Molecular Cancer

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# The Biology of the Ets1 Proto-Oncogene

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Published: 20 August 2003

*Molecular Cancer* 2003, **2**:29

Received: 21 July 2003

Accepted: 20 August 2003

This article is available from: http://www.molecular-cancer.com/content/2/1/29



## Abstract

The Ets1 proto-oncoprotein is a member of the Ets family of transcription factors that share a unique DNA binding domain, the Ets domain. The DNA binding activity of Ets1 is controlled by kinases and transcription factors. Some transcription factors, such as AML-1, regulate Ets1 by targeting its autoinhibitory module. Others, such as Pax-5, alter Ets1 DNA binding properties. Ets1 harbors two phosphorylation sites, threonine-38 and an array of serines within the exon VII domain. Phosphorylation of threonine-38 by ERK1/2 activates Ets1, whereas phosphorylation of the exon VII domain by CaMKII or MLCK inhibits Ets1 DNA binding activity. Ets1 is expressed by numerous cell types. In haemotopoietic cells, it contributes to the regulation of cellular differentiation. In a variety of other cells, including endothelial cells, vascular smooth muscle cells and epithelial cancer cells, Ets1 promotes invasive behavior. Regulation of MMP1, MMP3, MMP9 and uPA as well as of VEGF and VEGF receptor gene expression has been ascribed to Ets1. In tumors, Ets1 expression is indicative of poorer prognosis.

## Introduction

Ets proteins comprise a family of transcription factors that share a unique DNA binding domain, the Ets domain [1–4]. The name "Ets" stems from a sequence that was detected in an avian erythroblastosis virus, E26, where it formed a transforming gene together with Δgag and *c-myb* [5,6]. The newly discovered sequence was called E26 transformation specific sequence or Ets. Later, a cellular homologue to the viral ets (*v-ets*) gene, *c-ets1*, was found suggesting that *v-ets* derived from *c-ets1* [7,8].

## Structure of c-Ets1

The c-Ets1 protein is closely related to c-Ets2. It is believed that these two proteins derived from the same ancestor gene by duplication [9,10]. The genomes of *Drosophila* and sea urchin contain only one Ets1-related gene, *D-ets2* (*Pointed*) and *SU-ets2*, respectively, whereas birds, reptiles, amphibians and mammals harbor both the *ets1* and *ets2* genes [11,12]. In humans, the *ets1* and *ets2* genes are located on two distinct chromosomes, *ets1* on chromosome #11, *ets2* on chromosome #21 [7]. No *ets1*-related gene has been found in the genome of the *Caenorhabditis elegans*, although this nematode worm does express a variety of other Ets proteins [13].

In birds and reptiles, the *c-ets1* locus contains two different start sites leading to the expression of $p68^{c\text{-}ets1}$ and $p54^{c\text{-}ets1}$. These two proteins differ in their N-terminal sequence [14]. Exons α (I) and β (II) code for the N-terminus of $p68^{c\text{-}ets1}$, whereas exon $I^{54}$ (A) encodes the N-terminus of $p54^{c\text{-}ets1}$ (Fig. 1). The exon β domain of $p68^{c\text{-}ets1}$ shows a high degree of homology to the N-terminus of c-Ets2 and was thus called the Ets1-beta/Ets2-conserved sequence (BEC). In mammals, only exon $I^{54}$ is present, therefore, $p68^{c\text{-}ets1}$ is not expressed.

The human TATA-less *ets1* gene contains eight exons (A, III-IX) [15]. Transcripts either harbor all exons or lack

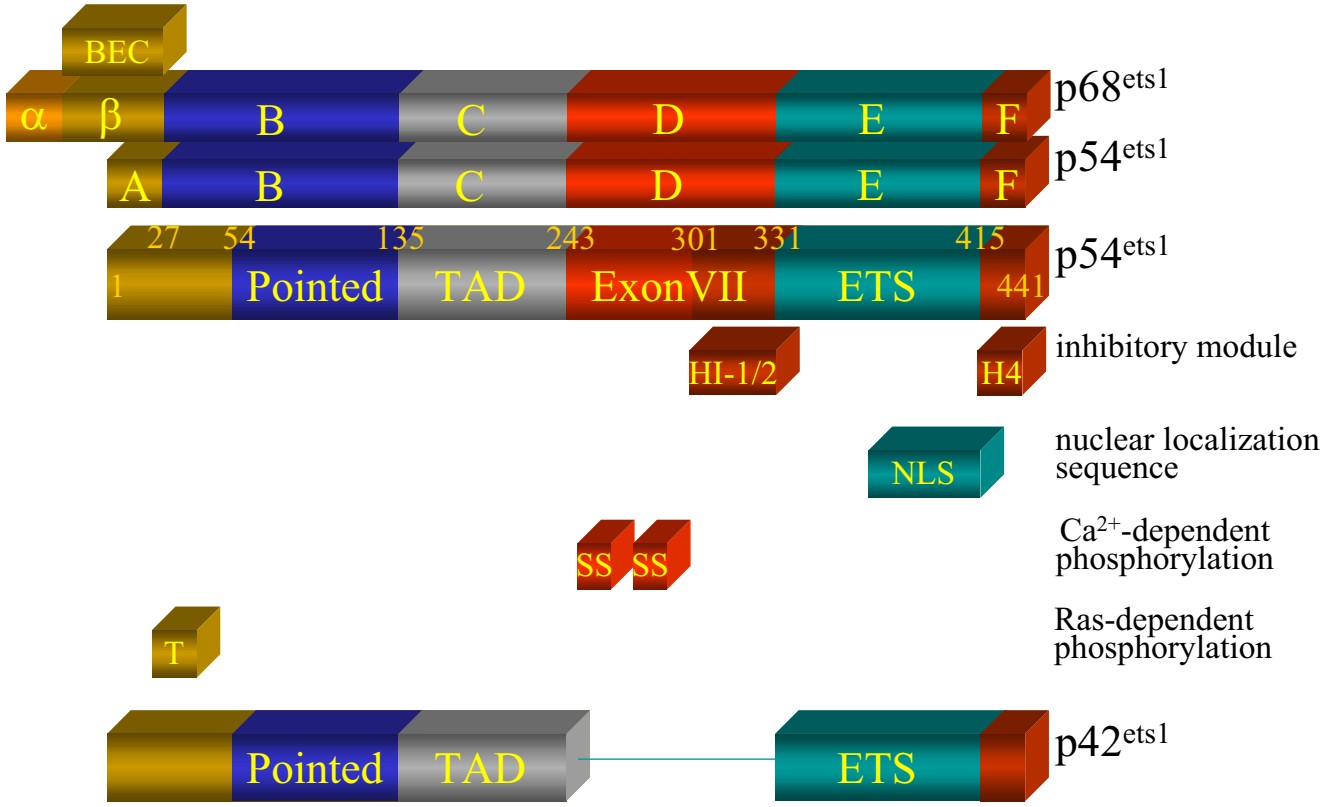


**Figure 1**
**The domains of the Ets1 protein.** BEC = Ets1-beta/Ets2-conserved sequence, TAD = transactivation domain, NLS = nuclear localization sequence, HI-1/2 = inhibitory α-helices 1/2, H4 = α-helix 4, S = serines, T = threonine-38.

exon IV- or exon VII- or exon IV/VII-specific sequence [16]. Only two proteins are generated from these RNAs, $p54^{c\text{-}ets1}$ (full length Ets1) and $p42^{c\text{-}ets1}$ (ΔVII-Ets1) (Fig. 1). The Ets1 protein sequence is highly conserved among species. E.g., the DNA and protein sequence of chicken Ets1 is 85% or 95%, respectively, identical to the corresponding sequence of human Ets1 [17]. The Ets1 protein can be divided into six domains, A-F (Fig. 1). The E-domain is the DNA binding Ets-domain. The adjoining D- (or exon VII-) domain and the F-domain are regulatory domains that control the activity of the E-domain. The A- und B- domains also function as regulatory units, whereas the C-domain is the activation domain of $p54^{c\text{-}ets1}$ and $p42^{c\text{-}ets1}$.

### *The Ets domain*

Ets domain proteins belong to the superfamily of winged helix-turn-helix (wHTH) DNA-binding proteins which includes also hepatocyte nuclear factor HFN-3γ, heat shock factor HSF and catabolite activator protein CAP [18]. The Ets domain, composed of 85 amino acids, comprises three α-helices and four β-strands that are arranged in the order H1-S1-S2-H2-H3-S3-S4 (Fig. 2). The Ets domain specifically recognizes DNA sequences that contain a GGAA/T core element [19]. In the Ets1 protein, the Ets domain stretches from residue 331 to residue 415. As revealed by NMR spectroscopy, the helix H3 of Ets1 interacts with the GGAA motif in the major groove, whereas the "wing", formed by the loop between strands 3 and 4 of the β-sheet, contacts the 5' minor groove [20]. Helices 1–3, the "turn" between helix H2 and helix H3 contribute to the interaction of the Ets1 domain with the 3' minor groove. Strikingly, the distantly related Ets factor PU.1 binds in a similar way to DNA [21] demonstrating that the Ets domain/DNA interaction is highly preserved. However, Ets proteins differ significantly in their preference for the sequence flanking the GGAA/T core motif. The consensus sequence for Ets1 is PuCC/a-GGAA/T-GCPy as determined by several rounds of selection and amplification of Ets1-binding sites [19]. Yet, many natural Ets1-responsive GGAA/T elements differ from this consensus sequence [22]. It is possible that other transcription

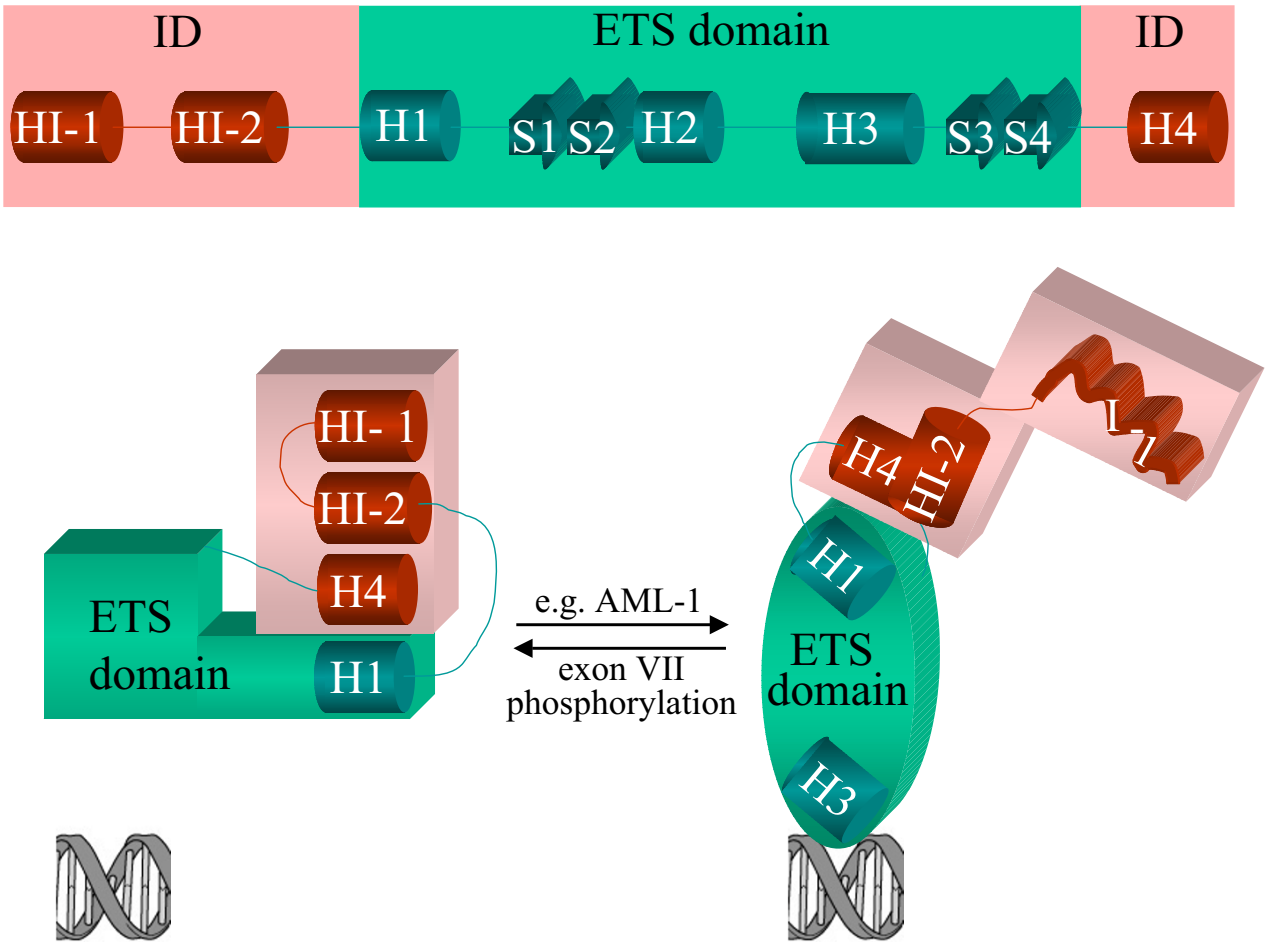


**Figure 2**
**The inhibitory module of Ets1.** Details are described in the text. For simplicity, only the major contact of the H3-helix with DNA is shown. ID = inhibitory domain, H = α-helix, S = β-sheet, HI= inhibitory α-helix, I-1 = HI-1 after having unfolded to a random coil, AML1 = acute-myeloid-leukemia-1

factors may facilitate Ets1 binding to unfavorable DNA sequences (see below).

Ets1 is predominantly found in the nucleus, though its presence in the cytoplasm of quiescent endothelial cells as well as of endometrial and ovarian cancer cells has also been demonstrated [23–25]. Deletion of the C-terminal part of the Ets domain excludes Ets1 from the nucleus [26] showing that the nuclear localization sequence of Ets1 resides on its Ets domain (Fig. 1).

### *The Pointed domain and the Ras-responsive phosphorylation site*

The Pointed (PNT) domain, named after the Ets1-related protein Pointed-P2 in *Drosophila*, is shared by many Ets proteins. The Ets1 PNT domain, located between amino acid 54 and 135, consists of five α-helices. Though originally thought to adopt a helix-loop-helix (HLH) conformation, NMR analysis revealed that the PNT domain forms a globular structure that does not resemble any other known protein- or DNA-binding interaction interface [27]. The N-terminal sequence of Ets1 contains also a Ras-responsive phosphorylation site at threonine-38 [28,29] (Fig. 1). Phosphorylation of this residue strongly increases the transcriptional activity of Ets1.

**Table 1: Ets1 interacting proteins**

| Proteins that cooperate with Ets1 | | | |
|---|---|---|---|
| ***protein*** | ***responsive gene(s)*** | ***comments*** | ***reference*** |
| AML-1 | *TCR$\alpha$*, *TCR$\beta$*, *osteopontin*, *GM-CSF* | mutual blockage of the negative regulatory domains induces cooperative binding of Ets1 and AML-1 | [47–52] |
| ATF-2 | *TCR$\alpha$* | binds stronger to Ets1 when it lacks dimerization domain | [48] |
| AP-1 (c-Jun/c-Fos) | *GM-CSF*, *TIMP-1*, *polyoma enhancer*, *MMP-1* | | [53–57] |
| CBP/p300 | *MMP-3* | | [42,58] |
| ERK1/2 | *uPA*, *MMP-3*, *prolactin* | phosphorylates threonine-38 | [28,29,59,60] |
| Ets1 | *MMP-3* | requires presence of a palindromic sequence | [61] |
| Estrogen receptor | *artificial promoter* | Ets1 cooperates with unliganded receptor | [62] |
| GATA3 | *human interleukin-5* | requires presence of PMA and ionomycin | [63] |
| HIF-2$\alpha$ | *VEGFR-2 (Flk-1)* | | [64] |
| HTLV-I Tax | *PTHrP (P3 promoter)*, *interleukin-5* | Tax forms ternary complex with Ets1 and Sp1, enhances GATA3/Ets1 cooperation | [63,65] |
| LEF-1 | *TCR$\alpha$* | LEF-1 induces DNA-bending that facilitates Ets1/ATF2 interaction | [48] |
| c-Myb | *$\alpha$4 integrin*, *mim-1*, *lck type I* | Ets1/c-Myb synergism blocks repression by ZEB | [66–68] |
| NFAT | *HIV-LTR* | | [69] |
| NF-$\kappa$B | *GM-CSF*, *HIV-LTR* | | [56,69] |
| Pax5 | *mb-1* | allows Ets1 to bind to unfavorable GGAG, changes contacts of Y395 (H3-helix) with DNA | [70–72] |
| Pit-1/GHF-1 | *prolactin* | | [73] |
| PKC$\alpha$ | *PTHrP (P3 promoter)* | constitutively active form increases Ets1 activity and induces phosphorylation of exon VII domain | [74] |
| mutant p53 | *MDR1* | wildtype p53 does not bind to and synergize with Ets1 | [75] |
| wildtype p53 | *mdm2*, *bax* | requires presence of CBP, and UV-radiation, Ets1 necessary for p53-dependent apoptosis in ES cells | [76] |
| Ras | *uPA*, *MMP-3, prolactin* | induces ERK1/2-dependent phosphorylation of threonine-38 | [28,29] |
| Smad3/4 | *PTHrP (P3 promoter)* | requires presence of TGF$\beta$ | [77] |
| SPBP | *synthetic promoter* | SPBP = stromelysin-1 PDGF responsive element binding protein | [78] |
| Sp1 | *PTHrP*, *MRG1*, *HTLV-I LTR*, *integrin $\alpha$IIb*, *P4 promoter of MVMp*, *FasL* | Ets1/Sp1 bind cooperatively to DNA | [79–83] |
| Sp100 | *MMP3*, *synthetic promoters* | does not activate $\Delta$VII-Ets1 | [45] |
| Stat5 | *GAS/Ets elements* | | [84] |
| TFE3 | *immunoglobulin $\mu$ heavy-chain gene* | basic helix-loop-helix domain of TFE3 sufficient for Ets1 binding, several Ets1 domains involved in binding | [46] |
| huUBC9 | *synthetic promoter* | | [85] |
| USF-1 | *HIV-LTR* | | [86] |
| Vitamin D receptor (VDR) | *artificial promoter* | Ets1 induces conformational change of VDR, in the presence of Ets1 activation by VDR does not require AF2-domain | [62] |
| **Proteins that repress Ets1 activity** | | | |
| ***protein*** | ***responsive gene*** | ***comments*** | ***reference*** |
| CaMKII | *GM-CSF* | blocks AML-1/Ets1 cooperative effect by phosphorylating exon VII domain | [47] |
| Daxx/EAPI (Ets1-associated protein) | *MMP1 and BCL2* | a nuclear protein that cooperates with Ets1 to repress transcription | [87] |
| EAPII (Ets1-associated protein) | *MMP1* | a nuclear protein that attenuates Ets1/AP1 cooperative effect, inhibits migration of epithelial cells | [55] |
| MafB (AP-1 likeprotein) | *transferrin receptor*, *porphobilinogen deaminase* | suppresses Ets1-dependent activation of erythoid-specific genes | [88] |
| ZEB | *$\alpha$4 integrin* | repression of Ets1 by ZEB is blocked when Ets1 synergizes with c-Myb | [67] |

### *The exon VII domain, the autoinhibitory module and calcium-dependent phosphorylation*

The D- or exon VII domain resides in the Ets1 protein between amino acids 243 and 331 (Fig. 1). It comprises two regulatory units. The C-terminal unit is part of an autoinhibitory module [30], the N-terminal sequence contains a calcium-responsive phosphorylation site [31]. The two units are functionally connected as phosphorylation within the N-terminal sequence increases the inhibitory effect of the C-terminal unit [32]. An inhibitory sequence within the exon VII domain was first proposed when studies showed that deletion of the exon VII domain results in an enhanced DNA binding activity [33]. Since a similar increase in DNA binding was also observed when the C-terminus (F-domain) was deleted or mutated [34–36] an interplay between the exon VII domain and the C-terminus of the Ets1 protein was suggested. Later, it became clear that the exon VII-specific sequences between 301 and 331 and the C-terminal sequence between 415 and 440 form a metastable autoinhibitory module [30]. The key structural unit was found to be formed by three inhibitory helices (HI-1 and HI-2 within exon VII domain and H4 at the C-terminus). These helices cooperatively block Ets DNA binding activity by interacting with the H1-helix of the Ets domain and, thereby, freeze the Ets domain in a closed confirmation [37,38]. The blockage is transient and can be relieved when the HI-1 helix spontaneously unfolds to form a random coil (Fig. 2). This structural switch allows other proteins, such as the transcription factor AML-1 (acute myeloid leukemia-1), to modulate Ets1 DNA binding activity [37].

Calcium-dependent phosphorylation of the exon VII domain also interferes with the activity of the Ets1 autoinhibitory module and decreases the stability of the Ets1 protein in T-lymphocytes [39]. It involves phosphorylation of a cluster of serines, including $Ser^{251}$, $Ser^{257}$, $Ser^{282}$ and $Ser^{285}$, within the N-terminal part of exon VII [31] (Fig. 1). It results in stabilization of the inhibitory structure and in a strongly reduced DNA binding activity [32]. The natural splicing variant ΔVII-Ets1 lacks an essential part of the autoinhibitory module and the calcium-sensitive serines. This confers the ability to ΔVII-Ets1 to form stable complexes with DNA [40] and renders this protein resistant to the negative action of calcium. The viral v-Ets1 protein contains a modified C-terminus which inactivates the autoinhibitory module as well and allows v-Ets1 to bind stably to DNA [36].

### *The activation domains*

The activation domain or C-domain is located between amino acids 130–242 and contains a high content of acidic residues. It is essential for $p54^{c\text{-}ets1}$ and $p42^{c\text{-}ets1}$ to activate transcription [41]. Chicken $p68^{c\text{-}ets1}$ harbors a second N-terminal transcriptional domain, BEC (as mentioned above), that it shares with Ets2 [10]. The C-domain is also necessary for the interaction of Ets1 with the CREB binding factor (CBP)/p300 [42]. CBP and p300 have dual functions as histone acetyl transferases and co-activators that facilitate cooperativity between transcription factors [43,44].

## Interaction with other proteins

Ets1 functionally and physically interacts with a variety of transcription factors and other proteins (Table 1). Most proteins that directly contact Ets1 bind to the Ets domain (Fig. 3). Others associate with the exon VII domain, the N-terminal part of Ets1 or the activation domain. Some proteins, such as Sp100 or TFE3, interact with several domains of the Ets1 protein [45,46].

### *Transcription factors*

A number of transcription factors have been shown to regulate the transcriptional activity of Ets1 by modulating Ets1 DNA binding affinity (Fig. 4). AML-1, Pit-1 (pituitary-specific transacting factor 1) and HIF-2α (hypoxia-inducible factor 2α) bind to the exon VII domain and block the inhibitory module of Ets1 [49,50,64,73]. In turn, Ets1 may also increase the DNA binding activity of its partner. E.g., Ets1 stimulates DNA binding activity of AML-1 by associating with its NRDB domain (negative regulatory domain for DNA binding) [50,89] (Fig. 4). In the presence of a palindromic Ets binding site, the exon VII domain also mediates homodimerization of Ets1 proteins [61]. The dimerization blocks the autoinhibitory mechanism allowing these proteins to mutually increase their DNA binding activities and to bind cooperatively to DNA. Pax5 (paired box containing gene 5), a transcription factor that contacts the Ets domain of Ets1, uses a different way to increase Ets1 affinity to DNA. It reorientates Tyr-395 of the DNA-contacting helix 3 such that Ets1 can even bind to an unfavorable GGAG containing element [70]. Often, synergism between Ets1 and another transcription factor require certain stimuli to induce activation and/or recruitment of the Ets1 interaction partner. Both phorbol ester and ionomycin are needed for the cooperative effect of Ets1 with GATA3 on the human interleukin-5 (IL-5) promoter, for the synergistic action of Ets1 with AP1 (activator protein 1)and NFκB (nuclear factor of κB) on the granulocyte-macrophage colony stimulating factor (GM-CSF) promoter and for the ability of Ets1 and Stat5 to cooperatively activate promoters through a GAS (interferon γ-activated sequence)/Ets combinatorial element [56,63,84]. Whereas, TGFβ (transforming growth factor β) is essential for the synergistic action of Ets1 and Smad3 (Sma/Mother against Decapentaplegic) on the PTHrP (parathyroid-hormone-related protein) P3 promoter [77]. However, the importance of vitamin D for the functional interaction of Ets1 with the vitamin D receptor (VDR) seems to depend on the target gene. While vitamin D was

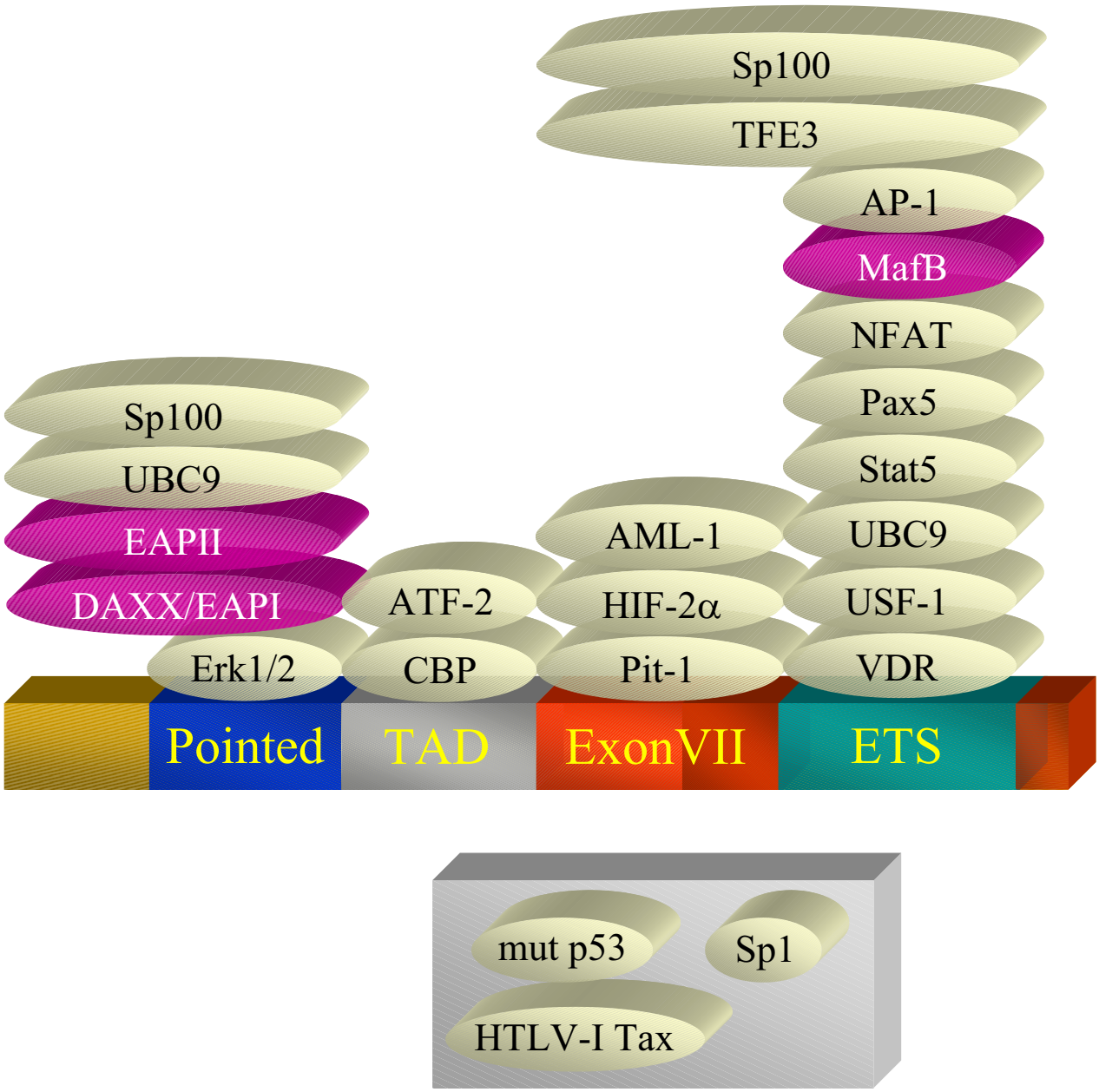


**Figure 3**
**Proteins that physically interact with Ets1.** Proteins that cooperate with Ets1 to activate genes are shown in light yellow, proteins that either repress Ets1 activity or cooperate with Ets1 to repress gene activity are shown in pink. Proteins whose specific sites of interaction on the Ets1 protein are not yet defined (Sp1, HTLV-I Tax, mut p53) are indicated below. AML1 = acute-myeloid-leukemia-1, AP-1 = activator protein 1, ATF2 = activating transcription factor 2, CBP = CREB binding protein, ERK1/2 = extracellular-signal-regulated kinase 1/2, HIF-2α = hypoxia-inducible factor 2α, HTLV-I Tax = human T-cell lymphotropic virus-I transactivating protein of region X, MafB = musculoaponeurotic fibrosarcoma B, NFAT = nuclear factor of activated T-cells, Pax5 = paired box containing gene 5, Pit-1 = pituitary-specific trans-acting factor 1, Sp100 = speckled, 100 kD, Stat5 = signal transducer and activators of transcription 5, EAP = Ets1-associated protein, UBC9 = ubiquitin-conjugating enzyme 9, USF-1 = upstream stimulatory factor 1, VDR = vitamin D receptor

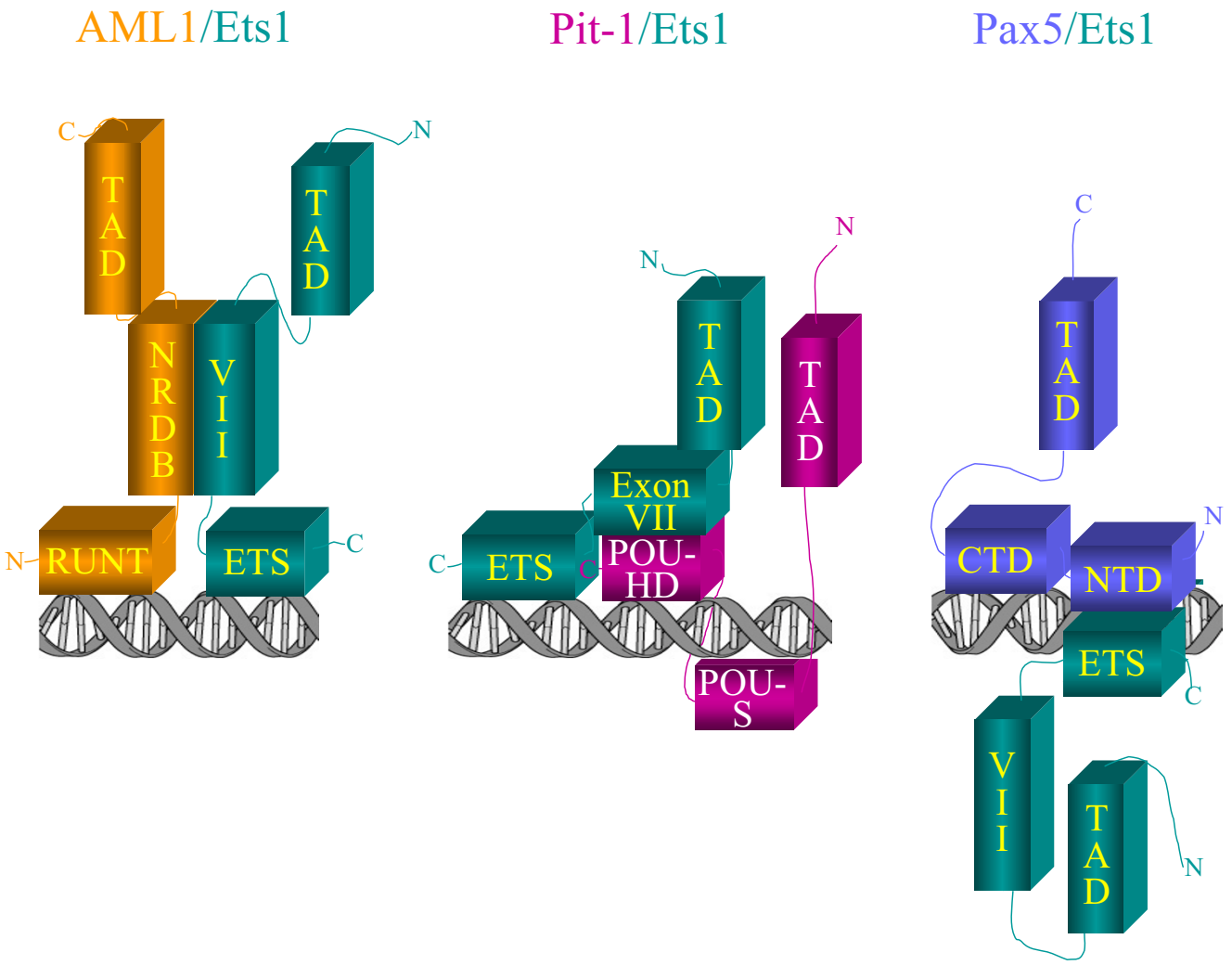


**Figure 4**
**Ternary complexes of Ets1 with AML1, Pit-1 or Pax5 and DNA.** TAD = transactivation domain, NRDB = negative regulatory domain for DNA binding (autoinhibitory domain of AML1), runt = DNA binding domain of AML1, POU-HD = POU homeo domain (C-terminal DNA binding domain of Pit-1), POU-S = POU-specific (N-terminal DNA binding domain of Pit-1), CTD = C-terminal DNA binding domain of Pax5, NTD = N-terminal DNA binding domain.

required for the Ets1/VDR synergistic effect on the rat cytochrome P450C24 gene promoter [90], it was dispensable for the Ets1/VDR combined effect on rat prolactin promoter [62]. The cooperative action of Ets1 with a transcription factor is sometimes mediated by a co-factor. This is the case for the Ets1/wildtype p53 synergism, where CBP is required for the interaction between these two proteins [76]. The combinatorial effect of Ets1 with another transcription factor can also lead to gene repression. This has been shown for the interaction of Ets1 with Daxx/EAPI (Ets1 associated protein I) which induces the suppression of the *bcl2* and *mmp1* genes [87].

Some nuclear proteins, EAPI/II, MafB and ZEB, are able to inhibit the transcriptional activity of Ets1 [55,67,88]. ZEB-induced Ets1 repression can be relieved by c-Myb, a protein that can synergize with Ets1 [67].

### *Kinases*

Several kinases have been demonstrated to phosphorylate Ets1 and modulate its activity. Among them is calmodulin-dependent kinase II (CaMKII) which can mimick calcium in its ability to induce phosphorylation of the exon VII domain [32,47]. This blocks Ets1 DNA binding activity and, hence, inhibits Ets1 transcriptional activity.

Recent data suggest that this inactivation may even convert Ets1 from an activator to a repressor protein. This has been shown for the GM-CSF (granulocyte/macrophage-colony stimulating factor) promoter, whose activity was found to be repressed when CaMKII-phosphorylated Ets1 was present [47]. It is thought that CaMKII-inactivated Ets1 may act as a dominant negative protein by interfering with the activity of related, CaMKII-resistant Ets proteins, such as ΔVII-Ets1. Besides CaMKII, also myosin light-chain kinase (MLCK) and a constitutively active form of protein kinase Cα (PKCα) are able to phosphorylate the exon VII domain [74,91]. Like CaMKII, MLCK was shown to inactivate Ets1 [91], whereas PKCα increased the transcriptional activity of Ets1 [74]. Whether exon VII phosphorylation and Ets1 activation by PKCα are independent events or whether the PKCα-mediated exon VII phosphorylation is different to that induced by CaMKII and MLCK remains to be seen. In support of the latter, PKCα-dependent exon VII phosphorylation was found to be independent of calcium [74], whereas, like CaMKII, MLCK mediates calcium-dependent exon VII phosphorylation [91]. Phosphorylation of the exon VII domain was also observed, when leukaemic cells go into mitosis [92].

The other known phosphorylation site on the Ets1 protein is threonine-38. Activation of the GTPase Ras leads to phosphorylation of threonine-38 and increased Ets1 activity [28,29]. The Ras effect is mediated by the mitogen activated kinases ERK1 and ERK2, the two effector kinases of the Ras/Raf/MEK(MAPK/ERK kinase 1)/ERK 1/2 (extracellular-signal-regulated kinase) pathway [28,29,59,93]. Phosphorylation of threonine-38 requires docking of ERK onto the PNT domain [60]. Typical Ras responsive genes harbor combinatorial Ets1/AP1 recognition motives through which Ets1 and AP1 synergistically activate transcription when stimulated by Ras [94]. Cooperative effects with Ras have also been reported for other Ets proteins, namely Ets2 and *Drosophila* Pointed. In both cases, a sequence similar to (114)LXLXXXF(120), which is crucial for ERK binding, is present within the PNT domain. Also a kinase-sensitive threonine is located exactly 16 amino acids upstream from the PNT domain. This suggests that the mechanism by which Ras induces phosphorylation of Ets1-related proteins is conserved.

#### *Acetyl transferases*

Ets1 interacts with the acetyl transferases CBP/p300 and ATF-2 (activating transcription factor 2) [42,48,58]. ATF-2, originally defined as a CRE (cAMP responsive element)-binding transcription factor, has recently also found to display acetyl transferase activity [95]. Interestingly, CBP and ATF-2 are the only Ets1-interacting proteins that so far have been demonstrated to associate with the transactivation domain of the Ets1 protein [42,48]. This may suggest that they serve a common function, such as acetylating histones in response to Ets1 recruitment to an Ets1-regulated promoter. ATF-2 has been demonstrated to support Ets1-dependent activation of the T-cell receptor α (TCRα) gene in cooperation with the transcription factors AML-1 and LEF-1 (lymphocyte enhancer-binding factor 1)/TCF (T-cell factor) [48]. CPB and p300 are required for the synergistic action of Ets1 and wildtype p53 on pro-apoptotic genes in embryonal stem cells [76] and increased induction of the MMP-3 (matrix metalloprotease-3) promoter by Ets1 [58]. Recently, Ets1 has been reported to be itself a target for acetyl transferases. When treated with TGFβ, human dermal fibroblasts were found to acetylate Ets1 [96]. This was accompanied by inhibition of TGFβ-dependent gene expression. Since Ets1 can also synergize with TGFβ to activate genes [77], it is possible that acetylation triggers Ets1 to display an anti-TGFβ activity.

## Ets1 expression

The Ets1 protein is produced by a variety of tissues. In most cases, its truncated form, ΔVII-Ets1, is expressed along with full-length Ets1. However, ΔVII-Ets1 is barely detectable in astrocytes, astrocytoma and invasive mammary carcinoma cells [91,97].

#### *The lymphoid and haematopoietic tissue*

In adults, expression of Ets1 was originally thought to be restricted to lymphoid tissues [8]. Here, Ets1 is present in T- and B-cells at all stages of their development [98]. Ets1 is also expressed in natural killer cells [99]. In chicken, it seems that resting peripheral blood mononuclear cells produce less Ets1 than cells of thymus, spleen and bursa [100]. In the same species, Ets1 expression increased when T-cells were activated and correlated well with the presence of CD-28. In contrast, the Ets1 level was reported to be downregulated in human T-cells upon activation [101,102]. Ets1 is also expressed in erythroid cells, where it is upregulated during differentiation [103].

#### *The vascular system*

Ets1 is also detected in other tissues. The two main blood vessel forming cell types, endothelial cells (EC) and vascular smooth muscle cells (VSMC), transiently produce Ets1 upon activation by angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiotensin II, endothelin-1, tumor necrosis factor α (TNFα) or hydrogen peroxide [104–108]. Activation of ECs and VSMCs results in proliferation, migration and invasion of these cells [109]. Inhibition of Ets1 expression by trans-dominant negative Ets1 mutant proteins or by anti-sense oligonucleotides directed against Ets1 abrogated the ability of ECs to migrate, to adopt an invasive behavior or to form tubes in response to angiogenic growth factors [107,110–112]. Conversely, constitutive Ets1 expression mimicked angiogenic stimulation and induced an invasive phenotype [113]. In addition,

**Table 2:** ***Ets1 expression in tumors***

| *tumor of* | *cancer type* | *tumoral expression* | *stromal(S)/vascular (V) expression* | *comments* | *reference* |
|---|---|---|---|---|---|
| brain | astrocytoma | 0% (grade II), 25% (III), 65% (IV) | high expression in glioma microvasculature | higher expression in recurrent vs. primary tumors; | [23,91,133] |
| | meningioma | benign (38%), invasive (86%) | | invasive tumor: correlation with uPA expression | [134] |
| breast | invasive carcinoma, DCIS, LCIS invasive cell lines | 62% | correlates with VEGF, MMP1 and MMP9 expression | prognostic marker for poor prognosis | [74,97,128,135, 136] |
| cartilage/bone (jaw) | chondro-sarcoma | 60% | | | [137] |
| | osteosarcoma | 0% | | | |
| cervix | cervical carcinoma | | correlates with TMD | correlates with poor prognosis | [127] |
| colon/rectum | adenomas | 0–44% | | | [126,138,139] |
| | colon cancer | 48–84% | 65% (V) correlates with TMD, 28% (S) correlated with lung metastasis | vascular Ets1: linked with LNM and poor prognosis | |
| endometrium | endometrial carcinoma | | correlates with TMD | associates with histological grade, detected in cytoplasm | [24,125] |
| esophagus | squamous carcinoma | | correlates with VEGF | heterogenous expression, higher at invasive sites | [140,141] |
| liver/biliary tract | hepatocellular carcinoma | 50–100% | | higher in poorly differentiated tumors | [142] |
| | bile duct carcinoma | 61% | | higher in well-differentiated tumors | [143] |
| | cholangio-cellular carcinomas | 22% | | | |
| lung | pulmonary adeno-carcinoma | | | linked to LNM | [130,144] |
| lymphoid tissue | T-leukemic cells (T-ALL, ATL) | | | | [132] |
| mouth | squamous cell carcinoma | 58% | | correlates with tumor stage and LNM | [131] |
| ovary | benign cystadenoma | 0% | | | [25,129,145] |
| | carcinoma | 42%, higher when stroma is invaded | 33% (S), correlates with MMP1 and MMP9 expression | associated with poor prognosis | |
| pankreas | adeno-carcinoma | 81% | | lower in poorly differentiated carcinoma | [146] |
| stomach | adenomas | 0% | | | [147,148] |
| | adeno-carcinoma | 64% | correlates with TMD | | |
| | mucosal carcinoma | 12% | | | |
| thymus | thymoma | | | higher in higher grade tumors | [149] |
| thyroid gland | thyroid carcinoma | 40% (adenomas), 50–98% (carcinoma) | | | [150] |
| vascular system (skin) | haemangioma | weak | | | [151] |
| | granuloma pyogenicum | weak | | | |
| | angiosarcoma | strong expression | | correlates with MMP1 expression | |

TMD = tumor microvessel density, LNM = lymph node metastasis, DCIS = ductal carcinoma *in situ*, LCIS = lobular carcinoma *in situ*

inhibition of Ets1 activity was shown to suppress hepatocyte growth factor (HGF) and FGF-2 induced angiogenesis in animals [114,115]. During the menstrual cycle, EC-dependent Ets1 expression increases in the proliferative phase and drops in the secretory phase, following changes in the VEGF level [116]. During pregnancy, Ets1 is found

in endothelial cells of the villous trophoblast, but not in ECs of maternal endothelial cells [117]. In the developing hypothalamo-hypophyseal system of the rat, Ets1 is detected during angiogenesis [118]. Under pathological conditions, Ets1 is produced by ECs that form new blood vessels in the synovial membrane of the joint in patients with active rheumatoid arthritis [119,120], by ECs of the ulcerous gastric mocusa during the early phase of the healing process [121], in ECs within arteriovenous malformations [122] and, in rats, by ECs in the kidney during glomerulonephritis [123]. Tumor-induced neo-angiogenesis also involves Ets1. Ets1 expression is found in the vascular stroma of cancerous lesions [124] and often correlates with tumor microvessel density [23,125–127]. In colon cancer, vascular Ets1 expression was associated with poor prognosis and higher incidence of lymph node metastasis [126].

#### *Tumors*

Ets1 is produced by a variety of solid tumors, including epithelial tumors, sarcomas and astrocytomas (Tab. 2). Dependent on the tumor type, Ets1 expression is either increased or exclusively found in invasive higher grade tumors. High Ets1 levels in breast, ovary and cervix carcinoma correlates with poorer prognosis [25,127–129]. Ets1 was found to be an independent prognostic marker of breast cancer that was not linked to other tumor markers, such as nodal status, tumor size, histological grade or estrogen receptor status [128]. In lung, colorectal and squamous cell carcinoma, Ets1 expression was associated with a higher incidence of lymph node metastasis [126,130,131]. In endometrial and ovarian cancer, the presence of Ets1 correlated with a higher histological grade [24,25]. In addition to advanced solid tumors, high Ets1 expression has also found in leukemic T-cells [132].

#### *Other tissues*

Ets1 is also expressed in astrocytes, in certain cells of the ovary, in hepatic stellate cells as well as in glandular epithelial cells and stromal cells of the endometrium during the menstrual cycle [91,152–154]. When murine breast epithelial cells were stimulated to form tubules, Ets1 mRNA was detected at their growing tips [155]. Fibroblasts in tumor-associated stroma, but not in normal tissue has also been found to be Ets1 positive [124]. High non-vascular stromal Ets1 level in colon cancers was found to be associated with a higher risk for developing metastastic lesions in the lung [51]. In rats, progression of glomerulonephritis was accompanied with Ets1 expression in glomerular epithelial cells and interstitial cells [123].

Ets1 is also expressed in a variety of tissues throughout the embryonal development [156]. During the first trimester of pregnancy, Ets1 was found in extravillous trophoblastic cells invading the uterine vessels [117]. At embryonic day 15 of murine embryo development, Ets1 could be detected in all organs [157], whereas, later, Ets1 was predominantly present in lymphoid tissues, brain and other organs during branching morphogenesis. In the developing nervous system, Ets1 could be detected in the neural pituitary and adenohypophysis at early stages and in the hypothalamic magnocellular nuclei at later stages [118] as well as in the hindbrain regions, neural tube, neural crest and in the first and second branchial arches [158]. Ets1 was also found in developing vascular structures, such as heart, arteries, capillaries and meninges. In the developing bone, Ets1 is found in mesenchymal cells, whereas Ets1 is not detected in the cartilage [159].

## Regulation of Ets1 expression

Ets1 expression can be modulated by a variety of factors (Fig. 5). The human Ets1 promoter is a TATA-less promoter containing both positive and negative regulatory elements [160]. Among the positive elements are binding sites for Ets1, AP1, AP2 and Sp1 in the proximal part of the promoter. More upstream, recognition sequence for Oct and another Ets site and the two negative regulatory elements are found. The Ets1/AP1 combinatorial element allows positive autoregulation of the Ets1 gene by a cooperative Ets1/AP1 interaction [161,162]. Recently, recognition elements for retinoic acid receptor and for hypoxia-inducible factor HIF-1 have also been identified, through which retinoic acid or hypoxia, respectively, activates Ets1 transcription [163–165]. Ets1 synthesis is also induced by HGF (hepatocyte growth factor), PDGF (platelet-derived growth factor) or TNFα (tumor necrosis factor α) via activation of the Ras/Raf/MEK1/ERK1/2 pathway [59,108,166]. In breast carcinoma, melanoma and osteosarcoma cells, Ets1 expression is controlled by protein kinase C (PKC) ([77] and J. Dittmer, unpublished results). In activated endothelial cells, the MAP kinase p38 is involved in Ets1 regulation [104]. In invasive breast cancer cells, Ets1 expression can be induced by TGFβ [167]. Some factors have been shown to repress Ets1 expression. Wildtype p53, but not mutant p53, was found to repress the Ets1 promoter [15] and, in endothelial cells, retinoic acid negatively modulates Ets1 expression [168]. Different regions of the Ets1 promoter drive Ets1 expression during murine embryonal development [169]. A 2.4 kbp 5'-flanking sequence is sufficient to allow Ets1 transcription in the neural tube at gestational day 8.5, whereas also the first exon and 9 kpb of the first intron are required for Ets1 expression in the developing vessels, meninges and choriod plexus. This suggest a rather complex regulation of Ets1 gene activity.

## Ets1 function

Numerous genes have been shown to respond to the transcription factor Ets1 [22]. However, whether a particular gene is indeed regulated by Ets1 or, alternatively, by a

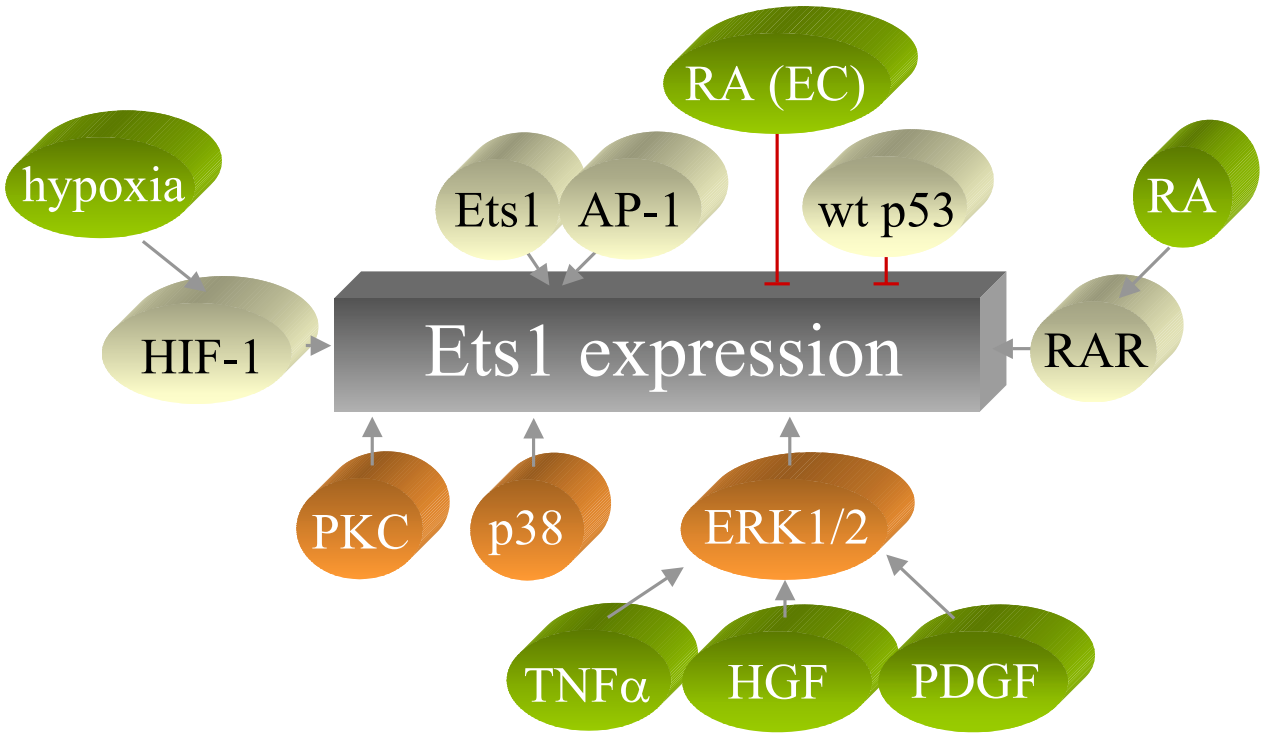


**Figure 5**
**Factors that modulate Ets1 expression.** Transcription factors that directly bind to the Ets1 promoter are shown in light yellow, kinases are in orange, growth factors and other factors are in green. AP-1 = activator protein 1, ERK1/2 = extracellular-signal-regulated kinase 1/2, HGF = hepatocyte growth factor, HIF-1 = hypoxia-inducible factor 1, PDGF = platelet derived growth factor, PKC = protein kinase C, RA = retinoic acid, RAR = retinoic acid receptor, TNFα = tumor necrosis factor α, wt p53 = wildtype p53.

related Ets factor can often not be answered with certainty. To address this problem, Ets1-specific anti-sense technology and trans-dominant negative protein treatments are frequently applied. However, neither method is as specific as desirable. Trans-dominant Ets1 proteins may potentially also interfere with the function of closely related Ets proteins, such as Ets2. Likewise, non-specific interactions of anti-sense oligonucleotides may also affect the expression of unrelated genes [170]. Nevertheless, based on data obtained by such approaches and by the knock-out technology in mouse a number of major functions has been ascribed to Ets1 (Fig. 6). Some of those are of more general importance, others are cell-type specific.

### *Haematopoietic development*

Ets1 plays an important role in the development of lymphoid tissues [156]. By applying the knock-out technology in mouse, a link between the presence of Ets1 and T-lymphocyte activation was established, showing that Ets1-deficient T-cells are defective in responding to activation signals and are more prone to undergo apoptosis [171,172]. Ets1 is also required for the development of natural killer cells [99]. In cooperation with AML-1, which is primarily expressed in T-cells [173], Ets1 activates a variety of T-cell specific genes, such as TCRα and β [48,50]. The transcription factors LEF-1/TCF and ATF-2 support the AML-1/Ets1 cooperative effect on TCRα expression [48]. The Ets1-Stat5 synergism plays a role in the activation of genes in response to T-cell activation [84]. In the Th2 subtype of T-helper cells, Th2-specific transcription factor GATA3 is able to functionally interact with Ets1 to transactivate the interleukin-5 promoter [63]. A function of Ets1 is also proposed for B-cell development. The *mb-1* (immunoglobulin alpha chain) gene which is involved in B-cell maturation has been demonstrated to be activated by a concerted action of Ets1 with the B-cell specific activator protein Pax5 [71,72]. A role for Ets1 in regulating the immunoglobulin heavy chain gene enhancer in B-cells

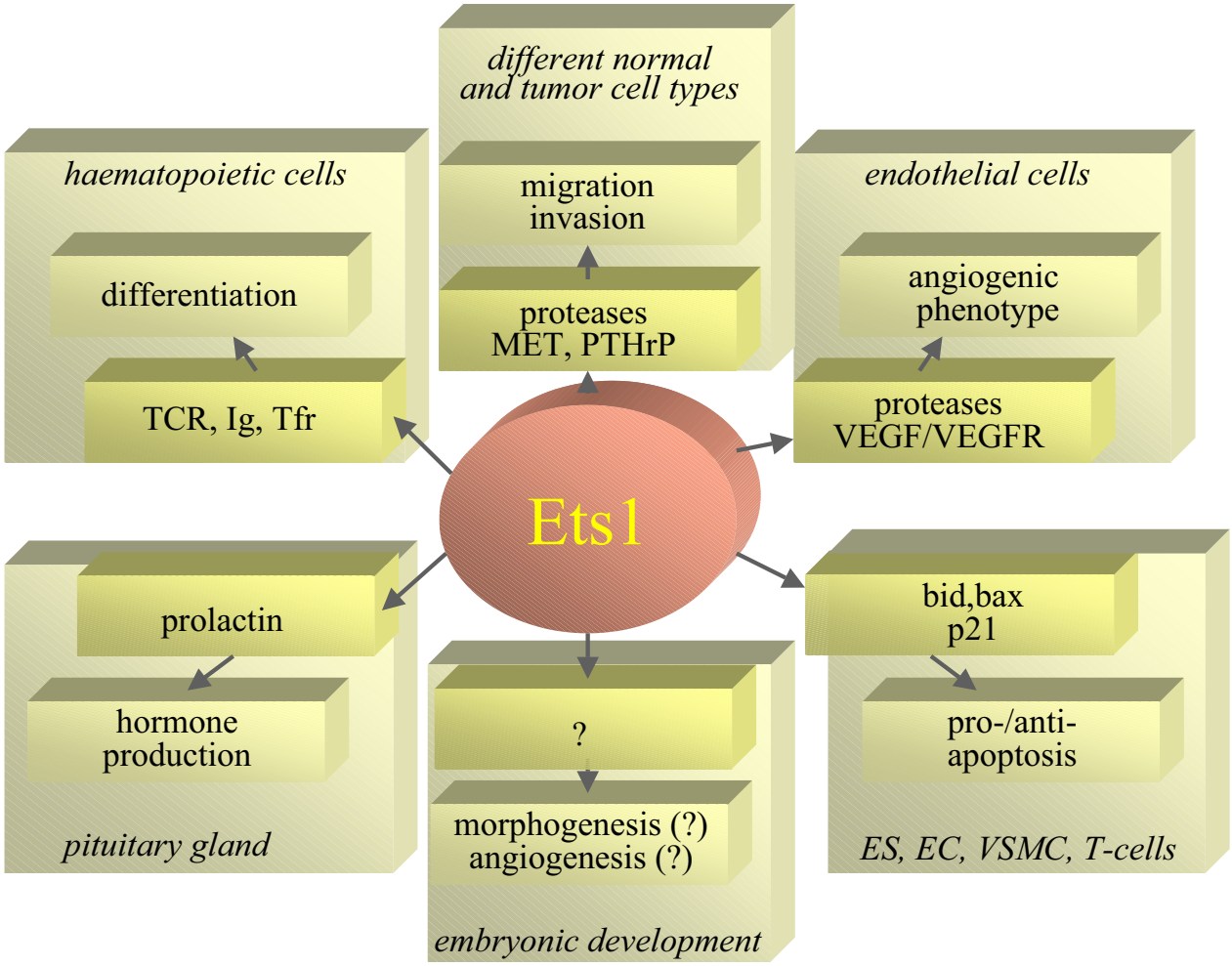


**Figure 6**
**Main proposed functions of Ets1 in mammals.** Only some of the potential Ets1 target genes are shown, ES = embryonic stem cells, EC = endothelial cells, VSMC = vascular smooth muscle cells, TCR = T-cell receptor, Ig = immunoglobulin, Tfr = transferrin receptor, VEGF = vascular endothelial growth factor, VEGFR = VEGF receptor

by cooperating with TFE3 and the distantly related Ets factor PU.1 has also been suggested [46]. In addition, it seems that Ets1 can inhibit the differentiation of B-cells to plasma cells [171,172]. In erythroid differentiation, Ets1 expression is linked to hemoglobinization [103]. Ets1 is suggested to regulate genes that are involved in heme synthesis [174]. Accordingly, Ets1-interacting protein MafB downregulates transferrin receptor gene and porphobilinogen deaminase expression by repressing Ets1 activity [88]. The haematopoietic transcription factor c-Myb cooperates with Ets1 to transactivate the promoters of important haematopoietic cell markers, such as mim-1 and α4 integrin [67,68]. Also janus kinase 3 (Jak3), a non-receptor tyrosine kinase that is predominantly expressed in haematopoietic cells and that is important for cytokine-mediated activation, is under the control of Ets transcription factors [175]. Chromatin immunoprecipitation assays revealed that Ets1/2 binds to the Jak3 promoter.

#### *Invasiveness and tumor progression*

The Ets1 protein has oncogenic potential. It is able to transform murine NIH3T3 cells [162] and it allows rat embryo fibroblasts to grow in serum-free medium [176]. There is also a growing body of evidence that Ets1 plays a key role in the acquisition of an invasive behavior. How could Ets1 regulate invasiveness? Among the genes that respond to Ets1 are those that code for certain proteases, such as matrix metalloproteases MMP-1, MMP-3, MMP-9,

and urokinase type plasminogen activator (uPA) [22]. These proteases are known to be involved in ECM (extracellular matrix)-degradion, a key event in invasion. In angiosarcoma of the skin, Ets1 is co-expressed with MMP-1 [151]. Ovarial carcinoma cells and stromal fibroblasts in breast and ovarian cancer produce MMP-1 and MMP-9 along with Ets1 [135,145]. In lung and brain tumors, Ets1 expression correlates with that of uPA [134,144,177]. Similarily, some maternal cell populations during mouse implantation and placentation were found to co-express uPA and Ets1 [178]. However, not always does Ets1 and uPA production overlap, as shown for trophoblasts that heavily synthesize uPA although they lack Ets1 [178]. By modulating Ets1 activity further evidence for a link between Ets1 and certain proteases could be accumulated. Inhibition of Ets1 expression in activated endothelial cells by anti-sense DNA reduced the synthesis of MMP-1 and uPA [110–112,115]. Introduction of a trans-dominant negative form of Ets1 into Ets1-expressing epithelial cell lines blunted the activity of uPA [155]. In invasive MDA-MB-231 breast cancer cells, RNA interference-mediated downregulation of Ets1 down-modulated the expression of MMP-1 and MMP-9, but not that of MMP-3 and uPA (J. Dittmer, unpublished results). When overexpressed in endothelial cells or hepatoma cells, Ets1 induced the production of MMP-1, MMP-3 plus MMP-9 or MMP-1, MMP-9 plus uPA, respectively [113,179,180]. It seems that the cellular context dictates whether a certain protease is regulated by Ets1 or not.

The integrin $\alpha_v$ subunit also promotes migration and invasion of cells and is involved in MMP activation [181]. In glioma cells, expression of the *integrin* $\alpha_v$ gene, which contains Ets binding sites [182], was found to be reduced in the presence of a transdominant-negative form of Ets1 [183]. There is also growing evidence demonstrating that Ets1 may be involved in the regulation of c-Met, the receptor of hepatocyte growth factor/scatter factor (HGF/SF). c-Met induces migration ("scattering"), proliferation and epithelial tube formation [184]. In esophageal cancer, Met expression was found to correlate with that of Ets1 [141]. In murine liver progenitor cells and hepatoma cell lines, Ets1 was shown to induce c-Met expression and to render hepatoma cells more susceptible to HGF/SF [180,185]. Also Epstein-Barr virus specific LMP-1 (latent membrane protein-1)-mediated induction of Ets1 expression in MDCK epithelial cells resulted in an increased production of c-Met, which could be prevented by addition of a trans-dominant negative form of Ets1 [186]. In endothelial cells, c-Met and HGF/SF expression could be reduced by anti-sense DNA directed against Ets1 [115] and high glucose treatment decreased both HGF/SF and Ets1 [187]. Conversely, c-Met also activates Ets1, as HGF/SF is able to stimulate Ets1 activity in MDCK cells through the Ras/Raf/MEK1/ERK1/2 pathway [59] and to induce Ets1 expression in endothelial cells [115]. Ets1 may even be an effector of c-Met. Not only was Ets1 found to mediate the effect of HGF/SF on MMP1 in human hepatic stellate cells [188], but it was also demonstrated to be capable of mimicking c-Met in its ability to induce "scattering" of liver progenitor cells [185].

A number of other Ets1-responsive genes are known to be involved in tumor progression. Among them is PTHrP [80,189], expressed by numerous tumors and inducer of hypercalcaemia of malignancy [190]. It is not only a potent angiogenic factor [191], but also promotes expansion of metastatic breast cancer cells in the bone by inducing bone degradation [192]. This leads to activation of TGFβ which further stimulates PTHrP gene expression. The TGFβ effect on PTHrP involves a synergistic action between Ets1 and Smad3 and is attentuated by inhibitors that abrogate Ets1 expression [77].

Overexpression of the multidrug resistance (MDR) gene increases aggressive behavior of MCF-7 cells [193]. Mutant, but not wildtype, p53 was found to cooperate with Ets1 to increase the transcription from the MDR gene [75]. The N-acetylglucosaminyltransferase V (GlcNAc-TV) has also been reported to be a target of Ets1 [194]. Expression of GlcNAc-TV, a Golgi enzyme that catalyzes β 1,6-GlcNAc-branching of N-glycans, is associated with metastatic activities of tumor cells [195].

An additional new function of Ets1 emerged when Ets1 was found to interact with Sp100 [45], a component of PML (promyelocytic leukemia protein) oncogenic domains (PODs), nuclear bodies, which harbor also a number of other proteins, such as p53, Rb and Ets1-interacting Daxx/EAPI [196]. It could be shown that Ets1 decreased the number of PODs in HeLa cells [45]. Since PML can act as a tumor suppressor [197], Ets1 may, by interfering with PODs, negatively influence PML function and promote tumorigenesis. Several important cellular processes, such as apoptosis, cell proliferation and senescence, are ascribed to PODs [196]. Ets1 may participate in controlling some of these events. In support of this notion, Ets1 has been shown to be involved in the regulation of apoptosis (see below). Ets1 has also been found to be overexpressed in senescent, but not in young normal human fibroblasts [198]. The effect of Ets1 on PODs may involve sumoylation. By sumoylation an approximately 100 kD protein, called SUMO (small ubiquitin-like modifier), is ligated to another protein [199]. This process requires ubiquitin conjugating enzyme Ubc9 which has been shown to interact with Ets1 and to enhance its transcriptional activity [85]. Sumoylation of TEL, an Ets repressor protein, leads to the formation of TEL nuclear bodies [200]. This process requires Ubc9 to bind to the POINTED domain of TEL. Sumoylation seems to be

important for packaging proteins in nuclear bodies and may effect the activity of transcription factors.

### *Angiogenesis*

It has been well documented that Ets1 is required for endothelial cells to adopt an angiogenic, blood vessel forming phenotype [110–113,201]. Since acquisition of invasive behavior is part of the endothelial activation program, Ets1 may be responsible for stimulating the necessary proteases [202]. However, it seems that Ets1 fulfills other functions in angiogenesis as well [203]. Antisense DNA directed against Ets1 downregulated VEGF expression in endothelial cells [115] suggesting that the VEGF production by endothelial cells is regulated by Ets1. Furthermore, VEGF was found to be co-expressed together with Ets1 in the vascular stroma of endometrial and colorectal carcinoma [125,126]. Ets1 was also found to activate the promoter of the endothelial VEGF receptor 1 gene, *flt-1*, [204]. Accordingly, production of Ets1 and of the VEGF receptor 1 (VEGFR-1) was co-induced in endothelial cells that was co-cultured with estrogen-treated breast cancer cells [201]. Also, endothelial expression of VEGFR-1 correlated with that of Ets1 in astrocytic cancers [23]. In addition, by cooperating with the hypoxia-inducible factor HIF-2α, Ets1 is able to induce transcription from the VEGFR-2 (VEGF receptor 2) gene (*flk-1*) promoter [64]. However, in contrast to VEGFR-1 production, VEGFR-2 expression was not associated with Ets1 expression in astrocytic cancers [23]. The RNA level of neuropilin, an enhancer of the VEGF-A effect, was also found to be linked to Ets1 activity. It was increased in the presence of Ets1 and decreased when Ets1 activity was suppressed [205]. Thus, Ets1 seems be involved in the regulation of activities of VEGF and its receptors. As was proposed for the c-Met/HGF/SF-Ets1 cooperativity, a positive feedback loop may exist for the Ets1/VEGF interaction. By inducing Ets1 expression, VEGF may trigger its own production leading to further Ets1 synthesis, finally resulting in endothelial cell activation. However, the function of Ets1 in endothelial cells may be more complex. Overexpression of Ets1 can also lead to reduced growth activity of endothelial cells at higher cellular density [206]. This is accompanied by an increased expression of VE-cadherin, which plays an important role in vascular morphogenesis and growth control.

### *Apoptosis*

There are conflicting data on the role of Ets1 in apoptosis. On the one hand, Ets1-deficiency increases T-cells apoptosis [171,172] and overexpression of Ets1 protects VSMCs from undergoing apoptosis by activating $p21^{WAF1/Cip1}$ [207]. On the other hand, deletion of the Ets1 gene cells render embryonic stem cells resistant to UV-induced apoptosis [76]. In this case, Ets1 acts synergistically with wildtype p53 to activate p53-responsive pro-apoptotic genes, such as *bax*. Similarly, Ets1 induces apoptosis in endothelial cells by stimulating the expression of pro-apoptotic genes, such as *bid* [202,208]. In colon cancer cells, Ets1 cooperates with Daxx/EAPI to suppress expression of the anti-apoptotic Bcl2 [87]. This effect may be linked to the ability of Ets1 to regulate the activity of PODs, since PODs contain Daxx/EAPI (see above). Also in colon cancer cells, the natural Ets1 variant ΔVII-Ets1 was found to induce the expression of caspase 1 and to render these cells more susceptible to Fas-mediated apoptosis [209]. When overexpressed in invasive breast cancer cells, ΔVII-Ets1 reduced the survival of these cells [97]. Invasive cancer cells that depend on Ets1 may, therefore, be forced to protect themselves against the pro-apoptotic effect of Ets1, in particular of ΔVII-Ets1. One way to do this is by adopting a mechanism that selectively suppress expression of ΔVII-Ets1 [97]. T-cells in the contrary, where Ets1 acts anti-apoptotically, may tolerate high protein levels of ΔVII-Ets1.

### *Other functions*

The presence of Ets1 in a variety of murine embryonic tissues, particular in the developing vascular system, in angiogenically active tissues and in those organs that undergo morphogenesis, suggest a fundamental role of Ets1 in embryonic development. Yet, Ets1 k.o. mice develop normally and are viable, although they show an increased perinatal mortality [171]. On the other hand, when Ets1 was downregulated together with Ets2, chicken embryos displayed defects in the epicardium, the coronary circulation and in the myocardium [210] that resembled those found in mice deficient in VCAM-1 (vascular cell adhesion molecule 1) and α4 integrin [211,212], two Ets-responsive genes [67,213]. Since no obvious effect on the vascular system was reported when the Ets2 gene alone was disrupted in mouse [214] it is likely that a double-knock down of Ets1 plus Ets2 is required to induce defects in the developing vascular system. Since Ets1 and Ets2 expression overlap in the developing embryo at some areas, such as in the developing heart [156], Ets2 may partially substitute for Ets1 in embryonic development. Several genes involved in embryonic development have been found to be Ets1-responsive. Among these are the gene that codes for transcription factors AP-2α (activating protein 2α) which plays a pivotal role in differentiation of the Ets1-expressing trophoblast [215].

The interaction of Ets1 with transcription factors, such as AML-1, Pit-1 or Pax5 whose expression is limited to certain tissues, predicts that Ets1 serves functions in specific tissues. As discussed above, AML-1/Ets1 and Pax-5/Ets1 cooperations may play important roles in T- and B-cell differentiation, respectively. Pit-1 is specifically produced by the anterior pituitary gland, where it is required for the expression of various genes and for the development of

this gland [216,217]. Pit-1 cooperates with pituitary Ets1 to stimulate prolactin synthesis [218,219] suggesting that Ets1 plays also a role in pituitary hormone secretion. Interaction of Ets1 with USF (upstream stimulatory factor) [86] seems to mediate Ets1-dependent transactivation of the DOR (δ-opioid receptor) gene through an EBS (Ets binding site)/E-box combinatorial element in neuronal cells [220].

Ets1 is also involved in viral transformation. Ets1 is important for mediating transcriptional activation of specific genes by the transforming viral protein Tax, a transactivator that is encoded by the region X of the human T-cell lymphotropic virus I (HTLV-I). Being unable to bind to DNA by itself, Tax interacts with transcription factors, to get in close contact with the transcriptional machinery [221]. Tax can bind directly to Ets1 and can form a ternary complex with Ets1 and Sp1 [65]. Tax/Ets1 synergism is involved in Tax-mediated activation of the PTHrP and interleukin-5 promoter as well as of the HTLV-I LTR (long terminal repeat) [63,189,222]. Ets1 was also shown to be required for efficient transcription from the LTR of the human immunodeficiency virus-1 (HIV-1) [69,86]. Accordingly, transdominant-negative Ets1 mutant protein was found to suppress HIV replication in T-cells [223].

## Conclusions

The currently available data demonstrate distinct roles for Ets1 in haematopoietic cell differentiation. Ets1 seems also to participate in the regulation of invasive behavior of many normal and tumor cells alike. Adoption of invasive behavior is an important step for endothelial cells to convert to an angiogenic phenotype. Accordingly, Ets1 is abundant in regenerating adult tissues or in areas of the developing embryo that require new blood vessel to be formed. Likewise, Ets1 is overexpressed in tissues under pathological conditions that involve angiogenic activities. The link between Ets1 and invasiveness is not limited to endothelial cells, also vascular smooth muscle, epithelial and fibroblastic cells seem to need Ets1 to become invasive. For epithelial cancer, Ets1 may fulfill a dual function; it may provide the cancer cells with nutrients and oxygen by inducing tumor vascularization and it may promote tumor invasion by activating ECM-degrading proteases in the cancer and/or in stromal cells. Consequently, high levels of Ets1 in tumors often correlates with poorer prognosis. Therefore, it would be desirable to develop therapies that target the Ets1 gene. It seems that PKC is a major regulator of Ets1 expression in cancer cells. Thus, PKC inhibitors, which already entered clinical trials [224], may be a useful tool to control Ets1 activity in cancer.

As mentioned above, the current understanding of the Ets1 function is based on data partially obtained by methods which may not clearly allow distinction between the involvement of Ets1 or that of another Ets protein. In future, more reliable results may be obtained by using the recently developed method of post-transcriptional silencing by RNA interference [225]. In addition, new DNA binding assays, such as chromatin immunoprecipitation assays, may help to demonstrate direct binding of Ets1 to the gene of interest *in vivo*.

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