Aberrant expression of ETS1 and ETS2 proteins in cancer

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Abstract

The ETS transcription factors regulate expression of genes involved in normal cell development, proliferation, differentiation, angiogenesis, and apoptosis, consisting of 28 family members in humans. Dysregulation of these transcription factors facilitates cell proliferation in cancers, and several members participate in invasion and metastasis by activating gene transcription. ETS1 and ETS2 are the founding members of the ETS family and regulate transcription by binding to ETS sequences. They are both involved in oncogenesis and tumor suppression depending on the biological situations used. The essential roles of ETS proteins in human telomere maintenance have been suggested, which has been linked to creation of new Ets binding sites. Recently, preferential binding of ETS2 to gain-of-function mutant p53 and ETS1 to wild type p53 (WTp53) have been suggested, raising the tumor promoting role for the former and tumor suppressive role for the latter. The oncogenic and tumor suppressive functions of ETS1 and 2 proteins have been discussed.

Introduction

The oncogene *v-ets* was originally discovered as a component of a chimeric gene along with a truncated *v-myb* gene, present in the genome of E26, an avian leukosis virus (Figure 1A) [1-3]. Members of the *ETS* gene family have been cloned and sequenced from a variety of species ranging from human to Drosophila. Ets proteins are highly conserved through different species [4].

The ETS family of transcription factors have the conserved primary sequence of their DNA-binding domains (Figure 1; ETS1/2, ERG, and FLI1 are shown). The human ETS factors are classified into 11 subgroups based upon ETS domain sequence homology: ETS1/2, ERG, PEA3 (ETV1/4/5), TCF (ELK1/3/4), GABP (GABPA: GA [purine] -binding protein alpha chain), ELF1/2/4, SPI1 (SPI1/B/C), TEL (ETV6/7), ERF (ERF, ETV3, ETV3L), FLI1 and FEV [4]. In addition, a subset of four ETS family genes (ELF3, ELF5, EHF, SPDEF) has been characterized as placenta-specific subgroup based upon their restricted expression to tissues with high epithelial cell content [4-6], totally 28 ETS proteins in humans.

The ETS transcriptions factors regulate numerous genes by binding to winged helix-turn-helix GGAA/T core of DNA-binding, and are involved in cell proliferation, differentiation, development, stem cell development, transformation, angiogenesis, and apoptosis [7-13]. ETS1 and ETS2 are representative members of the ETS family of transcription factors and are downstream effectors of the RAS/RAF/ERK pathway [14-19] (Figure 2). The pointed domain is at the N-terminal end (Figure 1, Pnt-P2 and Yan domain [PNT] in [20]). Overexpression of dominant-negative forms of several ETS factors, including ETS1 or ETS2, block *Ras* transformation [16,21,22] suggesting that ETS family members play a crucial role in this process. However, specific deletions of ETS family members are more accurate approaches for the understanding of functions of ETS members in *Ras* transformation [23]. It has been reported that aberrant activation of the ETS family transcription factors play roles throughout all stages of tumorigenesis [24]. Specifically in solid tumors, gene rearrangement/amplification, feed-forward growth factor signaling loop, formation of gain-of-function co-regulatory complexes, and novel cis-acting

mutations in ETS target gene promoters can result in increased ETS activity [24]. The ETS signaling enhances tumorigenesis through different mechanisms such as lineage specification, increased self-renewal, and genomic instability confirming the proto-oncogenic roles of ETS proteins in cancer.

It was reported that that the *ETS2* gene was located about 17 cM from the breakpoint of common t(8;21) translocation found in acute myelogenous leukemia (AML) [25,26], esp. M2 subclass. Then the chimeric gene at t(8;21) translocation was *AML1-ETO* was cloned and characterized [27,28]. Although the chimeric gene responsible for acute myeloid leukemia was did not contain *ETS*, the ETS family *ERG1* was situated just proximal to *ETS2* at 21q22.3 [29,30] (Figure 1B). They found that the *ERG1* gene was translocated from chromosome 21 to chromosome 8 in the t(8;21)(q22;q22) [30]. The ERG protein was considerably more stable than the short-lived ETS1 and ETS2 proteins with a half-life of 21 hours. It was then reported that *ERG2* is a nuclear phosphoprotein bound to purine-rich sequences [31]. The ERG2 protein was a sequence-specific, DNA-binding protein and is expressed at higher levels in early myeloid cells than in mature lymphoid cells, acting as a regulator of genes required for maintenance and differentiation of early hematopoietic cells [31].

Human telomerase reverse transcriptase gene (*TERT*) encodes a rate-limiting catalytic subunit of telomerase essential for genomic integrity [11,32-34]. TERT expression is repressed in somatic cells except for self-renewing proliferative cells and tumor cells [32]. Thus, immortality associated with cancer cells has been thought to be caused by telomerase overexpression. Importantly, the newly described germline and recurrent somatic mutations in melanoma [35] and other cancers [36] in the *TERT* promoter that create *de novo* ETS/
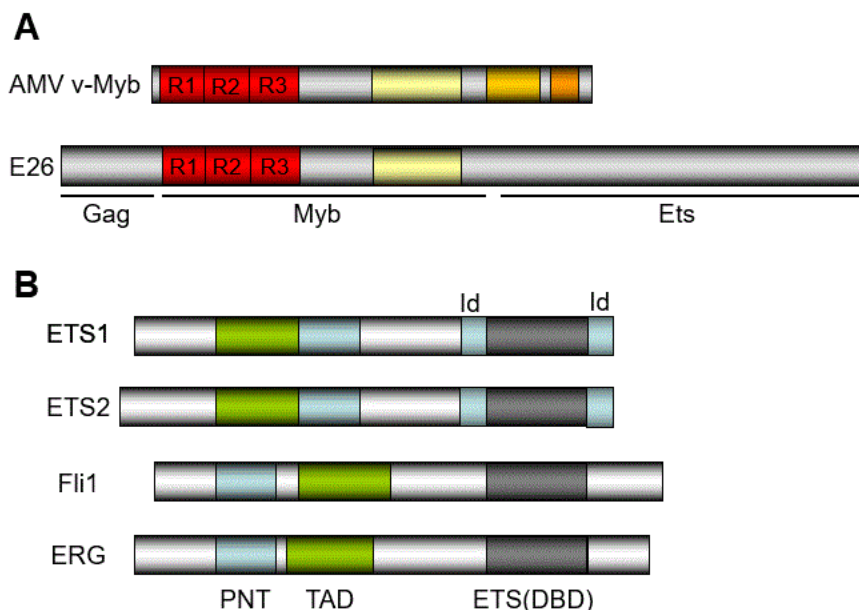
Figure 1. The structure of ETS proteins. **A)** The structure of AMV v-Myb and E26 retrovirus. R1, R2, and R3 are Myb-like repeats. The E26 virus consists with Gag, Myb, and Ets-like regions (1-3, 52-54). **B)** The structure of ETS proteins reviewed in this article. The domain structures for ETS1, ETS2, Fli1, and ERG are shown. The ETS domain that is essential for DNA-binding is shown in dark box. The DNA-binding by the ternary complex factor (TCF) subfamily of ETS-domain transcription factors (4) is tightly regulated by intramolecular and intermolecular interactions. The helix-loop-helix (HLH) -containing Id proteins (138) are *trans*-acting negative regulators of DNA binding by the TCFs. Inhibition is mediated by direct interactions of the Ids with the ETS DNA-binding domain of the TCFs. PNT: pointed domain, TAD: transactivation domain, ID: Id-interaction domain.
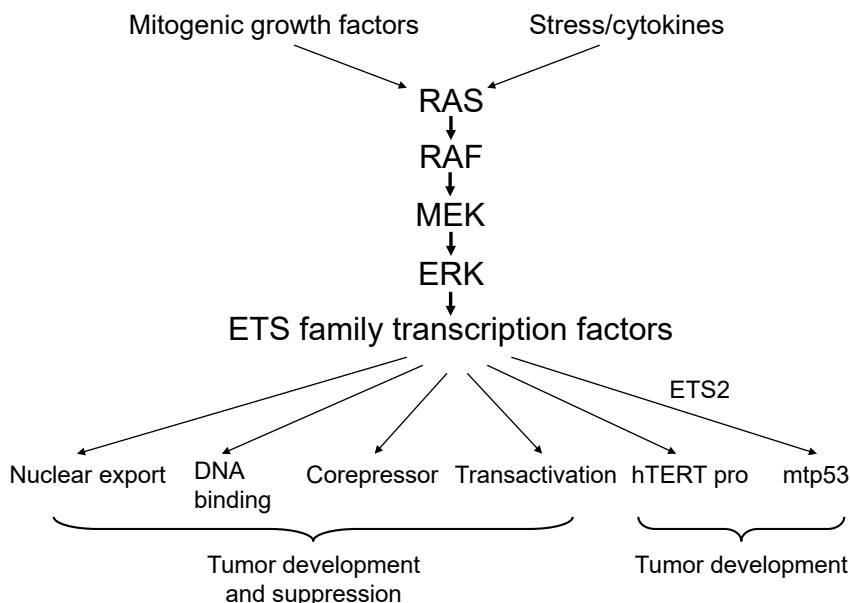
Figure 2. Oncogenic and tumor suppressive activities of ETS proteins. Both mitogenic signals stemming from growth factors and stress signals stimulate the RAS-RAF-MEK-ERK pathway to activate the ETS transcription factors. ETS proteins show oncogenic or tumor suppressive activities by increased nuclear transport [178,179], enhanced DNA binding of nuclear proteins [180,181], recruitment of co-repressors [182,183], and gene transactivation [184,185] involved in cell proliferation, apoptosis, angiogenesis. Mutation of *hTERT* promoter created novel ETS binding sites, which contributes increased telomerase activity in cancer cells. Mutant p53 which is overexpressed in half of human cancer cells, bind to ETS2 to stabilize the protein and reveal its oncogenic activity.

TCF binding sites, provide an insight into the possible cause of tumor-specific increased TERT expression [32,33,37].

Most mutant p53 (mtp53) expressed in cancer cells has gain-of-function (GOF) associated with genomic instability, increased tumorigenicity and stem cell-ness, invasiveness/metastasis, and resistance to chemo/radiation therapy [38]. In cancer cells expressing GOF mutant p53, groups of genes that belong to the CREB1 [39], NRF2 [40], c-Myc [41,42], Ets1/2 [43,44], networks as well as those that are involved in chromatin methylation/acetylation (MLL1/2, MOZ; 45) are transactivated (mtp53 reviewed in [38, 44, 46]. Preferential binding of ETS1 to WTp53 and ETS2 to mtp53 have been reported, suggesting tumor-suppressive function for the former and oncogenic function for the latter, which is discussed in this review.

The *ETS* gene often fuses with others as a result of translocation. For instance, *ETS1* is found in breakpoints such as t(9;11)(p22;q23) and t(4;11)(q21;q23) that are often found in human leukemias [47,48]. It is often overexpressed in human cancer with Cyclin D1 [49], p16[INK4a] [50] and telomerase [51] overexpression, and thus is heavily involved in human carcinogenesis). Human chromosomal translocations involving *ETS* family genes have been amply reported on *ERG:FUS* (acute leukemia), *EWS:FLI1* (Ewing sarcoma), *ERG:TMPRSS2* (prostate cancer), which will be reviewed in a different paper since Ets is such a big family. Here we will focus on Ets1 and Ets2 in carcinogenesis.

Ets1 and Ets2

Gene cloning and the protein structure

Watson et al. cloned and sequenced *ETS1* and *ETS2* cDNA clones obtained from human and mouse [52] (Figure 1B). In mammals, the Ets sequences are located on two separate chromosomal loci, called *Ets1* and *Ets2*. To determine the structure and open reading frames of these two genes, they sequenced human *Ets1* and *Ets2* cDNA clones obtained from both human and mouse. The human *ETS1* gene encodes a protein of 441 amino acids. This protein is greater than 95% identical to the chicken *c-Ets1* gene product. Thus, the human *ETS1* gene is homologous to the chicken *c-Ets1* gene, the proto-oncogene that the E26 virus. Human and mouse *Ets2* cDNA clones are closely related to *Ets1* having open reading frames for proteins of 469 and 468 residues, respectively. By *in situ* hybridization, de Taisne, *et al.* [53] mapped human oncogene *ETS1* to chromosome 11q23-q24. Next year, Watson et al. identified 2 distinct genomic DNAs homologous to the *Ets* region of the transforming gene of avian erythroblastosis virus, E26 [54] (Figure 1A).

The *ETS1* and *ETS2* genes are located on the same chromosome and are coordinately transcribed in avians [54]. Whereas the chicken Ets protein distributes equally between the cytoplasm and nucleus, the human ETS1 protein is cytoplasmic while the ETS2 protein is nuclear suggesting that they have different biologic functions [55]. ETS1 is a 441-amino acid protein that contains an N-terminal domain with PNT implicated in the self-association of oncoproteins and a C-terminal DNA-binding domain (Figure 1B). Of note, it has a MAPK phosphorylation site at Thr38 that mediates transcriptional regulation [56]. Splice variants of *ETS1* has been characterized: the full-length *ETS1* encodes a 51-kD protein (ETS1-p51) while ETS1ΔVII lacks exon 7 and encodes ETS1-p42 [57]. The latter lacks the N-terminal inhibitory domain found in ETS1-p51, but has unique DNA-binding and transcriptional properties and regulates different target genes [57]. Laitem, *et al.* identified a novel variant of Ets1Δ(III–VI), resulting from the alternative splicing of exons III to VI encoding a 27 kDa isoform [57]. Ets1-p27 lacks the Thr38 residue, PNT, and the transactivation domain, all of which are required for the transactivation by Ets1. They also showed that Ets1-p27 binds DNA in the same way as Ets1-p51 does and induces the translocation of Ets1-p51 from the nucleus to the cytoplasm thereby constituting a dominant-negative of Ets1-p51 [57].

Watson, *et al.* [54] mapped the *ETS2* gene to chromosome 21q22.1-q22.3 by *in situ* hybridization. Mavrothalassitis [58] reported that the *ETS2* gene has no TATA box or CAAT box in its promoter. Then Owczarek et al. determined that the *ETS2* gene spans 17.6 kb and has a major CpG island at its 5' untranslated region [59]. The product, ETS2 protein consists of 469 amino acids with an N-terminal pointed domain and a C-terminal DNA-binding domain [60] (Figure 1B). It also has a MAPK phosphorylation site at Thr72 that likely mediates transcriptional regulation. In short, the structure for ETS1 and ETS2 is very similar (Figure 1B).

Mice model for *Ets1*-deficiency

T cells go through a number of stages before final differentiation into single-positive CD4 (+) or CD8 (+) α (TCRα) T lymphocytes. The pre-T-cell receptor (TCR) stages include four CD4(-)/CD8(-): double-negative (DN) stages. Namely, DN1 (CD44(+)/CD25(-)), DN2 (CD44(+)/CD25(+)), DN3 (CD44(-)/CD25(+)), and DN4 (CD44(-)/CD25(-)) before differentiation to the double-positive (DP) (CD4(+)/CD8(+)) stage. Eyquem, *et al.* [61] created mice deficient for the *Ets1* transcription factor to determine its role in transition from DN3 to DN4, inhibition of DN cell apoptosis, cellular expansion, and allelic exclusion at the *TCRβ* locus. Although *Ets1*^{-/-} embryos were present up to day 18.5 postcoitus in a Mendelian ratio, only 2% of mice were *Ets1*^{-/-} by 3 weeks of age suggesting most of *Ets1*-deficiency was embryonic lethal [61]. They found that inactivation of *Ets1* impaired the development of DN3 into mature thymocytes and induced an elevated rate of cell death in the DN4 subset [61]. This defect was specific to the α/β lineage because γ/δ T cells matured efficiently. The percentage of thymocytes co-expressing two different TCRβ chains was increased in the *Ets1*-deficient background. These data identified Ets1 as a critical transcription factor for pre-TCR functioning and for allelic exclusion at the *TCRβ* locus [61].

In 2005, Zhan, *et al.* reported significantly reduced arterial wall thickening, perivascular fibrosis, and cardiac hypertrophy *Ets1*-deficient mice in response to angiotensin II [62]. The induction of two known targets of ETS1, *CDKN1a (p21[CIP1])* and *Plasminogen Activator 1*, by angiotensin II was markedly decreased in the aorta of *Ets1*-null mice compared with wild types [62]. The expression of Monocyte Chemotactic Protein 1 was also reduced, resulting in significantly diminished recruitment of T cells and macrophages to vessel walls. They concluded that ETS1 has an essential role as a transcriptional mediator of vascular inflammation and remodeling in response to angiotensin II [62].

Recent researches have demonstrated positive roles of aberrant splicing in carcinogenesis [63-71]. The full-length *Ets1* (p51-Ets1) and *Ets1*[ΔVII] (p42-Ets1) isoforms [57] have key distinctions regarding protein-protein interactions, DNA-binding kinetics, and transcriptional target specificity [72]. Splice variant-specific gene targeting studies showed that the *Ets1*[ΔVII] mutants demonstrate lymphocyte maturation defects associated with aberrant regulation of p16[Ink4a], p27[Kip1], and CD44. Thus, a balance in the differential regulation of Ets1 isoforms represents a potential mechanism in the control of lymphoid maturation and homeostasis [72,73].

Mice model for *Ets2* overexpression and deficiency

Expression of ETS2 is observed in a variety of cell types. During murine development, it is highly expressed in newly forming cartilage including skull precursor cells, and vertebral primordia [74]. Sumarsono, *et al.* created transgenic mice to investigate the consequences of overexpression of *Ets2* [74]. The mice with less than 2-fold Ets2 overexpression in particular organs developed neurocranial, visceral cranial, and cervical skeletal abnormalities [74]. Of note, these abnormalities had similarities with those found in trisomy 16 humans with Down's syndrome, in which the gene dosage of *ETS2* was increased. Thus, ETS2 has a role in skeletal development and that overexpression is contributed skeletal abnormalities found in Down's syndrome [75]. This is a nice study that link ETS2 with human disease.

They also reported that overexpression of *ETS2* resulted in apoptosis [75]. Transgenic mice overexpressing *ETS2* developed a smaller thymus and lymphocyte abnormalities, similar to features observed

in Down's syndrome. Importantly, increased apoptosis correlated with increased expression of p53, and alterations in downstream targets in the p53 pathway [75] suggesting that ETS2 stimulates p53. In HeLa cells, transfection with wild type (WT) p53 enabled ETS2 overexpression to induce apoptosis suggesting crosstalks between ETS2 and p53. Furthermore, crossing the *ETS2*-transgenic mice with *p53-/-* mice rescued the thymic apoptosis phenotype [75]. These are extremely important findings *in vivo* since Ets2 binds to mtp53 and is an essential transcription factor for mtp53 to revel its oncogenic activity (explained later in detail). In conclusion, overexpression of ETS2 induces apoptosis dependent on p53.

Inactivation of *Ets2* in mice by deletion of the Ets domain (*Ets2db1*) caused embryonic lethality before E8.5 [76]. Analysis of the mutant *Ets2db1/db1* conceptuses (products of conception) revealed deficiencies in trophoblastic tissues, including a smaller ectoplacental cone and absence of chorion formation. Further analysis has revealed that many *Ets2db1/db1* conceptuses fail to form extraembryonic ectoderm [77]. Then Wen, et al. [78] created an *Ets2* conditional knockout allele (*Ets2flox*) in mice. An epiblast-specific Cre line, Mox2-Cre, was crossed with *Ets2flox/wt* mice to delete *Ets2* in germ and embryonic cells [79]. However, the resulting recombined allele designated *Ets2db2*, caused recessive embryonic lethality around E8 when bred to homozygosity. *Ets2db2/db2* embryos at E7.5 were very similar to the *Ets2db1/db1* mutants [76] with much smaller conceptuses and unusual cone-shaped yolk sacs. They showed that inactivation of *Ets2* resulted in trophoblast stem (TS) cell slower growth, increased expression of differentiation-associated genes, and decreased expression of genes implicated in TS self-renewal [77]. In conclusion, Ets2 contributes to the regulation of genes important for maintaining the undifferentiated state of TS cells and is essential for embryonic development [78,79].

The *ETS1* gene function in tumorigenesis

Using two different highly tumorigenic human colon cancer cell lines DLD-1 and HCT116 that do not express endogenous ETS1 protein, Suzuki, *et al.* [80] found that ETS1 reversed the transformed phenotype in a dose-dependent fashion, indicating its tumor-suppressive potential [80]. The result is very important since Ets1 bind to WT p53 for tumor suppression (mentioned later in this review). It is a good model for studying suppression of tumorigenicity by ETS1 at transcriptional level, leading to the design and development of novel drugs for cancer therapy [80].

Hashiya et al. expressed human *ETS1* into rat hindlimb and found that it stimulated angiogenesis, as measured by increased capillary density and blood flow [81]. Overexpression of ETS1 upregulated the concentrations of hepatocyte growth factor and vascular endothelial growth factor in rat hindlimb. Their study demonstrated that ETS1 regulated angiogenesis through the induction of angiogenic growth factors. Blocking ETS1 expression may thus provide a new therapeutic strategy to treat peripheral arterial disease [81,82].

Alteration of *ETS1* in human cancer

The ETS family of transcription factors regulates the expression of genes involved in normal cell development, proliferation, and differentiation [4-6,83-85]. Therefore, the dysregulation of these transcription factors facilitates cell proliferation in many cancers, and several ETS members have been shown to participate in invasion and metastasis by activating gene transcription [83-85]. In three patients with acute monocytic leukemia (AMoL) and t(9;11)(p22;q23) showed that the breakpoint on 9p split the *interferon* genes and that

the *Interferon β1* gene was translocated to chromosome 11 [86]. As a consequence, the *ETS1* gene was translocated from chromosome 11 to 9p adjacent to interferon genes. They suggested that juxtaposition of interferon and *ETS1* genes might be involved in the pathogenesis of AMoL [86].

ETS1 overexpression is also found in human breast cancer (BC) associated with invasiveness and poor prognosis [87,88]. By overexpressing Ets1 or a dominant-negative mutant in BC cells, it was shown that ETS1 plays a key role in coordinating multiple invasive features of cancer cells [87]. They noticed that ETS1 decreased the density of BC cells cultured in three-dimensional extracellular matrix gels. ETS1 overexpression was deleterious to anchorage-independent growth of cells in soft agar. The role of ETS1 was confirmed *in vivo*, during primary tumor growth and metastatic assay of lung colonization [87] associated with epithelial-to-mesenchymal transition features [89] and high Ki67 [90] - positive cells. siRNA-mediated knockdown of ETS1 in human BC cell lines decreased colony growth, both in anchorage-independent assays and 3D extracellular matrix cultures. These observations suggested an oncogenic role of ETS1 in breast tumorigenesis [87]. In contrast to *ETS1*, loss of the Myb-like transcription factor *DMP1α* (*DMTF1α*) binds to the Ets site in the *Arf* promoter [91] is associated with low Ki67 and is associated with favorable prognosis [92] for Dmp1 [93-101; 67, 102-106 for reviews]. Dmp1α also binds directly to p53 for activation in *Arf*-deficient cells [100, 101]. It was reported that expression of neuNT, but not normal neu, caused transcriptional activation of Ets, AP1, or NF-κB-dependent reporter genes [107]. Co-transfection of dominant inhibitory Ets2 mutants specifically blocked neuNT-mediated activation of Ets-dependent reporter genes [107]. Thus, Ets activation is required for neuNT-mediated cellular transformation [105,108-110].

Previous reports have demonstrated that *ETS1* is required for activation of the RAS/ERK pathway and migration of RAS/ERK-activated cells by transcriptional activation through ETS/AP1 sites [111,112]. In invasive BC, upregulation of *ETS1* was associated with high aggressiveness and poor prognosis. Consistent with this observation, ETS1 regulates the expression of important angiogenetic and extracellular matrix remodeling factors such as *VEGF,* matrix metalloproteinases [113,114]. Furthermore, ETS1 represses genes such as *DUSP4, DUSP6,* and *SPRY4,* all of which provide negative feedback to the RAS/ERK pathway [85]. Statins, small molecule inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR), exert anti-tumor effects by altering the RAS/RAF/EK/ERK and RAS/PIK3CA/AKT signaling pathways [115,116]. Lipophilic statins inhibit the growth and proliferation of BC cells, especially hormone receptor-negative, basal-like BC cells [117-120]. Based on these studies, Jung et al. hypothesized that statins suppress TNBC growth by altering the expression of *DUSP4* and *ETS1* [121]. They demonstrated that *ETS1* mRNA and protein were overexpressed in TNBC cells compared with other BC cell lines, where *DUSP4* mRNA was downregulated. In addition, simvastatin restored *Dusp4*-deficiency and suppressed ETS1 expression in TNBC. Moreover, they found that depletion of DUSP4 overcame the anti-tumor activity of statins. MAPK pathway inhibitor, U0126 and PI3KCA inhibitor LY294002 also decreased levels of Ets1, phosphor-ERK and phosphor-AKT on Western blot assays. Taken together, these studies suggest the oncogenic function of Ets1 in cancer, and simvastatin potentially affects the activity of transcriptional factors such as ETS1 and DUSP4 through the MAPK pathway [121]. If ETS1 is a critical mediator for the RAS/MEK/ERK signaling, it could act as a tumor promoter or repressor dependent on the biological condition

Expression of ETS2 in human cancer: influences on *Cyclin D1* and *p16^INK4a* expression

One important function of ETS2 is the *Cyclin D1* promoter regulation [49,126]. Cyclin D1, the regulatory subunit of several cyclin-dependent kinases (CDK2/4/6), is required for, and capable of shortening, the G1 phase of the cell cycle [127,128]. The transforming mutants of p21^{Ras} (Ras^{Val12}, Ras^{Leu61}) induces the *Cyclin D1* promoter in human trophoblasts, mink lung epithelial, and in Chinese hamster ovary fibroblast cell lines [49]. Site-directed mutagenesis of AP1-like sequences at -954 abolished p21^{Ras}-dependent activation of Cyclin D1 expression. The AP1-like sequences were also required for activation of the *Cyclin D1* promoter by c-Jun [49]. In electrophoretic mobility shift assays, several AP1 proteins (Fos/Jun) bound the cyclin d1-954 region. The *Cyclin D1* promoter was stimulated by overexpression of mitogen-activated protein kinase (p41^{MAPK}) or c-Ets2 through the proximal 22 base pairs [49]. Conversely, expression of plasmids encoding either dominant negative MAPK (p41^{MAPKi}) or dominant-negatives of ETS activation, antagonized MAPK-dependent induction of *Cyclin D1* promoter activity. In summary Ets2 plays critical role in Cyclin D1 induction in response to mitogenic pathways mediated by Ras and MAPK [49,126-130].

The p16^{INK4a} cyclin-dependent kinase inhibitor (CDKN2a) has been implicated in replicative senescence, the state of permanent growth arrest driven by cell divisions or constitutive Ras-Raf-MEK signaling [131-134] (Figure 2). The p16^{INK4a} cyclin-dependent kinase inhibitor is implicated in replicative senescence, the state of permanent growth arrest caused by cumulative cell divisions or constitutive Ras-Raf-MEK signaling [50]. p16^{INK4a} is an essential tumor suppressor that prevents the emergence of incipient cancer cells [134]. The gene expression is regulated at the transcription level and thus the promoter analysis is critical [135,136]. Regulation of p16^{INK4a} expression occurs at the transcriptional level, and involves epigenetic control and multiple transcription factors. PRC1 (Pombe repressor complex 1) and PRC2 (Pombe repressor complex 2) proteins, and histone deacetylases play an important role in the promoter hypermethylation for suppressing p16^{INK4a} expression by YY1 [137] and Id1 [138]. Ohtani, *et al.* [50] demonstrate a role for the Ets1 and Ets2 transcription factors based on their ability to activate the *p16^INK4a* promoter through the consensus sequences, and their patterns of expression in human diploid fibroblasts. The induction of p16^{INK4a} by Ets2, which was abundant in young human diploid fibroblasts, was potentiated by signaling through the Ras-Raf-MEK kinase cascade, and inhibited by a direct interaction with the helix-loop-helix protein Id1 [50,138] (Figure 1B). In senescent cells, where the ETS2 levels and MEK signaling decline, they saw a marked increase in p16^{INK4a} expression consistent with the reciprocal reduction of Id1 and accumulation of ETS1 [50,138] (Figure 2). These results indicated the opposing effects of Ets and Id proteins on p16^{INK4a} expression during cellular senescence.

The tumor suppressor PTEN has both lipid and protein phosphatase activities [139-141]. Its lipid phosphatase activity is essential for its tumor-suppressive function via the phosphoinositide 3-kinase (PI3K) and AKT1 pathways. Weng et al. showed that overexpression of wild type PTEN in the MCF7 breast cancer cells resulted in a phosphatase activity-dependent decrease in the phosphorylation of ETS2 [142]. Interestingly, exposure of MCF7 cells to insulin, insulin-like growth factor-1, or epidermal growth factor lead to the phosphorylation

of ETS2. They also reported that the MAP2K1 inhibitor PD590089 cancelled insulin-stimulated phosphorylation of ETS2 [142]. Overexpression of PTEN abrogated activation of the Ras-responsive enhancer, a target of ETS2 action, in a phosphatase-dependent manner [142]. In short, PTEN blocks insulin-stimulated ETS2 phosphorylation through inhibition of the MAP kinase independently of PI3K, which may be mediated through PTEN's protein phosphatase activity [142].

ETS proteins in telomere maintenance

Telomere repeats at chromosomal ends, critical to genome integrity, are maintained through complicated network of proteins and pathways [11,32,33]. A specialized ribonucleic protein, telomerase, maintains telomere homeostasis through repeated addition to counter intrinsic shortcomings of DNA replication that leads to gradual sequence shortening (telomere erosion) in successive mitoses [143,144]. The telomerase is composed of telomerase reverse transcriptase (TERT) subunit and an RNA component [143]. Telomerase, while present in stem cells, is deactivated due to epigenetic silencing of the rate-limiting TERT upon differentiation in most of somatic cells [11]. However, in most cancer cells, telomerase reactivation remains a ubiquitous process and constitutes one of the major hallmarks [143-145]. Discovery of mutations within the core promoter of the *TERT* gene that create *de novo* binding sites for ETS transcription factors [32,33] provided a mechanism for cancer-specific telomerase reactivation [11] (Figures 2 and 3). In human cancers, the *TERT* promoter mutations have been shown to define subsets of patients with poor prognosis with increased transcription of *TERT*, telomerase reactivation, resulting in increased telomere length. Thus, the *TERT* promoter mutations have the potential for therapeutic target for cancer [11,32,33].

The Wilms' tumor 1 gene product, which can either be tumor suppressive [146] or oncogenic [147-151], can regulate h*TERT* gene expression and hence telomerase activity in cancer cells via multiple pathways [12] (Figure 3). It has been reported that WT1 directly bound to p53, and could be either transcriptional repressor or activator dependent on the status of p53 [152,153]. Thus it is likely that WT1 is a transactivator for h*TERT* rather than repressor in cancer stem cells where p53 in mutated. Indeed, most cases of leukemia show both the *TERT* and *WT1* gene overexpression [154]. Consistently, the *TERT* and *WT1* gene expression analyses provided useful pieces of information for the understanding of chronic myelogenous leukemia progression and their possible usefulness as surrogate markers for treatment monitoring [154].

Ets1/2 and mutant p53

To investigate the basis of its GOF activities of mtp53, Martinez et al. and other labs conducted genome-wide analysis of mtp53 binding,
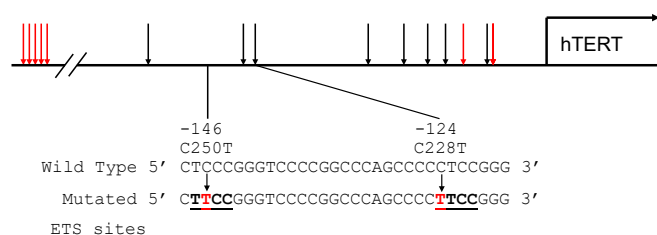
Figure 3. The human *TERT* promoter [11,143-145]. The proximal promoter has both WT1 (black arrows) and E2F1 (red arrows) -binding sites for regulation. In addition, E2F-binding sites are clustered at 600–1100 base pairs from the transcription initiating G. The *hTERT* promoter is often mutated to create new ETS sits for transcription factor-binding, which contributes increased telomerase activity in tumor cells.

and determined that it could be recruited to promoters through interactions with other transcription factors [44,46,155-164]. Many of the transcription factors that bind to mtp53 have also been shown to interact with wild type p53 (WTp53) (E2F1:DP [104], NF-Y, vitamin D3-responsive element [VDR], and SP1). Early studies showed that mtp53 regulates gene expression *via* recruitment of transcription factors on the *MDR1* promoter [165,166]. Although Sampath study showed that Ets1 interacted with mutant p53, later studies found specific interaction of Ets1 with WTp53 [167,168] which has become a generalized understanding.

Data from ChiP [99], Chip on Chip [169], and Chip-Seq [170] revealed that ~50% of promoters occupied by mtp53 contained ETS-binding sites, suggesting that physical binding with ETS proteins is an essential mechanism by which mtp53 regulates gene expression for oncogenic transformation [159,160]. Importantly, these mtp53-bound genomic regions do not have a WTp53 response element 5′-PuPuPuC(A/T)(A/T)GPyPyPy-3 [171], indicating that the mtp53 proteins do not cause transformation through direct binding to DNA [159]. Although the ETS1 might be important for the regulation of some mtp53 target genes [160], side-by-side comparison using recombinant proteins revealed that mtp53 preferentially associates with ETS2 [44,158]. All structural and DNA contact p53 mutants bound to ETS2 [44,158,162]. Whereas ETS1 knockdown had no effect on mtp53 target gene expression, ETS2 depletion reproduced changes in gene expression that occur on mtp53 depletion suggesting close relationship between mtp53 and ETS2. The observation that ETS2 interacts with various mtp53 (R175H, R248Q, R248W, R249S, R273H, R273L, and R280K) suggests that through ETS2 binding, different mtp53 proteins reveal oncogenic potential through common mechanisms consistent with the recent MD Anderson study [172].

The VCU group studied the mechanism for mtp53 binding by mapping the human genomic binding sites for p53^{R273H} (one of GOF mtp53) using ChIP-seq and showed that the protein was localized to ETS DNA sequence motifs with ETS1 and GABPA binding, both within promoters and distal to promoters [160]. p53^{R273H} showed significant and substantial binding to bidirectional promoters, which were enriched for inverted repeated ETS DNA sequence motifs [160]. p53^{R273H} showed an exponential increase in probability of binding to promoters with a higher number of ETS motifs [160]. The same group reported that in cells expressing p53^{R273H} GOF mtp53 simultaneously upregulates genes from multiple signaling pathways by recognizing promoters containing distinct transcription factor binding sites [161]. Thus, inhibition of one GOF mtp53-induced signaling cascade would be insufficient to inhibit tumor growth, but rather global inhibition of GOF mtp53 activity in necessary to fond a novel anti-cancer therapeutic agents [161].

It should be remembered that ETS2 binds to the tetramerization domain of p53 [173-175], which is thought to be functionally intact in both WTp53 and mtp53. Then the question is how ETS2 distinguishes between mtp53 and WTp53. One possibility is that p53 mutants adopt a different structure from normal p53 that enables their interaction with ETS2. However, some GOF p53 proteins have similar structures to normal p53 which raises the question for this. The 2nd possibility is when WTp53 in searching for DNA sequences throughout the genome, it might not be able to interact with ETS2 [175]. When WTp53 is bound to genomic DNA, it might alter the structure in a way that not compatible with binding to ETS2 [175]. The 3rd possibility is

that the expression of some WTp53 target genes prevents the normal protein interactions found in mtp53 [175]. Whatever the scenario is, it is of paramount importance to elucidate the mechanisms of activation/stabilization of mt53 since it is mutated in 50% of human cancers associated with malignant potential of tumors cells, hence worse prognosis of patients.

Very recently, the Lozano's group elucidated how GOF mtp53 drove cancer metastasis by developing a traceable osteosarcoma mouse model [172]. They showed that mtp*53* mice developed osteosarcomas with increased metastasis as compared with *p53*-null mice. The RNA-seq analysis of tumors identified a cluster of small nucleolar RNAs (snoRNAs) that were highly up-regulated in mtp53 mutant tumors with enrichment of Ets2 transcription factor-binding site [172]. Consistently, homozygous deletion of *Ets2* in p53 mutant mice resulted in significant down-regulation of snoRNAs with simultaneous reversion of the pro-metastatic phenotype of tumors induced my mtp53, which had no effect on the growth of primary osteosarcoma. Thus Ets2 inhibition is a potential therapeutic vulnerability in mtp53 mutant osteosarcomas [172].

Future directions

Ets1 shows both oncogenic and tumor suppressive activity dependent on the assay conditions. It is generally considered an oncogene by aberrant overexpression in human cancers. The role of Ets1 in embryonic maintenance or inhibition of cell proliferation for lymphoid cells is clear from gene knockout studies. Accumulating pieces of evidence has suggested that Ets1 accelerates angiogenesis, and has an oncogenic role mediated by MAPK signaling. Conversely, Ets2 has a tumor-suppressive role by upregulation the transcription of *p16^{INK4a}* although it stimulates the transcription of *Cyclin D1*. The situation is completely reversed when the cells have mutant p53; ETS2 reveals oncogenic function by stabilizing mutant p53 as evidenced by the recent study employing gene engineered mice [172]. The authors concluded that blocking Ets2 activity is essential to prevent lung metastasis of osteosarcoma. Thus the search for ETS2 blocker is of paramount importance to prevent cancer metastasis. Both ETS1 and ETS2 stimulate p53; however, neither ETS1 nor ETS2 is in the list of target genes for p53 [176,177]. Currently, the role of other ETS family proteins on activation/inhibition of mutant p53 is unknown. Thus extensive studies should be done in this direction since p53 is mutated in half of human cancers with GOF functions and that ETS have so many other family members.

It is very intriguing to know that *human telomerase* promoter have novel ETS sites when they are mutated [11]. That may be the reason why many human cancers have increased telomerase activity with long telomeres where the inhibitors are in clinical trials to cancer. Since human *TERT* promoter has both E2F1 and WT1 binding sites, it will be important how these transcription factors regulate the h*TERT* promoter. It will be also essential to analyze which ETS proteins bind to the *hTERT* promoter to translate the findings of ETS mutation to clinical levels.

Declarations

Conflicts of interest: The authors declare no conflicts of interest.

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