Review Article

ETS transcription factors: Possible targets for cancer therapy

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Ets family (ETS) transcription factors, characterized by an evolutionally conserved Ets domain, play important roles in cell development, cell differentiation, cell proliferation, apoptosis and tissue remodeling. Most of them are downstream nuclear targets of Ras-MAP kinase signaling, and the deregulation of *ETS* genes results in the malignant transformation of cells. Several *ETS* genes are rearranged in human leukemia and Ewing tumors to produce chimeric oncoproteins. Furthermore, the aberrant expression of several *ETS* genes is often observed in various types of human malignant tumors. Considering that some ETS transcription factors are involved in malignant transformation and tumor progression, including invasion, metastasis and neo-angiogenesis through the activation of cancer-related genes, they could be potential molecular targets for selective cancer therapy. (Cancer Sci 2004; 95: 626–633)

The *Ets* family (*ETS*) in mammals consists of approximately 30 genes homologous to *Ets-1*, the first of the cellular homologues of the viral oncogene v-*ets* in the avian transforming retrovirus E26. The gene products of this family are transcription factors controlling various cellular functions in cooperation with other families of transcription factors and co-factors. All members have an activation or a repression domain for transcription and an evolutionarily conserved Ets domain for DNA binding. The Ets domain was shown to bind the 5′-GGAA/T-3′ core motif of DNA through its wHTH (winged helix-turn-helix) structure, as determined by NMR analysis. Which of the ETS transcription factors interacts with specific binding sequences depends on the adjacent DNA sequences and binding of transcriptional partners.

ETS transcription factors are divided into several subfamilies based on homology within the Ets domain. Some subfamilies have the Ets domains at the C-terminal end, and some at the N-terminal end. Several members are expressed predominantly in certain types of tissues, and some ubiquitously. A schematic illustration of the protein structures and tissue distributions of representative ETS transcription factors is shown in Fig. 1.

The target genes for ETS transcription factors include oncogenes, tumor suppressor genes, apoptosis-related genes, differentiation-related genes, angiogenesis-related genes, and invasion and metastasis-related genes.[1–3] Thus, it is not surprising that the aberrant expression of *ETS* genes contributes to malignant transformation and tumor progression.

In the first part of this review, I will briefly describe the involvement of ETS transcription factors in carcinogenesis. In the second part, I will discuss the theoretical feasibility of ETS-targeted cancer therapy.

1. ETS transcription factors in signal transduction and apoptosis

Abnormalities in signal transduction pathways are often observed in tumors. Some of the final nuclear targets of signaling pathways are ETS transcription factors.[4] Several ETS transcription factors directly control the expression of the immediate-early response genes. It has been proposed that expression of the c-*fos* and *egr-1* genes is repressed by Net (new *ets* factor), a member of the TCF (ternary complex factor) subfamily of ETS transcription factors, through its recruitment of HDACs (histone deacetylases) to their promoters when the Ras-MAP kinase pathway is not activated. When this pathway is activated, other members of the TCF subfamily, Elk-1 (*Ets* like protein-1) and Sap-1 (serum responsive factor accessory protein-1), are phosphorylated and activate gene expression in cooperation with SRF (serum responsive factor) through the recruitment of CBP/p300, HATs (histone acetyltransferases), to the promoters.[5] Under these conditions, Net is also phosphorylated, and converted from a repressor to an activator.

Not only the TCF subfamily but also some ETS transcription factors including Ets-1 and Ets-2 are phosphorylated as a result of the activation of the Ras-MAP kinase signaling pathway and play important roles in regulating the expression of growth- and cell-cycle-related genes, such as c-*myc*, *junB*, *cdc2* and *cyclin D1*.[1, 6] Thus, some ETS transcription factors function as critical transcription factors to convert the Ras signal to the expression of a set of several growth-related genes.

Furthermore, the aberrant expression of apoptosis-related genes as well as growth-related genes has been observed in many human tumors. In general, the expression of anti-apoptotic genes such as *bcl-2* and *bcl-XL* is up-regulated, while that of pro-apoptotic genes such as *bax* is down-regulated in tumors with a poor prognosis. It has been reported recently that Ets-binding sites located in the promoters of *bcl-2* and *bcl-XL* are critical to the expression of these genes in hematopoietic cells.[7, 8] Thus, some of the ETS transcription factors regulate the expression of not only several growth-related genes but also some apoptosis-related genes.

2. Aberrant expression of ETS transcription factors in solid tumors

There are reports that the overexpression of a certain growth factor receptor in several types of human solid tumors leads to the constitutive activation of tyrosine kinases in the cells. The expression of some of these growth factor receptor genes is regulated by ETS transcription factors. For example, Ets-binding sites are located in the promoter of the human epidermal growth factor receptor-2 (*HER2/ErbB2/neu*) gene, the expression of which is regulated by ETS transcription factors, such as Ets-1, PEA3/E1AF and ESX/ESE-1.[9] The *HER2* gene is one of several oncogenes encoding receptor-type tyrosine kinases and is amplified in 20–30% of breast, ovarian and gastric cancers. The tumors having this change are generally more aggres-

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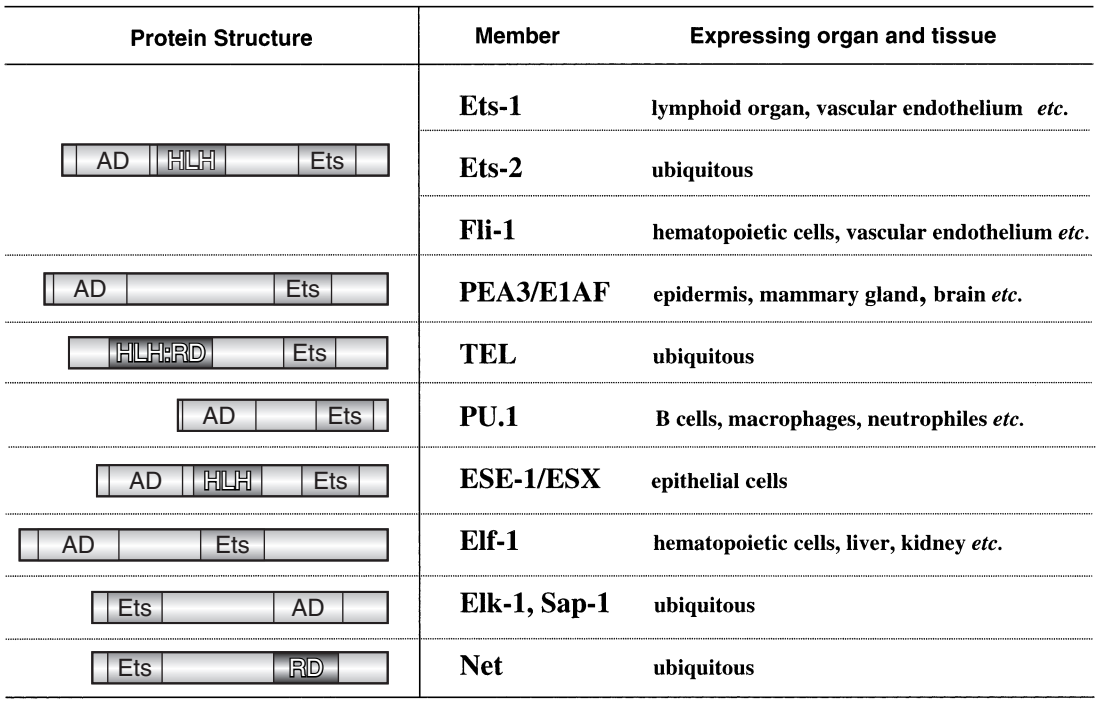

Fig. 1. Protein structures and tissue distributions of major ETS transcription factors. ETS transcription factors are divided into several subfamilies based on homology within the Ets DNA binding domain. Some subfamilies have the Ets domain at the C-terminal end, and some at the N-terminal end. Some ETS transcription factors have an HLH domain necessary for protein-protein interactions. Several members are expressed predominantly in certain types of tissues, and some ubiquitously.

Table 1. Aberrant expression of ETS transcription factors in human cancer

Member	Abnormality	Type of tumor
Ets-1	Overexpression	Many invasive tumors
Fli-1	Chromosome translocation (*EWS-Fli-1*)	Ewing tumor [t(11;22)]
Erg	Chromosome translocation (*EWS-Erg*)	Ewing tumor [t(21;22)]
	Chromosome translocation (*FUS-Erg*)	AML [t(16;21)]
E1AF/PEA3	Chromosome translocation (*EWS-E1AF*)	Ewing tumor [t(17;22)]
	Overexpression	Breast cancer etc.
ER81	Chromosome translocation (*EWS-ER81*)	Ewing tumor [t(7;22)]
ESE-1/ESX	Overexpression	Breast cancer etc.
PU.1	Mutation	AML (~7%)[1]
	Downregulation	AML [t(8;21)]
TEL	Chromosome translocation (*TEL-PDGFRβ*)	CMMoL [t(5;12)]
	Chromosome translocation (*TEL-AML1*), *del*	AML [t(12;21)]
	Chromosome translocation (*TEL-TrkC*)	Congenital fibrosarcoma [t(12;15)]

1) Conflicting. *del*, deletion; AML, acute myelogenous leukemia; CMMoL, chronic myelomonocytic leukemia.

sive, with a poor prognosis, indicating that HER2 is a useful prognostic marker for cancer.[10] It has been reported that overexpression of the *HER2* gene in less malignant mammary tumor cells resulted in neo-angiogenesis, invasion and metastasis, and the acquisition of resistance to chemotherapy, hormone therapy and radiation therapy. In tumors overexpressing HER2, the ETS proteins themselves are activated by phosphorylation, suggesting that they are targets for signal transduction via the HER2 receptor. Expression of the M-CSF receptor, c-Kit and, VEGF receptors is also regulated by ETS transcription factors and the activation of these receptors also leads to the phosphorylation of ETS transcription factors.

Enhanced expression of *Ets-1* is observed in many types of human tumors (Table 1). The level of Ets-1 has been associated with the grade of malignancy and prognosis in several types of tumors including breast cancer, lung cancer and colorectal cancer. *Smad4* encoding a downstream target of the TGF-β signaling pathway is often deleted in colon cancer and pancreas cancer. Transduction of *Smad4* in pancreatic cancer cells with deletion of *Smad4* resulted in inhibition of tumor growth in immunodeficient mice accompanied with down-regulation of VEGF and MMP-9 expression.[11] Interestingly, pancreatic tumor cells with a homozygous deletion of *Smad4* are more aggressive in terms of invasion, with a high expression level of Ets-1, suggesting a negative reciprocal regulation between *Smad4* and *Ets-1*.

Ewing tumors are characterized by specific chromosome translocations involving the *EWS* and *ETS* genes (Table 1).

These translocations are t(11;22)(q24;q12) (*EWS-Fli-1*), t(21;22)(q22;q12) (*EWS-Erg*), t(17;22)(q12;q12) (*EWS-E1AF*) and t(7;22)(p22;q12) (*EWS-ER81*). It is clear that these aberrant EWS-ETS fusion products are responsible for the malignant transformation, since the fusion proteins have the ability to transform HIH/3T3 cells.[12] The most prevalent chromosome translocation in Ewing tumors is t(11;22), whereby the N-terminal region of EWS including the transactivation domain fuses with the C-terminal region of Fli-1 including the DNA binding domain. It has been reported that the expression of the chimeric protein EWS-Fli-1 leads to a strong up-regulation of c-*myc* and a considerable down-regulation of $p57^{KIP2}$ expression in several cell lines including HeLa and neuroblastoma cells.[13] Furthermore, up-regulation of *Id2* (inhibitor of DNA binding 2) and down-regulation of $p21^{WAF1}$ gene expression has been documented in Ewing family tumors.[14] Moreover, the introduction of *EWS-Fli-1* suppresses transcription of the *TGF-β RII* (transforming growth factor β type II receptor) gene by binding to its promoter and reduces sensitivity to TGF-β, whereas the introduction of antisense oligonucleotides against *EWS-Fli-1* into cells positive for this fusion gene restores TGF-β RII expression and TGF-β sensitivity.[15] Thus, TGF-β RII is a direct target of the EWS-Fli-1 fusion protein. EWS-Fli-1 acts as a dominant negative form of Fli-1 in the TGF-β RII promoter. On the other hand, EWS-Fli-1 acts as a more potent transcriptional activator than the wild type of Fli-1 in several promoters. These differences might be due to cooperation with other transcription factors and co-factors involving different DNA sequences in the regulatory region of the target genes.

2.1 ETS transcription factors in tumor invasion and metastasis

Several ETS transcription factors are involved not only in the malignant transformation of cells but also in the promotion and progression of tumors by activating invasion and metastasis-related genes. Ets-1 is co-expressed with MMP-1 (matrix metalloproteinase-1) and uPA (urokinase-type plasminogen activator) in various types of tumors. Co-expression of Ets-1, MMP-2 and MMP-9 has been reported in pancreatic cancer and high expression levels of these genes are a poor prognostic marker in these tumors.[11] As shown in Fig. 2, there are Ets binding sites adjacent to AP1 binding sites in the promoter and/or enhancer regions of the *uPA* gene and several *MMP* genes including the *MMP-1/type I collagenase*, *MMP-3/stromelysin*, *MMP-7/matrilysin* and *MMP-9/type IV collagenase* genes. The expression of these genes is mainly regulated by the transcription factors ETS and AP1. We also reported that expression of the *uPA* gene was augmented by exogenous expression of PU.1 in HT1080 fibrosarcoma cells.[16] The region containing an ETS binding site adjacent to an AP1 binding site is called a Ras responsive element (RRE), since such elements are often found in the regulatory regions of the genes responsive to Ras signaling.[17] It has been reported recently that the promoter activity of MMP-7, highly expressed in colon cancer, is synergistically regulated by the PEA3 subfamily of ETS transcription factors and c-Jun, a component of AP1, in cooperation with β-catenin-LEF-1.[18] In liver cancer, Ets-1 is involved in invasiveness and metastasis by up-regulating the expression of MMP-7 and GnT-V (*N*-acetylglucosaminyl transferase). The expression level of the *osteopontin* (*OPN*) gene is also a prognostic marker in several tumors.[19] The RRE in the regulatory region is critical for the expression of the *OPN* gene. Thus, some ETS transcription factors regulate many invasion and metastasis-related genes in various types of tumor cells.

2.2 ETS transcription factors involved in tumor angiogenesis

Several ETS transcription factors are expressed in vascular endothelial cells and are thought to play central roles in angiogenesis. When vascular endothelial cells are stimulated with angiogenic factors such as VEGF, bFGF (basic fibroblast growth factor) and TNFα (tumor necrosis factor α), expression of Ets-1 is temporarily induced, followed by the expression of cell cycle-related genes such as c-*myc* and of the genes involved in the degradation of the extracellular matrix (ECM), such as the *uPA* and *MMP* genes, to induce the migration and invasion of endothelial cells. The gene expression of αv and β3 integrins, VE cadherin, ICAMs (intracellular adhesion molecules) and vWF (von Willebrand factor), necessary for cell attachment and the migration of cells, is controlled by Ets-1.[20] ETS binding sites are located in the regulatory regions of the VEGF receptor genes, *flt-1* and *flk-1*, and the angiopoietin receptor gene, *Tie-2*. ETS binding sites are also conserved in the regulatory regions of the mouse and human *VEGFR-3* gene, which is involved in lymphangiogenesis. Two different ETS transcription factors, Fli-1and Elf-1, in cooperation with GATA-2, are responsible for activation of the *SCL* (stem cell leukemia) enhancer in hemangioblasts, the bipotential precursors for hematopoietic stem cells and endothelium.[21]

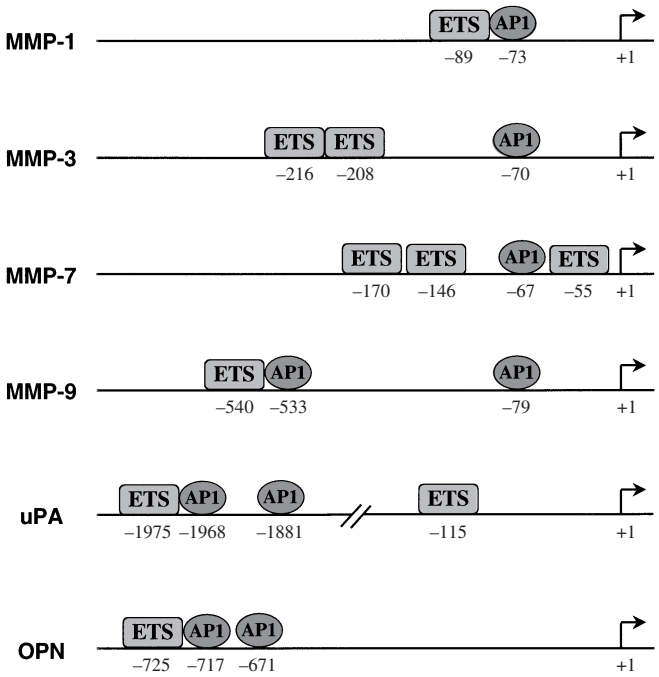

Fig. 2. Involvement of ETS transcription factors in regulation of the expression of invasion and metastasis-related genes. A Ras responsive element (RRE) consisting of an ETS binding site adjacent to an AP1 binding site is located in the promoters or enhancers of several invasion and metastasis-related genes, such as the *matrix metalloproteinase* (*MMP*), *urokinase-type plasminogen activator* (*uPA*) and *osteopontin* (*OPN*) genes.

Mice in which *Net*, a member of the TCF subfamily of *ETS* genes, was knocked out have been reported to die soon after birth with an abnormality of lymphatic vessels, suggesting that Net is involved in lymphangiogenesis. On the other hand, despite the importance of Ets-1 in angiogenesis, knockout of *Ets-1* caused no abnormality in blood vessels and lymphatic vessels in mice, although the mice showed a reduced number of T cells with B cell dysfunction. It is speculated that other ETS transcription factors compensate for the function of Ets-1 in vascular endothelial cells, since several members including Ets-1, Ets-2, Fli-1, Erg, Elf-1, NERF and TEL are expressed in vascular endothelial cells.

Neo-angiogenesis is necessary for supply of nutrition and oxygen to and for removal of metabolic waste from tumors, since the center of the tumor mass is hypoxic. Intratumoral hypoxia

leads to the overexpression of HIF-1α, a component of HIF (hypoxia-inducible factor), resulting in the subsequent induction of VEGF expression.[22] Interestingly, hypoxia induces Ets-1 expression via the activity of HIF-1. Thus, several ETS transcription factors participate in angiogenesis and lymphangiogenesis.

2.3 ETS transcription factors as prognostic markers of cancer

The expression levels of some ETS transcription factors are prognostic markers for cancer patients. In breast cancer, a high level of Ets-1 expression in stroma as well as in tumor cells has significant prognostic value for relapse-free survival, suggesting that Ets-1 is a strong predictor of poor prognosis in breast cancer.[23] Similar results have been observed in ovarian cancer with a good correlation between the expression of *Ets-1* and invasion-related genes, such as *VEGF*, *bFGF* and *MT1-MMP* (*membrane-type 1-MMP*). The correlation between the expression level of PEA3/E1AF and tumor progression has been documented in ovarian cancer, indicating that PEA3/E1AF is another prognostic marker in ovarian cancer. A relationship between expression of Ets-1 and tumor prognosis has also been reported in gastric and other cancers.

The expression levels of apoptosis-related genes are also of prognostic value in many types of human tumors. Tumor cells positive for Bcl-2 expression and/or negative for Bax expression are resistant to various cancer therapies., It has been reported that Fli-1 directly regulates and activates expression of the *bcl-2* gene in mouse erythroblasts.[7] The EWS-Fli-1 chimeric protein is anti-apoptotic in Ewing tumors. Similarly, expression of the *bcl-XL* gene is regulated by Ets-2 in mouse myeloid leukemia cells. Expression of the c-*rel* gene is regulated by Spi-B, highly homologous to PU.1,[24] in B cells and is involved in blocking apoptosis through the activation of NF-κB.[25] Knockout mice of Ets-1 exhibit a reduced number of T cells, suggesting that Ets-1 might also control cell death. Which of the ETS transcription factors regulates a given apoptosis-related gene appears to depend on the type of cell. Thus, some ETS transcription factors contribute to the inhibition of apoptosis and, therefore, it is possible that resistance to cancer therapy is partly due to the high levels of ETS transcription factors in tumor cells.

3. Aberrant expression of ETS transcription factors in leukemia/lymphoma

Several ETS transcription factors are preferentially expressed in certain lineages of hematopoietic cells and play crucial roles in their development and differentiation.[2, 26, 27]

PU.1 is expressed in B cells, macrophages and neutrophils, but not in mature T cells or erythroid cells. PU.1 controls expression of several lymphoid-specific genes such as the IL-7Rα and immunoglobulin genes, as well as many myelomonocytic cell-specific genes, such as the *M-CSF receptor*, *CD11b/CD18* (*Mac-1*), *myeloperoxidase* (*MPO*) and *neutrophil esterase* genes. *PU.1* knockout mice exhibit a block of cell differentiation in B cells, macrophages and neutrophils, suggesting that PU.1 is necessary not only for their functions, but also for their development.[28] Deregulated expression of *PU.1* is responsible for development of mouse erythroleukemia (MEL) induced by provirus insertion of SFFV (spleen focus forming virus).[29] We showed that overexpression of PU.1 in MEL cells resulted in blockade of erythroid differentiation accompanied with inhibition of cell growth and induction of apoptosis.[30] The PU.1-induced differentiation block in MEL cells seemed to be partly due to due inhibition of *β-globin* gene expression through recruitment of PU.1 with mSin3A/HDACs and MeCP2 complex at the IVS2 region, a candidate regulatory region of the *β-globin* gene.[31, 32] Loss of DNA binding activity of GATA-1, a critical transcription factor for erythroid differentiation, by overexpression of PU.1 might also be a reason for the inhibition of erythroid differentiation.[33, 34] Our results, taken together with those of others, suggest that sustained expression of PU.1 contributes to erythroleukemogenesis in mice at least through blocking cell differentiation. PU.1-induced growth inhibition and apoptosis in MEL cells were probably due to down-regulation of c-Myc and Bcl-2 expression,[35] induction of CKLiK (calcium-dependent calmodulin kinase like kinase) expression,[36] and sequestration of CBP by a large amount of PU.1.[37]

Introduction of PU.1 in multipotent hematopoetic progenitors promotes myeloid lineage commitment, suggesting that PU.1 is a master regulator for myeloid differentiation.[38] This was also true even in our system where phagocytic activity was induced with expression of many myeloid-specific genes in PU.1-overexpressing MEL cells that are committed to the erythroid lineage.[39] In this case, PU.1 probably binds with CBP on the ETS binding sites of the regulatory region of myeloid-specific genes.[40] Thus, it is reasonable to speculate that aberrant expression of PU.1 could be involved in human leukemogenesis as well as development of murine leukemia. Indeed, mutations in PU.1 have been reported in a small fraction of human myeloid leukemias, although the results are still conflicting.[41] In humans, AML-M2 having the chromosome translocation t(8;21) and down-regulation of the expression of PU.1 and C/EBPα,[42] both necessary for myeloid differentiation,[43] was observed as a result of a dominant negative function of the chimeric oncoprotein AML1-ETO acting against the wild-type AML1. Very recently, the importance of PU.1 as well as AML1 and C/EBPα in leukemogenesis in humans has been proposed, since knockdown of the expression of PU.1 induced AML in mice (Tenen DG, personal communication). This is consistent with the many reports indicating that PU.1 is essential for the normal differentiation of myelomonocytic lineages.

TEL (translocation Ets leukemia) is important for angiogenesis in the yolk sac and adult hematopoiesis. Overexpression of TEL has been shown to inhibit megakaryocytic differentiation, but to promote erythroid differentiation, presumably due to protein-protein interaction between TEL and Fli-1.[44, 45] In leukemia and lymphoma, specific chromosome translocations result in the fusion of two genes that sometimes produce chimeric proteins responsible for malignant transformation. The *TEL* gene is often a target for chromosome translocations in human leukemia (Table 1). Many genes including tyrosine kinase genes, such as the *PDGFβ* receptor, *ABL*, *JAK2* genes and *ARG* (*ABL*-related gene),[46] and differentiation-related transcription factor genes, such as the *AML1/RUNX1* gene, have been isolated as partner genes fused with *TEL*.[47] In the former, tyrosine kinases of the fusion partner are constitutively activated by homodimerization through the pointed domain of the TEL protein, which is followed by activation of MAP kinases and STAT5. In the latter, chimeric fusion proteins act as dominant negative mutants against the wild type of AML1. The remaining normal *TEL* allele is often deleted in pre-B cell leukemia harboring *TEL-AML1*, suggesting that *TEL* is a putative tumor suppressor gene.

4. Targeting Ets transcription factors for cancer therapy

ETS transcription factors, such as Ets-1 and PEA3/E1AF, could be candidate molecular targets for selective cancer therapy, since they play important roles in maintenance of the transformed phenotypes of tumor cells as stated above. Ets-1 seems to be one of the most promising candidates, because the targeting of this protein possibly not only inhibits directly the proliferation and resistance to apoptosis of tumor cells, but also inhibits indirectly tumor growth and progression, including invasion and metastasis, through the inhibition of tumor angiogenesis (Fig. 2). Several tyrosine kinase inhibitors such as Glivec (imatinib/ST1571) have been developed for molecular

target therapy in cancer. However, it appears difficult to develop drugs that directly block the action of ETS transcription factors. Accordingly, the following methods have been developed experimentally.

4.1 Use of dominant negative mutants

An experimental approach to the use of dominant negative forms of oncogenes for cancer therapy has been tried in a variety of oncogenes, including *ras*. Such an approach is also applicable to the *ETS* genes. As stated above, ETS transcription factors are targets for Ras signaling. The introduction of an expression plasmid containing the ETS domain of Ets-1 or Ets-2 into NIH/3T3 cells transformed with the *ras* oncogene resulted in the inhibition of transformed phenotypes as monitored in terms of the morphology of cells, serum requirement and anchorage-dependent cell growth in culture, and tumor growth, invasion and metastasis in immunodeficient mice.[48] It has also been reported that growth inhibition and apoptosis were induced by introduction of an expression plasmid of the ETS domain of Ets-2 in prostate cancer and thyroid cancer. It is suggested that the mutant protein of the ETS domain alone acted as a dominant negative form against the wild types of ETS transcription factors acting on the promoters of growth- and apoptosis-related genes. Similar inhibitory effects have been reported in signal transduction via the HER2/ErbB2/neu and M-CSF receptors in breast cancer using Ets-1 and Ets-2 dominant negative mutants.

A recent report showed that the introduction of a dominant negative mutant of Ets-1 effectively inhibited neo-angiogenesis *in vivo* as well as the growth of tumor cells in culture. Neo-angiogenesis induced by local inoculation of FGF in mouse ears was significantly inhibited by intravenous injection of a retroviral expression vector for Ets-1 with deletion of its activation domain.[49] This dominant negative mutant inhibited not only growth factor-induced but also tumor-induced neo-angiogenesis, which led to growth inhibition of tumor cells in mice. Therefore, such an approach may be applicable to human cancer treatment, since no severe side effects were observed in other organs or blood vessels in the whole body, except for tumor microvessels, at least in this experimental system.

4.2 Use of repressive ETS transcription factors

TEL, a member of the ETS transcription factors, has been suggested to function as a tumor suppressor protein as stated above. This is supported by the experimental findings that the introduction of an expression vector for *TEL* in *ras*-transformed NIH/3T3 cells caused inhibition of cell growth, colony formation in soft agar and MMP-3/stromelysin expression.[50] Furthermore, TEL protein has been shown to undergo homodimerization with itself and heterodimerization with Fli-1, another of the ETS transcription factors, to repress the function of Fli-1. Fli-1 has been shown to be one of the transcription factors that activate the promoters of anti-apoptotic genes.[7] Thus, it is possible that the introduction of an expression vector for *TEL* enhances sensitivity to apoptotic cell death in tumor cells. Indeed, it has recently been reported that apoptosis induced by serum depletion was enhanced by the introduction of exogenous *TEL* in NIH/3T3 cells through the suppression of *bcl-XL* mRNA and protein expression.[51]

Among ETS transcription factors, ERF (Ets-2 repressor factor) and METS (mitogen Ets transcription suppressor) function as repressors to inhibit growth-related genes such as c-*myc* and *cdc2* but they do not inhibit differentiation-related genes.[52] Thus, it is possible that they could be used as antagonists against ETS transcriptional activators. Since the phosphorylation of ERF changes its subcellular localization from the nucleus to cytoplasm, mutant forms of ERF having mutations at the phosphorylation sites may augment the suppressive effect on cell proliferation and tumorigenicity. Chimeric proteins fused with the DNA binding domains of ETS transcriptional activators and the transrepression domains of ETS transcriptional repressors may also augment the inhibitory effects of the dominant mutants of ETS transcription factors.

Another approach to inhibiting tumor growth is to use the wild type *ETS* genes, since the introduction of some *ETS* genes unexpectedly inhibited cell growth in several tumors. Forced expression of a p42 variant of Ets protein in colon cancer promoted apoptosis by increasing the expression of caspase 1 and decreasing the expression of Bcl-2.[53] The introduction of the *PEA3* expression vector with liposomes into breast cancer cells expressing high levels of *HER2/neu* resulted in suppression of HER2 expression and prolonged survival with inhibited tumor growth in mice.[54] However, even if this approach is effective, it may have limitations for clinical application, because most of the wild type *ETS* genes are probably oncogenic.

4.3 Use of antisense oligonucleotides

The use of antisense oligonucleotides was developed as a method to inhibit specifically the translational process for target gene transcripts via hybridization with a single strand DNA of around 20mer. Antisense oligonucleotides for the c-*myc*, *myb* and *mdm2* oncogenes or *bcl-2* and *bcl-XL* anti-apoptotic genes have been used experimentally in a variety of tumors.[55] Treatment of tumor cells with antisense oligonucleotides against oncogenes inhibits the transformed phenotypes and increases apoptosis and their sensitivity to drugs. Treatment of glioma cells with antisense oligonucleotides against *Ets-1* inhibited cell migration and invasion, accompanied with down-regulation of *Ets-1* and *uPA* expression.[56] Treatment of squamous carcinoma cells with antisense oligonucleotides against *E1AF* inhibited the invasive capacity of tumor cells accompanied with down-regulation of HGF-induced MMP-9 expression. Moreover, treatment with antisense oligonucleotides against Ets-1 effectively inhibited the expression of VEGF, HGF and c-*met* in human endothelial cells and vascular smooth muscle cells. So far, however, satisfactory results in cancer treatment have not been obtained by the *in vivo* use of antisense oligonucleotides due to their fragility and toxicity, although many successful examples *in vitro* have been reported. Therefore, artificial ribozymes were developed instead of antisense oligonucleotides to cut out specific sequences in the substrate mRNA of target genes. Furthermore, RNA interference using small double-stranded siRNA (small interfering RNA) has also been applied.

4.4 Use of RNA interference

The knockdown of mRNA from specific genes is easily carried out using the method of RNA interference. This is a process whereby the introduction into the cells of a 20–25mer siRNA against the specific DNA sequences of a target gene effectively inhibits the translation of the target gene by cutting the mRNA. The effectiveness of this method has recently been shown in the treatment of severe hepatitis induced by anti-Fas antibody in mice as an animal model of fulminant hepatitis. In this experiment, about 80% of mice treated with siRNA against Fas survived longer than 10 days, while untreated mice died of severe hepatitis within 3 days.[57] The use of siRNA is applicable to various types of human diseases, including cancer as well as viral infections.

As already stated, the formation of a chimeric oncoprotein due to a specific chromosome translocation is one step in carcinogenesis. Therefore, in these cases, the use of siRNA against DNA sequences at the breakpoint of a specific chromosome translocation, which does not exist in normal cells, could be expected to inhibit production of the chimeric protein and so kill only tumor cells, with no damage to normal cells. At least *in vitro*, the effectiveness of this strategy has been proven in

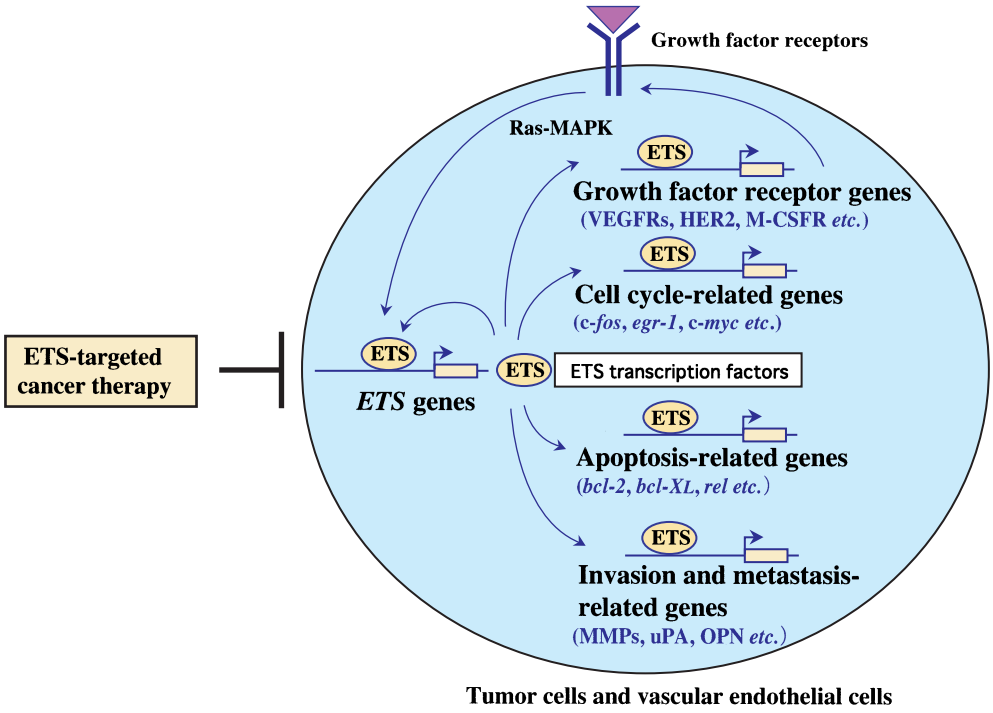

Fig. 3. The theoretical feasibility of ETS-targeted cancer therapy. ETS transcription factors are critical for the regulation of expression of several growth factor receptor genes and cell cycle-, apoptosis-, angiogenesis-, and invasion and metastasis-related genes. Thus, ETS-targeted cancer therapy could be a novel approach to inhibit expression of these cancer-related genes.

chronic myelogenous leukemia (CML) having the *BCR-ABL* chimeric gene with t(9;22) translocation and acute myelogenous leukemia (AML-M2) having the *AML1-ETO* chimeric gene with t(8;21) translocation.[58, 59] In Ewing tumors, siRNA has been used to target the chimeric gene *EWS-Fli-1*. Since *TEL*, a member of the *ETS* genes, is often involved in chromosome translocations in human leukemia as mentioned in Section 3, a similar approach could be applicable to these cases.

Not only such fusion genes, but also the *ras* oncogene, anti-apoptotic *bcl-2* gene and *telomerase* gene could be targets of RNA interference for cancer treatment, since they are activated in most tumors.[60, 61] Several *ETS* genes could also be potential targets in this sense. At the present time, however, it is necessary to verify the effectiveness of siRNA, because effectiveness depends on the design of the siRNAs. For clinical applications, it is also necessary to develop several kinds of expression vectors for the stable, cell type-specific expression of siRNA.

5. Conclusion and perspectives

There are several clinical reports that Glivec and Herceptin (a recombinant humanized anti-HER-2 monoclonal antibody), which were developed for molecular therapy targeting tyrosine kinases, are effective against certain types of tumors.[62, 63] However, there may be restrictions to the use of this new therapy, since several signal transduction abnormalities are observed in tumor cells and the suppression of one of these signals does not always inhibit all of the transformed phenotypes of tumor cells.

On the other hand, the targeting of a transcription factor might inhibit several cancer-related genes, since it regulates several downstream target genes. However, there are very few reports that the actions of transcription factors and co-factors are inhibited by drugs, compared with the reports of therapy targeting the molecules involved in signal transduction. This may be attributed to the difficulty in developing inhibitors specific for a certain type of transcription factor or co-factor, rather than tyrosine kinase inhibitors. Tricostatin A (TSA) or a structurally related substance, which inhibits histone deacetylases (HDAC), and retinoic acid (RA) or its derivatives, which in turn regulate nuclear hormone receptors, were re-evaluated from the point of view of molecular targeting therapy.[64, 65] Furthermore, novel immunotherapies against some transcription factors have recently been documented. Peptide-vaccine therapy targeting the transcription factor WT-1 in leukemia and DNA-vaccine therapy targeting the chimeric oncogenic transcription factor PML-RARα in acute promyelocytic leukemia (APL) are being watched with keen interest.[66, 67]

Attempts at cancer therapy targeting transcription factors have been carried out as gene therapy by transducing tumor suppressor genes or genes that antagonize the actions of oncogenes. Indeed, clinical application of the tumor suppressor gene *p53* has been tried in various human tumors including lung cancer in Japan, as well as other countries.[68] So far, however, no clinical trials of gene therapies targeting ETS transcription factors have been reported, although there are many experimental reports showing that some ETS transcription factors, especially Ets-1, are effective at inhibiting cell growth, metastasis and tumor angiogenesis.

Considering that some ETS transcription factors regulate growth-, apoptosis-, angiogenesis-, and invasion and metastasis-related genes in tumor cells, these transcription factors could be molecular targets for cancer gene therapy (Fig. 3). A molecular targeting therapy against ETS transcription factors could be a novel approach to selective cancer treatment in the near future.

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