Chapter 14
Fusion Oncogenes of Sarcomas

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Contents

Abstract Fusion oncogenes are reported in many types of sarcomas. They encode protein products acting as growth factor ligands, their receptors, signal transduction components and transcription factors. The oncogenes discussed here act as regulators of transcription. FET family and *PAX3/7-FOXO1* oncoproteins bind DNA with target gene specificity causing specific tumour types with limited morphological variation. Tumours expressing *SS18-SSX* fusion proteins show larger morphological variability, perhaps reflecting that the oncoproteins act through interactions with general chromatin regulators.

Mesenchymal stem cells are suspected target cells for transformation by fusion oncogenes in sarcomas.

Keywords Fusion oncogenes • Sarcoma • Tumour type specificity • Chromosome • Rearrangements

14.1 Fusion Oncogenes of Sarcomas

The formation of fusion genes requires at least two chromosome breaks coincident in time and space. Such events yield gene fragments that may fuse into new functional constellations. The transcriptional orientation of the two partner genes must

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be compatible, and the open reading frames maintained in the fusion transcripts. Simple translocations may thus lead to functional fusion genes only if partner genes are originally transcribed in the same centromeric/telomeric direction [1, 2]. Other gene orientations require additional chromosome breaks for inversion of partner genes and are probably less frequent. Translocations and gene fusions in lymphomas and some lymphoid leukaemia are most likely caused by errors during the rearrangements of immunoglobulin and T-cell receptor genes. This process involves cell type specific rearrangement mechanisms acting at specific target genes and sequences. No such mechanisms have been reported in sarcomas although sequences related to the lymphoid rearrangement points have been reported in, or close to the translocation breakpoints of some sarcomas. The frequency of tumours caused by fusion oncogenes is further determined by the oncogenic potency. Several observations suggest that the formation of fusion oncogenes is more frequent than the incidence of the corresponding tumour, indicating that they are necessary, but not sufficient, for malignant transformation. Escape from stress responses leading to apoptosis, or oncogene induced senescence, cell or tissue type dependence, or interactions with other genetic variables are important for the final outcome after formation of a new fusion oncogene [1, 3–5].

14.1.1 Cytogenetic Analysis of Sarcomas

Chromosome translocations and gene rearrangements were first described in leukaemias and lymphomas. These early results encouraged the cytogenetic analysis of solid tumours, including sarcomas, and the identification of recurrent translocations [6–10]. Sarcomas are tumours that show morphological similarities with mesenchymal cells of different lineages and stages of development. Mesenchymal stem or precursor cells are the most likely cells of origin for this group of tumours. Close to 100 entities of sarcomas are described based on morphological and genetic criteria [11]. Tumour cells from many sarcoma cases grow relatively well in short-term cultures, and this made early cytogenetic studies possible. Most of the common sarcoma entities, were found to be characterized by complex chromosome aberrations typical for tumours with advanced genomic instability. Entities that carry simple recurrent aberrations such as translocations were also reported, and followed up on the molecular level. These studies led to the early discovery of the chromosome translocation, t(12;16), resulting in the *FUS-DDIT3* fusion oncogene (also known as *TLS-CHOP)* in myxoid liposarcoma and the t(11;22) and *EWSR1-FLI1* fusion oncogene in Ewing sarcoma [7, 9, 12–15]. A long line of fusion oncogenes have subsequently been reported in many more forms of sarcoma, and new fusions are continuously reported as next-generation methods are employed in the analysis of more tumours. Most of the currently described sarcoma fusion oncogenes encode rearranged transcription factors, but genes encoding ligands, membrane receptors and signal transduction molecules are also represented [16–18]. This chapter will focus on a discussion of a few of the transcription factors encoding sarcoma fusion oncogenes.

14.1.2 Tumour Type specificity of Sarcoma Fusion Oncogenes

A surprisingly large proportion of the sarcoma fusion oncogenes are tumour-type specific. This specificity could be explained by several models, including cell type-specific mechanisms for chromosome rearrangements, cell/tissue type dependence for survival/oncogenic activity, and phenotype instructive activity of the fusion oncogene [1, 2]. Capacity to direct the tumour phenotype has been shown for several sarcoma fusion oncogenes [19–22]. Specific cell type requirements and interactions with differentiation programmes are also important factors [19–21, 23–28]. In contrast to the specific rearrangement mechanisms behind immunoglobulin and T-cell receptor translocations in lymphoma and leukaemia, no cell type-specific rearrangement mechanisms, or target sequences have been identified in sarcoma fusion oncogenes. Furthermore, several experimental transgenic animal or cell models with cDNA copies of sarcoma fusion oncogenes at random genomic loci recapitulate the tumour phenotypes[20, 22–24, 27, 29–31]. These observations rule out cell type-specific rearrangements as a necessary mechanism behind the observed tumour type specificity of sarcoma fusion oncogenes. Cell type associated localization of chromosome territories are, however, most likely important as a risk factor for rearrangements. Formation of fusion oncogenes is discussed elsewhere in this book.

14.2 The FET Group of Fusion Oncogenes

The FET group of fusion oncogenes are found primarily in human sarcomas and leukaemia. They encode fusion oncoproteins that are considered to be primary tumour-initiating and driving factors [4, 19–22, 32]. The resulting fusion protein products contain an N-terminal domain (NTD) from one of the FET family proteins, FUS, EWSR1 or TAF15, juxtaposed to the DNA binding domains from one of many alternative transcription factor (TF) partners (Fig. 14.1).

The FET fusion oncogenes are, with a few exceptions, tumour type-specific and therefore used as diagnostic tumour markers. The FET family of fusion oncogenes continue to grow as new variant FET oncogenes, involved in more tumour entities, are continuously discovered.

There is considerable variation with regard to the breakpoint location in some FET fusion oncogenes [33–35]. Fusion transcripts of individual tumours may thus differ with regard to number of exons included from the parental genes. The N-terminal domains of the FET partners and the DNA binding domains of the TF partners are, however, always present indicating that these parts are critical for the oncogenic effect. *FUS*, *EWSR1* and *TAF15* may also functionally replace each other as fusion partners in some tumour types (Fig. 14.1). The FET fusion oncoproteins most probably act as abnormal transcription factors with FET NTDs reported to function as transactivation domains [32, 36, 37].

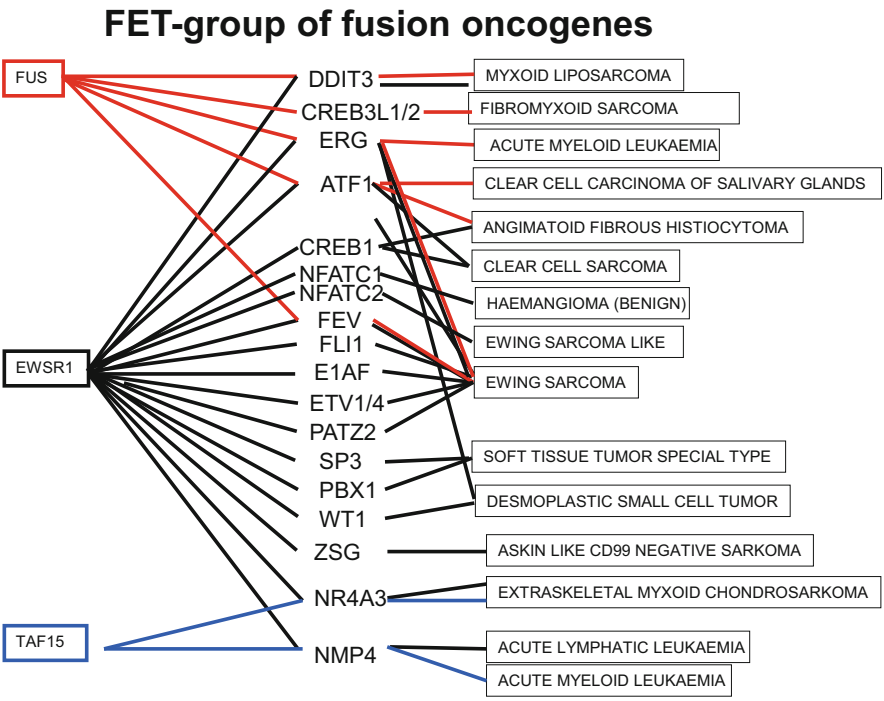

Fig. 14.1 The FET group of fusion oncogenes. The 5′ sequences of FET family genes *FUS, EWSR1* and *TAF15* are fused to genes encoding various transcription factors (*middle column*). The respective fusion genes are found in the tumour types as indicated in the right column (Note that the FET genes may replace each other in some fusion combinations. The figure is not complete as additional FET gene fusions are continuously discovered in more tumour entities)

The normal FET genes encode RNA and DNA binding proteins involved in transcriptional regulation [38–40], pre-mRNA splicing [41–43], RNA transport [44], translation [45] and DNA-repair [46, 47]. However, these functions involve the central and C-terminal protein domains that are lost in most of the fusion proteins. Analysis and structure predictions of the FET NTDs show that their structures are intrinsically disordered. Such domains are reported to function as mediators of protein-protein interactions [48]. Many proteins have also been shown to interact with the FET fusion proteins and their NTDs, including chromatin modifiers, transcription factors and cyclin dependent kinases [41, 49–55].

The full-length FET proteins were recently found as major interaction partners in pull-down experiments with FET NTDs and FUS-DDIT3. A common evolutionarily conserved "FET Binding Motif 1" (FETBM1) is present in the NTDs of all three FET proteins [56]. Specifically placed tyrosine residues, that have been reported to facilitate homotypic complex formation of FUS and similar sequences, are present in EWSR1 and TAF15 enabling formation of heterocomplexes [57]. A detailed study of the sequence requirement of the transactivating and transforming activity of EWSR1 NTD further pointed out the importance of the tyrosine residues and underscored the potential effects of their phosphorylation [58].

Experiments with deletion mutants of the full-length FUS showed that binding to the FUS NTD required amino acids 176–284, whereas residues 1–175 showed no significant affinity [56]. This observation rules out direct binding between FETBM1 sequences and indicates target structures elsewhere in the FET proteins. Combined results from several studies suggest that the FUS sequence between residues 176 and 214 forms an important target structure for FETBM1. Alignment experiments with this part of FUS, TAF15 and EWSR1 suggested that stretches containing repeats of G and RGG might form common target structures for FETBM1.

The normal recruitment of FET proteins to chromatin, genes and RNA molecules, is probably dependent on their RNA and DNA binding domains [38]. Each of the normal FET proteins binds thousands of different RNA species of several classes [59], and they also bind important protein components of transcription and RNA processing complexes [41, 51–55]. Recruitment of normal FET proteins to promoters targeted by oncogenic FET proteins may thus be an important part in deregulation of target genes.

Forced expression of FET fusion oncogenes in normal or various tumour cell lines most often results in apoptosis or cell senescence. Riggi et al. [19, 21] showed that FET oncogene- transduced mouse mesenchymal stem cells maintain proliferative capacity and are tumourigenic in mouse. Although FET oncogene-transduced human mesenchymal stem cells fail to form tumours after xenografting in mice, these data show that mesenchymal stem cells can survive and grow while expressing the fusion oncogenes. These observations suggest that FET fusion oncogenes lead to tumour formation only if they are formed in compatible cell types such as mesenchymal stem cells.

The most frequent FET oncogene-carrying tumour types are Ewing sarcoma (reviewed elsewhere in this book) and myxoid liposarcoma/round cell liposarcoma (MLS/RCLS), the latter with an incidence of 0,2 per 100,000/year [11]. The *DDIT3* partner of the myxoid liposarcoma fusion gene encodes a stress response protein induced under several stress conditions. It also has a role in the regulation of adipocyte differentiation, and forced expression of DDIT3 protein in fibrosarcoma cells has been reported to change the tumour morphology into liposarcomas [20]. *DDIT3* is also overexpressed as a result of the typical gene amplifications in well differentiated/dedifferentiated liposarcomas and may contribute to the liposarcoma phenotype of these tumours. *DDIT3* is by itself not considered an oncogene, whereas *FUS-DDIT3* transforms 3T3 cells and can turn transfected mesenchymal stem cells to liposarcoma-initiating cells in mice [19, 32].

14.3 Alveolar Rhabdomyosarcoma (ARMS)

Alveolar rhabdomyosarcoma make up around 20 % of childhood rhabdomyosarcoma cases [60]. Early cytogenetic studies, reported the recurrent t(2;13)(q36.1;q14.1) in Rhabdomyosarcomas [6, 10]. A detailed mapping of the chromosomal breakpoint regions led to the discovery of a rearranged *PAX3* gene and subsequently identified the *PAX3-FOXO1* (also known as *PAX3-FKHR*) fusion

oncogene [61–63]. Further studies showed that the less frequent t(1;13), also recurrent in ARMS, resulted in the variant *PAX7-FOXO1* fusion [63]. In the fusion proteins, the C-terminal transcriptional activation domains of PAX3 or PAX7 are replaced by the bisected forkhead transacting domain of FOXO1 retaining the N-terminal PAX3 paired box and homeodomains. The chimeric protein product of this translocation has been shown to promote cell proliferation and tumour formation by acting as an aberrant transcription factor with oncogenic properties. The fusion oncogenes have, however not been shown to cause ARMS by themselves in mesenchymal stem cells, but additional gene changes affecting *TP53* and the RAS pathway are needed [64, 65]. A number of PAX3-FOXO1 target or downstream genes have been identified. Some data suggest that the fusion oncoprotein promotes a myogenic differentiation pathway [64], and the formation of ARMS outside muscle tissue suggest that cell types other than myo-precursors may be reprogrammed by the fusion protein. Other studies show that the fusion protein blocks myocyte differentiation, partially by interfering with regulation of MyoD activity [23, 24, 27]. The normal PAX3 and PAX7 proteins have several isoforms with partially different functions and effects in myogenic differentiation, and the abnormal PAX3/7-FOXO1 proteins may cause dysregulation of this delicate system and arrest cells in a proliferative stage [66]. Normal myoblasts, with forced expression of the fusion oncoprotein, were still capable of cell fusion and myotube formation with wild type myoblasts [29]. Formation of such mixed myotubes and fibres was IL-4 receptor dependent [29]. Co-injection of myoblasts with tumour cells in mice, enhanced tumour take and growth/metastasis in an IL-4 receptor dependent manner. This suggests that cell fusions and complex interactions with normal cells are involved in the development of ARMS.

14.4 Synovial Sarcoma

Synovial sarcomas account for up to 10 % of soft-tissue sarcomas. A characteristic chromosomal translocation, t(X;18)(p11.2;q11.2), was originally reported by Turc-Carel in 1986 [8]. Molecular mapping and analysis of the breakpoint regions showed at least two different breakpoint regions on the X chromosome [67]. The translocations were subsequently shown to result in several alternative fusion genes. In the first described fusion oncogene, the 5′ end and major parts of *SS18* (also known as *SYT*) from chromosome 18 is fused to the 3′ partner *SSX1* on X [68]. Further studies showed that *SSX1* belongs to a large family of highly homologous genes and pseudogenes [69], and that several of them form fusion oncogenes with *SS18* [70]. The different *SSX* partner genes are highly similar. Still, the alternative fusion genes are associated with several morphological tumour variants, indicating functional differences in the SSX partners. This also shows that the SS18-SSX fusion oncogenes have some instructive activity that determine the tumour phenotype. As for other sarcoma fusion oncogenes, the *SS18-SSX* genes appear to be tumourigenic only in specific cell types [71, 72].

SS18–SSX encoded proteins lack DNA binding functions, but are shown to function as aberrant transcriptional regulators. *SS18* encodes a subunit of the SWI-SNF chromatin remodelling complex, and the SS18 containing fusion oncoprotein disrupts the normal formation and function of this complex [73–78]. The SSX partners interact with polycomb group protein complexes, and this activity is maintained in SS18-SSX fusion proteins [77]. Through these interactions with general chromatin remodelers and transcriptional repressors, the fusion protein may deregulate a very large numbers of genes. This very broad effect may explain the potency of this oncogene. Many direct target genes and downstream deregulated genes and functions, have been shown to be important for the oncogenic activity [73, 79, 80] and thus been proposed as therapeutic targets.

14.5 Summary

The sarcoma fusion oncogenes discussed in this chapter function as abnormal transcriptional regulators. The FET family and *PAX3/7-FOXO1* oncogenes carry DNA binding domains with sequence and target gene specificity and they cause specific tumour types with limited morphological variation. Tumours expressing *SS18-SSX* fusion proteins show larger morphological variability, perhaps reflecting the fact that these oncoproteins lack DNA binding parts but instead act by interactions with general chromatin regulators.

Stem cells of mesenchymal tissues have been proposed as the cell of origin for sarcomas. In experimental systems with forced expression of sarcoma fusion oncogenes, stem cells proliferate and form tumours, whereas other cell types fail to survive or grow. This highlights the importance of stem cells as targets for fusion oncogene-induced transformation.

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