

Double Agent: *SPDEF* Gene with Both Oncogenic and Tumor-Suppressor Functions in Breast Cancer

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Abstract: The dichotomy of cancer-regulatory genes into “oncogenes (OCGs)” and “tumor-suppressor genes (TSGs)” has greatly helped us in learning molecular details of tumor biology. *SPDEF*, known as the prostate-derived ETS factor, is reported to play a pivotal role in normal cell development and survival, which has also been endowed with dual characteristics in cancers. Breast cancer (BC) is a highly heterogeneous disease which becomes the leading reason for cancer-related fatality among women worldwide. The involvement of *SPDEF* in many aspects of BC has been postulated, whereas the mechanism governing the regulation of the pro- and anti-oncogenic activities of *SPDEF* in BC state remains poorly defined. In this review, we summarized *SPDEF* as the double agent involving in expression profiles, the regulatory mechanism in BC progression, as well as the role in diagnosis, treatment and prognosis of BC. The understanding of *SPDEF* duality has contributed to gain insight into the tumor biology and also add a new dimension to the new therapy targets for BC.

Keywords: *SPDEF*, double agent, oncogenes, tumor-suppressor genes, transcription factor, breast cancer

Introduction

As one of the most prevalent cancers, BC is the leading reason for cancer-related fatality among women worldwide.¹ Moreover, BC is a highly heterogeneous disease which not only brings difficulty to mechanisms research but also hinders the development of molecularly targeted drugs in clinical practice. In order to organize this heterogeneity, multiple BC classification systems have been developed. Especially, BC can be clustered into four subtypes including triple-negative, human epidermal growth factor receptor 2 positive (Her2⁺), luminal A and luminal B tumors according to gene expression profiling.² Triple-negative breast cancer (TNBC) is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and Her2, which exhibits commonly more aggressive and invasive. Since luminal A and luminal B are both typically ER⁺, the major difference of the two types is the higher ki-67 expression in luminal B BC.³ As for Her2⁺ BC, it is driven by overexpression of Her2 and genes associated with related pathways or the Her2 amplicon on chromosome 17q12,⁴ which has been shown to have a worse outcome than ER⁺ BC.⁵

More recently, the double-agent nature of genes has gradually been discovered to be one of the main reasons behind the heterogeneity, as well as the main mechanism underlying these in-tumor adversities.⁶ Targeted treatment for one

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gene or gene product may be effective for certain cancer cells, but it may also promote the survival or progression of other cells leading to tumorigenesis. Therefore, understanding the duality genes will further help us in selecting target molecules for the treatment of different BC subpopulations. Dichotomy of cancer-regulatory genes into two opposed classes in the tumorigenesis: “oncogenes (OCGs)” and “tumor-suppressor genes (TSGs)”. OCGs can induce a series of gene expressions related to cell growth and differentiation, leading to uncontrolled growth of normal cells, and eventually become cancer cells. On the contrary, TSGs protect normal cells from degradation into cancer cells. They appear to be two opposite gene classes in the tumorigenesis. Paradoxically, some certain genes exhibit carcinogenic and tumor-suppressing functions at the same time.^{7–10} For instance, p53 is recognized as a tumor suppressor which inhibits the malignant growth of tumor cells.^{11–15} Conversely, germline mutation of p53 causes Li-Fraumeni syndrome, which is a familial cancer syndrome including BC, soft tissue sarcoma and various other types of cancer.¹⁶ And mutant p53 (p53-R248Q) functions as an oncogene in promoting endometrial cancer by up-regulating REGγ.¹⁷ KMT2D epigenetic regulator in B cell lymphoma acts as a tumor suppressor,¹⁸ while its knockdown slows the growth and sensitizes to PI3K inhibitor treatment in BC cells.¹⁹ Further, ARID1A is hypothesized to be tumor suppressive, but the same protein has opposing and stage-dependent roles within the same tissue, as Arid1a deletion can both impair²⁰ and promote²¹ the initiation of liver tumorigenesis and accelerates progression and metastasis in established disease.²¹ In order to promote the rapid development of cancer research and ensure the emergence of new concepts that is conducive to research, it is necessary to provide a highly flexible data classification such as “double-agent genes”.

SPDEF gene is vital for normal cell development and survival, the role of which in BC has been controversial over the years. Firstly, current studies have shown that the dual function of *SPDEF* is mainly dependent on molecular subtypes of BC. In TNBC, *SPDEF* is confirmed as a TSG. The invasive inhibition of *SPDEF* mediated is related to the regulation of downstream target genes, such as decreasing the expression of epithelial-mesenchymal transformation (EMT) related gene urokinase-type plasminogen activator (uPA)²² and SLUG,²³ and elevating the expression of cell cycle regulation-related protein p21.²⁴ In Her2⁺ subtype, *SPDEF* can promote proliferation, migration and invasion of SK-BR-3 cells by AR-*SPDEF* pathway²⁵ or *SPDEF*-

CEACAM6 oncogenic axis²⁶ as an OCG. Noteworthy, for luminal BC, *SPDEF* has dual behavior by promoting oncogenesis and progression through ER/FOXA1/GATA3 network,²⁷ and growth suppressed in MCF 7 cells^{28,29} as a TSG. In addition, the researchers have also found *SPDEF* to be a biomarker of poor prognosis in ER⁺ primary BC.^{27,30} Secondly, both the pro- and anti-oncogenic activities of *SPDEF* have been demonstrated and are stage-dependent. From benign breast to ductal carcinoma in situ (DCIS), the protein expression of *SPDEF* gradually increased,³¹ and then lost in invasive BC (IBC).^{32,33} Given all that, *SPDEF* mainly plays the role of OCG in luminal BC and Her2⁺ BC, plays the role of TSG in TNBC, and even shows a dual role switch during the malignant progression of BC. Therefore, the purpose of this review is to provide compelling evidence that *SPDEF* can be presented as both TSG and OCG in BC.

SPDEF introduction

The Structure of SPDEF

SPDEF gene is located at chromosome 6p21.31 and encodes for 6 exons with the length of the coding sequence of 1005 nucleotides (Figure 1A). Alternatively, spliced transcript variants encoding different isoforms have been found for *SPDEF* gene, and exon 4 skipping is one predominant alternative splicing event (Figure 1B). Two isoforms have been produced by alternative splicing so far. Isoform 1 has been chosen as the canonical sequence with the missing of the amino acid sequence from 212 to 227 of isoform 2. And the function of isoform 2 has not been described in the published literature in detail. *SPDEF* protein is composed of 335 amino acids (Figure 1C). Unlike other ETS proteins, *SPDEF* mainly contains a pointed domain and a conserved 88 amino acid ETS domain. Moreover, the ETS domain of *SPDEF* protein prefers binding to GGAT to binding to GGAA core compared with other ETS TFs.³⁴

Phosphorylation Sites

ETS family members are regulated generally by phosphorylation and rarely by other post-translational modifications.^{35,36} Potential phosphorylation sites present in *SPDEF* include a protein kinase C site, two tyrosine kinase phosphorylation sites, two AKT phosphorylation sites, and eight MAPK phosphorylation sites,³⁴ whereas are little verified yet. One is the activation of extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) through Her2 and

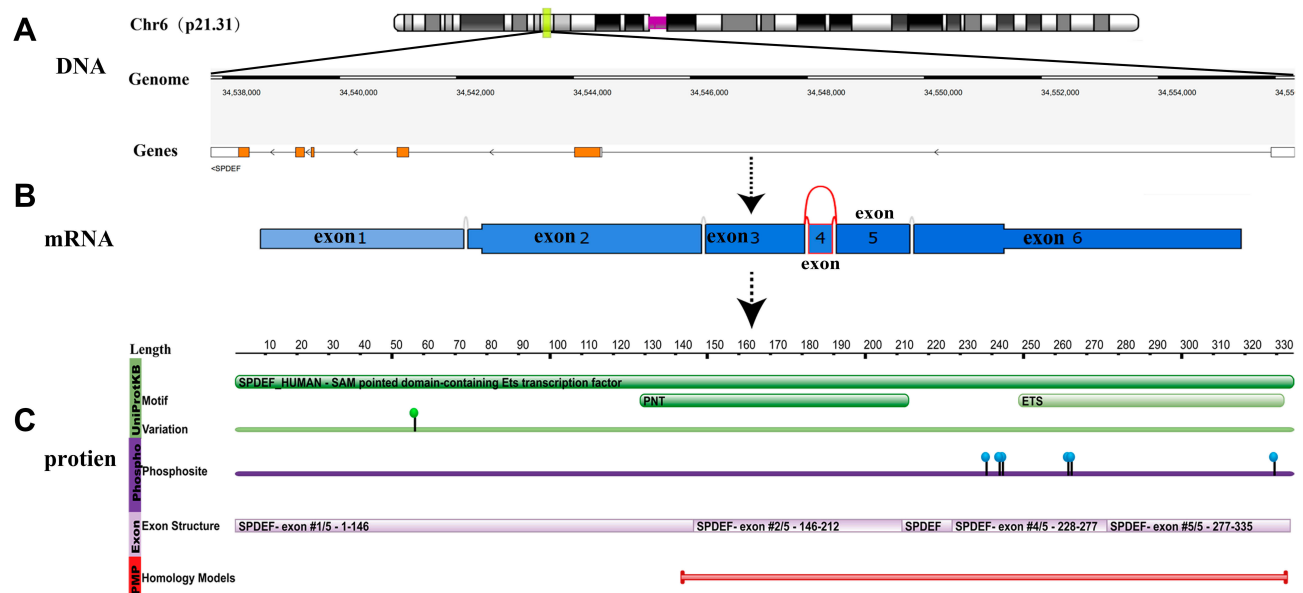


Figure 1 Schematic diagram of the *SPDEF* structure at the DNA, mRNA and protein level.

Notes: (A) The gene track represents the gene-structure on the genome: white boxes represent untranslated regions; orange: protein-coding regions; the black lines connecting boxes represent introns; (B) Exon 4 skip yield the major isoform of *SPDEF* at the mRNA level; (C) The green bar shows the motif of *SPDEF* mainly including EST and PNT. And the green point displays the variation data (sourced from UniProt) with non-genetic variation. Data in purple show phosphorylation sites; Data in lilac represent the genomic exon structure; Data in red indicate combined ranges of homology models. (A and C) are obtained from the RCSB PDB database, (B) is obtained from the TCGA SpliceSeq database.

colony-stimulating factor 1 receptor/colony-stimulating factor 1 (CSF-1R/CSF-1), which may regulate *SPDEF* phosphorylation, and thus promote MCF-10A movement and invasion.³⁷ The other is that the cell cycle kinase CDK11p5 directly interacts with and phosphorylates *SPDEF* on serine residues, leading to subsequent ubiquitination and degradation of *SPDEF* via the proteasome pathway. Then, the migration and invasion of prostate cancer cells increased due to the loss of *SPDEF* protein.³⁸ Future proofs will be needed to discover the full extent of *SPDEF* phosphorylation and the role phosphorylation plays in the regulation of *SPDEF* activity.

Expression Profiles of *SPDEF*

Unlike the majority of ETS family members, another feature of *SPDEF* is expressed exclusively in tissues with a high epithelial content such as prostate, breast, colon, and trachea.^{31,34,39,40} Such limited expression suggests that *SPDEF* plays a crucial role in the normal development and/or function of these tissues. Contrary to normal tissues where the limited tissue-specific *SPDEF* expression is generally accepted, the controversial expression of *SPDEF* in BC is not easy to understand. In general, analysis of the expression characteristics of *SPDEF* in tumors arising from basal versus luminal epithelial lineage showed widespread *SPDEF* expression in tumors arising

from the luminal epithelial lineage, and these included luminal, Her2⁺, and apocrine subtypes of luminal BC. In contrast, little *SPDEF* expression is observed in TNBC.³⁰ Table 1 summarizes the expression profiles of *SPDEF* in different BC cell lines and tissues reported over the years.

The following points are summed up based on Table 1: (i) *SPDEF* mRNA and protein are over-expressed in early low-grade malignant tumors and cell lines compared with normal tissues and cell lines;^{26,27,31,39-41} (ii) *SPDEF* mRNA and protein expression levels are too low or even undetectable in the highly malignant, advanced IBC and cell lines compared with early low-grade malignant tumors and cell lines;³² (iii) mRNA levels are not necessarily related to protein levels;²⁹ (iv) In IBC, a weak *SPDEF* staining is detected mainly in the cytosol.⁴²

Taken together, a large number of literatures have revealed that *SPDEF* expression alone is not enough to induce invasive activity in BC. However, an increased *SPDEF* expression levels may initiate transformation activity and/or sensitize these cells, resulting in other outcomes such as receptor tyrosine kinase (RTK) amplification or mutation activation to promote tumor progression.³⁷ *SPDEF* can promote progression in all aspects of the tumor when it works with oncogenes and/or carcinogenic-related genes, including increased cellular mobility, invasiveness, and non-anchored

Table I Differential Expression of *SPDEF* in Normal and Breast Tumor Specimens

Objects and Methods	Expression Profile of <i>SPDEF</i>	Ref.
cell lines		
RT-qPCR, NB, WB	a. Detectable mRNA and protein: MCF7, BT474, MDA-MB-361/468, T47D, HCC1428, BT-20, and Zr75.1; Detectable mRNA but limited or undetectable levels of protein: CAMA-1, HCC202, SK-BR-3, HCC-1428, and MDA-MB-415/453/175VII; Limited detectable or undetectable levels of mRNA or protein: HBL100, Hs578t, HCC1806, MDA-MB-231/157, BT549, MDA-MB-157/436/231, and MDA-MB-4355; b. The expression of <i>SPDEF</i> in ER ⁺ BC cell lines was higher than that in normal cell lines; c. The expression of <i>SPDEF</i> in ER ⁻ BC/BLBC cell lines was lower than that in ER ⁺ BC or non-BLBC cell lines; d. Protein expression is not necessarily detected following mRNA levels.	[28,29,32,40,41,49]
Tissues		
IHC	Protein expression reduced in all seven of examined IDCs compared with the CANT.	[32]
RT-qPCR	64 of 86 BC tissues expressed mRNA at a level at least equal to MCF7 cell line, and higher than the normal breast average expression level.	[40]
RT-qPCR, IHC	21 of 27 BC tissues expressed mRNA; The IBC showed reduced or negative protein expression compared with the CANT. The percentage of loss of protein expression appeared to be increased in the higher BRG tumors (3/6, 50%) as compared with the lower BRG tumors (0/6, 0%).	[28]
RT-qPCR	mRNA was strongly expressed in 4 of 5 PBCs, and the MBC was strongly positive; mRNA overexpression was varied in tumors over a wide range.	[39]
RT-qPCR	mRNA was overexpressed in majority of BC, and a 2- to 10-fold increasing in more than 75% of samples collected from ADH, DCIS, and IDC tumor samples.	[37]
IHC	A high nuclear <i>SPDEF</i> staining existed in most BC cases, while in IDC, a weak <i>SPDEF</i> staining was detected mainly in the cytosol.	[42]
IHC, RT-qPCR	Among the 246 ER ⁻ BC, 150 cases showed <i>SPDEF</i> ⁺ ; Among randomly selected 15 ER ⁻ BC, 14 cases showed that mRNA expression was higher than that in the CANT.	[74]
IHC, WB	Of the 100 ER ⁻ BC samples, 60% cases reported <i>SPDEF</i> ⁺ ; Randomly selected 8 cases showed mRNA expression was higher than that in the CANT.	[25]
IHC	In 90% (8 of 9) BC cases, the tumors reflected an increase in the number and/or the intensity of <i>SPDEF</i> staining as compared with the CANT.	[31]
IHC	In TMA, 18% of benign breast tissues scored <i>SPDEF</i> ⁺ ; The much higher percentages of <i>SPDEF</i> ⁺ such as 50%, 46%, and 51% has been revealed in DCIS, IDC, and ILC, respectively.	[31]
Data sets		
Bioinformatic methods	Compared with BLBC, mRNA expression was significantly overexpressed in luminal and Her2 ⁺ tumors.	[27,30]
DDD	<i>SPDEF</i> expression level was about 11-fold higher in the cDNA libraries from BC than that in the normal breast tissue.	[39]

Abbreviations: RT-qPCR, reverse transcription and quantitative real-time polymerase chain reaction; NB/WB, northern/Western blot; IHC, immunohistochemical; BLBC, basal-like breast cancer; IDC/ILC, invasive ductal/lobular carcinoma; ADH, atypical ductal hyperplasia; PBC/IBC/MBC, primary/invasive/metastatic BC; DCIS, ductal carcinoma in situ; CANT, corresponding adjacent normal tissue; ±, positive/negative; TMA, tissue microarrays; DDD, digital differential display.

growth of breast epithelial cells. It is revealed that *SPDEF* may involve in the occurrence or development of early BC⁴⁰ and may facilitate phenotypic effects in different stages of tumorigenesis.³⁷ Additionally, *SPDEF* mRNA expresses lower in advanced tumors than early tumors, which is usually consistent with the down-regulation of *SPDEF* and the detection of low protein in the highly malignant, advanced tumors, and also reconciles with a scheme of epithelium to mesenchymal transition to neoplasia.⁴⁰ In short, *SPDEF* expression

tends to be reduced or lost during tumor progression,^{32,43} suggesting that *SPDEF* may have diverse functions in different stages of BC development, which further supports the dual function of *SPDEF*. Moreover, mRNA levels are not always associated with protein levels. Test studies indicated that low *SPDEF* mRNA expression still translates into high protein nuclear detection in normal breasts.³² Instead, high *SPDEF* mRNA expression can be detected in some BC cell lines, with barely detectable protein, like HCC-1428.⁴⁰ It

may be because of a post-translation modification mechanism such as miRNAs regulation²⁹ or the rapid degradation of *SPDEF*.³⁴ It is worth noting that low *SPDEF* mRNA expression can be connected with sizeable protein expression such as MDA-MB-231.²⁹ Furthermore, a high nuclear *SPDEF* stain is usually reflected in most tumor tissues, but the cytoplasmic pool of *SPDEF* expression indicates that there may be non-functioning *SPDEF* protein or may represent the stored *SPDEF* for use in the nuclei when needed. However, since many members of the ETS family still express in the cytoplasm, non-specific reactions cannot always be excluded.

The Role of Double-Sided Gene *SPDEF* in the Progress of BC

Over the past few years, several studies have shown that the highly conservative ETS family of TFs regulate a variety of biological processes including cell proliferation, differentiation, apoptosis, transformation, migration, and invasion and are thought to play a momentous role in oncogenesis.^{44–47} *SPDEF*, as one of these ETS TFs, has been observed to be involved in these biological processes in BC in many studies. All of these studies concluded that *SPDEF* has dual functions. Researchers usually describe OCGs and TSGs with dominant mutation activation of proto-oncogenes and silent mutation inactivation of TSGs, respectively. However, *SPDEF* gene activation and inactivation mutations are rarely described in hundreds of BC that have been sequenced so far.⁴⁸ According to the studies about the effect of *SPDEF* on the biological behavior of BC cells, as well as the related underlying mechanisms of it, a summary of the dual function of *SPDEF* can be found in Figure 2.

SPDEF, an OCG: Promoting Growth and Proliferation

SPDEF appears to be a critical factor in regulating tumor growth and proliferation, and the molecular mechanism underlying the oncogenic effect of it on BC cells clarified below.

A research showed that *SPDEF* levels strongly correlate with ER⁺ luminal BC, and ER is recruited at the *SPDEF* gene locus in an estrogen-dependent manner. ER-cooperating factors, FOXA1 and GATA3 are also directly recruited at *SPDEF* gene locus. It is confirmed that *SPDEF* is a direct target of ER, FOXA1 and GATA3 in ER⁺BC cells. The researchers further reported that *SPDEF* is co-expressed with ER and regulated by GATA3 and FOXA1. GATA3

inhibits ER-mediated *SPDEF* expression and plays a negative role in the regulation of *SPDEF* expression. However, FOXA 1 promotes ER-mediated expression of *SPDEF* and further promotes cell growth.²⁷

Cao et al have proposed that androgen receptor (AR) can directly up-regulate the expression of *SPDEF* when AR and *SPDEF* are co-expressed, resulting in activation of *SPDEF* in ER[−]AR⁺BC cells. Activated *SPDEF* directly down-regulates the expression of MAD1, a transcriptional repressor of oncogene MYC, and promotes the degradation of MAD1 and the separation of MAD1 from MAX. In the absence of MAD1 competition, MAX and MYC form the largest heterodimer, which in turn induces MYC mediated gene transcription and promotes the proliferation, migration and invasion of ER[−]BC cells in vitro. Rescue experiment has also verified that the up-regulation of MAD1 expression significantly inhibits *SPDEF*-induced proliferation, migration and invasion of SKBR-3 cells. Moreover, pulmonary metastasis has been found in the mice inoculated with *SPDEF*-overexpressing cells instead of in the mice inoculated with MAD1-overexpressing cells.²⁵

SPDEF, an OCG: Promoting Migration, Invasion and Metastasis

The pro-cancer function of *SPDEF* can not only promote the survival of cancer cells but also accelerate the malignant transformation of cancer cells. Studies have demonstrated that the signal adaptor p62 is overexpression in metastatic BC. *SPDEF* upregulates p62 transcription by directly binding to p62 promoter at least two sites, so *SPDEF* may act as p62 co-activating factor and motivate the overexpression of p62 in BC.^{49,50} Although no studies have confirmed the effect of *SPDEF*-upregulated p62 on the biological behavior of BC cells so far, it has been proved that p62 promotes the invasive phenotypes of BC cells in vitro by interacting with intermediate filament vimentin. It also has been proved that p62 depletion inhibits BC metastasis and reduces the tumorigenicity in vivo.⁵⁰ In TNBC, patients with p62 overexpression have a higher risk of lymph node-positive and lymphoid metastasis.⁵¹

Gunawardane et al have illustrated that with the exception of MDA-MB-231 cells, *SPDEF* can induce migration in normal and cancer cell lines including non-metastatic BC cell lines and melanoma cell lines. Although *SPDEF* independent expression induced limited motility of MCF-10A cells, the co-expression of *SPDEF* with the RTK Her2 and CSF-1R/CSF-1 significantly enhanced MCF-10A motility and

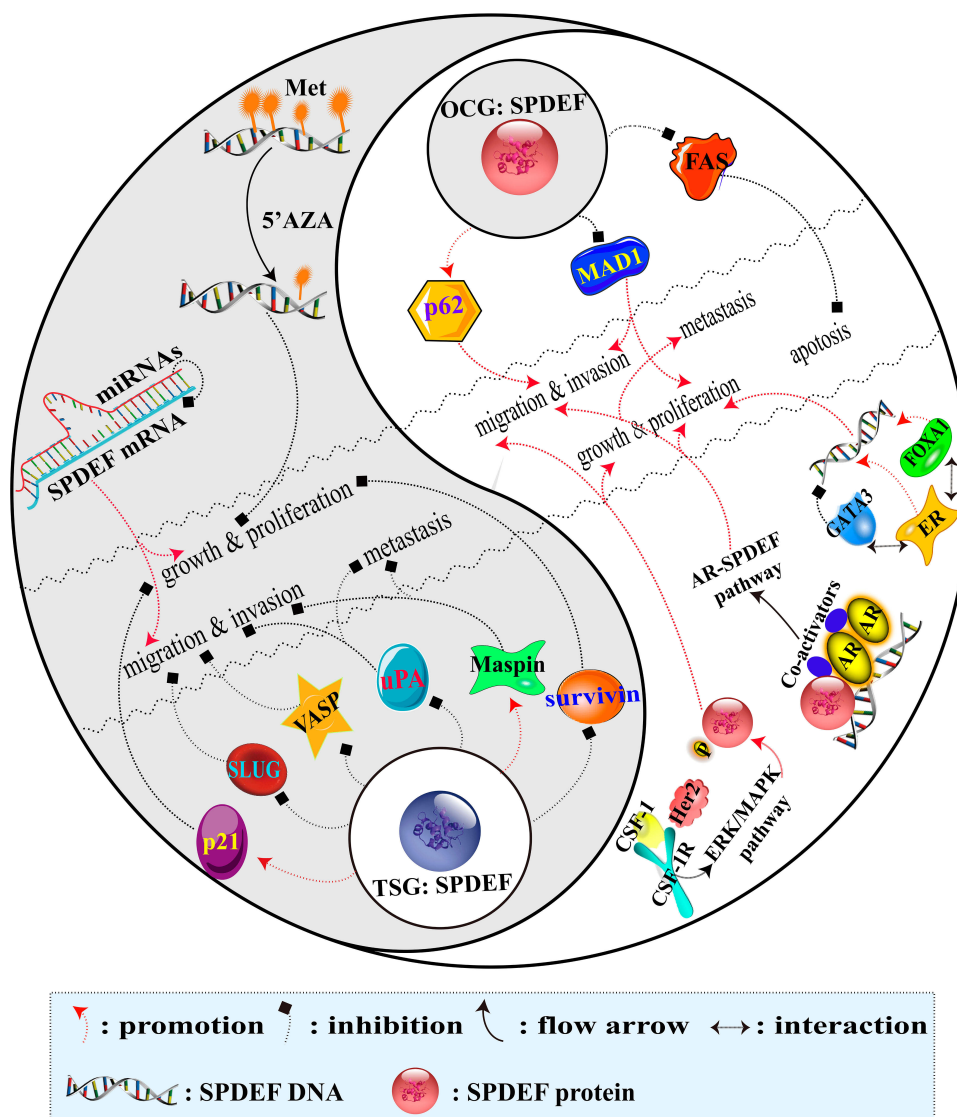


Figure 2 Regulatory mechanism diagram of double-agent *SPDEF* in BC.

Notes: The darker part of the Tai Chi diagram illustrates the network of *SPDEF* involved in tumor-inhibiting effect as a TSG. Conversely, the lighter part of the Tai Chi diagram shows the network of *SPDEF* involved in tumor-promoting effect as an OCG.

anchorage-independent growth and induced a dramatic invasive phenotype in three-dimensional cultures. Then, Her2 and CSF-1R/CSF-1 may activate ERK/MAPK, which can cooperate with *SPDEF* to promote motility and invasion.³⁷ However, the specific molecular mechanism remains largely undiscovered.

SPDEF, an OCG: Inhibition of Apoptosis

To date, many researchers have revealed that the proapoptotic genes including *FAS* directly are regulated by *SPDEF* in BC. However, the mechanism by which *SPDEF* inhibits the apoptosis pathway to stimulate the progression of BC remains unclear. *FAS* receptor, a member of the

tumor necrosis family involved in the extrinsic apoptosis pathway,⁵² is a direct *SPDEF* transcriptional target. High-level expression of *FAS* is detected in non-transformed mammary epithelial cell lines, but the expression of it is reduced in several BC cell lines.⁵³ *SPDEF* suppresses the apoptosis of MCF-7 cells by down-regulating the expression of the *FAS* gene, especially under stress conditions such as hormone depletion.²⁷

SPDEF, a TSG: Inhibition of Growth and Proliferation

As it is mentioned above, many researchers have confirmed the role of *SPDEF* in promoting cancer in BC,

but some studies have shown that *SPDEF* exhibits a clear tumor-suppressor function. The effects of *SPDEF* on the growth and proliferation of BC cells through the regulation of target genes and methylated modification are elucidated as follows.

A study has indicated that *SPDEF* directly binds to cyclin-dependent kinase (CDK) inhibitor p21 promoter and then up-regulates the expression levels of p21 in PyV-mT (polyoma virus middle-T) mouse mammary tumor cell line. Elevated p21 leads to the reduction of CDK2 activity, which ultimately results in decreased PyV-mT cells growth and proliferation under non-stress/damaged conditions both in vitro and in vivo. *SPDEF* expression partially blocks the cell cycle progression in G1/S phase without an effect on apoptosis has been reflected using cell cycle analysis. Nevertheless, p21 silencing eliminates the *SPDEF* growth inhibition in vitro and in vivo.²⁴

Another study has also proved that the inhibitor of apoptosis survivin is the direct transcriptional target of *SPDEF* in prostate cancer,⁵⁴ and ectopic *SPDEF* expression can down-regulate survivin promoter activity and endogenous survivin expression in BC. The down-regulated survivin can inhibit the growth of MCF 7 BC cells in vitro and xenograft tumor formation in vivo. Conversely, *SPDEF* silencing can up-regulate survivin expression, which triggers the growth of MCF 7 BC cells in vitro and xenograft tumor formation in vivo.²⁸

DNA methylation is a specific change in tumorigenesis. *SPDEF* with DNA methylation as an epigenetic alteration in MDA-MB-468 cells, indicating that the functions of *SPDEF* are selective in BC cells. Increased *SPDEF* expression shows a dose-dependent manner following DNA methylation inhibitor 5' azacytidine (5'AZA) treatment decreased or the growth or proliferation rate of BC cells slowed. Meanwhile, an enhanced p21 expression is found following the same expression pattern as *SPDEF*.⁵⁵

SPDEF, a TSG: Inhibition of Migration, Invasion and Metastasis

Many direct downstream target genes of *SPDEF* have been found during these years, which are participated in the negative regulation of migration, invasion and metastasis of BC including but not limited to uPA, Maspin, VASP and SLUG.

uPA ligand and its membrane-bound receptor uPAR are responsible for tumor development such as cell

proliferation, migration and adhesion.^{56,57} Plenty of related researches have shown that uPA is a direct transcriptional target of *SPDEF*-negative regulation.^{22,54,58} The negative regulation of uPA by *SPDEF* can change the migration ability of the cancer cells through a variety of possible mechanisms.²² First of all, uPA is activated on the surface of MDA-MB-231 cells⁵⁹ and can convert surface plasminogen into plasmin. The plasmin can not only directly degrade various extracellular matrix (ECM) components,⁶⁰ but also directly or indirectly activate matrix metalloprotease (MMP) that can further enhance the degradation of ECM.⁶¹ The process of degradation of the basement membrane might be inhibited by *SPDEF*-downregulated uPA. Second, in IBC cells, the *SPDEF*-downregulated uPA leads to compensatory increased expression of uPAR mRNA. Intriguingly, increased soluble uPAR may damage many functions of urokinase system such as proteolysis and tumor growth to limit the potential of metastasis in BC cells.⁵⁸ Third, the uPA/uPAR system is also very important in intracellular signal transduction, including interaction with tyrosine kinase,⁶² EGFR signaling pathway⁶³ and signal-related integration family members.⁶⁴ *SPDEF*-downregulated uPA may reduce the binding of uPAR and change the intracellular signal pattern and then inhibit cell migration.

Maspin is often down-regulated in the development of BC.^{32,65} It has been revealed that *SPDEF* can regulate Maspin promoter^{28,32} and positively regulate the expression of Maspin.³² In IBC, the decrease of Maspin expression is largely due to the deletion of *SPDEF*, which may contribute to the invasion and metastasis of tumor cells. Moreover, several studies have mentioned that Maspin is a type II TSG, which can inhibit tumor growth, motility, invasion and metastasis when expressed in some cancers, including BC.^{66–68}

VASP, an action-binding protein, participates in linking signaling pathways to the remodel actin cytoskeleton.⁶⁹ Bioinformatics initially analysis shows that VASP is a presumptive target gene for *SPDEF*. Turner et al subsequently have verified that VASP is up-regulated directly by *SPDEF* in vitro.²² Multiple lamellipodia can be produced when the up-regulated VASP is located on the cell membrane, which can retard cell migration and its phenotype is similar to the re-expression of *SPDEF*.⁷⁰

SLUG is a member of the SNAIL superfamily, and its high expression associates with the aggressive basal phenotype in breast tumors.⁷¹ Besides, SLUG is also inversely correlated with E-cadherin expression and is a critical

event-promoting EMT in many tumor types.⁷² As the study identified that *SPDEF* is able to regulate downstream targets of SLUG in both SLUG-dependent and -independent manners, suggesting a critical role for regulating EMT.²³ E-cadherin is also known to be a transcriptional target of SLUG during carcinogenesis. Low expressed *SPDEF* is able to relieve the repression of E-cadherin through direct inhibition of SLUG, which is a critical interaction in inhibiting the migratory phenotype.²³

miRNAs are endogenous 19–25 nucleotide noncoding RNAs that also have dual functions in tumor progression.⁷³ To date, although there have been many studies on miRNAs in BC, only few studies have focused on the miRNAs that interact with *SPDEF* mRNA. Fortunately, Findlay et al have discovered that *SPDEF* is directly regulated by two kinds of miRNAs (miRNA-204 and miRNA-510), which can prevent the translation of *SPDEF* mRNA, resulting in the loss of *SPDEF* protein expression and promoting tumors to gain a more aggressive phenotype. In addition, extrinsic *SPDEF* expression can inhibit the overexpression phenotype of miRNAs.²⁹

The Role of Dual-Functional *SPDEF* in Diagnosis and Prognosis of BC

Oncologists have looking for new BC related molecules as the markers for early diagnosis or prognosis. Dual-functional *SPDEF* is one of the most potential candidate markers at present. As described above, *SPDEF* is necessary for ER⁺BC survival, and there is a strong positive correlation between the expression of *SPDEF* and ER. In order to evaluate the sensitivity and specificity at which *SPDEF* expression could predict an association of the ER positivity, the expression of *SPDEF* has been detected in 86 clinical specimens and then analyzed by receiver-operator curves (ROCs). The result showed that ER positivity with 98.3% sensitivity (58/59) and 76.9% specificity (20/26) when *SPDEF* expression is at or above the MCF7 level, with the area under the curve (AUC) 0.902.⁴⁰ In another word, predicting ER⁺BC by *SPDEF* markers seems to be reliable. Moreover, numerous studies have demonstrated that the loss of *SPDEF* protein expression and high expression of *SPDEF* mRNA may be a powerful indicator to estimate the migration promoting characteristics of advanced BC.

Prognosis of BC is also influenced by *SPDEF*. There is a correlation between higher *SPDEF* expression and shorter overall survival (OS) in 246 patients with

ER⁺BC, but *SPDEF* expression is not associated with disease-free survival (DFS). Meanwhile, the multivariate analysis confirmed that *SPDEF* expression is a significant independent prognostic variable affecting OS.^{25,74} Moreover, three independent data sets were downloaded from GEO and ArrayExpress databases, and analyzed by Kaplan-Meier. The results showed that the high *SPDEF* expression is related to the poor OS in ER⁺BC patients. In the Cox regression model, *SPDEF* is an important predictor of survival when *SPDEF* is a continuous variable in ER⁺BC, which is consistent with the previous reports.^{27,30} In summary, *SPDEF* can be used as a valuable indicator to evaluate the prognosis of BC.

The Functional Duality of *SPDEF* Arouses New Considerations on BC Therapies

The targeted therapy model has opened up a new field of tumor chemotherapy, which has been widely used in the treatment of various molecular subtypes of BC. In Her2⁺ BC population, Her2-blocking therapies and the achievement of trastuzumab have considerably modified the prognosis of these patients.⁷⁵ Further, the addition of pertuzumab to trastuzumab and docetaxel demonstrated an improved progression-free survival (PFS) and OS, as well as validating the dual Her2 blockade concept by CLEOPATRA trial.^{76,77} Endocrine therapy is still the main treatment for luminal BC and selective adjuvant chemotherapy. More recently, the addition of a targeted therapy to endocrine treatment has offered new therapeutic options for luminal BC patients. Indeed, the addition of everolimus to exemestane has improved DFS in this population.⁷⁸ For TNBC, there is no target to easily block yet, and the anthracycline and paclitaxel schemes remain mainstream. Specifically, in the Phase 3 trial of IMPASSION-130, atezolizumab combined with nab-paclitaxel significantly improved PFS in patients with metastatic TNBC and is more pronounced in PDL1-positive tumors.⁷⁹ Since tumor cells are interindividual heterogeneity and increase with the treatment, these lead to poor therapeutic effect or serious toxicity. And the dual-functional characteristics of genes are related to tumor heterogeneity. Hence, finding the most suitable tumor candidate antigen based on this dual nature will benefit the development of tumor-targeted immunotherapy.

At present, Her2 is the only antibody-mediated immunotherapy target for BC,⁸⁰ which is critical for BC

treatment. Current therapeutic applications have shown that targeting OCG and its related pathways are expected to develop new drugs, including antibodies and small synthetic molecules.⁸¹ Moreover, the overall limited expression of *SPDEF* in normal human tissues suggests that *SPDEF*-based anti-tumor therapies will have minimal toxicity against vital normal tissues.⁸² These observations support *SPDEF* as a highly desirable novel candidate antigen against luminal BC. Meanwhile, Sood found that *SPDEF* might be immunogenic and intolerant in female BC patients, and the *SPDEF* sequence appears to contain HLA-A2-binding peptides that are potentially capable of eliciting HLA-A2-restricted T cell responses.⁸³ Additionally, *SPDEF* as TF can significantly affect the biological characteristics of tumors by inducing large-scale changes in gene expression. And these alter genes may encode the cell surface and/or secreted molecules capable of influencing the behavior of the neighboring tumor and/or stromal cells.³¹ This concept is supported by the characteristics of ER expression in BC and its implication for endocrine therapy. Similarly, the eliminating *SPDEF* expressed cells from BC by *SPDEF*-targeted vaccines/immunotherapy should not only elicit the killing of *SPDEF* expressing tumor cells but also modulate the tumor microenvironment and inhibit tumor progression.⁸³ Furthermore, *SPDEF* is essential for luminal BC cell survival and models of endocrine resistance, which may have therapeutic value for BC patients treated with endocrine therapy.²⁷ Therefore, *SPDEF* should be a useful novel target for co-targeting with endocrine therapy to minimize endocrine resistance in BC.⁸² Although there are no reports of clinical trials on *SPDEF* targeted therapy, these considerations provide a compelling rationale to evaluate the potential of *SPDEF* as a novel luminal breast tumor antigen. In future work, to redefine “*SPDEF* gene” by considering each mRNAs, regulatory RNA, protein isoform, and posttranslational modification from the same genomic locus⁶ instead one-sided thinking that *SPDEF* is an OCG or a TSG in BC, may be conducive to better understand tumor biology and to select targets for different cancer subtypes for individualized treatment.

Conclusions

SPDEF discussed in this review has attracted much attention because of its role in oncogenesis and progression. The definition of *SPDEF* in the dichotomy of cancer-regulatory genes has been controversial. It is more reasonable to consider *SPDEF* is a dual-functional gene by

thorough summarizing the current knowledge, which will contribute to comprehend the heterogeneity of tumors and facilitate future research. The mechanism underlying the regulation of *SPDEF* must be investigated due to a better understanding of the dual-functional nature of *SPDEF* and is of great significance to the development of targeted therapy for each BC subgroup. Therefore, *SPDEF* has the potential of being a new diagnostic and therapeutic target in tumor biology in the near future.

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Disclosure

The authors report no conflicts of interest in this work.

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