Function of p21 and its therapeutic effects in esophageal cancer (Review)

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Abstract. Esophageal cancer (EC) is the eighth most common type of cancer worldwide and ranks sixth among the causes of cancer-related mortality. Due to the high mortality rate and poor treatment efficacy for EC, millions of individuals succumb to this disease; thus, the identification of novel treatment targets is of utmost importance and urgency. In recent years, there have been advances if therapies targeting cell cycle regulators. p21 is a type of cell cycle regulator that plays a dual role in tumor cells, as it can not only regulate the cell cycle, induce apoptosis and inhibit cell proliferation, but can also protect cells from apoptosis. It has been found that p21 often exerts a tumor-suppressive effect on EC, which provides a basis for its use as a treatment target for EC. Therefore, the aim of the present study was to review the function of p21 and its potential value as a therapeutic target for EC.

Contents

1. Introduction
2. p21 regulates the cell cycle, DNA replication and apoptosis
3. p21 may be regulated through p53-dependent and -independent pathways
4. Role of p21 in the treatment of esophageal cancer
5. Conclusion

1. Introduction

According to the 2018 Global Cancer Statistics, the number of new cases of esophageal cancer (EC) in 2018 accounted

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for 3.2% of the total number of cancer cases, with EC-related fatalities accounting for 5.3% of the total cancer deaths (1). There are two main histological types of EC, namely esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EADC). ESCC is the most common histological type of EC worldwide, particularly in high-risk areas, such as China. China is one of the areas exhibiting a high incidence of EC, which may be associated with genetics and lifestyle habits. Smoking, excessive alcohol consumption and the excessive intake of red meat and spicy foods are considered as high-risk factors for ESCC (2-4). Previous studies on EC cases in China, particularly in Linxian county, which has the highest ESCC-related mortality rate worldwide, demonstrated that a polymorphism in the TP53 gene (the main regulator of p21) is associated with the risk of developing ESCC (5,6). Intestinal metaplasia in Barrett's esophagus is a major risk factor for EADC, and the inactivation of p53 and p21 may be implicated in the progression of Barrett's esophagus to cancer (7,8).

Radical surgery is the only curative method currently available for the treatment of EC; however, as the early symptoms of EC are not evident, the majority of patients are already at an advanced stage at the time of diagnosis. Radiotherapy and chemotherapy are the main treatment strategies for patients with advanced EC. However, chemotherapeutic resistance and adverse events may occur, which are the main causes of treatment failure in EC. Immunotherapy for EC has recently emerged; PD-1/PD-L1 inhibitors have been proven to be effective in the treatment of EC (9). Furthermore, angiogenesis inhibitors and inhibitors of epidermal growth factor receptor, such as bevacizumab, cetuximab, panitumumab and erlotinib, have been reported to be useful for the treatment of EC (10). However, only limited progress has been made in targeting drugs specific to EC. Therefore, the identification of novel therapeutic targets for EC is the focus of ensuing research.

It has been >20 years since p21 was discovered as a cell cycle inhibitor regulated by p53. In 1993, el-Deiry *et al* identified p21 by using subtractive hybridization technology, which is downstream of wild-type p53, a 21-kDa protein encoded by wild-type p53-activated fragment gene 1 (WAF1) (11). In addition, Harper *et al* demonstrated that p21 is a type of cyclin-dependent kinase (CDK) inhibitor through a two-hybrid system (12). In recent years, the therapeutic potential of targeting the cell cycle has been widely acknowledged. The new generation of selective CDK4/6 inhibitors, such as palbociclib, has been approved for the treatment of breast cancer (13). This

has put forward a new approach to the treatment of EC via targeting the cell cycle. p21, as a cell cycle kinase inhibitor, can regulate cell cycle progression in EC (14). Subsequent research has identified that p21 also plays an important role in inducing cell senescence and apoptosis, promoting DNA repair and maintaining genome stability (15). Therefore, it is necessary to examine the role of p21 in EC and actively develop drugs targeting p21.

2. p21 regulates the cell cycle, DNA replication and apoptosis

p21 regulates the cell cycle by inhibiting the cyclin-CDK complex. Cyclin is a type of protein that is ubiquitous in eukaryotic cells and can appear and disappear regularly during the cell cycle (16). Cyclin regulates the cell cycle by binding to and activating CDK. As shown in Table I, the phosphorylation of specific targets by the cyclin-CDK complex sets in motion different processes that drive the cell cycle in a timely manner (17). Over the past two decades, numerous studies have demonstrated that cell cycle disorders are implicated in human cancers; the overactivation of CDK may promote tumor development by inducing unprogrammed cell division of stem or progenitor cells.

p21 is an effective CDK inhibitor that can bind to CDK and block the cell cycle (12). p21 can be combined with and inhibit almost every cyclin-CDK complex, such as cyclin D-CDK4, cyclin E-CDK2 and cyclin A-CDK2 (11). p21 inhibits the cyclin E-CDK2 complex, thereby inhibiting the phosphorylation of RB and the sequestration of E2F1 and arresting the cell cycle in the G_1 phase. p21 also inhibits the kinase activity of cyclin A-CDK1/2, which is necessary for entering the G_2 phase through the S phase. In addition, p21 inhibits the kinase activity of cyclin B-CDK1, thereby inhibiting progression through the G_2/M phase. Under certain conditions, p21 promotes the activity of CDK4 or CDK6, thereby promoting progression through the G_1 phase (18). The aforementioned findings indicate that p21 plays multiple roles in the regulation of the cell cycle. A summary of the role of p21 in cell cycle regulation by combining with different complexes is illustrated in Fig. 1.

p21 inhibits the binding of proliferating cell nuclear antigen (PCNA) to polymerase δ to affect DNA replication. The control of DNA replication is a key factor for maintaining normal cell function, as it can affect the stability of the genome. DNA replication that occurs in the S phase of the cell cycle is essential for cell division (19). PCNA is an evolutionarily well-preserved protein that exists in all eukaryotes. PCNA was first found to be a processing factor for DNA polymerase δ, which is required for DNA synthesis during replication (20). PCNA recognizes the primer-template junction and conjugates with replication factor C (RFC) to assist in the positioning of polymerase δ, and it also strengthens the advancement of polymerase in the extension process.

Podust *et al* (21) found that p21 first inhibited the RFC-catalyzed loading of PCNA onto DNA and that, second, it prevented the binding of the DNA polymerase δ core to the PCNA clamp assembled on the DNA; the latter was the most potent inhibitory effect on polymerase δ enzyme activity. p21 can form a stable complex with PCNA on DNA, preventing

further interaction with the replication proteins, RFC and DNA polymerase δ (22). In addition, Zhang *et al* (23) discovered the existence of PCNA-p21/cyclin-CDK quaternary complexes, which may be associated with the regulation of the cell cycle.

p21 can inhibit apoptosis by suppressing stress-activated protein kinase (SAPK) and apoptotic signal-regulated kinase 1 (ASK1). SAPK is a member of the subfamily of the mitogen-activated protein (MAP) kinases, which can be activated in response to DNA damage. During cellular stress, the N-terminus of p21 binds to and inhibits the activity of SAPK, prevents c-Jun phosphorylation and Ap-1 activation, thus participating in the regulation of apoptosis. p21 located in the cytoplasm can interact with SAPK and ASK1 to inhibit their catalytic activity, thereby inhibiting apoptosis (24,25). ASK1, also known as MAP kinase 5 (MAP3K5), has the potential to induce cellular apoptosis under various physiological conditions. Zhan *et al* (26) demonstrated that p21 interacts with ASK1 and downregulates its kinase activity. These findings may indicate some of the mechanisms through which p21 inhibits apoptosis.

3. p21 may be regulated through p53-dependent and -independent pathways

p53-dependent pathway. Numerous studies have confirmed that p21 expression is dependent on p53 and performs part of the functions of p53 as its downstream mediator (11,12,27,28). When cells are exposed to radiation and DNA damage occurs, p53 and p21 are often highly expressed in p53 wild-type cells, while p53-deficient cells usually do not exhibit a high p21 expression, and the cells do not undergo cell cycle arrest. At present, p21 is known as the cell cycle inhibitory protein with the most extensive kinase inhibitory activity. Amino acids 21-26 and 49-72 of p21 bind to cyclin and CDK, respectively, so that the kinase activity of cyclin-CDK complex is lost. In summary, when cells suffer DNA damage due to environmental stimuli, p21 can inhibit the activity of the cyclin-CDK complex as a CDK inhibitor through the p53-dependent pathway, so that Rb protein cannot be phosphorylated, thus arresting the cell cycle in the G_1/S phase (11). In addition, it was previously demonstrated that p53 not only induces the expression of p21 and exerts a tumor-suppressive effect, but also plays a role by directly forming a complex with p21 (29,30). For example, p53 and p21 can bind to Bcl-2 family proteins, such as Bcl-w and Bcl-xL, and release the Bax protein through the formation of the p53/p21/Bcl-w complex to promote tumor cell apoptosis.

In EC cells, the p53/p21 pathway is also crucial for the regulation of cell proliferation. For example, researchers previously used mibefradil in EC cells to inhibit the function of calcium channels, and found that p21 increased in the p53-dependent pathway, which ultimately inhibited the proliferation of EC cells (31). In addition, SOX6, EC-related gene (ECRG)4, KLF4/5 and other genes were shown to regulate the proliferation of EC cells through the p53/p21 pathway (32-35).

p53-independent pathway
Transcriptional regulation of p21. The regulation of p21 in cells is highly complex and diverse and p21 can also be regulated
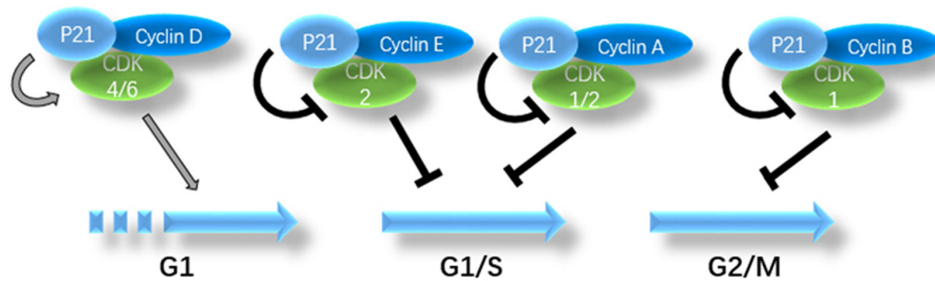
Figure 1. p21 combines with different cyclin-CDK complexes to exert its effects. CDK, cyclin-dependent kinase.

Table I. Cyclin-CDK complex can act at different times in the cell cycle.

Cell cycle	Cyclin-CDK complex
G1 phase	Cyclin D-CDK4, cyclin D-CDK6
G1/S phase	Cyclin E-CDK2
S phase	Cyclin E-CDK2, cyclin A-CDK1, cyclin A-CDK2
G2/M phase	Cyclin B-CDK1

CDK, cyclin-dependent kinase.

through a p53-independent pathway. The Krüppel-associated box zinc-finger protein 545 (ZNF545) has been proven to directly regulate p21 independently of p53 (36). It was previously demonstrated that, in p53-mutant EC cell lines, the expression of ZNF545 upregulated the protein expression levels of the pivotal effectors, p21 and Bax, thereby inhibiting tumor cell proliferation and promoting apoptosis. Human riboflavin transporter 2 and ECRG1 have also been confirmed to directly regulate the expression of p21, thereby regulating the proliferation of EC cells (37,38).

Post-transcriptional control of p21. p53 activates the transcription of p21 in the case of DNA damage and creates an unstable p21, the stability of which may improve by interacting with WISp39 and Hsp90. As previously demonstrated, p21 cannot be upregulated in response to DNA damage due to the lack of WISp39, suggesting that p21 transcriptional control is insufficient to upregulate p21 protein expression following DNA damage in the absence of p21 stability (39,40). A 2018 study demonstrated that the loss of USP11 may cause p21 instability and induce G_1/S transition in cells; in addition, the accumulation of p21 due to DNA damage was completely eliminated in cells lacking USP11, which led to the abolition of the G_2 checkpoint and the induction of apoptosis (41). p21 is mainly degraded through the ubiquitination pathway (42). As an important regulator of the cell cycle, Skp2 can specifically recognize phosphorylated substrates and perform ubiquitin-mediated degradation. p21 is one of the substrates of the ubiquitin proteasome pathway; it is phosphorylated at the Ser-130 site with the participation of CDK2-cyclin E and enters the ubiquitin degradation pathway under the action of the accessory protein, CKS1 (43).

In addition, affecting mRNA stability and the control of the translation of p21 mRNA are also an important part of the p21 post-transcriptional regulatory mechanism. In 2017, Li *et al* (44) silenced methyltransferases (NSUN2, METTL3 or METTL14) and found that the protein level of p21 decreased, although its mRNA expression was not altered; thus, they came to the conclusion that NSUN2, METTL3 and METTL14 may affect p21 expression levels by regulating p21 translation. Another study found that HuR and AUF1 may competitively bind with the p21 3'-untranslated region and regulate p21 mRNA stability (45). These two RNA-binding proteins competitively bind to p21 mRNA, but lead to opposite results; HuR can enhance the stability of mRNA, while AUF1 accelerates p21 mRNA decay. The regulation of p21 mRNA and protein stability has not yet been extensively investigated in EC cells. The aim of the present review was mainly to explain the diversity of the mechanisms implicated in the regulation of p21 and the key role of p21 in complex networks. The aforementioned regulation mechanism is shown in detail in Fig. 2.

4. Role of p21 in the treatment of esophageal cancer

p21 holds promise as a prognostic indicator and for the evaluation of treatment efficacy. From the aforementioned description of the function of p21 and its regulatory mechanisms, it may be inferred that p21 is a key regulatory factor in EC; thus, the study of p21 is of utmost clinical significance. In fact, some studies have confirmed that p21 plays an important role in the prognosis of EC. A previous meta-analysis revealed that a low p21 expression was associated with clinicopathological characteristics such as poor differentiation, lymph node metastasis, aggressiveness, higher grade and clinical stage, which are associated with a poor outcome of patients with EC (46). Apart from this latter study, there is ample evidence indicating that high expression of p21 is an important indicator of good prognosis for patients with EC (47-49). These studies suggested that low p21 expression is an indicator of a highly malignant EC. The expression of p21 is often regulated by p53; thus, comprehensive consideration is needed when determining the prognosis of patients with EC. A previous study demonstrated that, in p53-positive patients with EC, the expression of p21 exerted no significant effect on the survival rate of the patients; however, the survival rate of patients with p21-positive tumors was significantly higher compared with that of patients with p21-negative tumors (50).
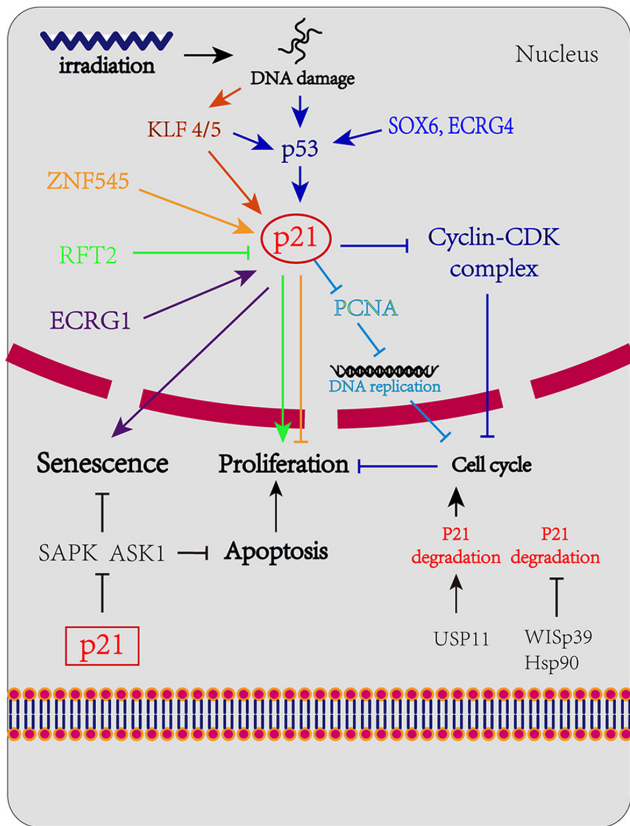
Figure 2. p21 is regulated by multiple pathways. It was found that p21 is a key factor in esophageal cancer cells. Through the control of p21, the regulation of cell proliferation, senescence and apoptosis may be achieved. The pathway indicated by the black line has not been confirmed in esophageal cancer cells. The main purpose was to highlight the double-sidedness of p21, and this double-sidedness is often associated with the localization of p21 in the cell.

The combination of chemotherapy and radiotherapy is the main treatment strategy for advanced EC. The expression of p53 and p21 has been proven to be an indicator of the efficacy of radiotherapy and chemotherapy (47,50,51) for ESCC as well as EADC. As shown in Fig. 3, a previous study indicated that the change in the p21 status from negative to positive during treatment was accompanied by an improvement in survival (52).

At present, p21 is known to play an important role in a variety of cancers and cannot be considered as a specific marker for the diagnosis of EC. However, p21 is important for evaluating prognosis and treatment efficacy in patients with EC.

p21 as a novel target for EC treatment

CDK4/6 inhibitors in clinical treatment. p21 is a classic cell cycle regulator that acts as a gene downstream of p53 to block the progression of the cell cycle. This provides a basis for its use as a target for cancer treatment. In fact, antitumor therapy targeting the cell cycle has gradually matured. Some antitumor drugs targeting the cell cycle have entered clinical practice, such as palbociclib, abemaciclib and ribociclib (53). Palbociclib is an oral targeted drug that directly acts on CDK4/6 and inhibits its function, thereby restoring cell cycle control and inhibiting tumor cell proliferation (54). At present, CDK4/6 inhibitors are only approved for the treatment of ER-positive

breast cancer and attempts are being made to further expand their use. Moreover, the identification of a novel target for EC is required. The present review aimed to address the possibility of using p21 as a novel therapeutic target.

Drugs targeting p21 exert therapeutic effects on EC. Some drugs in trials have been shown to promote p21 expression, thereby inhibiting EC cell growth. A number of drugs targeting p21 are currently under development. Diallyl disulfide (DADS) is a lipid-soluble organic compound derived from garlic. A previous study used various concentrations of DADS to treat cells, and found that the proportion of EC cells in the G_2/M phase continued to increase, and the mRNA level of p21 also increased with increasing DADS concentration. The same study confirmed that DADS was an effective anticancer drug by suppressing cell viability, blocking the cell cycle at the G_2/M phase and inducing the apoptosis of EC cells (55). Molecular analysis revealed that cell cycle arrest may be due to the reduction of cyclin B1, cdc2, p-cdc2 and cdc25c, and the activation of the p53/p21 pathway. DADS was shown to activate caspases, alter the Bax/Bcl-2 balance and inhibit the MEK/ERK pathway to induce apoptosis (55).

Another study found that obatoclax (a type of BH3-mimetic) induced G_1/G_0 arrest of EC cells through the p38/p21 signaling pathway. Obatoclax is an indole bipyrrole compound that can inhibit all known antiapoptotic Bcl-2 family members. Obatoclax did not alter the expression of CDKs, including CDK2, CDK4 and CDK6, but significantly increased the protein level of the CDK inhibitor, p21. The findings of that study may shed new light on the anticancer activity of obatoclax and its potential application in clinical practice (56).

Cinobufagin, isolated from traditional Chinese herbs, exerts antitumor, anesthetic, analgesic and anti-inflammatory effects. A previous study by Deng *et al* (57) indicated that cinobufagin induced ESCC cell cycle arrest at the G_2/M phase and promoted apoptosis by regulating the expression of p21, indicating that traditional Chinese medicine may prove useful for the treatment of EC.

Combined use of drugs targeting p21 can enhance the sensitivity to radiotherapy and chemotherapy. It was recently reported that crocetin combined with cisplatin exert a synergistic anti-ESCC effect by upregulating the p53/p21 pathway. The researchers demonstrated that the combination of crocetin and cisplatin treatment markedly affected the expression of p53 and p21 compared with treatment with cisplatin alone. This type of combined treatment inhibited KYSE-150 cell proliferation and promoted cell apoptosis *in vitro* (58).

Liu *et al* (59) demonstrated that *Antrodia cinnamomea* mycelial fermentation broth blocked the cell cycle progression of EC cells in the G_2/M phase by upregulating the expression of p21, thus rendering the cells more sensitive to the subsequent dose of radiation. The aforementioned simple examples demonstrate that p21-regulated drugs have exhibited potent antitumor effects in experiments; a number of other studies have also proven this effect (58,60-67). The mechanisms of some of these drugs are summarized in Table II.

Table II. Substances used to treat esophageal cancer by regulating p21 expression in previous experiments.

Substance	Mechanism	(Refs.)
DADS	p53/p21 pathway	(55)
Obatoclax	p38/p21 pathway	(56)
Cinobufagin	p73/p21 pathway	(57)
Crocetin	p53/p21 pathway	(58)
AC-MFB	Upregulation of p21 expression	(59)
Ruthenium (II) complex	p53/p21 pathway	(60)
Costunolide	p53/p21 pathway	(61)
Oridonin	p53/p21 pathway	(62)
Licochalone C	Upregulation of p21/p27 expression	(63)
Suberoylanilide hydroxamic acid	Upregulation of p21/p27/Rb expression	(65)
Tanshinone IIA	p53/p21 pathway	(66)
Thymoquinone	p53/p21 pathway	(67)

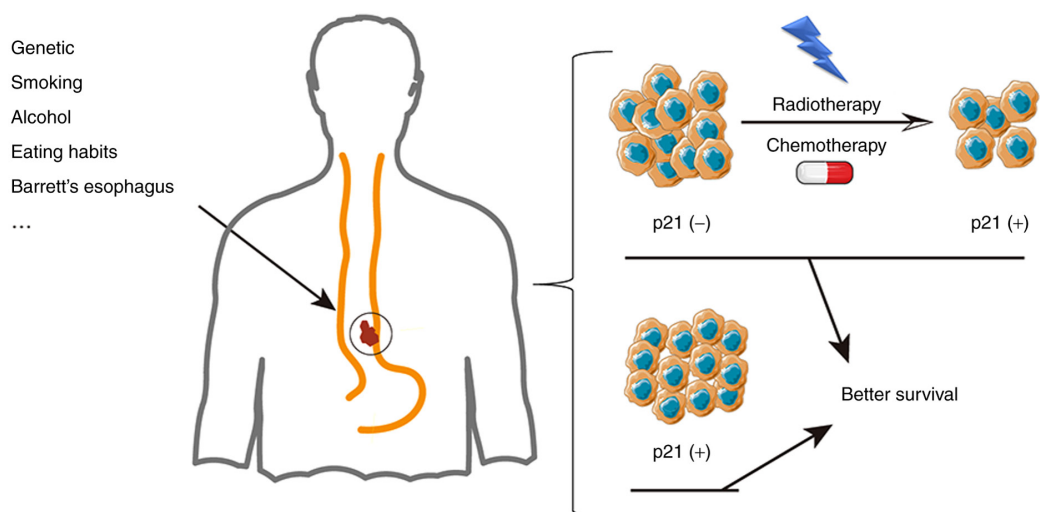DADS, diallyl disulfide; AC-MFB, *Antrodia cinnamomea* mycelial fermentation broth.

Figure 3. A number of pathogenic factors are implicated in esophageal cancer. Hereditary and daily living habits are considered as the main carcinogenic factors. Barrett's esophagus is a high-risk factor for esophageal adenocarcinoma. Previous studies have demonstrated that a high expression of p21 is an indicator of a favorable prognosis for patients with esophageal cancer. Following treatment, the expression of p21 changes from negative to positive, which also indicates a favorable prognosis.

5. Conclusion

The therapeutic potential of p21 in EC warrants further investigation. At present, the drugs targeting p21 have not been used in clinical practice. All of the aforementioned agents have been proven effective in the treatment of EC *in vitro*. These drugs cause cell cycle arrest and induce apoptosis by increasing the expression of p21. Furthermore, some substances obtained from Chinese medicine have been shown to exert therapeutic effects on EC, which also proves the value of Chinese medicine in antitumor therapy (57).

It has been previously indicated that patients with p21-deficient EC have a shorter survival time (46). For this group of patients, it would be of interest to determine whether a type of p21 analog could be produced in order to take advantage of the tumor-suppressive effects of p21. Similar to the use of p16 as a template for the development of palbociclib, a similar drug may

be created using p21 as the template. To date, there is no study available on p21 analogues, at least to the best of our knowledge. If p21 protein analogs can be developed to regulate the cycle of EC cells, this may benefit a greater number of patients with EC.

Georgakilas *et al* (15) described p21 as a 'two-faced genome guardian'; this indicates that it has different functions in different conditions in tumors. In the absence of p53, the long-term overexpression of p21 enables some cells to escape senescence (68). There are a number of difficulties encountered in the identification of the mechanisms of p21 due to its multiple roles. However, current research indicates that p21 mainly plays a role in inhibiting cell proliferation in EC. In addition, the prognosis of patients with EC can be evaluated and the efficacy of chemotherapy and radiotherapy can be determined by detecting the expression of p21 (69). Thus, investigating the mechanism of p21 in EC should be the focus of future research. If p21 can be used as a prognostic indicator

for EC, the treatment efficacy of EC may improve, and more patients will reap the benefits.

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Availability of data and materials

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Authors' contributions

LW and HH were involved in designing the study, in the literature review and in the drafting of the manuscript. LW was also responsible for designing the figures. LD and ZW participated in the acquisition and analysis of data, and in the discussion of the manuscript. YQ designed the study and critically revised the manuscript. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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