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REVIEW ARTICLE

p21$^{Waf1/Cip1}$: its paradoxical effect in the regulation of breast cancer

Samir F. Zohny[1,2] · **Abdulrahman L. Al-Malki**[1] · **Mazin A. Zamzami**[1] · **Hani Choudhry**[1]

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Abstract

p21$^{Waf1/Cip1}$, the cyclin-dependent kinase (CDK) inhibitor belonging to the KIP/CIP family, was initially regarded as a tumor suppressor protein because it was recognized as the chief mediator of p53-dependent cell cycle arrest elicited by DNA damage. Conversely, it has been proposed that p21$^{Waf1/Cip1}$ may also function as an oncogene because it can inhibit apoptosis. Thus, p21$^{Waf1/Cip1}$ is regarded as a protein with a dual behavior, as its expression might cause potential benefits or dangerous effects in breast cancer. Consequently, careful planning is required in targeting p21$^{Waf1/Cip1}$ expression for therapy of breast cancer patients. This review illustrates the discovery and mechanisms of induction of p21$^{Waf1/Cip1}$. Then, we focus on elucidating the paradoxical effect of p21$^{Waf1/Cip1}$ expression on human breast carcinogenesis and explaining how the subcellular localization (nuclear or cytoplasmic) of p21$^{Waf1/Cip1}$ has an impact on both determining its fate as either cell-growth inhibitor or antiapoptotic molecule and, its effect on clinicopathological factors and prognosis of breast cancer patients. Moreover, we explore how the pattern of the p21$^{Waf1/Cip1}$ could affect the responsiveness of human breast cancer to chemotherapy. Furthermore, the pharmacological approaches to target p21$^{Waf1/Cip1}$ expression for therapy of breast cancer are clarified.

Keywords p21$^{Waf1/Cip1}$ · p53 · Prognosis · Pharmacological approaches · Breast cancer

Introduction

Human carcinogenesis, including breast cancer, originates from an imbalance of cell proliferation and cell death. Numerous cell cycle mediators involving cyclins, CDKs and CDK inhibitors (CDKIs) have been recognized that precisely control various phases of the cell cycle and hence govern this balance. This trio should cooperate closely for assuring orderly progression through the cell cycle. CDKIs are divided into two major families, namely, the inhibitor of CDK4 (INK4) family and the kinase inhibitory protein/CDK-interacting protein (KIP/CIP) family, reliant on their structure and CDK specificity. The members of INK4 family (CDKN2A/p16^{INK4a}, CDKN2B/p15^{INK4b}, CDKN2C/p18^{INK4c} and CDKN2D/p19^{INK4d}) particularly inhibit cyclin D/CDK4 or CDK6. On the other hand, the KIP/CIP family members (CDKN1A/p21$^{Waf1/Cip1}$, CDKN1B/p27^{Kip1} and CDKN1C/p57^{Kip2}) are more indistinctive and generally hinder the activities of cyclin D/CDK4 or CDK6, cyclin E/CDK2, cyclin A/CDK1 (Cdc2) or CDK2 and cyclin B/CDK1 [1–3]. In this review, we elucidate the contradictory effect of p21$^{Waf1/Cip1}$ expression on progression and outcome of breast cancer. Many reports in the literature have suggested that nuclear p21$^{Waf1/Cip1}$ has tumor-suppressor activity and consequently, loss expression and/or dysfunction of p21$^{Waf1/Cip1}$ leads to breast cancer [4–8]. However, there is some evidence indicating that cytoplasmic p21$^{Waf1/Cip1}$ has antiapoptotic/oncogenic properties and in turn, it can promote breast cancer [9–14]. Moreover, regarding the clinical outcomes of p21$^{Waf1/Cip1}$, the experiments carried out on breast cancer samples have failed to reach an agreement and the results obtained are indecisive [4–10, 12, 15, 16]. Keeping in mind that p21$^{Waf1/Cip1}$ can function as tumor suppressor or antiapoptotic molecule; we describe the various protocols employed by the researchers to either positively or negatively targeting p21$^{Waf1/Cip1}$ expression for therapy of breast cancer.

✉ Samir F. Zohny
sfmz@hotmail.com

[1] Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia

[2] Biochemistry Department, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt

p21$^{Waf1/Cip1}$: discovery and mechanisms of induction

In diploid fibroblasts, cyclin D1 can form a complex with catalytic subunits of CDK2, CDK4 and CDK5. Moreover, 21 kDa protein (p21) and 36 kDa-proliferating cell nuclear antigen (PCNA) can associate with cyclin D1. Similarly, cyclin D3 combines with numerous CDKs, PCNA and p21. A quaternary complex of D cyclin, CDK, PCNA, and p21 may assemble in vivo [17]. Subsequently, it was established that p21 acts as a universal inhibitor of each member of the cyclin/CDK family [18]. In 1993, two studies concurrently published the cloning of *p21* gene. El-Deiry et al. [19] searched for proteins that could act as mediators for tumor growth suppressive function of p53. Applying the subtractive hybridization technique, they identified *p21* gene whose upregulation occurred by expression of wild-type, but not mutant *p53* gene in a human brain tumor cell line. They named this gene wild-type p53 activated fragment 1 (*Waf1*). The *Waf1* gene is located on chromosome 6p21.2. Transfection of *Waf1* cDNA caused suppression for the growth of human colon, lung and brain tumor cell lines. Using a yeast enhancer trap, a p53 responsive element is recognized at 2.4 kb upstream of *Waf1* gene. In the existence of wild-type p53, the *Waf1* promoter and p53 responsive element stimulated the expression of a reporter gene. Meanwhile, Harper et al. [20] applied a yeast two-hybrid system to recognize proteins that could interact with CDKs. They identified a human gene encoding p21 and dubbed this gene CDK-interacting protein 1 (*Cip1*). The Cip1 can suppress the phosphorylation of pRB by cyclin D/CDK4 and cyclin E/CDK2 complexes. Because the discovery of p21 is made concomitantly and independently by these two groups, p21 is often referred to as p21$^{Waf1/Cip1}$. In their studies to identify mediators of senescence, Noda et al. [21] isolated a gene they named senescent cell-derived inhibitor 1 (*Sdi1*). They found that *Sdi1* cDNA caused inhibition of DNA synthesis when transfected into young fibroblast cycling cells. Expression of *Sdi1* cDNA was upregulated 10–20-fold in senescent cells in comparison to young cells and the raise in RNA mostly matched with the loss of cell proliferation and onset of the senescent phenotype. By comparing the protein and DNA sequences, it was realized that all of these groups are independently discovered p21.

When DNA damage occurs, p53 is activated and initiated a cascade of events leading to arrest in G1. p53 activation occurs through a series of post-translational modifications mediated by activated ataxia-telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR). ATM and ATR are serine/threonine protein kinases, which belongs to the phosphatidylinositol 3-kinase-related kinase protein family. Whereas, ATM responds exclusively to double-strand break (DSB) caused by ionizing radiation (IR), ATR responds to stalled DNA replication forks and DSB caused by IR and UV. Autophosphorylation of ATM on S1981 caused the conversion of inactive dimmer or higher order multimer ATM to an active monomeric form. Serine 15 of p53 is phosphorylated directly by ATM and ATR, while S20 of p53 is phosphorylated by ATM-activated checkpoint kinase 2. ATM also phosphorylates Mdm2 on S395. Phosphorylation of p53 on S20 interferes with p53's association with its inhibitor, Mdm2. Consequently, p53 degradation by Mdm2 is prohibited resulting in accumulation of p53. Functioning as a transcription factor, p53 stimulates transcription of *p21*$^{Waf1/Cip1}$ gene, which encodes p21$^{Waf1/Cip1}$. The p21$^{Waf1/Cip1}$ protein binds to the G1-to-S checkpoint cyclin E/CDK2 complexes and inhibits their activity. Therefore, entry into S is blocked and the cell arrests in G1 [22, 23]. Moreover, as a response to genetic damage, p21$^{Waf1/Cip1}$ binds to PCNA inhibiting its role in DNA replication, but not in nucleotide excision repair of DNA [24]. p53 and p21$^{Waf1/Cip1}$ appear to be necessary for G2-to-M checkpoint control as cells are arrested in G2 phase following DNA damage induced by γ radiation, while after disruption of the *p53* or the *p21*$^{Waf1/Cip1}$ gene, γ radiated cells progressed into mitosis [25]. Recently, it has been obvious that p53-mediated cell cycle arrest is controlled by the p53–p21$^{Waf1/Cip1}$–DREAM–E2F/CHR pathway [26]. Several studies have demonstrated that p21$^{Waf1/Cip1}$ can be induced by p53-independent pathways [27–30]. It was shown that fetal bovine serum or purified growth factors such as fibroblast growth factor, platelet-derived growth factor and epidermal growth factor (EGF) could induce p21$^{Waf1/Cip1}$ in quiescent embryonic fibroblasts from p53 "knock-out" mice [27]. Expression of p21$^{Waf1/Cip1}$ is upregulated in p53-null human leukemic cell lines exposed to okadaic acid, 12-*O*-tetradecanoylphorbol-13-acetate or interferon γ [28]. Treatment of HaCaT cells, which contain two mutant alleles of *p53*, with transforming growth factor-β results in elevation of p21$^{Waf1/Cip1}$ expression [29]. Moreover, retinoid was found to induce the expression of p21$^{Waf1/Cip1}$ in breast cancer cell lines having a wild type (MCF-7 cells) or mutated (MDA-MB-468 cells) p53 [30].

Fluctuating expression of nuclear p21$^{Waf1/Cip1}$ in breast cancer: explaining how nuclear p21$^{Waf1/Cip1}$ functions as a tumor suppressor protein

Several immunohistochemical studies revealed low nuclear p21$^{Waf1/Cip1}$ expression in breast cancer tissues [4–6]. Low expression of p21$^{Waf1/Cip1}$, Mdm-2 and p53 was detected in 68.1%, 53.7% and 65.0% of the breast cancer specimens, respectively. Downregulated expression of p21$^{\mathrm{Waf1/Cip1}}$ was associated with p53 overexpression. On the contrary to p21$^{\mathrm{Waf1/Cip1}}$, overexpression of Mdm-2 was correlated with high p53 expression. Low expression of p21$^{\mathrm{Waf1/Cip1}}$ and overexpression of p53 and Mdm-2 were significantly associated with grade 3 tumors and the onset of lymph node metastasis. Low expression of p21$^{\mathrm{Waf1/Cip1}}$ was correlated with shortened overall survival. However, p21$^{\mathrm{Waf1/Cip1}}$ expression did not display any relationship to the status of estrogen receptor (ER) and different tumor histotypes [4]. p21$^{\mathrm{Waf1/Cip1}}$ expression was quite low in breast carcinomas and its expression was low in lobular carcinomas compared to ductal carcinomas. p21$^{\mathrm{Waf1/Cip1}}$ positive tumors were mostly p53 positive but expression of p21$^{\mathrm{Waf1/Cip1}}$ did not associate with Ki-67 or AP-2 expression in the malignant group. High nuclear p21$^{\mathrm{Waf1/Cip1}}$ positivity was correlated with poor differentiation. Moreover, p21$^{\mathrm{Waf1/Cip1}}$ expression had no association with stage, ER, progesterone receptor (PR), lymph node status, recurrence-free survival or breast cancer-related survival [5]. In previous study, 67.8% of breast cancer tissues were considered p21$^{\mathrm{Waf1/Cip1}}$ negative. p21$^{\mathrm{Waf1/Cip1}}$ expression was correlated only with increased PCNA expression, but not with age, menopausal status, tumor size, lymph node status, tumor grade, ER, PR, Her-2/neu expression, p53 expression, relapse-free survival, and overall survival [6].

According to Wakasugi et al. [7], nuclear immunoreactivity of p21$^{\mathrm{Waf1/Cip1}}$ was expressed in 49% of invasive breast carcinomas, and p53 in 32% tumors. Expression of these two proteins was inversed in 76 patients (73%), where 47 were positive for p21$^{\mathrm{Waf1/Cip1}}$ and negative for p53, and 29 were negative for p21$^{\mathrm{Waf1/Cip1}}$ and positive for p53. Four patients were positive for both p21$^{\mathrm{Waf1/Cip1}}$ and p53, whereas 24 were negative for both. Regarding clinicopathological features, p21$^{\mathrm{Waf1/Cip1}}$-positive tumors associated with negative lymph nodes, a low histologic grade and positive ER status. While p21$^{\mathrm{Waf1/Cip1}}$-negative tumors correlated with shortened recurrence-free survival. Using nuclear immunohistochemical staining, Somlo et al. [8] detected nuclear p21$^{\mathrm{Waf1/Cip1}}$ and p53 in 51% and 26% of the breast cancer tissues, respectively. Using real-time RT-PCR, *p21*$^{\mathit{Waf1/Cip1}}$ and *EGF* receptor (*EGFR*) mRNA levels were found to be expressed in 35% and 28% of the malignant breast tumors, respectively. Additionally, low expression of p21$^{\mathrm{Waf1/Cip1}}$ and high expression of *EGFR* mRNA in breast cancer patients were accompanied with worse prognosis as the periods of disease-free survival and overall survival were shortened.

In a previous study, p21$^{\mathrm{Waf1/Cip1}}$ nuclear immunoreactivity was detected in 82% of infiltrating breast carcinomas and high p21$^{\mathrm{Waf1/Cip1}}$ expression was associated with large tumor size, lymph node involvement, high mitotic count and poorly differentiated tumors. Moreover, p21$^{\mathrm{Waf1/Cip1}}$ negative/p53 positive breast carcinomas had the worst prognosis (lowest disease free survival and overall survival) compared to p21$^{\mathrm{Waf1/Cip1}}$ negative/p53 negative, p21$^{\mathrm{Waf1/Cip1}}$ positive/p53 negative and p21$^{\mathrm{Waf1/Cip1}}$ positive/p53 positive tumors [15]. Applying immunohistochemistry technique, another study declared that nuclear p21$^{\mathrm{Waf1/Cip1}}$ was found in 90% of breast cancer and p21$^{\mathrm{Waf1/Cip1}}$ overexpression was related to high tumor grade and short relapse-free survival, but it had no correlation with MIB-1 expression, tumor size, lymph node status, tumor histotypes, ER status and p53 expression [16].

Overall, it is obvious that the reports concerning the pattern of nuclear p21$^{\mathrm{Waf1/Cip1}}$ expression and its influence on the clinicopathological parameters and prognosis of breast cancer patients are inconsistent [4–8, 15, 16]. Breast cancer patients with low p21$^{\mathrm{Waf1/Cip1}}$ expression matched with the idea that p21$^{\mathrm{Waf1/Cip1}}$ is being a tumor suppressor protein [18, 20, 21] and consequently, loss or reduction in its function could be a contributing factor in the incidence of breast cancer as it loses its capability to inhibit cyclin/CDK complexes, phosphorylation of pRb, and DNA replication. On the other hand, the p21$^{\mathrm{Waf1/Cip1}}$-positive pattern in breast cancer patients might be attributed to mutant non-functional forms of p21$^{\mathrm{Waf1/Cip1}}$. Balbín et al. [31] have investigated the sequence of the PCR-amplified exons 2 and 3 of *p21*$^{\mathit{Waf1/Cip1}}$ gene in 36 primary breast carcinomas and they discovered a case with C to T transition in codon 94 that caused the substitution of arginine with a tryptophan (p21$^{\mathrm{R94W}}$). This mutation was attributed to a tumor-specific alteration as it is not observed in DNA extracted from peripheral blood cells of the same patient or in a PCR-restriction fragment length polymorphism of unrelated individuals. Functional analysis of the p21$^{\mathrm{R94W}}$ protein produced in different expression systems revealed that this mutation cause impairment of the ability of p21$^{\mathrm{Waf1/Cip1}}$ to inhibit cyclin A/CDK2, cyclin B/Cdc2, cyclin D/CDK4 and cyclin D1/CDK6. These data suggest that p21$^{\mathrm{R94W}}$ protein may participate in breast carcinogenesis as it becomes unable to inhibit a series of cyclin/CDK complexes.

Using immunohistochemistry, previous studies [4–8, 15, 16] have reported varying patterns regarding the association between expression of nuclear p21$^{\mathrm{Waf1/Cip1}}$ and p53 in breast carcinoma. There are different possible explanations for these findings. Firstly, high p53 accompanied with low p21$^{\mathrm{Waf1/Cip1}}$ could be due to mutational inactivation of p53 function [32]. It was demonstrated that p53 immunopositivity largely correlates with the detection of p53 mutations in a variety of malignancies including breast cancer [33]. Secondly, patients with mutant non-functional p53 and high p21$^{\mathrm{Waf1/Cip1}}$ give the notion that p53 could be implicated in the induction of p21$^{\mathrm{Waf1/Cip1}}$ expression in certain circumstances but the overall p21$^{\mathrm{Waf1/Cip1}}$ regulation seems to be more complicated as it might be stimulated by p53-independent pathways [27–30]. Thirdly, patients with wild-type p53 and high expression of $p21^{Waf1/Cip1}$ augment the crucial role of wild-type p53 in upregulating $p21^{Waf1/Cip1}$ [19]. However, low $p21^{Waf1/Cip1}$ expression might be occurred if the function of wild-type p53 is suppressed. In fact, the ability of a cell to degrade p53 in either the nucleus or cytoplasm adds another layer of complexity to p53 control. For example, it is thought that poly-ubiquitination of p53 by Mdm2 serves as a signal for proteasomal degradation of p53 at later stages of the DNA damage response [34].

Matching with the role of $p21^{Waf1/Cip1}$ as tumor suppressor protein, it was reported that transfection of ERα-positive breast cancer cell lines (MCF-7 and T47D) with $p21^{Waf1/Cip1}$ caused growth suppression as cells succumbed to apoptosis. Moreover, upregulation of $p21^{Waf1/Cip1}$ increased efficacy of anti-estrogen treatment where the number of drug-resistant colonies are reduced [35]. In accordance with the previous data, Pellikainen et al. [5] showed that overexpression of $p21^{Waf1/Cip1}$ in breast carcinomas predicted response to adjuvant hormonotherapy with antiestrogens in postmenopausal patients with metastatic lymph node, and ER and/or PR positive tumors.

Cytoplasmic $p21^{Waf1/Cip1}$ expression: its antiapoptotic/oncogenic properties and association with incidence and poor prognosis of breast cancer

The subcellular localization of $p21^{Waf1/Cip1}$, cyclin B1 and Cdc2 have examined by immunohistochemistry in primary infiltrating breast cancer. $p21^{Waf1/Cip1}$ was mostly positioned in the cytoplasm in a lot of the tumors and it was associated with cytoplasmic cyclin B1 and overexpression of nuclear p53. Increment of cytoplasmic Cdc2 expression was correlated with poorly differentiated tumors. Cytoplasmic $p21^{Waf1/Cip1}$ and cytoplasmic cyclin B1 levels were significantly associated with decreased overall survival. Moreover, cytoplasmic cyclin B1 level was correlated with shortened relapse-free survival [9]. It was declared that HER-2/neu-positive breast carcinomas showed proportionately higher cytoplasmic $p21^{Waf1/Cip1}$ staining in comparison to HER-2/neu-negative breast cancer. HER-2/neu positivity and cytoplasmic $p21^{Waf1/Cip1}$ were shown to predict worse prognosis [10]. It was demonstrated that activated Akt is mandatory for HER2/neu-mediated proliferation of breast cancer cell line as transfection of cells with dominant negative Akt caused slowness of cell-growth. Activated Akt physically binds to and phosphorylates $p21^{Waf1/Cip1}$ at T145 leading to cytoplasmic localization of $p21^{Waf1/Cip1}$. Blocking Akt signaling by overexpression of dominant negative Akt retrieves nuclear localization and cell-growth inhibiting activity of $p21^{Waf1/Cip1}$ [11]. The level of HER2/neu expression was greatly correlated with the phospho-Akt expression level, while the overexpression of phospho-$p21^{Waf1/Cip1}$ (T145) was strongly associated with cytoplasmic $p21^{Waf1/Cip1}$ in infiltrating breast carcinoma. High expression of phospho-$p21^{Waf1/Cip1}$ (T145) and cytoplasmic $p21^{Waf1/Cip1}$ were correlated with overexpression of HER2/neu and phospho-Akt. Upregulated expression of HER2/neu, phospho-Akt, phospho-$p21^{Waf1/Cip1}$ (T145) and cytoplasmic $p21^{Waf1/Cip1}$ were associated with worse overall survival. Investigating the pattern of HER2/neu and $p21^{Waf1/Cip1}$ together affords precise prediction of patients' survival. In order of worst to best, the 5-year survival rate was 16% in high HER2/neu and cytoplasmic $p21^{Waf1/Cip1}$ patients, 29% in low HER2/neu and cytoplasmic $p21^{Waf1/Cip1}$ patients, 60% in high HER2/neu and negative/nuclear $p21^{Waf1/Cip1}$ patients and 79% in low HER2/neu and negative/nuclear $p21^{Waf1/Cip1}$ patients [12]. Regarding the clinicopathological parameters of breast cancer patients, no significant relationship was found between the cytoplasmic $p21^{Waf1/Cip1}$ and tumor size, lymph node status [9, 12], stage, tumor grade, ER and PR [12].

To explore whether Akt-phosphorylated/activated form of $p21^{Waf1/Cip1}$ ($p21^{Waf1/Cip1}$D) expedite mammary tumorigenesis, transgenic mice expressing $p21^{Waf1/Cip1}$D in mammary epithelium was generated. The $p21^{Waf1/Cip1}$D was predominantly expressed in the cytoplasm. Overexpression of $p21^{Waf1/Cip1}$D in cytoplasm has an oncogenic role in promoting the development of mammary tumors and lung metastasis in MMTV/*neu* mice [13]. It was declared that expression of oncogenic Akt in MCF-7 breast cancer cell line caused an increase in endogenous $p21^{Waf1/Cip1}$ protein stability. $p21^{Waf1/Cip1}$ was degraded in nontransfected MCF-7 after the addition of cycloheximide. Similarly, the addition of taxol to either nontransfected cells or cells expressing the kinase dead form of Akt prevents the induction of $p21^{Waf1/Cip1}$. In contrast, $p21^{Waf1/Cip1}$ appears to be stabilized in cells expressing the oncogenic form of Akt in the presence of either cycloheximide or taxol, indicating that elevated $p21^{Waf1/Cip1}$ induction is linked to chemoresistance. Moreover, Akt was found to block the endogenous interaction between PCNA and $p21^{Waf1/Cip1}$; consequently, this enables PCNA to achieve its role in DNA replication [36]. Furthermore, the immunoreactivity expression of IκB kinase β (IKKβ) and $p21^{Waf1/Cip1}$ in a retrospective study involving 128 primary breast cancer samples was studied. The IKKβ level was correlated with total $p21^{Waf1/Cip1}$ expression. In addition, IKKβ expression was associated with cytoplasmic $p21^{Waf1/Cip1}$ expression, but not with nuclear $p21^{Waf1/Cip1}$ expression. Moreover, applying Western blot analysis the protein level of $p21^{Waf1/Cip1}$ was measured in cell lysates of breast cancer cell lines MCF-7 and MDA-MB-453, stably transfected with flag-tagged IKKβ or pCDNA3 vectors. $p21^{Waf1/Cip1}$ expression was apparently high in the IKKβ transfectants comparing to the pCDNA3 transfectants. Furthermore, cells are fractionated to analyze p21$^{Waf1/Cip1}$ protein level by Western blotting in different cellular compartments. p21$^{Waf1/Cip1}$ expression was obviously upregulated in the cytoplasm, but not in the nucleus of IKKβ transfectants compared to vector controls. Using RT-PCR, they investigated whether p21$^{Waf1/Cip1}$ expression may be regulated at the mRNA level. The expression of p21$^{Waf1/Cip1}$ mRNA was highly detected in the IKKβ stable cell lines relative to pCDNA3 vectors in MCF-7 and MDA-MB-453 cell lines. Western blotting was applied to evaluate the expression of total Akt and phospho-Akt (S473) in MDA-MB-453 stable cells. The expression of phosphorylated Akt (S473) but not total Akt level was apparently higher in the IKKβ stably transfected cell line than in the vector controls. To examine whether the upregulation of cytoplasmic p21$^{Waf1/Cip1}$ expression by IKKβ could be reduced by inhibiting the activity of Akt, the MDA-MB-453 cell line was treated with LY294002, which specifically blocks PI-3 kinase-dependent Akt phosphorylation. Cytoplasmic p21$^{Waf1/Cip1}$ was apparently downregulated in the IKKβ stable transfectants compared to pCDNA3 vectors demonstrating that Akt activation is implicated in IKKβ-mediated cytoplasmic p21$^{Waf1/Cip1}$ localization [14]. Collectively, Akt is phosphorylated and activated by HER2/neu and/or IKKβ. Activation of Akt causes translocation of p21$^{Waf1/Cip1}$ into the cytosol where it acts as an oncogene and associates with poor prognosis of breast cancer patients [11–14].

p21$^{Waf1/Cip1}$ overexpression: its association with poor responsiveness to chemotherapy in breast cancer

Taxol induces G2/M arrest and apoptosis in MDA-MB-435 breast cancer cell line by activation of cyclin B/Cdc2. The use of a dominant-negative mutant of Cdc2 or olomoucine, an inhibitor of Cdc2 impeded taxol-induced apoptosis in these cells. Moreover, HER2/neu-overexpressing 435.eB cells showed upregulated p21$^{Waf1/Cip1}$ expression, which subsequently associates with Cdc2, preventing taxol-induced Cdc2 activation. In turn, these cells become unable to enter G2/M phase resulting in prohibition of taxol-induced apoptosis. Transfection of MDA-MB-435 cells with antisense p21$^{Waf1/Cip1}$ abolishes the ability of Her2/neu to inhibit taxol-induced apoptosis. These data clarify the indispensable role of p21$^{Waf1/Cip1}$ in the regulation of a G2/M checkpoint as it contributes to resistance to taxol-induced apoptosis in Her2/neu-overexpressing 435.eB cells [37]. HER2/neu overexpression was associated with upregulation of p21$^{Waf1/Cip1}$ and increasing Cdc2-Y15 phosphorylation in breast cancer. The combined use of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) is one of the most broadly used chemotherapy regimens involved in the treatment of breast carcinomas. Overexpression of either HER2/neu or p21$^{Waf1/Cip1}$ was found to be correlated with poor response to CMF and worse disease free survival [38].

The antisense p21$^{Waf1/Cip1}$ oligodeoxynucleotide as therapy of breast cancer

In consideration of the antiapoptotic/oncogenic effect of p21$^{Waf1/Cip1}$ in breast cancer, previous studies have studied the possibility of using antisense p21$^{Waf1/Cip1}$ oligodeoxynucleotide (ODN) as therapy of breast cancer [39, 40]. It was revealed that nude mice that are transplanted with metastatic breast cancer cell line Met-1and then subjected to daily subcutaneous injection of a phosphorothioate antisense p21$^{Waf1/Cip1}$ ODN showed inhibition of breast cancer growth and angiogenesis [39]. Moreover, the human breast cancer cell lines, MCF-7 (adenocarcinoma) and T47D (ductal carcinoma), whether serum starved or grown in serum containing medium, showed high levels of p21$^{Waf1/Cip1}$. Transfection of MCF-7 and T47D cells with antisense p21$^{Waf1/Cip1}$ ODN caused remarkable, dose-dependent attenuation of p21$^{Waf1/Cip1}$, nuclear morphological changes consistent with apoptosis and marked poly (ADP-ribose) polymerase cleavage. Furthermore, T47D cells showed obvious dose-dependent caspase-3 cleavage after transfection with the antisense p21$^{Waf1/Cip1}$ ODN [40].

Conclusions, challenges and perspectives

In conclusion, the duality role of p21$^{Waf1/Cip1}$ in the regulation of breast cancer greatly depends on its subcellular localization. The tumor suppressor activity of p21$^{Waf1/Cip1}$ might be associated with its nuclear localization; whereas cytoplasmic p21$^{Waf1/Cip1}$ inhibited apoptosis [4–14]. Several laboratory findings confirmed the correlation between high cytoplasmic p21$^{Waf1/Cip1}$ and poor prognosis [9, 10, 12].

Many challenges exist that obstacle rely on p21$^{Waf1/Cip1}$ as reliable biomarker and therapeutic target for breast cancer. The frequent inconsistency regarding the expression of nuclear p21$^{Waf1/Cip1}$ in breast cancer tissues is a huge hindrance against validating nuclear p21$^{Waf1/Cip1}$ as trustworthy breast cancer biomarker. Moreover, we still lack understanding the contradictory reports concerning the relation between nuclear p21$^{Waf1/Cip1}$ expression level and clinicopathological factors as well as clinical outcomes in breast cancer patients.

Combining the expression of p21$^{Waf1/Cip1}$ with other parameters could result in obtaining a more accurate diagnosis for breast cancer. Recently, our lab investigated the expression pattern of p21$^{Waf1/Cip1}$ and p57^{Kip2} in breast cancer as well as their possible association with clinicopathological features. The expression of p21$^{\mathrm{Waf1/Cip1}}$ was obviously upregulated whereas p57$^{\mathrm{Kip2}}$ level was apparently downregulated in patients with breast cancer in comparison to patients having benign breast lesions. The sensitivity and specificity were 64.71% and 77.78% for p21$^{\mathrm{Waf1/Cip1}}$, whereas the values were 65.88% and 80.56% for p57$^{\mathrm{Kip2}}$. Interestingly, combination of p21$^{\mathrm{Waf1/Cip1}}$ and p57$^{\mathrm{Kip2}}$ increased sensitivity and specificity to 82.35% and 86.11%, respectively. Regarding the clinicopathological factors, the positivity rates of p21$^{\mathrm{Waf1/Cip1}}$ and p57$^{\mathrm{Kip2}}$ together were found to be associated with late stages, lymph node metastasis and poorly differentiated tumors in breast cancer patients. Taken together, our data revealed that combining p21$^{\mathrm{Waf1/Cip1}}$ and p57$^{\mathrm{Kip2}}$ could be a reliable diagnostic tool for breast cancer [41].

The promising results showing the capability of antisense p21$^{\mathrm{Waf1/Cip1}}$ ODN in attenuating breast cancer growth in mice [39], and the induction of apoptosis after transfection of human breast cancer cell lines with antisense p21$^{\mathrm{Waf1/Cip1}}$ ODN [40] suggest the possibility for the use of antisense p21$^{\mathrm{Waf1/Cip1}}$ ODN as future therapy in human breast cancer. In fact, achieving efficient and site-specific delivery of antisense p21$^{\mathrm{Waf1/Cip1}}$ ODN to breast cancer is highly challenging, as it is crucially important to avoid side effects associated with ODN delivery systems. In the near future, p21$^{\mathrm{Waf1/Cip1}}$ might be a therapeutic target in combination with other therapeutic regimens in breast cancer treatment.

Briefly, it is obvious that the impact of p21$^{\mathrm{Waf1/Cip1}}$ on breast cancer is tremendous. With more researches, p21$^{\mathrm{Waf1/Cip1}}$ could become one of the favorable clinical candidates that serve as a diagnostic, prognostic and therapeutic biomarker for breast cancer.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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