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TITLE: Gene Targets in Prostate Tumor Cells that Mediate
Aberrant Growth and Invasiveness

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Our hypothesis that the human PPC-1 prostate tumor cell lines with experimentally altered Ets transcription factor function, which show a reduction in the transformed phenotype, do so because of altered expression patterns in important genes downstream of Ets factors. We proposed to analyze global differences in gene expression between these cell lines, and assess the functional significance of changes in gene expression. Altered Ets function was found to delay xenograft tumor onset, and tumors from Ets2 overexpressing cells had dramatically reduced tumor microvasculature. Expanded microarray expression analysis has now identified over 65 potential Ets target genes in PPC-1 cells, including genes whose products can contribute to the observed changes in motility, invasiveness, survival, tumorigenicity, and tumor angiogenesis. Regulation of Ets target gene expression in prostate tumor cells was quite different than in other cell types, and prostate Ets family expression relative to other tissues was characterized. Follow-up analysis of Ets target genes has implicated IL-8 in cell motility, PKC delta in survival, CC3 in tumor angiogenesis, and MT-MMP-1 in invasiveness. This characterization of Ets factor signaling and targets in prostate cell transformation has elucidated new potential therapeutic targets and provides new insights on the molecular basis of aggressive prostate tumor cell behavior.

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INTRODUCTION

The proposed studies will broadly characterize the changes in gene expression that take place in a cell line model system for androgen-independent tumor progression, and assess their functional consequences. Differences in gene expression resulting from experimentally altered Ets transcription factor function, which reduces the tumor cells cancerous behavior, will be identified in gene microarray experiments, and correlated with their altered phenotype. Observed alterations in gene expression will be followed up to confirm regulation and assess potential functional consequences. Identifying gene products whose altered expression is involved in resistance to cell death, increased invasiveness, as well as tumor growth and angiogenic signaling by prostate tumor cells can identify important new therapeutic targets in androgen-independent prostate cancer.

BODY

1. Characterize differences in gene expression between PPC-1 prostate tumor cells and the Ets-construct altered PPC-1 lines with altered phenotypes.

In initial experiments, to assess the role of Ets transcription factors in the transformed characteristics of the PPC-1 prostate tumor cell line, we established sublines which stably expressed either a dominant inhibitor of Ets family function, the Ets2 DNA binding domain (Ets2DBD) or full-length Ets2, a prototypical transcriptional activator in the 27 member Ets transcription factor family. These cell lines with altered Ets activity(hereafter referred to as PPC1-Ets2 and PPC1-E2DBD) had lost many of their *in vitro* transformed characteristics, including substantial reductions in anchorage-independent growth, motility, invasiveness, and survival. We previously reported results from initial microarray and quantitative PCR (q-PCR) analysis, where 21 potential Ets target genes in PPC-1 cells were identified. This analysis was performed with probe made from RNA from PPC-1 cells as the reference standard, and probe made from PPC1-E2DBD or PPC1-Ets2 cells. The analysis was performed on arrays representing approximately 5,000 genes. To more fully characterize Ets-mediated changes in gene expression that led to loss of the transformed phenotype, we have utilized microarrays with oligonucleotide probes for over 20,000 known genes. The results of this analysis are summarized in Figure 1. Applying the cutoffs of a minimum signal and significant upregulation of over 2-fold, or downregulation to less than 0.6 fold in the majority of replicates (criteria we determined to leads to >80% validation by extensive q-PCR analysis), the expression of sixty-five different genes was found to be altered in either PPC1-E2DBD or PPC1-Ets2 cells.

Interestingly, expression of only 15 of these Ets target genes was found altered in both lines. Additionally, only five of these common target genes exhibited reciprocal activation of expression by Ets2 and repression by Ets2DBD, the pattern anticipated from standard reporter gene assays. Analysis of the direction of regulation of target genes in PPC1-E2DBD cells revealed that the Ets2DBD "dominant inhibitor" actually activated the expression of more genes than it repressed (Fig. 1). This suggests a unique balance of Ets family members in prostate tumor cells, with Ets family repressors predominating and being displaced by the Ets2DBD, rather than displacing the activating Ets family members which predominant in most cell types. This stimulated the comparison of Ets family member expression in normal prostate, prostate tumors, and prostate tumor cell lines described in aim 4. A further unexpected result was that expression of full-length Ets2 in PPC1-Ets2 cells caused an equal number of genes to be upregulated or downregulated (Fig. 1). Despite the unanticipated directions of Ets target gene

expression, the finding that only 8/65 Ets target genes were changed in the same direction in PPC1-Ets2 and PPC1-E2DBD cells, supports our hypothesis that the similar but not identical reversal of multiple transformed features in these two lines likely results from distinct sets of altered target genes. The potential role of the identified Ets target genes in the reversal of specific aspects of the transformed phenotype, and the follow-up analysis, is described in aim 6.

2. Generate PPC-1 cell lines with inducible Ets2DBD or Ets2 expression and determine their *in vitro* phenotype.

As stated in the previous reports, generation and analysis of PPC-1 cells with inducible Ets2 or Ets2DBD constructs became a low priority. In a separate parallel project, breast tumor cell lines with inducible Ets2 or Ets2DBD constructs exhibited the same *in vitro* and *in vivo* phenotypes (loss of transformed characteristics) as cells with stably expressed Ets2 constructs. Thus, it appears in a tumor cell context, that the observed reversal of transformation by these Ets2 constructs is not an artifact of clonal selection. In addition, for target gene analysis, rapid changes in Ets target genes suggests a direct role for Ets factors, but ultimately promoter-based analysis is still required to demonstrate direct targeting for each individual target gene.

3. In vivo analysis of the tumorigenic , angiogenic, and metastatic potential of the altered PPC-1 lines.

We previously reported the results of initial tumor analysis, where palpable xenograft tumor onset from either PPC1-E2DBD or PPC1-Ets2 cells was significantly delayed relative to the parental PPC-1 cells ($p= 0.0008$ and 0.0003 , respectively). Due to the large fluctuation in xenograft tumor size and growth, the clear 2-fold average reduction in tumor size from the PPC1-E2DBD or PPC1-Ets2 cells was not found to be statistically significant by t-test. Immunohistochemistry was used to compare the tumor microvasculature in the PPC1-E2DBD or PPC1-Ets2 cells to tumors from the parental PPC-1 cells. Size-matched tumors (rather than age-matched) generated from these three cell lines were sectioned and immunostained with an antibody to CD31, an endothelial cell marker, and counterstained with hematoxylin. Figure 2 shows representative fields of such analysis. There was a dramatic reduction of the (brown) anti-CD31 stained blood vessels specifically in the PPC1-Ets2 cells. This reduced vasculature was particularly apparent towards the centers of the tumors, which also appeared necrotic. The inhibition of signaling to tumor angiogenesis by Ets2 in prostate carcinoma cells represents a novel phenotypic role for Ets signaling, and two biologically relevant Ets target genes whose expression pattern correlates to the specific inhibition of tumor angiogenesis have been identified (aim 6). While *in vitro* invasiveness (see results aim 4) and enhanced angiogenesis are strongly linked to metastatic potential, direct assessment of the metastatic potential of the PPC1-E2DBD and PPC1-Ets2 cells using the tail vein injection assay would be complicated by the reduced survival and growth rate of these cells with altered Ets activity, and their strongly impaired ability to form tumors and grow *in vivo*. Thus, it was not undertaken.

4. Expand the analysis of altered gene expression in prostate cells to PC-3 lines that show reduced or increased invasiveness, and to non-malignant prostate tissue.

The PPC1-E2DBD and PPC1-Ets2 cells were found to exhibit very different invasiveness in the Matrigel assays, with PPC1-Ets2 cells exhibiting the highly invasive phenotype of the parental cells, whereas invasiveness was almost entirely lost in the PPC1-E2DBD cells. We have used these genetically nearly identical cell lines to focus on the Ets target genes mediating

invasiveness in prostate tumor cells, and two proinvasive candidate mediators, MT-MMP1/MMP14 and MMP3 were identified as specifically exhibiting downregulation in the PPC1-E2DBD line.

The primary reason that the Ets2DBD alters the expression of many genes not regulated by Ets2 overexpression, is that it acts as a broad inhibitor of Ets family function by displacing Ets factors from promoter binding sites. However, the expression status of most of the 27 Ets family members in prostate tumors or tissue is unknown. To understand how Ets targets are regulated in prostate tumor cell lines and in non-malignant prostate tissue, we determined the mRNA expression profiles for the entire Ets family in these contexts, and compared it to other types of cells. Figure 3 shows the results of this analysis, with the expression of each Ets factor in normal prostate, the hormone-dependent and less aggressive LNCaP prostate tumor line, and several more invasive prostate tumor cell lines. In addition, in collaboration with Dr. Robert Abraham, we also assessed the impact of hypoxia, a condition found in advanced tumors, on Ets family expression in PC-3 cells. Relative to the mixed cell line "Universal RNA" standard, a variety of Ets factors are more abundant in prostate tissue. This included the epithelial-specific family of Ets/PSE, as well as Ets2. A number of immune-specific Ets factors were also increased, likely due blood in the prostate tissue. The prostate tumor cell lines, unlike the complex prostate tissue composed of many cell types, expressed lower levels of many Ets factors. However, in PPC-1 cells, 16 different Ets factors were still expressed at >20% of mixed tissue, demonstrating unexpected complexity in which Ets family members are mediating transformation. It is likely that some of the differences between normal prostate tissue and the tumor cells are not tumor-specific but rather cell-type specific, and we are obtaining normal prostate epithelial cell RNA to compare with the tumors. Strong expression of several Ets family repressors was observed, but they were not elevated sufficiently at the level of mRNA to explain the unique repressive state of Ets-mediated gene expression in PPC-1 cells. Overall, the pattern of Ets factor expression was quite similar in the PPC-1, PC-3, and DU-145 cells, with contrasting results in the LNCaP. Interestingly, a similar complexity and pattern of Ets factor expression in mouse normal prostate and prostate tumors was observed in a collaborative study with Dr. Robert Oshima. This suggests that analysis of the role of Ets factors in mouse models of prostate cancer will be relevant to the observed roles in human tumors.

An example of follow-up analysis of Ets target gene expression in normal prostate tissue and various prostate tumor cell lines is shown in figure 4. Quantitative real-time PCR (Q-PCR) was used to determine the expression of several genes, relative to expression of the housekeeping gene Cph/PPIA in the same cDNA sample. The data are expressed as percent expression of the same gene in normal prostate tissue. Expression of NK3.1 is often lost in advanced prostate tumors, and its expression was reduced in LNCaP cells, and absent in the more invasive tumor cell lines. Expression of MMP3 and MT-MMP1, Ets target genes that we have implicated in PPC-1 cell invasiveness, were specifically downregulated in the PPC1-E2DBD line, relative to the parental cells and DU145 cells. In contrast, TFPI2, a potential anti-angiogenic factor, was specifically upregulated in the PPC1-Ets2 cells, which exhibit reduced tumor angiogenesis. The constant signal from the GAPDH housekeeping gene for all of the cell lines shows that normalization to Cph was appropriate. Overall, this follow-up analysis extends the correlation of identified Ets target gene expression to normal and tumor cells, further indicating a biological role for these targets in features such as invasiveness and angiogenesis.

5 Bioinformatic analysis of altered gene expression

Several kinds of comparisons with our data set have been performed. These include matching functional annotation (e.g. GO terms and functional descriptions) of identified targets with observed changes in phenotype. This has led to a list of targets that “make sense”. While it might seem circular to look for targets with known roles, many of these functions are from other tumor types, or inferred from protein sequence. Thus, they represent novel targets in prostate tumors. A second kind of analysis involved comparison of the direction of change in Ets target genes in PPC-1 cells relative to what has been reported in the literature and our array analysis of breast tumor cells. While genes downregulated in PPC1E2DBD cells corresponded well to other results, genes upregulated in these cells (e.g. MMP1 or KLKB1) were the opposite of other findings. Similarly, Ets targets were also often regulated in the opposite direction in the PPC1Ets2 cells relative to other cells overexpressing Ets2 (e.g. TFPI2, IL-8, THBS1, S100A4). These results highlight the value of assessing Ets target genes in prostate tumor cells to identify important and potentially unique regulators of transformation. During the course of this work, global informatic analysis resources for prostate normal/tumor cell gene expression comparison have become available through the NCI, with the CGAP project (<http://cgap.nci.nih.gov/>) and the CMAP project (<http://cmap.nci.nih.gov/Profiles/ProfileQuery>) .

6 Determine functional significance of observed changes in prostate tumor cell line gene expression.

We previously reported follow-up analysis on one target gene that we identified, IL-8. Protein levels for IL-8, a interleukin implicated in several functions including cell motility, were substantially reduced in the PPC1E2DBD cells. These cells displayed strongly reduced motility relative to the parental cells, and addition of exogenous IL-8 to the motility assay significantly enhanced their motility, but did not alter motility of the parental cells. This indicates that IL-8 functionally contributes to the motility of PPC-1 prostate tumor cells, and highlights a new role for IL-8 in prostate tumors beyond tumor angiogenesis.

A second target gene that was followed up is PKC delta. Expression of PKC delta mRNA and of protein levels (fig. 5) were strongly upregulated in PPC1-E2DBD cells. PKCs have a wide role in cellular responses, and upregulation of PKC delta is associated with impaired cell survival, consistent with the increased apoptosis observed in PPC1-E2DBD cells. Addition of rottlerin, a selective inhibitor for PKC delta activity, enhanced PPC1-E2DBD cell attachment and growth, and changed their rounded morphology back to that of the parental PPC-1 cells. This suggests that PKC delta is a functional Ets target in PPC-1 cells, and that modulating Ets signaling and PKC expression could be used sensitize tumor cells to killing by other agents.

Other targets that warrant eventual follow-up as mediators of the impaired growth and apoptotic phenotype of the PPC1-Ets2 line, include upregulation of the cell cycle inhibitor p16INK4A, downregulation of the anti-apoptotic v-myb homolog MYBBL2, and upregulation of DUSP5, a dual specificity phosphatase that inhibits MAP kinase signaling.

Ets targets that are candidates for the specific downregulation of PPC1-Ets2 cell xenograft tumor angiogenesis are CC3/TIP30, a recently characterized anti-angiogenic factor, and the strongly

upregulated TFPI2, an inhibitor of MMPs, which are required for blood vessel invasion into the tumor. The role of CC3 in the observed phenotype was a subject of a grant application.

An Ets target gene whose upregulated expression may contribute to the reduced growth rate of both PPC1-E2DBD and PPC1-Ets2 cells is MAP2K6, which activates p38 signaling leading to stress induced cell cycle arrest. The S100A4 calcium binding protein is also a common Ets target in the two cell lines, and its strong downregulation in both cell lines and its function in cell motility likely contribute to reduced motility in these cell lines. Finally, several identified Ets targets are candidates for mediators of PPC-1 invasiveness, based on their reduced expression in the less invasive PPC1-E2DBD cells. Specific downregulation of MMP3, MMP7, and MT-MMP-1 were seen in this cell line. In addition, production of HGF, an autocrine and motility factor for prostate tumor cells, is also downregulated in PPC1-E2DBD cells. The biological significance and generality of these findings will be the subject of further investigation.

KEY ACCOMPLISHMENTS

Microarray analysis has identified 65 Ets target genes whose expression is altered in PPC-1 prostate tumor cells that have lost many hallmarks of oncogenic transformation.

In vivo xenograft tumor analysis has revealed a new role for Ets2 signaling in prostate tumors – an anti-angiogenic function. Candidate Ets target genes with anti-angiogenic activity (e.g. CC3 and TFPI2) have been identified.

PKC delta has been identified as an Ets target gene whose altered expression impacts on tumor cell survival, and other candidates (e.g. p16INK4A, MYBBL2, DUSP5) mediating survival have been identified.

Candidates for mediating PPC-1 cell invasiveness were identified by differential expression in the cell lines, including MMP3, MMP7, and MT-MMP-1.

Expression analysis in normal prostate and other prostate tumor cell lines has demonstrated the significance and generality of some identified Ets target genes.

Analysis of the expression of the entire Ets family in normal prostate and multiple prostate tumor cell lines has revealed unexpected complexity in the number of Ets factors present, and has highlighted which Ets factors are more abundant in prostate tissues and tumor cells. These are important data for interpreting how Ets2DBD targets may be regulated and modulated.

A similar complexity and pattern of Ets factor expression was observed in a mouse model of prostate cancer, suggesting results on the role of Ets factors obtained in mouse models will correspond to human tumors.

REPORTABLE OUTCOMES

Foos G, Hauser CA (2004) The role of Ets transcription factors in mediating cellular transformation. In: Handbook of Experimental Pharmacology, Vol. 166: Transcription Factors. Springer, Heidelberg and New York, pp 259-275. (follows as appendix)

Foos, G. and Hauser, C. Molecular targets of Ets transcription factor signaling in prostate tumor cells. (Manuscript in preparation)

The phenotypically characterized PPC-1 prostate tumor cells with altered Ets functions are distributed on request.

Results from these studies were the basis for part of an NIH RO1 application proposing a detailed analysis of how Ets factors mediate transformation.

Results from these studies also partial basis for submitted DOD Idea development award PC041153.

Career Development: involvement with, and development of the technology needed for these studies (microarrays, real-time PCR, histology, etc.) contributed to appointment of PI as Cancer Center Associate Director, Shared Resources, as well as an Institutional appointment as the Director of Research Support.

CONCLUSIONS

This study is based on the hypothesis that PPC-1 prostate tumor cell lines with experimentally altered Ets transcription factor function, which show a reduction in the transformed phenotype, do so because of altered expression patterns in important downstream genes. Thus, we proposed to further validate this cell model in vivo, analyze global differences in gene expression between these cell lines, and assess the functional significance of the observed changes. We have accomplished many of these goals. The completed tumorigenicity studies demonstrate that the Ets2DBD and Ets2Full expressing cell lines exhibit a highly significant delay in tumor formation. In addition, a strong inhibition of angiogenesis was specifically observed in xenograft tumors from the cells overexpressing Ets2. These data extend the in vitro results, and make this a more compelling model system for understanding the genes whose altered expression cause reduced tumorigenicity. Indeed, a new signaling pathway which one can modulate to attack tumor angiogenesis has exciting therapeutic implications, and we are following up on Ets targets implicated in this signaling, including CC3 and TFPI2.

The broad microarray analysis of altered gene expression has identified 65 Ets targets in PPC-1 cells, many of which have been confirmed by quantitative PCR. These target genes are associated with the regulation of many important aspects of cancer cell behavior, and many of the changes in are unique to prostate cancer. We now have identified promising Ets targets which can modulate PPC-1 cell survival, growth, motility, invasiveness, and the above-mentioned tumor angiogenesis. Analysis of altered protein expression and functional assays have now been completed for several target genes, including IL-8 and PKC delta. Real-time PCR analysis was used to analyze the expression of the entire 27 member Ets family in normal prostate tissue and various prostate tumor cell lines, and to compare this to other cell types. Fairly striking differences were observed in the expression of several Ets factors, and parallel changes were seen in a mouse model of prostate cancer.

While it is premature for such analysis to lead to new drugs or gene therapy, the identification of Ets target genes that modulate the cancerous phenotype promises to yield new therapeutic targets for androgen-independent tumors (e.g. upregulating anti-angiogenic regulators). Approaches to identify important changes that take place in prostate tumors based on cell lines have both advantages and disadvantages. In our system, we are looking at events that occur in the reversal of cancerous behavior. The advantage we hypothesized and our results demonstrate, is that a fairly defined number of changes can and have been identified in these nearly genetically identical cells. The characterization of how these changes in gene expression act to reverse prostate cell transformation, has provided important new knowledge on the molecular basis of aggressive prostate tumor cell behavior.

REFERENCES CITED none, but full citations in attached review.

APPENDIX

Figure 1. Genes whose expression was significantly changed in PPC-cells with altered Ets activity.

Downregulated in Ets2DBD		Upregulated in Ets2	
Gene	Accession #	Gene	Accession #
MYADM	AK027693	UCHL1	AK055249
MYADML	AK057470	CDK6	AK055922
EIF1A	NM_001412	HSPCA	AK056446
EIF2S3	NM_001415	HLA-C	NM_002117
EIF4A2	NM_001967	CDKN2A/p16INK4A	NM_000077
RBBP7	NM_002893	ARHGDI2/Rho GDI 2	NM_001175
DUSP5	NM_004419	CCNA2	NM_001237
HGF	NM_000601	MAP2K6	NM_002758
MMP7	NM_002423	DUSP5	NM_004419
MT-MMP1/MMP14	NM_004995	TFPI2	NM_006528
IL-7R	NM_002185	ATX/ENPP2	NM_006209
MGCA	NM_139321	KLKB1	NM_000892
S100A4	NM_002961	CD73/NTSE	NM_002526
HLA-DRg	NM_019111	IL-8	NM_000584
THBS1	NM_003246	HXB/TNC	NM_002160
MMP3	NM_002422	CC3/HTATIP2	NM_006410
		Bcl2L1	NM_138578
		IL-7R	NM_002185
		MT-MMP1/MMP14	NM_138578
		HGF	NM_000601
		ITGB3	NM_000212
		NCOA/SRC1	NM_147223
		TIMP3	NM_000362
Upregulated in Ets2DBD		Downregulated in Ets2	
Gene	Accession #	Gene	Accession #
SHD	AK056268	HLA-DMB	NM_002118
EPHB1	AL133099	TNC	NM_002160
CSE1L	NM_001316	MMP1	NM_002421
EEF1A2	NM_001958	MYBL2	NM_002466
EIF5	NM_001969	NFIX	NM_002501
FGF7	NM_002009	PRSS11	NM_002775
GAB1	NM_002039	TFF1	NM_003225
MMP1	NM_002421	TFF3	NM_003226
NFIX	NM_002501	IKBKG	NM_003639
MAP2K6	NM_002758	NRP1	NM_003873
VEGFC	NM_005429	ELF3	NM_004433
IPO7	NM_006391	ITGBL1	NM_004791
PIAS1	NM_016166	GFRA1	NM_005264
P38IP	NM_017569	TRIP	NM_005879
KLK12	NM_019598	SKP2	NM_005983
RASL10B	NM_033315	IPO7	NM_006391
KLKB1	NM_000892	PIK3CD	NM_005026
CD73	NM_006410	MGCA	NM_139321
ENPP2/ATX	NM_006209	THBS	NM_003246
Bcl2L1	NM_138578	HLA-DRg	NM_019111
NCOA/SRC1	NM_147223	S100A4	NM_002961
PKRCD	NM_006254		

Combined data from replicate microarray analysis (5K and 20K human arrays), with many of these target genes confirmed by quantitative PCR analysis. Genes are grouped by regulatory pattern, with significant upregulation a ≥ 2.0 fold average increase, or downregulation to ≤ 0.6 fold less in the indicated PPC-1 lines (stably expressing Ets2DBD or Ets2) relative to expression of the same gene in the parental PPC-1 prostate tumor cells. There are a total of 65 different genes, with 15 significantly altered in both the Ets2 and Ets2DBD cell lines.

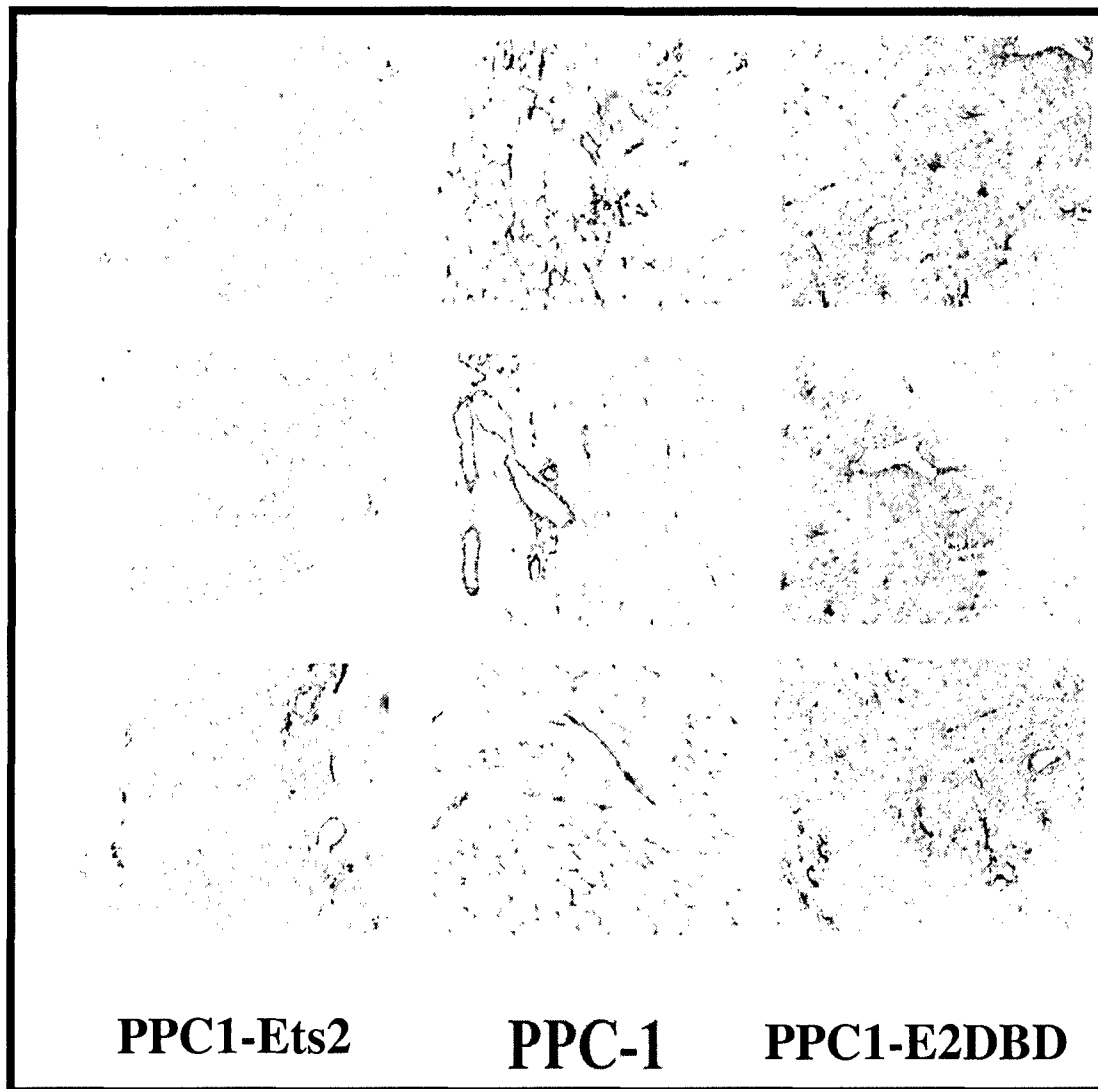


Figure 2. Representative CD31 stained frozen sections from xenograft tumors derived from the indicated cell line

Ets Factor	Prostate tissue	LNCaP	PPC-1	Normal PC-3	Hypoxic PC-3	DU-145
Ets2			65			153
Ets1	171				154	
ER71			58	167		
GABPa	121		56	115	69	
PEA3			108	166	88	
ER81	144		144	88	63	
ERM	90					
Fli						
ERG						
ERF			81	56		
PE1	155			73		
Elk1		66		134	104	
Elk3	184		119	153	80	
Elk4	107		65			109
Elf1				66		
Elf2						
Elf4	182		90		80	
Ese1			98	80		
Ese2						
Ese3						
PSE		173		97		
TEL1				72	64	
TEL2				185		
Spi1						
SpiB						
SpiC						
FEV						

Figure 3. Expression of the entire Ets family in normal human prostate tissue and human prostate tumor cell lines. Expression was quantitated by q-PCR, and normalized to Cph/PPIA levels. Data shown are the percent expression for each Ets factor, relative to its expression in a mixture of RNAs from 10 diverse cell lines (Stratagene Universal RNA). Red and green denote 2-fold up- or down-regulation, respectively

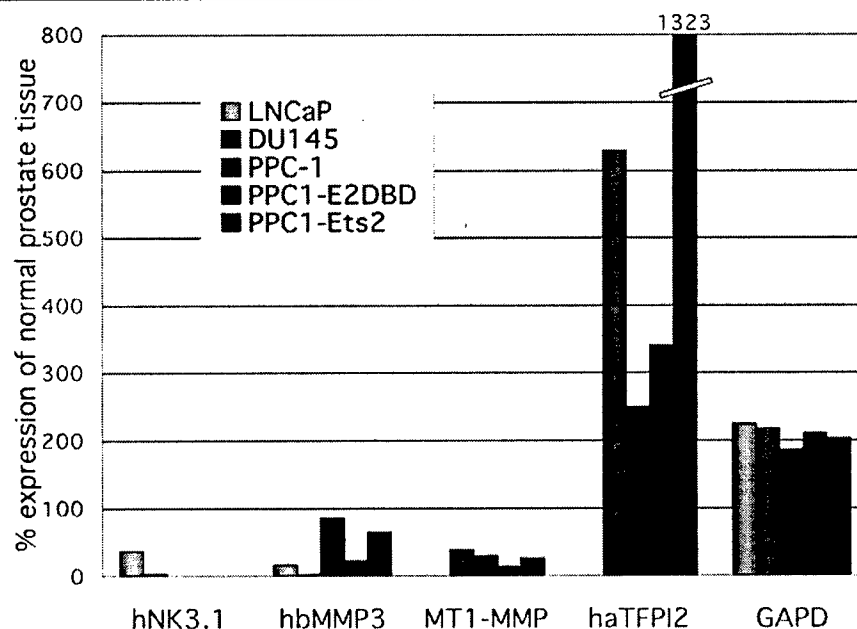


Figure 4. Regulation of Ets target genes in human prostate tumor cell lines, relative to normal prostate tissue. Gene expression was determined by q-PCR and normalized to Cph/PPIA mRNA levels in each sample.

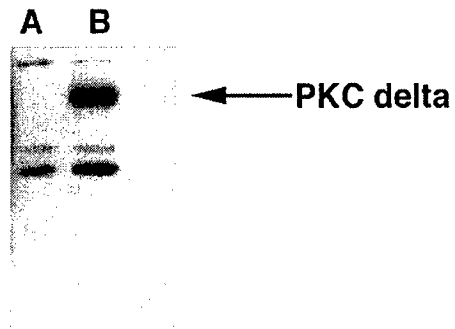


Figure 5. Immunoblot analysis of PKC delta protein expression. Whole cell extract from PPC-1 cells (lane A) and PPC1-E2DBD (lane B).

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Part 2: Transcription Factors in Pathophysiology

The Role of Ets Transcription Factors in Mediating Cellular Transformation

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1 Introduction to the Ets Family of Transcription Factors

1.1 The Ets Gene Families of Mice and Humans

1.2 Ets Family Nomenclature

1.3 Ets Family Functions

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

1.5 Ets Target Genes

2 Evidence Implicating Ets Factors in Cellular Transformation and Cancer

2.1 Ets Factor Overexpression Resulting from Proviral Insertion

2.2 Chromosomal Translocations of Ets Genes Associated with Human Cancers

2.3 Mutations in Ets Genes Associated with Human Cancers

2.4 Signaling to Ets Factors from Oncogenes

2.5 Expression Correlation of Ets Factors with Tumors

2.6 Reversal of Cellular Transformation by Altered Ets Factor Function

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

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

References

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 Duterrque-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 homology) 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.

[Table 1. will appear here. See end of document.]

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 Waslylyk 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 activated 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 (Athanasίου 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 Ets2/Elf5 and Ets3/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 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 (Athanasίου 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; Athanasίου 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, Ets1, 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 hematopoietic 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, Vandebunder 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* 279:11281-11292
- 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 ELFI-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, et al (1990) The ETS-domain: a new DNA-binding motif that recognizes a purine-rich core DNA sequence [letter]. *Genes Dev* 4:1451-1453
- Klamt 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, Dutourque-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, Gégonne 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, Grall F., 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, Jermini 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

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 ↓	<u>Ets2</u>	c-ets-2	yes	NM_011809	Mm.22365	16	NM_005239	Hs.292477	21
	<u>Ets1</u>	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	<u>Etv4</u> , (h)E1AF	no	NM_008815	Mm.5025	11	NM_001986	Hs.434059	17
	ER81	<u>Etv1</u> , (m)EtsRP81	no	NM_007960	Mm.4866	12	NM_004956	Hs.150011	7
	ERM	<u>Etv5</u>	no	NM_023794	Mm.155708	16	NM_004454	Hs.43697	3
Erg ↓	<u>Fli1</u>	(h)ERGB, EWSR2	yes	NM_008026	Mm.258908	9	NM_002017	Hs.257049	11
	<u>Erg</u>		yes	NM_133659	Mm.164531	16	NM_182918	Hs.45514	21
	<u>Fev</u>	(h)HSRNAFEV, Pet1	no	NM_153111	Mm.150496	1	NM_017521	Hs.234759	2
ERF ↓	<u>Erf</u>	(h)PE-2	no	NM_010155	Mm.8068	7	NM_006494	Hs.440332	19
	PE1	<u>Etv3</u> , METS	no	NM_012051	Mm.34510	3	NM_005240	Hs.352672	1
Elk/TCF ↓	<u>Elk1</u>		no	NM_007922	Mm.3064	X	NM_005229	Hs.181128	X
	<u>Elk3</u>	Net, Sap-2, ERP	no	NM_013508	Mm.4454	10	NM_005230	Hs.288555	12
	<u>Elk4</u>	Sap1	no	NM_007923	Mm.195050	1	NM_021795	Hs.129969	1
Elf/Ese ↓	<u>Elf1</u>	Elf-1	no	NM_007920	Mm.24876	14	NM_172373	Hs.124030	13
	<u>Elf2</u>	(h)NERF	no	NM_023502	Mm.46503	3	NM_006874	Hs.82143	4
	<u>Elf4</u>	MEF, ELFR	no	NM_019680	Mm.154274	X	NM_001421	Hs.151139	X
	<u>Ese1</u>	<u>Elf3</u> , ESX, jen, Ert	yes	NM_007921	Mm.3963	1	NM_004433	Hs.67928	1
	<u>Ese2</u>	<u>Elf5</u>	yes	NM_010125	Mm.20888	2	NM_001422	Hs.11713	11
	<u>Ese3</u>	<u>Ehf</u>	yes	NM_007914	Mm.10724	2	NM_012153	Hs.200228	11
	<u>Pse</u>	(m)Spdef, (h)PDEF	yes	NM_013891	Mm.26768	17	NM_012391	Hs.79414	6
TEL ↓	TEL	<u>Etv6</u> , TEL1	yes	NM_007961	Mm.269995	6	NM_001987	Hs.171262	12
	TEL2	<u>Etv7</u> , TELB	yes	None	None	-	NM_016135	Hs.272398	6
Spi ↓	PU.1	(m) <u>Sfp1</u> , (h) <u>SPI1</u>	no	NM_011355	Mm.1302	2	NM_003120	Hs.157441	11
	<u>SpiB</u>	Spi-B	no	U87620	Mm.8012	7	NM_003121	Hs.437905	19
	<u>SpiC</u>	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 symbol. Table data are compiled from the 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 number

^d UniGene Cluster

^e Chromosomal location