MINI-REVIEW

# Roles of PTEN (Phosphatase and Tensin Homolog) in Gastric Cancer Development and Progression

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## Abstract

**Gastric cancer is highly invasive, aggressively malignant, and amongst the most prevalent of all forms of cancer. Despite improved management strategies, early stage diagnosis of gastric cancer and accurate prognostic assessment is still lacking. Several recent reports have indicated that the pathogenesis of gastric cancer involves complex molecular mechanisms and multiple genetic and epigenetic alterations in oncogenes and tumor suppressor genes. Functional inactivation of the tumor suppressor protein PTEN (Phosphatase and Tensin Homolog) has been detected in multiple cases of gastric cancer, and already shown to be closely linked to the development, progression and prognosis of the disease. Inactivation of PTEN can be attributed to gene mutation, loss of heterozygosity, promoter hypermethylation, microRNA- mediated regulation of gene expression, and post-translational phosphorylation. PTEN is also involved in mechanisms regulating tumor resistance to chemotherapy. This review provides a comprehensive analysis of PTEN and its roles in gastric cancer, and emphasizes its potential benefits in early diagnosis and gene therapy-based treatment strategies.**

**Keywords:** PTEN - gastric cancer - inactivation - chemoresistance - gene therapy

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## Introduction

Gastric cancer is one of the most fatal forms of malignant cancers, and the second leading cause of cancer- related death (Guggenheim et al., 2013). It is far more prevalent in developing than in developed nations. The highest incidence of gastric cancer is found in East Asia, South America, and Central and Eastern Europe, accounting for approx. 70% of the total number of cases reported worldwide. Although traditional treatments such as surgery, radiotherapy, and chemotherapy can prolong the life of patients by 20-35% when administered at an early stage, they have achieved limited success in treating advanced gastric cancer, where the median survival time is 6-11 months (Wagner et al., 2010; Jemal et al., 2011). Due to the complexity of the molecular mechanisms and signaling pathways implicated in carcinogenesis and the lower incidence of gastric cancer in the developed western nations, research on targeted therapies for gastric cancer has been slower than other types of cancer. It is known that accumulation of genetic alterations, such as the activation of an oncogene or the loss of a tumor suppressor gene, can cause gastric carcinoma (Deng et al., 2012). Hence, it is important to study the consequence of genetic alterations on the properties of malignant tumors.

PTEN (Phosphatase and Tensin Homolog) is a tumor suppressor gene, discovered in 1997 (Li and Sun, 1997; Li et al., 1997; Steck et al., 1997). It functions as a dual-specificity protein and phospholipid phosphatase, and regulates a variety of cellular processes and signal transduction pathways in a complex network system. For example, PTEN can induce apoptosis and suppress proliferation by antagonizing the phosphatidylinositol 3- kinase (PI3K)/Akt signaling pathway. It also controls cell adhesion, migration and tumor invasion by downregulating the activity of focal adhesion kinases (FAKs) (Tamura et al., 1998). Additionally, it can restrict cellular differentiation by negatively regulating mitogen-activated protein kinase (MAPK)- mediated signaling (Besson et al., 1999; Ciuffreda et al., 2012). Genetic modification- induced inactivation of PTEN has been detected frequently in different forms of cancers, including glioblastoma multiforme, endometrial carcinoma, skin, prostate and breast cancer (Baig et al., 2011; Romano et al., 2012; Cancer Genome Atlas Research Network et al., 2013; Moon et al., 2013; Patel et al., 2013). Different mechanisms have been implicated in PTEN inactivation in gastric cancer; its decreased expression is closely related to the incidence, progression and prognosis of the disease. This review summarizes the various kinds of cancer-associated genetic alterations in PTEN, its diverse function specifically in gastric cancer, and the wide prospect it offers for early diagnosis and gene therapy- mediated treatment.

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## Characteristics of PTEN

*Structure*

PTEN gene, also known as MMAC1 (muted in multiple advanced cancers) or TEP1 (TGF-β-regulated and epithelial cell enriched phosphatase) is a tumor suppressor gene isolated and identified by three laboratories in 1997. PTEN is a 200 kb gene located on chromosome 10q23.3. It is composed of 9 exons and 8 introns, and encodes a 403 amino acid long protein with a relative molecular mass of approx. 47 kDa. The following features of its structure are likely to contribute to its function as a tumor suppressor: (1) N- terminal of the phosphatase domain, which carries the conserved phosphatase motif HCSSGSSR. This domain is highly homologous to the catalytic domains of tyrosine phosphatase and serine/threonine phosphatase, indicating its ability to dephosphorylated tyrosine and serine/threonine residues, and facilitating the tumor suppressor function of PTEN; (2) C- terminal of the C2 domain, which combines with phospholipids in a Ca2+- independent manner, and functions to localize PTEN in cell membranes so it can mediate in vivo signal transduction. One study suggested that phosphorylation of the C- terminal tail of PTEN could modulate the molecular interaction between the phosphatase and C2 domains, which dictates the percentage of membrane-associated PTEN and the level of Phosphatidylinositol (3,4,5)- triphosphate (PtdIns(3,4,5)- P3) (Rahdar et al., 2009); (3) The PDZ protein- binding domain and two PEST sequences (rich in Proline (P), Glutamic acid (E), Serine (S) and Threonine (T)) in the tail region, which regulate the stability and biological activity of PTEN.

*Biological function*

PTEN is a tumor suppressor gene, mutations in which are associated with a large number of tumors, second only to p53. Recent work has shown that PTEN can regulate a variety of cellular signal transduction pathways or molecules to form a complex network system that can control proliferation, apoptosis, migration, adhesion and genetic stability. PTEN can function both as a lipid and a protein phosphatase. Also, it can regulate signal transduction by both phosphatase- dependent and independent mechanisms. Unlike most tumor suppressor genes, PTEN can function in the nucleus as well as the cytoplasm. Also, its precise intracellular localization can greatly impact its stability and function. The most detailed study of PTEN is focused on its negative regulation on PI3K/Akt signaling pathway, which is important in controlling cell survival, promoting proliferation and inhibiting apoptosis (Ye et al., 2012). Through phosphorylation of its 3' hydroxyl group, secondary messenger PIP3 transfers Akt kinase to the cell membrane, and changes its conformation such that Akt can now be phosphorylated and activated by PI3K 1/ 2, the transduction of anti-apoptotic signals is thus achieved. In the cytoplasm, however, PTEN can dephosphorylate PIP3 to PIP2 by removing the 3' phosphoric acid moiety of the inositol ring of PIP3 (Maehama and Dixon, 1998). Under normal conditions, PI3K phosphorylates and PTEN dephosphorylates PIP2 and PIP3, respectively, to maintain the dynamic equilibrium between the two secondary messengers. Under conditions where PTEN function is inactivated, PIP3 can no longer be dephosphorylated into PIP2, resulting in an accumulation of PIP3, activation of Akt kinases, and altered expression of p27, Bad (Bcl-2 antagonist of cell death), foxo (forkhead box protein O), mTOR (mechanistic target of rapamycin), GSK-3 (glycogen synthase kinase-3), and other factors downstream of Akt signaling. This prevents apoptosis, and promotes cell survival and proliferation. Bad, a pro-apoptotic factor of the Bcl-2 family, competes with other pro- apoptotic factors like BAX (Bcl-2-associated X protein) and displaces it from anti-apoptotic Bcl-2 and Bcl-xL complexes, thereby promoting cell death (Yang et al., 1995). Akt- mediated phosphorylation of Bad at the Ser136 locus causes loss of function (Datta et al., 1997; Sakamaki et al., 2011). GSK-3 can block cell cycle progression by regulating the proteolysis and subcellular localization of cyclin D1 (Diehl et al., 1998). Akt- mediated phosphorylation of GSK-3α and GSK-3β at Ser21 and Ser9 loci, respectively, causes loss of function (Cross et al., 1995). In addition to Akt, cytoplasmic PTEN also regulates EGF signaling by inhibiting phosphorylation of SHC adaptor proteins, which in turn suppress activation of the Ras/MAPK pathway (Gu et al., 1998). Finally, PTEN can control cell migration, stretching and adhesion by regulating the activity of FAKs (through dephosphorylation) and other membrane channels (Tamura et al., 1999).

Like other tumor suppressor genes, nuclear PTEN has important biological functions. The level of functional PTEN in the nucleus is regulated through dynamic changes in its ubiquitylation. Monoubiquitylation of PTEN is mediated by the E3 ubiquitin- protein ligase NEDD4; deubiquitylation is mediated by the signaling complex of deubiquitylating enzyme USP7 (ubiquitin carboxyl-terminal hydrolase 7), and PML nuclear bodies (Trotman et al., 2007; Yang et al., 2012). Nuclear PTEN can reduce the level of cyclin D1, induce G0- G1 cell cycle arrest and, through its non- enzymatic function, inhibit tumor growth by regulating the activity of the anaphase-promoting complex/ cyclosome (APC/C) (Song et al., 2011). A recent study showed that nuclear PTEN binds to the centromeric protein CENP- C in a phosphatase- independent manner to maintain centromeric stability. It also interacts with transcription factor E2F1 to in turn activate the transcription of Rad51, that encodes a DNA repair and recombination protein, thus promoting DNA double-strand break (DSB) repair, and inhibiting chromosomal instability induced by DSBs. This guardian role of PTEN in maintaining chromosome structure and function seems to be independent of its lipid phosphatase activity (Shen et al., 2007; Kim et al., 2011). Also, since nuclear PTEN has a longer half-life and is more stable than its cytoplasmic counterpart, it is likely that the former plays a greater role in suppressing tumor formation.

*Regulation of PTEN expression*

Due to its principal function as a tumor suppressor, maintaining a stable level of PTEN expression is critical. Studies have shown that the expression and enzymatic

activity of PTEN can be regulated at transcriptional, microRNA (miRNA), and post-translational levels. Positive regulators of PTEN gene expression include, EGR1 (early growth response protein 1), PPARγ (peroxisome proliferator- activated receptor γ) and Tp53 (tumor protein 53; also known as p53); these were shown to directly bind to the PTEN promoter region (Patel et al., 2001; Stambolic et al., 2001; Virolle et al., 2001; Martelli et al., 2011). Negative regulators include MKK-4 (mitogen activated protein kinase kinase- 4), TGF-β (transforming growth factor beta), NFκB (nuclear factor of kappa light polypeptide gene enhancer in B-cells), transcriptional cofactor c-JUN and oncogene BMI1; these were shown to suppress PTEN expression in several cancer models (Gericke et al., 2006; Lau et al., 2011; Meng et al., 2012). Many miRNAs are also known to impact on PTEN expression and function in both normal and pathological conditions (Tian et al., 2013). Post-translational modifications such as active site oxidation, acetylation, phosphorylation, and ubiquitination can also regulate PTEN activity (Gericke et al., 2006; Tamguney and Stokoe, 2007). For example, the phosphatase activity of PTEN can be negatively regulated by oxidation of cystein residues C71 and C124, acetylation and phosphorylation. Proteolytic degradation of PTEN is mediated by the E3 ubiquitin ligase NEDD4-1 ( Wang et al., 2007). Finally, genetic and epigenetic mechanisms such as point mutations, chromosomal deletions, and promoter methylation status, can also modulate the expression and activity of PTEN (Waite and Eng, 2002). Aberrations in any of these regulatory mechanisms are likely to alter PTEN expression levels. When the sample size is small, genetic anomalies are more likely due to alterations in single regulatory mechanisms, but in a large sample, these mutations might be the combined outcome of multiple regulatory aberrations.

## Inactivation of PTEN in Gastric Cancer

Inactivation of PTEN has been shown to occur in a variety of tumor cells and tissues. Studies have also reported that inactivation of PTEN is closely associated with the incidence and progression of gastric cancer. The following mechanisms are involved: gene mutations, loss of heterozygosity, promoter hypermethylation, miRNA- mediated regulation and post- translational phosphorylation.

*PTEN gene mutations*

The primary cause of inactivation of PTEN function is gene mutations. It has been detected in various types of malignancies, at different stages of disease progression. PTEN mutations are frequently and specifically found in late stage glioblastoma, melanoma and prostate cancer, and in the early stages of thyroid cancer and cervical endometrial cancer. PTEN mutations were not detected in other types of tumors. Currently available studies report that PTEN mutation rate is low in gastric cancer. Sato et al. (2002) examined 10 gastric cancer cell lines and 58 patients diagnosed with primary gastric cancer, and found a silent genetic mutation in only one patient- a deletion of 5 nucleotides in the seventh intron of PTEN that did not change the mRNA sequence. Lima et al. (2005) analyzed samples from 48 patients diagnosed with gastric adenocarcinoma, and found only one patient with a chromosomal translocation, and concluded that genetic mutations in PTEN occur rarely in gastric cancer. Several independent studies determined the same (Chang et al., 1999). In contrast, Wang et al. (2003) observed that 28.3% (17/60) patients diagnosed with advanced gastric cancer had PTEN gene mutations, that included 8 missense mutations, 5 silent mutations, 2 nonsense mutations, 1 deletion and 1 intron splice donor site mutation.

*Loss of heterozygosity of PTEN gene*

Loss of heterozygosity (LOH) is a mechanism commonly found in malignant glioma, endometrial cancer, prostate cancer, malignant melanoma and other types of tumor cell lines and tissues. Research in recent years demonstrated that although PTEN mutations in gastric cancer are rare, LOH is more frequent. Byun et al. (2003) found decreased expression of PTEN and upto 47% LOH in 33% (5/15) gastric cancer cell lines and 36% (22/55) gastric cancer tissue samples. LOH rate was significantly higher in advanced than in early gastric cancer (63% to 18%); it was also significantly higher in poorly differentiated than highly and moderately differentiated gastric cancer (69% to 29%). Additionally, no genetic mutation was detected in the remaining allele. This suggests that complete functional inactivation of PTEN is not necessary to cause gastric carcinogenesis, loss of one allele is sufficient. Li et al. (2005) examined gastric mucosal injury tissues for PTEN mutation and LOH. They found that the occurrence of LOH increased with the progression of gastric carcinogenesis, with rates of 10% (3/30), 10% (3/30), 13.3% (4/30), 20% (6/30) and 33.3% (9/30) in atrophic gastritis, intestinal metaplasia, dysplasia, early and advanced gastric cancer, respectively. In this study, genetic mutations were detected in 10% advanced gastric cancer tissues, but none in precancerous lesions and early gastric cancer tissues. Thus, while loss of heterozygosity occurs in different stages of gastric cancer, genetic mutations could be detected only in advanced gastric cancer. Both gene mutations and LOH can promote tumor invasion and metastasis. Some studies have shown that the LOH status of PTEN can impact sensitivity to chemotherapy and hence, the prognosis in patients with gastric cancer. Hence, it may also be used as an independent prognostic tool (Oki et al., 2005; Oki et al., 2006; Chong et al., 2013).

*PTEN promoter methylation*

DNA methylation, especially of the CG- rich promoter regions (called CpG islands), is a highly prevalent mechanism of gene regulation. Abnormal methylation of CpG islands in the promoters of tumor suppressor genes can inhibit their expression. This has been detected in brain tumors, hematological malignancies, malignant melanoma and other tumors. It is known that promoter hypermethylation is an important mechanism used to suppress PTEN expression. Kang et al. (2002) screened 310 cases of gastric cancer, and found that 62 lacked PTEN

expression due to methylation of it's promoter. This is turn was significantly associated with increased tumor depth and size, disease progression (increased proportion of advanced stage cancer), lymphatic invasion and reduced patient survival. Absence of PTEN function thus has important consequences in the pathogenesis of gastric cancer. According to this study, loss of PTEN activity is a relatively late event in the course of gastric carcinogenesis, and hence is more important in disease progression than onset. Hino et al. (2009) found that 43% Epstein-Barr Virus (EBV)- positive gastric cancer cases lacked PTEN expression, a significantly higher proportion than the EBV-negative cases (10%). They also found better correlation between PTEN promoter methylation status and the incidence of gastric cancer in the EBV- positive form. On the contrary, Sato et al. (2002) examined 10 gastric cancer cell lines and 20 gastric cancer patient samples, and found neither a decrease in PTEN mRNA level or hypermethylation of the CpG islands in PTEN promoter. Other investigators observed that the methylated PTEN gene is a quasi gene, and not the gastric cancer- associated PTEN gene (Byun et al., 2003).

*Regulation of PTEN expression by miRNAs*

miRNAs are a class of small non-coding RNAs, 18-25 nucleotides long. They influence gene expression on a broad scale, by mechanisms such as post-transcriptional repression and translational suppression. For example, they can down-regulate target gene expression by complexing with the 3'-UTR of mRNAs, thereby repressing their translation (Hobert, 2008). miRNAs play a crucial regulatory role in multiple biological processes, including differentiation, and stress responses (Schoof et al., 2012). Current studies have determined that aberrations in miRNA- mediated gene regulation can lead to various diseases including gastric cancer (Cheung et al., 2011; Zhang et al., 2011; Yu et al., 2012). miR-21, together with its known targets and other associated genes, forms a complex regulatory network that plays a crucial role in the etiology and progress of gastric carcinogenesis. Guo et al. (2009) first identified PTEN as a target of miR-21. Chun et al. (2010) and Wang et al. (2011) identified PTEN as a target of miR-221 and miR-222, and found that suppression of the miR-221/222 cluster in gastric cancer cells restricted cell proliferation, induced apoptosis, decreased cell invasion and increased their sensitivity to radiation, by upregulating PTEN expression. Further, Yang et al. (2013) demonstrated that miR-214 can also post-transcriptionally regulate PTEN expression by binding to the 3'-UTR of its mRNA. miR- 214 expression is upregulated in gastric cancer, while its knockdown in gastric cancer cells can suppress proliferation and tumor invasion, by altering PTEN- mediated signaling.

*Post- translational phosphorylation of PTEN*

Recent studies have reported that PTEN activity can be regulated at the protein level by phosphorylation, oxidation, and by its lipid ligands and protein- binding partners. Among these, phosphorylation is the most significant method of post-translational modification of PTEN. Casein kinase 2 (CK2) and GSK3β, both, are shown to phosphorylate PTEN on its C-terminal tail. CK2 phosphorylation on S370 and S385 causes stabilization of PTEN (Torres et al., 2001). However, phosphorylation on S362 and T366 by GSK3β negatively regulates PTEN/PI3K signaling. Thus far, a variety of phosphorylation sites (Ser380, Thr382, and Thr383) have been identified in the C2 domain of PTEN, which when phosphorylated cause decreased phosphatase activity and increased stability (Vazquez et al., 2000). This can in turn enhance susceptibility to cancer by reducing the tumor suppressor activity of PTEN. The most important phosphorylation site for regulating PTEN function is Ser380 (Vazquez et al., 2000). Our previous work showed that the ratio of phosphorylated PTEN (pPTEN) to PTEN (called phosphorylation ratio) is significantly higher in fresh gastric carcinoma than in adjacent tissues. We also found it to be higher in four kinds of human gastric cancer cell lines- MKN28, MKN45, SGC-7901 and AGS, when compared to the non- cancerous gastric epithelial cell line GES-1. During the course of gastric cancer, PTEN expression level decreased progressively, accompanied by a concomitant increase in the level of pPTEN. These results suggest that post- translational phosphorylation is a novel and important process for PTEN inactivation during gastric carcinogenesis (Yang et al., 2013).

## Roles of PTEN in Gastric Cancer

Current studies have shown that inactivation of PTEN in gastric cancer is a multi- process event closely linked to incidence and disease progression. Zheng et al. (2003) analyzed PTEN expression in 113 samples of gastric carcinoma compared to adjacent normal tissues, and found significantly lower PTEN expression in gastric cancer tissue (54.9%), than in adjacent tissue (89.4%). Yang et al. (2003) and Zheng et al. (2003) systematically determined PTEN expression in different gastric mucosal injury tissues. It is established that gastric carcinogenesis evolves according to the following developmental sequence-, normal mucosa, atrophic gastritis, intestinal metaplasia, dysplasia, early and advanced gastric cancer. Accordingly, they found a gradual decrease in PTEN expression with advancing stage of gastric cancer. The expression of PTEN during lymph node metastasis or in advanced gastric cancer was significantly lower than at the non-lymph node metastatic stage or early gastric cancer; also, it was lower in the diffuse-type than the intestinal-type gastric cancer. Lowest expression was reported in signet ring cell carcinoma, much lower than the moderately differentiated gastric cancer tissue. These studies indicated that reduction or absence of PTEN expression is a dynamic process during the course of gastric cancer progression, and that PTEN levels can be used as an indicator to diagnose the pathological state of gastric cancer. Other studies exploring the role and mechanism of action of PTEN in gastric cancer, found similar results (Li et al., 2012; Bai et al., 2013; Li et al., 2013).

*Mechanism of action of PTEN in apoptotic cell death*

PTEN has been shown to promote apoptotic cell death, which in turn could suppress tumor growth (Carson et al.,

1999). Caspase-3 is a proteinase and a crucial effector in the apoptotic pathway. Its expression in human tissues was first described by Krajewska et al. (1997). They showed that caspase-3 is detectable in almost all cell types, emphasizing its role in modulating cell survival and death. Schwartzbauer and Robbins (2001) discovered that the addition of recombined adenoviruses to cultured neonatal rat primary cardiomyocytes could enhance PTEN expression in these cells, leading to increased caspase-3 activity and apoptosis. In addition, some studies have indicated that PTEN could enhance Fas/Fasl or cytochrome c- mediated apoptosis that involves activation of caspase-3 (Carson et al., 1999; Wick et al., 1999; Wick et al., 2012). From these studies, we inferred that in gastric cancer, decreased PTEN expression could downregulate caspase-3, thus disrupting the apoptotic pathway in tumor cells, which in turn could support cancer progression. This could be the contribution of PTEN to gastric oncogenesis.

*Mechanism of PTEN function in metastasis*

In a study by Hwang et al. (2001), it was determined that by acting on matrix metalloproteinases (MMPs) and VEGF, PTEN can enhance tumor metastasis. Another report showed that PTEN- mediated dephosphorylation of FAKs can influence cell adhesion (Maehama et al., 2001). Reduced or lost expression of PTEN decreases cell adhesion and increases synthesis of MMPs and VEGF, which could subsequently promote tumor invasion and angiogenesis.

*Mechanism of PTEN function in angiogenesis*

Angiogenesis is essential for tumor growth, invasion and metastasis. The interaction between tumor and host can induce tumor- secreted angiogenic factors like VEGF and MMPs to stimulate intratumoral and peritumoral neovascularization (Kim et al., 1999; Gyenge et al., 2013). Huang et al. (2002) showed that when endogenous PTEN in cultured endothelial cells is suppressed by adenovirus-mediated expression of a dominant negative mutant form, multiple VEGF and PIP3-kinase- mediated cellular responses and signaling pathways are strongly enhanced, inducing cell survival and migration. As a corollary, these effects of VEGF were inhibited by overexpression of wildtype PTEN. In angiogenesis models, overexpression of wildtype PTEN caused oriented endothelial tube formation and vascular sprouting. Furthermore, reduction and deletion of wildtype PTEN cause increased expression of MMPs, that play a crucial role in angiogenic processes like proliferation and migration of endothelial cells, and disruption of the extracellular matrix (Cox and O'Byrne, 2001). These observations suggest that decreased PTEN expression in gastric cancer can promote angiogenesis by upregulating the levels of VEGF and MMPs.

## PTEN Inactivation and Chemoresistance

Surgery is the only way to cure cancer completely, but since patients tend to be at an advanced stage at the time of diagnosis, it is difficult for surgery alone to uproot gastric cancer and prevent a relapse. Hence, chemotherapy has become an almost unavoidable alternative treatment for gastric cancer. However, primarily due to the decreased sensitivity or complete resistance of tumor cells to chemotherapeutic drugs, current treatments either fail or are not satisfactory. Oki et al. (Oki et al., 2005) found that gastric cancer patients who carry a loss of heterozygosity in PTEN are less sensitive to chemotherapy. Hwang et al. (Hwang et al., 2005) demonstrated that overexpression of PTEN in SNU-5 or SNU-216 gastric cancer cell lines could increase apoptosis induced by etoposide or doxorubicin. In a study by Yu et al. (Yu et al., 2008), overexpression of PTEN in transfected human gastric cancer cell line BGC-823, was shown to reduce the basal and post- chemotherapy activity of Akt kinases while increasing cell sensitivity to etoposide or doxorubicin. This inhibited tumor growth and promoted apoptotic cell death, implying that in gastric cancer, PTEN inactivation, and consequently activation of Akt pathway, may be the main cause of tumor resistance. Numerous mechanisms have been proposed to date, to explain the specific role of PTEN in imparting tumor cells resistance to chemotherapeutic drugs. First, activation of the PI3K signaling pathway, specifically, PI3K3A and pAkt, which were found responsible for PTEN-induced drug resistance possibly by inducing expression of multidrug resistance protein-1 (MRP1) (Lee et al., 2004; Esteva et al., 2010; Xu et al., 2012) . In addition, Zhang et al. (Zhang et al., 2011) recently discovered that the non-receptor tyrosine kinase SRC is a key regulator of trastuzumab (chemotherapeutic drug) response because of its ability to activate Akt. More importantly, they found that PTEN could dephosphorylate and restrict SRC kinase activity. Hence, reduced PTEN expression in cancer cells could result in increased phosphorylation and activation of SRC kinases and thus contribute to resistance to chemotherapeutic agents like trastuzumab.

Due to the significant role of PTEN in gastric carcinogenesis, several studies were conducted to investigate whether administration of exogenous PTEN could improve sensitivity to chemotherapy, as well as directly impact proliferation, apoptosis and cell cycle regulation in gastric cancer cells. Several lines of evidence were uncovered to confirm this hypothesis. For example, in a study by He et al. (He et al., 2007), when exogenous PTEN was introduced into the gastric cancer cell line SGC7901, the doubling time increased significantly; the rate and size of colony formation decreased and the proportion of cells in G1 phase also increased significantly. Concomitantly, they also detected a reduction in VEGF and MMP-9 concentration in the culture supernatant, and inferred that exogenous PTEN may inhibit the growth and proliferation of SGC7901 cells by suppressing the expression of VEGF and MMP-9. In other studies, Hwang et al. (Hwang et al., 2005) and Hang et al. (Hang et al., 2005) found that exogenous PTEN significantly suppressed proliferation, and induced apoptosis and cell cycle arrest in gastric cancer cells. Hang et al. also demonstrated that exogenous PTEN could inhibit Akt, FAK and p44/42 MAPK pathway in gastric cancer cells, but had no effect on normal cells, suggesting that PTEN may have a selective apoptotic effect on tumor cells. Administration of exogenous PTEN could thus become

a new strategy in gastric cancer treatment (Hang et al., 2005).

## Concluding Remarks

Following more than ten years of research into the tumor suppressor protein PTEN, its critical role in gastric carcinogenesis is now being unraveled. Currently available evidence suggests that reduction or absence of PTEN expression is a multi- process event, closely associated with the incidence, progression and chemotherapy-response of gastric cancer. However, several aspects of PTEN function in gastric cancer are still unknown and more research is needed to explore them. Due to its multiple role in various cellular processes, it is important to examine how PTEN mediates the interaction between these processes, their dynamics and homeostatic balance in response to pathological conditions. Additionally, although PTEN gene mutation is a proven mechanism of PTEN inactivation in gastric cancer, it occurs rarely, and mostly only in advanced gastric cancer. On the other hand, loss of heterozygosity and promoter methylation are likely to be more common, suggesting they might be the primary etiological mechanism. More research is needed to further investigate these processes. The cause of these genetic alterations is unclear; also, it is not known whether diversity in sample size, lesion progress phase, histological subtype, and patient ethnicity can affect the findings on the role of PTEN in gastric cancer. Besides, PTEN is a promising tool to use as an indicator of pathogenic state or disease prognosis and possibly also as a target for treatment of gastric cancer. Further research to address these issues will help clarify the specific role of PTEN in tumor suppression and oncogenesis and provide new avenues for early diagnosis of gastric cancer, and for identification of novel gene therapy approaches for treatment.

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