Chromosomal Translocations in Sarcomas: New Perspectives

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Introduction

The sarcomas are a heterogeneous group of cancers derived from the connective tissue lineage. The etiology of these tumors is unknown and the vast majority of the cases occur without known hereditary factors.

Cells, Genes, Proteins, and Chromosomes: This article deals, in part, with cells, genes, proteins, and chromosomes. Some readers may find it useful to review these biological entities. There is an excellent publication discussing many of them provided by the National Institute of General Medical Sciences (NIGMS) website. It is called "Inside the Cell." NIGMS is part of the National Institutes of Health (NIH). It is a large PDF document and you can download it by clicking here. Click on any of the following links, cell, gene, protein, chromosome, DNA, RNA, protein synthesis, cell cycle, cell proliferation, cell differentiation, or transcription, to access a useful discussion of the selected topic. Articles describing other related topics discussed in this article are also found on the BookRags.com website; click here to view the complete index of articles. Lastly, the National Human Genome Research Institute's "Talking Glossary of Genetic Terms" can be accessed by clicking here. The RealAudio player is required to play the expanded explanations (this is a free download from their website).

In the last two decades, the finding of specific acquired chromosomal alterations in sarcomas has helped in many cases to understand the underlying genetic basis of these tumors. These studies have allowed researchers to classify sarcomas into two main groups: (1) sarcomas with specific genetic alterations on a background of relatively few other chromosomal changes, and (2) sarcomas with no specific genetic alterations on a complex background of numerous chromosomal changes. One third of sarcomas fall in the first group and are characterized by specific recurrent genetic changes known as chromosomal translocations (Table 1). As the molecular basis of these chromosomal translocation events are identified for each sarcoma, important new information has been provided that is changing how these sarcomas are diagnosed and how the prognosis of these sarcoma patients is being determined. Finally, this information will hopefully be useful in changing how these cancers are treated in the future.
What is a chromosomal translocation?

Chromosomal translocations are structural alterations of chromosomes in which pieces of two chromosomes are swapped. (Fig. 1). This swapping creates chimeric or derivative chromosomes, which are new chromosomes consisting of joined portions of the two original chromosomes. The mechanism underlying these events is not understood but is hypothesized to be the result of either damage to chromosomal DNA (e.g., by radiation or chemicals) or mistakes in normal recombination processes. In most sarcomas with translocations, cytogenetic studies have found balanced or reciprocal exchange of pieces between two chromosomes, resulting in two derivative chromosomes, with no net gain or loss of chromosomal material. In a few instances, only one derivative chromosome is formed (or remains in the cell after division), and thus there is a net loss of chromosomal material corresponding to the two pieces of the involved chromosomes that were not retained.

Figure 1: Generation of a chromosomal translocation. Breaks occur in the blue and purple chromosomes in the vicinity of two cellular genes indicated by the green and orange rectangles. Portions of the broken blue and purple chromosomes are exchanged to create chimeric chromosomes with generation of fusion genes (shown as the juxtaposed green and orange rectangles) at the breakpoints.

For photographs of actual sarcoma chromosomal translocations, see the Atlas of Genetics and Cytogenetics in Oncology and Haematology.

Chromosomal translocations in cancer: what are the consequences of chromosomal translocations?

Each recurrent chromosomal translocation in a sarcoma introduces breaks within two cellular genes and results in the joining of portions of the two genes to create a novel chimeric or fusion gene (Fig. 2).
Figure 2: Generation of gene fusion and downstream effects. In Gene 1 and Gene 2, the rectangles represent exons, the portions that will be transcribed and contribute to the final mRNA after processing. The line segments represent introns, the intervening portions that will be transcribed and removed during RNA processing. In these genes, the chromosomal translocations generally introduce breaks (shown as black arrowheads) in the introns and result in the formation of a fusion gene (or chimeric gene) following joining of the portions of the two broken chromosomes. The fusion gene is transcribed and processed into a fusion mRNA transcript, which in turn is translated into a fusion protein. This fusion protein is an oncoprotein that might impact multiple cellular properties to contribute to the cancer process. These processes include cell proliferation (division of one cell into daughter cells), apoptosis (programmed cell death), differentiation (development of specialized characteristics), and motility (cell movement).

The fusion gene is transcribed into a fusion transcript and then is translated into a fusion protein, which combines functional domains from the two corresponding normal proteins to create a novel protein with new functional properties. In many cases, these translocations generate novel transcription factors in which the DNA binding domain is derived from one gene and one or more transcriptional regulatory domains are derived from the other gene. An example is the PAX3-FKHR gene fusion resulting from the 2;13 chromosomal translocation in alveolar rhabdomyosarcoma (Refs. 1 and 2). This gene fusion encodes a transcription factor in which the DNA binding domain is derived from the PAX3 gene and the transcriptional activation domain is derived from the FKHR gene. The fusion transcription factors generated by various translocations either inappropriately regulate the expression of their usual target genes or possibly gain the ability to interact with other transcription factors to regulate expression of novel target genes. The resulting changes in gene expression modify key cellular pathways involved in processes such as cell division and cell death, and thereby contribute to tumor initiation and/or tumor progression.

**Transcription factors** are nuclear proteins that participate in and regulate transcription, the expression of RNA from genes in the nuclear DNA. These proteins contact the DNA of each target gene by means of a portion of the protein called the **DNA binding domain**. In addition, these proteins interact with other transcription factors by means of a portion of the protein called the **transcriptional regulatory domain**. Often this domain serves to increase the overall level of expression of the associated gene and is referred to as a **transcriptional activation domain**. Click here for more details on gene expression and transcription factors.

In several of these sarcoma categories, variant translocations may be found in subsets of cases (Table 1). These variant translocations join one of the genes usually involved in the common...
translocation to a gene closely related to the other partner involved in the common translocation. The fusion proteins resulting from these variant translocations have similar structure and function to the fusion proteins from the common translocation. An example is the 1;13 translocation in alveolar rhabdomyosarcoma that fuses FKHR from chromosome 13 to PAX7 from chromosome 1, a gene closely related to PAX3, to generate a PAX7-FKHR fusion product (Ref. 3).

The close association between a chromosomal translocation and a sarcoma category

Two very important properties of these chromosomal translocations (and their associated fusion products) are their consistency and specificity. Multiple studies have indicated that the same translocation (or in some cases, one of a related group of translocations) occurs in the far majority of cases of a given sarcoma, and thus a translocation or group of translocations is consistent within a sarcoma category. Furthermore, this translocation or one of a related group of translocations does not occur in any other type of sarcoma, and thus the translocation is specific for that sarcoma category. Therefore, there is a very close relationship between the translocation or its fusion product and that sarcoma category.

Two theories have been put forward to explain this very close relationship between the translocation and sarcoma subtype.

- Theory 1 (The seed determines the soil): The translocation occurs in a connective tissue progenitor cell and the fusion product resulting from the translocation influences the pattern of proteins produced by the cell, thereby helping to determine the specific tumor type. A progenitor cell is a specialized cell type that divides and gives rise to differentiated cells, more mature cells with specific functional properties, during development of a tissue.
- Theory 2 (The soil determines the seed): Only a specific type of connective tissue cell is capable of forming a specific fusion product or is susceptible to and/or permissive of the effects of a fusion protein generated by a specific chromosomal translocation due to constraints associated with that environment. These constraints can include the ability to generate the fusion gene, express the fusion protein, tolerate toxicity of the fusion protein, or respond to the oncogenic effects of the protein.

Molecular diagnosis and chromosome translocations

One of the important consequences of the close relationship between chromosomal translocations and sarcoma categories is that these translocations are very useful diagnostic markers. Application of such genetic tools is particularly useful in cases where the pathologic presentation is not straightforward and the differential diagnosis includes several possible tumors, including sarcomas which are associated with specific chromosomal translocations. Beyond simple detection of the translocation event by routine cytogenetic examination of chromosome spreads in tumor cells, much more powerful diagnostic evaluation is possible when the presence of the fusion gene, fusion transcript, or fusion protein is evaluated by molecular methodologies (Refs. 4-7). In particular, clinically applicable strategies for molecular diagnosis of soft tissue sarcomas have been developed to detect the fusion transcripts in RNA isolated from tumor samples by reverse transcriptase polymerase chain reaction (RT-PCR) or to detect the fusion gene in tumor cells or tumor nuclei by fluorescence in situ hybridization (FISH). Both of these assays can be applied to relatively small amounts of material, and have also more recently been shown to be applicable to formalin-fixed paraffin-embedded specimens. Depending on the specific assay design, there may be instances where FISH is preferable to RT-PCR or where RT-PCR is preferable to FISH, but in general, both technologies are quite robust, and can provide useful molecular diagnostic information to assist in the differential diagnostic process.
What defines a tumor, morphology or genetics?

In large studies of sarcomas comparing molecular and histologic findings, there is very strong concordance between these two sets of data (Ref. 4 and Refs. 8-11). Tumors diagnosed as sarcomas in the categories associated with translocations generally demonstrated gene fusions while tumors diagnosed as sarcomas in categories not associated with translocations did not generally demonstrate any gene fusions. However, there were circumstances where the molecular and histologic "diagnoses" were not concordant.

In these comparisons of molecular and histologic findings, there is usually a small subset of tumors in each sarcoma category that is negative for the specific associated gene fusion. In the alveolar rhabdomyosarcoma category, 20% of the cases are negative for the PAX3-FKHR or PAX7-FKHR fusion resulting from the 2;13 or 1;13 chromosomal translocation (Ref. 12). In the Ewing's sarcoma and synovial sarcoma categories, various studies indicate that approximately 10% of the cases do not express the EWS-FLI1 or EWS-ERG fusion transcripts characteristic of the former category and SYT-SSX1 or SYT-SSX2 fusion transcripts characteristic of the latter category (Refs. 4, 10, 13). There are several technical issues that could explain some of these results: incorrect histologic diagnosis, poor quality sample for molecular diagnosis, or too few tumor fusion-positive tumor cells to be detected by the assays. In addition to these technical explanations, there are a few cases within any sarcoma category with rare variant translocations. Examples of such variant translocations are found in Ewing's sarcoma and result in the fusion of EWS on chromosome 22 to genes encoding additional members of the ETS family of transcription factors on chromosomes 2, 7, and 17 (Refs. 14-16). Finally, as indicated by recent studies of fusion-negative cases of alveolar rhabdomyosarcoma, there may be a true subset of discordant cases that points to an area where future clinical correlative research is necessary to determine how the clinical phenotype of this subset compares to that of fusion-positive alveolar rhabdomyosarcomas (Ref. 17).

In a second situation, fusion-positive cases were found in tumors diagnosed as a category that was not associated with recurrent chromosomal translocations. One such example of these fusion-positive cases was found in the analysis of the SYT-SSX fusions, which are associated with synovial sarcoma, in another tumor type, malignant peripheral nerve sheath tumor (Refs. 18 and 19). The finding of fusion-positive cases in this spindle cell tumor that can have a very similar morphology to a subset of synovial sarcomas (monophasic) demonstrates the difficulties encountered in the differential diagnosis of certain cases. Application of histologic criteria alone may not be sufficient to diagnose difficult cases and therefore the application of an assay for the SYT-SSX gene fusions (specific for synovial sarcoma) will provide a useful tool to aid in the accurate classification of these difficult lesions.

Another important set of lessons regarding positive findings in a second tumor category is exemplified with the ETV6-NTRK3 gene fusion resulting from the 12;15 translocation in infantile fibrosarcoma, a soft tissue tumor with a spindle morphology (20). In this case, analysis of a kidney tumor known as cellular mesoblastic nephroma, which also has spindle cell morphology, identified the ETV6-NTRK3 gene fusion in nearly all cases of this second tumor type (Ref. 21). Based on the recurrent nature of the gene fusion in this kidney tumor, this finding does not represent a false positive finding or a misdiagnosis. Instead, this finding of the same gene fusion links these two lesions together into a common "histogenetic" entity because of the very similar histologic appearance of the two lesions despite the difference in sites of occurrence. In contrast to this finding in cellular mesoblastic nephroma, the ETV6-NTRK3 gene fusion was subsequently
found to be consistently expressed in a rare subset of breast cancer termed secretory carcinoma (Ref. 22). This finding indicates that this translocation and the associated gene fusion can occur and are oncogenic (promotes the formation of cancer) in two unrelated lineages (connective tissue and epithelial). Since there is not a diagnostic problem distinguishing breast cancer and these fibroblastic lesions, this finding does not pose a molecular diagnostic problem but does present a situation where the gene fusion is not absolutely specific for one lineage.

What can these gene fusions tell us about patient outcome?

Despite the fact that initial studies indicated that variant fusion products within sarcoma categories generally have similar structure and function, clinical studies of tumors with these variant fusions demonstrated clinical differences. For alveolar rhabdomyosarcoma, one study has provided evidence that the PAX7-FKHR fusion is associated with a significantly better outcome than the PAX3-FKHR fusion in patients with metastatic disease (Ref. 12). Several studies of synovial sarcoma have indicated that in patients with localized disease, the SYT-SSX2 fusion gene is associated with a better outcome than the SYT-SSX1 fusion (Refs. 7, 23, 24). Finally, in Ewing's sarcoma, there appears to be no difference in outcome between the EWS-FLI1 and EWS-ERG fusions (Ref. 25). However, there is significant diversity in the location of the breakpoints of the 11;22 translocation in both the EWS and FLI1 genes, and different combinations of exons from EWS and FLI1 encode different fusion transcripts (Ref. 26). The most common combinations are the type 1 fusion (EWS exon 7-FLI1 exon 6), which occurs in 65% of cases, and type 2 fusion (EWS exon 7-FLI1 exon 5), which occurs in 20% of cases. In two large studies, the presence of type 1 transcripts in tumors in patients with localized disease is associated with improved outcome (Refs. 27-29).

The risk of local tumor recurrence and the development of distant metastasis are significant clinical problems for patients with sarcomas. Several studies have shown that even patients with localized disease can relapse at distant sites. Clinical observations often suggest that spread of tumor cells occurred by the time of diagnosis and in some patients these metastases cannot be detected by conventional methods, including microscopic examination, and in many cases represent submicroscopic disease. The sensitivity and specificity of PCR-based technology to detect fusion-positive cells provides a tool to detect these small numbers of disseminated tumor cells (Refs. 30 and 31). In one important study of patients with Ewing's sarcoma, a high-sensitivity RT-PCR assay for the EWS-FLI1 and EWS-ERG fusion transcripts was successful in detecting submicroscopic evidence of tumor cells in 18 of 92 bone marrow specimens from patients with clinically localized disease. Furthermore, the presence of fusion positive cells in the bone marrow was associated with an adverse outcome in these patients with localized disease. Evidence was also provided that the presence of fusion-positive cells tumor cells in the peripheral blood is also linked with a poor prognosis (Ref. 32).

Targeted therapy - does a magic bullet really exist?

Though combined treatment with surgery, chemotherapy, and/or radiotherapy has increased overall survival in many categories of soft tissue sarcomas, conventional treatment has not resolved the problem of metastatic disease and many situations of disease relapse. Another important concern is the side effects of conventional chemotherapy and radiotherapy. Since most current therapies are not specifically targeted to the tumors cells, a variety of normal cells in the body may also be affected by these therapies, resulting in undesired side effects. Therefore, the goal of much of modern cancer research is to identify new molecular targets to which new therapies can be directed with enhanced efficacy and fewer side effects. Ultimately, we hope to identify therapies that are sufficiently specific and efficient and thus may truly be "magic bullets."
What is a molecular target?

A molecular target is a molecule in the tumor cell to which a specific therapeutic approach is developed. This molecular target must be present in all cases of the disease considered for targeted therapy. The patient's tumor tissues must express the target at sufficient levels for the corresponding protein to be functional in the tissue. Furthermore, the function of the molecular target must be critical for the maintenance of the tumor phenotype. This role in tumor phenotype in inhibiting the tumorigenic phenotype should be shown in alternative experiments by mutation or inactivation of the molecular target. Finally, for pharmaceutical development, the target should not be present or required for normal tissue functions.

Can we target the gene fusion?

Based on the premise presented above, the fusion proteins in the translocation-positive sarcomas are excellent candidates for molecular targets. As discussed, these fusion proteins are generated from translocations that are specific for the tumor cells. In addition, the presence of these fusion proteins is necessary for the survival of the tumor cells. A variety of methodologies can thus be applied to inhibit either the expression or function of the fusion protein, and thereby represent potential therapeutic tools. In addition to directing these agents directly at the fusion protein, other strategies can also target downstream products (expression targets) of these fusion proteins.

Decreasing fusion product expression

The first logical option for a sarcoma targeted therapy is to decrease or eliminate expression of the fusion product. Several studies have used different methodologies to modulate fusion gene expression and showed that when the fusion gene is no longer expressed, the tumor cells usually stop growing. In Ewing’s sarcoma, the expression of the EWS-FLI1 fusion product has been decreased in cell culture and xenograft studies by strategies, including antisense expression constructs, antisense DNA oligodeoxynucleotides, and siRNA (33-35). In each of these strategies, DNA or RNA constructs complementary to the mRNA transcript are introduced into the cell and inhibit expression either by binding to and destabilizing the mRNA or by inhibiting translation of the mRNA into protein. However, an important practical issue is how to deliver these agents when moving these experiments from a simple cell culture system to a more complex tumor-bearing organism, such as a test animal or patient. Studies using nanotechnology in which constructs were packaged in targeted, non-viral delivery systems consisting of small particles with surface receptors for targeting have shown promising results in initial mouse xenograft experiments (Ref. 36).

For more information on nanotechnology in cancer research, see, "An Overview of Some Recent Cancer-Related Nanotechnology Research" by Bruce Shriver, Electronic Sarcoma Update Newsletter, October 2005, Vol. No. 5.

Inhibiting downstream targets

As described above, many of the fusion proteins in sarcomas alter expression of a variety of downstream genes involved in numerous pathways. Another possible strategy is to take advantage of specific downstream gene products whose expression is changed in these tumor cells and which are able to be targeted by specific drugs. For example, in Ewing’s sarcoma, uridine phosphorylase is a downstream target of the EWS-FLI1 fusion protein (Ref. 37). When cells increase expression of this enzyme, cell culture and xenograft studies indicate that the cells become sensitive to treatment with the DNA base analog, 5'-deoxy-5'fluorouridine. Therefore, treatment with this agent represents a novel therapy based on expression of a downstream target of the fusion protein in
Ewing’s sarcoma.

**Immune-based therapies**

There is increasing evidence for an immune response to cancer in humans, demonstrated in part by the identification of autoantibodies against a number of intracellular and surface antigens detectable in sera from patients with different cancer types. Therefore, it has been proposed that the fusion proteins generated by these chromosomal translocations are ideal targets for immunotherapy. However, recent studies have indicated that the problem is more complex than initially thought. Some studies have found that the fusion proteins in sarcomas can serve as antigenic peptides while other studies did not demonstrate evidence of such an effect (Refs. 38-41). One study demonstrated the ability of peptides corresponding to the fusion breakpoint sequences associated with several of these sarcomas to be appropriately recognized and, in the case of SYT-SSX, also demonstrated stimulation of a cellular immune response in normal donor lymphocytes (Ref. 42). In addition, a second study focused on the SYT-SSX fusion protein and identified evidence of primed lymphocyte precursors in synovial sarcoma patients (Ref. 38). The identification of specific reactive SYT-SSX peptides offered the opportunity to design peptide-based immunotherapy.

**Intracellular signaling pathways**

The signal transduction inhibitor Imatinib is a prototype of a successful targeted therapy (Refs. 43-45). This drug, which has been extensively studied in leukemias with the 9;22 translocation (generating a BCR-ABL fusion protein) and in gastrointestinal stromal tumors with KIT mutations (the first targeted therapy in sarcomas) is active against a number of tyrosine kinase-containing cell surface receptors. Among the translocation-positive sarcomas, the 17;22 translocation in dermatofibrosarcoma protuberans results in a PDGFB-COL1A1 fusion protein. This fusion protein is ultimately processed to wild-type PDGF beta, which is a secreted signaling protein that binds to the PDGF receptor (46). Since the PDGF receptor is a similar tyrosine kinase-containing cell surface receptor that is targeted by Imatinib, studies have examined the efficacy of this drug for treating dermatofibrosarcoma protuberans. Both animal studies and then actual use in patients have demonstrated clear and sometimes dramatic activity of Imatinib against this translocation-positive sarcoma (Refs. 47-50).

You can search for trials using Imatinib in our Clinical Trials listings.

**Final perspectives**

The fusion proteins generated by the chromosomal translocations in one third of sarcomas provide a set of tools for both research and ultimately clinical application. These fusion proteins are consistently and specifically associated with sarcoma categories, and thus provide powerful new markers for diagnosis and prognosis, and exciting new molecular targets for novel directed therapeutics. Finally, the discovery of these fusion proteins has opened up new frontiers in the investigation of the basic biology of these tumors, and as the underlying mechanisms are better worked out, the strategies for molecular therapeutics will be continually revised and refined.

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References


