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Molecular mechanisms of ETS transcription factor mediated tumorigenesis

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Abstract

The ETS family of transcription factors is critical for development, differentiation, proliferation and also has a role in apoptosis and tissue remodeling. Changes in expression of ETS proteins therefore have a significant impact on normal physiology of the cell. Transcriptional consequences of ETS protein deregulation by overexpression, gene fusion, and modulation by RAS/MAPK signaling are linked to alterations in normal cell functions, and lead to unlimited increased proliferation, sustained angiogenesis, invasion and metastasis. Existing data show that ETS proteins control pathways in epithelial cells as well as stromal compartments, and the crosstalk between the two is essential for normal development and cancer. In this review we have focused on ETS factors with a known contribution in cancer development. Instead of focusing on a prototype, we address cancer associated ETS proteins and have highlighted the diverse mechanisms by which they affect carcinogenesis. Finally, we discuss strategies for ETS factor targeting as a potential means for cancer therapeutics.

Introduction

The ETS family of transcription factors positively and/or negatively regulates the expression of genes involved in signaling pathways, development, cell proliferation, differentiation, migration, apoptosis, invasion and metastasis. Thus, ETS factors have a significant impact on various disease states, particularly cancer, which results from multiple events involving initiation, promotion, transformation and metastatic growth. Since cancer cells acquire

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Conflicts of Interest
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similar biological properties considered as hallmarks of cancer, such as proliferative signaling, resisting cell death, evading growth suppressors, and activating invasion and metastasis, it is critical to understand the molecular mechanisms underlying such transformation events. In this review, we will focus on the current understanding of the molecular mechanisms that lead to ETS dysregulation and how specific ETS factors cause tumorigenesis, with tumorigenesis referring to the formation of solid tumors, such as carcinomas, sarcomas and lymphomas, and liquid tumors, such as leukemias and multiple myeloma.

Remarkably, ETS mediated tumorigenesis is largely dependent on the cellular context, and apart from the cell/tissue type and cooperative mutations/lesions, the tissue specific role of ETS proteins is controlled by the ETS regulatory code, involving co-factor interactions, ETS factor post-translational modifications, DNA-binding specificity, and subcellular localization. All ETS proteins are defined by their highly conserved DNA binding domain (DBD) that recognizes a GGAA core sequence, but the tissue-specific role of ETS proteins in part is dictated by distinct DNA binding specificities conferred by protein interactions and post-translational modifications, which also result in switching of activation and repression activities. In cancer, however, the pathophysiological functions of ETS proteins are not always governed solely by ETS DNA binding ability. To this end, we have broadly divided the mechanisms of ETS protein-mediated oncogenesis into seven different categories. These include: (1) activation by elevated expression; (2) activation by amplification; (3) ETS gene fusions as a consequence of chromosomal translocation; (4) transformation resulting from cytoplasmic expression; (5) transformation by stromal expression of ETS proteins; (6) oncogenesis by binding to genome regulatory regions; (7) increased transcription potency as a result of protein interaction, post-translational modification or protein stabilization; and, (8) switch from repressor to activator (Table 1). The first section of this review will present an overview of ETS family structure, DNA sequence preference, and regulation of DNA binding, to provide a basic understanding of ETS protein biochemistry. We then address the role of each of the above mechanisms in ETS mediated transformation and malignancy, and end by summarizing the role of ETS factors as tumor repressors or tumor suppressors. Table 2 shows the cancer-relevant ETS factors discussed in this review, listing their alternative names, and the section of the review in which they are discussed.

A. The ETS family: Structure, DNA sequence preference, and regulation of DNA binding

A.1 ETS Transcription Factor Structure

The ETS (E26 transformation-specific) is one of the largest transcription factor families, consisting of 27 ETS genes in humans, 26 in mice, 10 in *Caenorhabditis elegans*, and 9 in *Drosophila*, which can be structurally categorized into 11 subfamilies (ETS, ERG, ELG, ELF, ESE, ERF, TEL, PEA3, SPI, TCF and PDEF) (Fig. 1) (Galang et al., 2004, Hollenhorst et al., 2004, Gutierrez-Hartmann et al., 2007). The oncogene v-ets was first identified in the leukemogenic E26 avian replication defective retrovirus as a fusion protein with v-Myb. Subsequently, v-ets related genes were identified in metazoans. Phylogenetic analysis shows that the ETS gene family is present throughout the metazoan phyla and its

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continuous expansion from invertebrates to vertebrates is likely the result of large-scale duplications of vertebrate genomic regions (Hsu et al., 2004, Oikawa and Yamada, 2003, Papas et al., 1986). The ETS proteins are defined by their conserved ETS domain, which is a conserved winged helix-turn helix DNA binding domain and is composed of three alpha helices and four stranded anti parallel beta sheets. Structural analysis showed that the ETS domain consists of 85 amino acids and recognizes a core 5′-GGA(A/T)-3′ core sequence, which is defined as the ETS binding site (EBS) (Wang et al., 1992, Donaldson et al., 1996, Papas et al., 1986). The two invariant amino acid residues in helix 3 and residues in the turn between helices H2 and H3, as well as those in the wing between beta strands 3 and 4, provide key ETS protein interactions with the major groove in DNA (Szymczyna and Arrowsmith, 2000, Agarkar et al., 2010). Many ETS proteins, like GA-binding protein alpha (GABPA), ETS-1, ETS-2, and ESE-1 have a regulatory domain, consisting of a short helix or an ordered coiled sequence flanking the ETS domain, which contributes to their stability and DNA binding ability. In the case of GABPA, the two C terminal helices H4 and H5 serve as a platform for binding the obligate partner, GA-binding protein beta 1 (GABPB1), which indirectly enhances DNA affinity. For Ets-1, the regulatory domain is part of the autoinhibitory module. All ETS proteins, with the exception of GABP alpha, are autoinhibited by virtue of two inhibitory regions that flank the ETS DNA binding domain, namely the inhibitory module and a serine rich region SRR (Hagman and Grosschedl, 1992, Wasylyk et al., 1992). A helical bundle, composed of helices HI-1, HI-2, H4 and H5, form the inhibitory module of Ets-1 is packed against the H1 of the ETS domain, and promotes the closed conformation of the ETS domain, reducing its DNA binding affinity by half. The adjacent SRR reduces DNA binding affinity by another 10-20 fold (Graves et al., 1998, Dittmer, 2003).

A subset of ETS proteins (ETS, ERG, ELG, ESE, TEL and PDEF) has yet another conserved domain known as the Pointed (PNT) domain, first characterized in *Drosophila*. A series of NMR and crystallographic studies on the ETS Pointed domain derived from *Drosophila* and several vertebrate proteins show that the core of this 80 residue conserved domain is formed by four alpha helices (H2-H5), along with a short alpha helix (H2′) (Slupsky et al., 1998). The Pointed domain is similar to the sterile alpha motif (SAM) domain, which is known for protein-protein or protein RNA interactions. Crystallography approaches, coupled with biochemical studies, indicate that the Pointed domain functions in homo-oligomerization, hetero-dimerization, and transcriptional repression. TEL and TEL derived oncogenic chimeric proteins, where the PNT domain genetically fuses with the catalytic tyrosine kinase domains of PDGFRβ and NTRK3, form homotypic oligomers *in vitro* and *in vivo*. Fusion of TEL with fragments of transcription factors, such as AML1, promotes transcriptional repression (De Braekeleer et al., 2012). In ETS-1 and ETS-2, preceding the Pointed domain lies an N-terminal Ras responsive phosphorylation site at threonine and serine residues. In such cases, the Pointed domain acts as an Erk docking site and helps to interact with a complementary docking site on Erk kinases, like ERK2, and thus enhances transcriptional activity of ETS-1 and ETS-2 (Seidel and Graves, 2002).

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A.2 ETS DNA Binding Site Sequences and Regulation of DNA Binding Ability

Genome wide occupancy studies using either ChIP-chip or ChIP-seq reveal that a single ETS protein can bind to hundreds to thousands of ETS binding sites in a particular cell type, but these studies also show that multiple ETS factors can occupy any given single ETS binding site within a particular cell type, indicating redundant binding (see Fig. 1 for high affinity binding site for each ETS subfamily) (Hollenhorst et al., 2007, Barski and Zhao, 2009, Farnham, 2009, Yu et al., 2010, Hollenhorst et al., 2011b). Importantly, many of the redundant binding sites are characterized by the consensus ETS sequence ACCGGAAGT and are mostly found on the proximal promoters of housekeeping genes (Barski and Zhao, 2009, Farnham, 2009, Hollenhorst et al., 2007). By contrast, nonredundant ETS sites are more often characterized by atypical, low affinity ETS binding site sequences that are often flanked by binding sites of other transcription factors, such as AP-1 or PAX5, with these proteins often providing a stabilizing binding surface (Wheat et al., 1999, Hollenhorst et al., 2011b)

Microwell binding assays or protein binding microarrays of 26 mouse and 27 human ETS proteins (Wei et al., 2010), and other *in vitro* binding studies, have found that the differences in consensus DNA binding sequences for most ETS proteins are minimal, and specificity is often determined by the amino acids surrounding the ETS domain in the ETS protein and by DNA sequences flanking the central GGAA/T core in the genome. Based on *in vitro* binding affinities, the ETS binding profiles for the 11 ETS subfamilies fall into four distinct classes (Figure 1): Class I contains most of the ETS subfamily members (ETS, TCF, ERG, PEA3) and displays a consensus sequence of ACCGGAAGT. Class II contains the TEL, ESE and ELF subfamilies and this class displays binding affinity for a consensus CCCGGAAGT, which differs mostly in the first nucleotide. Class III is composed of only the SPI family and has affinity for binding sites with an adenine rich sequence 5′ to the GGAA core sequence. Class IV includes only SPDEF and displays a higher affinity for a GGAT core sequence instead of GGAA (Barski and Zhao, 2009, Farnham, 2009, Hollenhorst et al., 2007).

Although all ETS DBDs are relatively highly conserved, specificity is dictated by attributes other than the central DNA binding core. Preference for different DNA flanking sequences for different ETS proteins helps them to differentially bind to specific DNA sites to regulate distinct biological functions. It has been found that members of Class II are sensitive to an adenine-to-thymine substitution at position 7 (Mo et al., 1998), while SPI preferably binds to core GGA sites preceded by an adenine-rich sequence (Bosselut et al., 1993, Hollenhorst et al., 2009, Oettgen et al., 2000). Much of our detailed understanding of this differential binding comes from biochemical and structural studies on ELK-1, a member of the ternary complex factor (TCF) subfamily of ETS domain transcription factors. Replacement of residues D38 and D69 in ELK-1 by corresponding residues Q37 and V68 from SAP-1 is sufficient to confer SAP-1 DNA binding specificity on ELK-1 (Shore et al., 1996, Mo et al., 1998), even though the position of neither Q37 or V68 explain hydrophobic or hydrogen bond contacts made with DNA. Crystal structure reveals that the different DNA binding specificities of TCF members, like ELK-1 and ELK-4, actually arise from conformational changes imposed by tertiary contacts of conserved residues in the DNA binding interface

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with adjacent non-conserved residues (Mo et al., 2000) and impose restriction on ELK-1 for binding variants of the core GGAT sequence.

Specificity in cells also derives from structural elements adjacent to the ETS domain, and interaction with partner transcription factors and cofactors. Autoinhibition in Ets-1 is orchestrated allosterically by increasing the level of phosphorylation in the SRR domain, which although distant from the DBD, drives the ETS domain and inhibitory module to a more rigid, inactive state and diminishes the flexible state competent for DNA binding (Garvie et al., 2002, Lee et al., 2005, Cowley and Graves, 2000). Disinhibition, resulting in enhancement of ETS DBD activity and target gene specificity, is achieved by ETS factor interaction with other transcription protein partners, like CBFalpha and PAX5 (Garvie et al., 2001, Goetz et al., 2000, Gu et al., 2000). In cases of ESE and TCF subfamilies, DNA binding is repressed by sequences distant from the ETS domain. Inhibition in TCF members ELK-1, ELK-3 and ELK-4 is controlled by the B box, the C-terminal transactivation domain, the Net inhibitory domain (NID), and the Id helix-loop-helix proteins (Sharrocks, 2002, Stinson et al., 2003), and can be reversed by phosphorylation of the transactivation domain mediated through MAPK and interaction with SRF (Yang et al., 1999). The ETS-domain transcription factor PEA3 is autoinhibited by an inhibitory module composed of a combination of two short motifs located on either side of the ETS DBD. But other helix-loop-helix containing Id proteins, like USF, inhibit PEA3 DNA binding via a trans-acting negative mechanism (Greenall et al., 2001).

Ternary complex formation between adjacent DNA bound transcription factors is another mechanism of controlling ETS protein DNA binding, whereby DNA binding is regulated by juxtaposition of the two protein's DNA binding sites. The recruitment of SRF by TCF family members, like ELK-1, allows ELK-1 to bind the *c-fos* promoter, reflecting an acquired DNA binding specificity mechanism. Cooperation with co-regulatory protein partners also allows combinatorial control of gene expression and enhances the specificity of action of ETS domain proteins. This is highlighted by studies on PU.1/SAPI-1 and its lymphoid-specific co-regulatory partner Pip. Covalently tethered PU.1-Pip chimeras regulate the expression of different genes to PU.1 alone (Brass et al., 1996). In addition to co-regulatory partners co-repressors like CtBP and mSin3a are also recruited to ETS domain proteins, like Net/SAP2 and ELK-1 (Mavrothalassitis and Ghysdael, 2000).

B. Mechanisms of ETS protein mediated oncogenesis

B.1 Oncogenesis by ETS Factor Overexpression

Elevated ETS expression has been documented in many invasive and metastatic solid tumors of breast, lung, colon, pancreatic and thyroid cancer (Table 1). The strongest evidence for oncogenic activation by ETS amplification or overexpression comes from breast, prostate and hematological cancers. Multiple ETS factors, including ESE-1/Elf3, PEA3, ETS-1, ETS-2, ERM, and ER81, are up-regulated in breast cancer and are associated with poor prognosis and metastasis (Seth and Watson, 2005). Functional studies demonstrate that such up-regulation affects genes like *HER2/NEU*, *UPA*, *MMP*s, *MET*, *BCL2*, *VEGF*, *maspin* and *survivin*, which are normally associated with proliferation, transformation, migration, invasion, anti-apoptosis, and angiogenesis (Turner et al., 2007). The molecular

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mechanism underlying oncogenic activation in such cases is a result of both gene amplification and increased transcriptional activity, and is very prominent in the ESE subfamily of ETS transcription factors.

The ESE subfamily of ETS transcription factors (ESE-1/Elf3, ESE-2/Elf5, and ESE-3/EHF) has important roles in human epithelial tumorigenesis and many reviews have summarized their function in cancer (Gutierrez-Hartmann et al., 2007, Seth and Watson, 2005). Here we will focus on some unique aspects of these factors. For example, ESE-1, which is overexpressed in lung and HER2/Neu-positive ductal carcinoma *in situ* (DCIS), has both tumor initiation and tumor maintenance functions (Fig. 2) (Eckel et al., 2003, Prescott et al., 2004, Darius M. Walker and Gutierrez-Hartmann, 2010). ESE-1 is able to initiate the transformed phenotype in ESE-1-negative nontransformed MCF10A and MCF12A mammary epithelial cells (MECs) via a cytoplasmic mechanism whereby a 40-amino acid serine-and aspartic rich domain is necessary and sufficient for this transformation process (Fig. 2A) (Prescott et al., 2004) (Prescott, 2011). This mechanism has been discussed at length in section B.4. In fully transformed breast cancer cells, a feed-forward loop exists between ESE-1 and HER2, with ESE-1 being required to maintain the transformed phenotype (Fig 2B) (Darius M. Walker and Gutierrez-Hartmann, 2010). Knockdown of ESE-1 in ZR-75-1 and MCF-7 luminal breast cancer cell lines inhibits clonogenicity and anchorage independent colony growth, revealing that once fully transformed, these breast cancer cells become dependent on ESE-1 (Darius M. Walker and Gutierrez-Hartmann, 2010). On the other hand, ESE-2, or murine Elf-5, another member of the ESE subfamily, is a key downstream effector of prolactin signaling and controls terminal MEC differentiation (Lee and Ormandy, 2012, Kalyuga et al., 2012). Current understanding of the role of ESE-2/Elf-5 in breast cancer reveals that ELF5 functions as an important transcriptional switch, serving to suppress estrogen sensitivity in luminal breast cancer cells and promoting basal characteristics in basal breast cancer cells (Kalyuga et al., 2012). Thus, ESE-2 could play a significant role in determining the breast cancer subtypes, and may be involved in aspects of tamoxifen resistance and tumor progression. ESE-3/EHF appears to function as a tumor repressor and will be discussed in Section C.

The PEA3 subfamily of ETS factors, consisting of PEA3, ERM and ER81, positively correlates with HER2/Neu expression in 25-30% of all HER2-positive human breast cancers. The molecular basis for the elevated PEA3 transcripts in these cancers is due to HER2/Neu receptor tyrosine kinase initiating an intracellular signaling cascade that results in increased PEA3 transcriptional activity (Scott et al., 1994, Benz et al., 1997). HER2 overexpressing breast cancer cells lines, like BT-474, MDA-453, and ZR-751, show a 30-fold enhanced activity of PEA3 for its binding site on the *HER2* promoter. Although the majority of evidence supports a model whereby PEA3 and HER2 are locked in a positive feedback loop, there is some controversy, since a few reports suggest an anti-tumor role of PEA3 in breast cancer and that there is no significant correlation of HER2/Neu and PEA3 expression (Xia et al., 2006, Yu et al., 2006). Evidence for a definitive role of PEA3 downstream of HER2 and in oncogenesis comes from studies using dominant-negative proteins to inhibit signaling steps downstream of HER2 (O'Hagan and Hassell, 1998, Shepherd et al., 2001). For example, overexpression of Rap1a, a protein capable of

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antagonizing Ras function, completely inhibits the ability of HER2/Neu to stimulate PEA3-dependent gene expression. Also, dominant inhibitory mutants of either ERK or JNK cascades partially inhibit HER2/Neu activation of PEA3-dependent gene expression, suggesting that HER2/Neu regulates PEA3 activity through two different Ras-dependent MAPK pathways (O'Hagan and Hassell, 1998). Shepherd et al (Shepherd et al., 2001) observed that expression of a dominant-negative *PEA3* transgene under the control of the MMTV promoter in mammary epithelial cells of MMTV-*neu* transgenic mice delays the onset of mammary tumors, and inhibition of PEA3 or ERM transcripts by siRNA in mouse mammary cells reduces tumorigenesis (Shepherd et al., 2001, Firlej et al., 2008). By contrast, increased PEA3 expression promotes tumor cell migration and invasion, mostly by increasing transcription of *MMPs, VEGF, and COX-2* genes, often by acting in concert with other cell surface receptors, coactivators or signaling molecules, like CXCR4, AIB1, SRC-1, and cAMP (Subbaramaiah et al., 2002, Fleming et al., 2004, Hua et al., 2009, Gu et al., 2011).

Overexpression of other ETS factors, like ETS-1 and ETS-2, has a similar impact on breast cancer metastasis and invasion (Table 1) (Dittmer, 2003). In human breast cancer cell lines, ETS-1 expression has been correlated with cell invasiveness and epithelial-to-mesenchymal transition (EMT), concomitant with expression of uPA, MMP1, MMP3, vimentin, and loss of E-cadherin. Similar to PEA3, the impact of ETS-1 and ETS-2 on phenotypes and molecular regulation of breast cancer cells has been studied using gain-of-function and loss-of-function studies. In Down's (trisomy 21) syndrome, overexpression of ETS-2 in brain and fibrobasts has been shown to cause an increase in apoptosis, which involves increased activation of caspase-3 (Ge et al., 2008, Baldus et al., 2004). Introduction of a DNA binding mutant form of ETS-1 into ETS-1-expressing epithelial cell lines inhibited the activity of uPA and resulted in decreased cell migration and invasion in two dimensional assays (Delannoy-Courdent et al., 1998). Other experiments showed that ETS-1 plays a significant role in angiogenesis. Comparison of angiogenic properties of high and low Ets-1 expressing murine endothelial cell lines showed co-relation between gene expression of matrix metalloproteases and invasiveness with Ets-1expression (Sato et al., 2000). Murine Ets-1 is also implicated in the regulation of the HGF receptor, c-Met. Ets-1 and c-MET function in a positive feedback loop, where Ets-1 induces c-Met expression to render hepatoma cells susceptible to HGF/SF and, conversely, c-Met also activates Ets-1 via HGF/SF through the Ras/ERK1/2 pathway (Jiang et al., 2001, Sato et al., 2000, Dittmer, 2003).

Advanced stages of prostate cancer are associated with increased expression of multiple ETS proteins, including ETS-1, ETS-2, FLI-1, ERG, ESE-2/ELF5, ELF-1, and ETV1 (Table 1) (Gavrilov et al., 2001, Alipov et al., 2005), and the decreased expression of PEA3, PDEF, and ESE-3 (Rostad et al., 2007, Gu et al., 2007, Cangemi et al., 2008). Apart from the fact that there is ETS-mediated transcriptional activation of multiple genes related to cancer, little is known about the molecular mechanism underlying ETS overexpression in prostate cancer for ETS-1, ETS-2 or ETV1 (independent of the TMPRESS:ETV1 fusion). Analysis of androgen receptor (AR) function in prostate cancer provides evidence for androgen-mediated robust induction of these ETS transcription factors. The molecular mechanism of ERG overexpression, an ETS factors which is the most prominent marker in prostate cancer,

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has been ascribed to a chromosomal rearrangement that results in fusion of the 5′-end of a prostate-specific, androgen-responsive, transmembrane serine protease gene (*TMPRSS2*) to *ERG* resulting in increased ERG expression (Tomlins et al., 2005). ChIP-seq of TMPRSS2-ERG, in both cell lines and tumors that overexpress TMPRSS2-ERG, shows a high level of about 44% co-occupancy with the AR (Yu et al., 2010).

ERG overexpression in acute myeloid leukemia (AML) with complex or normal karyotypes is associated with a poor prognosis (Baldus et al., 2004, Marcucci et al., 2005), but ERG overexpression is not always related to genomic amplification in these cases. There is also little information on downstream targets of ERG that contribute to malignant leukemogenesis. A genome wide screen of ERG target genes conducted with ChIP-chip in Jurkat cells included the WNT signaling genes *WNT11, WNT2, WNT9A, CCND1*, and *FZD7* (Mochmann et al., 2011). Sampling of patient diagnostic material showed that ERG and WNT1 were co-expressed in 80% of AML, whereas knockdown of ERG1 by siRNA lead to WNT1 transcript down-regulation and WNT1 transcripts could be stimulated with tet-on ERG inducible assay. This study also confirmed that a WNT1 agonist, 6-bromouridine-3-oxime, resulted in an ERG-dependent proliferative growth advantage leading to morphological transformation, which could be effectively inhibited by siRNA knockdown of WNT1. Thus, taken together, these results suggest that ETS transcriptional networks in leukemia could converge on the WNT pathway.

B.2 Oncogenesis Associated with ETS Factor Amplification

Amplification of ETS genes is not a dominant mechanism driving cancer. The few cases identified to date occur in leukemia and lymphoma (Table 1). Amplification and rearrangement of the *c-ETS1* sequence is found in one case of acute myelomonocytic leukemia in which a homogeneously staining region occurred on 11q23 (Rovigatti et al., 1986, Baldus et al., 2004) and in one case of small lymphocytic cell lymphoma with an inverted insertion that also involved band 11q23. Amplification of *ETV6* is found in one case of B lymphoblastic leukemia, and amplification of *ETS-2* encoded by chromosome 21 is observed in patients with AML and acute non lymphoblastic leukemia (Chae et al., 2010, Baldus et al., 2004).

B.3 Oncogenesis by ETS Gene Fusions

B.3.a ETS fusions in prostate cancer—In contrast, malignant cellular transformation occurs often via chromosomal translocations, resulting in ETS gene fusions and abnormal regulation of ETS gene expression (Table 1). There is a spectrum of cancers where balanced reciprocal translocation results in fusion proteins that contains the ETS DNA binding domain. In prostate cancer, the most abundant and clinically relevant dominant oncogenes are ETS fusion genes resulting from chromosomal translocation of 5′ *TMPRSS2* to the ETS genes *ERG*, *ER81*, or *PEA3*. The resulting TMPRSS:ERG fusion protein is found in 50% of the prostate cancers, and chromosomal rearrangements between TMPRSS2 (21q21.3) and Er81 (7p21.2) or PEA3 (17q21) are found in about 30%. These fusions result in androgen-mediated robust induction of specific ETS factors, which in turn activate multiple ETS responsive genes with roles in transformation, as shown in Fig 3A (Sun et al., 2008, Tomlins et al., 2005). Insights into mechanism of TMPRSS-ERG mediated oncogenic transformation

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were provided by observations that TMPRSS-ERG activates MYC, abrogates prostate epithelial differentiation (Sun et al., 2008), and is capable of inducing an invasive-associated transcriptional program without an increase in cellular proliferation or anchorage-independent growth in primary or immortalized benign prostate epithelial cells. This latter result suggests that TMRSS-ERG may not be sufficient in inducing transformation in the absence of pre-existing secondary lesions (Tomlins et al., 2008). Nevertheless, somatic TMPRSS2-ETS fusions have become one of the most common cancer causing fusions and emphasizes the importance of ETS factors in the oncogenic process (Sun et al., 2008, Tomlins et al., 2005).

B.3.b ETS fusions in leukemia—ETS gene fusions occur often in human leukemia (Table 1). TEL, otherwise known as ETS translocation variant, is responsible for more than 40 different translocations in human hematological cancers where the PNT domain, the ETS domain, or both domains are fused in-frame with transcription partners such as AML1 (Romana et al., 1995b, Romana et al., 1995a) and EVI1 (Peeters et al., 1997), as well as with tyrosine kinases, such as PDGFβR (Golub et al., 1994), TRKc (Eguchi et al., 1999), ABL (Golub et al., 1996, Papadopoulos et al., 1995), and JAK2 (Lacronique et al., 1997). In most cases involving fusions with tyrosine kinase partners, the PNT domain of TEL oligomerizes and is required for the constitutive activation of the protein tyrosine kinase, and resultant transformation and pathogenesis. TEL-PDGFβR, TEL-TRKc, TEL-ABL, and TEL-JAK2 fusion proteins are each capable of causing myeloproliferative disease in murine bone marrow transplant models of leukemia and activate downstream targets, such as STAT5, which can then transactivate a number of genes, including *oncostatin M, Bcl-Xl*, cyclin D1and *Pim1*, all of which regulate cellular proliferation and survival (Fig. 3B) (Schwaller et al., 2000, Matsumura et al., 1999, Nosaka et al., 1999). Translocation t(12;21)(p13;q22) and t(8;21)(q22;q22), resulting in fusion protein TEL-AML1/RUNX and AML1-ETO, respectively, result in modification of the original function of the transcription factor and is frequently found in B-precursor ALL (Salomon-Nguyen et al., 2000). The RUNT domain of AML1 forms a heterodimeric complex with CBFβ, which confers increased DNA binding and stability to the complex (Bartfeld et al., 2002). AML1 can function as transcriptional activator or repressor, based on the promoter/enhancer tested. In contrast the TEL-AML1 fusion converts AML1 to a predominantly negative transcriptional regulator by recruiting co-repressor molecules like N-CoR and mSin3A and impedes differentiation (Fig. 3C) (Hiebert et al., 1996, Guidez et al., 2000, Chakrabarti and Nucifora, 1999, Zelent et al., 2004).

Experiments by Ford et al (Ford et al., 2009) suggest that TEL-AML1 expression also provides selective advantage to candidate preleukemic stem cells in the presence of TGF-β. In cancer, TGF-β mutants lose the Smad-dependent inhibitory control mechanism by which c-Myc is inhibited, and this loss of inhibitory function leads to deregulation of $p15^{INK4b}$/p15 and p21/$p27^{KIP1}$ (Massague et al., 2000, Mishra et al., 2005), and progression of disease and metastasis. In murine progenitor cells, regulated expression of TEL-AML1 protein inhibits activation of $CD79^{a}$ and Igα target promoters and blocks the ability of TGF-β to suppress proliferation via the activation of $p27^{KIP1}$ (Kamesaki et al., 1998). Although the precise mechanism by which TEL-AML1 inhibits TGF-β signaling has not been established,

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experiments by Ford et al (Ford et al., 2009) suggest that TEL-AML1 binds to principal TGF-β effectors, such as Smad3, and compromises Smad3′s ability to activate target promoters. Other evidence of TEL-AML1's oncogenic ability comes from miRNA expression arrays, which suggest that TEL-AML1 exerts in anti-apoptotic action, in part, by suppressing miRNA-494 and miRNA320, lowering their expression and causing enhanced survivin expression (Diakos et al., 2010).

AML1-ETO, TEL-ARNT and MN1-TEL fusions are yet other examples where fusion alters transcriptional activity. A commonly accepted model is that AML1-ETO contributes to leukemogenesis by acting as a dominant inhibitor of CBFβ function mediated through altered DNA binding (Meyers et al., 1995). Extensive mutation studies on the RUNT domain that disrupts heterodimerization of the RUNT domain with CBFβ show that CBFβ is necessary and essential for AML1-ETO mediated leukemogenesis (Roudaia et al., 2009). AML1-ETO can also act as a constitutive repressor by recruiting co-repressors to the genomic targets of RUNT through its ETO domain (Meyers et al., 1995, Wildonger and Mann, 2005). However, the actual mechanism of AML1-ETO mediated leukemogenesis could be quite complex. AML1-ETO mutation alone is insufficient to promote leukemogenesis, and development of acute myeloid leukemia is highly accelerated in presence of additional mutations that affect co-repressor interactions with the C-terminal of AML1-ETO protein (Yuan et al., 2001, Yan et al., 2004). It is therefore thought that the ETO domain has an inhibitory effect on AML1-ETO leukemogenesis (Wang et al., 1998). The presence of other splice variants of AML1-ETO (AML1-ETO9a) that impair co-repressor binding and deregulate cell cycle progression also accelerates the leukemogenic potential (Yan et al., 2006, Peterson et al., 2007). Other evidence from mutational analysis and 3D solution based structure on cell line models show that the eTAFH domain of ETO interacts with many E proteins and can inhibit E transcription factor transactivation (Plevin et al., 2006). Since E proteins are key players in T and B cell development, inhibition of E protein activation by AML1-ETO was also considered to be a major contributing factor in leukemogenesis. This, however, is disputed, and additional investigation based on a solution structure of the complex between the AML1-ETO eTAFH domain and an interacting E protein like HEB, reveals that the E protein has a relatively small contribution to AML1-ETO induced clonogenecity or proliferation in primary mouse bone marrow cells (Park et al., 2009).

In acute myeloblastic leukemia (AML-M2), the PNT domain of TEL is fused to the aryl hydrocarbon receptor nuclear translocator (ARNT) protein (Salomon-Nguyen et al., 2000, Otsubo et al., 2010). ARNT is a partner of several heterodimeric transcription factors, including those containing AhR and hypoxia inducible factor (HIF1α), suggesting that altering activity of AhR and HIF1α can contribute to leukemogenesis.

The MN1-TEL fusion results in an transcription factor with altered MN1 and TEL functions and is associated with leukeomogenesis (Buijs et al., 2000). MN1 has a N-terminal transcription activation domain (TAD) and functions as either an inhibitory or stimulatory transcription cofactor for retinoic acid receptor/retinoic-X-receptor (van Wely et al., 2003). The fusion protein MN1-TEL acts as a dominant-negative mutant of MN1 and prevents MN1 from stimulating the transcriptional activity of RAR/RXR (van Wely et al., 2007).

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Haar et al (ter Haar et al., 2012b) hypothesized that leukemia and lymphoid tumors in mice, resulting from forced expression of MN1-TEL, occur because of the repression of RAR/RXR-mediated transcription by the MN1-TEL fusion protein and also because of interference of the repressive effect of TEL caused by MN1-TEL binding to ETS responsive elements (Kawagoe and Grosveld, 2005). Recent findings support the notion that the MN1-TEL fusion protein can block TEL-specific DNA recognition sequences, preventing the binding of other factors for proper transcriptional regulation, which may contribute to leukemogenesis (Ter Haar et al., 2012a). Other TEL fusions, such as TTL-ETV6, ETV6-BAZ2A, ETV6-FCHO2, ETV6-MDS2, mediate oncogenesis through loss-of-function of fusion genes affecting *TEL* and the partner gene, and likely contributes to haplo-insufficiency, inducing a lack or decrease in homo and hetero-dimerization with other TEL proteins. The deletion of the normal *TEL* allele in the presence of a translocation affecting *TEL* is quite frequent, and is particularly found in children suffering from pre-B ALL with t(12;21)(p13;q22) and an TEL-AML1 fusion. Also, rearrangements of *TEL* with *CHIC2*, *MDS2*, or *PER1* genes do not produce any unique or functional proteins, and evaluation of genes located around these breakpoints reveal that these are ectopically expressed in leukemia cells compared to normal cells and interfere with growth, differentiation and apoptosis of hematopoietic cells (De Braekeleer et al., 2012).

B.3.c ETS fusions in sarcomas—In Ewing sarcoma, a childhood cancer of the soft tissue, the ETS gene *FLI1* translocates from its original position of 11q24 to chromosome 22, which generates transcripts resulting in the fusion of the amino-terminal region of the *EWS* gene with the carboxyl-terminal DNA binding domain of the *FLI1* gene (Table 1) (Riggi and Stamenkovic, 2007). Upon fusion, EWS-FLI1 becomes a more potent transactivator than FLI1 itself. Many targets of EWS-FLI1, both direct and indirect, have been found to be involved in EWS tumor maintenance. EWS-FLI1 partners with several proteins to modulate mRNA transcription and splicing (Knoop and Baker, 2000). Biochemical studies and computer algorithms have revealed that the EWS-FLI1 molecule is intrinsically disordered, from the amino-terminus of EWS up to the DNA binding domain of FLI1, and that unique aromatic side-chains within a disordered structure of EWS-FLI1 are critical for the transcription and transforming activity of the EWS family oncoproteins (Ng et al., 2007). Although direct transcriptional targets of EWS-FLI1 have been analyzed using the ChIP/miRNA approach and have led to discovery of target genes, few have been validated for differential expression, EWS-FLI1 binding, and gene regulation in Ewing sarcoma family tumors (ESFT) (Siligan et al., 2005). Consistent with its transcriptional activation function, EWS-FLI1 associates with several proteins of the basal transcriptional machinery, such as RNA polymerase II, HsRBP7, CREB binding protein CBP/p300, and RNA helicase A (Erkizan et al., 2010, Knoop and Baker, 2000). There is also interaction with the putative tumor suppressor gene *BARD1*, which interacts with *BRCA1*, linking EWS-FLI1 with DNA repair and check-point control. The *FOXO1* gene, which encodes another potential tumor suppressor, is directly suppressed by EWS-FLI1, and therefore possibly contributes to cellular transformation in many Ewing family tumors.

A direct transcriptional target of EWS-FLI1 is NROB1/DAX1 and it is a key effector in EWS-FLI1 mediated oncogenesis. siRNA silencing of DAX1 in EWS-FLI1 cell lines results

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in growth arrest and inhibition of tumor formation in immunodeficient mice. DAX1 also physically interacts with EWS-FLI1 to control gene expression and to modulate the transformed phenotype of Ewing sarcoma (Gangwal et al., 2008, Garcia-Aragoncillo et al., 2008, Kinsey et al., 2009, Anderson et al., 2012, Kovar, 2012). Other known direct transcriptional targets of EWS-FLI1 consist of glutathione-S-transferase M4 (GSTM4), protein tyrosine phosphatase L1 (PTPL1), and GLI1 (Abaan et al., 2005, Beauchamp et al., 2009, Luo et al., 2009). Similar to the inhibition of PTPL1, pharmacological inhibition of GLI1, a known oncogenic mediator in the Hedgehog pathway, decreases proliferation and soft agar colonies in ESFT cells. In many cases, however, pathogenesis of EWS-FLI1 depends on cooperative DNA binding with other transcription factors, such as AP-1, which have tandem binding sites close to the EWS-FLI1 target promoters (Kim et al., 2006).

Pathogenesis of Ewing sarcoma also depends on other cellular functions that indirectly control EWS-FLI1 activity. Among these functions are the p53/INK4a pathway, hypoxia, IGF-1/IGF-1R pathway, and microRNAs (Erkizan et al., 2010). Loss of tumor suppressor genes, such as p53 and $p16^{INK4a}$, greatly accelerate tumorigenesis in EWS-FLI1 transgenic mice, and studies show that loss of p16 pathways stabilizes EWS-FLI1 expression and helps in EWS-FLI1 mediated transformation (Huang et al., 2005, Deneen and Denny, 2001, Lessnick et al., 2002). EWS-FLI1 also significantly inhibits p300-mediated acetylation of p53, and thus suppresses its transcriptional activity and enhances MDM2-mediated p53 degradation (Li et al., 2012b). In ESFT, hypoxia has also been shown to contribute to resistance of apoptosis via HIF1α (Aryee et al., 2010, Knowles et al., 2010). EWS-FLI expression increases transiently under hypoxic conditions in a HIF-1α-dependent manner, and there is co-localization of HIF-1α and necrotic areas in an ESFT tissue array, suggesting a role of hypoxia in the induction of HIF1α (Aryee et al., 2010, Knowles et al., 2010).

Autocrine loops encompassing IGF-1/IGFR also play an important role in proliferation and survival of ESFT via activation of AKT and ERK1/2 pathways (Toretsky et al., 1997, Scotlandi et al., 2011). In mouse embryonic fibroblasts, expression of IGFR is required for EWS-FLI1-mediated cellular transformation, and several lines of evidence indicate that inhibition of this pathway decreases tumor growth *in vitro* and *in vivo*, along with decreased angiogenesis and increased cell death (Toretsky et al., 1997, Scotlandi et al., 2011). A search for genes by gene array techniques specifically activated in Ewing sarcoma cells, but not in other tumor cell lines, identified high level expression of Id2, an oncogenic helix-loop-helix protein, and threw light on the possible role of the c-myc/Id2 pathway in tumorigenesis induced by EWS/ETS fusion proteins (Fukuma et al., 2003).

Current studies have also implicated microRNAs influencing EWS-FLI1 activity. It is estimated that 30% – 40% of the genome is regulated by miRNAs, and regulated genes include those contributing to tumorigenesis, proliferation, differentiation, and invasion. One of the best characterized miRNA in Ewing sarcoma is mir145, which is consistently repressed by EWS-FLI1 (Ban et al., 2011). Reconstitution of mir145 expression resulted in decreased EWS-FLI1 expression, and consequently reduced cell growth and soft agar colony formation (Ban et al., 2011). A global miRNA study in A673 cells identified other miRNAs (miR100, MiR125, MiR29a) repressed by EWS-FLI1, with these miRNAs targeted IGF signaling pathways (McKinsey et al., 2011). Another miRNA significantly repressed in

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Ewing sarcoma is miRNA708. Studies show that EWS-FLI1 regulates the DNA repair protein and s transcriptional cofactor EYA3 via modulation of miRNA708, resulting in increased cell survival and chemoresistance (Robin et al., 2012). Moreover, global miRNA expression profiling of human mesenchymal stem cells (hMSC) and ESFT cell lines show that EWS-FLI1 induces the repression of the oncogenic miRNA17-92 cluster and represses the tumor suppressor let-7a family (De Vito et al., 2011). The tumor suppressor let-7a regulates ESFT growth via target gene HMGA2. Inhibition of tumor growth by let-7a overexpression is associated with a concomitant decrease in the let-7a target gene, HMGA2, in Ewing tumor cell lines A673 and TC252.

B.4 Transformation Initiation by Cytoplasmic Localization of ESE-1

While it has been presumed that ETS factors function exclusively in the nucleus as transcription factors regulating gene expression, a cytoplasmic role has been identified for the ETS factor ESE-1 (Fig. 2 and Table 1). Specifically, ESE-1 initiates transformation of MCF12A and MCF10A human mammary epithelial cells (MECs) via a novel cytoplasmic mechanism (Prescott et al., 2004, Schedin et al., 2004, Prescott et al., 2011). ESE-1 is located on chromosome 1q32.1, in a region that is amplified in 50% of the breast cancers. ESE-1 is overexpressed in human breast ductal carcinoma in situ (DCIS) and there is a positive feedback loop between the *HER2* proto-oncogene and ESE-1, whereby expression of HER2 and ESE-1 is positively co-related (Feldman et al., 2003, Eckel et al., 2003). A unique 40 amino acid serine and aspartic rich (SAR) domain is necessary and sufficient for the cytoplasmic-mediated transformation of ESE-1 (Fig. 2A) (Prescott et al., 2004, Schedin et al., 2004, Prescott et al., 2011). Enforced nuclear expression of either full-length ESE-1 or of the SAR domain alone abrogates ESE-1's ability to initiate transformation. In contrast, nuclear function of ESE-1 is required to maintain the transformed phenotype in already transformed MCF-7, ZR-75 and T47D breast cancer cells (Fig. 2B) (Eckel et al., 2003, Darius M. Walker and Gutierrez-Hartmann, 2010). Taken together, these data suggest that nuclear-cytoplasmic shuttling of ESE-1 is required for initiation of transformation in nontransformed MECs. A single NES (nuclear export signal) lies in the ESE-1 DBD and is required for ESE-1 mediated initiation of MCF-12A transformation (Prescott et al., 2011). The discovery that ESE-1 functions in the cytoplasm to initiate transformation of mammary epithelial cells throws light into subcellular function of ETS transcription factors and defined a novel NES-mediated transformation initiation mechanism independent of nuclear transcriptional function.

B.5 Transformation by ETS Factor Expression in Stromal Tissue

Fibroblasts in the tumor microenvironment contribute to the progression of many tumors by stimulating growth, sustaining angiogenesis, and promoting invasion and metastasis. Early evidence of the role of stromal ETS expression to invasion and metastasis comes from studies where a single targeted *Ets2* allele blocked mammary tumors in transgenic mice carrying the polyoma virus middle T oncogene (Table 1) (Neznanov et al., 1999). Although the exact mechanism by which Ets-2 overexpression promotes oncogenesis remains unknown, there is evidence pointing to the possible role of ERK1/2 phosphorylation in ETS transcriptional activation (Svensson et al., 2005). Generation of female mice expressing a mutated homozygous *Ets2* gene, $Ets2^{A72/A72}$, which precludes ERK phosphorylation of

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Thr72, blocks mammary tumor development by transplantation of Neu- and polyoma virus middle-T-initiated mammary tumors in the mammary fat pads of *Ets2*$^{A72/A72}$ homozygous mice (Man et al., 2003). Restriction in tumor size also correlated with fewer stromal cells expressing MMP9. Further investigation showed that Ets-2 supports mammary tumors exclusively via Ets-2 expression in the microenvironment (Tynan et al., 2005). Inactivation of Ets-2 within MEC tumor cells has no effect on tumor appearance, but complete inactivation of Ets-2 in both epithelial and stromal cells affects the early hyperplastic phase of tumor development and the time of tumor appearance, but has no effect on tumor growth or occurance (Tynan et al., 2005). Surprisingly, study of ETS-2 phosphorylation in human epithelial cells suggests that both ERK1/2 and ETS-2 phosphorylation inversely correlates with tumor size in breast cancer patients (Baker et al., 2003). But this could be in line with the fact that ETS-2 acts in concert with the SWI/SNF complex to functionally repress the transcription of the tumor suppressor gene, *BRCA1* (Baker et al., 2003). Moreover, genetic inactivation of the tumor suppressor Pten in stromal fibroblasts of mouse mammary cells accelerated initiation, progression of mouse mammary tumors, and was linked to an increase in Ets-2 expression, and activation of Ets-2 target promoters. Inactivation of the *Ets2* gene in *Pten*-deleted stroma reduced the size of tumors, thereby directly establishing a role of Pten-Ets-2 signaling in oncogenic stromal tissue (Trimboli et al., 2009).

Comparing the expression of another ETS subfamily member, c-ETS-1, with invasiveness and expression of vimentin, E-cadherin, uPA, MMP-1 and MMP-3 also demonstrates an association of c-ETS-1 with an invasive, EMT-derived phenotype in human breast cancer cells lines (Gilles et al., 1997). Other studies reveal that target genes of ETS-1, like MMP1 and MMP9, are switched on and localize to both endothelial and stromal fibroblasts in carcinoma in situ of the breast (Behrens et al., 2001). Furthermore, an antisense oligonucleotide to ETS-1 decreased VEGF-induced expression of both MMP 1 and uPA (urokinase-type plasminogen activator) in endothelial cells (Behrens et al., 2001).

B.6 Oncogenesis Associated with ETS Factor Binding to Genome Regulatory Regions

Generation of ETS protein binding sites on promoter elements of target genes controlled by hormone receptors, or other transcription factors, also assists transformation and oncogenesis (Table 1). The androgen receptor initiates important male sexual developmental pathways, but is also responsible for the onset and progression of prostate cancer. While the classical example is the TMPRSS2:ETV fusion, where TMPRSS2:ETS DBD fusion protein over-expression is driven by androgen responsiveness on the TMPRSS2 promoter, there is also evidence for ETS-1 and AR interaction in a subset of additional AR promoter target genes (Massie et al., 2007). A ChIP-on-chip assay in the androgen-responsive LNCaP prostate cancer cell line identified ~1500 genomic AR binding sites and revealed that 50% of these AR sites are atypical 6-bp, rather than 15-bp, AR binding elements. This study also confirmed an enrichment of ETS transcription factor family binding sites in a subset of these AR promoter targets and showed a direct interaction between AR and ETS-1 (Massie et al., 2007). Finally, ChIP-seq analysis revealed that AREs co-localize with TMPRSS2-ERG fusion binding sites and that ETS proteins inhibit AR-responsive genes by several mechanisms in prostate cancer cells, resulting in an interference of the AR-dependent

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prostate differentiation and induction of dedifferentiated, transformed phenotype, as discussed previously (Yu et al., 2010).

Elucidation of ETS factor-mediated regulation of human telomerase reverse transcriptase (*hTERT)* gene expression driving oncogenesis has provided key insights into novel mechanisms of ETS-regulated tumorgenesis (Xu et al., 2008, Huang et al., 2013, Horn et al., 2013). For example, ETS-2 interacts with c-Myc on a composite ETS/Myc DNA binding site on the *hTERT* promoter, and both ETS-2 and c-Myc are required to maintain breast cancer cell proliferation (Xu et al., 2008). A particularly interesting mechanism by which ETS factors drive tumorigenesis is via gain-of-function, single-base mutations, whereby novel ETS DNA bindings sites are generated (Huang et al., 2013, Horn et al., 2013, Killela et al., 2013). Analysis of whole genome sequencing data from patient-derived malignant melanomas reveal that 71-75% of these melanomas harbor two somatic mutations localized 100-bp upstream from the transcriptional start site of *hTERT* gene. Both mutations resulted in a cytidine-to-thymidine transition and generated an identical nucleotide stretch bearing a consensus binding site for ETS transcription factors (Huang et al., 2013, Horn et al., 2013, Killela et al., 2013). A reporter assay system confirmed the capability of each of these mutations to augment transcriptional activity from the *hTERT* promoter 2-3-fold, suggesting that these promoter mutations may function as driver events that contribute to onocogenesis by TERT dysregulation. In fact, TERT promoter mutations have been found with a surprisingly high frequency in a large number of cancers, further revealing the overall importance of ETS factors in human cancer (Killela et al., 2013).

The Ewing sarcoma family of tumors provides another example wherein ETS factors binding to a DNA regulatory region to promote transformation. Specifically, cooperative DNA binding of ETS fusion proteins, such as EWS-ETS, and of Ewing's-relevant ETS factors, such as FLI1, ERG, ETV1, with AP-1 proteins, to tandem binding sites on the *uridine phosphorylase* (*UPP*) gene, appear to mediate transformation by the EWS-ETS fusion protein (Kim et al., 2006). Furthermore, *NROB1* and *GSTM4*, two ETS fusion target genes required for the Ewing's transformed phenotype, contain microsatellites bearing tandem repeats of the GGAA core ETS binding sequence (Luo et al., 2009, Gangwal et al., 2008). Indeed, multiple ETS proteins can bind to GGAA microsatellites, and ETS proteins, such as ETV6 and ETV7, bind as homo-oligomers to neighboring binding sites. But whether or how these microsatellites contribute to the cooperativity of ETS factor binding with other neighboring factors to tandem DNA binding sites is not clear.

B.7 Increased ETS Factor Transcription Potency as a Result of Transcription Factor Interactions, Post-translational Modifications, and Protein Stabilization in Cancer

B.7.a Protein-protein interactions contributing to ETS mediated tumorigenesis—A key mechanism by which ETS factors contribute to the control of cell proliferation, transformation, differentiation and growth factor responsiveness is via combinatorial interactions with other transcription factors, often on tandem or composite DNA binding sites. Such interactions result in functional cooperativity, due to release of ETS DNA binding auto-inhibition, via MAPK phosphorylation, or both, resulting in increased ETS factor transcription factor potency (Table 1) (Wasylyk et al., 1998, Wasylyk et al., 1992,

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Garvie et al., 2002). While there is a fair bit of complexity in the number of ETS-interacting proteins identified to date, SRF, AP-1 and NF-κB appear to be key factors in the ETS cancer interactome (Li et al., 2000).

The importance of ETS factor co-operation with other transcription factors in driving oncogenesis is underscored by the interaction of ETS and c-Jun, a component of AP-1 (Yang et al., 1996, Hollenhorst et al., 2011b). Composite ETS and AP-1 binding sites are found in promoter enhancer elements called Ras responsive elements (RRE) (Wasylyk et al., 1998). In a large number of genes, ETS and AP-1 elements mediate induction by a repertoire of oncogenes associated with the Ras signaling pathway. Ras-MAPK specific phosphorylation of ETS-1 and ETS-2 are essential for the ETS and AP-1 synergistic activation of the RREs (Yang et al., 1996) and the controlled expression of many genes, including cytokines, glutathione-S-transferase, and MMPs related to invasiveness and metastasis (Li et al., 2000). For example, the enhancing action of ETS and AP-1 is required for the expression of maspin, a tumor suppressor gene in normal mammary epithelial cells, and this enhanced action is lost in invasive tumor cells, like MB-MDA-231, resulting in loss of maspin expression during tumor progression (Zhang et al., 1997). In liver cancer, Ets-1 is involved in invasiveness and metastasis by up-regulating expression of MMP-7 and N-acetyl glucosaminyl transferase, and promoter activity of MMP-7 is highly up-regulated in colon cancer and is synergistically regulated by PEA-3 and c-Jun, in conjunction with β-catenin LEF-1 (Crawford et al., 2001, Tootle and Rebay, 2005). However, a subset of ETS factors when overexpressed, can by-pass RAS-MAPK phosphorylation to activate RAS-MAPK target genes by binding to the ETS/AP-1 sequence (Hollenhorst et al., 2011a). Regulation of cancer cell metastasis and invasion in such cases is dictated by the transcriptional consequence of the particular ETS protein that bind to the ETS/AP-1 sequence and is independent of Ras/MAPK pathway (Hollenhorst et al., 2011a)

Transcriptional regulatory elements of many T cell genes. and of certain genes in human immunodeficiency virus types 1 and 2, also have adjacent or overlapping ETS and NF-κβ family proteins binding sites (Bassuk et al., 1997). NF-κβ is a ubiquitous transcription factor involved in inflammation and stress response. Even though there is no known significance of this physical interaction in malignancy or transformation, an evolutionary conserved interaction like this presents potential target for developing immunosuppressive drugs and antiviral therapies (Bassuk et al., 1997).

Interactions of AML-1, HIF-2α, with Ets-1 are other examples where transcription factor interaction leads to an increase in ETS factor potency and possibly affects carcinogenesis. AML-1 and HIF-2α bind to the regulatory domain of ETS-1, and counteract ETS-1 auto-inhibition (Goetz et al., 2000, Kim et al., 1999, Elvert et al., 2003, Augustijn et al., 2002). AML1 (also known as RUNX1/CBFalpha2) is a potent transcriptional factor involved in B cell development, and rearrangement of the AML1 gene is often the cause of myeloid and lymphoid leukemias. In the case of the ETS-1 and AML1 interaction, co-operativity of AML1 with ETS-1 leads to 10-fold decrease in auto-inhibition of ETS-1 DNA binding, suggesting that co-operativity results from a relief of auto-inhibition (Wasylyk et al., 1992, Garvie et al., 2002). ETS-1 in turn stimulates DNA binding activity of AML-1 by associating with its NEDB (negative regulatory domain for DNA binding) domain.

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Mutations and translocations in AML1 lead to the disruption of cooperative gene activation of AML-1 with another ETS factor SPI1, contributing to aberrant repression of myeloid differentiation genes, like macrophage and granulocyte macrophage colony-stimulating factor receptors (*MCSFR* and *GMCSFR*) and leukemogenesis (Hu et al., 2011). In the case of ETS-1 and HIF-2α, cooperation of ETS-1 and HIF-2α is required for the full transcriptional activation of the angiogenic protein, VEGF-2, and further stresses the importance of such interactions in tumor progression.

Other examples of well-characterized interactions between ETS factors and other transcription factors include those of ETS-1 and PAX5, serum response factor (SRF) and ELK-1, and SRF and SAP-1. Although, there is no current evidence to support their contribution to malignancy, these interactions increase the DNA binding ability of oncogenic ETS factors on promoters of genes relevant in cell differentiation. Oncogenic ETS-1 can inhibit the differentiation of B cells to plasma cells, and can stimulate c-Met, which is also a transcriptional target for PAX5 (O'Brien et al., 2011). Ternary complex formation involving PAX-5 and ETS-1 allows ETS-1 to bind the promoter of the *mb-1* (Ig-alpha/immunoglobin associated alpha) gene implicated in B cell development and maturation with higher affinity (Garvie et al., 2001, Fitzsimmons et al., 2009). Interaction of SRF with the ETS domain of ELK-1 increases its ability to bind to sub-consensus DNA sequence, and the recruitment of ELK-1 by SRF allows ELK-1 to bind the serum response element (SRE) motif of the c-fos promoter to form a ternary complex (Sharrocks, 2002). FLI1 and the oncogenic fusion protein EWS-FLI1, along with ELK-1 and SAP-1a, can also function as TCFs forming ternary complex with SRF on SREs of the *c-fos* promoter and the early growth response gene *Egr1* (Watson et al., 1997).

Another ETS protein, ELF-1, contains a motif that is highly related to the Rb binding site in several viral oncoproteins and binds to the hypo-phosphorylated form of Rb. Notably, ELF-1 fails to bind to Rb mutants derived from patients of retinoblastoma and over-expression of a phosphorylation-defective form of Rb inhibits ELF-1 dependent transcription during T cell activation, showing that this interaction might be important for lymphokine production with cell cycle progression in activated T cells (Wang et al., 1993). FLI1, on the other hand, regulates erythropoietin (Epo) induced erythroid proliferation and differentiation, and has binding sites in the promoter of the Rb gene (Tamir et al., 1999). The interaction of FLI1 and Rb could be a determinant in erythroid progenitor cell regulation, which is deregulated in Friend murine leukemia virus (F-MuLV) induced erythroleukemia. Furethermore, FLI 1 modulates the response of erythroid cells to Epo by negative regulation of Rb (Tamir et al., 1999). Constitutive expression of FLI-1 in HB60 cells, similar to retroviral insertional activation of FLI-1 observed in F-MuLV-induced erythroleukemia, blocks Epo-induced differentiation, while promoting Epo-induced proliferation and this response correlates with expression level and phosphorylation status of Rb (Tamir et al., 1999).

B.7.b Post-translational modifications of ETS factors in cancer—Subtype, order, and combinations of post-translational modifications are yet additional levels of ETS protein regulation (Table 1). Table 3 is brief summary of the distinct post-translational modifications on ETS proteins and their functional consequences related to oncogenesis.

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Kinase-mediated phosphorylation of certain ETS factors serves a key regulatory mechanism affecting transcription potency, stability, and DNA-binding affinity. Phosphorylation of Ets-1 is essential for Ets-1 to recruit the co-activator p300/CBP and to form a ternary complex with AP-1, leading to transcriptional activation of *uPA* and *MMP* genes and promotion of extracellular matrix (ECM) breakdown (Yang et al., 1996, Wasylyk et al., 1997).

Phosphorylation of ESE-1 by p21 activated kinase 1 (Pak1) increases the stability of ESE-1, which enhances its transformation potency of MCF-10A and MCF-12A human mammary epithelial cells through its 40-amino acid serine-and-aspartic rich (SAR) domain (Manavathi et al., 2007, Prescott et al., 2004). In the unphosphorylated state, ESE-1 is a relatively unstable protein, degraded via the βTrCP-dependent ubiquitin proteasome pathway (Manavathi et al., 2007). However, upon Pak1 phosphorylation of ESE-1 at Ser207, which is located within the SAR domain, ESE-1 degradation is diminished, and its transcription and transformation potency are increased (Manavathi et al., 2007).

Other examples, where post-translational modifications have resulted in transcriptional activation affecting transformation include that of ELK-1 and ELF-1. Since ELK-1 is controlled both positively and negatively by phosphorylation, and represents a major instance of activator-to-repressor switch, it will be discussed in the next section. ELF-1 on the other hand is positively regulated by glycosylation and phosphorylation (Wang et al., 1993, Juang et al., 2002). Up-regulation of ELF-1 is observed in variety of cancers, such as prostate, ovarian, breast and leukemia/lymphoma (Table 1). ELF-1 is dynamically distributed between the cytoplasm and nucleus. The 80-kDa form of Elf-1 is sequestered in the cytoplasm via interactions with Rb. For example, upon T cell activation, phosphorylation and glycosylation converts ELF-1 to a 98-kDa form, whereupon it dissociates from Rb and translocates to the nucleus and tightly binds the TCR zeta chain gene.

TEL, a strong transcriptional repressor (Kim et al., 2001, Lopez et al., 1999), is de-repressed by MAPK-mediated phosphorylation and sumoylation (Maki et al., 2004, Chakrabarti et al., 1999). TEL oligomers repress transcription in the absence of MAPK signaling, and MAPK-mediated phosphorylation and sumoylation of TEL removes TEL oligomers from DNA, preventing transcriptional repression. SUMO-modified TEL localizes to cell cycle-specific nuclear speckles named TEL bodies, which help in nuclear export of TEL (Chakrabarti and Nucifora, 1999). A TEL mutant that cannot be sumoylated at lysine 99 does not localize at TEL bodies and cannot be exported to the cytoplasm (Chakrabarti and Nucifora, 1999). This mutant also acts as a superior transcriptional repressor compared to the wild type TEL. Moreover there is additional evidence that SUMO-modified TEL-AML1 fusion protein localizes to TEL bodies in a pattern different from normal nuclear compartment localization of AML1 (Chakrabarti et al., 2000). Taken together, these findings suggest that variation in post-translational modifications of TEL fusion proteins and distinct subcellular localization serve as additional attributes that govern TEL-mediated functions, and thus likely serve in establishing leukemogenesis.

Acetylation of ETS proteins also regulates their function. For example, TGFβ signaling leads to prolonged acetylation of Ets-1, resulting in p300/CBP-Ets-1 complex dissociation

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(Czuwara-Ladykowska et al., 2002). Dissociation of p300/CBP allows it to become available for interaction with other transcription factors, such as SMADs, which are downstream of TGFβ signaling, and thus TGFβ promotes ECM maintenance (Czuwara-Ladykowska et al., 2002). Another example where acetylation affects ETS protein function is provided by post-translational modification of the oncogenic fusion protein EWS-FLI1 (Schlottmann et al., 2012). Acetylation of multiple relevant acetylation sites in the C-terminus of the fusion protein increases EWS-FLI1's DNA binding ability, protein stability, and transcriptional potency (Schlottmann et al., 2012). These studies reveal that cancer invasiveness and metastasis mediated by certain the ETS proteins are carefully regulated by post-translational modifications, such that an antagonistic mechanism results whereby acetylation inhibits ETS factor function (Czuwara-Ladykowska et al., 2002).

B.8 Oncogenic Activation by Switching ETS Factor Function from an Activator to a Repressor

Individual ETS factors function as an activator or repressor depending on the target DNA-binding sequence, presence of additional tissue-specific factors, and combinatorial control by other transcription factors. Even though there is sufficient evidence for the role of ETS factors as activators or repressors in development and organogenesis, few cases of oncogenic activation by this process have been reported. The ETS factor PU.1/SPI-1 can act as a repressor or activator, and its dysregulation is associated with leukemia. SPI-1 binds to the purine-rich sequence known as a PU-box near the promoters of target genes, and activates gene expression during myeloid and B-lymphoid cell development in conjunction with other transcription factors and co-factors (Li et al., 2000). PU.1 also functions as a repressor by interacting with GATA-1, a zinc finger transcription factor required for erythroid differentiation. GATA-1 and PU.1 bind to each other on DNA, and control lineage commitment during hematopoiesis (Yamada et al., 2001), but a stoichiometric imbalance between the two could lead to acute leukemias in mice. In murine erythroleukemia (MEL), cells are blocked at the blast stage, and gene expression arrays have identified two key hematopoietic transcription factors, C/EBPalpha and cbfb, that are direct targets for PU.1-mediated activation and GATA-1 mediated repression (Burda et al., 2009). Interestingly, both of these target genes are repressed in MEL by the inhibitory action of GATA-1, and it has been found that an increase in PU.1 levels can reverse this repression and lead to MEL differentiation. Examples of decreased PU.1 function are also evident in acute pro-myelocytic leukemia (APL) (Zou et al., 2012). In APL, the associated fusion protein pro-myelocytic leukemia/retinoic acid receptor alpha (PML/RARα), can reduce PU.1 serine phosphorylation and promoter binding (Zou et al., 2012). This causes a decrease in PU.1-dependent transcriptional activation and promotes leukemogenesis. In human AML, there is evidence of co-operation between mutated RUNX1 (eg, N-terminal truncated RUNX1, called RUNT) or translocated RUNX1, and wild type PU.1 (Zou et al., 2012). This cooperation leads to persistent recruitment of co-repressors, which are otherwise excluded in a wild type RUNX1 and wild type PU.1 interaction (Hu et al., 2011).

Alterations in the ETS Pointed domain, which can switch its activator and repressor functions, have been linked to alterations in ETS factor-mediated transforming activity or cancer progression. As discussed previously, the fusion proteins EWS-ERG and EWS-FLI

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retain the ETS DBD but have lost the Pointed domain, and this results in a strong transcriptional activator function compared to ERG or FLI1 alone (Riggi and Stamenkovic, 2007). Part of this is attributed to the ability of the fusion protein EWS-FLI 1 to interact with human RNA polymerase II (hsRPB7), which may influence promoter selectivity. In contrast, the TEL-AML1 fusion converts AML1 from an activator to a transcriptional repressor (Chakrabarti and Nucifora, 1999). Here, unlike EWS-FLI1, the gain of the Pointed domain has led to a switch from activation to repression, impeding differentiation by involving the recruitment of co-repressor molecules, such as NcoR and Sin3A. In ETS-2, the Pointed domain directly interacts with Brg-1, the ATP hydrolyzing component of SWI/SNF complex (Baker et al., 2003). The interaction of ETS-2 and Brg-1 allows ETS-2 to repress the BRCA-1 promoter in MCF-7 transfection assays, thereby establishing a role for ETS-2 as transcriptional repressor in addition to its well-defined transcriptional activator function.

Other examples of switching between activation and repression comes from the ETS factor, ELK-1 (Yang et al., 2001). The equilibrium between activation and repression of ELK-1 is altered in favor of activation following stimulation of the MAPK cascade. ELK-1 consists of a C-terminal transcriptional activation domain (TAD) and an N-terminal transcriptional repression domain. MAPK pathway activation allows multiple phosphorylation events on ELK-1, which causes an ELK-1 conformational change, and results in increased DNA binding and transcriptional activation (Li et al., 2003). Absence of MAPK signaling allows the repressor domain to recruit co-repressor molecules, such as mSin3a-histone de-acetylase, which results in shutting off one of the ELK-1 target promoters, *c-fos* (Yang et al., 2001). Therefore, in addition to being an activator, ELK-1 also functions as a transcriptional repressor and undergoes activator-repressor switching in response to growth signals. It has been observed that reducing the level of ELK-1 phosphorylation reduces the transcriptional activity of ELK-1 and inhibits human colon cancer growth by suppressing gene expression of EGFR (Chen et al., 2006). An additional level of ELK-1 control comes from ELK-1 sumoylation, where sumoylation represses ELK-1 basal transcriptional activity and expressing SUMO specific proteases increases Elk-1 transcriptional activity, in the absence of any MAPK signaling (Yang et al., 2003).

ER81 is an ETS factor that has been implicated in breast cancer, and multiple post-translational events modulate protein-protein interactions, protein DNA interactions and protein stability to convert ER81 from an activator to a repressor. ER81 has been implicated in mammary tumor development in HER2 transgenic mice and maximal transcription activation of ER81 is achieved by post-translational modifications initiated by HER2/Neu signaling. HER2 signaling activates MAPK, which phosphorylates ER81 on multiple residues (Bosc et al., 2001, Janknecht, 2001). ER81 is additionally phosphorylated by mitogen and stress activated protein kinase 1 (Msk1), turned on by HER2 signaling events (Bosc et al., 2001, Janknecht, 2001). Msk1 in turn activates PKA, which phosphorylates ER81 at an additional site to stabilize ER81. In contrast, by a negative feedback loop, HER2 signaling leads to phosphorylation of map activated protein kinase 2 (Mk2), which phosphorylates two serines in the inhibitory domain of ER81 and thereby converts ER81 into a repressor (Baert et al., 2002, Janknecht, 2003).

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C. Mechanisms of ETS protein mediated tumor suppression

While it has been presumed that the primary function of ETS factors in tumorigenesis is that they initiate and/or promote the malignant phenotype, in certain tissues ETS factors actually serve to repress or suppress tumorigenesis (Sussan et al., 2008, Jedlicka et al., 2009, Albino et al., 2012). For example, overexpression of Ets-2 in a mouse model of trisomy 21 results in reduction of GI tumors in APC^{min} mice, most likely due to Ets-2-mediated repression of a gene network contributing to the GI tumor phenotype (Sussan et al., 2008). In this case, excess Ets-2 protein functions as a repressor of gene transcription, rather than as a genetic tumor suppressor (Sussan et al., 2008). Again, in the GI system, transgene targeting of an Ets dominant repressor (Engrailed/Erm DBD) to the small intestinal epithelium resulted in an increase in tumor numbers in Apc^{min} mice, with tumors showing greater stromal invasiveness (Jedlicka et al., 2009). Finally, in prostate epithelium, knock down of Ese3 induced epithelial-to-mesenchymal transition (EMT), stem like features, and metastatic properties; whereas re-expressing Ese3 inhibited the stem-like properties and tumorigenic potential of prostate cancer cells (Albino et al., 2012). This study also showed that loss of Ese3 could be linked to a group of prostate tumors with distinct molecular characteristics showing increased expression of EMT genes (Albino et al., 2012). Taken together, these reports reveal that in GI and prostate tissues, certain ETS factors function to repress tumor-promoting genes and/or induce tumor suppressor genes, thus inhibiting tumorigenesis in these tissues.

Conclusions and Outlook

This review underscores the importance of ETS factors in oncogenesis, such that during the process of cellular transformation in different tissues, dysregulation of ETS genes and their products becomes a frequent event, resulting in multiple mechanisms driving tumorigenesis by acting on distinct, yet redundant ETS genes (Fig. 4). Examining in detail the various mechanisms by which ETS factors mediate tumorigenesis helps to draw attention the wide range and scope of ETS protein involvement in cancer. In Fig. 4, we have summarized the various mechanisms by which ETS transcription factors drive tumorigenesis. Indeed, when one considers the total number of cancers cause by ETS fusions in leukemias, ETS fusions in prostate cancer, and of the many cancers that display gain-of-function ETS DNA binding mutations in the hTERT promoter, it becomes clear the ETS factors play a dominant role in human tumorigenesis (Fig. 4) (Yu et al., 2010, Huang et al., 2013, Killela et al., 2013, De Braekeleer et al., 2012) .

Thus, the redundancy itself provides an increased number of potential targets that can be misregulated to initiate, promote and maintain tumorigenesis. Implicit is that downstream target genes are key in the tumorigenic process, and that the genes that are commonly regulated by these distinct ETS factors causing cellular transformation are the most important effectors in ETS-mediated carcinogenesis. Taken together, precisely because specific ETS factors are key drivers of leukemia, prostate and breast cancer, and downstream effectors are very likely to be in common, then these individual ETS factor and/or their common oncogenic effectors provide novel drug targets for the treatment of these deadly malignancies. Redundancy in ETS protein DNA binding sequence and

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functionality has made it difficult to assess the precise role of an individual ETS factor in specific functions *in vivo*. Current data addressing the occupancy pattern of ETS proteins reveals that multiple ETS proteins can occupy the same high affinity consensus ETS binding site, but this mostly occurs for promoters of housekeeping genes, and it is likely that this mode of binding provides consistent regulation of these ubiquitously expressed genes (Hollenhorst et al., 2007). Nevertheless, nonredundant binding also occur, and ETS factor binding to these sites appears to be mediated via protein-protein interactions, with partner proteins stabilizing ETS factor binding to atypical, low affinity sites. In this regard, overexpression of ETS factors that partner with AP-1 result in constitutive activation of otherwise Ras-responsive, ETS/AP-1-regulated genes (Hollenhorst et al., 2007, Hollenhorst et al., 2011a). While these ETS/AP-1 gene targets have functions in normal cell biology, this persistent activation of ETS/AP-1 target genes appears to contribute to oncogenesis (Hollenhorst et al., 2011a)These findings stress the need for extending genome wide occupancy studies to other cell types, tissues and tumors. There is also a requirement for high throughput functional assessment of genes that are being predicted to be associated with these ETS binding sites.

Given the fact that a single, specific ETS factor or ETS factor fusion appears to be a key driver in a number of cancers (leukemia, prostate), these oncogenic ETS factors become obvious targets for targetd therapy. Thus, developing novel therapies, such as siRNA, DNA decoys, targeting a specific DNA sequence, or peptides to disrupt transcription factor homodimerization or heterodimerization is critical. For example, siRNA has been successfully used to target the *BCR-ABL* chimeric gene in CML and against the *AML1-ETO* in AML-M2 (Scherr et al., 2003, Heidenreich et al., 2003). Similarly, shRNA-mediated knockdown of ESE-1 in luminal breast cancer cell lines has reversed the transformed phenotype (Walker, 2010). These studies reveal that targeting a single ETS factor is sufficient to reverse transformation and thus underscore the notion that therapeutic targeting of oncogenic ETS factors should be pursued. However, it is critical to keep in mind that certain ETS factors maybe oncogenic in one tissue and a tumor repressor in another (Sussan et al., 2008, Jedlicka et al., 2009), and thus tissue-specific targeting will need to be carefully considered. In this regard, controlled tissue-specific delivery agents targeting oncogenic factors would be ideal. The development of synthetic carrier systems, like nanoparticles, liposomes or lipid nanoparticles, coupled with receptor-mediated or biophysical (ultrasound) delivery approaches, leads to organ specific complex delivery (Schroeder et al., 2012). Alternatively, exosomes are being used to deliver siRNAs, mRNAs, receptors and enzymes (van den Boorn et al., 2011, Alvarez-Erviti et al., 2011). Thus, research needs to expand to further develop and refine novel approaches to target single specific transcription factors in a tissue-specific manner, and thus generate new treatments for these deadly cancers.

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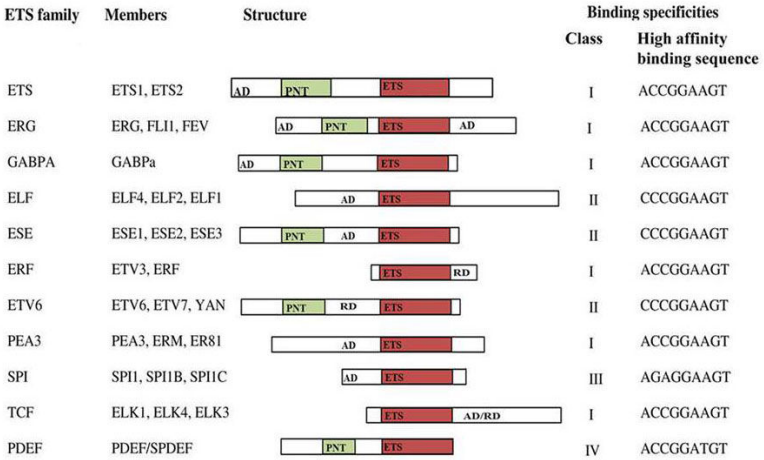

Figure 1.

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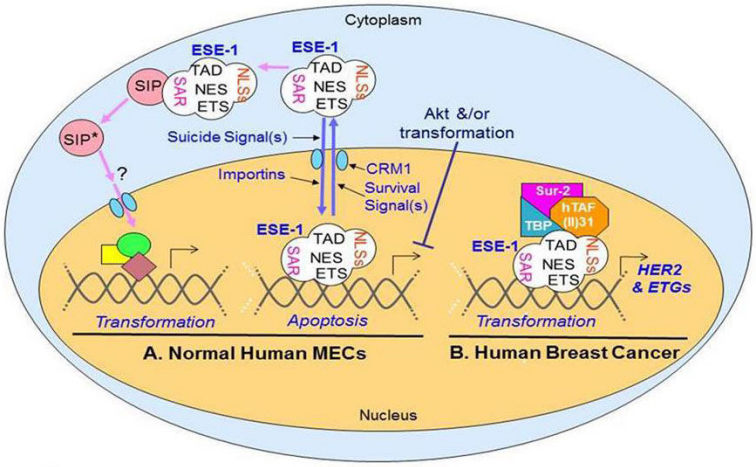

Figure 2.

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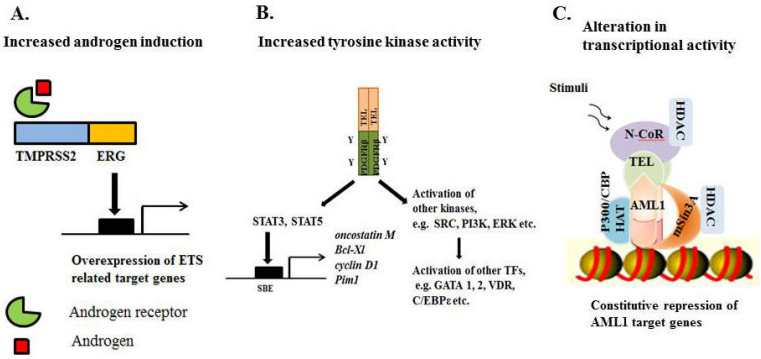

Figure 3.

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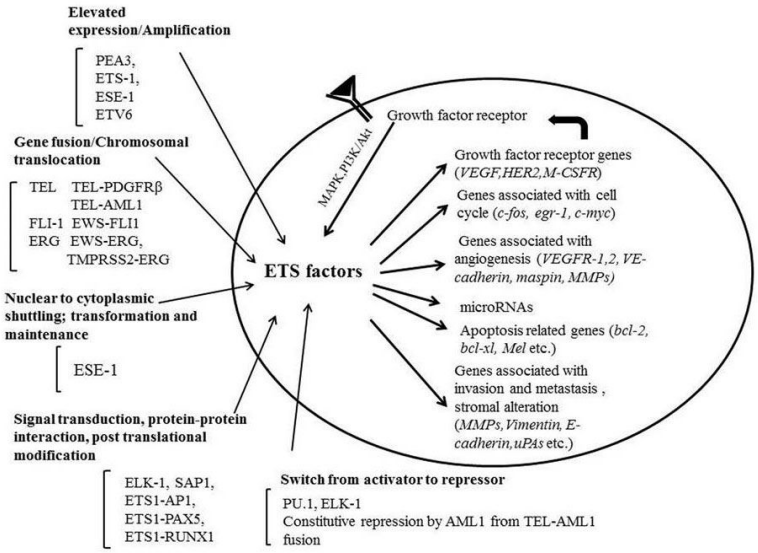

Figure 4.

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Table 1

A summary of mechanism of tumorigenesis and tumor types caused by prototype ETS proteins

Subfamily	Members	Mechanism of Tumorigenesis	Type of tumors	Primary Organs /Tissues affected
ETS	ETS-1	Overexpression. ETS-1also shows enhanced transactivation and increased DNA binding affinity with partner proteins upon MAPK mediated phosphorylation in the pointed domain; expression correlates with expression of uPA, MMPs, and with ECM degradation. Murine Ets-1 induces c-Met expression and Ets-1 and c-Met function in a positive feedback loop. Amplification and rearrangement of the *c-ETS1* sequence is found in one case of acute myelomonocytic leukemia in which a homogeneously staining region occurred on 11q23. ETS-1 binds to genome regulatory region to assist transformation and oncogenesis; there is direct interaction of AR and ETS-1in a subset of AR promoter targets in the androgen responsive LNCaP prostate cancer cells. ETS-1 is capable of inducing synergistic activation of specific promoters with interacting partner proteins (AP-1, NF-Kβ, CBP, Sp100, RUNX1).	Meningioma (Okuducu et al., 2006, Kitange et al., 2000), invasive carcinomas of breast (Dittmer et al., 2004, Li et al., 2012a, Ghosh et al., 2012, Behrens et al., 2003), colorectal carcinoma (Nakayama et al., 2001), pancreatic carcinoma (Ito et al., 1998) adenocarcinoma (Ito et al., 2002), thyroid carcinoma (Nakayama et al., 1999), thymoma (Sasaki et al., 2002) and angiomas (Naito et al., 2000)	Brain, breast, ovary, colon, pancreas, thymus, thyroid, prostate
ETS	ETS-2	Overexpression of ETS-2 in brain and fibroblasts in Down syndrome patients increase apoptosis. Overexpression of ETS-2 observed in human breast cancers. In human breast cancers, ETS-2 transcriptionally regulate *hTERT* gene and silencing of ETS-2 reduces *hTERT* gene expression and increases apoptosis of cancer cells. Ets-2 acts in the stroma to regulate mouse mammary tumors. Blocking of MAPK mediated phosphorylation of Th72 on the *Ets2* allele by alanine substitution ($ETS2^{A72}$), restricts the development of	Breast cancer (Xu et al., 2008, Man et al., 2003)	Breast

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Subfamily	Members	Mechanism of Tumorigenesis	Type of tumors	Primary Organs /Tissues affected
ERG	ERG	mammary tumors transplanted into fat pads of the *Ets2*$^{A72/A72}$ homozygous mice. Overexpression of TMPRSS2: ERG fusion in prostate; fusion leads to androgen dependent robust induction of ERG fusion, and activation of ETS responsive genes. Fusion also activates MYC and abrogates prostate epithelial cells differentiation. Overexpression of ERG noticed in AML; ERG is co-expressed with WNT1. ERG fuses with EWS in soft tissue sarcomas.	AML (Hamalainen et al., Hermkens et al.), Ewing tumors (Tomlins et al., 2013), Prostate cancers (Tomlins et al., 2005)	Soft tissues like bone, cartilage and prostate, blood
ERG	FLI1	*FLI1* gene Translocates to chromosome 22 and generates EWS-FLI1, a fusion transcript. EWS-FLI1 becomes a more potent transactivator than FLI1 alone and acts on EWS-FLI1 target genes for direct transcriptional activation or repression.	Ewing tumor (Riggi and Stamenkovic, 2007)	Soft tissues like bone, cartilage, and fat
PEA3	PEA3	Overexpression, there is growing evidence for Her2/Neu activation of PEA3. Increase in PEA3 expression correlates with increased transcription of *MMPs*, *VEGF*, *COX-2* and *NOTCH* genes.	Invasive breast carcinoma (de Launoit et al., 2000, Bieche et al., 2004)	Breast
	ER81	*EWS* gene translocates to *ER81* gene to produce EWS-ER81 fusion transcripts. Overexpressed in breast carcinomas, ER81 transcriptional activity is increased by HER2/Neu overexpression and ER81 can upregulate HER2 expression in breast tumors suggesting a feed forward loop. In contrast, by a negative feedback loop Her2 signaling can convert ER81 from an activator to a repressor	Ewing tumors (Fuchs et al., 2003), prostate carcinoma (Shin et al., 2013), breast carcinoma(de Launoit et al., 2000, Bosc et al., 2001)	Prostate, soft tissues, breast
ELF	ELF-1	Upregulated in prostate, ovarian, and breast cancers, leukemia and lymphoma. ELF-1 is a HER2 regulating ETS factor candidate and is capable of binding and transactivating HER2 promoter at levels found in BT474, SKBR3, ZR-75-1 but cannot compete with other ETS factors like PEA3 or ESE-1 and the net cellular expression of HER2 depends on other limiting promoter regulators.	Prostate carcinoma (Shaikhibrahim et al., 2011), breast carcinoma(Scott et al., 1994), ovarian carcinoma (Takai et al., 2003)	Prostate, ovary, breast

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Subfamily	Members	Mechanism of Tumorigenesis	Type of tumors	Primary Organs /Tissues affected
TEL	TEL/ETV6	Balanced reciprocal chromosomal translocations resulting in fusion protein in which the PNT domain of TEL is fused in frame with partners like PDGFβR, TRKc, ABL and JAK2 and causes myeloproliferative disease in murine bone marrow transplant models of leukemia. For TEL/AML1 leukemias, there is loss of function of both *TEL* alleles. Also TEL-AML1 fusion converts AML1 to a negative transcriptional regulator, TEL-AML1 fusion provides selective advantage to pre-leukemic stem cells in presence of TGF-β.	Haematological malignancy like leukemia (Romana et al., 1995a, Buijs et al., 2000, Zelent et al., 2004), fibro sarcoma (Knezevich et al., 1998).	Blood, soft tissue.
SPI	PU.l/SPI 1	Can act as a repressor or activator. PU.1 target genes *C/EBPalpha* and *CBFB* are repressed in MEL. In human AML, there is cooperation between RUNT and wild type PU.1 to trigger PU.1 mediated repression events affecting differentiation of genes.	Promyelocytic leukemia (Zou et al., 2012), Acute myelocytic leukemia (Hu et al., 2011)	Blood

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Table 2

List of ETS factors in tumors

Subgroup	Members	Alternate names	Review section
ETS	ETS-1		A.2, B.1, B.2, B.7b
	ETS-2		B.1, B.5, B.6, C
TCF	ELK-1		A.2, B.7a
	NET	ELK-3, SAP-2	A.2
ERG	FLI-1	ERGB	B.3c
	ERG		B.3a, B.8
PEA3	PEA3	ETV4, E1AF	B.1
	ER81	ETV1	B.1, B.8
ELF1	ELF-1	E74 like factor 1	A.2, B.8
SPI1	SPI-1	PU.1, SPI-A	A.2,
TEL	TEL	ETV6	B.3b
ESE	ESE-1	ESX, ELF-3, ERT	B.1, B.4
	ESE-2	ELF-5	B.1
	ESE-3	EHF	C
PDEF	SPDEF		A.1

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Table 3

Post translational modification on ETS transcription factors and functional effects related to oncogenesis

ETS protein	Modifying agents/Signaling events	Type of post translational modification	Effects	Pathology involving changes in post translational modification	Reference
TEL	MAPK	Phosphorylation	Loss of repression and export to nucleus	Leukemia	(Wood et al., 2003)
	SUMO conjugating enzyme UBC9	Sumoylation			
Ets-1	CAMKII	Phopshorylation	Inhibits DNA binding and transcriptional activation		(Nelson et al., 2010, Foulds et al., 2004)
	MAPK	Phosphorylation	Allows for cooperative transcriptional activation of Ets-1 and AP1, recruitment of p300/CBP to Ets-1, and transcriptional activation of *MMP* and *uPA* genes		
	TGFβ	Acetylation	Dissociation of p300/CBP, relieving ETS mediated activation, and making p300/CBP available for interaction with SMADs to activate transcription of ECM maintenance proteins	Invasive breast carcinoma	(Czuwara-Ladykowska et al., 2002)
Ets-2	MAPK	Phopshorylation	Increases activation and protein stability	Invasive breast cancer, Leukemia	(Nelson et al., 2010, Foulds et al., 2004, Yang et al., 1996)
ELK-1	MAPK	Phosphorylation	Increased DNA binding affinity and ELK-1/SRF ternary complex formation, increase transcriptional activation	Cervical cancer (HeLa)	(Lee, 2005, Latinkic et al., 1996, Tian and Karin, 1999)
	SUMO conjugating enzyme UBC9	Sumoylation	Recruitment of HDACs, repression of transcriptional activation		(Yang and Sharrocks, 2004, Yang et al., 2003)
ELF-1	Agents unidentified	Phosphorylation	Maximal activation of the ELF-1 promoter,converts ELF-1 from 80KDa to 98KDa,dissociates from Rb in cytoplasm and translocates to nucleus		(Juang et al., 2002)
		Glycosylation			
Er81	HER2/Neu signaling; MAPK	Phosphorylation	Activates transcription	Breast cancer	(Bosc et al., 2001, Janknecht, 2001, Wang et al., 2011)
	MSK1, PKA, Mk2		Suppresses transcriptional activity		
	p300;P/CAF	Acetylation	Increases DNA binding affinity and potency of Er81 TAD; inhibits ubiquitin mediated degradation.		(Goel and Janknecht, 2003)
SAP-1	MAPK	Phosphorylation	Increases DNA affinity, promotes ternary complex, increases transcriptional		(Strahl et al., 1996)

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ETS protein	Modifying agents/Signaling events	Type of post translational modification	Effects	Pathology involving changes in post translational modification	Reference
			activation		
NET	Ras-ERK	Phosphorylation	Converts repressor to activator, stimulates angiogenesis		(Giovane et al., 1994)
	JNK	Phosphorylation			
	SUMO conjugating enzyme UBC9	Sumoylation	Increases repression		(Wasylyk et al., 2005)