ELSEVIER

European Journal of Cancer 41 (2005) 2513–2527

European Journal of Cancer

www.ejconline.com

Chromosome translocations in sarcomas and the emergence of oncogenic transcription factors

Shujuan J. Xia, Frederic G. Barr *

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, 505C Stellar Chance Laboratories, 422 Curie Boulevard, Philadelphia, PA 19104-6082, USA

Available online 6 October 2005

Abstract

A subset of sarcomas is characterised by recurrent chromosome translocations that generate novel fusion oncoproteins. One or both of the genes involved in these translocations often encode transcription factors, and the resulting fusion proteins have aberrant transcriptional function compared to their wild-type counterparts. These fusion transcription factors disrupt multiple biological pathways by altering expression of target genes, and thereby result in a variety of altered cellular properties that contribute to the tumourigenic process. However, experimental data indicate that the fusion gene alone is not sufficient for transformation in primary cells (EWS–FLI1) or tumourigenesis in the mouse (PAX3–FKHR, FUS–CHOP), suggesting that additional collaborating genetic alterations are required. In addition to improving our understanding of the etiology of these tumours, this accumulating knowledge of the oncogenic properties of these fusion proteins, their downstream targets, and cooperating genetic alterations will permit the development of a variety of novel approaches to improve the therapy of these cancers.

Keywords: Chromosomal translocation; Fusion protein; Transcription factor; Sarcoma

1. Introduction

Cytogenetic analysis has frequently demonstrated chromosomal rearrangements in cancers. Non-random chromosome translocations have been detected in fourteen sarcoma categories (Table 1), which comprise about one third of all sarcomas [1]. In general, these translocations occur in the majority of cases of the sarcoma category and do not occur in other tumour types. As a result of the translocation, two protein-coding regions (or portion of these regions) are fused in frame, producing a chimeric protein. Molecular genetic studies identified that genes encoding transcription factors are often involved at one or both of the breakpoints in these chromosomal translocations. Therefore, the fusion proteins are aberrant transcription factors, usually demonstrating higher activity than their wild-type counterparts. For many of these gene fusions, only one of the two chimeric products is consistently and highly expressed, and is therefore presumed to be the major product of the translocation.

It is widely accepted that fusion proteins resulting from chromosome translocations are oncogenic, based on evidence demonstrating they are able to transform cells in culture, mostly using the immortalised mouse NIH3T3 fibroblast cell line as a model system. The fusion proteins with evidence of transforming activity in this system are PAX3–FKHR from the t(2;13)(q35;q14) in alveolar rhabdomyosarcoma (ARMS), EWS–FLI1 from the t(11;22)-(q24;q12) in Ewing's sarcoma, SYT–SSX1 from the t(X;18)(p11;q11) in synovial sarcoma, and FUS–CHOP from the t(12;16)(q13;p11) in myxoid liposarcoma [2–5]. Injection of these transformed cells into immunodeficient mice, usually as subcutaneous xenografts, form tumours

* Corresponding author. Tel.: +1 215 898 0884; fax: +1 215 898 4227.
E-mail address: barrfg@mail.med.upenn.edu (F.G. Barr).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.08.003

Table 1
Chromosome translocations in sarcomas[a]

Tumour	Translocation	Fusion product	Reference
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	**PAX3**–FKHR	[19]
	t(1;13)(p36;q14)	**PAX7**–FKHR	[20]
Alveolar soft-part sarcoma	t(X;17)(p11;q25)	**TFE3**–ASPL	[119]
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	FUS–**ATF1**	[120]
Clear-cell sarcoma	t(12;22)(q13;q12)	EWS–**ATF1**	[89]
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	COL1A1–PDGFB	[121]
Desmoplastic small-round cell tumour	t(11;22)(p13;q12)	EWS–**WT1**	[93]
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZF1–JJAZ1	[122]
Ewing's tumour family	t(11;22)(q24;q12)	EWS–**FLI1**	[123]
	t(21;22)(q22;q12)	EWS–**ERG**	[124]
	t(7;22)(p22;q12)	EWS–**ETV1**	[125]
	t(2;22)(q33;q12)	EWS–**E1AF**	[126]
	t(17;22)(q12;q12)	EWS–**FEV**	[127]
	t(16;21)(p11;q22)	FUS–**ERG**	[59]
Infantile fibrosarcoma	t(12;15)(p13;q25)	ETV6–NTRK3	[128]
Inflammatory myofibroblastic tumour	t(1;2)(q22;p23)	TPM3–ALK	[129]
	t(2;19)(p23;p13)	TPM4–ALK	[129]
	t(2;17)(p23;q23)	CLTC–ALK	[130]
Low grade fibromyxoid sarcoma	t(7;16)(q33;p11)	FUS–**CREB3l2**	[131]
Myxoid chondrosarcoma	t(9;22)(q22;q12)	EWS–**CHN**	[132]
	t(9;17)(q22;q11)	TAF2N–**CHN**	[133]
	t(9;15)(q22;q21)	TCF12–**CHN**	[134]
Myxoid liposarcoma	t(12;16)(q13;p11)	FUS–**CHOP**	[104]
	t(12;22)(q13;q12)	EWS–**CHOP**	[135]
Synovial sarcoma	t(X;18)(p11;q11)	SYT–SSX1	[136]
		SYT–SSX2	[136]
		SYT–SSX4	[137]

[a] Bold part contains DNA binding domain.

supporting their tumourigenic function [5,6]. Furthermore, a transgenic mouse model expressing FUS–CHOP revealed liposarcoma formation, and a conditional PAX3–FKHR knock-in mouse model displayed ARMS formation, further demonstrating the link between the fusion gene and these sarcomas [7,8]. The finding of a low tumour frequency in these animals that can be increased by crosses with animals carrying mutations in other loci indicates that the fusion gene is not sufficient for tumourigenesis [8], but rather additional genetic events are needed as collaborating events during multistep tumourigenesis.

The potential activities of these fusion proteins that contribute to oncogenesis include increasing cell proliferation, promoting anchorage-independent cell growth, abrogating cell contact inhibition, inhibiting apoptosis, enhancing invasion, and suppressing terminal differentiation. As aberrant transcription factors, the fusion proteins perform their oncogenic functions mainly through deregulating downstream target gene expression, resulting in changes of biological signalling pathways and ultimately cellular changes, such as in cell cycle control, apoptosis and differentiation. In addition to their role as DNA binding proteins, some fusion proteins interact directly with other transcription factors. For example, EWS–ATF1 interacts with the coactivator CBP to suppress p53 mediated trans-activation [9]. In addition to transcriptional control, some fusion proteins also interfere with post-transcriptional processes, such as EWS–FLI1, which interacts with splicing factors and modulates the splicing process [10].

Variant translocations, which occur less frequently than the characteristic translocations, have been reported in at least six sarcoma categories (Table 1). Molecular genetic analysis has determined that, in these variants, one of the two fusion partners is identical to a gene involved in the common translocation whereas the other partner is usually closely related to the other gene involved in the common translocation. The resulting variant fusion proteins have similar structure and function as the more common fusion proteins, with similar target genes and comparable tumourigenic phenotypes [11,12].

Even though the fusion proteins generated by these chromosome translocations have oncogenic potential, it is unlikely that a single genetic change is sufficient for human malignancy. Studies of cellular transformation and tumourigenesis support the notion that introduction of multiple oncogenes and/or inactivation of tumour suppressor genes are required to transform mouse cells. In addition to these events, telomerase activation is also required to transform human cells [13]. In fact, amplification of MYCN (an oncogene) is found in ARMS, and deletion of p16 (a tumour suppressor) is frequently detected in Ewing's sarcoma [14,15], suggesting that such oncogene activating and tumour suppressor gene inactivating events are candidates for collaborators in oncogenesis induced by the sarcoma-associated fusion proteins. Use of modern genomic strategies to identify additional collaborating events will contribute to a better understanding of the mechanisms underlying tumourigenesis in sarcomas.

As the biology of these fusion proteins and collaborating factors are elucidated and refined, investigations are beginning to pursue potential applications for therapy. At first, many efforts were directed towards targeting the fusion protein itself since it plays a fundamental role in oncogenesis. Secondly, the downstream targets of the fusion protein and the collaborating factors also provide promising molecular targets for therapeutic pursuit. Finally, manipulating a combination of more than one target may lead to optimal result for sarcoma therapeutics.

This review will focus on the findings that establish these fusion proteins in sarcomas as oncogenic transcription factors. In addition to discussing the structure of these proteins, this review will consider their effects on downstream target genes, the cellular changes evoked by expression of these proteins, and the putative cooperating genetic alterations required for tumourigenesis. Finally, the potential for translating these recent advances into novel therapeutic strategies will also be explored.

2. Alveolar rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is a common childhood soft tissue tumour associated with the skeletal muscle lineage. There are two subtypes of RMS based on histopathologic features: ARMS and embryonal RMS (ERMS). ARMS typically occurs in the age group of adolescents and young adults, frequently in the sites of trunk and extremities, and portends a poorer prognosis; whereas ERMS often occur in children younger than ten years old, most frequently in the head and neck, genitourinary tract, and the retroperitoneum, and has a more favorable prognosis [16].

2.1. Chromosome translocation in ARMS

Of the two RMS subtypes, recurrent chromosome translocations were found in the ARMS subtype [17]. The most prevalent finding is a translocation involving chromosomes 2 and 13, t(2;13)(q35;q14), which was detected in 70% of published ARMS cases. In addition, there are several reports of a t(1;13)(p36;q14) variant translocation. These two translocations are not associated with any other tumour type and thus appear to be specific markers for ARMS, providing a molecular diagnostic tool to distinguish these two RMS subtypes.

Based on physical mapping studies, PAX3 was localised to the 2;13 translocation breakpoint on chromosome 2, and PAX7 was found on the 1;13 translocation breakpoint on chromosome 1 [18–20]. These two genes encode highly related members of the paired box transcription factor family that are organised with N-terminal DNA binding domains and C-terminal transcriptional activation domains [21]. PAX3 and PAX7 constitute a subfamily within the paired box (PAX) transcription factor family characterised by an N-terminal DNA binding domain consisting of a paired box, intervening octapeptide and complete homeodomain.

Studies of PAX3 have demonstrated an essential role in early skeletal muscle development. Though the heterozygous mutation of Pax3 in Splotch mice produces a phenotype of abnormal pigmentation due to neural crest defects, the homozygous mutation is an embryonic lethal condition due to defects in skeletal muscle in addition to abnormalities of neural tube and neural crest derivatives. The skeletal muscle defect is characterised by an absence of limb musculature which results from a failure of myogenic precursor cells to migrate from the lateral dermomyotome to the limbs [22]. PAX3 mutations are found in the human disease Waardenburg syndrome, which is characterised by pigmentation abnormalities, deafness, and other neural crest defects [23]. Functional studies revealed that the mutations in Splotch and Waardenburg syndrome alter or abolish PAX3 transcriptional activity, and result in a loss of function [24]. Gene transfer studies demonstrate that PAX3 initiates myogenesis cascades in embryonic cell lines [25].

PAX7 has a distinct and essential role in later postnatal skeletal muscle development. In mouse embryogenesis, Pax7 and Pax3 share expression domains in the dorsal neural tube and the somite, but with spatial and temporal differences. Though the precise role in postnatal myogenic satellite cell development is controversial, it is clear that the absence of Pax7 leads to a loss of muscle satellite cells during postnatal development and impaired muscle regeneration in Pax7 null mice [26,27]. However, the relatively normal phenotype of homozygous Pax7 null mutation mice at birth suggests that there is functional redundancy, perhaps with Pax3 [28]. In a further test of this functional redundancy, Pax3

was replaced with Pax7 in a gene targeting experiment, demonstrating that Pax7 can substitute for Pax3 in neural tube, neural crest and somite development, but not in limb muscle development and muscle precursor cell migration [29].

The FKHR (FOX01A) gene is located at the 2;13 translocation breakpoint on chromosome 13. The FKHR protein is a member of the forkhead transcription factor family and is the prototype of a subfamily of forkhead transcription factors which play roles in cellular metabolism, cell cycle progression, and apoptosis [30]. As a transcription factor, FKHR contains an N-terminal forkhead DNA binding domain and a C-terminal transcriptional activation domain. In addition, FKHR contains three AKT phosphorylation sites responsible for phosphoinositide 3-kinase mediated signal transduction [30]. Signalling pathways, such as insulin and insulin-like growth factor (IGF), regulate the transcriptional activity of FKHR by modulating the phosphorylation status of these sites and thereby regulate the cellular localisation of the protein. However, a phosphorylation independent mechanism of FKHR subcellular localisation appears to be operative during myotube fusion in myoblasts [31].

The 2;13 or 1;13 chromosome translocation generates a fusion protein that contains the intact DNA binding domain of PAX3 or PAX7 in the N-terminal region fused in frame with a C-terminal FKHR segment containing the transactivation domain (Fig. 1). A series of molecular biology studies demonstrated that the chromosomal changes in ARMS result in high level expression, potent transcriptional activity, and constitutive nuclear localisation of the PAX3–FKHR and PAX7–FKHR fusion products [32–36]. The end result is exaggerated activity at multiple biological levels that converges to inappropriate activation of PAX3/PAX7 target genes and ultimately contributes to tumourigenic behavior.

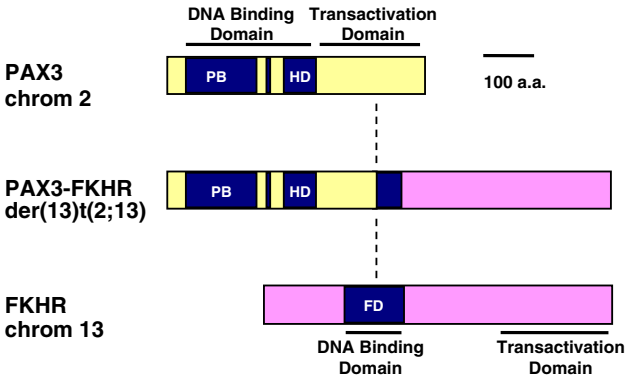

Fig. 1. Fusion protein generated by the 2;13 chromosome translocation in ARMS. The wild-type and fusion proteins are shown. The paired box (PB), octapeptide, homeodomain (HD) and fork head domain (FD) are shown as solid boxes and the overall DNA binding domains and transactivation domains are shown as horizontal lines. The vertical line indicates the fusion point.

2.2. Effects of PAX3–FKHR oncogene on cellular process

Gene transfer studies provided evidence to support the oncogenic role of this tumour-specific fusion protein. In chicken embryo fibroblasts and NIH3T3 fibroblasts, expression of PAX3–FKHR but not wild-type PAX3 led to cellular transformation, including morphological changes, anchorage-independent growth and focus formation [2,37]. In another study, stable transfection of PAX3–FKHR into two ERMS cell lines resulted in increased proliferative rate and cell growth in low serum conditions [38]. This study also showed that tumours formed by implanting the PAX3–FKHR transfected ERMS cells in immunodeficient mice were more aggressive than tumours arising from control transfectants.

Downregulation of fusion gene expression in an ARMS cell line using antisense oligonucleotides resulted in cell death, presumably by promoting apoptosis [39]. Further studies were performed to address the relationship between PAX3–FKHR and the anti-apoptotic protein BCL-XL. Following introduction of PAX3–FKHR into cultured cells, BCL-XL mRNA was elevated [40]. Moreover, BCL-XL was able to rescue tumour cells from apoptosis induced by PAX3–FKHR antisense incubation and DNA binding and reporter studies supported the premise that BCL2L1 (BCL-XL) is a direct transcriptional target of PAX3–FKHR. These findings indicate that PAX3–FKHR is required to maintain cell viability by preventing apoptosis, involving a BCL-XL-dependent mechanism.

2.3. PAX3–FKHR upregulates downstream targets enhancing metastasis in ARMS

MET is the receptor for hepatocyte growth factor/scatter factor (HGF/SF) and a member of the tyrosine kinase family of signalling molecules [41]. MET–HGF/SF signalling induces cell proliferation and motility in normal cells, and is also implicated in invasion and metastasis in cancer. During embryogenesis, Met is expressed in the Pax3-expressing cells that migrate from the lateral dermomyotome to the limb buds and form the limb musculature. The Met homozygous mutant mouse has a very similar muscle phenotype as the Pax3/Splotch homozygous mutant mouse, indicating that both proteins function in a common pathway involving myogenic precursor development [42]. The relationship was clarified when Pax3 was shown to bind to the MET promoter and upregulate Met expression [43]. Additional studies indicate that MET is also a downstream target for PAX3–FKHR [44]. The overexpression of MET in ARMS is associated with altered cell behavior of ARMS cells in the presence of HGF, including increased locomotion, chemotaxis, adhesion to other cells, and invasion through matrigel [45]. The propensity of ARMS cells to metastasise to the bone marrow can be

at least partly explained by the bone marrow's secretion of HGF, which is a potent chemotactic agent for the ARMS cells.

CXCR4 is a G-protein coupled chemokine receptor whose ligand is stromal derived factor-1 (SDF1), which is also secreted by bone marrow stromal cells [46]. The CXCR4–SDF1 signalling pathway is proposed to play an important role in the homing of normal cells to hematopoietic sites and the corresponding metastasis of tumour cells to the same sites [47]. As evidence that CXCR4 is a downstream expression target of PAX3–FKHR, the expression level of CXCR4 was directly correlated with the expression level of PAX3–FKHR in stable long-term RD ERMS subclones constitutively expressing PAX3–FKHR and in a population of RD cells expressing a tamoxifen-inducible form of PAX3–FKHR [48]. To explore the influence of CXCR4–SDF1 signalling on the metastatic behavior of ARMS cells, SDF1 treatment of ARMS cell lines was shown to induce cell motility, adhesion, invasion and protease (MMP2) secretion [49]. Furthermore, T140, a CXCR4-specific inhibitor, blocked SDF1 directed adhesion and chemotaxis in ARMS cells, thus providing a model for a therapeutic strategy aimed at inhibition of this signalling pathway.

2.4. PAX3–FKHR disrupts developmental processes

Several studies indicate that PAX3–FKHR also interferes with the myogenic pathway. Introduction of PAX3–FKHR in C2C12 myoblasts and MyoD-transfected C3H10T1/2 fibroblasts, which undergo myogenic differentiation under low serum conditions, resulted in inhibition of myogenic differentiation [50]. Using microarray profiling to compare the expression patterns in NIH3T3 cells transduced with either PAX3 or PAX3–FKHR, PAX3–FKHR, but not PAX3, was found to induce expression of a large set of genes involved in myogenesis, including the transcription factors MyoD, Myogenin, Six1 and Slug [51]. The apparent contrast of the findings of these two studies may be explained by the hypothesis that PAX3–FKHR may initiate the early steps of the myogenesis pathway, but block this process at the late phase. This aberrant regulation of myogenic differentiation by PAX3–FKHR may contribute to the relative lack of differentiated markers and inability to undergo terminal differentiation seen in this tumour.

To study the impact of PAX3–FKHR on mouse development, mouse models expressing Pax3–Fkhr were created [8,29,52,53]. In several models, Pax3–Fkhr was expressed from Pax3 regulatory elements, either by a transgenic approach with a Pax3 regulatory cassette or gene targeting of Fkhr sequences into the Pax3 locus. The experiments expressing Pax3–Fkhr in Pax3 expression domains generally resulted in loss of viability during embryogenesis or soon after birth, or selection for low Pax3–Fkhr expression in surviving animals. The embryos demonstrated a variety of developmental abnormalities, some of which are similar to loss of Pax3 function seen in the Splotch mice while other findings suggest a gain of function phenotype. Examples of the latter include ectopic delamination, ectopic neuroprogenitor cell proliferation, and abnormal migration of myogenic precursors. At least some of these gain of function effects could be attributed to PAX3–FKHR expression inducing upregulation and ligand-independent signalling of the Met receptor [29]. Despite these gain of function effects and the finding that strains with low Pax3–Fkhr expression survived, no mice developed tumours, suggesting that additional collaborative events are required for PAX3–FKHR induced tumourigenesis.

2.5. Collaboration of other genetic alterations is required for PAX3–FKHR transformation

Studies of the oncogenic role of PAX3–FKHR in NIH3T3 cells revealed an inverse correlation of PAX3–FKHR expression level and transformation efficiency [54]. This inverse relationship is explained by the finding that high level PAX3–FKHR expression, which is comparable to that in ARMS cells, suppresses the growth of immortalised murine cell lines. The inability of these cell lines to tolerate the high PAX3–FKHR expression levels characteristic of ARMS cells suggests that additional genetic alterations are required for a cell to abrogate the growth suppressive effects of the gene fusion and thereby become tolerant. As candidate collaborating events, comparative genomic hybridisation studies have shown that gene amplification occurs frequently in ARMS but not ERMS cells [55,56]. In particular, the most frequently amplified chromosomal regions are 2p24 (involving MYCN) and 12q13-15 (involving MDM2 and CDK4) [55]. These alterations may result in overexpression of proteins that increase the tolerance of cells for PAX3–FKHR toxicity, thereby permitting PAX3–FKHR to exert its oncogenic effects.

The Pax3–Fkhr knock-in mouse models described above revealed that widespread early embryonic expression of Pax3–Fkhr in Pax3 expression domains is toxic whereas low level expression is tolerated and results in developmental abnormalities but does not cause tumours [29]. Several possibilities can be explored to further examine the potential of Pax3–Fkhr as an oncogene in this mouse model. First, additional genetic events may be necessary to permit the cells to tolerate the high level of Pax3–Fkhr expression. Alternatively, the gene fusion could be introduced into a smaller number of cells to avoid affecting entire lineages with its deleterious consequences. Finally, Pax3–Fkhr expression could be delayed until late in development. To explore this latter possibility, a mouse strain was generated with

a conditional Pax3–Fkhr knock-in allele activated in Myf6-expressing cells, which are predominantly terminally differentiated skeletal muscle cells. In these Pax3–Fkhr heterozygous mice, ARMS tumours developed in 1 of 228 mice at 12 months [8]. Because Ink4a/ARF and Trp53 mutations are common in malignant tumours, these mutations may cooperate with Pax3–Fkhr to cause tumour progression. When animals homozygous for INK4a/ARF knockout allele in combination with Pax3–Fkhr knock-in allele were generated, soft tissue tumours consistent with ARMS developed in 4 of 14 mice with a median age of onset of 2.4 months. Furthermore, to distinguish whether the INK4a/ARF protein effect was due to its interference with the pRB or the Trp53 pathway, mice with the Pax3–Fkhr knock-in and Trp53 knockout alleles were generated. Tumours with ARMS histology were found in these animals, suggesting the disruption of Trp53 function collaborated with the activation of Pax3–Fkhr function in ARMS tumourigenesis.

3. Ewing's family tumour

The Ewing's family of tumours (EFTs) include Ewing's sarcoma, peripheral primitive neuroectodermal tumour and Askin tumour [57]. These lesions constitute a spectrum of morphologic differentiation spanning from relatively undifferentiated to a readily detectable neural differentiation pattern. These highly malignant tumours occur mainly in soft tissue and bone of children, adolescents and young adults.

3.1. Chromosome translocation in EFT

The genetic hallmark of these tumours is the translocation, t(11;22)(q24;q12), resulting in the EWS–FLI1 fusion gene. This translocation accounts for 80–85% of the cases of EFT and a variant translocation t(21;22)(q22;q12) , which results in an EWS–ERG fusion, accounts for 5–10% of the cases. In addition, there are additional rare variant translocations in which EWS is fused to other members of the ETS family, such as ETV1, E1A, or FEV (Table 1) [58]. Finally, in another recently reported rare variant, FUS, a gene related to EWS, is joined to ERG by a t(16;21)(p11;q22) chromosomal translocation [59].

The wild-type genes involved in the 11;22 translocation are EWS on chromosome 22 and FLI1 on chromosome 11. The EWS coding region can be divided into two functional domains: the N-terminal domain shares homology with the large subunit of RNA polymerase II, and the C-terminal portion consists of an RNA binding domain and other regions homologous to other ribonucleoproteins [60,61]. EWS is ubiquitously expressed both in the normal and tumour cells, yet its function is not clear [62]. FLI1 along with ERG, ETV1, E1AF and FEV belongs to the ETS transcription factor family, which is characterised by transcription factors with structurally related ETS-type DNA binding domains that recognise a conserved DNA sequence [63]. FLI1 is organised with the DNA binding domain in its C-terminal region and a transcriptional activation domain in its N-terminal region.

As a result of the 11;22 translocation, the N-terminal region of EWS is fused in-frame to the C-terminal region of FLI1, producing a chimeric protein EWS–FLI1 containing the intact ETS-type DNA binding domain of FLI1 (Fig. 2). Although the fusion protein and FLI1 share the same DNA binding specificity [64], EWS–FLI1 acts as a stronger transcription activator than FLI1 [65]. These and other studies thus indicate that a region within the N-terminus of EWS is capable of acting as a transcriptional activation domain. The aberrant activity of EWS–FLI1 may result in more efficient activation or suppression of FLI1 target genes, thus contributing to EFT oncogenesis.

3.2. EWS–ETS is oncogenic

There is compelling evidence that the EWS–FLI1 fusion protein has oncogenic activity. When transduced into NIH3T3 fibroblasts, the EWS–FLI1 expressing cells formed colonies in soft agar, foci in cell culture, and tumours in nude mice, and were capable of serum-independent growth in bulk culture. In comparable experiments, FLI1 did not induce these activities, indicating that the EWS–FLI1 fusion gains transforming and tumourigenic activity [3,66]. Furthermore, mutation of either the EWS or FLI1 domain abolished transforming activity, indicating that both domains are required for its oncogenic effect [3].

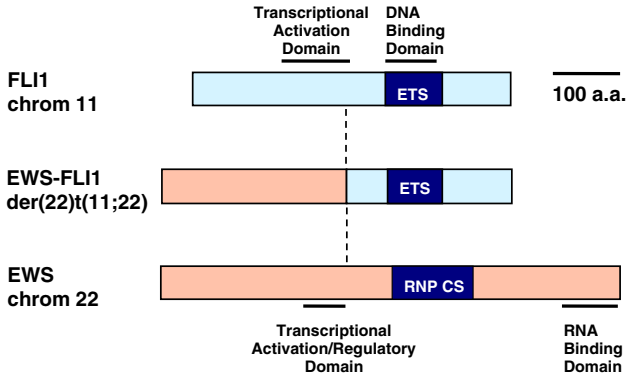

Fig. 2. Fusion protein generated by the 11;22 chromosome translocation in Ewing's sarcoma. The wild-type and fusion proteins are shown. The ETS DNA binding domain and a sequence with homology to other ribonucleoproteins (RNP CS) are shown as solid boxes and the overall DNA binding and transcriptional activation domains are shown as horizontal lines. The vertical line indicates the fusion point.

The complementary studies using specific repressors, antisense and RNA interference (RNAi) to abrogate EWS–FLI1 expression also revealed reduction of cell growth and increasing apoptosis, decreased invasion, loss of anchorage-independent colony formation, and loss of tumourigenic phenotype in EWS–FLI1 transfected cells and EFT cell lines [66–71]. These studies demonstrate that EWS–FLI1 is required for oncogenic characteristics and establish the potential for therapeutic approaches directed against the fusion product for treatment of this cancer. A variant EWS–ETS fusion product, EWS–ETV1, also revealed similar oncogenic properties as EWS–FLI1 in tumour formation in immunodeficient mice, suggesting that they share common biological pathways in tumourigenesis despite the structure differences in ETS domains [12].

3.3. EWS–ETS effects on cell cycle regulatory proteins

Several lines of evidence demonstrated that the EWS–FLI1 fusion affects cell proliferation by altering the expression of key regulators of the cell cycle. In several studies, transfection of EFT cell lines with antisense reagents directed against EWS–FLI1 resulted in a loss of endogenous EWS–FLI1 protein expression and suppression of cell growth [67,68,72]. One study examined in detail the downstream effects of this growth suppression [73]. Cell cycle was arrested at G0/G1 phase, and expression of numerous G1 regulatory genes was altered in the treated cells in association with downregulation of EWS–FLI1 protein expression. In particular, cyclin D1 and E, positive regulators in G1–S transition, were downregulated and cyclin-dependent kinase inhibitors p21 and p27, negative regulators in cell cycle were induced. Furthermore, the DNA binding and reporter studies support the premise that CDKN1A (p21) is a direct target of this fusion protein [74]. These data indicate that alteration of G1–S regulatory genes caused by antisense-mediated suppression of EWS–FLI1 expression results in G1 growth arrest in EFT cell lines and suggest that EWS–FLI1 is normally a growth-promoting gene in this cancer.

As a complementary study to the antisense experiments, the expression of cell cycle regulators following expression of EWS–FLI1 in various cell lines has been investigated. One study of EFT cells and EWS–FLI1-transfected HeLa cells revealed overexpression of cyclin D1 in EWS–FLI1 expressing cells. In addition, this study also showed that Myc, an oncogene promoting cell cycle progression, was upregulated; and p57, an inhibitor of the cyclin-CDK complex, was repressed by the EWS–FLI1 protein. In a second study, initial expression analysis of 7 EFT cases and 13 RMS cases revealed overexpression of cyclin D1 in all EFT cases, whereas higher levels of cyclin D2, D3 and E1 were found in RMS [75]. Introduction of an EWS–FLI1 expression construct into an ERMS cell line increased cyclin D1 but decreased cyclin D3 expression. This reversal of the cyclin D expression pattern by EWS–FLI1 indicates that this fusion protein mediates the expression of D-type cyclins independent of cellular context.

Additional methodologies, such as chromatin immunoprecipitation, provided evidence for the transcriptional relationship between EWS–ETS fusion proteins and the genes encoding some of these cell cycle regulators. EWS–FLI1 was found to interact with MYC and CCND1 (cyclin D1) promoters directly [11]. Of note, additional evidence suggests that DNA binding by EWS–FLI1 may not be required at the CCND1 promoter but rather the fusion protein may interact with the Sp1 protein at Sp1 DNA binding sites [76]. The Id2 protein, which promotes cell proliferation and inhibits cell differentiation via binding to basic HLH transcription factors, was also shown to be a direct target of EWS–FLI1 [77]. Taken together, aberrant transcription activity of EWS–ETS fusion protein causes deregulation of cell cycle regulators, disturbs normal cell cycle control and thereby contributes to EFT tumourigenesis.

3.4. EWS–ETS disrupts signal transduction pathways

Based on previous data indicating that ETS transcription factors regulate expression of the TGF-β type II receptor gene (TGFBR2), this receptor gene was examined as a potential target of EWS–FLI1 [78]. EFT cells expressing the EWS–FLI1 fusion protein demonstrated low or undetectable expression of TGFBR2 and reduced sensitivity to TGF-β. Reporter transfection analysis showed that FLI1 induced TGFBR2 promoter activity, whereas EWS–FLI1 suppressed activity. Introduction of EWS–FLI1 into fusion-negative cells reduced expression of TGFBR2, and antisense targeted to EWS–FLI1 in an EWS–FLI1 positive cell line restored this expression. Furthermore, overexpression of TGFBR2 in an EFT cell line restores TGF-β sensitivity and inhibited tumourigenicity in immunodeficient mice. Moreover, study of the less common variants, EWS–ERG and EWS–ETV1, also revealed downregulation of TGFBR2 expression and reduced TGF-β sensitivity in NIH3T3 cells, whereas the corresponding wild-type ETS proteins induced TGFBR2 promoter activity [79]. Therefore, repression of TGFBR2 expression by EWS–FLI1 fusion proteins appears to plays a role in EFT oncogenesis.

Additional signal transduction pathways altered by the EWS–FLI1 protein were identified using an RNA interference strategy to target EWS–FLI1 in EFT cells [71]. As the expression of EWS–FLI1 was inhibited, cell growth was suppressed and apoptosis was increased. Expression profiling of these siRNA-treated cells revealed that insulin-like growth factor binding protein 3 (IGFBP3), a regulator of the IGF signal transduction

pathway, which plays a key role in cell proliferation and apoptosis, was induced dramatically. Parallel studies also demonstrated that IGFBP3 expression is low in 12 EFT cell lines, suggesting that EWS–FLI1 normally represses IGFBP3 expression. Further studies indicate that IGFBP3 is a direct target of the EWS–FLI1 fusion protein and that binding of the fusion protein to the IGFB3 promoter results in repression of IGFBP3 expression. Additional siRNA studies have provided evidence that IGFBP3 repression is critical for tumour cell viability in EWS–FLI1-expressing cells.

As further indication of the involvement of the IGF pathway, the insulin-like growth factor-I receptor (IGF1R) was reported to play a role in EWS–FLI1 induced transformation in fibroblasts [80]. As indicated by the soft agar assay, introduction of EWS–FLI1 is capable of transforming fibroblasts expressing endogenous IGF1R, but not IGF1R knockout cells. Therefore, IGF1R appears to be required for EWS–FLI1 induced cellular transformation. Moreover, following stimulation with the ligand IGF-1, EWS–FLI1 expressing cells revealed greater phosphorylation of the target insulin receptor substrate-1 compared to control fibroblasts, indicating that the fusion protein alters the IGF1R signalling pathway.

3.5. EWS–ETS affects telomerase in EFT

Like most tumour cells, telomerase activity is detected in EFT cells, but not in somatic human cells [81,82]. Telomerase activity was increased, and hTERT mRNA, encoding the catalytic subunit of telomerase, was elevated in NIH3T3 cells expressing the EWS–FLI1 or EWS–E1AF fusion protein. As further proof that hTERT is a direct target, reporter transfection analysis showed that the fusion proteins increased activity of reporter constructs containing the hTERT promoter region. Furthermore, silencing EWS–FLI1 by siRNA decreased hTERT mRNA level and telomerase activity in an EFT cell line. This data is consistent with the premise that hTERT is a downstream target of EWS–ETS oncogenesis [82].

3.6. EWS–FLI1 affects cell differentiation

Most cases of EFT arise in bone, whereas the majority of other cases occur in soft tissue sites, suggesting that the target cell may involve multilineage potential. The murine myoblast C2C12 cell line, which is able to differentiate into muscle, bone or fat in defined conditions, was used as a model system to study biologic function of the ETS-FLI1 fusion protein [83]. EWS–FLI1-expressing C2C12 cells did not undergo muscle differentiation in low serum medium in which C2C12 cells normally formed multinucleated myotubes. This block in myogenic differentiation could not be rescued by overexpression of the myogenic transcription factors MyoD or myogenin and appears to be the result of EWS–FLI1-induced inhibition of the transcriptional activity of these myogenic transcription factors. Since C2C12 cells can potentially be induced into an osteoblastic differentiation pathway, EWS–FLI1-expressing cells were examined for bone lineage markers. Though alkaline phosphatase, a common marker for bone lineage, was found in EWS–FLI1-expressing cells, as well as in EFT cell lines, but not in control C2C12 cells, cells expressing EWS–FLI1 did not express other markers consistent with bone differentiation. These findings indicate that EWS–FLI1 expression in a multipotential cell type will inhibit some differentiation pathways, such as myogenesis, and alter other pathways, such as osteogenesis, thereby providing clues to the pattern of marker expression in EFT.

Further analysis of the interaction of the EWS–FLI1 protein with differentiation pathways provided another example in which this fusion protein switched off one pathway and potentially turned on another. In this case, the fusion protein was expressed in cell lines derived from neuroblastoma, a pediatric cancer derived from the sympathetic nervous system [84]. Following transduction into cell lines, EWS–FLI1 suppressed neuroblastoma-specific markers, such as MYCN, chromogranin A, and ShcC. In addition to these negative effects, the EWS–FLI1-expressing neuroblastoma cells up-regulated markers characteristic of EFT, including MIC2, MYC and ShcA. In addition, microarray analysis revealed that the gene expression patterns in EWS–FLI1-transduced neuroblastoma cells were similar to the pattern in EFT cell lines and different from the patterns in neuroblastoma cells. Therefore, EWS–FLI1 significantly altered the overall differentiation program of these cells.

3.7. EWS–FLI1 changes the status of p53

One study assessed the relationship of the EWS–FLI1 fusion protein and the p53 tumour suppressor. In particular, this study compared features of p53 before and after introduction of EWS–FLI1 into a neuroblastoma cell line [85]. p53 protein was localised in the nuclei of EWS–FLI1 expressing cells in contrast to the cytoplasmic localisation in parental neuroblastoma cells. Moreover, p53 protein expression is increased in EWS–FLI1 expressing cells, which is attributed to increased protein stability. The mechanistic basis for this increased stability is hypothesised to be the result of phosphorylation of p53 on Ser-15, which blocks MDM2 binding [86]. Additional analysis demonstrated that p53 is not functional in EWS–FLI1 expressing cells, as assessed by response to genotoxic stress, whereas this response is normal in the parental cells. Taken together, these findings indicate that the EWS–FLI1 oncoprotein abrogates the p53 pathway, thus contributing to tumourigenesis.

3.8. EWS–FLI1 is cooperative with other genetic alterations for its oncogenic features

Though its transforming capability is well documented in NIH3T3 cells, EWS–FLI1 can not transform murine or human primary cells, but instead causes cell growth arrest or cell death [87,88]. Based on the premise that cancer is the result of the accumulation of multiple mutations that alter multiple pathways, EWS–FLI1 is hypothesised to be essential but not sufficient for the malignant phenotype. Therefore, additional mutations are proposed to be necessary to collaborate with EWS–FLI1 during tumourigenesis. Approximately 30% of EFT show homozygous loss of p16, which regulates cell cycle progression, and thus the effects of transduction of EWS–FLI1 into p16-deficient mouse embryonic fibroblasts were examined [87]. The degree of growth arrest was attenuated in the p16-deficient cells expressing EWS–FLI1 compared to that in wild-type cells expressing the fusion gene. Similar growth kinetics and EWS–FLI1 tolerance were also observed in p53- and ARF-deficient mouse embryonic fibroblasts, indicating loss of these pathways at least partially rescue the cells from EWS–FLI1-induced cell death. However, these EWS–FLI1-expressing p16-, p53- and ARF-deficient cells had no or only limited tumourigenic potential in immunodeficient mice, suggesting that additional genetic alterations are required for EWS–FLI1 induced tumourigenesis.

To study the effect of EWS–FLI1 on a primary human cell, a conditional EWS–FLI1 expression system was developed in telomerase-immortalised human primary fibroblasts [88]. Expression of EWS–FLI1 again caused cell growth arrest in this system. Gene expression profiling of the EWS–FLI1-expressing cells identified overexpression of p53, and this increased p53 was shown to be due to transcriptional upregulation. To explore if EWS–FLI1-induced p53 expression is critical to the growth arrest following EWS–FLI1 expression, human papillomavirus HPV16 E6 (an inhibitor of p53 function) was introduced into EWS–FLI1-expressing cells. The growth arrest induced by EWS–FLI1 was diminished in the E6-expressing cells, but the abrogation of this p53 effect plus EWS–FLI1 expression is not sufficient to transform the fibroblasts, as assessed by the soft agar assay of anchorage independence.

4. Other sarcomas

4.1. Clear cell sarcoma

In clear cell sarcoma, the t(12;22)(q13;12) translocation fuses the EWS gene on chromosome 22 to the ATF1 gene on chromosome 12, resulting in expression of an EWS–ATF1 fusion product [89]. ATF1 is a member of the CREB transcription factor family, and contains a basic leucine zipper DNA binding and dimerisation domain in its C-terminal end and a protein kinase A phosphorylation control site in its N-terminal end. This latter phosphorylation site allows for regulation of this transcription factor by cAMP. In the fusion protein, the N-terminal end of EWS is fused to the C-terminal region of ATF1, including its DNA binding and dimerisation region. The loss of its phosphorylation site releases this protein from cAMP regulation and converts it into a constitutive transcriptional activator dependent on the activation domain within EWS [90]. EWS–ATF1 was shown to be a transcriptional activator on several promoters in cell culture systems. Using chromatin immunoprecipitation, several upregulated targets such as ARNT2 were identified and then validated by DNA binding and reporter studies [91]. In addition, one region was isolated for which EWS–ATF1 suppressed reporter activity; this region was derived from the POSH gene, which is proapoptotic, and EWS–ATF1 downregulates endogenous expression of this gene. In another mechanism of EWS–ATF1 action, based on the ability of wild-type ATF1 to bind to the coactivator CBP, the fusion also interacts with CBP and represses p53/CBP-mediated transactivation by sequestration of CBP [9]. Finally, as a complementary study to investigate the contribution of EWS–ATF1 to clear cell sarcoma biology, an anti-ATF-1 antibody fragment was expressed in a clear cell sarcoma cell line and resulted in cell death, demonstrating that EWS–ATF1 is essential to maintain tumour cell viability [92].

4.2. Desmoplastic small round cell tumour

The t(11;22)(p13;q12) translocation in desmoplastic small round cell tumour (DSRCT) generates a fusion protein consisting of the N-terminal portion of EWS joined to the C-terminal portion of the WT1 protein [93,94]. WT1 is best known as a transcription factor with tumour suppressor function involved in a subset of Wilms' tumour. It has a DNA binding domain in it C-terminal end that consists of four zinc finger motifs, three of which are contained in the region fused to EWS in DSRCT. There is also alternative splicing in the C-terminal end of both EWS–WT1 and WT1, which includes or excludes a three amino acid sequence of lysine, threonine and serine (KTS) between zinc fingers 3 and 4. The ratio of the −KTS and +KTS isoforms of EWS–WT1 is approximately 1:2. The EWS–WT1(−KTS) isoform binds to a CG-rich site similar to the WT(−KTS) protein. By introducing EWS–WT1(−KTS) into cell lines, such as osteosarcoma cells, this fusion protein has been found to upregulate a number of genes relevant to tumourigenesis, including the interleukin-2 receptor beta chain (IL2RB), the IGF-1 receptor (IGF1R), BAIAP3 (a protein implicated in

regulated exocytosis), and platelet derived growth factor A chain (PDGFA) [95–98]. Corresponding DNA binding sites were identified in the promoter regions of these genes and appropriate expression patterns were found in DSCRT tumour specimens. In contrast, there was no known recognition site for wild-type WT1(+KTS) and it was hypothesised that this protein had a role in mRNA processing. To determine if the abundant EWS–WT1(+KTS) had any role in gene expression, a cDNA subtractive hybridisation strategy was used and identified the gene LRRC15 in osteosarcoma cells with EWS–WT1(+KTS) inducible expression [99]. LRRC15, which is expressed in primary DSCRT cells, belongs to a leucine-rich repeat family, which encodes transmembrane proteins involved in cell–cell interaction [100]. Studies revealed that the fusion protein binds to a specific sequence upstream of LRRC15 gene, and this sequence is transactivated by EWS–WT1(+KTS). High level expression of LRRC15 was found in highly invasive breast carcinoma cells and RNAi treatment reduced the LRRC15 level and decreased invasion in a matrigel assay. These findings indicate that both isoforms of EWS–WT1 function as transcription factors with oncogenic targets that contribute to DSRCT tumourigenesis.

4.3. Myxoid and round cell liposarcoma

In myxoid and round cell liposarcoma, the t(12;16)(q13;p11) results in a fusion between the CHOP gene on chromosome 12 and FUS gene on chromosome 16 [101–103]. This gene fusion produces a chimeric protein containing the N-terminal portion of FUS fused in-frame with the entire CHOP coding region. FUS is a member of the RNA binding protein family that also includes EWS, and similarly has an RNA binding domain in its C-terminal region that is not present in the fusion protein. The CHOP gene encodes a member of the C/EBP transcription factor family, which is involved through dimer formation in the regulation of adipocyte differentiation [104]. In a murine preadipocytic cell culture system, introduction of the FUS–CHOP fusion inhibits adipocytic differentiation induced by insulin and thiazolidinedione [105]. Additional analysis indicated that, in the presence of the FUS–CHOP fusion, the CEBP family member CEBP alpha was either not upregulated in these cells or, when expressed, was not functionally active. Finally, this fusion protein also scored positive in standard assays of transforming activity including focus formation, soft agar colony formation, and tumour formation in immunodeficient mice. To further study the oncogenic effect of FUS–CHOP, a transgenic mouse was produced which expressed the fusion gene from the elongation factor 1α promoter [106]. Despite widespread expression of the transgene, the transgenic mice developed only one type of tumour, liposarcoma. In contrast, transgenic mice with only the CHOP gene failed to develop any tumour and had normal adipose tissue [107]. These data indicate a direct link between the fusion gene and liposarcoma development.

5. Prospects for therapy

As a better understanding is achieved of the oncogenic roles of these fusion proteins in sarcoma tumourigenesis, the potential will exist to design more specific therapeutic approaches than the currently available chemotherapy and radiation therapy. Silencing the specific fusion gene and/or protein, which is considered fundamental for growth and other activities of the tumour, is a promising strategy. Second, blocking downstream targets of these fusion transcription factors may inhibit strategic outputs necessary for their ultimate phenotypic activity. Finally, therapeutic approaches may be targeted to interrupt collaborating events necessary for fusion protein function or tolerance. Combination therapy to repress more than one of these areas may provide the most powerful potential to treat these sarcomas.

5.1. Targeting fusion protein expression

Based on the oncogenic function of these proteins, specific inhibition of fusion protein expression is an obvious therapeutic pursuit. A commonly used approach to repress fusion gene expression is introduction of an antisense oligonucleotide or expression construct producing antisense RNA (complementary to the target gene mRNA) to block expression inside the tumour cells. A successful example is the use of antisense oligonucleotides directed against EWS–FLI1 delivered in nanoparticles, which inhibits EWS–FLI1 expression in xenografted tumour and inhibits tumour growth [108]. An example in cell culture is application of antisense oligonucleotides directed against PAX3–FKHR to an ARMS cell line, resulting in apoptosis [39].

The recent discovery of RNA interference (RNAi) provides another powerful tool to silence expression of a specific gene [109]. This technology has been adapted to silence targets of choice with synthesised double-stranded RNA, and thus the potential exists to target endogenous fusion genes generated from chromosome translocations in sarcoma cells. There are several successful examples in which this technology has been applied to EFT. In one study, synthetic siRNA targeting the FLI1 portion of EWS–FLI1 was introduced into an EFT cell line, and resulted in suppression of EWS–FLI1 expression. The downregulation of EWS–FLI1 was correlated with decreased cell proliferation, increased apoptosis and abrogated invasion in an *in vitro* matrigel assay [70]. Another study used the siRNA approach but targeted the junctional sequence between EWS and FLI1 and revealed loss of EWS–FLI1 induced

cell cycle arrest and apoptosis in a different EFT cell line [71].

5.2. Inhibition of fusion protein downstream targets

As an aberrant transcription factor, these fusion proteins exert their oncogenic effects through deregulation of downstream targets, and therefore manipulation of these target proteins provides an avenue for attenuating or abrogating oncogenic function. As described earlier, the TGFBR2 gene is directly downregulated by EWS–FLI1, resulting in insensitivity of EFT cell lines to TGF-β treatment. Introduction of a TGFBR2 expression construct into EFT cell lines restored TGF-β signalling and suppressed tumourigenic potential of the cells when inoculated into immunodeficient nice [78]. In other previously discussed findings, EWS–FLI1 downregulates expression of IGFBP3, a regulator of IGF signalling important for cell proliferation and survival. To demonstrate the importance of this regulatory mechanism, introduction of IGFBP3 into an EFT cell line resulted in induction of apoptosis [71]. Finally, overexpression of MET is found in ARMS as well as other cancers, and provides a promising molecular target. As a first step in the development of targeted therapeutics against this protein, antibodies against MET or HGF/SF have been developed for inhibition of the MET signalling pathway [41].

Another potential strategy takes advantage of metabolic pathways that are connected to the presence of a target protein and the use of these pathways to convert a prodrug to a toxic form. In the case of EFT, the EWS–ETS fusion protein was found to upregulate expression of the metabolic enzyme uridine phosphorylase in EWS–ETS transduced cells. This enzyme can convert 5′-deoxy-5′fluorouridine (5dFUrd) to a cytotoxic form (5-FUra), that will inhibit cell growth [110]. As proof of this principle, EWS–FLI1 expressing NIH3T3 cells displayed higher sensitivity to 5dFUrd than control cells, though both cells were sensitive to 5-FUra. As further evidence that this target gene provides a potentially useful drug against tumour growth, 5dFUrd delayed tumour formation in a xenograft tumour model in which EWS–FLI1 expressing or vector only NIH3T3 cells were injected into immunodeficient mice.

5.3. Blocking collaborative factors

Several lines of evidence strongly support the premise that additional genetic alterations are required for these fusion proteins to fully achieve their oncogenic potential. Blocking these collaborative factors would thus provide another potential target for therapeutic intervention. In ARMS, possible candidates for these collaborating factors are the frequent amplification events on 2p24 (potentially involving MYCN) and on 12q13-15 (potentially involving CDK4 and MDM2). For MYCN, both an antisense strategy which downregulates MYCN expression and small-molecule antagonists that inhibit MYC–MAX dimerisation, essential for MYC function, are promising tool for cancer therapy [111], and may have relevance in the context of ARMS. In EFT, loss of p16 is frequently found, and as discussed previously, the ability of p16 mutation to attenuate the growth suppressive influence of EWS–FLI1, suggests a cooperative role for p16 mutation in the mechanism of EWS–FLI1-induced tumourigenesis [87]. Restoring p16 function by adenoviral transduction has been performed experimentally and shown to induce cell cycle arrest and increase apoptosis in p16-deficient tumour cell lines [112]. In another study, injection of a p16-derived synthetic peptide coupled with a peptide vector into tumour-bearing mice resulted in inhibition of tumour growth in the mice with evidence of apoptosis [113].

Alterations of the retinoblastoma (Rb) pathway occur in most of human malignancies, as evidenced in these tumours by CDK4 amplification and p16 mutations. As a tumour suppressor, pRb regulates cell cycle progression through G1 by binding to and regulating transcription factors such as those of the E2F family. Phosphorylation of pRb by cyclin-dependent kinases (CDK) disables its function, and identification of small molecules that block the phosphorylation of pRb in tumours would provide possible reagents for cancer treatment. A high-throughput screen was developed to identify small molecule inhibitors that block pRb phosphorylation [114]. Modulating CDK activity is another way to prevent phosphorylation of pRb for therapeutic intervention. For example, the small molecule flavopiridol binds to the ATP binding site of CDK2 thus inhibiting its activity, and this compound also depletes cyclin D1, a cofactor of CDK activation [115]. Flavopiridol treated cells reveal a cell cycle block, promoting differentiation, inducing apoptosis and inhibition of angiogenesis.

Inactivation of p53, a tumour suppressor, is also common in cancers, including many of these sarcomas. Gene therapy to reintroduce a wild-type p53 gene or restore its function in cancer cells would be a possible therapeutic tool. In one study, the p53 gene was transferred into cell lines with either a p53 deletion or p53 mutation using glucosylated polyethylenimine and photochemical internalisation, resulting in induction of apoptosis [116], thus showing the feasibility of p53 gene therapy to treat cancers with p53 mutations. Some tumours retain wild-type p53, but the function is inactivated by binding to MDM2, leading to its degradation. Small molecules that disrupt the interaction of MDM2 and p53 and stabilise p53 have been developed as a potential strategy for these scenarios [117]. A successful example is the small molecule RITA, which binds to p53, blocks the interaction of p53 and MDM2, and restores p53 function in tumours [118].

6. Conclusions

This review summarises the molecular and cellular biology of the fusion proteins resulting from chromosome translocations in sarcomas. Many of these chimeric proteins are transcription factors with aberrant transactivation capability compared to their wild-type counterparts. The aberrant transcription factors modulate the expression of a constellation of target genes, altering multiple cellular pathways, and ultimately changing the phenotypic properties of the cell to contribute to the tumourigenic pathway. Although the fusion proteins play important roles in oncogenesis, additional collaborative genetic alterations are required to transform cells in culture or form tumours in animals. As more oncogenic properties of the fusion protein and cooperative events are elucidated and refined, therapeutic strategies can be further developed to interrupt these tumourigenic process. Silencing the specific fusion gene that plays fundamental roles for the corresponding tumour, blocking targets of fusion proteins, and repressing the cooperating events are all promising strategies that need to be further explored. Ultimately, a combination of these approaches may provide a powerful new ability to treat these fusion-positive sarcomas.

Conflict of interest statement

None declared.

Acknowledgements

This work was supported in part by NIH Grants CA64202 and CA89461; the Sarcoma Foundation of America; and the Alveolar Rhabdomyosarcoma Research Fund.

References

1. Borden EC, Baker LH, Bell RS, *et al*. Soft tissue sarcomas of adults: state of the translational science. *Clin Cancer Res* 2003, **9**(6), 1941–1956.
2. Scheidler S, Fredericks WJ, Rauscher III FJ, *et al*. The hybrid PAX3–FKHR fusion protein of alveolar rhabdomyosarcoma transforms fibroblasts in culture. *Proc Natl Acad Sci USA* 1996, **93**(18), 9805–9809.
3. May WA, Gishizky ML, Lessnick SL, *et al*. Ewing sarcoma 11;22 translocation produces a chimeric transcription factor that requires the DNA-binding domain encoded by FLI1 for transformation. *Proc Natl Acad Sci USA* 1993, **90**(12), 5752–5756.
4. Nagai M, Tanaka S, Tsuda M, *et al*. Analysis of transforming activity of human synovial sarcoma-associated chimeric protein SYT-SSX1 bound to chromatin remodeling factor hBRM/hSNF2 alpha. *Proc Natl Acad Sci USA* 2001, **98**(7), 3843–3848.
5. Schwarzbach MH, Koesters R, Germann A, *et al*. Comparable transforming capacities and differential gene expression patterns of variant FUS/CHOP fusion transcripts derived from soft tissue liposarcomas. *Oncogene* 2004, **23**(40), 6798–6805.
6. Arvand A, Welford SM, Teitell MA, *et al*. The COOH-terminal domain of FLI-1 is necessary for full tumourigenesis and transcriptional modulation by EWS/FLI-1. *Cancer Res* 2001, **61**(13), 5311–5317.
7. Perez-Mancera PA, Perez-Losada J, Sanchez-Martin M, *et al*. Expression of the FUS domain restores liposarcoma development in CHOP transgenic mice. *Oncogene* 2002, **21**(11), 1679–1684.
8. Keller C, Arenkiel BR, Coffin CM, *et al*. Alveolar rhabdomyosarcomas in conditional Pax3:Fkhr mice: cooperativity of Ink4a/ARF and Trp53 loss of function. *Genes Dev* 2004, **18**(21), 2614–2626.
9. Fujimura Y, Siddique H, Lee L, *et al*. EWS–ATF-1 chimeric protein in soft tissue clear cell sarcoma associates with CREB-binding protein and interferes with p53-mediated trans-activation function. *Oncogene* 2001, **20**(46), 6653–6659.
10. Knoop LL, Baker SJ. The splicing factor U1C represses EWS/FLI-mediated transactivation. *J Biol Chem* 2000, **275**(32), 24865–24871.
11. Fukuma M, Okita H, Hata J, *et al*. Upregulation of Id2, an oncogenic helix–loop–helix protein, is mediated by the chimeric EWS/ets protein in Ewing sarcoma. *Oncogene* 2003, **22**(1), 1–9.
12. Thompson AD, Teitell MA, Arvand A, *et al*. Divergent Ewing's sarcoma EWS/ETS fusions confer a common tumourigenic phenotype on NIH3T3 cells. *Oncogene* 1999, **18**(40), 5506–5513.
13. Rangarajan A, Hong SJ, Gifford A, *et al*. Species- and cell type-specific requirements for cellular transformation. *Cancer Cell* 2004, **6**(2), 171–183.
14. Maitra A, Roberts H, Weinberg AG, *et al*. Aberrant expression of tumour suppressor proteins in the Ewing family of tumours. *Arch Pathol Lab Med* 2001, **125**(9), 1207–1212.
15. Dias P, Kumar P, Marsden HB, *et al*. N-myc gene is amplified in alveolar rhabdomyosarcomas (RMS) but not in embryonal RMS. *Int J Cancer* 1990, **45**(4), 593–596.
16. Xia SJ, Pressey JG, Barr FG. Molecular pathogenesis of rhabdomyosarcoma. *Cancer Biol Ther* 2002, **1**(2), 97–104.
17. Barr FG. Molecular genetics and pathogenesis of rhabdomyosarcoma. *J Pediatr Hematol Oncol* 1997, **19**(6), 483–491.
18. Barr FG, Galili N, Holick J, *et al*. Rearrangement of the PAX3 paired box gene in the paediatric solid tumour alveolar rhabdomyosarcoma. *Nat Genet* 1993, **3**(2), 113–117.
19. Galili N, Davis RJ, Fredericks WJ, *et al*. Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. *Nat Genet* 1993, **5**(3), 230–235.
20. Davis RJ, D'Cruz CM, Lovell MA, *et al*. Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. *Cancer Res* 1994, **54**(11), 2869–2872.
21. Tremblay P, Gruss P. Pax: genes for mice and men. *Pharmacol Ther* 1994, **61**(1–2), 205–226.
22. Mansouri A. The role of Pax3 and Pax7 in development and cancer. *Crit Rev Oncog* 1998, **9**(2), 141–149.
23. Tassabehji M, Read AP, Newton VE, *et al*. Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene. *Nature* 1992, **355**(6361), 635–636.
24. Chalepakis G, Goulding M, Read A, *et al*. Molecular basis of splotch and Waardenburg Pax-3 mutations. *Proc Natl Acad Sci USA* 1994, **91**(9), 3685–3689.
25. Ridgeway AG, Skerjanc IS. Pax3 is essential for skeletal myogenesis and the expression of six1 and Eya2. *J Biol Chem* 2001, **276**, 19033–19039.
26. Seale P, Sabourin LA, Girgis-Gabardo A, *et al*. Pax7 is required for the specification of myogenic satellite cells. *Cell* 2000, **102**(6), 777–786.
27. Oustanina S, Hause G, Braun T. Pax7 directs postnatal renewal and propagation of myogenic satellite cells but not their specification. *EMBO J* 2004, **23**(16), 3430–3439.

28. Mansouri A, Stoykova A, Torres M, *et al*. Dysgenesis of cephalic neural crest derivatives in Pax7−/− mutant mice. *Development* 1996, **122**(3), 831–838.
29. Relaix F, Polimeni M, Rocancourt D, *et al*. The transcriptional activator PAX3–FKHR rescues the defects of Pax3 mutant mice but induces a myogenic gain-of-function phenotype with ligand-independent activation of Met signalling in vivo. *Genes Dev* 2003, **17**(23), 2950–2965.
30. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* 2004, **117**(4), 421–426.
31. Bois PR, Grosveld GC. FKHR (FOXO1a) is required for myotube fusion of primary mouse myoblasts. *EMBO J* 2003, **22**(5), 1147–1157.
32. Davis RJ, Barr FG. Fusion genes resulting from alternative chromosomal translocations are overexpressed by gene-specific mechanisms in alveolar rhabdomyosarcoma. *Proc Natl Acad Sci USA* 1997, **94**(15), 8047–8051.
33. Bennicelli JL, Advani S, Schafer BW, *et al*. PAX3 and PAX7 exhibit conserved cis-acting transcription repression domains and utilize a common gain of function mechanism in alveolar rhabdomyosarcoma. *Oncogene* 1999, **18**(30), 4348–4356.
34. Bennicelli JL, Edwards RH, Barr FG. Mechanism for transcriptional gain of function resulting from chromosomal translocation in alveolar rhabdomyosarcoma. *Proc Natl Acad Sci USA* 1996, **93**(11), 5455–5459.
35. Fredericks WJ, Galili N, Mukhopadhyay S, *et al*. The PAX3–FKHR fusion protein created by the t(2;13) translocation in alveolar rhabdomyosarcomas is a more potent transcriptional activator than PAX3. *Mol Cell Biol* 1995, **15**(3), 1522–1535.
36. del Peso L, Gonzalez VM, Hernandez R, *et al*. Regulation of the forkhead transcription factor FKHR, but not the PAX3– FKHR fusion protein, by the serine/threonine kinase Akt. *Oncogene* 1999, **18**(51), 7328–7333.
37. Lam PY, Sublett JE, Hollenbach AD, *et al*. The oncogenic potential of the Pax3–FKHR fusion protein requires the Pax3 homeodomain recognition helix but not the Pax3 paired-box DNA binding domain. *Mol Cell Biol* 1999, **19**(1), 594–601.
38. Anderson J, Ramsay A, Gould S, *et al*. Pax3–fkhr induces morphological change and enhances cellular proliferation and invasion in rhabdomyosarcoma. *Am J Pathol* 2001, **159**(3), 1089–1096.
39. Bernasconi M, Remppis A, Fredericks WJ, *et al*. Induction of apoptosis in rhabdomyosarcoma cells through down-regulation of PAX proteins. *Proc Natl Acad Sci USA* 1996, **93**(23), 13164–136169.
40. Margue CM, Bernasconi M, Barr FG, *et al*. Transcriptional modulation of the anti-apoptotic protein BCL-XL by the paired box transcription factors PAX3 and PAX3/FKHR. *Oncogene* 2000, **19**(25), 2921–2929.
41. Birchmeier C, Birchmeier W, Gherardi E, *et al*. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003, **4**(12), 915–925.
42. Bladt F, Riethmacher D, Isenmann S, *et al*. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995, **376**(6543), 768–771.
43. Epstein JA, Shapiro DN, Cheng J, *et al*. Pax3 modulates expression of the c-Met receptor during limb muscle development. *Proc Natl Acad Sci USA* 1996, **93**(9), 4213–4218.
44. Ginsberg JP, Davis RJ, Bennicelli JL, *et al*. Up-regulation of MET but not neural cell adhesion molecule expression by the PAX3–FKHR fusion protein in alveolar rhabdomyosarcoma. *Cancer Res* 1998, **58**(16), 3542–3546.
45. Jankowski K, Kucia M, Wysoczynski M, *et al*. Both hepatocyte growth factor (HGF) and stromal-derived factor-1 regulate the metastatic behavior of human rhabdomyosarcoma cells, but only HGF enhances their resistance to radiochemotherapy. *Cancer Res* 2003, **63**(22), 7926–7935.
46. Moore MA. The role of chemoattraction in cancer metastases. *Bioessays* 2001, **23**(8), 674–676.
47. Murphy PM. Chemokines and the molecular basis of cancer metastasis. *N Engl J Med* 2001, **345**(11), 833–835.
48. Tomescu O, Xia SJ, Strezlecki D, *et al*. Inducible short-term and stable long-term cell culture systems reveal that the PAX3–FKHR fusion oncoprotein regulates CXCR4, PAX3, and PAX7 expression. *Lab Invest* 2004, **84**(8), 1060–1070.
49. Libura J, Drukala J, Majka M, *et al*. CXCR4–SDF-1 signalling is active in rhabdomyosarcoma cells and regulates locomotion, chemotaxis, and adhesion. *Blood* 2002, **100**(7), 2597–2606.
50. Epstein JA, Lam P, Jepeal L, *et al*. Pax3 inhibits myogenic differentiation of cultured myoblast cells. *J Biol Chem* 1995, **270**(20), 11719–11722.
51. Khan J, Bittner ML, Saal LH, *et al*. cDNA microarrays detect activation of a myogenic transcription program by the PAX3–FKHR fusion oncogene. *Proc Natl Acad Sci USA* 1999, **96**(23), 13264–13269.
52. Anderson MJ, Shelton GD, Cavenee WK, *et al*. Embryonic expression of the tumour-associated PAX3–FKHR fusion protein interferes with the developmental functions of Pax3. *Proc Natl Acad Sci USA* 2001, **98**(4), 1589–1594.
53. Lagutina I, Conway SJ, Sublett J, *et al*. Pax3–FKHR knock-in mice show developmental aberrations but do not develop tumours. *Mol Cell Biol* 2002, **22**(20), 7204–7216.
54. Xia SJ, Barr FG. Analysis of the transforming and growth suppressive activities of the PAX3–FKHR oncoprotein. *Oncogene* 2004, **23**(41), 6864–6871.
55. Gordon AT, Brinkschmidt C, Anderson J, *et al*. A novel and consistent amplicon at 13q31 associated with alveolar rhabdomyosarcoma. *Genes Chromosomes Cancer* 2000, **28**(2), 220–226.
56. Bridge JA, Liu J, Qualman SJ, *et al*. Genomic gains and losses are similar in genetic and histologic subsets of rhabdomyosarcoma, whereas amplification predominates in embryonal with anaplasia and alveolar subtypes. *Genes Chromosomes Cancer* 2002, **33**(3), 310–321.
57. West DC. Ewing sarcoma family of tumours. *Curr Opin Oncol* 2000, **12**(4), 323–329.
58. Kim J, Pelletier J. Molecular genetics of chromosome translocations involving EWS and related family members. *Physiol Genom* 1999, **1**(3), 127–138.
59. Shing DC, McMullan DJ, Roberts P, *et al*. FUS/ERG gene fusions in Ewing's tumours. *Cancer Res* 2003, **63**(15), 4568–4576.
60. Ohno T, Rao VN, Reddy ES. EWS/Fli-1 chimeric protein is a transcriptional activator. *Cancer Res* 1993, **53**(24), 5859–5863.
61. Zinszner H, Albalat R, Ron D. A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. *Genes Dev* 1994, **8**(21), 2513–2526.
62. Kovar H, Jug G, Hattinger C, *et al*. The EWS protein is dispensable for Ewing tumour growth. *Cancer Res* 2001, **61**(16), 5992–5997.
63. Wasylyk B, Hahn SL, Giovane A. The Ets family of transcription factors. *Eur J Biochem* 1993, **211**(1–2), 7–18.
64. Mao X, Miesfeldt S, Yang H, *et al*. The FLI-1 and chimeric EWS–FLI-1 oncoproteins display similar DNA binding specificities. *J Biol Chem* 1994, **269**(27), 18216–18222.
65. Bailly RA, Bosselut R, Zucman J, *et al*. DNA-binding and transcriptional activation properties of the EWS–FLI-1 fusion protein resulting from the t(11;22) translocation in Ewing sarcoma. *Mol Cell Biol* 1994, **14**(5), 3230–3241.
66. Chan D, Wilson TJ, Xu D, *et al*. Transformation induced by Ewing's sarcoma associated EWS/FLI-1 is suppressed by KRAB/FLI-1. *Br J Cancer* 2003, **88**(1), 137–145.
67. Kovar H, Aryee DN, Jug G, *et al*. EWS/FLI-1 antagonists induce growth inhibition of Ewing tumour cells in vitro. *Cell Growth Differ* 1996, **7**(4), 429–437.

68. Ouchida M, Ohno T, Fujimura Y, *et al*. Loss of tumourigenicity of Ewing's sarcoma cells expressing antisense RNA to EWS-fusion transcripts. *Oncogene* 1995, **11**(6), 1049–1054.
69. Lambert G, Bertrand JR, Fattal E, *et al*. EWS fli-1 antisense nanocapsules inhibits ewing sarcoma-related tumour in mice. *Biochem Biophys Res Commun* 2000, **279**(2), 401–406.
70. Chansky HA, Barahmand-Pour F, Mei Q, *et al*. Targeting of EWS/FLI-1 by RNA interference attenuates the tumour phenotype of Ewing's sarcoma cells in vitro. *J Orthop Res* 2004, **22**(4), 910–917.
71. Prieur A, Tirode F, Cohen P, *et al*. EWS/FLI-1 silencing and gene profiling of Ewing cells reveal downstream oncogenic pathways and a crucial role for repression of insulin-like growth factor binding protein 3. *Mol Cell Biol* 2004, **24**(16), 7275–7283.
72. Dohjima T, Ohno T, Banno Y, *et al*. Preferential down-regulation of phospholipase C-beta in Ewing's sarcoma cells transfected with antisense EWS–Fli-1. *Br J Cancer* 2000, **82**(1), 16–19.
73. Matsumoto Y, Tanaka K, Nakatani F, *et al*. Downregulation and forced expression of EWS–Fli1 fusion gene results in changes in the expression of G(1)regulatory genes. *Br J Cancer* 2001, **84**(6), 768–775.
74. Nakatani F, Tanaka K, Sakimura R, *et al*. Identification of p21WAF1/CIP1 as a direct target of EWS–Fli1 oncogenic fusion protein. *J Biol Chem* 2003, **278**(17), 15105–15115.
75. Zhang J, Hu S, Schofield DE, *et al*. Selective usage of D-Type cyclins by Ewing's tumours and rhabdomyosarcomas. *Cancer Res* 2004, **64**(17), 6026–6034.
76. Fuchs B, Inwards CY, Janknecht R. Vascular endothelial growth factor expression is up-regulated by EWS–ETS oncoproteins and Sp1 and may represent an independent predictor of survival in Ewing's sarcoma. *Clin Cancer Res* 2004, **10**(4), 1344–1353.
77. Nishimori H, Sasaki Y, Yoshida K, *et al*. The Id2 gene is a novel target of transcriptional activation by EWS–ETS fusion proteins in Ewing family tumours. *Oncogene* 2002, **21**(54), 8302–8309.
78. Hahm KB, Cho K, Lee C, *et al*. Repression of the gene encoding the TGF-beta type II receptor is a major target of the EWS–FLI1 oncoprotein. *Nat Genet* 1999, **23**(2), 222–227.
79. Im YH, Kim HT, Lee C, *et al*. EWS–FLI1, EWS–ERG, and EWS–ETV1 oncoproteins of Ewing tumour family all suppress transcription of transforming growth factor beta type II receptor gene. *Cancer Res* 2000, **60**(6), 1536–1540.
80. Toretsky JA, Kalebic T, Blakesley V, *et al*. The insulin-like growth factor-I receptor is required for EWS/FLI-1 transformation of fibroblasts. *J Biol Chem* 1997, **272**(49), 30822–30827.
81. Shay JW. Telomerase in human development and cancer. *J Cell Physiol* 1997, **173**(2), 266–270.
82. Takahashi A, Higashino F, Aoyagi M, *et al*. EWS/ETS fusions activate telomerase in Ewing's tumours. *Cancer Res* 2003, **63**(23), 8338–8344.
83. Eliazer S, Spencer J, Ye D, *et al*. Alteration of mesodermal cell differentiation by EWS/FLI-1, the oncogene implicated in Ewing's sarcoma. *Mol Cell Biol* 2003, **23**(2), 482–492.
84. Rorie CJ, Thomas VD, Chen P, *et al*. The Ews/Fli-1 fusion gene switches the differentiation program of neuroblastomas to Ewing sarcoma/peripheral primitive neuroectodermal tumours. *Cancer Res* 2004, **64**(4), 1266–1277.
85. Rorie CJ, Weissman BE. The Ews/Fli-1 fusion gene changes the status of p53 in neuroblastoma tumour cell lines. *Cancer Res* 2004, **64**(20), 7288–7295.
86. Shieh SY, Ikeda M, Taya Y, *et al*. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell* 1997, **91**(3), 325–334.
87. Deneen B, Denny CT. Loss of p16 pathways stabilizes EWS/FLI1 expression and complements EWS/FLI1 mediated transformation. *Oncogene* 2001, **20**(46), 6731–6741.
88. Lessnick SL, Dacwag CS, Golub TR. The Ewing's sarcoma oncoprotein EWS/FLI induces a p53-dependent growth arrest in primary human fibroblasts. *Cancer Cell* 2002, **1**(4), 393–401.
89. Zucman J, Delattre O, Desmaze C, *et al*. EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. *Nat Genet* 1993, **4**(4), 341–345.
90. Fujimura Y, Ohno T, Siddique H, *et al*. The EWS–ATF-1 gene involved in malignant melanoma of soft parts with t(12;22) chromosome translocation, encodes a constitutive transcriptional activator. *Oncogene* 1996, **12**(1), 159–167.
91. Jishage M, Fujino T, Yamazaki Y, *et al*. Identification of target genes for EWS/ATF-1 chimeric transcription factor. *Oncogene* 2003, **22**(1), 41–49.
92. Bosilevac JM, Olsen RJ, Bridge JA, *et al*. Tumour cell viability in clear cell sarcoma requires DNA binding activity of the EWS/ATF1 fusion protein. *J Biol Chem* 1999, **274**(49), 34811–34818.
93. Ladanyi M, Gerald W. Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumour. *Cancer Res* 1994, **54**(11), 2837–2840.
94. Gerald WL, Rosai J, Ladanyi M. Characterization of the genomic breakpoint and chimeric transcripts in the EWS–WT1 gene fusion of desmoplastic small round cell tumour. *Proc Natl Acad Sci USA* 1995, **92**(4), 1028–1032.
95. Wong JC, Lee SB, Bell MD, *et al*. Induction of the interleukin-2/15 receptor beta-chain by the EWS–WT1 translocation product. *Oncogene* 2002, **21**(13), 2009–2019.
96. Soldatenkov VA, Trofimova IN, Rouzaut A, *et al*. Differential regulation of the response to DNA damage in Ewing's sarcoma cells by ETS1 and EWS/FLI-1. *Oncogene* 2002, **21**(18), 2890–2895.
97. Palmer RE, Lee SB, Wong JC, *et al*. Induction of BAIAP3 by the EWS–WT1 chimeric fusion implicates regulated exocytosis in tumourigenesis. *Cancer Cell* 2002, **2**(6), 497–505.
98. Lee SB, Kolquist KA, Nichols K, *et al*. The EWS–WT1 translocation product induces PDGFA in desmoplastic small round-cell tumour. *Nat Genet* 1997, **17**(3), 309–313.
99. Reynolds PA, Smolen GA, Palmer RE, *et al*. Identification of a DNA-binding site and transcriptional target for the EWS–WT1(+KTS) oncoprotein. *Genes Dev* 2003, **17**(17), 2094–2107.
100. Kobe B, Deisenhofer J. Proteins with leucine-rich repeats. *Curr Opin Struct Biol* 1995, **5**(3), 409–416.
101. Knight JC, Renwick PJ, Cin PD, *et al*. Translocation t(12;16)(q13;p11) in myxoid liposarcoma and round cell liposarcoma: molecular and cytogenetic analysis. *Cancer Res* 1995, **55**(1), 24–27.
102. Kanoe H, Nakayama T, Hosaka T, *et al*. Characteristics of genomic breakpoints in TLS–CHOP translocations in liposarcomas suggest the involvement of Translin and topoisomerase II in the process of translocation. *Oncogene* 1999, **18**(3), 721–729.
103. Panagopoulos I, Mertens F, Isaksson M, *et al*. A novel FUS/CHOP chimera in myxoid liposarcoma. *Biochem Biophys Res Commun* 2000, **279**(3), 838–845.
104. Crozat A, Aman P, Mandahl N, *et al*. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature* 1993, **363**(6430), 640–644.
105. Kuroda M, Ishida T, Takanashi M, *et al*. Oncogenic transformation and inhibition of adipocytic conversion of preadipocytes by TLS/FUS–CHOP type II chimeric protein. *Am J Pathol* 1997, **151**(3), 735–744.
106. Perez-Losada J, Pintado B, Gutierrez-Adan A, *et al*. The chimeric FUS/TLS–CHOP fusion protein specifically induces liposarcomas in transgenic mice. *Oncogene* 2000, **19**(20), 2413–2422.
107. Perez-Losada J, Sanchez-Martin M, Rodriguez-Garcia MA, *et al*. Liposarcoma initiated by FUS/TLS–CHOP: the FUS/TLS domain plays a critical role in the pathogenesis of liposarcoma. *Oncogene* 2000, **19**(52), 6015–6022.

108. Maksimenko A, Lambert G, Bertrand JR, *et al*. Therapeutic potentialities of EWS–Fli-1 mRNA-targeted vectorized antisense oligonucleotides. *Ann N Y Acad Sci* 2003, **1002**, 72–77.
109. Hannon GJ. RNA interference. *Nature* 2002, **418**(6894), 244–251.
110. Deneen B, Hamidi H, Denny CT. Functional analysis of the EWS/ETS target gene uridine phosphorylase. *Cancer Res* 2003, **63**(14), 4268–4274.
111. Lu X, Pearson A, Lunec J. The MYCN oncoprotein as a drug development target. *Cancer Lett* 2003, **197**(1–2), 125–130.
112. Blasco F, Penuelas S, Cascallo M, *et al*. Expression profiles of a human pancreatic cancer cell line upon induction of apoptosis search for modulators in cancer therapy. *Oncology* 2004, **67**(3–4), 277–290.
113. Hosotani R, Miyamoto Y, Fujimoto K, *et al*. Trojan p16 peptide suppresses pancreatic cancer growth and prolongs survival in mice. *Clin Cancer Res* 2002, **8**(4), 1271–1276.
114. Barrie SE, Eno-Amooquaye E, Hardcastle A, *et al*. High-throughput screening for the identification of small-molecule inhibitors of retinoblastoma protein phosphorylation in cells. *Anal Biochem* 2003, **320**(1), 66–74.
115. Senderowicz AM. Small molecule modulators of cyclin-dependent kinases for cancer therapy. *Oncogene* 2000, **19**(56), 6600–6606.
116. Ndoye A, Merlin JL, Leroux A, *et al*. Enhanced gene transfer and cell death following p53 gene transfer using photochemical internalisation of glucosylated PEI–DNA complexes. *J Gene Med* 2004, **6**(8), 884–894.
117. Vassilev LT, Vu BT, Graves B, *et al*. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 2004, **303**(5659), 844–848.
118. Issaeva N, Bozko P, Enge M, *et al*. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumours. *Nat Med* 2004, **10**(12), 1321–1328.
119. Ladanyi M, Lui MY, Antonescu CR, *et al*. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. *Oncogene* 2001, **20**(1), 48–57.
120. Waters BL, Panagopoulos I, Allen EF. Genetic characterization of angiomatoid fibrous histiocytoma identifies fusion of the FUS and ATF-1 genes induced by a chromosomal translocation involving bands 12q13 and 16p11. *Cancer Genet Cytogenet* 2000, **121**(2), 109–116.
121. Simon MP, Pedeutour F, Sirvent N, *et al*. Deregulation of the platelet-derived growth factor B-chain gene via fusion with collagen gene COL1A1 in dermatofibrosarcoma protuberans and giant-cell fibroblastoma. *Nat Genet* 1997, **15**(1), 95–98.
122. Koontz JI, Soreng AL, Nucci M, *et al*. Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumours. *Proc Natl Acad Sci USA* 2001, **98**(11), 6348–6353.
123. Delattre O, Zucman J, Plougastel B, *et al*. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature* 1992, **359**(6391), 162–165.
124. Zucman J, Melot T, Desmaze C, *et al*. Combinatorial generation of variable fusion proteins in the Ewing family of tumours. *EMBO J* 1993, **12**(12), 4481–4487.
125. Jeon IS, Davis JN, Braun BS, *et al*. A variant Ewing's sarcoma translocation (7;22) fuses the EWS gene to the ETS gene ETV1. *Oncogene* 1995, **10**(6), 1229–1234.
126. Kaneko Y, Yoshida K, Handa M, *et al*. Fusion of an ETS-family gene, EIAF, to EWS by t(17;22)(q12;q12) chromosome translocation in an undifferentiated sarcoma of infancy. *Genes Chromosomes Cancer* 1996, **15**(2), 115–121.
127. Peter M, Couturier J, Pacquement H, *et al*. A new member of the ETS family fused to EWS in Ewing tumours. *Oncogene* 1997, **14**(10), 1159–1164.
128. Knezevich SR, McFadden DE, Tao W, *et al*. A novel ETV6-NTRK3 gene fusion in congenital fibrosarcoma. *Nat Genet* 1998, **18**(2), 184–187.
129. Lawrence B, Perez-Atayde A, Hibbard MK, *et al*. TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumours. *Am J Pathol* 2000, **157**(2), 377–384.
130. Bridge JA, Kanamori M, Ma Z, *et al*. Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumour. *Am J Pathol* 2001, **159**(2), 411–415.
131. Panagopoulos I, Tiziana Storlazzi C, Fletcher CD, *et al*. The chimeric FUS/CREB3l2 gene is specific for low-grade fibromyxoid sarcoma. *Genes Chromosomes Cancer* 2004, **40**(3), 218–228.
132. Labelle Y, Zucman J, Stenman G, *et al*. Oncogenic conversion of a novel orphan nuclear receptor by chromosome translocation. *Hum Mol Genet* 1995, **4**(12), 2219–2226.
133. Sjogren H, Meis-Kindblom J, Kindblom LG, *et al*. Fusion of the EWS-related gene TAF2N to TEC in extraskeletal myxoid chondrosarcoma. *Cancer Res* 1999, **59**(20), 5064–5067.
134. Sjogren H, Wedell B, Kindblom JM, *et al*. Fusion of the NH2-terminal domain of the basic helix–loop–helix protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9;15)(q22;q21). *Cancer Res* 2000, **60**(24), 6832–6835.
135. Panagopoulos I, Hoglund M, Mertens F, *et al*. Fusion of the EWS and CHOP genes in myxoid liposarcoma. *Oncogene* 1996, **12**(3), 489–494.
136. Crew AJ, Clark J, Fisher C, *et al*. Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. *EMBO J* 1995, **14**(10), 2333–2340.
137. Skytting B, Nilsson G, Brodin B, *et al*. A novel fusion gene, SYT-SSX4, in synovial sarcoma. *J Natl Cancer Inst* 1999, **91**(11), 974–975.