The Impact of Genetics on Sarcoma Diagnosis: An Evolving Science

Frederic G. Barr and Paul J. Zhang

Although current standards for cancer diagnosis and classification are based primarily on histologic characteristics, progress in molecular genetics during the last few decades has enabled the development of new markers beyond the cellular level. Based on these studies, specific genetic changes have been detected in some groups of tumors with a common morphologic phenotype. Bone and soft tissue sarcomas are one such tumor category with recurrent genetic alterations (1). In one-third of sarcomas, recurrent chromosomal translocations characteristic of specific subtypes have been detected. These translocations generate gene fusions that are expressed as fusion transcripts encoding fusion oncoproteins. By using *in situ* hybridization or reverse transcription-PCR technology, molecular diagnostic testing has been instituted to detect the fusion products associated with these sarcoma subtypes. This molecular genetic testing has led to enhanced diagnostic accuracy and a deeper understanding of these disease processes. However, this testing has also continually provided findings that require the re-examination of the underlying biological and clinical tenets, thereby resulting in the evolution of the translational science.

Early molecular diagnostic studies of these sarcoma subtypes soon revealed cases with discordant molecular and histologic findings. In particular, there were cases with the characteristic fusion-positive histology that lacked the gene fusion. Further analysis of many of these cases established the phenomenon of variant gene fusions. In Ewing's sarcoma, the characteristic 11;22 translocation fuses the gene encoding the TET family RNA-binding protein EWSR1 (EWS) to the gene encoding the ETS family transcription factor FLI1. However, in smaller subsets of Ewing's sarcoma, *EWSR1* is fused to *ERG*, *ETV1*, *ETV4*, or *FEV*, which are genes encoding other ETS family transcription factors (2). Finally, in another small subset of cases, *FUS* (*TLS*), a gene encoding another TET family RNA-binding protein related to EWSR1, is joined to *ERG* (3). As further demonstration of the interchangeability of these RNA binding proteins, in myxoid liposarcoma, *FUS* is usually joined to the gene encoding the transcription factor DDIT3 (*CHOP*), but in a smaller subset of cases, *EWSR1* is joined to *DDIT3* (4). From an overall phenotypic perspective, the finding of variant fusions in tumors with a common histology shows that family members with similar function can be substituted for the constituents in these fusion oncoproteins and provide a comparable phenotypic contribution. However, comparisons of tumor subsets with different fusion subtypes have suggested that more subtle pathologic or clinical differences may be associated with different fusion subtypes (5–7).

The next set of discordant data are the findings of the same gene fusion in different tumor types. Several examples involve the *ETV6-NTRK3* fusion, which was originally detected in the soft tissue tumor infantile fibrosarcoma (8). When this fusion was subsequently identified in the renal tumor congenital mesoblastic nephroma, the finding of similar histologic features in renal and soft tissue lesions suggested that these lesions are manifestations of the same biologic process in different anatomic locations. However, this fusion was then found in secretory breast carcinoma and cases of acute myeloid leukemia. Therefore, the same fusion can occur in mesenchymal, epithelial, and hematopoietic cell types. Similarly, the *FUS-ERG* fusion, which occurs in Ewing's sarcoma, is also found in acute myeloid leukemia (9). Therefore, some fusions are not specific for a single tumor category, and thus, are not unambiguous diagnostic markers but must be interpreted in the context of the existing histology. From a biological perspective, these findings indicate that some fusions can occur and contribute to oncogenicity in more than one target cell type. As the differentiated phenotypes of the associated cancers differ, the fusion does not appear to provide a common set of differentiation instructions. These observations are consistent with the finding of significant differences in downstream expression modulated by these fusion proteins in different cellular environments (10). However, other experiments show that some fusion proteins can program an unrelated cell type to differentiate along a specific pathway (11). Based on this data, the question arises of the relationship of the gene fusion to the target cell. Some data support the hypothesis that the gene fusion occurs in an established lineage that imposes constraints such that the target cell "selects" the fusion. In contrast, other data suggest that the fusion occurs in an undifferentiated precursor and influences differentiation such that the fusion "creates" the lineage.

Clear cell sarcoma (CCS) is a prime example of a tumor system for which our understanding of tumor biology and classification is evolving as these recurrent gene fusions are studied. In 1965, based on histologic findings, Enzinger first recognized a group of distinct soft tissue tumors as CCS of tendons and aponeuroses (12). Due to the presence of immunoreactivity for the S100 protein and other evidence of melanocytic differentiation in the far majority of these lesions, the tumors were also later referred to as malignant melanoma of soft parts (13). Subsequently, the 12;22 translocation was

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discovered to be a recurrent genetic alteration in the majority of these tumors and the gene fusion was subsequently identified to be *EWSR1-ATF1* (14), thus permitting a distinct entity to be recognized and especially distinguished from cutaneous melanoma.

The identification of variant fusions related to *EWSR1-ATF1* has introduced complexities not previously encountered in the study of sarcoma-associated gene fusions. First, *FUS*, again substituting for *EWSR1*, is joined to *ATF1* in two cases of angiomatoid fibrous histiocytoma, a soft tissue lesion histologically distinct from CCS and negative for the S100 antigen and melanocytic markers (15, 16). Second, as reported in this issue of *Clinical Cancer Research*, *EWSR1* is joined to *CREB1*, a gene encoding a bZIP transcription factor related to ATF1, in three S100-immunopositive tumors with histologic patterns resembling CCS, but lacking other evidence of melanocytic differentiation (17). In contrast to the usual soft tissue location of CCS, these three *EWSR1-CREB1*-positive tumors occurred in the gastrointestinal tract. Although these findings may suggest that these variant fusions define novel entities, additional reports in the literature indicate that the *EWSR1-ATF1* fusion also can occur in these novel entities. First, *EWSR1-ATF1* has been identified in a case of angiomatoid fibrous histiocytoma (18). Second, eight CCS or CCS-like tumors have been reported in the gastrointestinal tract with the t(12;22) and/or *EWSR1-ATF1* fusion (17). Like the *EWSR1-CREB1*-positive cases, most of these EWSR1-ATF1-expressing gastrointestinal cases are S100-immunopositive but otherwise lack melanocytic differentiation.

Based on these findings, it is postulated that these differing phenotypes are not due to the fusion subtypes but rather to the cell types in which the fusion is expressed. Therefore, three distinct cell types are proposed in which these fusions can be formed and expressed. There are two putative soft tissue cell types, one permissive for S100 expression and melanocytic differentiation, and resulting in a CCS histologic pattern, and another that is not permissive for S100 expression nor melanocytic differentiation, and resulting in an angiomatoid fibrous histiocytoma histologic pattern. Finally, a third cell type in the gastrointestinal tract is permissive for S100 expression but not melanocytic differentiation, and results in a CCS histologic pattern. The question arises whether the EWSR1-ATF1 protein induces S100 expression and melanocytic differentiation in one soft tissue cell type or whether these features are endogenous aspects of this cell. As evidence in favor of the former hypothesis, a recent publication has shown that, in CCS cells, the EWS-ATF1 protein binds and activates the promoter of the MITF gene which encodes a transcription factor of melanocytic genes (19).

Finally, we return to where we started and must consider the diagnostic ramifications of this combined gene fusion and histologic data. In this group of lesions expressing EWSR1-ATF1 and related fusions, where do the final diagnostic lines get drawn to distinguish one entity from the next? In the previously discussed example of ETV6-NTRK3-expressing lesions, diagnostic categories of secretory carcinoma, infantile fibrosarcoma, and acute leukemia were easily distinguishable. However, based on the gene fusion data, should the soft tissue lesions of CCS and angiomatoid fibrous histiocytoma be merged into one fusion-positive soft tissue diagnostic category, or should the two categories remain distinct? Most would argue for separate diagnostic categories. Even more problematic, should the soft tissue and gastrointestinal CCS-like lesions still be grouped into a single fusion-positive category or should the gastrointestinal lesion be separated into a distinct category. Clearly, there is data both for and against each side of the argument, and further discussion and investigation will be needed before experts in the field reach a consensus. In the end, it is important to acknowledge that the fusion gene data should not be considered the final answer but only one facet of a multidisciplinary process to classify the overall clinical reality.

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