-helix DNA binding domain (DBD) is indicated in yellow, and the hydrophobic heptad repeat domain required for dimerization is shown in green. Other domains required for interaction with cyclin A/cdk2 and Rb family members are also indicated.

factor 1 (DRTF1). The consensus DNA-binding site of DRTF1 was later found to be the same as that of E2F2, and DRTF1 was also found to interact with Rb (*12*). Evidence is now clear that DRTF1 and E2F2 are the same factor (*13*) and that E2F proteins regulate complex cellular functions by forming heterodimeric protein complexes with a member of the DP family of proteins (DP-1 or DP-2).

3. Role and function

3.1. Interaction between E2F2 and cell cycle proteins

E2F2 plays a significant role in promoting the cell cycle (*14*), which is regulated by CDKs and CDK inhibitors (CKIs). E2F2 is often referred to as an activator because it transcriptionally activates certain target genes, such as cyclin E. Analysis of the cell cycle regulatory machinery has indicated that expression of E2F2 can greatly induce cyclin A and E while not affecting the expression of CKIs such as p21. For example, E2F2-mediated expression of cyclin A and E can induce limited proliferation of cardiomyocytes (*15,16*). In addition, E2F2 has nuclear localization signals adjacent to its cyclin A-binding domain. This ensures its movement into the nucleus, thereby modulating E2F2 activity in a cell cycle-dependent manner.

The "pRb pathway" is one of the most significant pathways in normal cell cycle control. Unphosphorylated pRb binds to E2F2 in G0/G1, forming a complex that actively represses E2F2-responsive genes (*17*). Once activated by mitogenic signals, CDKs phosphorylate pRb, p107, and p130, causing the release and accumulation of sequestered E2F2 (*18*). In many cell types, pRb family members play an important role in regulating terminal differentiation by directly controlling cell division through regulation of E2F-dependent promoters. For example, TNF-α stimulates proliferation of vascular smooth muscle cells by activating the Raf-1/MEK/ERK pathway and stimulating Rb-Raf-1, resulting in high expression of E2F2 that regulates cell proliferation (*19*). Rb-mediated control of E2F2 creates complex signaling and regulatory loops. For example, Rb-E2F2 controls angiogenesis by regulating VEGF (vascular endothelial growth factor A) receptors. In a hypoxic environment, E2F2 is released and activated, ultimately regulating angiogenesis *via* interaction with hypoxia-inducible factor 1 to activate *VEGF*, allowing the secretion of VEGF and eventual interaction with its receptor on endothelial cells (*20*). Rb-E2F2 also regulates angiogenesis with other mediators – for example, p53 binds to E2F2 to form a transcriptional complex that inhibits VEGF expression (*21*).

Mice with targeted deletion of *E2f2* exhibit impaired liver regeneration, and their hepatocytes display delayed cell-cycle entry from quiescence. In addition, E2F2-mediated transcription promotes adult hepatocyte proliferation and liver regeneration (*22*). Overexpression of E2F2 in cultured cells stimulates their entry into the S phase (*23*), indicating that E2F2 promotes cell cycle progression. In light of this, E2F2-responsive genes are potent transcriptional activators (*24,25*), and overexpression of E2F2 is sufficient to induce quiescent cells to re-enter the cell cycle (*26-28*) by promoting activation of target genes that are important to the G1/S transition (*29*). Knockdown of E2F2 expression reduces the proliferation of glioma and GSCs (cancer stem-like cells), while overexpression of E2F2 partially reverses the inhibitory effect of Let-7b (a member of the Let-7 microRNA family) on the proliferation of glioma and GSCs (*30*). These findings are consistent with the positive role E2F2 presumably plays in progression from the G1 to the S phase (*31*).

3.2. E2F2 can promote or inhibit proliferation

Current evidence suggests that E2F2 may act both as a suppressor and promoter of proliferation, depending on the cellular context (Table 1).

3.2.1. Promoting proliferation

Studies of multiple E2Fs have revealed both redundant and specific roles for E2F2 in proliferation. $E2F1^{-/-}$ $E2F2^{-/-}$ T cells exhibit profound defects in homeostatic proliferation (*31*). Intriguingly, *E2F2* and *E2F1* double knockout (DKO) mice are severely impaired in all hematopoietic cell lineages because of defective S phase progression in progenitor populations (*32*). E2F2 is expressed in a cell cycle-regulated manner and is highest in the late G1 and S phases (*33*). In cultured neonatal rat cardiomyocytes, directed expression of E2F2, but not E2F1, E2F3, or E2F4, stimulates cell division without affecting apoptosis, indicating that E2F2 offers promise as a specific candidate for regenerating cardiomyocytes (*34*). Moreover, a combination of *E2f1*, *E2f2*, and *E2f3* mutations is sufficient to completely block proliferation of mouse embryonic fibroblasts (MEFs) (*35*).

3.2.2. Inhibiting proliferation

Table 1. Effect of E2F2 on cell proliferation

Genotype	Cell type or tissue	Effect on proliferation	Phenotypic consequences
E2F2$^{-/-}$	T cells	Increases antigen-dependent proliferation	
	Cardiomyocytes	Inhibits proliferation	
	Glioma cells	Inhibits proliferation	
	GSCs	Inhibits proliferation	
	hESCs	Inhibits proliferation	
	ECs	Promotes proliferation	
Elevated E2F2	Adult hepatocytes	Promotes proliferation	liver regeneration
	Cardiomyocytes	Promotes proliferation	
E2F1$^{-/-}$	T cells	Promotes antigen-dependent proliferation	
E2F2$^{-/-}$	Exocrine pancreas	Promotes endoreduplication	polyploidy, exocrine de-generation, and diabetes
	T cells	Inhibits homeostatic prolifera-tion	T-cell lymphopenia
	Hematopoietic progenitors	Impairs S-phase progression	defective hematopoiesis, anemia, and leukopenia
E2F1$^{-/-}$ *E2F2*$^{-/-}$ *E2F3*$^{-/-}$	MEFs	Arrests the cell cycle throughout	

Table 2. The effect of E2F2 on apoptosis

Genotype	Cell type or tissue	Effect on apoptosis	The reason
E2F2$^{-/-}$	Myc-induced T cell lymphomagenesis	Inhibits apoptosis	p53-dependent apoptosis
	cone cells	Inhibits apoptosis	p53-dependent apoptosis
	MEF	Inhibits apoptosis	p53-dependent apoptosis
	melanocytes	Inhibits apoptosis	p53-independent apoptosis
dE2F2$^{-/-}$	wing proliferative tissue of Drosophila	Inhibits apoptosis	p53-independent apoptosis
	peripheral nervous system of Drosophila	Inhibits apoptosis	p53-independent apoptosis
E2F2$^{-/-}$	cardiomyocytes	Promotes apoptosis	p53-dependent apoptosis
	melanoma cells	Promotes apoptosis	p53-dependent apoptosis
E2F1$^{-/-}$ *E2F2*$^{-/-}$	differentiating cells	Promotes apoptosis	p53-dependent apoptosis

Conversely, a study has indicated that E2F1/E2F2 transcription factors play a key role in slowing the rate of proliferation during terminal cell differentiation (*36*). T lymphocytes deficient in E2F2 proliferate with TCR stimulation. An E2F2 deficiency, or more significantly loss of both E2F1 and E2F2, results in increased proliferation of T cells in peripheral blood, which is consistent with a reduced threshold of antigen activation (*37,38*). In addition, hind-limb ischemia was surgically induced in E2F2$^{-/-}$ mice and their wild-type littermates; two weeks later, laser Doppler perfusion measurements, capillary density, and endothelial cell proliferation were significantly enhanced in E2F2$^{-/-}$ mice (*16*). Zhou *et al.* found that loss of E2F2 expression improved endothelial cell growth, proliferation, gene expression in the G1/S phase, and neovascularization after myocardial infarction (*16,39*).

Since E2Fs can both suppress and stimulate proliferation, the balance between various signal intensities is what determines whether cells proliferate or differentiate (*40*). In the microenvironment of the body, E2F-mediated control of cell proliferation results from the balance between repressor and activator E2F proteins. Therefore, E2F2 may upset this balance and induce different regulation of proliferation in different *in vivo* microenvironments.

3.3. E2F2 can promote or inhibit apoptosis

Apoptosis, the process of programmed cell death, occurs throughout the lifespan of multicellular organisms. Apoptosis acts to maintain homeostasis of tissues and organs by removing unwanted or damaged cells. Depending on the cells or tissues, E2F2 can have pro- or anti-apoptotic effects. Indeed, E2F2 expression induces apoptosis in different proliferative tissues, but this effect is not observed in differentiated post-mitotic cells. This phenomenon suggests that the regulation of apoptosis by E2F2 may be related to cell type and developmental status (Table 2).

3.3.1. Promoting apoptosis

The alternative expression or combination of any of the eight *E2F* family genes can induce strong apoptotic activity. This apoptosis is widely is believed to be a result of high levels of E2F activity because ectopic expression of E2F2 can induce both p53-dependent and p53-independent apoptosis (*41*).

p53-dependent apoptosis: Removing one allele of *E2f2* reduces apoptosis and promotes the formation of Myc-driven murine T-cell lymphomas (*42*). Mechanistically, this may be due to the E2f2-dependent

Ser15 phosphorylation of P53 (*43*), which in cone cells leads to concomitant induction of the p53 targets Noxa and Siva. Deleting p53 in a context of elevated E2f2 ($Rb^{-/-}$) inhibits the induction of apoptosis in cone cells, indicating the process is p53-dependent. E2f2 also maintains levels of Tradd, which inhibits Trip12/Ulf-mediated Arf ubiquitylation and degradation (*44,45*). Removing E2f2 reduces Arf levels, increases the Arf target Mdm2, and ultimately activates the p53 pathway (*46*).

p53-independent apoptosis: Rbf1, the *Drosophila* homolog of Rb, is pro-apoptotic in proliferative tissue. In flies, E2F2 is the main partner of Rbf1 (*47*). In that study, Clavier *et al.* found that dE2F2 and dDP are required for Rbf1-induced apoptosis. Moreover, Rbf1 and dE2F2 reduce the expression of two major anti-apoptotic genes in *Drosophila*: buffy, an anti-apoptotic member of the Bcl-2 family; and diap1, possibly encoding a caspase inhibitor. Rbf1/dE2F2 represses buffy at the transcriptional level, contributing to cell death. In addition, Rbf1 and dE2F2 upregulate HOW expression. HOW is an RNA binding protein involved in diap1 regulation. HOW is essential for cell survival and key to mRNA degradation (*48*). In summary, Rbf1 appears to coordinate with dE2F2 and some complexes to downregulate the anti-apoptotic genes buffy and diap1, thereby promoting cell death in proliferating tissues (*47*). In *Drosophila*, Rovani *et al.* found that the dREAM complex, which includes dE2F2, cooperates with the proapoptotic factor Grim to induce cell death in the peripheral nervous system (*49*). In melanocytes, Raj *et al.* found that E2F2 binds to the *Survivin* (an anti-apoptotic factor) promoter and that mutation of either the p53 or E2F2-binding sites within the promoter is sufficient to increase transcription (*50*).

3.3.2. Inhibiting apoptosis

Expression of E2F2 in cardiomyocytes reduces expression of various apoptosis-related genes, including *p53*, *p21CIP/WAF*, and *mdm2*, and it represses the activity of proapoptotic pathways (*34*). Iglesias *et al.* found that $E2f1^{-/-}$/$E2f2^{-/-}$DKO mice exhibited apoptosis of pancreatic cells and mitochondria (*51,52*). Providing insight into the mechanisms underlying apoptosis, typical p53 direct transcriptional target genes involved in intrinsic (*Bax*, *Puma*, *Apaf-1*, and *Pidd*) and extrinsic (*Dr5*) pathways are significantly overexpressed in DKO pancreatic samples, and disruption of p53 in *E2f1/E2f2*-deficient mice prevents apoptosis and restores a normal pancreatic phenotype (*52*). Interestingly, p21 was found to be optional for the aberrant pancreatic phenotype developed by cells lacking E2F1/E2F2. In melanoma, however, E2F2 regulates SIRT1 to inhibit p53-dependent apoptosis (*53*).

3.4. E2F2 can promote or inhibit inflammation

The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissue damage arising from the original insult or the inflammation process itself, and initiate tissue repair. According to a recent study, E2F2 has both pro-inflammatory and anti-inflammatory action in mammals (Table 3).

3.4.1. Promoting inflammation

Silencing E2F2 significantly decreases expression of the inflammatory cytokine IL-6 in J774A.1 macrophages and MES 13 mesangial cells (*54*). A previous gene chip analysis by the current authors revealed that expression of E2F2 is higher in synovial tissue from patients with rheumatoid arthritis (RA) than from patients with osteoarthritis (OA) (*55*). The current authors previously found that its increased expression contributes to the abnormal proliferation, invasion, and cytokine production of RA synovial fibroblasts (RASFs). Further research revealed that TNF-α can facilitate the nuclear translocation of E2F2, NF-κB can bind to the E2F2 promoter, and E2F2 can directly bind to the IL-6 promoter (*56*). Moreover, E2F2 affects the formation of the STAT1/MYD88 complex by directly binding to STAT1 and MYD88 promoters. This, in turn, influences entry of STAT1 into the nucleus and activation of the PI3K/AKT/NF-κB pathway, which ultimately regulates expression of inflammatory cytokines including IL-1α, IL-1β, and TNF-α (*55*). Moreover, Wu *et al.* demonstrated that *E2F1/E2F2* DKO significantly reduces neuronal death, neuroinflammation, and associated neurological deficits (*57*), which is consistent with results from the current authors.

Table 3. The effect of E2F2 on inflammation

Genotype	Cell type or tissue	Effect on inflammation	Mechanism	Consequence
$E2F2^{-/-}$	J774A.1 macrophages, MES 13 mesangial cells	Inhibits inflammation	decreased let-7a expression	decreased IL-6 production
	RASFs	Inhibits inflammation	PI3K/AKT/NF-κB	decreased IL-1α, IL-1β, and TNF-α production
	T lymphocytes	Promotes inflammation		inflammatory infiltrates
$E2F1^{-/-}$ $E2F2^{-/-}$	neuronal cells	Inhibits inflammation		reduced neuronal death, neuroinflammation, and tissue damage

3.4.2. Inhibiting inflammation

E2F2 functions as a negative regulator of the immune response in mice by suppressing cellular proliferation of activated lymphocytes. As they age, *E2f2*$^{-/-}$ animals develop an autoimmune disorder with features of splenomegaly, multiorgan inflammatory infiltrates, glomerulonephritis, and serum anti-DNA antibodies, and the animals die prematurely. In these mice, E2F2-deficient T cells are hyperresponsive to TCR stimulation, responding with increased proliferation to lower concentrations of ligand. Thus, low levels of self-ligands may be sufficient to trigger autoimmune disease in these mice, resulting in a dramatic, abnormal expansion of the CD44^{hi}/CD69-effector/memory population of T cells (*58*).

3.5. Migration and invasion

Cell migration and invasion are central processes in the development and maintenance of multicellular organisms. Wound healing, immune response, and tissue formation and shaping during embryonic development all require orchestrated movement of cells. Cell migration is usually in response to specific external signals, both chemical and mechanical. Errors during this process have serious consequences, including intellectual disability, vascular disease, and tumor metastasis. Zhang *et al.* silenced E2F2 to suppress the migration and invasion of RASFs *in vitro* (*56*). Yoon *et al.* found that expression of an E2F2 mutant deficient in DNA-binding interfered with H-RAS dependent invasion of SUM-159 cells, suggesting transcriptionally active E2F2 is required for this process (*59*). Active E2F2 may promote H-RAS-dependent invasion in part by increasing expression of the B4 integrin subunit, a component of the A6B4 integrin that is known to enhance carcinoma invasion. Specifically, expression of E2F2 increases B4 mRNA, protein, and cell surface expression. These aspects link active H-RAS, transcriptionally active E2F2, and A6B4 integrins in a common pathway that enhances A6B4-dependent invasion. H-RAS also can activate E2F2 and A6H4 integrins through a common pathway, ultimately enhancing A6H4-dependent invasion.

4. E2F2 in malignancies

The effect of E2F2 on tumors cannot be ignored; it seems to be more inclined to promote tumor progression. There is scant evidence that E2F2 inhibits tumor progression (Table 4).

4.1. Promoting malignant tumors

4.1.1. Breast cancer

Aberrant E2F2 expression is associated with cancer progression and metastasis (*60*). Fujiwara *et al.* found that activation of the E2F2 pathway is associated with a lower relapse-free survival (RFS) rate in patients with breast cancer (*61*). In particular, E2F2 expression impacts cell-matrix adhesion, with potential consequences for metastatic colonization during breast cancer (*62*). Moreover, levels of gene expression have revealed that tumors from *E2f2* knockout mice have reduced expression of genes associated with the epithelial-mesenchymal transition (EMT), corresponding with a reduced probability of Ras activation. A study has found that the low likelihood of E2F2 pathway activation in human breast cancer is related to longer recurrence-free survival (*61*). That finding also illustrates the unique genetic requirements of individual E2Fs in mediating tumorigenesis in human breast cancer. Li *et al.* analyzed Oncomine data and found that *E2F2* mRNA levels are higher in breast cancer ($p < 0.001$) than in normal tissues (*63*).

4.1.2. Lung cancer

Immunohistochemical analysis of lung cancer biopsies from 119 patients detected E2F2 expression in 18% of patient samples and predominantly in patients with adenocarcinoma rather than squamous cell carcinoma (*64*). Sun *et al.* examined *E2F2* transcription and data on the survival of patients with lung cancer using the Oncomine, GEPIA, Kaplan-Meier Plotter, and cBioPortal databases. Analyses indicated that levels of *E2F2* expression were higher in lung adenocarcinoma and squamous cell lung carcinoma tissues than in

Table 4. The effect of E2F2 on cancer

Genotype	Type of cancer	Effect on cancer	Consequence
E2F2$^{-/-}$	breast cancer	Inhibits cancer	increased latency fewer tumors with EMT inhibited cell-matrix adhesion
	lung cancer	Inhibits cancer	decreases the CSC population reduces cell viability and colony formation
	liver cancer	Inhibits cancer	
E2F2 polymor-phisms	squamous cell carcinoma of oropharynx	Alters the risk of SCCOP recurring	
High E2F2 expression	ovarian cancer	Promotes cancer	worsens overall survival
	gastric cancer	Promotes cancer	
E2F2$^{-/-}$	lymphoma	Promotes cancer	

normal lung tissues, and levels of *E2F2* expression correlated with a tumor in an advanced stage. Moreover, survival analysis using the Kaplan-Meier Plotter database revealed that high levels of *E2F2* mRNA are associated with a low RFS rate in the patients with lung cancer that were studied (*65*). In addition, Chen *et al.* immunohistochemically analyzed 86 non-small cell lung cancer (NSCLC) samples and found that E2F2 expression was markedly increased in 62.8% (54/86) of samples compared to that in non-tumor lung tissue. Further studies have found that E2F2 expression is closely related to clinical stage ($p = 0.039$) and tumor size ($p = 0.045$). E2F2 acts as an activator in the progression of NSCLC and may serve as a promising indicator of prognosis for patients with NSCLC (*66*).

4.1.3. Liver cancer

Bioinformatic analysis of TCGA data revealed that the expression of E2F2 in HCC samples was significantly correlated with histological grade, clinical stage, and tumor status. Therefore, elevated E2F2 can be used as an independent prognostic marker and therapeutic target for liver cancer (*67*). Moreover, HCC tumor tissues exhibited overexpression of the BRD4-E2F2-cell cycle regulation axis, and E2F2 overexpression was significantly associated with a poor prognosis in those patients with HCC (*68*). Thus, E2F2 overexpression appears to play a central role in dysregulation of the cell cycle in HCC.

4.1.4. Squamous cell carcinoma of the oropharynx

Li *et al.* investigated associations between genetic variants in five *E2F2* promoter polymorphisms and the risk of recurrence of squamous cell carcinoma of the oropharynx (SCCOP) in 1,008 patients (*69,70*). Compared to patients with the variant *E2F2* genotypes rs2742976 and rs3218123, patients with the common homozygous genotypes had better disease-free survival (both log-rank, $p < 0.001$) and lower risk of SCCOP recurrence (HR: 0.4; 95% CI: 0.3-0.6; and HR: 0.3: 95% CI: 0.2-0.5, respectively) after multivariable adjustment. This finding suggests that *E2F2* polymorphisms may individually or jointly modify the risk of SCCOP recurrence.

4.1.5. Ovarian cancer

Xie *et al.* examined 308 ovarian cancer samples and found that E2F2 is significantly upregulated in ovarian cancer epithelial cells (CEPIs) (*71*). That study also indicated that increased E2F2 expression significantly enhances MCM4, CCNE2, and WHSC1 transcription in the SKOV3 and A2780 ovarian cancer cell lines. In addition, high levels of E2F2 and CCNE2 expression were associated with poorer overall survival. The high level of E2F2 expression offsets the effect of an LBX2-AS1 knockdown in ovarian cancer cells (*72*). LBX2-AS1 is a new type of lncRNA that promotes the progression of ovarian cancer. Therefore, E2F2 promotes ovarian cancer.

4.1.6. Gastric cancer

miRNA chip analysis indicated that miR-31 decreased in gastric cancer. E2F2 is the direct target of miR-31. E2F2 expression is up-regulated in gastric cancer tissues and is inversely proportional to the level of miR-31. miR-31 plays a vital role as a tumor suppressor by inhibiting the expression of E2F2s (*73*). Bioinformatic analysis using multiple databases revealed that the level of E2F2 expression in GC tissue was significantly higher than that in normal tissues and that the expression of E2F2 was related to survival (*74*). E2F2 is a potential biomarker and therapeutic target for the treatment of differentially expressed genes in GC (*75*).

4.2. Inhibiting malignant tumors

Opavsky *et al.* used a bitransgenic mouse model of Myc-induced T cell lymphomagenesis and analyzed tumor progression in E2F-deficient mice (*42*). Interestingly, the targeted inactivation of E2F1 or E2F3 has no significant effect on tumor progression while the loss of E2F2 accelerates the development of lymphoma. The loss of a single copy of E2F2 also accelerates the development of tumors, albeit to a lesser extent. In terms of its mechanism, E2F2 acts as a tumor suppressor through its ability to regulate apoptosis.

5. E2F2 in other diseases

Huntington's disease (OMIM 143100) is a neurodegenerative disorder characterized by movement abnormalities (chorea and hypokinesia), cognitive decline, and psychiatric symptoms, which are usually noticeable at ages 35-50 (*76*). Valcárcel-Ocete *et al.* found that presence of the *E2F2* rs2742976 T allele is associated with onset age of Huntington's disease and the level of *E2F2* expression. This highly significant E2F2 signal warrants further investigation. Moreover, Valcárcel-Ocete *et al.* speculated that a lower level of *E2F2* expression in symptomatic patients with Huntington's disease could be associated with a delay in the age of onset (*77*).

In degenerative diseases such as Stargardt disease and age-related macular degeneration, the leading cause of blindness in the developed world, retinal pigmented epithelial (RPE) cell loss is followed by photoreceptor cell death. RPE cells can proliferate upon *E2F2* gene transfer, suggesting an intrinsic regenerative potential. These findings provide proof-of-concept for an *E2F2*-mediated strategy to induce in situ regeneration of RPE

to treat degeneration (*78*).

6. Conclusion and perspectives for the future

Like other members of the E2F family, E2F2 typically binds to the promoter region of genes to control gene expression, playing a vital role in controlling the cell cycle. Mounting data suggest that *E2F2* plays different roles in the body and is an indispensable gene. E2F2 is not only involved in cell cycle progression but also in apoptosis, inflammation, and cell migration and invasion. As discussed in this review, E2F2 is a complex molecule that can promote the proliferation of hepatocytes and cardiomyocytes but that can also inhibit the proliferation of peripheral blood T cells; E2F2 induces the apoptosis of cone photoreceptor cells but also can inhibit the apoptosis of pancreatic cells. These and other findings have revealed the complex role of E2F2 in the human body and have challenged the traditional view that E2F2 is consistently and solely an "activator" of cell cycle gene expression. This view does not reflect the complexity of E2F2's function in the human microenvironment—its function seems to depend on the tissue and state of development.

E2F2 may have the opposite effect in even the same phenotype or disease. Whether E2F2 promotes or inhibits apoptosis depends on the tissue and state of development. In cone cells, E2F2 promotes apoptosis, and this promotion is dependent on P53. The promotion of apoptosis by E2F2 sometimes does not depend on P53. In melanocytes, E2F2 can directly bind to anti-apoptotic factors to promote apoptosis. However, an interesting aspect is that E2F2's anti-apoptotic action seems to depend on P53. Therefore, E2F2 is both an "activator" and an "inhibitor" of apoptosis. Inflammation seems to be a symptom of all diseases, so the effect of E2F2 on inflammation is also an issue that cannot be ignored. A number of studies have found that E2F2 can both promote and inhibit inflammation. In RA and neuroinflammation, E2F2 acts as an "activator" of inflammation to promote inflammation. However, E2F2 acts as a negative regulator of the immune response in mice by inhibiting the proliferation of activated lymphocytes. There is, nonetheless, an interesting phenomenon in terms of the effect that E2F2 has on tumors. E2F2 is more inclined to be an "activator" of the development and progression of tumors. A large amount of the literature indicates that E2F2 can promote the progression of tumors such as breast, lung cancer, and liver cancer, but the loss of E2F2 in transgenic mouse models of lymphoma accelerates the development of lymphoma. This seems to be the only evidence that E2F2 acts as an "inhibitor" of tumors. Therefore, E2F2 acts as an "activator" of the cell cycle as well as an "activator" of cell proliferation, apoptosis, and inflammation. Because of the tissue and state of development, it can inhibit the cell cycle, apoptosis, inflammation, and other processes. In short, the role of E2F2 in the human microenvironment is very complicated. Thus, it should not be simply labeled as an "activator" or "inhibitor." Its effect on the biological activity of cells depends on the tissue and state of development.

Although modern molecular technology and experimental models have revealed many functions of the intriguing transcription factor E2F2, there is still much to learn about its roles in the body. Further research on E2F2 needs to be conducted to clarify its role in diseases so that its potential as a diagnostic and therapeutic target can be fully realized. In fact, numerous studies have focused on its potential prognostic value in different types of diseases because of the importance of E2F2 in both normal homeostasis and tissue pathologies. E2F2 is also an attractive drug target, particularly with regard to cancer, and we may yet discover that E2F2 has other valuable functions, warranting many more years of study.

Funding: This work was supported by the National Natural Science Foundation of China (Grant No. 81671624), The Innovation Project of the Shandong Academy of Medical Sciences, and the program to promote academics of the Shandong First Medical University (LJ001).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Kumin D, Hofmann C, Uckert W, Both GW, Loser P. Identification of an ovine atadenovirus gene whose product activates the viral E2 promoter: Possible involvement of E2F-1. Virology. 2004; 318:79-89.
2. Chen HZ, Tsai SY, Leone G. Emerging roles of E2Fs in cancer: An exit from cell cycle control. Nat Rev Cancer. 2009; 9:785-797.
3. Fischer M, Müller GA. Cell cycle transcription control: DREAM/MuvB and RB-E2F complexes. Crit Rev Biochem Mol Biol. 2017; 52:638-662.
4. Ebelt H, Liu Z, Müller-Werdan U, Werdan K, Braun T. Making omelets without breaking eggs: E2F-mediated induction of cardiomyoycte cell proliferation without stimulation of apoptosis. Cell Cycle. 2006; 5:2436-2439.
5. Lammens T, Li J, Leone G, De Veylder L. Atypical E2Fs: New players in the E2F transcription factor family. Trends Cell Biol. 2009; 19:111-118.
6. Lin SY, Black AR, Kostic D, Pajovic S, Hoover CN, Azizkhan JC. Cell cycle-regulated association of E2F1 and Sp1 is related to their functional interaction. Mol Cell Biol. 1996; 16:1668-1675.
7. Karlseder J, Rotheneder H, Wintersberger E. Interaction of Sp1 with the growth- and cell cycle-regulated transcription factor E2F. Mol Cell Biol. 1996; 16:1659-1667.
8. Martínez-Balbás MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T. Regulation of E2F1 activity by

acetylation. EMBO J. 2000; 19:662-671.
9. Marzio G, Wagener C, Gutierrez MI, Cartwright P, Helin K, Giacca M. E2F family members are differentially regulated by reversible acetylation. J Biol Chem. 2000; 275:10887-10892.
10. Harbour JW, Luo RX, Dei Santi A, Postigo AA, Dean DC. Cdk phosphorylation triggers sequential intramolecular interactions that progressively block Rb functions as cells move through G1. Cell. 1999; 98:859-869.
11. Halaban R, Cheng E, Smicun Y, Germino J. Deregulated E2F transcriptional activity in autonomously growing melanoma cells. J Exp Med. 2000; 191:1005-1016.
12. Bandara LR, La Thangue NB. Adenovirus E1a prevents the retinoblastoma gene product from complexing with a cellular transcription factor. Nature. 1991; 351:494-497.
13. Girling R, Partridge JF, Bandara LR, Burden N, Totty NF, Hsuan JJ, La Thangue NB. A new component of the transcription factor DRTF1/E2F. Nature. 1993; 365:468.
14. Attwooll C, Lazzerini Denchi E, Helin K. The E2F family: Specific functions and overlapping interests. Embo j. 2004; 23:4709-4716.
15. Suzuki DE, Nakahata AM, Okamoto OK. Knockdown of E2F2 inhibits tumorigenicity, but preserves stemness of human embryonic stem cells. Stem Cells Dev. 2014; 23:1266-1274.
16. Zhou J, Cheng M, Wu M, Boriboun C, Jujo K, Xu S, Zhao TC, Tang YL, Kishore R, Qin G. Contrasting roles of E2F2 and E2F3 in endothelial cell growth and ischemic angiogenesis. J Mol Cell Cardiol. 2013; 60:68-71.
17. Cobrinik D. Pocket proteins and cell cycle control. Oncogene. 2005; 24:2796-2809.
18. Roskoski R Jr. Cyclin-dependent protein serine/threonine kinase inhibitors as anticancer drugs. Pharmacol Res. 2019; 139:471-488.
19. Davis R, Pillai S, Lawrence N, Sebti S, Chellappan SP. TNF-α-mediated proliferation of vascular smooth muscle cells involves Raf-1-mediated inactivation of Rb and transcription of E2F1-regulated genes. Cell Cycle. 2012; 11:109-118.
20. Yang D, Wang J, Xiao M, Zhou T, Shi X. Role of Mir-155 in controlling HIF-1α level and promoting endothelial cell maturation. Sci Rep. 2016; 6:35316.
21. Deshpande R, Mansara P, Kaul-Ghanekar R. Alpha-linolenic acid regulates Cox2/VEGF/MAP kinase pathway and decreases the expression of HPV oncoproteins E6/E7 through restoration of p53 and Rb expression in human cervical cancer cell lines. Tumour Biol. 2016; 37:3295-3305.
22. Delgado I, Fresnedo O, Iglesias A, Rueda Y, Syn WK, Zubiaga AM, Ochoa B. A role for transcription factor E2F2 in hepatocyte proliferation and timely liver regeneration. Am J Physiol Gastrointest Liver Physiol. 2011; 301:G20-G31.
23. Maldonado EN, Delgado I, Furland NE, Buqué X, Iglesias A, Aveldaño MI, Zubiaga A, Fresnedo O, Ochoa B. The E2F2 transcription factor sustains hepatic glycerophospholipid homeostasis in mice. PLoS One. 2014; 9:e112620.
24. Helin K, Lees JA, Vidal M, Dyson N, Harlow E, Fattaey A. A cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. Cell. 1992; 70:337-350.
25. Lees JA, Saito M, Vidal M, Valentine M, Look T, Harlow E, Dyson N, Helin K. The retinoblastoma protein binds to a family of E2F transcription factors. Mol Cell Biol. 1993; 13:7813-7825.
26. Johnson DG, Schwarz JK, Cress WD, Nevins JR. Expression of transcription factor E2F1 induces quiescent cells to enter S phase. Nature. 1993; 365:349-352.
27. Qin XQ, Livingston DM, Kaelin WG, Jr., Adams PD. Deregulated transcription factor E2F-1 expression leads to S-phase entry and p53-mediated apoptosis. Proc Natl Acad Sci U S A. 1994; 91:10918-10922.
28. Lukas J, Petersen BO, Holm K, Bartek J, Helin K. Deregulated expression of E2F family members induces S-phase entry and overcomes p16INK4A-mediated growth suppression. Mol Cell Biol. 1996; 16:1047-1057.
29. DeGregori J, Leone G, Miron A, Jakoi L, Nevins JR. Distinct roles for E2F proteins in cell growth control and apoptosis. Proc Natl Acad Sci U S A. 1997; 94:7245-7250.
30. Song H, Zhang Y, Liu N, Zhang D, Wan C, Zhao S, Kong Y, Yuan L. Let-7b inhibits the malignant behavior of glioma cells and glioma stem-like cells *via* downregulation of E2F2. J Physiol Biochem. 2016; 72:733-744.
31. DeGregori J. The genetics of the E2F family of transcription factors: Shared functions and unique roles. Biochim Biophys Acta. 2002; 1602:131-150.
32. Li FX, Zhu JW, Hogan CJ, DeGregori J. Defective gene expression, S phase progression, and maturation during hematopoiesis in E2F1/E2F2 mutant mice. Mol Cell Biol. 2003; 23:3607-3622.
33. Cam H, Dynlacht BD. Emerging roles for E2F: Beyond the G1/S transition and DNA replication. Cancer Cell. 2003; 3:311-316.
34. Ebelt H, Hufnagel N, Neuhaus P, Neuhaus H, Gajawada P, Simm A, Müller-Werdan U, Werdan K, Braun T. Divergent siblings: E2F2 and E2F4 but not E2F1 and E2F3 induce DNA synthesis in cardiomyocytes without activation of apoptosis. Circ Res. 2005; 96:509-517.
35. Wu L, Timmers C, Maiti B, Saavedra HI, Sang L, Chong GT, Nuckolls F, Giangrande P, Wright FA, Field SJ, Greenberg ME, Orkin S, Nevins JR, Robinson ML, Leone G. The E2F1-3 transcription factors are essential for cellular proliferation. Nature. 2001; 414:457-462.
36. Iglesias-Ara A, Zenarruzabeitia O, Fernandez-Rueda J, Sánchez-Tilló E, Field SJ, Celada A, Zubiaga AM. Accelerated DNA replication in E2F1- and E2F2-deficient macrophages leads to induction of the DNA damage response and p21(CIP1)-dependent senescence. Oncogene. 2010; 29:5579-5590.
37. DeGregori J, Johnson DG. Distinct and overlapping roles for E2F family members in transcription, proliferation and apoptosis. Curr Mol Med. 2006; 6:739-748.
38. Azkargorta M, Fullaondo A, Laresgoiti U, Aloria K, Infante A, Arizmendi JM, Zubiaga AM. Differential proteomics analysis reveals a role for E2F2 in the regulation of the Ahr pathway in T lymphocytes. Mol Cell Proteomics. 2010; 9:2184-2194.
39. Zhou J, Wu M, Xu S, Cheng M, Ding C, Liu Y, Yan H, Biyashev D, Kishore R, Qin G. Contrasting roles of E2F2 and E2F3 in cardiac neovascularization. PLoS One. 2013; 8:e65755.
40. Müller H, Bracken AP, Vernell R, Moroni MC, Christians F, Grassilli E, Prosperini E, Vigo E, Oliner JD, Helin K. E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. Genes Dev. 2001; 15:267-285.
41. Stravopodis DJ, Karkoulis PK, Konstantakou EG,

Melachroinou S, Thanasopoulou A, Aravantinos G, Margaritis LH, Anastasiadou E, Voutsinas GE. Thymidylate synthase inhibition induces p53-dependent and p53-independent apoptotic responses in human urinary bladder cancer cells. J Cancer Res Clin Oncol. 2011; 137:359-374.
42. Opavsky R, Tsai SY, Guimond M, Arora A, Opavska J, Becknell B, Kaufmann M, Walton NA, Stephens JA, Fernandez SA, Muthusamy N, Felsher DW, Porcu P, Caligiuri MA, Leone G. Specific tumor suppressor function for E2F2 in Myc-induced T cell lymphomagenesis. Proc Natl Acad Sci U S A. 2007; 104:15400-15405.
43. Chen D, Chen Y, Forrest D, Bremner R. E2f2 induces cone photoreceptor apoptosis independent of E2f1 and E2f3. Cell Death Differ. 2013; 20:931-940.
44. Chen D, Shan J, Zhu WG, Qin J, Gu W. Transcription-independent ARF regulation in oncogenic stress-mediated p53 responses. Nature. 2010; 464:624-627.
45. Morris EJ, Michaud WA, Ji JY, Moon NS, Rocco JW, Dyson NJ. Functional identification of Api5 as a suppressor of E2F-dependent apoptosis *in vivo*. PLoS Genet. 2006; 2:e196.
46. Iaquinta PJ, Aslanian A, Lees JA. Regulation of the Arf/p53 tumor surveillance network by E2F. Cold Spring Harb Symp Quant Biol. 2005; 70:309-316.
47. Clavier A, Baillet A, Rincheval-Arnold A, Coléno-Costes A, Lasbleiz C, Mignotte B, Guénal I. The pro-apoptotic activity of Drosophila Rbf1 involves dE2F2-dependent downregulation of diap1 and buffy mRNA. Cell Death Dis. 2014; 5:e1405.
48. Reuveny A, Elhanany H, Volk T. Enhanced sensitivity of midline glial cells to apoptosis is achieved by HOW(L)-dependent repression of Diap1. Mech Dev. 2009; 126:30-41.
49. Rovani MK, Brachmann CB, Ramsay G, Katzen AL. The dREAM/Myb-MuvB complex and Grim are key regulators of the programmed death of neural precursor cells at the Drosophila posterior wing margin. Dev Biol. 2012; 372:88-102.
50. Raj D, Liu T, Samadashwily G, Li F, Grossman D. Survivin repression by p53, Rb and E2F2 in normal human melanocytes. Carcinogenesis. 2008; 29:194-201.
51. Iglesias A, Murga M, Laresgoiti U, Skoudy A, Bernales I, Fullaondo A, Moreno B, Lloreta J, Field SJ, Real FX, Zubiaga AM. Diabetes and exocrine pancreatic insufficiency in E2F1/E2F2 double-mutant mice. J Clin Invest. 2004; 113:1398-1407.
52. Iglesias-Ara A, Zenarruzabeitia O, Buelta L, Merino J, Zubiaga AM. E2F1 and E2F2 prevent replicative stress and subsequent p53-dependent organ involution. Cell Death Differ. 2015; 22:1577-1589.
53. Zhao H, Tang W, Chen X, Wang S, Wang X, Xu H, Li L. The NAMPT/E2F2/SIRT1 axis promotes proliferation and inhibits p53-dependent apoptosis in human melanoma cells. Biochem Biophys Res Commun. 2017; 493:77-84.
54. Chafin CB, Regna NL, Caudell DL, Reilly CM. MicroRNA-let-7a promotes E2F-mediated cell proliferation and NFκB activation *in vitro*. Cell Mol Immunol. 2014; 11:79-83.
55. Wang S, Wang L, Wu C, Sun S, Pan JH. E2F2 directly regulates the STAT1 and PI3K/AKT/NF-κB pathways to exacerbate the inflammatory phenotype in rheumatoid arthritis synovial fibroblasts and mouse embryonic fibroblasts. Arthritis Res Ther. 2018; 20:225.
56. Zhang R, Wang L, Pan JH, Han J. A critical role of E2F transcription factor 2 in proinflammatory cytokines-dependent proliferation and invasiveness of fibroblast-like synoviocytes in rheumatoid arthritis. Sci Rep. 2018; 8:2623.
57. Wu J, Sabirzhanov B, Stoica BA, Lipinski MM, Zhao Z, Zhao S, Ward N, Yang D, Faden AI. Ablation of the transcription factors E2F1-2 limits neuroinflammation and associated neurological deficits after contusive spinal cord injury. Cell Cycle. 2015; 14:3698-3712.
58. Murga M, Fernández-Capetillo O, Field SJ, Moreno B, Borlado LR, Fujiwara Y, Balomenos D, Vicario A, Carrera AC, Orkin SH, Greenberg ME, Zubiaga AM. Mutation of E2F2 in mice causes enhanced T lymphocyte proliferation, leading to the development of autoimmunity. Immunity. 2001; 15:959-970.
59. Yoon SO, Shin S, Mercurio AM. Ras stimulation of E2F activity and a consequent E2F regulation of integrin alpha6beta4 promote the invasion of breast carcinoma cells. Cancer Res. 2006; 66:6288-6295.
60. Gao Y, Ma X, Yao Y, Li H, Fan Y, Zhang Y, Zhao C, Wang L, Ma M, Lei Z, Zhang X. miR-155 regulates the proliferation and invasion of clear cell renal cell carcinoma cells by targeting E2F2. Oncotarget. 2016; 7:20324-20337.
61. Fujiwara K, Yuwanita I, Hollern DP, Andrechek ER. Prediction and genetic demonstration of a role for activator E2Fs in Myc-induced tumors. Cancer Res. 2011; 71:1924-1932.
62. Bollig-Fischer A, Marchetti L, Mitrea C, Wu J, Kruger A, Manca V, Drăghici S. Modeling time-dependent transcription effects of HER2 oncogene and discovery of a role for E2F2 in breast cancer cell-matrix adhesion. Bioinformatics. 2014; 30:3036-3043.
63. Li Y, Huang J, Yang D, Xiang S, Sun J, Li H, Ren G. Expression patterns of E2F transcription factors and their potential prognostic roles in breast cancer. Oncol Lett. 2018; 15:9216-9230.
64. Feliciano A, Garcia-Mayea Y, Jubierre L, *et al.* miR-99a reveals two novel oncogenic proteins E2F2 and EMR2 and represses stemness in lung cancer. Cell Death Dis. 2017; 8:e3141.
65. Sun CC, Zhou Q, Hu W, *et al.* Transcriptional E2F1/2/5/8 as potential targets and transcriptional E2F3/6/7 as new biomarkers for the prognosis of human lung carcinoma. Aging (Albany NY). 2018; 10:973-987.
66. Chen L, Yu JH, Lu ZH, Zhang W. E2F2 induction in related to cell proliferation and poor prognosis in non-small cell lung carcinoma. Int J Clin Exp Pathol. 2015; 8:10545-10554.
67. Zhan L, Huang C, Meng XM, Song Y, Wu XQ, Miu CG, Zhan XS, Li J. Promising roles of mammalian E2Fs in hepatocellular carcinoma. Cell Signal. 2014; 26:1075-1081.
68. Hong SH, Eun JW, Choi SK, Shen Q, Choi WS, Han JW, Nam SW, You JS. Epigenetic reader BRD4 inhibition as a therapeutic strategy to suppress E2F2-cell cycle regulation circuit in liver cancer. Oncotarget. 2016; 7:32628-32640.
69. Li Y, Sturgis EM, Zhu L, Cao X, Wei Q, Zhang H, Li G. E2F transcription factor 2 variants as predictive biomarkers for recurrence risk in patients with squamous cell carcinoma of the oropharynx. Mol Carcinog. 2017; 56:1335-1343.
70. Li Y, Sturgis EM, Yuan Y, Lu M, Cao X, Wei Q, Li G.

Effect of human papillomavirus seropositivity and E2F2 promoter variants on risk of squamous cell carcinomas of oropharynx and oral cavity. Carcinogenesis. 2016; 37:1070-1078.
71. Xie L, Li T, Yang LH. E2F2 induces MCM4, CCNE2 and WHSC1 upregulation in ovarian cancer and predicts poor overall survival. Eur Rev Med Pharmacol Sci. 2017; 21:2150-2156.
72. Cao J, Wang H, Liu G, Tang R, Ding Y, Xu P, Wang H, Miao J, Gu X, Han S. LBX2-AS1 promotes ovarian cancer progression by facilitating E2F2 gene expression *via* miR-455-5p and miR-491-5p sponging. J Cell Mol Med. 2020; 25:1178-1189.
73. Wang H, Zhang X, Liu Y, Ni Z, Lin Y, Duan Z, Shi Y, Wang G, Li F. Downregulated miR-31 level associates with poor prognosis of gastric cancer and its restoration suppresses tumor cell malignant phenotypes by inhibiting E2F2. Oncotarget. 2016; 7:36577-36589.
74. Yu J, Fang C, Zhang Z, Zhang G, Shi L, Qian J, Xiong J. H19 rises in gastric cancer and exerts a tumor-promoting function *via* miR-138/E2F2 axis. Cancer Manag Res. 2020; 12:13033-13042.
75. Liu X, Hu C. Novel potential therapeutic target for E2F1 and prognostic factors of E2F1/2/3/5/7/8 in human gastric cancer. Mol Ther Methods Clin Dev. 2020; 18:824-838.
76. McColgan P, Tabrizi SJ. Huntington's disease: A clinical review. Eur J Neurol. 2018; 25:24-34.
77. Valcárcel-Ocete L, Alkorta-Aranburu G, Iriondo M, *et al.* Exploring genetic factors involved in huntington disease age of onset: E2F2 as a new potential modifier gene. PLoS One. 2015; 10:e0131573.
78. Kampik D, Basche M, Luhmann UFO, Nishiguchi KM, Williams JAE, Greenwood J, Moss SE, Han H, Azam S, Duran Y, Robbie SJ, Bainbridge JWB, Larkin DF, Smith AJ, Ali RR. In situ regeneration of retinal pigment epithelium by gene transfer of E2F2: A potential strategy for treatment of macular degenerations. Gene Ther. 2017; 24:810-818.

Received February 17, 2021; Revised April 8, 2021; Accepted April 25, 2021.

**Address correspondence to:*
Jihong Pan, Biomedical Sciences College & Shandong Medicinal Biotechnology Centre, Shandong First Medical University, No. 6699 Qingdao Road, Ji'nan 250117, China.
E-mail: panjihong@sdfmu.edu.cn

Released online in J-STAGE as advance publication April 29, 2021.