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ETS transcription factors and their emerging roles in human cancer

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Abstract

Cancer can be defined as a genetic disease, resulting as a consequence of multiple events associated with initiation, promotion and metastatic growth. Cancer results from the loss of control of cellular homeostasis. Cell homeostasis is the result of the balance between proliferation and cell death, while cellular transformation can be viewed as a loss of relationship between these events. Oncogenes and tumour suppressor genes act as modulators of cell proliferation, while the balance of apoptotic and anti-apoptotic genes controls cell death. All cancer cells acquire similar sets of functional capacities: (1) independence from mitogenic/growth signals; (2) loss of sensitivity to "anti-growth" signals; (3) evade apoptosis; (4) Neo-angiogenic conversion; (5) release from senescence; and (6) invasiveness and metastasis [Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000, **100**, 57–70]. One of the goals of molecular biology is to elucidate the mechanisms that contribute to the development and progression of cancer. Such understanding of the molecular basis of cancer will provide new possibilities for: (1) earlier detection as well as better diagnosis and staging of disease with detection of minimal residual disease recurrences and evaluation of response to therapy; (2) prevention; and (3) novel treatment strategies. We feel that increased understanding of ETS-regulated biological pathways will directly impact these areas. ETS proteins are transcription factors that activate or repress the expression of genes that are involved in various biological processes, including cellular proliferation, differentiation, development, transformation and apoptosis. Identification of target genes that are regulated by a specific transcription factor is one of the most critical areas in understanding the molecular mechanisms that control transcription. Furthermore, identification of target gene promoters for normal and oncogenic transcription factors provides insight into the regulation of genes that are involved in control of normal cell growth, and differentiation, as well as provide information critical to understanding cancer development. This review will highlight the current understanding of ETS genes and their role in cancer.

Keywords: ETS transcription factors; Cancer; Oncogene; Tumour suppressor gene; Translocation

1. The ETS family

The oncogene v-ets was originally discovered as part of the gag-myb-ets transforming fusion protein of an avian replication-defective retrovirus, E26. The v-ets oncogene transforms fibroblasts, myeloblasts, and erythroblasts *in vitro* and causes mixed erythroid–myeloid and lymphoid leukaemia *in vivo*. The identification of v-ets related genes from a variety of metazoan species has established the ETS family as one of the largest families of transcriptional regulators, with diverse functions and activities (for reviews, see [2–5]). To date, 27 human ETS family members have been identified (Table 1). All ETS genes retain a region of conserved sequence, the ETS domain. This 85 amino acid region forms the winged helix-turn-helix (wHTH) DNA binding domain composed of three alpha helices and a four-stranded,

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Table 1
List of the known human ETS genes, including gene names and alternative nomenclature, GenBank accession number, chromosomal location, size of protein (amino acids), approximate boundaries of the Ets domain (85 amino acids) and approximate boundaries of the Pointed domain (65–80 amino acids, if present)

	Name	GenBank	Locus	Size	ETS	Pointed
A1	ETS1	J04101	11q23.3	441	331–416	54–135
2	ETS2	J04102	21q22.3	469	369–443	88–168
3	ERG2	M17254	21q22.3	462	290–375	120–201
4	ELK1	M25269	Xp11.2	428	Jul-92	None
5	SPI1 (PU.1)	X52056	11p12-p11.22	264	168–240	None
6	FLI1 (ERGB)	M98833	11q24.1-q24.3	452	277–361	115–196
7	SAP1 (ELK4)	M85165	1q32	431	Apr-89	None
8	ELF1	M82882	13q13	619	207–289	None
9	SPIB	X96998	19q13.3-q13.4	262	169–251	None
10	ETV2 (ER71)	NM_014209	19q13	370	265–350	None
11	E4TF1 (GABP)	D13318	21q21-q22.1	454	318–400	171–249
12	E1AF (PEA3, ETV4)	D12765	17q21	462	315–399	None
13	PE1 (ETV3)	L16464	1q23.3-q23.4	250	56–140	None
14	ERM (ETV5)	X76184	3q28	510	368–449	None
15	TEL (ETV6)	U11732	12p13	452	340–419	38–119
16	NET (SAP2, ERP. ELK3)	Z36715	12q23	407	May-85	None
17	ERF	U15655	19q13	548	26–106	None
18	ETV1 (ER81)	X87175	7p22	458	314–397	None
19	NERF2	U43188	4	581	198–277	None
20	MEF (ELF4, ELFR)	U32645	Xq26	663	204–290	None
21	ESX (JEN, ESE1,ERT,ELF3)	AF110184	1q32.2	371	275–354	47–132
22	FEV	Y08976	2q23	238	43–126	None
23	EHF (ESE3)	AF170583	11p12	300	209–288	42–112
24	ELF5 (ESE2)	AF049703	11p14	255	165–243	46–115
25	PDEF (ESF, PSE)	AF071538	6p21.3	335	248–332	138–211
26	TREF (TEL2)	AF147782	6p21	264	149–228	49–114
27	SPIC	NM_152323	12q23	248	111–193	None

beta sheet that recognises a core GGAA/T sequence (Ets Binding Site, EBS) [6]. The HTH motif is formed by helices H2 and H3. The third alpha helix makes major groove contacts with the DNA (GGAA/T core). Two invariant arginine residues present in helix H3 make contact with the two guanine residues of the EBS. The DNA recognition sequence preference for several family members has been determined by *in vitro* selection of randomised oligonucleotides and indicates that target site recognition is dependent on sequences flanking the core motif. It is clear that ETS proteins often interact *in vivo* with EBS sequences that do not conform to the consensus binding site defined by *in vitro* selection experiments. Binding of ETS proteins to sub-consensus sequences is facilitated by the binding of other transacting factors to *cis*-elements in proximity to the EBS. Indeed, binding is often mediated by synergistic interaction with transcriptional partners on composite DNA elements.

The second conserved domain found in a subset of ETS genes is the pointed (PNT) domain. This 65–85 amino acid domain is found in 11 of 27 human ETS genes and has in some cases been shown to function in protein–protein interaction and oligomerisation. Structural analysis of this domain indicates that it forms an independent structure with unique architecture of a monomeric five-helix bundle.

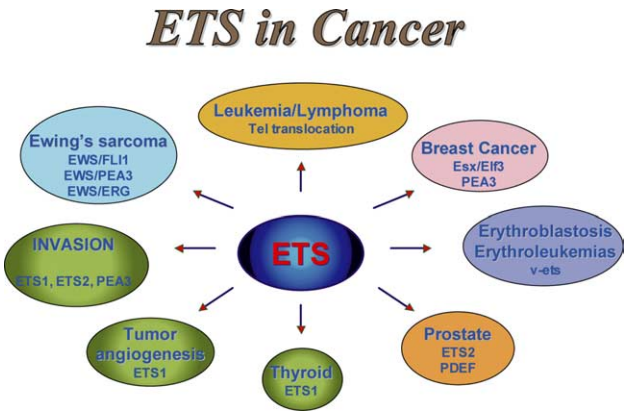

Fig. 1. ETS transcription factor involvement in cancer.

ETS factors are known to act as positive or negative regulators of the expression of genes that are involved in various biological processes, including those that control cellular proliferation, differentiation, hematopoiesis, apoptosis, metastasis, tissue remodeling, angiogenesis and transformation (Fig. 1). Our earlier literature survey enabled identification of over 200 ETS target genes [7], and, to date, over 400 ETS target genes have been defined based upon the presence of functional EBS in their regulatory regions (Watson, unpublished). While most ETS factors were initially characterised as transcriptional

activators or repressors, it has become evident that several ETS factors can function as either activators or repressors, depending upon the type of promoter and cellular context.

2. Modulation of ETS function

ETS functional activity is modulated at multiple levels. ETS factors are dependent on interaction with other factors for precise transcriptional regulation. Second, specific intracellular signalling pathways and post-translational modification directly affect the activity of several ETS proteins by regulating subcellular compartmentalisation, DNA-binding activity, transactivation potential or stability.

2.1. Regulation by protein–protein interactions

Transcriptional regulation is dependent upon the combinatorial interactions between multiple nuclear proteins. ETS proteins form complexes with many transcription factors and such interactions may strengthen the transcriptional activity and/or define target gene specificity. Tissue-specific combination of ETS with other co-factors also provides a mechanism for proper regulation of relevant target genes in a particular cell type. Many transcription factors have their DNA binding sites adjacent to EBS (for reviews, see [8,9]). Depending on the precise sequence context, binding of an ETS protein near other transcription factors results in higher affinity interaction, synergistic activation and/or repression of specific target genes. Among the first characterised interactions were studies demonstrating cooperativity between ETS factors and the AP1 transcriptional complex to activate cellular responses by increasing the transcriptional activities of promoters containing AP1-EBS binding sites, including MMP1 (matrix metalloprotease-1/collagenase), uPA (urokinase plasminogen activator), GM-CSF (granulocyte-macrophage colony stimulating factor), maspin, and TIMP-1 (tissue inhibitor of metalloproteinase-1). In contrast, MafB, an AP1 like protein, inhibits ETS1-mediated transactivation of the AP1-EBS sites [10]. Another well-characterised interaction involves SRF and ELK1 (or SAP1, SAP2/NET, FLI1, EWS–FLI1) that together form a ternary complex with the SRE motif present in several genes, including c-fos, Egr-1, pip92 Mcl-1 and SRF [11]. RUNX1/AML1 and ETS1 interaction counteracts auto-inhibition of DNA binding activity [12] and homotypic ETS1 interaction enhances binding to palindromic EBS [13]. Interaction with Pax-5 allows ETS1, as well as other family members, to bind to a non-consensus EBS present in the early B-cell-specific mb-1 promoter [14].

Several proteins that modulate ETS function have been identified, including Daxx [EAP1 (ETS1 Associated Protein 1)], EAPII and SP100 [15–17]. The suggestion that loss of corepressor protein expression is relevant to cancer was recently tested using the NCoR corepressor protein and the coregulators SRC-1 and AIB1, all of which interact with both ETS1 and ETS2 [18]. The strongest clinical association was in breast cancer showing NCoR downregulation in more aggressive hormone-unresponsive tumours [18].

2.2. Regulation by post-translational modification

ETS function is controlled by phosphorylation-mediated effects on DNA binding, protein–protein interaction, transcriptional activation and subcellular localisation (reviewed in [19]). A common feature of many tumours is deregulation of signal transduction pathways, resulting in constitutive and often ligand independent activation. As end effectors of these pathways, ETS factor function is significantly altered in cancer. In addition to being downstream of many RTKs (e.g. HER2/neu), ETS factors regulate the expression of multiple receptors, including HER2/neu, M-CSF receptor, MET, c-kit, and VEGF receptor [7].

Erk, JNK, and p38 MAP kinases are downstream components of kinase cascades. Erks are activated in response to mitogenic signals, while JNKs and p38/SAPKs (stress-activated protein kinases) respond to stress signals. Specific ETS factors are substrates for some or all of these signal transduction pathways. Phosphorylation of a mitogen-activated protein (MAP) kinase site adjacent to the PNT domain has been shown to positively regulate transcriptional activities of ETS1 and ETS2. Although MAP kinase phosphorylation of ETS1 does not affect DNA binding, calcium-induced phosphorylation of ETS1 occurs at serine residues present adjacent to the DNA binding domain and inhibits ETS1 DNA binding activity without affecting nuclear localisation. ETS1 and ETS2 activity may also be activated by PKC in invasive breast cancer cells [20,21]. In contrast, TEL (Translocation Ets Leukaemia) activity is negatively regulated by phosphorylation by MAPK, as phosphorylation of TEL results in its nuclear export and decreased DNA binding activity. Processes that are reversibly controlled by protein phosphorylation require a balance between protein kinase and protein phosphatase (PP) activities. Thus, it will be important to assess whether specific PP(s) are associated with dephosphorylation of ETS proteins.

While protein acetylation has been best characterised for histones, where it is correlated with activation state of chromatin, recent focus has been placed upon the effects of acetylation on transcription factor activity. In addition to phosphorylation, acetylation can regulate ETS gene function. Acetylation of ER81 enhances its DNA binding activity and ability to transcriptionally activate target genes [22]. Recently, we found that

ETS1 is acetylated, in response to TGFβ signalling [23]. The possible consequences of acetylation on ETS1 stability, interaction with other proteins, subcellular localisation, and DNA-binding affinity and target genes selectivity remain to be determined.

Sumoylation has been shown to affect the stability, activity, and localisation of its targets. SUMO modification has been found to alter the function of several transcription factors, including ETS family members. For example, ELK1 is modified by SUMO, and this modification is reversed by ERK–MAP kinase pathway activation. Mechanistically, it has recently been shown that sumoylation of ELK1 facilitates recruitment of histone deacetylase-2 (HDAC-2) activity to promoters. This recruitment leads to decreased histone acetylation and altered chromatin structure, resulting in transcriptional repression at Elk-1 target genes [24]. In contrast, sumoylation within the PD of TEL inhibits TEL mediated repression [25], associated with sequestering to subnuclear compartments. Mutation of SUMO acceptor results in increased transcriptional repression, presumably because of decreased nuclear export [26]. Other ETS factors (e.g., ETS1, ELF1, PU.1) interact with UBC9, suggesting that they may also be modified by SUMO.

Future studies will help elucidate the functional impact of specific post-translational modifications on the activity of ETS transcription factors. As specific antibodies are developed, it will be possible to determine the temporal relationships between specific post-translational events, as has been observed for other transcription factors, such as the tumour suppressor gene, p53. Through such analyses, it will also be possible to determine whether specific events work cooperatively or antagonistically.

3. ETS genes in cancer

Early studies demonstrated that overexpression of ETS1, ETS2 and ERG results in cellular transformation *in vitro* and transfected fibroblasts expressing high levels of ETS1 or ETS2 gene products are tumourigenic *in vivo* [27–29]. ELF3 (ESX, ESE1) expression in non-transformed MCF-12A breast epithelial cells confers a transformed phenotype and increased cell adhesion, motility and invasion [30]. The importance of ETS genes in human carcinogenesis is further supported by the observations that ETS genes have altered expression patterns in leukaemias and solid tumours, are chromosomally amplified or deleted, or are located at translocation breakpoints in leukaemias and solid tumours.

3.1. Oncogenic activation

Oncogenic activation of cellular genes can occur through multiple mechanisms, including: (1) amplification and/or overexpression; (2) activation by insertions of new regulatory sequences following retroviral integration; (3) fusion with other proteins as a consequence of chromosomal translocations or (4) point mutations.

3.2. Amplification/elevated expression

ETS1 is amplified and rearranged in leukaemia and lymphoma [31]. In cases of acute myeloid leukaemia (AML) with 11q23 amplification, the amplicon encompassed both ETS1 and FLI1 genes [32]. ETS2 amplification has also been demonstrated in patients with acute nonlymphoblastic leukaemia and t(6; 21; 18) [33]. Both ETS2 and ERG are amplified in AML patients with complex karyotype and abnormal chromosome 21; in this study, genomic amplification and ETS overexpression were correlated [34].

Elevated ETS1 expression has been observed in many invasive and metastatic solid tumours, including breast [35], lung [36], colon, pancreatic and thyroid cancer (Table 2). ETS2 is expressed at elevated levels in breast, hepatic, cervical [37], prostate [38] and esophageal cancer. Significantly, ETS2 function is necessary for the transformed state in breast and prostate cancer cells *in vitro* [39–42]. Furthermore, *in vivo* studies demonstrate that a single targeted Ets2 allele restricts development of mammary tumours in MMTV-Polyoma middle T-transgenic mice [43].

EIAF/PEA3, ERM, PDEF, and ELF3 (ESX, ESE1) transcripts are each elevated in human breast tumours [44,45]. Each member of the PEA3 subfamily (PEA3, ER81, and ERM) was found to be overexpressed in mammary tumours of MMTV-neu transgenic mice. Significantly, expression of an inhibitory (dominant negative) PEA3 transgene in mammary epithelial cells of MMTV-neu transgenic mice was correlated with increased time to tumour formation concomitant with reduced tumour size and number [46].

3.3. Point mutations

Multiple point mutations within PU.1 have been identified in AML patients [47]. The majority of the mutations are in the DNA binding domain of PU.1 and these genetic events decreased the ability of PU.1 to regulate target gene expression as well as its ability to interact with other transcription factors.

3.4. Translocations

To maintain normal cellular physiology, genes must be expressed in the correct temporal and tissue-specific manner. Cancer involves many chromosomal aberrations; the most well studied being non-random chromosomal translocations resulting in recombinant

Table 2
ETS expression in tumours

Subfamily	Members	Unigene name	Other names	Cancer
Ets	ETS1	ETS1	V-ets erythroblastosis virus E26 oncogene homolog 1; ETS1, EWSR2	Thyroid [68,69,167], colon [84–87], liver, [72,73], pancreas [78], prostate [79], breast [98–100,168–170], lung [36], gastric and esophageal [119,171]
	ETS2	ETS2	V-ets erythroblastosis virus E26 oncogene homolog 2 (avian); ETS2	Liver [74], colon [83], pancreas [71], thyroid [69], prostate, [38,40]
TCF	Elk-1	ELK1		
	Sap-1	ELK4	ETS-domain protein (SRF accessory protein 1) (ELK4); SRF accessory protein 1A (SAP-1, SAP1);	
	Sap-2/Net	ELK3	ETS-domain protein (SRF accessory protein 2) (ELK3); Net transcription factor; Sap-2 (SRF accessory protein 2); ERP, NET, SAP2	
Erg	Fli-1	FLI1	Friend leukaemia integration 1 transcription factor; EWSR2, SIC-1, ERGB	Ewing's sarcoma
	Erg	ERG	V-ets erythroblastosis virus E26 oncogene like (avian) (ERG); erg-3, p55	Ewing's sarcoma
		ERG2		TLS/FUS fusion in CML
PEA3	PEA3	ETV4	PEA3, polyoma enhancer binding; E1A enhancer binding protein (E1AF); Pea-3, Pea3 ets variant	Breast [91,94,95], Ewing's sarcoma, lung [89], gastric [148]
	ERM	ETV5	gene 5; ets-related molecule (ERM); Ets variant	Breast [96]
	Er81	ETV1	gene 1 (ETV1); (ER81)	Ewing's sarcoma
GABP	GABPa	GABPA	GA binding protein transcription factor; E4TF1-60, E4TF1A, NFT2, NRF2, NRF2A	
Elf	Elf-1	ELF1	E74-like factor 1 (ets domain transcription factor);	Prostate [75], endometrial [163], ovarian [164]
	NERF-1/-2	ELF2	E74-like factor 2; EU32, NERF, NERF-1A, NERF-1B, NERF-1a,b, NERF-2	
	MEF	ELF4	E74-like factor 4 (ets domain transcription factor); myeloid elf-1 like factor (MEF); ELFR	
Spi	PU.1	SPI1	Spleen focus forming virus (SFFV) proviral integration oncogene spi1 (SPI1); OF, PU.1	Leukaemia
	SpiB	SPIB	Spi-B transcription factor (Spi-1/PU.1 related)	
	SpiC	SPIC	Spi-C transcription factor (Spi-1/PU.1 related)	
Yan	TEL	ETV6	Ets variant gene 6 (TEL oncogene) (ETV6); TEL/ABL fusion protein	Multiple fusions with other genes in leukaemias
	TEL2	ETV7	Ets variant gene 7 (TEL2 oncogene) (ETV7); TEL-2b	
Erf	ERF	ERF	Ets2 repressor factor; PE-2	
	PE-1	ETV3	Ets variant gene 3; Ets transcriptional suppressor METS; PE-1, PE1, bA110J1.4	
Others	ESE-2	ELF5	E74-like factor 5 r; epithelium-specific Ets transcription factor 2 (ESE2)	
	ESE-3	EHF	Ets homologous factor (EHF); epithelium-specific ets factor 3, ESE3, ESEJ	
	PDEF	SPDEF	SAM pointed domain containing ets transcription factor; PDEF, bA375E1.3	Breast [76,158], prostate [77]
	ER71	ETV2	Ets variant gene 2 (LOC339321); ER71	
		ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific); EPR-1, ERT, ESE-1, ESX	Breast [172,173]
		FEV	FEV (fifth Ewing variant); HSRNAFEV, PET-1	Ewing's sarcoma

chromosomes. ETS genes can be translocated to new chromosomal positions in the absence of any apparent gene rearrangement. The ETS genes are found at chromosomal loci close to breakpoints occurring in certain childhood-associated leukaemias. The ETS1 and FLI1 are on chromosome 11 in the band region of 11q23-24, a region that has also been implicated in a number of monocytic and certain childhood-associated leukaemias. The FLI1 gene also co-translocates with ETS1 in the t(4;11)(q21;q23) translocation noted for acute non-lymphocytic leukaemias (ANLL). In similar fashion, both the ETS2 and ERG genes are located

in the region of chromosome 21 (21q22.3) associated with Down syndrome, are part of a group of genes amplified in Down Syndrome (DS), and are associated with translocations characteristic of certain types AML. ELK1 maps to chromosome Xp11.2 near the translocation breakpoint seen in t(X; 18) (p11.2; q11.2) and is characteristic of synovial sarcoma. Several chromosomal breakpoints result in a chimeric ETS protein that may be expressed in the wrong cell type, allowing for clonal selection and tumour formation. Activation by chromosomal translocation results in abnormal regulation of ETS genes resulting in carcinogenic transformation.

3.5. EWS–ETS fusions

Tumour cell formation results from the translocation associated production of FLI1 chimeric proteins as has been shown for Ewing's sarcomas (EWS) and related primitive neuroectodermal tumours (PNET) (reviewed in [48]). In this instance, the FLI1 gene is translocated from its normal 11q24 position to chromosome 22, which generates the formation of chimeric transcripts resulting in the fusion of the amino terminal region of the EWS gene with the carboxyl terminal DNA-binding domain of the FLI1 gene [49]. The chimeric fusion protein lacks the putative RNA-binding domain of EWS and one the transactivation domains of FLI1. It has also been shown that the EWS–FLI1 fusion is a more potent transcriptional activator than the FLI1 protein. While initial investigations demonstrated that both the transactivation domain of EWS and the ETS (DNA-binding) domain are required for transformation, subsequent studies have indicated that EWS–FLI1 also controls oncogenic pathways that are independent of DNA binding [50]. Significantly, the transformed phenotype of Ewing's sarcoma cells can be suppressed by blocking EWS–FLI1 production.

In other Ewing's sarcoma and primitive neuroectodermal tumours, translocations fuse the EWS gene to other members of the ETS family, including ERG [t(21;22)], ETV1 [t(7,22)], E1AF [t(17;220] and FEV [t(2;22)].

Based on these associations, elevated or unscheduled expression of EWS-ETS gene products in the inappropriate cell types may thus be causally related to the generation of malignancy. These fusion proteins would likely be unable to modulate ETS-targeted genes appropriately; other targeted gene products might be activated by the unscheduled, and therefore inappropriate, specific interactions with the EWS binding domain. These inappropriate actions could trigger autonomous growth of these tumour cells. Such a hypothesis is consistent with a study that identified transcripts from distinct target genes that were differentially activated in cells expressing EWS–FLI1 or FLI1 [51].

In addition to altered transcriptional regulation, several studies have demonstrated a role for EWS and ETS fusions in post-transcriptional mRNA processing. For example, EWS–FLI1 and TLS–ERG inhibit RNA splicing mediated by YB-1 protein [52]. Both EWS and EWS–FLI interact with U1C, snRNP protein important in the early stages of spliceosome formation and co-expression of U1C represses EWS/FLI-mediated transactivation [53]. EWS–FLI, but not EWS, interfered with hnRNP A1-dependent splice site selection of E1A and the ability to affect pre-mRNA splicing correlated with transforming activity [54]. It has also been suggested that the wild-type EWS protein is an adapter molecule that controls transcription and RNA splicing based upon its ability to bind hyperphosphorylated RNA polymerase II (RNAP II) through the N-terminal domain of EWS as well as the serine-arginine (SR) splicing factors through the C-terminal domain of EWS. While EWS–FLI1 retains the ability to bind to RNAP II, it is not able to bind SR proteins due to replacement of the C-terminal domain of EWS by FLI1 [55]. As noted above, ERG–TLS fusion products are found in CML presenting with t(16, 21). TLS (translocated in liposarcoma)/FUS is another docking protein for RNAP II and SR splicing factors, further supporting the model that chimeric ETS proteins may affect post-transcriptional processes, such as mRNA splicing.

EWS and EWS–FLI1 have been shown to interact via their common NH2 terminus with the COOH terminus of BARD1, a putative tumour suppressor. BARD1 associates via its NH2-terminal RING domain with the breast cancer susceptibility gene BRCA1 that provides a platform for interactions with proteins involved in DNA repair and checkpoint control, suggesting a possible link between the Ewing's sarcoma gene product and the genome surveillance complex [56].

3.6. TEL–ETS fusions

TEL (Translocation Ets Leukaemia; also known as ETV-6) was originally identified by its rearrangement in specific cases of chronic myelomonocytic leukaemia (CMML) presenting a t(5,12)(q33;p13) chromosomal translocation [57]. TEL is rearranged in CMML, acute myelogenous leukaemia (AML), acute myeloblastic leukaemia (AML-M2), myelodysplastic syndrome (MDS) and acute lymphoblastic leukaemia (ALL). Either the PNT domain or ETS domain or both domains of TEL have been identified in over 20 different translocations observed in human leukaemia and, more rarely solid tumours (reviewed in [58]). These translocations often result in the expression of fusion proteins. Fusions involving the PNT domain of TEL often lead to oligomerisation that is necessary for constitutive activation of kinase activity of receptor or protein tyrosine kinases (e.g. PDGFRβ, ABL1, ABL2/ARG, JAK2, TRK-C,

Syk, FGFR3) and resultant transformation. Fusions that retain the DNA binding domain of TEL are expected to result in aberrant regulation of ETS target genes.

The most frequent chromosomal translocation found in childhood leukaemia is the t(12;21)(p13;q22) in B-precursor ALL. This translocation results in the formation of a TEL–AML1/RUNX1 fusion protein, in which the PNT domain and the central repression domain of TEL are fused to nearly the complete AML1. AML1/RUNX1 functions either as transcriptional activator or repressor, depending on the promoter/enhancer tested. In contrast, the TEL–AML1 fusion protein appears to function as a transcriptional repressor, suggesting that TEL–AML1 may repress genes that are normally activated by AML1 that lead to leukaemia. Recent studies suggest that TEL–AML1 exerts dominant-interfering effects on both AML1 and TEL [59]. Based on a number of studies in this area, TEL t(12;21) gene rearrangements may be able to serve as a new prognostic marker in ALL.

Another TEL fusion arises from the t(1;12)(q21;p13) translocation in acute myeloblastic leukaemia (AML-M2). In this case, the oligomerisation domain (PNT) of TEL is fused to most of the aryl hydrocarbon receptor nuclear translocator (ARNT) protein. ARNT protein belongs to a subfamily of the basic region helix–loop–helix (bHLH) protein and contains an additional region of similarity called the PAS (Per, ARNT, SIM) domain. ARNT is a partner of several heterodimeric transcription factors, including those containing the aryl hydrocarbon (dioxin) receptor (AhR) and the hypoxia-inducible factor 1α (HIF1α), suggesting that altering the expressed activity of AhR or HIF1α can contribute to leukaemogenesis [60].

The t(12;22)(p13;q11) translocation found in myeloid leukaemia leads to the expression of a MN1–TEL fusion protein in which the DNA binding domain of TEL is fused to MN1 sequences. The transforming activity of MN–TEL depends on both N-terminal MN1 sequences and a functional TEL DNA binding domain. Based upon the presence of proline residues and polyglutamine stretches in the MN1 region and the ability of MN1 to activate transcriptional activity of the Moloney sarcoma virus, it was suggested that MN1–TEL acts as a deregulated transcription factor [61]. More recently, it has been shown that MN1 may act as a coactivator rather than a sequence-specific transcription factor, synergising with coactivators to stimulate RAR/RXR-mediated transcription [62].

The amino-terminal end of the homeobox gene HSXB9 is fused to the PNT and DNA binding domains of TEL in infant acute myeloid leukaemias with t(7;12)(q36;p13) [63]. Similarly, the amino-terminal region of PAX5 is fused to most of the TEL gene in acute lymphoblastic leukaemia with a t(9;12)(q11;p13) translocation. The resulting chimeric protein would retain the PAX5 paired-box domain and both the PNT/helix-loop-helix and DNA binding domains of TEL [64].

The role of TEL fusions in oncogenesis has been further established by mouse model systems. Irradiated mice repopulated with bone marrow cells transduced with retroviral TEL–AML1 develop ALL [65]. TEL-PDGFβR, introduced into bone marrow by retroviral transduction, caused a fatal myeloproliferative disease that closely recapitulated human CMML [66]. Similar retroviral bone marrow transduction and transplantation studies demonstrated that TEL–ABL has leukaemogenic properties *in vivo* [58].

4. ETS genes in specific neoplasms

Functional inhibition by ETS transdominant mutants has revealed a causal role for Ets-dependent activity in the transformation essential to malignancy [39,41,42,67]. Correlation of Ets gene expression levels with tumour progression occurs in human neoplasias such as thyroid, pancreas, liver, prostate, colon, lung, and breast carcinomas and leukaemias (Table 2).

4.1. Cancers of the thyroid

ETS family genes are implicated in the development of thyroid cancer. Two highly related ETS genes, ETS1 and ETS2, are expressed at much higher levels in neoplasias of the thyroid relative to benign and normal tissues [68,69]. Functional roles of ETS1 and ETS2 proteins in thyroid cell transformation were tested by transfecting an Ets-dominant negative construct, leading to programmed cell death in various thyroid carcinoma cell lines [69]. In contrast, overexpression of the wild-type ETS1 gene in normal thyroid cells did not significantly modify growth requirements, and expression of ETS gene family members did not differ between thyroid benign adenomas and normal thyroid [68,69].

4.2. Neoplasms of the pancreas and liver

ETS gene overexpression is associated with early stages of pancreatic and liver cancers. The PEA3 transcription factor protein correlates with advancing hepatocellular carcinoma (HCC) [70]. Pancreatic cancers with aggressive phenotype have significantly higher ETS2 expression than normal pancreas ducts; pancreatic cancer lines transfected ETS2 induced larger tumours in nude mice [71]. The high levels of ETS1 expression seen in the early stages of HCC and in extrahepatic bile duct carcinomas are significantly decreased in advanced stages; with cases showing high levels of ETS1 having a better disease-free survival outcome [72,73]. A similar pattern of high ETS2 protein levels in early stages of

hepatocellular carcinoma has also been found [74]. ETS target genes induced during liver carcinogenesis may be the basis of these observed events, which appear to be involved with initiating the progression to invasion and metastasis.

4.3. Prostate tumours

Advanced stages of prostate cancer are associated with expression of FLI1, ELF1, PDEF, ETS1, and ETS2. Immunohistochemistry has shown that FLI1 and ELF1 proteins are highly expressed in prostatic adenocarcinomas relative to normal cells [75]. Although the PDEF gene is expressed almost exclusively in the normal prostate where it activates androgen-independent expression of the prostate-specific antigen, its overexpression at the mRNA level is a characteristic of aggressive prostate tumours [76,77]. Similarly, ETS1 expression between benign and malignant, as well as different clinical stages of prostatic cancer show significant differences but no associations with clinical prognostic indicators such as lymph node status [78,79]. Relative to normal prostatic epithelium, ETS2 is overexpressed in high Gleason-score primary and metastatic prostate cancers [38,80]. Triplex-DNA reduction of ETS2 or dominant negative ETS2 expression reduces androgen independence, tumourigenicity, and nude mouse tumour formation in prostate cancer cell lines [40,81]. Transcriptional activation of ETS genes is essential for upregulation of extracellular matrix-degrading proteins including MMP-1, MMP-9, uPA, and the uPA receptor, many of which are associated with clinical features such as lymph node status and prognosis in prostate cancer. Interestingly, although ERG1 is highly expressed in microdissected prostate tumour cells relative to benign tissues, its expression is higher in less aggressive prostate cancers than in more aggressive tumours as shown by GeneChip analysis, real-time qRT-PCR and *in situ* hybridisation [82].

4.4. Cancers of the colon

In colon cancer, the expression of ETS genes are found at high levels in advanced stages of the disease. Expression of ETS1 and ETS2 is not observed in normal colon and hyperplastic polyps, however their expression is associated with advancing tumour grade and correlates with lymph node metastasis in colon cancer [83]. ETS1 is pro-apoptotic in colon cancer cells where its ectopic expression reverses the transformed phenotype and tumourigenicity in a dose-dependent manner [84]. A naturally occurring splice variant of ETS1, p42-ETS1, induces apoptosis in epithelial cancer cells by rescuing the damaged Fas-apoptotic pathway that exists in these tumour cells [85,86]. ETS1 also correlates with integrin beta3 expression during lung metastasis from colon cancer, indicating that ETS1 target genes are major players during metastasis and invasion of stromal cells [87]. Some insight into the specificity of ETS genes in colon cancer is provided by the observation that the incidence of most solid tumours, including colon, is reduced in Down's syndrome, a condition in which ETS2 is overexpressed as part of congenital trisomy 21 [88].

4.5. Lung cancers

Non-small-cell lung cancers (NSCLCs) are characterised by dissemination to regional lymph nodes and distant metastasis resulting from aggressive invasion of the primary tumour. Transcripts of the E1AF gene are frequently expressed in NSCLCs tumours and E1AF is upregulated by HGF/Met signalling in NSCLC cell lines [89]. ETS1 is also upregulated by HGF signalling in NSCLC cell lines, as well as having a positive correlation with MMP7 expression in primary lung carcinomas [36,89]. ETS2 was identified as one of 50 genes in mice that are involved in the progression of lung cancer from adenoma to carcinoma [90].

4.6. Breast cancers

Several ETS factors are deregulated in the development of breast cancers. From a survey of ductal carcinoma *in situ* (DCIS) and invasive human breast tumours overexpression of PEA3 was linked to increased HER2/Neu receptor tyrosine activity, an early event in the genesis of human breast cancer [91]. Both PEA3 and its family member ER81 are downstream targets of HER2/Neu which is a predictor of the aggressiveness and lethality of breast cancers [91–93]. Immunohistochemical PEA3-positivity was significantly associated with HER2/neu gene overexpression in 41 DCIS cases and 33 invasive ductal carcinomas. Less than half of HER2/Neu-negative tumours and 93% HER2/Neu-positive tumours were positive for PEA3, and its expression in normal breast epithelial tissue was undetectable. ETS2 was not associated with HER2/Neu in the same samples, indicating the specificity of HER2/Neu association with PEA3 [91]. On the other hand, high PEA3 mRNA levels relative to normal ductal cells correlate with histopathological grade but not with poor prognosis [94]. PEA3 is significantly upregulated in malignant effusions compared to primary tumours [95]. The related PEA3 family gene ERM may also be clinically relevant as its expression is an independent predictor of prognosis [96].

PDEF, an Ets-family gene highly expressed in the normal prostate, is overexpressed at the mRNA level in breast cancers, and correlates with advancing tumour grade [44,76]. Present at low levels in hormone regulated normal tissues such as the mammary gland and the ovary and in tissues with a high epithelial cell content

such as the breast and colon, PDEF mRNA is up to 20-fold higher in breast cancer as compared to fibrocystic tissue [76,77,97].

Two groups used *in situ* hybridisation, mRNA, and/or immunohistochemistry to show that ETS1 is increased in invasive breast cancers relative to *in situ* lesions [35,98]. ETS1-producing invasive breast cancer cells may specifically downregulate the p42 isoform of Ets1, which may adversely affect the survival of these cells [99]. Clinically, ETS1 overexpression has been found to have a strong, independent association with poor prognosis in breast cancer [100]. Regulation of ETS1 gene expression in response to growth factors and tumour grade has been related to disease-free survival in breast cancer [18]. Both ETS1 and ETS2 protein levels are significantly associated with time to disease recurrence in breast tumour patients [18]. Down's syndrome people have trisomy 21 induced overexpression of ETS2, however, breast cancer is underrepresented in the uniquely different tumour profile of this population [101].

4.7. Leukaemias

ETS genes are essential contributors to the genesis of Ewing's sarcomas and several leukaemias as described above. Although ETS2 plays no part in EWS or TEL leukaemias, its overexpression in Down's syndrome patients underlies the 20-fold excess of leukaemias found in this population [88]. ETS genes are also translocated with partners other than EWS in chronic myelomonocytic leukaemia (CML) and acute myeloid leukaemia (AML). In CML the ETS domain of ERG2 is joined to the TLS/FUS gene with the fusion protein retaining the ability to bind RNA polymerase II at the N-terminal TLS region and to bind DNA through the ETS domain of ERG2 [102]. ETV6/TEL is translocated with the meningioma 1 (MN1) gene in AML, resulting in fusion of the transcriptionally active domain of MN1 with the DNA binding region of the ETS gene [103]. Unlike the other ETS genes involved in tumourigenic chromosomal translocations, the N-terminal region of TEL is fused with a nearly complete AML1 protein in pediatric B progenitor acute lymphoblastic leukaemia (ALL) [104].

5. Invasion, angiogenesis, and tumour suppression

5.1. Epithelial–stromal cell interactions

While most human tumours are derived from epithelial cells that have undergone multiple genetic alterations, it is quite evident that alterations in the tumour microenvironment contribute significantly to tumour progression [105]. Epithelial cells are in close contact with the stromal compartment, which is composed of endothelial cells, infiltrating blood cells, fibroblasts, nerves and intervening ECM (extracellular matrix). Tumour metastases are the result of a complex process that involves remodeling of the surrounding tissue, cellular migration, tumour vascularisation, intravasation into blood or lymphatic vessels, and survival at distant sites. Stromal cells play a critical role in tumour growth and progression. For example, stromal cell functions influence the growth, differentiation, survival and invasiveness of epithelial cells. In addition, specific interactions between tumour epithelial cells and endothelial cells are also required for neo-vascularisation of the tumour [106]. Stromal cells produce most of the connective tissue ECM as well as many of the ECM-degrading enzymes. Excessive degradation and remodeling of ECM is a characteristic of reactive stroma. Such ECM remodeling is required for cancer cells to turn into invasive and metastatic cancer cells. ETS targets, such as the MMPs and other proteases are responsible for activation of some of the essential enzymes that dissolve the protein components of ECM, which is crucial for the onset of metastasis (reviewed in [107]). Significantly, recent studies have identified two additional ETS targets that further implicate ETS functions in stroma remodeling: tenascin-C and collagen type I [108,109].

It is becoming evident that ETS factors control pathways not only in epithelial cells, but also within the stromal compartment and the precise regulation of ETS function in epithelial and stromal cells affects their interaction, both in normal development and cancer. Previous studies have indicated that ETS factors are abnormally expressed in both tumour and stromal compartments, which frequently correlates with tumour progression. For example, increased vascular ETS1 expression is correlated with higher microvessel density, upregulated VEGF expression and poor prognosis of colon cancer patients [110]. Coordinate upregulation of ETS1, MMP1 and MMP9 is observed in the stroma of invasive ductal and lobular breast cancer [35] and also in invasive HNPCC and sporadic colon cancer [111], suggesting an important role for ETS1 in tumour invasion and angiogenesis. Stromal cell expression of an ETS target gene, MMP9, appears critical for angiogenesis and subsequent growth of human ovarian tumours in mice [106]. More direct evidence for the contribution of stromal ETS expression in tumourigenesis is provided by transplantation of Neu- and polyomavirus middle-T-initiated mammary tumours into mammary fat pads of mutant Ets2 mice. In this *in vivo* study, the resultant increase in tumour size was dependent on Ets2 activity and expression of MMP9 in the stromal compartment [112].

ETS factors likely contribute to the control mechanisms that regulate the dynamic interplay between epithelial and stromal cells critical for normal tissue homeostasis. Their deregulation and response to

abnormal signalling contribute significantly to the aberrant interactions between cancer cells and the reactive stroma that are required for tumour metastasis. Collective experimental findings demonstrate that ETS regulated target gene expression is cell-type specific. Recent expression profiling of specific cell types in normal breast and breast cancer demonstrates that extensive and complementary gene expression changes occur in each cell type [113]. Since the stromal microenvironment is critical for properties of epithelial cells, it will be important to characterise ETS-regulated pathways in these different cell types and how each cell type contributes to the overall phenotype of cancer.

5.2. Tumour invasion

ETS factors regulate transcription of ECM degrading enzymes including MMPs, uPA, and interstitial collagenases [114]. Three structurally distinct ETS transcription factors, ETS1, FLI1 and PU.1, differently modulate AP-1-dependent upregulation of MMP-1 gene expression [75]. Similarly, ETS1 and ETS2 overexpression potentiates uPA and MMP-9 promoter activation in response to epidermal growth factor (EGF) signalling [115]. Urokinase plasminogen activator, its receptor (uPAR), and the type IV collagenase are associated with invasion and metastasis in many cancers including breast cancer [116]. The expression of these proteases is regulated by growth factors, receptor-type tyrosine kinases, like EGF, TGFβ, HGF, IGFa, and cytoplasmic oncoproteins such as ras [115]. ETS1 and ETS2 genes are highly expressed in invasive breast tumour cells, and they may be the transcriptional link between EGF stimuli and the upregulated expression of uPAR, and MMP9 (type IV collagenase) [115]. ETS family genes promote transcription of MET, the receptor of HGF (a growth factor regulating cell motility) thereby controlling invasive growth; MET is often seen overexpressed in a significant percentage of human cancers [117–119]. MET activation also induces transcription of ETS1 mRNA, illustrating further that this product and its protein can act both upstream and downstream of MET. HGF has also been reported to upregulate the expression of the ETS-related PEA3 transcription factor gene whose product in turn activates a number of MMP genes, which then leads to enhanced cancer cell invasion in oral (squamous carcinoma cell) cancers [120].

5.3. Angiogenesis

An essential step in malignancy is angiogenesis, the process by which new blood vessels are formed allowing growth, progression and metastasis of invasive tumours [121,122]. The expression of several members of the ETS family, ETS1, ERG and FLI1, correlates with the occurrence of angiogenesis during normal and pathological development [123]. ETS1 is highly expressed during tumour angiogenesis as well as during normal angiogenic processes such as wound healing and neoangiogenesis in developing human embryos [124]. However neoangiogenesis in the adult is most frequently associated with the occurrence of malignant invasion [125–127]. This expression occurs in the stroma of surrounding malignant tissue where ETS1 is one of the first transcription factors detectable *in situ*, during the invasive process.

Tumours often produce VEGF, which in turn induces ETS1 production in endothelial cells active during malignancy induced neovascularisation [100,125,128]. In turn, ETS1 is involved in the transcriptional regulation of the VEGF receptor, Flt-1, that is upregulated during tumour angiogenesis [129,130]. VEGF-induced invasiveness is inhibited by ETS1 antisense oligonucleotides, but not by sense oligonucleotides [125]. The ETS-related factor TEL is also regulated by VEGF [131,132]. ETS1 is sufficient to convert endothelial cells to the angiogenic phenotype by inducing u-PA, MMP-1, MMP-3, MMP-9, and integrin beta 2, implicating these as target genes of ETS1 [127]. In addition to ETS1 regulation of the VEGF receptor Flt-1, the expression of the ETS family of transcription factors, ERG and FLI1, has been correlated with Tie2 gene expression that is also involved in the formation and remodeling of normal vascular networks [123]. One of three distinct isoforms of the novel ETS transcription factor NERF, (NERF2 specifically), is expressed in endothelial cells and could transactivate the regulatory regions of the Tie2 gene; this is in contrast to other ETS factors, which have little or no effect [133,134].

5.4. Tumour suppression

While considerable effort has been directed towards the elucidation of ETS as oncogenic transcription factors, some recent studies support a role for ETS factors functioning to suppress cellular growth, suggesting a possible tumour suppressor function. The majority of human cancers are of epithelial origin. A subset of four ETS factors (ESE1, ESE2, ESE3 and PDEF) are expressed in epithelial tissues including breast, prostate, and colon, tissues commonly susceptible to cancer. Several studies have indicated that the expression of three of these ETS factors is reduced during tumourigenesis. In contrast to ESE1 whose expression is increased in DCIS [135], ESE2 mRNA is absent in primary breast carcinomas when compared to normal adjacent epithelium. Furthermore, ESE2 mRNA is not detected in the majority of cancer-derived cell lines examined [109]. The ESE3 protein is absent in invasive bladder, oral, prostate, and breast cancer [136]. Prostate and colon cancer-derived cell lines are known to express ESE3 mRNA, however the level of its protein expression has not yet been

reported. PDEF protein is reduced or absent in prostate and breast cancer [76,137] and significantly PDEF expression inhibits cell proliferation, migration and invasion, consistent with its role as a class II tumour suppressor gene [76]. The functional significance of reduced ESE2 and ESE3 expression remains to be determined.

MEF has properties consistent with tumour suppressor function. Expression of MEF in lung carcinoma cells reduced anchorage independent growth *in vitro* and tumourigenesis *in vivo*. The tumours that formed in the MEF expressing A549 cells were more differentiated and less angiogenic than those from the parental cells. Furthermore, MEF repressed transcription of MMP-2 and MMP-9 in these tumours [138].

6. ETS target genes

ETS proteins carry out multiple critical roles in many biological processes, including lymphoid development, hematopoiesis, differentiation, proliferation, apoptosis, oncogenesis and embryonic development. This importance necessitates the identification of downstream cellular target genes of specific ETS proteins. Although some overlap in the biological function of different ETS proteins may exist, the presence of a family of closely related transcription factors suggests that individual ETS members may have evolved unique roles, manifested through the control and interaction of specific target genes. As noted above, it is possible that ETS-regulated pathways specific to cancer cells of a given type may themselves form the foundation for the development of new therapeutics.

ETS target gene expression contributes to oncogenesis, promoting tumour invasiveness, cell migration and metastasis as well as angiogenesis. Several studies have demonstrated co-expression of ETS factors and presumptive ETS target genes, including MMP1 (type I collagenase), MMP2 (gelatinase B), MMP9, and MMP7 (matrilysin). For example, EIAF/PEA3 and HER2/neu have coordinated expression in breast cancer [91]. ETS1 expression was positively correlated with uPA [98] and VEGF and PAI-1 [100] in breast cancer. ETS1, MMP11 (Stromelysin 3) and VEGF receptor expression have been correlated with microvessel density, progression and prognosis of oral cancer [139].

In a comparison of global gene expression between a high-grade invasive breast tumour and a high grade ductal carcinoma *in situ*, known ETS factor target genes Egr-1, c-Fos, and keratin-18 were found to be downregulated more than 2.5-fold in the high-grade invasive breast tumour sample. ETS target genes upregulated in the invasive breast tumour included TIMP-1, NME1, and TIE-2 [140].

A single nucleotide polymorphism (SNP) in the MMP1 promoter (1G/2G) creates a functional EBS. Several studies have demonstrated a significant correlation between individuals with this SNP and cancer progression and/or poor patient prognosis. The 1G/2G polymorphism is correlated with colorectal cancer invasiveness [141], melanoma tumour invasion and metastasis[142] and clinical stage of cervical cancer [143]. MMPs are implicated in proliferative, invasive, angiogenic and metastatic properties of tumours. Although synthetic, low molecular weight MMP inhibitors (MMPIs) have been developed, treating patients with advanced cancers using these agents have been consistently disappointing. These older trials utilised broad-spectrum MMPIs and new trials testing the efficacy of selective MMP inhibitors alone or in combination with other chemotherapeutics is now being examined.

7. ETS genes as therapeutic targets and diagnostic factors

Ets factors are associated with cellular transformation and cancer progression through regulation of target genes that control migration and invasion, metastasis, escape from senescence and apoptosis and angiogenesis. Thus, ETS factors and/or the genetic pathways that they regulate could be targets for cancer therapy. Multiple approaches have been used successfully to demonstrate the biological importance of specific ETS factors. Loss of function studies have been performed by expression of antisense, siRNA, and dominant negative (competitive inhibitor) molecules. While these studies have already yielded insight into ETS regulation of biological processes, their success suggests potential modes for therapy. In this section, we will briefly review some of the more promising studies.

Several laboratories have assessed the molecular and biological impact following expression of the antisense oligonucleotides, antisense cDNA, small interfering RNA (siRNA) or the DNA binding domain of ETS factors (sometimes termed dominant negative, DN). Indeed, the first demonstration that ETS1 and ETS2 were downstream of, and necessary for ras-activated pathways, was accomplished by expression of a lacZ fusion with the DNA binding domain of ETS2 [144]. Antisense E1AF/PEA3 expression resulted in reduced MMP activity and diminished invasion of oral cancer cell [145]. Expression of antisense ETS2 or DN-ETS2 inhibited anchorage independent growth of prostate cancer-derived cells [40] and DN-ETS2 induced apoptosis in thyroid cancer cell lines [69]. Antisense-ESX and DN-ESX expression in T47D breast cancer cells resulted in reduced colony formation [146]. Antisense-E1AF expression reduced matrilysin expression, associated with inhibition of invasion of HT29 colon carcinoma [147] and MKN45 gastric cancer [148] cells *in vitro*. The potential utility of targeting ETS fusion proteins has been demonstrated. Antisense EWS–FLI1 inhibited

proliferation of Ewing's sarcoma and PNET cell lines [149]. Growth suppression and induction of apoptosis were observed following expression of siRNA in Ewing's sarcoma cells [150].

Expression of ETS1 antisense oligonucleotides inhibited VEGF-induced endothelial cell migration [151] and DN-ETS1 inhibited angiogenesis [152,153].

Triplex forming oligonucleotides (TFOs) bind to homopurine:homopyrimidine sequences, forming a stable, sequence specific complex. Direct inhibition of ETS2 transcription by TFOs resulted in growth inhibition and induction of apoptosis in human prostate cancer cells [81].

As with any gene or anti-gene therapy, complexities associated with specific targeting to cancer cells remains a major problem. While new delivery systems are being developed to enhance target cell specificity, identification of ETS-regulated pathways specifically deregulated in cancer cells (either epithelial or stromal cells) may provide another opportunity to affect cancer, but not normal, cell populations.

7.1. Combination therapy

Novel insights provided by molecular biology have led to the development of selective molecular targeting agents for genes involved in cell proliferation, apoptosis, and angiogenesis in cancer cells. For example, trastuzumab (Herceptin) is a chimeric monoclonal antibody to HER-2 that has been used for successful treatment of breast and other cancer patients whose tumours show HER2/neu overexpression. Gefinitib (ZD1839, Irresa), a tyrosine kinase inhibitor of EGFR, has been used in treatment of non-small cell lung cancer. Given that ETS factors are downstream effectors of signalling from these and other receptors, blocking ETS function may enhance therapeutic benefit when used in combination therapy.

Delivery of inhibitors against activated kinases has also yielded successful clinical results. Imatinib mesylate (Glivec, formerly STI571) was originally developed as an inhibitor of for treatment of chronic myeloid leukaemia patients with the BCR/ABL fusion protein. Glivec selectively blocks cellular proliferation and induces apoptosis of cells harboring the BCR–ABL tyrosine kinase. Glivec is also able to inhibit the functional activity of other kinases. Relevant to ETS, pre-clinical studies demonstrate that ST1571 inhibits the *in vitro* growth of HT93A cells, which were derived from a AML-M3 patient with the t(1;12) translocation and resultant TEL–ABL2/ARG fusion protein [154].

7.2. Differentiation therapy

Promotion of differentiation, associated with induction of apoptosis, has multiple therapeutic applications. Many hematological malignancies arise from failure of normal differentiation processes and agents to induce differentiation have made significant impact on the treatment of specific cancers. As known regulators of cellular differentiation, modulation of ETS genes or ETS-regulated pathways may provide complementary approaches for differentiation therapy. For example, PU.1 is found to block differentiation of murine erythroleukaemia (MEL) cells, in part by promoting transcription of CDK6 [112]. Downregulation of PU.1 is required for differentiation and thus therapeutic interference with PU.1 may promote erythroid differentiation.

7.3. Diagnostics

As discussed above, several ETS factors (TEL, FLI1, ERG, ETV1, E1AF, and FEV) are oncogenically activated by specific disease-associated translocations. Therefore, ETS factors may provide the basis for highly sensitive molecular detection of fusion proteins or chimeric RNAs associated with cancer-specific translocations. Detection of EWS–FLI1 gene rearrangement allows definitive diagnosis of Ewing's sarcoma from other forms of childhood small round cell tumours (SRCT) [155]. Furthermore, the type of EWS/ETS fusion affects clinical biology [156]. The presence of the TEL–AML1 fusion gene highlights a subgroup of children with good prognosis and can be used to monitor relapse after treatment for B-precursor ALL [157].

Direct examination and expression profiling have demonstrated increased expression of several ETS factors in cancer. Among the interesting applications of these observations is the potential utility of ETS factors as tumour markers. PDEF mRNA is overexpressed in breast cancer [44] and has utility as a marker for detection of metastatic breast cancer [158]. In addition, PDEF expression correlated better with ovarian cancer than other cancer-related markers [159].

7.4. Patient prognosis

Recent studies have determined that expression of several ETS factors is correlated with patient prognosis. Elevated ETS1 expression was found to be a predictor of poor prognosis in breast [100] and ovarian [160] cancer. In oral squamous cell carcinoma, ETS1 expression was associated with tumour stage and lymph node metastasis [161]. ETS1 expression was associated with microvessel density and it was significantly higher in metastatic nonseminomatous testicular germ cell tumours [162].

Similar associations have been documented for other ETS factors. For example E1AF/PEA3 expression correlated with progression of ovarian cancer. ERM gene expression was found to be an independent negative

prognostic factor for overall survival in breast cancer patients [96]. Increased ELF1 expression has been reported in prostate cancer, breast cancer, and osteosarcoma and the malignant potential of endometrial and ovarian cancer has been correlated with ELF1 overexpression [163,164].

8. Conclusions

The ETS genes encode transcription factors that have independent activities but are likely to be part of an integrated network. Although it was originally observed that specific ETS factors function either as positive or negative regulators of transcription, it is now evident that the same ETS factor may function in either fashion, reflecting promoter and cell context specificities. Furthermore, the precise balance between cancer promotion and inhibition by ETS factors, which may differentially regulate specific target genes, can thus control its progression. This concept forms the basis of the hypothesis that "Ets conversion" plays a critical role during tumour progression and coordination of multiple ETS gene functions also mediates interactions between tumour and stromal cells [2].

While previous studies have focused on single ETS factors in the context of specific promoters, future studies should consider the functional impact of multiple ETS present within a specific cell type. The pattern of ETS expression within a single tissue [165], as well as in multiple tissues and specific cell lines [166] is, not surprisingly, quite complex. Multiple ETS factors may be able to regulate the same genes, albeit at different magnitude or in different directions. In addition, functional antagonism between different ETS factors and between ETS and other transcription factors has been observed and contributes to the final nature of the transcriptome. Additional parameters that need to be carefully examined are the magnitude and kinetics of ETS expression, which have already been shown experimentally to affect hematopoietic lineage selection. Complete system analysis assessment of the regulatory network of ETS factors will also require knowledge of their upstream and downstream effectors. When available, it should be possible to determine if such a "systems network" approach represents an improved biomarker set for tumourigenesis and cancer progression. Through such approaches, upstream and downstream effectors of ETS functions critical for metastasis will be identified, many which may represent novel therapeutic targets.

Conflict of interest statement

None declared.

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