HEP (2004) 166:259–275

The Role of Ets Transcription Factors in Mediating Cellular Transformation

G. Foos · C. A. Hauser (✉)

La Jolla Cancer Research Center, The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA
CHauser@burnham.org

1 Introduction to the Ets Family of Transcription Factors 260
1.1 The Ets Gene Families of Mice and Humans 260
1.2 Ets Family Nomenclature 262
1.3 Ets Family Functions 262
1.4 Many Different Ets Factors Can Be Present in a Specific Tissue or Cell Type . 263
1.5 Ets Target Genes 264

2 Evidence Implicating Ets Factors in Cellular Transformation and Cancer . . 264
2.1 Ets Factor Overexpression Resulting from Proviral Insertion 264
2.2 Chromosomal Translocations of Ets Genes Associated with Human Cancers . 265
2.3 Mutations in Ets Genes Associated with Human Cancers 266
2.4 Signaling to Ets Factors from Oncogenes 266
2.5 Expression Correlation of Ets Factors with Tumors 267
2.6 Reversal of Cellular Transformation by Altered Ets Factor Function 267
2.7 Genetic Loss-of-Function Studies of Ets Factors in Cancer 269

3 Future Perspectives for Understanding the Role of Ets Factors in Transformation 269

References 270

Abstract The Ets family of transcription factors in mouse or humans is comprised of around 27 unique family members that contain an evolutionarily conserved DNA-binding domain called the Ets domain. The Ets family includes both transcriptional activators and repressors. The normal cellular Ets transcription factors have been implicated as mediators of a wide range of cellular processes, including oncogenic transformation. This chapter provides an overview of the Ets family, and describes each of the multiple lines of evidence that Ets transcription factors are mediators of cellular transformation. This evidence includes: (a) cancers resulting from Ets factor overexpression or chromosomal translocations that generate fusion proteins containing Ets factor domains; (b) signaling from oncogenes to Ets factors; (c) expression correlation of Ets factors with tumor formation; (d) reversal of cellular transformation by dominant inhibitory Ets constructs; (e) delayed tumor development after genetic disruption of an Ets factor; and (f) the potential role of many Ets target genes in transformation. A better understanding of the role of Ets factors and their target genes in cancer should provide the basis for more specific novel therapeutic approaches for the treatment of cancers.

Keywords Ets gene family · Transcription factors · Cellular transformation

1
Introduction to the Ets Family of Transcription Factors

The first Ets family member v-ets, was identified as part of a fusion oncogene in the E26 avian transforming retrovirus. The name 'ets' came simultaneously from E26 transformation-specific (Nunn et al. 1983) or E-twenty-six (Leprince et al. 1983). Since the initial identification of a v-ets cellular homolog in chickens (Leprince et al. 1983), and the recognition that other proteins have a related domain (Karim et al. 1990), Ets transcription factor families have been identified in a variety of organisms. The Ets family size ranges from 10 putative Ets factors in *Caenorhabditis elegans* (Hart et al. 2000) to 27 characterized Ets family members in humans (Oettgen et al. 2000). The Ets transcription factor family is defined by the presence of an evolutionarily conserved domain of about 85 amino acids—the Ets domain. The Ets domain mediates binding of Ets family members to DNA sequences containing a GGAA/T core sequence. While there is some specificity conferred by the nucleotides flanking the core sequence, there is considerable overlap of Ets factor DNA binding specificity. The functional specificity of Ets factors is thought to derive from a combination of their tissue-specific expression patterns, post-transcriptional modifications, and interactions with a variety of partner proteins (reviewed in Ghysdael and Boureux 1997; Graves and Petersen 1998; Sharrocks 2001; Verger and Duterque-Coquillaud 2002; Oikawa and Yamada 2003).

1.1
The Ets Gene Families of Mice and Humans

Mammalian Ets factors have been organized into subfamilies by several criteria, the most common based on the similarity of their Ets domains. Table 1 lists the 26 currently characterized human/mouse Ets family orthologs, and one human Ets factor (TEL2) where a mouse ortholog has not yet been reported. This subfamily grouping is based on the Ets domain molecular phylogeny analysis of (Laudet et al. 1999), with the addition of several more recently characterized Ets factors, as tabulated in (Oettgen et al. 2000). A bioinformatic study of the mouse genome sequence suggests that few additional Ets domain-containing genes remain to be discovered (Xuan et al. 2002). A second conserved domain found in 11 of the Ets family members is the pointed domain, named for the *Drosophila pointed* gene where this domain was first identified (Klambt 1993). The presence or absence of a pointed domain is indicated for each of the Ets factors in Table 1. Pointed domains are associated with highly divergent Ets domains (e.g., the Ets1/2 and TEL subfamilies), and thus arranging the Ets family by pointed domain homology would lead to an organization quite different from that shown in Table 1. The Ets1/2, Erg, and Elf/Ese subfamilies (based on Ets domain homol-

Table 1 The mouse and human Ets families

Ets Subfamily	Ets Gene[a]	Alternative Names[b]	Pointed Domain	Mouse UGRepAcc[c]	Mouse UGCluster[d]	Mouse Chrom.[e]	Human UGRepAcc[c]	Human UGCluster[d]	Human Chrom.[e]
Ets1/2	Ets2	c-ets-2	yes	NM_011809	Mm.22365	16	NM_005239	Hs.292477	21
↓	Ets1	c-ets-1	yes	NM_011808	Mm.14115	9	NM_005238	Hs.18063	11
	ER71	(m)ETSRP71, (h)ETV2	no	NM_007959	Mm.4829	7	XM_290831	Hs.194061	19
GABPa	Gabpa	E4TF1A	yes	NM_008065	Mm.18974	16	NM_002040	Hs.78	21
PEA3	PEA3	Etv4, (h)E1AF	no	NM_008815	Mm.5025	11	NM_001986	Hs.434059	17
↓	ER81	Etv1, (m)EtsRP81	no	NM_007960	Mm.4866	12	NM_004956	Hs.150011	7
	ERM	Etv5	no	NM_023794	Mm.155708	16	NM_004454	Hs.43697	3
Erg	Fli1	(h)ERGB, EWSR2	yes	NM_008026	Mm.258908	9	NM_002017	Hs.257049	11
↓	Erg		yes	NM_133659	Mm.164531	16	NM_182918	Hs.45514	21
	Fev	(h)HSRNAFEV, Pet1	no	NM_153111	Mm.150496	1	NM_017521	Hs.234759	2
ERF	Erf	(h)PE-2	no	NM_010155	Mm.8068	7	NM_006494	Hs.440332	19
↓	PE1	Etv3, METS	no	NM_012051	Mm.34510	3	NM_005240	Hs.352672	1
Elk/TCF	Elk1		no	NM_007922	Mm.3064	X	NM_005229	Hs.181128	X
↓	Elk3	Net, Sap-2, ERP	no	NM_013508	Mm.4454	10	NM_005230	Hs.288555	12
	Elk4	Sap1	no	NM_007923	Mm.195050	1	NM_021795	Hs.129969	1
Elf/Ese	Elf1	Elf-1	no	NM_007920	Mm.24876	14	NM_172373	Hs.124030	13
↓	Elf2	(h)NERF	no	NM_023502	Mm.46503	3	NM_006874	Hs.82143	4
	Elf4	MEF, ELFR	no	NM_019680	Mm.154274	X	NM_001421	Hs.151139	X
	Ese1	Elf3, ESX, jen, Ert	yes	NM_007921	Mm.3963	1	NM_004433	Hs.67928	1
	Ese2	Elf5	yes	NM_010125	Mm.20888	2	NM_001422	Hs.11713	11
	Ese3	Ehf	yes	NM_007914	Mm.10724	2	NM_012153	Hs.200228	11
↓	Pse	(m)Spdef, (h)PDEF	yes	NM_013891	Mm.26768	17	NM_012391	Hs.79414	6
TEL	TEL	Etv6, TEL1	yes	NM_007961	Mm.269995	6	NM_001987	Hs.171262	12
↓	TEL2	Etv7, TEL.B	yes	None	None	–	NM_016135	Hs.272398	6
Spi	PU.1	(m)Sfpi1, (h)SPI1	no	NM_011355	Mm.1302	2	NM_003120	Hs.157441	11
↓	SpiB	Spi-B	no	U87620	Mm.8012	7	NM_003121	Hs.437905	19
	SpiC	Spi-C, Prf	no	NM_011461	Mm.21642	10	NM_152323	Hs.511791	12

[a] Names of Ets family members used in this work, based on wide usage or to emphasize subfamily relationships. Underline indicates current mouse UniGene symbols. Table data are compiled from Stanford SOURCE site (http://source.stanford.edu) and from UniGene (http://www.ncbi.nlm.nih.gov/UniGene).

[b] Other common names used for each Ets gene, with current UniGene symbols underlined. Names used primarily for mouse or human orthologs are designated (m) or (h), respectively. Where mouse/human UniGene symbols differ beyond capitalization, (human symbols are all capitals) both are shown.

[c] UniGene representative mRNA accession numbers

[d] UniGene Cluster

[e] Chromosomal location

ogy) are examples of Ets subfamilies of which only some subfamily members contain a pointed domain. Four of the seven Elf/Ese subfamily members contain a pointed domain, and this observation along with their epithelial pattern of expression, has led to the grouping of the Ese/Pse family as a distinct subfamily (Feldman et al. 2003b). The roles of Ets factor pointed domains in oncogenesis are discussed below.

1.2 Ets Family Nomenclature

One of the confounding problems of understanding the extensive literature on Ets transcription factors (currently more than 2000 publications) is the multiple names in use for each Ets factor. Table 1 includes alternative names used for the human and/or mouse Ets family members including their UniGene symbols, representative transcript accession numbers, and cluster number. Additionally, both in common usage and even in UniGene symbols, there are sometimes different names for mouse and human orthologs. Finally, several UniGene symbols, particularly those based on involvement of seven sometimes unrelated Ets factors identified in chromosomal translocations (ETV 1–7), are not used by most researchers in the field. An example of the challenges in nomenclature is PEA3/E1AF/ETV4. PEA3 started out as a generic term for factors that bound to what later would be called an Ets-binding site in the polyoma enhancer (Gutman and Wasylyk 1990; Leprince et al. 1992). Subsequently, the name PEA3 was given to a specific Ets family member (Xin et al. 1992). Later, the human ortholog of PEA3 (with 94% total sequence identity) was discovered, but was named E1AF (Higashino et al. 1993). Finally, PEA3/E1AF was found to be the fourth Ets factor involved in chromosomal fusions with EWS (Kaneko et al. 1996), and was designated ETV4 in UniGene. A literature search revealed that for PEA3, E1AF, and ETV4, there were 136, 26, and 3 citations respectively, and this ratio has not substantially changed in the last 2 years.

1.3 Ets Family Functions

Ets transcription factors have been implicated in the regulation of virtually all cellular functions, including growth, development, differentiation, survival, and oncogenic transformation (reviewed in Dittmer and Nordheim 1998; Maroulakou and Bowe 2000; Oikawa and Yamada 2003). Gene products associated with all of these cellular functions are among the hundreds of putative Ets factor target genes already identified by a variety of criteria (reviewed in Sementchenko and Watson 2000). The involvement of some of these target genes in cellular transformation is discussed below. Despite the potential functional redundancy of Ets factors, gene disruption of most Ets

factors studied thus far results in embryonic or perinatal lethality (Bartel et al. 2000; Oikawa and Yamada 2003). Such early lethality in knockout mice reveals essential early roles for Ets factors, but complicates the study of the role of individual Ets factors in oncogenesis.

The majority of Ets factors are transcriptional activators, which serve as downstream effectors for a variety of signal transduction pathways, as discussed below. However, at least five mammalian Ets factors have been reported to have repressor activity, including Erf, PE1/METS, Elk3/Net, TEL, and TEL2. (Mavrothalassitis and Ghysdael 2000; Gu et al. 2001; Klappacher et al. 2002). In addition, depending on the signaling inputs, several additional Ets factors possess both transcriptional activation and repression activities (reviewed in Sharrocks 2001). The mixed transcriptional role of Ets factors has been evolutionarily conserved from *Drosophila*, where several of the Ets factors are transcriptional activators (Hsu and Schulz 2000), but Yan is a negative regulator (O'Neill et al. 1994) which opposes the action activators such as pointed (Brunner et al. 1994; Gabay et al. 1996). The *C. elegans* Lin-1 Ets factor may also possess negative regulatory activity (Tan et al. 1998). Overall, in normal mammalian cells, there is a balance between positive and negative regulation of Ets-dependent gene expression, and there are multiple lines of evidence that changes in this balance can have a significant impact on oncogenic transformation.

1.4
Many Different Ets Factors Can Be Present in a Specific Tissue or Cell Type

Because of the similar DNA binding specificity of Ets factors, to understand how Ets target genes are regulated in a particular cell type, it is important to know which Ets factors are present. The normal course of gene discovery is that a new Ets factor is found, and its expression is analyzed in several tissues. Subsequently, other investigators may examine the expression of this Ets factor in tissues of their interest. The resulting expression data for each Ets factor is therefore rather anecdotal. When our studies led to the question of which Ets factors act as crucial mediators of cancer, we were surprised to find that the expression status of less than half of the Ets family members was known in any single cell type or tissue (Maroulakou and Bowe 2000; Barrett et al. 2002). Thus, we undertook a comprehensive study to determine which of the Ets factor mRNAs are expressed in normal mammary, mammary tumors, and mammary related cell lines. The unexpected result of this analysis was that 24 of the 25 mouse Ets factors analyzed were expressed in normal mammary tissue, and even in clonal cell lines, between 14 and 20 of the Ets factors were significantly expressed (Galang et al. 2004). These data show that identifying which Ets factors are regulating specific target genes is more complex than previously appreciated.

1.5
Ets Target Genes

There is substantial interest in Ets transcription factor target genes, in part, because of the potential role of these genes in the transformed phenotype. Over 200 genes with Ets factor-binding sites in their promoters have been established as Ets target genes by various criteria. The products of these target genes are associated with every aspect of cellular regulation, including growth, adhesion, motility, invasion, angiogenesis, and apoptosis (Sementchenko and Watson 2000). In addition, correlative evidence connects expression of various Ets factors to these cellular functions, and Ets factor-binding sites are found in the promoter of nearly every matrix metalloproteinase, molecules important in invasive behavior (Sato 2001; Singh et al. 2002; Oikawa and Yamada 2003). Clearly, gene products involved in controlling these diverse cellular functions are likely to be important downstream targets of oncogenic signaling. Because most of the Ets target genes have been characterized by reporter gene analysis upon overexpression of a few Ets factors, the physiological role of individual Ets factors in regulating these target genes remains unclear, as does the contribution of this observed regulation to the transformed phenotype.

2
Evidence Implicating Ets Factors in Cellular Transformation and Cancer

A variety of lines of evidence support the role of Ets factors as mediators of cellular transformation and tumor progression. These include: (a) erythroleukemias from viral-induced overexpression of mouse Ets factors; (b) chromosomal translocations involving at least six different Ets genes generate fusion proteins associated with a variety of tumors; (c) mutations in some Ets factors are associated with tumor development; (d) many Ets factors are downstream signaling targets for oncogenes; (e) correlation of Ets factor expression with tumor progression; (f) reversal of cellular transformation by dominant negative and positive Ets constructs or other reagents that interfere with Ets factor function; (g) impaired tumor development in mice with genetically altered function of a specific Ets factor. These seven lines of evidence are described below.

2.1
Ets Factor Overexpression Resulting from Proviral Insertion

There are two examples in where overexpression of mouse Ets factors due to nearby viral integration contributes to erythroleukemias. The Spi-1/PU.1 Ets factor was first identified in erythroleukemias as an oncogene frequently ac-

tivated by Friend spleen focus forming virus insertion (Moreau-Gachelin et al. 1988). Similarly, elevated Fli1 expression resulting from Friend murine leukemia virus insertion was also found in erythroleukemias (Ben-David et al. 1991). The viral insertions did not alter the coding sequence of PU.1 or Fli1, but proximity of the strong viral enhancer elevated the transcription of these Ets factors. Transgenic mouse models were subsequently used to show that overexpression of PU.1, but not Fli1, was sufficient to induce erythroleukemia (Zhang et al. 1995; Moreau-Gachelin et al. 1996). In addition to these naturally occurring examples, experimental overexpression of several Ets factors has been reported to transform rodent cells (reviewed in Dittmer and Nordheim 1998).

2.2 Chromosomal Translocations of Ets Genes Associated with Human Cancers

Fusions of the N-terminal portion of EWS with the Ets domain (DNA-binding domain) of at least five different Ets factors (Fli, Erg, ER81, PEA3, FEV) are associated with Ewing's family tumors (reviewed in Arvand and Denny 2001). The ability of so many different Ets DNA-binding domains (Ets DBDs) to participate in these fusions with similar outcomes, suggests that the EtsDBD have similar DNA-binding specificities, and that critical Ets target gene expression is being altered by fusion to EWS. This is likely due in part to the enhanced transactivation activity of the Ets fusion proteins (Ohno et al. 1993; Bailly et al. 1994). Indeed, experimentally interfering with Ets-dependent gene expression by expression of the Fli1 EtsDBD fused to a repressor domain reverses the transformed phenotype of Ewing Sarcoma cells (Athanasiou et al. 2000). However, there is emerging evidence that other activities of EWS also mediate transformation, as the Ews–Fli1 fusion proteins can also negatively regulate Ets-dependent gene expression (Im et al. 2001) and EWS–Ets fusions exhibit both DNA-binding-dependent and -independent transformation mechanisms (Jaishankar et al. 1999; Knoop and Baker 2001; Welford et al. 2001).

The TEL gene is involved in several kinds of cancer associated gene fusions, which reveal distinct contributions of three different domains of this Ets family member. One type of TEL fusion associated with leukemias is the Ets domain of TEL fused to a transactivation domain of transcription factor MN1 (Buijs et al. 2000). This fusion protein presumably leads to inappropriate activation of Ets-dependent gene expression. A unique feature of TEL (and the recently discovered TEL2) among the Ets family members is its ability to homodimerize through its pointed domain. Fusions of the TEL dimerization domain to the kinase domain of variety of tyrosine kinase genes leads to dimerized and constitutively activated tyrosine kinases associated with leukemias (Golub et al. 1996). In addition to leukemias, such fusions can also lead to lymphomas (Yagasaki et al. 2001) and fibrosarcomas

(Knezevich et al. 1998). Finally, TEL also contains a repressor domain (Chakrabarti and Nucifora 1999), and gene fusion of this domain with AML1 generates a protein that may repress critical AML1 target genes leading to leukemias (Hiebert et al. 2001). Overall, the Ets fusion genes associated with cancers highlight the function of several Ets factor domains. These data, along with induction of erythroleukemias from elevated expression of PU.1 or Fli1, strongly suggest that altered regulation of Ets target genes contributes to a variety of malignancies.

2.3 Mutations in Ets Genes Associated with Human Cancers

There is not strong evidence that mutation of Ets family members is a widespread event in human cancers. Nonetheless, there are a few suggestive examples. In addition to participation of TEL in gene fusions, TEL also maps to a chromosomal region (12p12-p13) found deleted in about 5% of children with acute lymphoblastic leukemia (ALL), suggesting a possible role as a tumor suppressor (Stegmaier et al. 1995). Further analysis of TEL loss of heterozygosity (LOH) in ALL patients has generated mixed results, but loss of the unfused TEL allele in TEL-AML1-induced ALL is quite common, suggesting there is selective pressure for this LOH (Raynaud et al. 1996). Heterozygous mutations in PU.1 were recently identified in 9 of 126 acute myeloid leukemia (AML) patients, with most of these mutations disrupting PU.1 DNA-binding function. It was postulated that such mutations could inhibit PU.1 function and block early myeloid differentiation (analogous to the differentiation block observed in PU.1$^{-/-}$ mice), contributing to development of AML (Mueller et al. 2002). The Ese2/Elf5 and Ese3/EHF genes are closely linked and map to human chromosome 11p13–15. This chromosomal region has been found to exhibit LOH in breast and prostate carcinomas, suggestive of a possible negative role for these Ets factors in tumors (Zhou et al. 1998; Tugores et al. 2001).

2.4 Signaling to Ets Factors from Oncogenes

Ets transcription factors are downstream targets of multiple signaling pathways, and their activity can be modulated by a variety of post-transcriptional modifications. The Ras signaling pathway alters the activity of many Ets factors, and other oncogenic signaling also converges on Ets transcription factors (for review see Dittmer and Nordheim 1998; Wasylyk et al. 1998; Yordy and Muise-Helmericks 2000; Oikawa and Yamada 2003). As an example, Ets2 is transcriptionally activated by oncogenic Ras or Neu/ErbB-2 signaling, and this activation requires mitogen activated protein kinase-mediated phosphorylation of an evolutionarily conserved Ets2 threonine residue

(Galang et al. 1996; Yang et al. 1996; McCarthy et al. 1997). Another evolutionarily conserved function of oncogenic signaling is relief of negative regulation by Ets family repressors. This is seen from Ras signaling in flies (Gabay et al. 1996) to oncogenic signaling in mammals (Le Gallic et al. 1999; Lopez et al. 2003). In addition to phosphorylation, other reported regulatory modification of Ets family members include acetylation of Ets1 (Czuwara-Ladykowska et al. 2002), glycosylation of Elf1 (Tsokos et al. 2003), and SUMO modification of TEL (Wood et al. 2003). Overall, modifications of Ets factors resulting from oncogenic signaling may strongly influence their activity, through mechanisms including altered DNA binding, interactions with partner proteins, protein stability, or subcellular localization.

2.5
Expression Correlation of Ets Factors with Tumors

There have been many correlative studies demonstrating differences in the expression of many of the Ets factors in normal and tumor tissue. A recent comprehensive review on Ets1 cited 35 correlative studies of the expression of just this one Ets factor in tumors (Dittmer 2003). Our recent analysis of expression of the entire Ets family in mouse mammary tumor development found that expression of the mRNAs of nine different Ets factors was significantly elevated in mammary tumors as compared with normal mammary tissue (Galang et al. 2004). Some of this altered Ets factor expression was found to reflect changes the in cellular composition from normal mammary tissue to tumors (e.g., an increased epithelial cell content), whereas other differences were found to represent actual tumor-specific events. Another complicating factor in interpreting expression Ets correlation studies is that one cannot distinguish whether changes in Ets factor expression contribute to the tumor phenotype, or simply result from altered signaling in the tumors. Nonetheless, there is a wealth of suggestive evidence that alterations in expression of specific Ets factors correlates with the development or progression of specific types of tumors (Oikawa and Yamada 2003).

2.6
Reversal of Cellular Transformation by Altered Ets Factor Function

One of the most compelling lines of evidence that Ets factors play a causal role in specifically mediating cellular transformation comes from experimental alteration of Ets family function in transformed cells. In mouse cells, broadly inhibiting Ets factor activity by expression of a dominant negative Ets construct consisting of just the DNA-binding domain (DBD) of Ets1, Ets2, or PU.1 inhibits or reverses the Ras or Neu/ErbB-2 transformation of murine fibroblasts (Langer et al. 1992; Giovane et al. 1994; Galang et al. 1996; Foos et al. 1998). Transgenic expression of a PEA3 DBD also inhibits

tumor formation in a mouse model (Shepherd et al. 2001), and cationic lipid delivery of a PEA3 DBD expression construct to tumors resulted in prolonged survival of the treated animals (Wang and Hung 2000). Additional evidence of the importance of Ets signaling in transformation came from reversal of Ras transformation by overexpression of an inhibitory mutant form of ERF (Le Gallic et al. 1999) or overexpression of TEL, a transcriptional repressor in the Ets family (Athanasiou et al. 2000).

Similar to rodent cells, reversal of aspects of the transformed phenotype was observed in human tumor cells upon interfering with Ets function in prostate, thyroid, breast, and Ewing sarcoma tumor cells (Kovar et al. 1996; Delannoy-Courdent et al. 1998; Sapi et al. 1998; Sementchenko et al. 1998; Athanasiou et al. 2000; Foos and Hauser 2000; de Nigris et al. 2001; G. Foos and C.A. Hauser, unpublished results). Interestingly, in either rodent or human tumor cells, while Ets DBD inhibition of cellular Ets function has strong effects on the transformed phenotype (e.g., loss of anchorage-independent growth) it does not usually impair normal cell growth. This indicates that cellular Ets factors mediate transformation-specific signaling not required for normal cell growth. Thus, intervening with this signaling could have the specificity desired for cancer therapy.

One must interpret the Ets factor DBD studies carefully with respect to which specific Ets factors are important. It has long been suspected that Ets DBD constructs (which bind to similar promoter sites) could broadly inhibit Ets family activity. We recently demonstrated such broad activity, showing that Ets2DBD expression strongly inhibits Ets-dependent gene expression even in an Ets2 knockout cell line (Hever et al. 2003). This study further showed that despite the ability the Ets2DBD to reverse Ras transformation in a variety of cells, that Ets2 knockout cells exhibited no defects in Ras transformation. Thus, due to the promiscuity of Ets domain DNA binding, Ets dominant negative experiments clearly do not identify which specific Ets factors mediate transformation, but they do reveal the critical role of the Ets family in mediating transformation-specific signaling.

Surprisingly, experimental overexpression of a variety of Ets family transcriptional activators can also reverse aspects of the transformed phenotype in mouse and human cells. Overexpression of Ets1, Ets2, PEA3, Ese1, or PDEF reverses aspects of the transformed phenotype in both Ras transformed NIH3T3 cells (Foos et al. 1998) and in human colon, prostate and breast tumor cell lines (Suzuki et al. 1995; Chang et al. 2000; Foos and Hauser 2000; Xing et al. 2000; Feldman et al. 2003a; G. Foos and C.A. Hauser, unpublished results). Such studies must also be carefully interpreted, as high-level expression of an Ets factor likely impacts on the physiological targets of other Ets family members. In summary, a balance of Ets function (mediated by one or more unidentified Ets factors) appears to be needed to provide signaling specifically required to maintain cellular transformation.

2.7 Genetic Loss-of-Function Studies of Ets Factors in Cancer

One of the most compelling ways that a gene product can be implicated in tumor formation or progression, is by genetic loss-of-function analysis. This approach has been difficult with Ets factors, because their homozygous disruption often leads to embryonic or perinatal lethality (Bartel et al. 2000; Oikawa and Yamada 2003). In light of the extensive literature connecting Ets transcription factors and cancer, it is surprising that only one Ets factor, Ets2, has been demonstrated to be specifically involved in tumor development in vivo. This analysis of Ets2 function was also complicated by embryonic lethality, but it was shown that heterozygote *ets2* (+/−) mice exhibited delayed tumor onset in a transgenic mouse mammary tumor model (Neznanov et al. 1999). It was subsequently shown that mice homozygous for a hypomorphic *ets2* allele (which could not be activated by Ras pathway signaling) also exhibited delayed mammary tumor formation (Man et al. 2003). Definitive genetic analysis of the requirement of Ets2 or the other 25 Ets family members may require the use of conditional gene disruption.

3 Future Perspectives for Understanding the Role of Ets Factors in Transformation

While there is fairly overwhelming evidence that Ets transcription factors are important mediators of cellular transformation, important questions still need to be addressed. One of these questions is which specific Ets family members mediate transformation? Given the size of the Ets family, identification of individual Ets factors mediating transformation in specific cellular contexts will likely require loss-of-function analysis. While several loss-of-function approaches are possible, the use of emerging RNA interference technologies holds great promise. If individual Ets members whose function is critical in transformation can be identified, then therapeutic approaches based on specifically interfering with their expression or interactions can be developed, or alternatively, approaches developed based on interfering with the signaling which modulates the Ets factor activity.

A second major question is what are the important target genes for the Ets-mediated transformation-specific signaling. One current problem is trying to determine which of the hundreds of identified putative Ets target genes are actually effectors of transformation. In addition, there may also be novel transformation-specific targets of Ets factors yet to be identified. Most of the broad functional analysis of Ets target genes by microarray analysis thus far, has focused on the role of Ets factors in differentiation. Such differentiation analysis includes targets of PU.1, TEL, and MEF in hematapoetic

cells and Ets1 and ERG in HUVEC (McLaughlin et al. 2001; Teruyama et al. 2001; Yamada et al. 2001; Sakurai et al. 2003; Hedvat et al. 2004). As a start to the identification of Ets targets important in cancer, we have applied microarray analysis to the human breast tumor cell line system, comparing gene expression in tumor cells to subclones reverted by dominant-acting Ets constructs. This approach has identified at least one functionally important Ets target gene (interleukin-8) in these tumor cells, with several other intriguing candidates (G. Foos and C.A. Hauser, unpublished results). Overall, it is anticipated that important insights into the molecular events in oncogenic transformation and tumor progression will be made from future studies of the role of Ets transcription factors in cancers.

References

Arvand A, Denny CT (2001) Biology of EWS/ETS fusions in Ewing's family tumors. Oncogene 20:5747–5754

Athanasiou M, LeGallic L, Watson DK, Blair DG, Mavrothalassitis G (2000) Suppression of the Ewing's sarcoma phenotype by FLI1/ERF repressor hybrids. Cancer Gene Ther 7:1188–1195

Bailly RA, Bosselut R, Zucman J, Cormier F, Delattre O, Roussel M, Thomas G, Ghysdael J (1994) DNA-binding and transcriptional activation properties of the EWS-FLI-1 fusion protein resulting from the t(11;22) translocation in Ewing sarcoma. Mol Cell Biol 14:3230–3241

Barrett JM, Puglia MA, Singh G, Tozer RG (2002) Expression of Ets-related transcription factors and matrix metalloproteinase genes in human breast cancer cells. Breast Cancer Res Treat 72:227–232

Bartel FO, Higuchi T, Spyropoulos DD (2000) Mouse models in the study of the Ets family of transcription factors. Oncogene 19:6443–6454

Ben-David Y, Giddens EB, Letwin K, Bernstein A (1991) Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the ets gene family, Fli-1, closely linked to c-ets-1. Genes Dev 5:908–918

Brunner D, Ducker K, Oellers N, Hafen E, Scholz H, Klambt C (1994) The ETS domain protein pointed-P2 is a target of MAP kinase in the sevenless signal transduction pathway. Nature 370:386–389

Buijs A, van Rompaey L, Molijn AC, Davis JN, Vertegaal AC, Potter MD, Adams C, van Baal S, Zwarthoff EC, Roussel MF, Grosveld GC (2000) The MN1-TEL fusion protein, encoded by the translocation (12;22)(p13;q11) in myeloid leukemia, is a transcription factor with transforming activity. Mol Cell Biol 20:9281–9293

Chakrabarti SR, Nucifora G (1999) The leukemia-associated gene TEL encodes a transcription repressor which associates with SMRT and mSin3A. Biochem Biophys Res Commun 264:871–877

Chang J, Lee C, Hahm KB, Yi Y, Choi SG, Kim SJ (2000) Over-expression of ERT(ESX/ESE-1/ELF3), an ets-related transcription factor, induces endogenous TGF-beta type II receptor expression and restores the TGF-beta signaling pathway in Hs578 t human breast cancer cells. Oncogene 19:151–154

Czuwara-Ladykowska J, Sementchenko VI, Watson DK, Trojanowska M (2002) Ets1 is an effector of the transforming growth factor beta (TGF-beta) signaling pathway and an antagonist of the profibrotic effects of TGF-beta. J Biol Chem 277:20399–20408

de Nigris F, Mega T, Berger N, Barone MV, Santoro M, Viglietto G, Verde P, Fusco A (2001) Induction of ETS-1 and ETS-2 transcription factors is required for thyroid cell transformation. Cancer Res 61:2267–2275

Delannoy-Courdent A, Mattot V, Fafeur V, Fauquette W, Pollet I, Calmels T, Vercamer C, Boilly B, Vandenbunder B, Desbiens X (1998) The expression of an Ets1 transcription factor lacking its activation domain decreases uPA proteolytic activity and cell motility, and impairs normal tubulogenesis and cancerous scattering in mammary epithelial cells. J Cell Sci 111:1521–1534

Dittmer J (2003) The biology of the Ets1 Proto-oncogene. Mol Cancer 2:29 (http://www.molecular-cancer.com/content/2/1/29)

Dittmer J, Nordheim A (1998) Ets transcription factors and human disease. Biochim Biophys Acta 1377: F1–F11

Feldman RJ, Sementchenko VI, Gayed M, Fraig MM, Watson DK (2003a) Pdef expression in human breast cancer is correlated with invasive potential and altered gene expression. Cancer Res 63:4626–4631

Feldman RJ, Sementchenko VI, Watson DK (2003b) The epithelial-specific Ets factors occupy a unique position in defining epithelial proliferation, differentiation and carcinogenesis. Anticancer Res 23:2125–2131

Foos G, García-Ramírez JJ, Galang CK, Hauser CA (1998) Elevated expression of Ets2 or distinct portions of Ets2 can reverse Ras-mediated cellular transformation. J Biol Chem 273:18871–18880

Foos G, Hauser CA (2000) Altered Ets transcription factor activity in prostate tumor cells inhibits anchorage-independent growth, survival, and invasiveness. Oncogene 19:5507–5516

Gabay L, Scholz H, Golembo M, Klaes A, Shilo BZ, Klambt C (1996) EGF receptor signaling induces pointed P1 transcription and inactivates Yan protein in the Drosophila embryonic ventral ectoderm. Development 122:3355–3362

Galang CK, Garcia-Ramirez J, Solski PA, Westwick JK, Der CJ, Neznanov NN, Oshima RG, Hauser CA (1996) Oncogenic Neu/ErbB-2 increases ets, AP-1, and NF-kappaB-dependent gene expression, and inhibiting ets activation blocks Neu-mediated cellular transformation. J Biol Chem 271:7992–7998

Galang CK, Muller WJ, Foos G, Oshima RG, Hauser CA (2004) Changes in the expression of many Ets family transcription factors and of potential target genes in normal mammary tissue and tumors. J Biol Chem 279:11281J. Biol. Chem. 11292

Ghysdael J, Boureux A (1997) The Ets family of transcriptional regulators. In: Yaniv M, Ghysdael J (eds) Oncogenes as transcriptional regulators, vo–6408

Hedvat CV, Yao J, Sokolic RA, Nimer SD (2004) Myeloid ELF1-like Factor Is a Potent Activator of Interleukin-8 Expression in Hematopoietic Cells. J Biol Chem 279:6395–6400

Hever A, Oshima RG, Hauser CA (2003) Ets2 is not required for Ras or Neu/ErbB-2 mediated cellular transformation in vitro. Exp Cell Res 290:132–143

Hiebert SW, Lutterbach B, Amann J (2001) Role of co-repressors in transcriptional repression mediated by the t(8;21), t(16;21), t(12;21), and inv(16) fusion proteins. Curr Opin Hematol 8:197–200

Higashino F, Yoshida K, Fujinaga Y, Kamio K, Fujinaga K (1993) Isolation of a cDNA encoding the adenovirus E1A enhancer binding protein: a new human member of the ets oncogene family. Nucl Acids Res 21:547–553

Hsu T, Schulz RA (2000) Sequence and functional properties of Ets genes in the model organism Drosophila. Oncogene 19:6409–6416

Im YH, Kim HT, Lee C, Poulin D, Welford S, Sorensen PH, Denny CT, Kim SJ (2001) EWS-FLI1, EWS-ERG, and EWS-ETV1 oncoproteins of Ewing tumor family all suppress transcription of transforming growth factor beta type II receptor gene. Cancer Res 60:1536–1540

Jaishankar S, Zhang J, Roussel MF, Baker SJ (1999) Transforming activity of EWS/FLI is not strictly dependent upon DNA-binding activity. Oncogene 18:5592–5597

Kaneko Y, Yoshida K, Handa M, Toyoda Y, Nishihira H, Tanaka Y, Sasaki Y, Ishida S, Higashino F, Fujinaga K (1996) Fusion of an ETS-family gene, EIAF, to EWS by t(17;22)(q12;q12) chromosome translocation in an undifferentiated sarcoma of infancy. Genes Chromosomes Cancer 15:115–121

Karim FD, Urness LD, Thummel CS, Klemsz MJ, McKercher SR, Celada A, Van Beveren C, Maki RA, Gunther CV, Nye JA, al. e (1990) The ETS-domain: a new DNA-binding motif that recognizes a purine-rich core DNA sequence [letter]. Genes Dev 4:1451–1453

Klambt C (1993) The Drosophila gene pointed encodes two ETS-like proteins which are involved in the development of the midline glial cells. Development 117:163–176

Klappacher GW, Lunyak VV, Sykes DB, Sawka-Verhelle D, Sage J, Brard G, Ngo SD, Gangadharan D, Jacks T, Kamps MP, Rose DW, Rosenfeld MG, Glass CK (2002) An induced Ets repressor complex regulates growth arrest during terminal macrophage differentiation. Cell 109:169–180

Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PH (1998) A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. Nat Genet 18:184–187

Knoop LL, Baker SJ (2001) EWS/FLI alters 5'-splice site selection. J Biol Chem 276:22317–22322

Kovar H, Aryee DN, Jug G, Henockl C, Schemper M, Delattre O, Thomas G, Gadner H (1996) EWS/FLI-1 antagonists induce growth inhibition of Ewing tumor cells in vitro. Cell Growth Differ 7:429–437

Langer SJ, Bortner DM, Roussel MF, Sherr CJ, Ostrowski MC (1992) Mitogenic signaling by colony-stimulating factor 1 and ras is suppressed by the ets-2 DNA-binding domain and restored by myc overexpression. Mol Cell Biol 12:5355–5362

Laudet V, Hanni C, Stehelin D, Duterque-Coquillaud M (1999) Molecular phylogeny of the ETS gene family. Oncogene 18:1351–1359

Le Gallic L, Sgouras D, Beal G, Jr., Mavrothalassitis G (1999) Transcriptional repressor ERF is a Ras/mitogen-activated protein kinase target that regulates cellular proliferation. Mol Cell Biol 19:4121–4133

Leprince D, Crepieux P, Stehelin D (1992) c-ets-1 DNA binding to the PEA3 motif is differentially inhibited by all the mutations found in v-ets. Oncogene 7:9–17

Leprince D, Gegonne A, Coll J, de Taisne C, Schneeberger A, Lagrou C, Stehelin D (1983) A putative second cell-derived oncogene of the avian leukaemia retrovirus E26. Nature 306:395–397

Lopez RG, Carron C, Ghysdael J (2003) v-SRC Specifically Regulates the Nucleo-cytoplasmic Delocalization of the Major Isoform of TEL (ETV6). J Biol Chem 278:41316–41325

Man AK, Young LJT, Tynan J, Lesperance J, Egeblad M, Werb Z, Hauser CA, Muller WJ, Cardiff RD, Oshima RG (2003) Ets2-dependent stromal regulation of mouse mammary tumors. Mol Cell Biol 23:8614–8625

Maroulakou IG, Bowe DB (2000) Expression and function of Ets transcription factors in mammalian development: a regulatory network. Oncogene 19:6432–6442

Mavrothalassitis G, Ghysdael J (2000) Proteins of the ETS family with transcriptional repressor activity. Oncogene 19:6524–6532

McCarthy SA, Chen D, Yang B-S, García-Ramírez JJ, Cherwinski H, Chen X-R, Klagsbrun ML, Hauser CA, Ostrowski MC, McMahon M (1997) Rapid phosphorylation of Ets-2 accompanies MAP kinase activation and the induction of HB-EGF gene expression by oncogenic Raf-1. Mol Cell Biol 17:2401–2412

McLaughlin F, Ludbrook VJ, Cox J, von Carlowitz I, Brown S, Randi AM (2001) Combined genomic and antisense analysis reveals that the transcription factor Erg is implicated in endothelial cell differentiation. Blood 98:3332–3339

Moreau-Gachelin F, Tavitian A, Tambourin P (1988) Spi-1 is a putative oncogene in virally induced murine erythroleukaemias. Nature 331:277–280

Moreau-Gachelin F, Wendling F, Molina T, Denis N, Titeux M, Grimber G, Briand P, Vainchenker W, Tavitian A (1996) Spi-1/PU.1 transgenic mice develop multistep erythroleukemias. Mol Cell Biol 16:2453–2463

Mueller BU, Pabst T, Osato M, Asou N, Johansen LM, Minden MD, Behre G, Hiddemann W, Ito Y, Tenen DG (2002) Heterozygous PU.1 mutations are associated with acute myeloid leukemia. Blood 100:998–1007

Neznanov N, Man AK, Yamamoto H, Hauser CA, Cardiff RD, Oshima RG (1999) A single targeted Ets2 allele restricts development of mammary tumors in transgenic mice. Cancer Res 59:4242–4246

Nunn MF, Seeburg PH, Moscovici C, Duesberg PH (1983) Tripartite structure of the avian erythroblastosis virus E26 transforming gene. Nature 306:391–395

O'Neill EM, Rebay I, Tjian R, Rubin GM (1994) The activities of two Ets-related transcription factors required for Drosophila eye development are modulated by the Ras/MAPK pathway. Cell 78:137–147

Oettgen P, Finger E, Sun ZJ, Akbarali Y, Thamrongsak U, Boltax J, GrallF., Dube A, Weiss A, Brown L, Quinn G, Kas K, Endress G, Kunsch C (2000) PDEF, a novel prostate epithelium-specific Ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. J Biol Chem 275:1216–1225

Ohno T, Rao VN, Reddy ES (1993) EWS/Fli-1 chimeric protein is a transcriptional activator. Cancer Res 53:5859–5863

Oikawa T, Yamada T (2003) Molecular biology of the Ets family of transcription factors. Gene 303:11–34

Raynaud S, Cave H, Baens M, Bastard C, Cacheux V, Grosgeorge J, Guidal-Giroux C, Guo C, Vilmer E, Marynen P, Grandchamp B (1996) The 12;21 translocation involving TEL and deletion of the other TEL allele: two frequently associated alterations found in childhood acute lymphoblastic leukemia. Blood 87:2891–2899

Sakurai T, Yamada T, Kihara-Negishi F, Teramoto S, Sato Y, Izawa T, Oikawa T (2003) Effects of overexpression of the Ets family transcription factor TEL on cell growth and differentiation of K562 cells. Int J Oncol 22:1327–1333

Sapi E, Flick MB, Rodov S, Kacinski BM (1998) Ets-2 transdominant mutant abolishes anchorage-independent growth and macrophage colony-stimulating factor-stimulated invasion by BT20 breast carcinoma cells. Cancer Res 58:1027–1033

Sato Y (2001) Role of ETS family transcription factors in vascular development and angiogenesis. [Review] [52 refs]. Cell Struct Funct 26:19–24

Sementchenko VI, Schweinfest CW, Papas TS, Watson DK (1998) ETS2 function is required to maintain the transformed state of human prostate cancer cells. Oncogene 17:2883–2888

Sementchenko VI, Watson DK (2000) Ets target genes: past, present and future. Oncogene 19:6533–6548

Sharrocks AD (2001) The ETS-domain transcription factor family. [Review] [112 refs]. Nat Rev Mol Cell Biol 2:827–837

Shepherd TG, Kockeritz L, Szrajber MR, Muller WJ, Hassell JA (2001) The pea3 subfamily ets genes are required for HER2/Neu-mediated mammary oncogenesis. Curr Biol 11:1739–1748

Singh S, Barrett J, Sakata K, Tozer RG, Singh G (2002) ETS proteins and MMPs: partners in invasion and metastasis. Curr. Drug Targets 3:359–367

Stegmaier K, Pendse S, Barker GF, Bray-Ward P, Ward DC, Montgomery KT, Krauter KS, Reynolds C, Sklar J, Donnelly M (1995) Frequent loss of heterozygosity at the TEL gene locus in acute lymphoblastic leukemia of childhood. Blood 86:38–44

Suzuki H, Romano-Spica V, Papas TS, Bhat NK (1995) ETS1 suppresses tumorigenicity of human colon cancer cells. Proc Natl Acad Sci USA 92:4442–4446

Tan PB, Lackner MR, Kim SK (1998) MAP kinase signaling specificity mediated by the LIN-1 Ets/LIN-31 WH transcription factor complex during C. elegans vulval induction. Cell 93:569–580

Teruyama K, Abe M, Nakano T, Takahashi S, Yamada S, Sato Y (2001) Neurophilin-1 is a downstream target of transcription factor Ets-1 in human umbilical vein endothelial cells. FEBS Lett 504:1–4

Tsokos GC, Nambiar MP, Juang YT (2003) Activation of the Ets transcription factor Elf-1 requires phosphorylation and glycosylation: defective expression of activated Elf-1 is involved in the decreased TCR zeta chain gene expression in patients with systemic lupus erythematosus. Ann NY Acad Sci 987:240–245

Tugores A, Le J, Sorokina I, Snijders AJ, Duyao M, Reddy PS, Carlee L, Ronshaugen M, Mushegian A, Watanaskul T, Chu S, Buckler A, Emtage S, McCormick MK (2001) The epithelium-specific ETS protein EHF/ESE-3 is a context-dependent transcriptional repressor downstream of MAPK signaling cascades. J Biol Chem 276:20397–20406

Verger A, Duterque-Coquillaud M (2002) When Ets transcription factors meet their partners. Bioessays 24:362–370

Wang SC, Hung MC (2000) Transcriptional targeting of the HER-2/neu oncogene. Drugs Today 36:835–843

Wasylyk B, Hagman J, Gutierrez-Hartmann A (1998) Ets transcription factors - nuclear effectors of the Ras-MAP-kinase signaling pathway [review]. Trends Biochem Sci 23:213–216

Welford SM, Hebert SP, Deneen B, Arvand A, Denny CT (2001) DNA binding domain-independent pathways are involved in EWS/FLI1-mediated oncogenesis. J Biol Chem 276:41977–41984

Wood LD, Irvin BJ, Nucifora G, Luce KS, Hiebert SW (2003) Small ubiquitin-like modifier conjugation regulates nuclear export of TEL, a putative tumor suppressor. Proc Natl Acad Sci USA 100:3257–3262

Xin JH, Cowie A, Lachance P, Hassell JA (1992) Molecular cloning and characterization of PEA3, a new member of the Ets oncogene family that is differentially expressed in mouse embryonic cells. Genes Dev 6:481–496

Xing X, Wang SC, Xia W, Zou Y, Shao R, Kwong KY, Yu Z, Zhang S, Miller S, Huang L, Hung MC (2000) The ets protein PEA3 suppresses HER-2/neu overexpression and inhibits tumorigenesis. Nat Med 6:189–195

Xuan Z, McCombie WR, Zhang MQ (2002) GFScan: a gene family search tool at genomic DNA level. Genome Res 12:1142–1149

Yagasaki F, Wakao D, Yokoyama Y, Uchida Y, Murohashi I, Kayano H, Taniwaki M, Matsuda A, Bessho M (2001) Fusion of ETV6 to fibroblast growth factor receptor 3 in

peripheral T-cell lymphoma with a t(4;12)(p16;p13) chromosomal translocation. Cancer Res 61: 8371–8374

Yamada T, Abe M, Higashi T, Yamamoto H, Kihara-Negishi F, Sakurai T, Shirai T, Oikawa T (2001) Lineage switch induced by overexpression of Ets family transcription factor PU.1 in murine erythroleukemia cells. Blood 97:2300–2307

Yang B-S, Hauser CA, Henkel G, Colman MS, Van Beveren C, Stacey KJ, Hume DA, Maki RA, Ostrowski MC (1996) Ras-mediated phosphorylation of a conserved threonine residue enhances the transactivation activities of c-Ets1 and c-Ets2. Mol Cell Biol 16:538–547

Yordy JS, Muise-Helmericks RC (2000) Signal transduction and the Ets family of transcription factors. Oncogene 19:6503–6513

Zhang L, Eddy A, Teng YT, Fritzler M, Kluppel M, Melet F, Bernstein A (1995) An immunological renal disease in transgenic mice that overexpress Fli-1, a member of the ets family of transcription factor genes. Mol Cell Biol 15:6961–6970

Zhou J, Ng AY, Tymms MJ, Jermiin LS, Seth AK, Thomas RS, Kola I (1998) A novel transcription factor, ELF5, belongs to the ELF subfamily of ETS genes and maps to human chromosome 11p13–15, a region subject to LOH and rearrangement in human carcinoma cell lines. Oncogene 17:2719–2732