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REVIEW

Genetics and Epigenetics of the TET-ETS Translocation Network

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Abstract: In the present paper we review the translocation network involving TET and ETS family members with special focus on the Ewing family of tumors. FUS (fusion, involved in t(12;16) in malignant liposarcoma = TLS, Translocated in liposarcoma), EWSR1 (Ewing sarcoma breakpoint region 1) and TAF15 (TATA box-binding protein-associated factor, 68-KD) are the three human members of the TET family of RNA binding proteins. In addition, two EWSR1 pseudogenes are present in the human genome. TET family members are involved in several oncogenic gene fusions. Five of the 18 known fusion partners belong to the E26 (E twenty-six, ETS) family of transcription factors. Gene fusions between TET or ETS family members and other fusion partners link these gene fusions to a large network of oncogenic gene rearrangements.

Keywords: Ewing family tumors, ETS transcription factors, TET family, gene fusions, pseudogenes

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Introduction

Several tumor entities are characterized by recurrent chromosomal aberrations which lead to the activation of oncogenes. Two types of oncogene activation can be distinguished: Firstly, regulatory elements of a gene can be juxtaposed to an oncogene, resulting in loss of physiological regulation of this oncogene. A well known example for this type of oncogene activation is Burkitt's lymphoma (BL). The majority of BL carry translocations between the MYC (v-myc myelocytomatosis viral oncogene homolog) oncogene and one of the immunoglobulin loci.[1] High expression of MYC in BL is driven by regulatory elements of the immunoglobulin loci[2] and deregulated expression of MYC in B cells is a major factor for the malignant phenotype of BL cells.[3–5] Formation of tumor specific fusion proteins represents the second type of oncogene activation. In the present paper, we focus on fusion proteins of the TET-ETS (Translocated in liposarcoma/Ewing sarcoma breakpoint region 1/TATA box binding protein-associated factor—avian erythroblastosis virus E26 oncogene homolog) family of aberrant transcription factors.

This class of oncogenes has been described in Ewing family tumors (EFT). Initially described as endothelioma,[6] EFT represent a group of bone and soft tissue sarcomas with uncertain histogenetic origin. Gene expression analyses indicate a relationship between EFT and endothelial, neuroectodermal, as well as mesenchymal stem cells.[7–10] The majority of EFT carry chromosomal translocations between chromosomes 11 and 22.[11] By molecular analysis of this translocation a gene fusion between EWSR1 (Ewing sarcoma breakpoint region 1) and FLI1 (Friend leukemia virus integration 1) was detected.[12] EWSR1-FLI1 is the proto-type of fusion proteins involving members of the TET family of RNA binding proteins and members of the ETS family of transcription factors. In addition to TET-ETS fusions, translocations between TET genes and other fusion partners have been identified (Fig. 1 and Table 1).[13–41] ETS transcription factors can be divided into several groups and sub-families.[42] All ETS transcription factors that have been identified as fusion partners for TET proteins are members of the sub-family ETS and are included in the groups PEA3 or ERG (PEA3 group: ETS variant 1 (ETV1), ETS variant 4 (ETV4 = PEA3, polyomavirus enhancer activator-3); ERG group: ETS related gene (ERG), fifth Ewing variant (FEV), FLI1). A third gene in

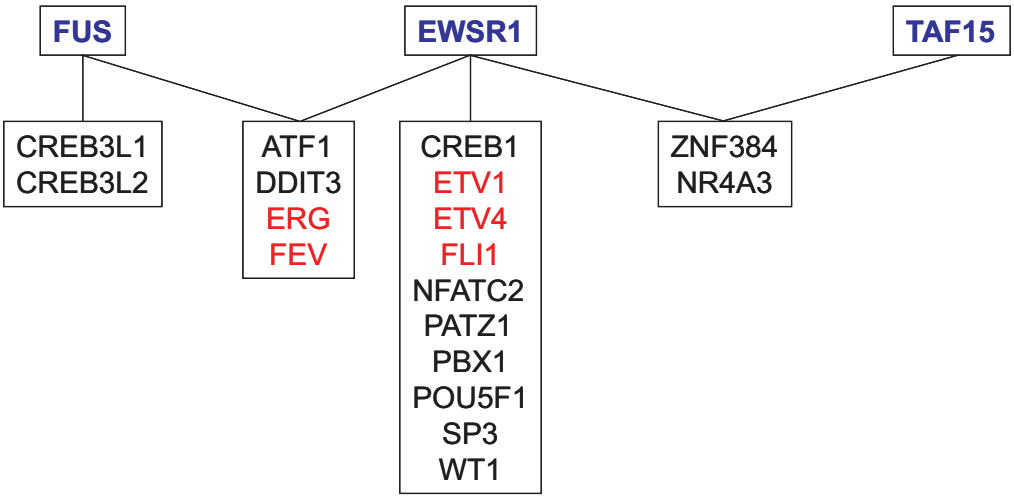

Figure 1. Translocations involving members of the TET family. The three members of the TET family (blue) are involved in different gene fusions involving members of the ETS family of transcription factors (red) and other genes. Each line represents a gene fusion. Genes that are involved in gene fusions with the same fusion partner(s) are grouped together in rectangles.
Abbreviations: ATF1, activating transcription factor 1; CREB1, cAMP responsive element binding protein 1; CREB3L1/2, cAMP responsive element binding protein 3-like 1/2; DDIT3, DNA-damage-inducible transcript 3; ERG, ETS related gene; ETV1/4, ETS variant 1/4; EWSR1, Ewing sarcoma breakpoint region 1; FEV, fifth Ewing variant; FLI1 , Friend leukemia integration 1; FUS, fusion involved in malignant liposarcoma; NFATC2, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2; NR4A3, nuclear receptor subfamily 4, group A, member 3; PATZ1, POZ (BTB) and AT hook containing zinc finger 1; POU5F1, POU class 5 homeobox 1; SP3, specificity protein 3 transcription factor; TAF15, TATA box binding protein-associated factor, 68 kDa; WT1, Wilms tumor 1; ZNF384, zinc finger protein 384. The corresponding chromosomal breakpoints and references are summarized in table 1.

the PEA3 group, ETV5 (ETS variant 5) has not been identified as fusion partner for TET proteins. However, ETV5 is up-regulated (together with ETV1) by CIC-DUX4 (capicua homolog-double homeobox, 4) oncofusion proteins which have been found in so called "Ewing-like sarcomas".[43] Gene fusions between ETV1, ETV4, ETV5 or ERG and different other fusion partners have also been detected in prostate cancer.[43–51] One of these fusion partners, SLC45A3 (solute carrier family 45, member 3), forms additional gene fusions with the ETS family member ELK4 (ETS-like transcription factor 4).[52] Like the other fusion partners, ELK4 is a member of the sub-family ETS, but represents the first member

Table 1. Gene fusions involving members of the TET family.

TET gene	Fusion partner	Location	Typical aberration	Disease	Ref.
EWSR1 (22q12.2)	**ATF1**	12q13	t(12;22)(q13;q12)	Clear cell sarcoma; angiomatoid malignant fibrous histiocytoma	13,14
	CREB1	2q34	t(2;22)(q33;q12)	Clear cell sarcoma; angiomatoid malignant fibrous histiocytoma	15,16
	DDIT3	12q13.1–q13.2	t(12;22)(q13;q12)	Liposarcoma	17
	ERG	21q22.3	t(21;22)(q22;q12)	EFT	18
	ETV1	7q21.3	t(7;22)(p21;q12)	EFT	19
	ETV4	17q21	t(17;22)(q21;q12)	EFT	20
	FEV	2q36	t(2;22)(q35;q12)	EFT	21
	FLI1	11q24.1–q24.3	t(11;22)(q24;q12)	EFT	12
	NFATC2	20q13.2–q13.3	r(20;22)	EFT	22
	NR4A3	9q22	t(9;22)(q31;q12)	Myxoid chondrosarcoma	23
	PATZ1	22q12.2	inv(22)(q12q12)	EFT	24
	PBX1	1q23	t(1;22)(q23;q12)	Myoepithelioma	25
	POU5F1	6p21.31	t(6;22)(p21;q12)	Undifferentiated sarcoma; mucoepidermoid carcinoma; hidradenoma	26,27
	SP3	2q31	t(2;22)(q31;q12)	Undifferentiated small round cell sarcoma	28
	WT1	11p13	t(11;22)(p13;q12)	Desmoplastic small round cell tumor	29
	ZNF384	12p12	t(12;22)(p13;q12)	ALL, acute undifferentiated leukemia	30
FUS (16q11.2)	**ATF1**	12q13	t(12;16)(q13;p11)	Angiomatoid malignant fibrous histiocytoma	13
	CREB3L1	11p11.2	t(11;16)(p11;p11)	Fibromyxoid sarcoma	31
	CREB3L2	7q34	t(7;16)(q34;p11)	Fibrosarcoma; Fibromyxoid sarcoma	32,33
	DDIT3	12q13.1–q13.2	t(12;16)(q13;p11)	Liposarcoma	34
	ERG	21q22.3	t(16;21)(p11;q22)	EFT, AML, ALL	35–39
	FEV	2q36	t(2;16)(q35;p11)	EFT	40
TAF15	**NR4A3**	9q22	t(9;17)(q31;q12)	Myxoid chondrosarcoma	41
(17q11.1–q11.2)	**ZNF384**	12p12	t(12;17)(p13;q12)	AML, ALL	30

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; EFT, Ewing family tumor. Gene abbreviations see legend to figure 1.

of the ELK group.[42] An unusual gene fusion between two ETS family members has been described in acute myeloid leukemia.[53] This fusion leads to the formation of fusion proteins between ERG and ELF4 (E74-like factor 4), a member of the ELF sub-family of ETS transcription factors.

Material and Methods

Cell line SK-N-MC[54] was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen (Braunschweig, Germany). Cells were cultured in RPMI1640 medium supplemented with 10% fetal calf serum and penicillin/streptomycin. For visualization of mitotic figures in living cells, cells were transfected with vector pBOS-H2BGFP[55,56] (Becton-Dickinson, Heidelberg, Germany) and stable transfectants were selected by treatment with 2 µg/ml blasticidin. Information about gene fusions was collected from the literature and the Mitelman Database of Chromosome Aberrations in Cancer.[57] For identification of EWSR1 pseudogenes we performed a BLAST search[58] using the mRNA sequence of EWSR1 exons 1–7 as query. For comparison of EWSR1 transcripts and pseudogenes, the open reading frame of EWSR1-FLI1 type I was amplified from cell line A-673[59] with EWSR1 and FLI1 specific primers (5'-TTG GAT CCG CTT CAG CTA GAA GGC CAC T-3'; 5'-AAA AGC TTA TGG CGT CCA CGG ATT AC-3') and sequenced by using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. Sequence alignment between the EWSR1 gene, EWSR1 transcripts and pseudogenes was visualized by using GeneDoc.[60]

Results and Discussion

Several of the fusion partners of TET and ETS family members are involved in additional chromosomal rearrangements. For example, PBX1 (pre-B-cell leukemia homeobox 1) and ZNF384 (zinc finger protein 384) are both involved in translocations with EWSR1 and the unrelated transcription factor TCF3 (transcription factor 3).[61,62] The observation that such translocations often occur in tumors of the same type as the corresponding translocations involving TET and/or ETS family members suggest that these translocations have similar pathophysiological effects. For instance, fusion proteins between the TET proteins EWSR1 or TAF15 and NR4A3 (nuclear receptor subfamily 4, group A, member 3; a member of the steroid/thyroid hormone and retinoid receptor super-family)[63] have been observed in myxoid chondrosarcoma.[23,41] In the same tumor type translocations between NR4A3 and TCF12 (transcription factor 12)[64] or TFG (neurotrophic tyrosine kinase, receptor, type 1-fused gene)[65] have been found. Interestingly, TFG was found initially by searching for TET family members,[66] but (like TCF12) has only low sequence similarity with EWSR1, FUS and TAF15. Such rearrangements link TET-ETS translocations with seemingly unrelated gene fusions, including fusions involving the ETS transcription factor ETV6 (ETS variant 6; Fig. 2). ETV6 (also known as TEL: translocation, ETS, leukemia) is the only ETS family member for which gene fusions have been described but which is not involved in gene fusions with TET family members or SLC45A3 (solute carrier family 45, member 3). ACSL3 (acyl-CoA synthetase-like 3)-ETV1[50] and ETV6-ACSL6 (acyl-CoA synthetase-like 6)[67] gene fusions both involve an ETS family member and a member of the long chain acyl-CoA synthetase family. However, whereas ASCL3-ETV1 gene fusions allow the expression of a truncated ETV1,[50] it seems that fusions between ASCL6 and ETV6 did not allow expression of ETV6 or fusion proteins. Therefore, these two gene fusions might exert different pathogenetic functions.

The formation of fusion genes involving members of the TET and/or ETS family has multiple oncogenic effects. One effect is the deregulated expression of the 3' fusion partner which is driven by the promoter of the 5' fusion partner. In prostate cancer, the formation of fusion proteins has been described,[46] but most gene fusions lead to up-regulation of ETS-factors by heterologous promoters without formation of fusion proteins.[44,50] Aberrant regulation of target genes can explain some of the oncogenic activity of gene fusions involving ETS family members or other transcription factors. The EFT specific EWSR1-FLI1 oncofusion protein has similar DNA binding specificity as FLI1.[68] In addition, up-regulation of ETS factors by unrelated oncogenic events in so called "Ewing-like sarcomas" suggest that activation of ETS factors without formation of novel fusion proteins is largely sufficient for induction of the EFT phenotype.[43]

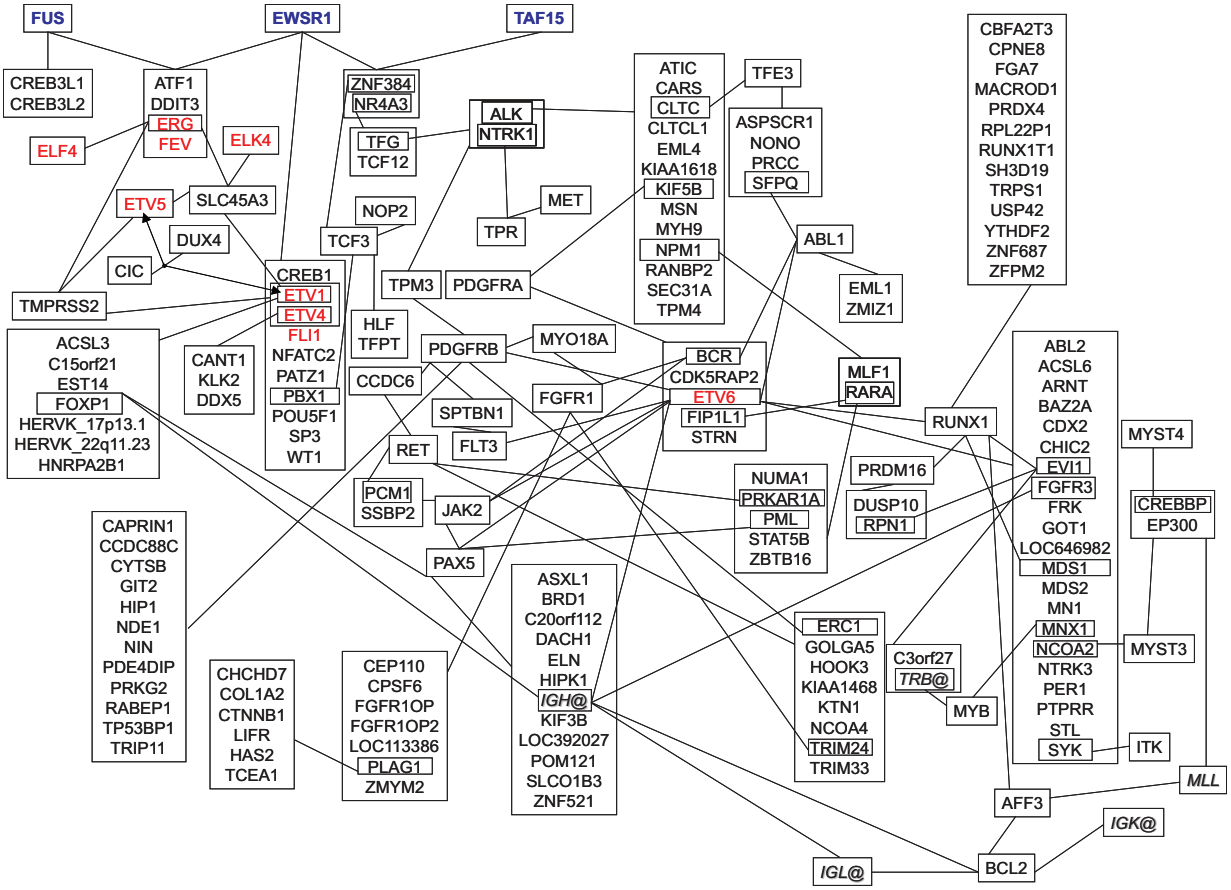

Figure 2. The extended TET-ETS translocation network. Fusion partners of TET family members (blue) are involve in several other gene fusions linking TET fusions to seemingly unrelated gene fusions. Members of the ETS family of transcription factors are colored in red. Each line represents a gene fusion. Genes that are involved in gene fusions with the same fusion partner(s) are grouped together in rectangles. Included is the CIC-DUX4 gene fusion which is not directly linked to the network but leads to up-regulation (arrows) of ETS transcription factors in tumors with similarities to Ewing family tumors.[43] The genes MLL (mixed lineage leukemia), TRB@ (T cell receptor beta locus), IGH@ (immunoglobulin heavy chain locus), IGK@ (immunoglobulin kappa light chain locus) and IGL@ (immunoglobulin lambda light chain locus) are involved in rearrangements with several other genes that have been not included for space limitations.

Symbol	Name	Locus
ABL1	V-abl Abelson murine leukemia viral oncogene homolog 1	9q34.1
ABL2	V-abl Abelson murine leukemia viral oncogene homolog 2	1q24–q25
ACSL3	Acyl-CoA synthetase long-chain family member 3	2q34–q35
ACSL6	Acyl-CoA synthetase long-chain family member 6	5q31
AFF3	AF4/FMR2 family, member 3	2q11.2–q12
ALK	Anaplastic lymphoma receptor tyrosine kinase	2p23
ARNT	Aryl hydrocarbon receptor nuclear translocator	1q21
ASPSCR1	Alveolar soft part sarcoma chromosome region, candidate 1	17q25.3
ASXL1	Additional sex combs like 1	20q11.1
ATF1	Activating transcription factor 1	12q13
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	2q35
BAZ2A	Bromodomain adjacent to zinc finger domain, 2A	12q24.3-qter
BCL2	B-cell CLL/lymphoma 2	18q21.3
BCR	Breakpoint cluster region	22q11.23
BRD1	Bromodomain containing 1	22q13.33
C15orf21	Chromosome 15 open reading frame 21	15q21.1
C20orf112	Chromosome 20 open reading frame 112	20q11.1–q11.23
C3orf27	Chromosome 3 open reading frame 27	3q21
CANT1	Calcium activated nucleotidase 1	17q25.3
CAPRIN1	Cell cycle associated protein 1	11p13
CARS	Cysteinyl-tRNA synthetase	11p15.5
CBFA2T3	Core-binding factor, runt domain, alpha subunit 2; translocated to, 3	16q24

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Symbol	Name	Locus
CCDC6	Coiled-coil domain containing 6	10q21
CCDC88C	Coiled-coil domain containing 88C	14q32.11
CDK5RAP2	CDK5 regulatory subunit associated protein 2	9q33.2
CDX2	Caudal type homeo box 2	13q12.3
CEP110	Centrosomal protein 110 kDa	9q33–q34
CHCHD7	Coiled-coil-helix-coiled-coil-helix domain containing 7	8q12.1
CHIC2	Cysteine-rich hydrophobic domain 2	4q11
CIC	Capicua homolog	19q13.2
CLTC	Clathrin, heavy chain (Hc)	17q11–qter
CLTCL1	Clathrin, heavy chain-like 1	22q11.21
COL1A2	Collagen, type I, alpha 2	7q22.1
CPNE8	Copine VIII	12q12
CPSF6	Cleavage and polyadenylation specific factor 6, 68 kDa	12q15
CREB1	cAMP responsive element binding protein 1	2q34
CREB3L1	cAMP responsive element binding protein 3-like 1	11p11.2
CREB3L2	cAMP responsive element binding protein 3-like 2	7q34
CREBBP	CREB binding protein	16p13.3
CTNNB1	catenin (cadherin-associated protein), beta 1, 88 kDa	3p21
CYTSB	cytospin B	17p11.2
DACH1	dachshund homolog 1	13q22
DDIT3	DNA-damage-inducible transcript 3	12q13.1–q13.2
DDX5	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	17q21
DUSP10	Dual specificity phosphatase 10	1q41
DUX4	Double homeobox, 4	4q35
ELF4	E74-like factor 4	Xq26
ELK4	ETS-like transcription factor 4	1q32
ELN	Elastin	7q11.23
EML1	Echinoderm microtubule associated protein like 1	14q32
EML4	Echinoderm microtubule associated protein like 4	2p22–p21
EP300	E1A binding protein p300	22q13.2
ERC1	ELKS/RAB6-interacting/CAST family member 1	12p13.3
ERG	ETS related gene (v-ets erythroblastosis virus E26 oncogene homolog)	21q22.3
EST14	EST from chromosome 14 (unknown)	14q21.1
ETV1	ETS variant 1	7p21.3
ETV4	ETS variant 4	17q21
ETV5	ETS variant 5	3q28
ETV6	ETS variant 6 (TEL1 oncogene)	12p13
EVI1	Ecotropic viral integration site 1	3q24–q28
EWSR1	Ewing sarcoma breakpoint region 1	22q12.2
FEV	Fifth Ewing variant	2q36
FGA7	Fused gene 7 to AML1(unknown)	4q28
FGFR1	Fibroblast growth factor receptor 1	8p11.2–p11.1
FGFR1OP	FGFR1 oncogene partner	6q27
FGFR1OP2	FGFR1 oncogene partner 2	12p11.23
FGFR3	Fibroblast growth factor receptor 3	4p16.3
FIP1L1	FIP1 like 1	4q12
FLI1	Friend leukemia virus integration 1	11q24.1–q24.3
FLT3	Fms-related tyrosine kinase 3	13q12
FOXP1	Forkhead box P1	3p14.1
FRK	Fyn-related kinase	6q21–q22.3
FUS	Fusion (involved in t(12;16) in malignant liposarcoma)	16p11.2
GIT2	G protein-coupled receptor kinase interacting ArfGAP 2	12q24.1
GOLGA5	Golgi autoantigen, golgin subfamily a, 5	14q32.12–q32.13
GOT1	Glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	10q24.1–q25.1
HAS2	Hyaluronan synthase 2	8q24.12
HERVK_17p13.1	Human endogenous retroviral family K	17p13.1
HERVK_22q11.23	Human endogenous retroviral family K	22q11.23
HIP1	Huntingtin interacting protein 1	7q11.23
HIPK1	Homeodomain interacting protein kinase 1	1p13.2
HLF	Hepatic leukemia factor	17q22
HNRPA2B1	Heterogeneous nuclear ribonucleoprotein A2/B1	7p15

(*Continued*)

Symbol	Name	Locus
HOOK3	Hook homolog 3	8p11.21
IGH@	Immunoglobulin heavy locus	14q32.33
IGK@	Immunoglobulin kappa locus	2p12
IGL@	Immunoglobulin lambda locus	22q11.1–q11.2
ITK	IL2-inducible T-cell kinase	5q31–q32
JAK2	Janus kinase 2	9p24
KIAA1468	LisH domain and HEAT repeat-containing protein KIAA1468	18q21.33
KIAA1618	KIAA1618 (hypothetical protein LOC57714)	17q25.3
KIF3B	Kinesin family member 3B	20q11.21
KIF5B	Kinesin family member 5B	10pter–q22.1
KLK2	Kallikrein-related peptidase 2	19q13.41
KTN1	Kinectin 1 (kinesin receptor)	14q22.1
LIFR	Leukemia inhibitory factor receptor alpha	5p13–p12
LOC113386	LOC113386 similar to envelope protein (human endogenous retroviral family K)	19q13.43
LOC392027	LOC392027 (ribosome-binding protein 1 pseudogene)	7p12.1
LOC646982	LOC646982 (twelve-thirteen translocation leukemia gene)	13q14.11
MACROD1	MACRO domain containing 1	11q11
MDS1	Myelodysplasia syndrome 1	3q26
MDS2	Myelodysplasia syndrome 2	1p36
MET	Met proto-oncogene (hepatocyte growth factor receptor)	7q31
MLF1	Myeloid leukemia factor 1	3q25.1
MLL	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog)	11q23
MN1	Meningioma (disrupted in balanced translocation) 1	22q12.1
MNX1	Motor neuron and pancreas homeobox 1	7q36
MSN	Moesin	Xq11.2–q12
MYB	v-myb myeloblastosis viral oncogene homolog	6q22-q23
MYH9	Myosin, heavy chain 9, non-muscle	22q13.1
MYO18A	Myosin XVIIIA	17q11.2
MYST3	MYST histone acetyltransferase (monocytic leukemia) 3	8p11
MYST4	MYST histone acetyltransferase (monocytic leukemia) 4	10q22.2
NCOA2	Nuclear receptor coactivator 2	8q13.3
NCOA4	Nuclear receptor coactivator 4	10q11.2
NDE1	nudE nuclear distribution gene E homolog 1	16p13.11
NFATC2	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	20q13.2–q13.3
NIN	Ninein (GSK3B interacting protein)	14q22.1
NONO	non-POU domain containing, octamer-binding	Xq13.1
NOP2	NOP2 nucleolar protein homolog	12p13
NPM1	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	5q35
NR4A3	Nuclear receptor subfamily 4, group A, member 3	9q22
NTRK1	Neurotrophic tyrosine kinase, receptor, type 1	1q21–q22
NTRK3	Neurotrophic tyrosine kinase, receptor, type 3	15q25
NUMA1	Nuclear mitotic apparatus protein 1	11q13
PATZ1	POZ (BTB) and AT hook containing zinc finger 1	22q12.2
PAX5	Paired box gene 5	9p13
PBX1	pre-B-cell leukemia homeobox 1	1q23
PCM1	Pericentriolar material 1	8p22–p21.3
PDE4DIP	Phosphodiesterase 4D interacting protein	1q12
PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide	4q11–q13
PDGFRB	Platelet-derived growth factor receptor, beta polypeptide	5q31-q32
PER1	Period homolog 1	17p13.1–p12
PLAG1	Pleiomorphic adenoma gene 1	8q12
PML	Promyelocytic leukemia	15q22
POM121	POM121 membrane glycoprotein	7q11.23
POU5F1	POU class 5 homeobox 1	6p21.31
PRCC	Papillary renal cell carcinoma (translocation-associated)	1q21.1
PRDM16	PR domain containing 16	1p36.23–p33
PRDX4	Peroxiredoxin 4	Xp22.11
PRKAR1A	Protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	17q23–q24
PRKG2	Protein kinase, cGMP-dependent, type II	4q13.1–q21.1
PTPRR	Protein tyrosine phosphatase, receptor type, R	12q15
RABEP1	Rabaptin, RAB GTPase binding effector protein 1	17p13.2

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Symbol	Name	Locus
RANBP2	RAN binding protein 2	2q12.3
RARA	Retinoic acid receptor, alpha	17q21
RET	Ret proto-oncogene	10q11.2
RPL22P1	Ribosomal protein L22 pseudogene 1	3q26.2
RPN1	Ribophorin I	3q21.3
RUNX1	Runt-related transcription factor 1 (AML1)	21q22.3
RUNX1T1	Runt-related transcription factor 1; translocated to, 1 (cyclin D-related, ETO)	8q22
SEC31A	SEC31 homolog A	4q21.22
SFPQ	Splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	1p34.3
SH3D19	SH3 domain containing 19	4q31.3
SLC45A3	Solute carrier family 45, member 3	1q32.1
SLCO1B3	Solute carrier organic anion transporter family, member 1B3	12p12
SP3	Sp3 (specificity protein 3) transcription factor	2q31
SPTBN1	Spectrin, beta, non-erythrocytic 1	2p21
SSBP2	Single-stranded DNA binding protein 2	5q14.1
STAT5B	Signal transducer and activator of transcription 5B	17q11.2
STL	Six-twelve leukemia	6q22.33
STRN	Striatin, calmodulin binding protein	2p22–p21
SYK	Spleen tyrosine kinase	9q22
TAF15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa	17q11.1–q11.2
TCEA1	Transcription elongation factor A (SII), 1	8q11.2
TCF12	Transcription factor 12	15q21
TCF3	Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	19p13.3
TFE3	Transcription factor binding to IGHM enhancer 3	Xp11.22
TFG	TRK-fused gene	3q12.2
TFPT	TCF3 (E2A) fusion partner (in childhood Leukemia)	19q13
TMPRSS2	Transmembrane protease, serine 2	21q22.3
TP53BP1	Tumor protein p53 binding protein 1	15q15–q21
TPM3	Tropomyosin 3	1q21.2
TPM4	Tropomyosin 4	19p13.1
TPR	Translocated promoter region (to activated MET oncogene)	1q25
TRB@	T cell receptor beta locus	7q34
TRIM24	Tripartite motif-containing 24	7q32–q34
TRIM33	Tripartite motif-containing 33	1p13.1
TRIP11	Thyroid hormone receptor interactor 11	14q31–q32
TRPS1	Trichorhinophalangeal syndrome I	8q24.12
USP42	Ubiquitin specific peptidase 42	7p22.1
WT1	Wilms tumor 1	11p13
YTHDF2	YTH domain family, member 2	1p35
ZBTB16	Zinc finger and BTB domain containing 16	11q23.1
ZFPM2	Zinc finger protein, multitype 2	8q23
ZMIZ1	Zinc finger, MIZ-type containing 1	10q22.3
ZMYM2	Zinc finger, MYM-type 2	13q11–q12
ZNF384	Zinc finger protein 384	12p12
ZNF521	Zinc finger protein 521	18q11.2
ZNF687	Zinc finger protein 687	1q21.3

On the other hand, EWSR1-FLI1 has a higher transformation activity than FLI1,[69] indicating that the fusion protein has oncogenic capacities that are not attributed to over-expression of the ETS translocation partner alone. Recent evidence indicate that the chimeric EWSR1-FLI1 transcription factor bind not only ETS consensus sites but also microsatellite sequences. The enrichment of such sequences in proximity to known EFT associated genes suggests that EWSR1-FLI1 fusion proteins regulate gene expression after binding to these microsatellite sequences.[70] In addition to the direct regulation of

gene expression, EWSR1-FLI1 exert transcription factor activity-independent oncogenic activities. EWSR1-FLI1 activates gene transcription,[70,71] but tumorigenicity of EWSR1-FLI1 is partially independent on DNA binding.[72] Interference of several TET fusion proteins with the splicing machinery has been observed.[73–76] Because TET proteins are involved in RNA splicing,[77–79] this might be indicative for an interference between wild type TET proteins and fusion proteins. Indeed, inhibition of wild type EWSR1 function by EWSR1-FLI has been described.[80] Interestingly, this inhibition leads to mitotic defects. Aberrant mitotic figures can be regularly observed in cultured EFT cells (Fig. 3) and might be responsible for the high frequency of secondary chromosomal aberrations seen in EFT.[81,82] Finally, the different activities of the fusion proteins lead to altered gene expression. Despite a similar histological appearance, TET-ETS positive EFT have a gene expression profile which clearly discriminates these tumors from other tumors of the family of so called small round blue cell tumors.[7,83,84] Several target genes of TET-ETS fusion proteins have been identified[85–99] and different TET-ETS fusion proteins induce a similar tumorigenic activity in transgenic cells.[100] On the other hand, the consequences of TET-ETS fusion protein activity depend on the cellular background[101,102] and fusion type dependent differences in the gene expression profile of EFT have been observed.[103,104] The fusion type as well as the genes expression profile have been identified

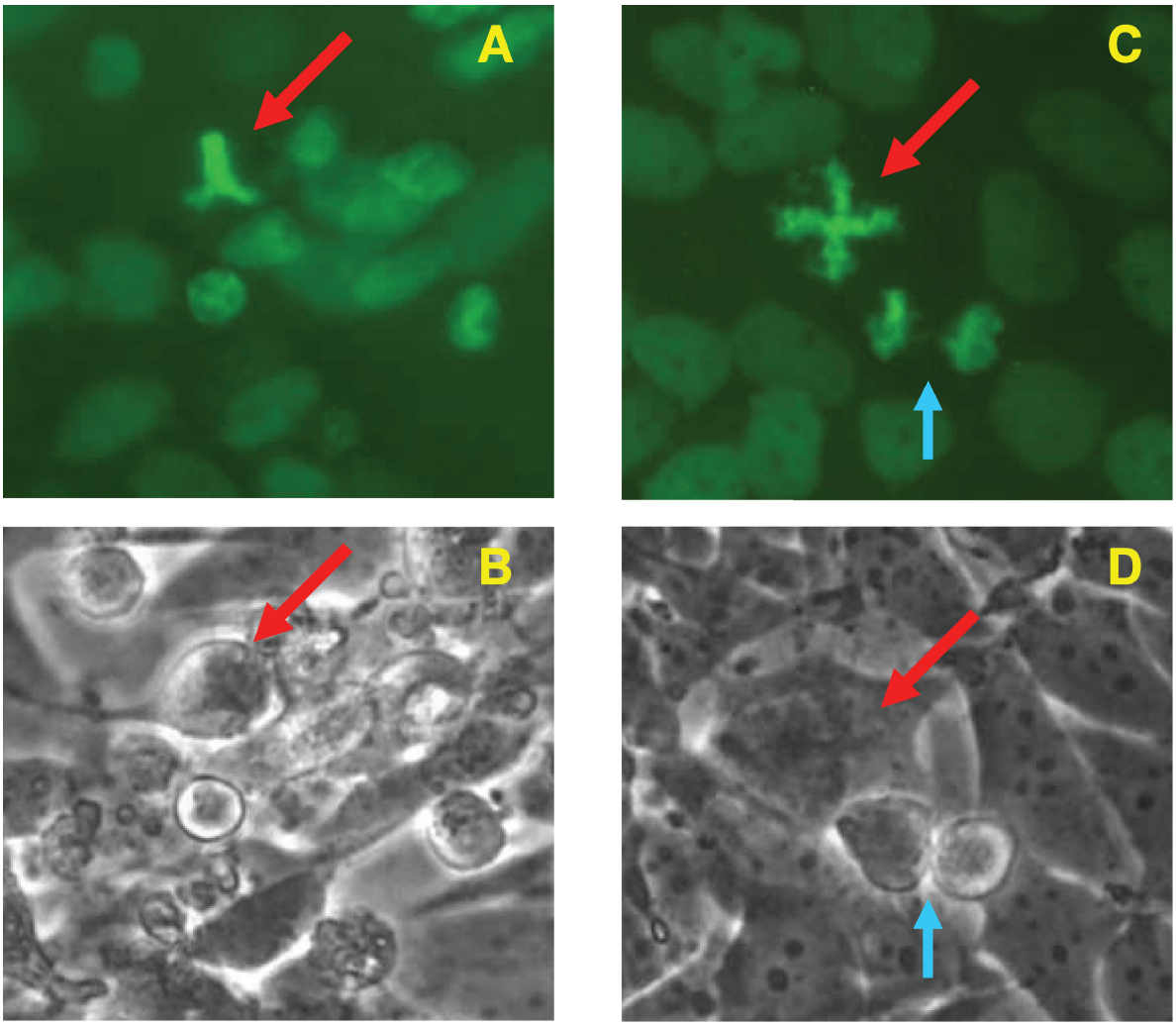

Figure 3. Visualization of aberrant mitosis in living EFT cells. SK-N-MC cells were transfected with an expression vector for a histone H2B-green fluorescent protein fusion as described in Material and Methods and mitotic figures were analyzed in living cells by fluorescence microscopy (**A**, **C**) and phase contrast microscopy (**B**, **D**) in the same visual field. x200, zoomed image. Aberrant mitotic figures are marked with red arrows. In panels **C** and **D** a second mitosis can be seen (blue arrow).

as prognostic factors for EFT patients.[105–109] The exact function of TET-ETS fusion proteins in cancer pathogenesis remains unclear. Case reports describing patients with two TET-ETS translocations in the same tumor might indicate that TET-ETS translocations are not the primary event leading to tumor formation.[110] Nevertheless, TET-ETS fusion proteins are required for growth of EFT and several strategies for the inactivation of TET-ETS fusion transcripts have been developed.[111–114] EFT have a neuronal phenotype which can partially be explained by the activity of EWSR1-ETS fusion proteins.[7,115–118] TET proteins are involved in neuronal biology and a link between TET proteins and neurodegenerative diseases have been established.[119,120] However, the function of wild type TET proteins is not restricted to the nervous system but is also required for hematopoiesis.[121] Surprisingly, EWSR1-FLI1 expressing transgenic animals did not develop EFT like sarcomas but leukemia.[122] Restricted expression of the fusion proteins in mesenchymal cells leads to sarcoma formation only in the setting of additional TP53 aberrations.[123] These observations indicate that TET-ETS fusion proteins are not sufficient to induce tumor formation. Similarly, TET-ETS fusion proteins are not sufficient to induce the complete gene expression program of EFT. Which factors are responsible for expression of EFT-associated but TET-ETS-independent genes (e.g. lipase member I, LIPI)[124] is unknown. Recently, tumor stem cells in EFT have been identified.[125] Interestingly, these cells are characterized by expression of transcription factors NANOG (Tir Na Nog) and POU5F1 (POU (Pituitary-specific 1, Octamer transcription factor, Uncoordinated-86) domain, class 5, transcription factor 1). Expression of these factors is usually not observed in mesenchymal stem cells but is a characteristic feature of embryonic stem cells.[126] Cell populations with the phenotype of embryonic stem cells have been identified in the adult body.[127,128] Whether such cell populations are permissive for EWSR1-FLI1 induced transformation and whether EFT are derived from these cell populations have to be determined.

Recent evidence indicates that epigenetic mechanisms play a major role in cancer pathogenesis mediated by TET and/or ETS gene fusions. Epigenetic inactivation of tumor suppressor genes have been observed in EFT[129] and inhibitors of histone deacetylation or DNA methylation have been shown to exert anti-tumor activity against EFT.[130,131] Similarly, TMPRSS2 (transmembrane protease, serine 2)-ETS translocations in prostate cancer are associated with increased histone deactetylase expression.[132] One of the target genes of EWSR1-FLI1, enhancer of zeste homolog 2 (EZH2), is involved in epigenetic inactivation of genes. Based on the observation of high expression of EZH2 in EFT[7] a model for epigenetic inactivation of differentiation inducing genes was proposed.[10,133] This model implicates that up-regulation of epigenetic silencers like EZH2 by TET-ETS fusion proteins fix the tumor cell in an un-differentiated state. In deed, inhibition of EZH2 allows differentiation of EFT cells and inhibits tumor growth.[10] Another implication of this model is that different primary oncogenic events might be fixed by TET-ETS fusion proteins leading to similar tumor morphology.

Today, only three members of the TET family have been identified in the human genome. Another RNA binding protein, RBM14 (RNA binding motif 14), with weak similarity to TET proteins and involvement in regulation of RNA transcription and splicing has been identified,[134] but translocations involving this gene have not been found. Interestingly, the human genome contains EWSR1 pseudogenes.[135] By using the cDNA sequence of the EWSR1 part from the EWSR1-FLI1 type I translocation as bait we found two human pseudogenes. The presence of multiple EWSR1 pseudogenes in the human genome indicates that EWSR1 sequences have repeatedly been involved in rearrangements. One pseudogene on chromosome 1 (LOC284685) contains an intronless copy of the complete open reading frame of EWSR1 corresponding to the longer (EWS) isoform (Fig. 4). Several point mutations did not allow the translation of a corresponding EWSR1 protein. The second copy on chromosome 14 (LOC644584) is again an intronless copy of the open reading frame of EWSR1. However, this copy contains only the 5' part (corresponding to exons 1 to 7) of EWSR1 and resembles EWSR1 in the most common type 1 EWSR1-FL1 translocation (Fig. 4). Again, several point mutations and deletions did not allow translation of a protein. In this pseudogene the 3' part of EWSR1 is replaced by a non-coding sequence from chromosome 3 (data not shown).

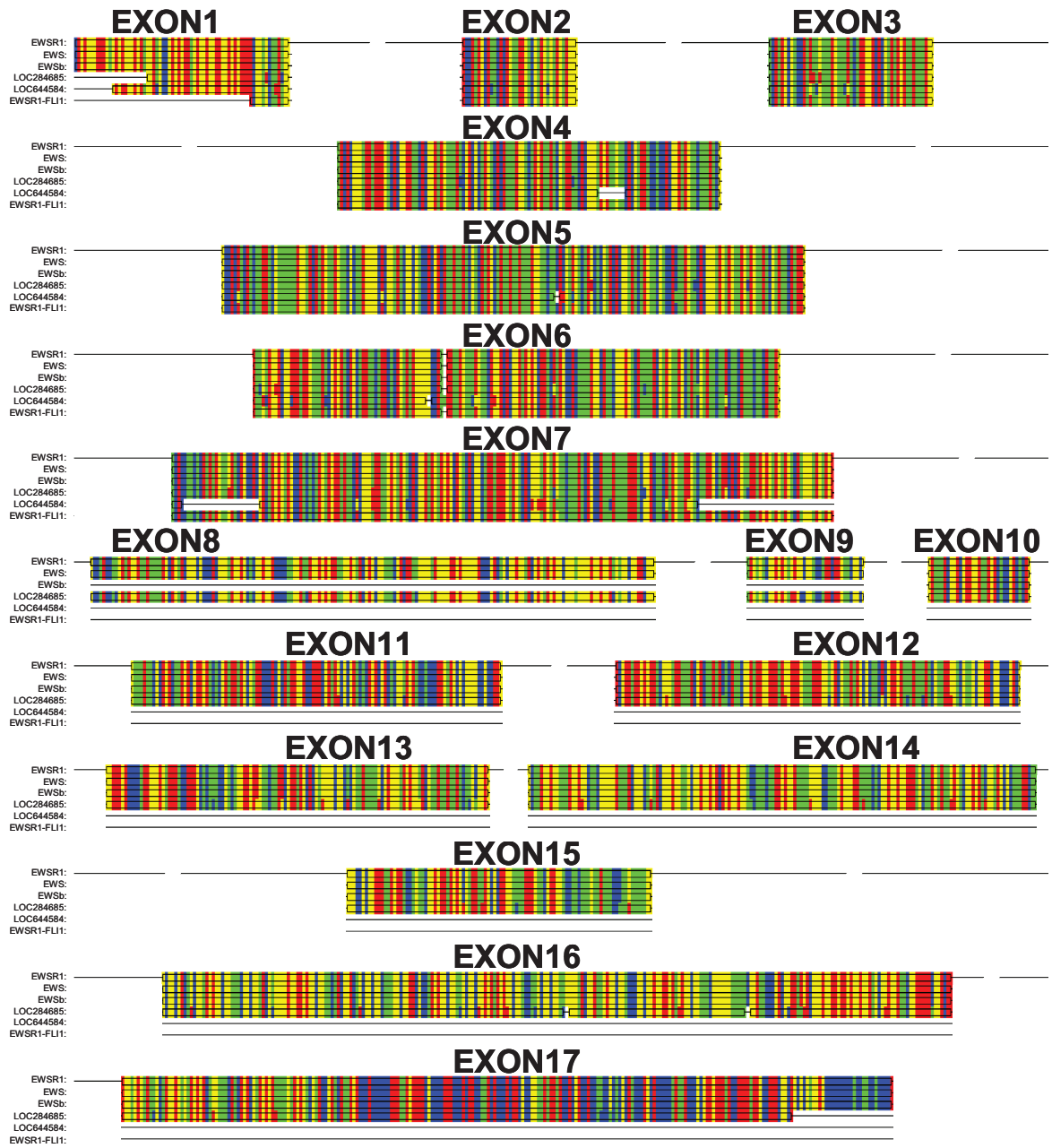

Figure 4. Structure of the human EWSR1 gene, transcripts and pseudogenes. mRNA sequences of EWSR1 (isoforms EWS and EWSb) and EWSR1-FLI1 (translocation type 1) were aligned with genomic sequences of EWSR1 and the two ESWR1 pseudogenes. Nucleic acids were colored red (**A**), blue (U/T), yellow (**G**), and green (**C**), respectively. Intron sequences of EWSR1 have been deleted and are represented by uncolored lines. Only the 3' part of the first exon of EWSR1 is shown. The start of the EWSR1-FLI1 sequence marks the position of the start ATG.

Taken together, gene rearrangements involving members of the TET or ETS families are part of a large network of oncogenic gene fusions. Whether these gene fusions can be targeted with clinical therapeutic benefit has to be shown.

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Disclosures

The authors report no conflicts of interest.

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