Identification of neural stem cell gene expression signatures associated with disease progression in alveolar soft part sarcoma by integrated molecular profiling

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Alveolar soft part sarcoma (ASPS) is a relatively enigmatic soft tissue sarcoma with poor prognosis. Apart from the recurrent, non-reciprocal (1;17)(p11.2;q25) translocation, there is little molecular evidence for the origin, initiation and progression of this cancer. We have applied FISH analysis, in conjunction with array comparative genomic hybridization (aCGH) and expression profiling, to examine 16 primary and metastatic ASPS samples, to derive candidate molecular pathways that may be involved in the cancer biology. FISH analysis identified the ASPS-TFE3 fusion in all cases where the translocation of TFE3 is replaced by the fused ASPL sequences, while retaining the TFE3 DNA-binding domain. High-resolution aCGH revealed a higher number of numerical aberrations in the metastatic tumors compared to the primary, but failed to identify any consistent alterations in either of the groups. Subsequent DASL analysis revealed 1,063 genes which were differentially expressed, among which, 207 were up-regulated in primaries, while 116 were up-regulated in metastases. Gene set enrichment analysis using a gene ontology from Gene Ontology, Molecular Signature Database, and Genesignificantly identified 18 enriched gene sets (p ≤ 0.1) associated with the differentially expressed genes. Notable among these were several stem cell gene expression signatures and pathways related to differentiation. In particular, the paired box transcription factor PAX6 was up-regulated in the primary tumors, along with several genes whose mouse orthologs have previously been implicated in Past-DNA binding during neural stem cell differentiation. Of these, the LIN-28 homolog protein LIN-6, which plays a key regulatory role in development of neural cells, is a known methylation marker in head and neck carcinomas. While aCGH did not show a metastatic signature, the finding is consistent with the biology of several other translocation-associated sarcomas where transcriptional deregulation from fusion genes is implicated in the pathogenesis of the tumors, rather than extensive chromosomal instability. Of interest was the identification of stem cell gene expression signatures and pathways related to differentiation. In addition to suggesting a neural origin for ASPS, these may reveal more accessible therapeutic targets.

INTRODUCTION

Alveolar soft part sarcoma (ASPS) is a rare, high-grade, mesenchymal malignancy with a distinctive histological and ultrastructural appearance, but still enigmatic in terms of differentiation and origin. It affects mainly adolescents and young adults in the second and third decade, with slight female predominance. In adults, ASPS occurs most commonly in the deep soft tissues of the thigh or buttock, whilst, in children and infants, the head and neck regions are often involved. Despite a relatively indolent clinical course, the prognosis is poor and is often characterized by late metastases. ASPS has been the subject of considerable interest for pathologists and clinicians, owing to its unique microscopic features, unusual time of differentiation and unpredictable clinical behaviour. The goal of this project is to gain better insights into the underlying pathogenesis of ASPS and possibly identify therapeutic molecular targets by performing gene and tissue microarrays.

Cytogenetic and Molecular Features:

ASPS is characterized by a specific unbalanced translocation: 1;17(q11.2;q25). The translocation is present as either type 1 or 2 variants involving the fusion of the first seven exons of the ASPS (ASPS) gene to either 6 (type 1) or 5 (type 2) of the TFE3 transcription factor gene. This creates a novel ASPS-TFE3/TFE3 fusion protein that seems to act as an oncoprotein, which is implicated in the pathogenesis of ASPS through transcriptional deregulation of TFE3-regulated genes.

Histopathology:

Histologically, ASPS has a distinctive appearance, usually consisting of nests of epithelioid or polygonal cells arranged in nests or bundles, sometimes with a central focus of cohesion leading to the characteristic pseudopapillary pattern for which it is named. Mitotic activity is usually low and necrosis is infrequent. These tumors also possess a dense capillary vasculature.

Differential Gene Expression Analysis

Using RNA extracted from 16 FFPE samples, we studied genome-wide expression based on the high-throughput DASL platform. 3 samples were discarded due to poor signal quality. We started with quantile-normalized data on 18,401 genes, as generated by Broad Institute’s DASL platform, and performed a 2-sample t-test to detect differential expression of every gene across 5 primary and 8 metastatic sarcomas with Broad Institute’s GenePattern platform. Based on FDR for 10,563 genes to be ≤ 0.05, which 3,063 genes were found to be significant at the nominal p-value level of 0.05, of which 323 genes with t-test score greater than 3 were most differentially expressed (DE). Among these top DE genes, 207 were up-regulated in primary sarcoma (21%) including ASPS and rare pediatric renal cell sarcoma (ASPSCR1) gene) is observed following break at 17q25.3. Nuclear expression TFE3 is seen almost consistently in over 90% of ASPS and rarely in other diseases. This study was made possible through the generous support of: iCureASPS

Diagnostic value of TFE3:

Nuclear expression TFE3 is seen almost consistently in ASPS, including ASPS and rare pediatric renal cell sarcoma (ASPSCR1) gene). Loss of nuclear expression of TFE3 is specific to ASPS, the presence of strong TFE3 nuclear staining along with morphologic characteristics would strongly support the diagnosis of ASPS.Paraffin human tumor tissue were sectioned at 5 microns and subsequently processed for immunohistochemical staining using a standard protocol. TFE3-antibody (Santa Cruz; goat polyclonal) was used at 1:500 dilution and the staining was visualized using DAB.

Summary of findings:

1. aCGH/FISH studies show results consistent with the characteristic 1;17(q11.2;q25) translocation.
2. Gene expression profiling reveal genes differentially regulated between primary tumors and metastases. Many of the enriched genes are involved in biological processes essential for tumor progression (e.g. growth, migration and proliferation). In addition, GOECA also reveals a unique stem cell-like signature, involving the Pax-6 regulated set of genes associated with neural stem-cell biology.

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