Fusion of PAX7 to FKHR by the Variant t(1;13)(p36;q14) Translocation in Alveolar Rhabdomyosarcoma

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Abstract

Although the t(2;13)(q35;q14) translocation has been found in most cases of the pediatric cancer alveolar rhabdomyosarcoma, several cases have been reported with a variant t(1;13)(p36;q14) translocation. Our findings indicate that this t(1;13) rearranges PAX7 on chromosome 1 and fuses it to FKHR on chromosome 13. This fusion results in a chimeric transcript consisting of 5' PAX7 and 3' FKHR regions, which is similar to the 5' PAX3-3' FKHR transcript formed by the t(2;13). The 5' PAX3 and PAX7 regions encode related DNA binding domains, and therefore we postulate that these translocations create similar chimeric transcription factors that alter expression of a common group of target genes.

Introduction

In a number of hematopoietic and solid tumors, consistent chromosomal translocations have been shown to result in protooncogene activation by altering transcriptional regulation or forming chimeric gene products (1). For some of these tumor types, less frequent variant translocations cause similar genetic changes. For example, in Burkitt's lymphoma, the t(8;14) translocation deregulates MYC expression by juxtaposition with the immunoglobulin heavy chain locus, and the variant t(2;8) and t(8;22) translocations cause similar deregulation by moving MYC into the immunoglobulin light chain loci. The t(11;22) translocation of Ewing's sarcoma and peripheral neuroepithelioma has been shown to result in a fusion of the NH2-terminal region of EWS and the COOH-terminal region of FLI1, which contains an ETS DNA binding domain (2). A variant t(2;22) has been described in these tumors and was recently shown to fuse EWS with ERG, which also contains an ETS domain (3–5).

The molecular basis of the characteristic t(2;13)(q35;q14) translocation in the pediatric soft tissue tumor alveolar rhabdomyosarcoma has been recently elucidated (6–8). This translocation joins PAX3, a chromosome 2 locus encoding a member of the paired box or PAX family, and FKHR (or ALV), a chromosome 13 locus encoding a member of the fork head transcription factor family and has been recently localized to chromosomal region ip36 (16–18). The only published human PAX7 sequence is from the 5' paired box-containing region (19). We designed an

Materials and Methods

Two cases of alveolar rhabdomyosarcoma with a t(1;13) translocation were studied. In case CW520, the tumor occurred as a thigh mass in an 11-month-old male (11). In case CW1181, the tumor presented as a gluteal mass in an 11-month-old male (12).

Tissue and tumor samples were frozen in liquid nitrogen and pulverized. Total RNA and DNA were isolated with RNA-DNA STAT 60 extraction reagents (TEL-TEST "B"). Prior to RT-PCR, RNA samples (500 ng) were treated at 37°C for 1 h with 3 units DNase I and 10 units RNasin in a 3.5-μl final volume containing 2 mM MgCl₂. RT-PCR reactions were then performed using the GeneAmp RNA PCR kit (Perkin-Elmer) as described (7, 14), except that the annealing temperature was raised to 65°C and extension time at 72°C was shortened to 2 min. The PAX3- and FKHR-specific primers have been reported previously (7). The 5' PAX3/PAX7 consensus and 5' PAX7 primers are: CCAACACAGCATCGACG and mGAGAGGACCCACGCCCC, respectively.

PCR reaction products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and blotted to nylon membranes (Hybond N+, Amersham). These blots were hybridized to labeled probes specific for PAX3 [AP fragment (6)], FKHR [610-base pair EcoRI fragment (7)], and PAX7 [235-base pair Bsp12861 fragment (15)].

PCR products were isolated from the agarose gel and subcloned into the plasmid pCR II using the TA Cloning Kit (Invitrogen). Double stranded sequencing of plasmid inserts was performed with Sequenase Version 2.0 (United States Biochemical).

Genomic DNA was isolated from cell lines, as described (14). Aliquots of genomic DNA were digested with restriction enzymes, electrophoresed, and blotted onto nylon membranes. Cloned fragments were isolated, labeled, and hybridized to membranes, as described previously.

Results

The molecular basis of the variant t(1;13) translocation was first investigated by RT-PCR analysis of RNA isolated from two t(1;13)-containing alveolar rhabdomyosarcomas. To determine whether the t(1;13) is a complex translocation which masks the involvement of 2q, we used PAX3- and FKHR-specific primers, which consistently detect a fusion product in t(2;13)-containing tumors. As a positive control for the presence of amplifiable intact RNA, we utilized an RT-PCR assay for the wild-type FKHR transcript. Analysis of t(1;13)-containing tumor RNA did not reveal a detectable PAX3-FKHR product (Fig. 1). This negative result was confirmed with higher sensitivity by hybridization of PAX3- and FKHR-specific probes to a Southern blot of the PCR products (data not shown).

We next explored the possibility that the t(1;13) results from juxtaposition of FKHR with a candidate gene from chromosome 1. PAX7 is a member of the paired box-containing transcription factor family and has been recently localized to chromosomal region 1p36 (16–18). The only published human PAX7 sequence is from the 5' paired box-containing region (19). We designed an

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oligonucleotide from the 3' end of this sequence that corresponds to a region of sequence identity between PAX3 and PAX7 (Fig. 2A). RT-PCR assay of RNA from t(1;13)- or t(2;13)-containing tumors with this PAX3/PAX7 consensus primer and the FKHR-specific primer generated detectable products (Fig. 1). The size of these products corresponds to the 870-base pair fragment predicted from the sequence of the PAX3-FKHR chimeric transcript (7, 8). Southern blot analysis revealed hybridization of the FKHR-specific probe to PCR products from both t(1;13)- and t(2;13)-containing tumors (data not shown). However, a PAX3-specific probe only hybridized to the PCR products from the t(2;13)-containing tumors. In contrast, a probe from the murine Pax-7 cDNA (15) hybridized specifically to the PCR products from the t(1;13)-containing tumors. These results suggest that the t(1;13) results in a PAX7-FKHR fusion.

To extend these findings, the RT-PCR products were cloned and sequenced (Fig. 2). The sequence of the PCR products from two t(2;13)-containing tumors corresponds to the previously determined

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Fig. 2. Nucleotide and predicted amino acid sequence of chimeric transcripts associated with t(1;13) and t(2;13) translocations. A, comparison of PAX7 cDNA sequence [derived from PCR product in t(1;i3)-containing tumors] with murine Pax-7 (15) and human PAX3 (7, 8) cDNA sequences. B, comparison of predicted amino acid sequence of DNA binding domains of PAX3-FKHR and PAX7-FKHR fusion products. Hyphens have been introduced to optimize the sequence alignments. Bold solid line, conserved domains; dashed lines, PCR primers; vertical lines and asterisks, conserved nucleotides and amino acids, respectively. The PAX3 and PAX7 sequences located NH2-terminal to the octapeptide domain were obtained from Ref. 19.
PAX3-FKHR chimeric transcript (7, 8). The 3' end of the PCR products from the t(1;13)-containing tumors reveals the FKHR sequence with the same fusion point found in the t(2;13). The sequence following the 5' primer in these t(1;13) PCR products exactly matches the final 18 nucleotides of published human PAX7 sequence (19).

Furthermore, the entire sequence shows 90% identity with the published mouse Pax-7 sequence (15) and only 70% identity with the corresponding human PAX3 (7, 8) or mouse Pax-3 sequence (20). Therefore, these findings confirm that PAX7 is fused to FKHR in these t(1;13)-containing rhabdomyosarcomas.

To develop a RT-PCR assay specific for the PAX7-FKHR fusion, we used our sequence data to design a PAX7-specific oligonucleotide primer (Fig. 2A). Assay of tumor RNA with the PAX7- and FKHR-specific primers revealed the predicted 695-base pair PCR product in both t(1;13)-containing tumors (Fig. 1). This product was not detected in 4 cases of alveolar rhabdomyosarcoma with a t(2;13) translocation. These results were confirmed by demonstrating hybridization of the PCR products to PAX7- and FKHR-specific probes but not to PAX3-specific probes (data not shown).

To assay tumor DNA for genomic rearrangements of the PAX7 gene, we first isolated a 3' PAX7-specific probe consisting of a primer (Fig. 2A). These breakpoints over a large genomic region, we used the PAX7 re

Structural alterations of PAX7 were investigated by Southern blot analysis of genomic DNA from the t(1;13)-containing rhabdomyosarcomas. Since previous studies of PAX3 rearrangements in t(2;13)-containing rhabdomyosarcomas have demonstrated distribution of breakpoints over a large genomic region, we used the PAX7 restriction map to identify the endonucleases (BclI and EcoRV) that cleaved farthest in the 3' direction. The 3' PAX7 probe hybridized to a novel fragment in BclI digests and EcoRV digests of CW1181 tumor DNA when compared with unrelated lymphoid control DNA (Fig. 4) or fibroblast DNA from patient CW1181 (data not shown). These findings confirm the presence of a genomic rearrangement and rule out the alternative possibility of restriction fragment length polymorphism. Furthermore, the absence of the wild-type BclI band in the tumor DNA is consistent with allelic loss of the wild-type PAX7 locus in this tumor.

PAX3-FKHR chimeric transcript (7, 8). The 3' end of the PCR products from the t(1;13)-containing tumors reveals the FKHR sequence with the same fusion point found in the t(2;13). The sequence following the 5' primer in these t(1;13) PCR products exactly matches the final 18 nucleotides of published human PAX7 sequence (19). These breakpoints over a large genomic region, we used the PAX7 re

Discussion

We have examined the molecular basis of the variant t(1;13) translocation in alveolar rhabdomyosarcoma. Possible hypotheses for this variant translocation are a complex translocation which fuses PAX3 and FKHR but masks involvement of chromosomal region 2q, juxtaposition of FKHR on chromosome 13 with a novel locus on chromosome 1, or a translocation between two completely different loci. Our Southern blot and RT-PCR experiments demonstrate rearrangement of the PAX7 locus on chromosome 1 and generation of a PAX7-FKHR fusion transcript, thereby confirming the second hypothesis.

Our sequence analysis indicates that, similar to the PAX3-FKHR chimeric transcript, PAX7 is fused in-frame with FKHR to produce a chimeric open reading frame. The PAX7-FKHR chimeric transcript would thus encode a fusion protein consisting of the PAX7 paired box and homeodomain, which constitute an intact DNA binding domain, joined to a truncated FKHR DNA binding domain and the acidic and proline-rich COOH-terminal FKHR region. This putative fusion protein is very similar in structure to the PAX3-FKHR fusion protein that was previously identified in t(2;13)-containing alveolar rhabdomyo-
sarcoma cells by immunoprecipitation experiments (7). We propose that both PAX3-FKHR and PAX7-FKHR fusion proteins function as transcription factors to aberrantly regulate transcription of genes controlled by PAX3 or PAX7 DNA binding sites. The predicted amino acid sequences of the PAX7 paired box and homeodomain show 94% (123 of 131) and 97% (59 of 61) identity with the sequences of the corresponding PAX3 domains. This very high sequence identity suggests that the PAX3 and PAX7 DNA binding domains recognize very similar target sites and thus may regulate a common set of target genes involved in the pathogenesis of alveolar rhabdomyosarcoma.

These findings on the fusion products formed by the t(2;13) and t(1;13) of alveolar rhabdomyosarcoma are very similar to recent data on the t(11;22) and t(21;22) of Ewing's sarcoma and peripheral neuroepithelioma (2–5). In the latter case, the EWS gene is fused to FLII or ERG which are two members of the ETS transcription factor family. The translocations thereby generate fusion proteins consisting of the NH2-terminal EWS region and the COOH-terminal FLII or ERG region which contains the ETS DNA binding domain. The amino acid sequences of the FLII and ERG DNA binding domains show 98% identity; therefore these fusion proteins may also aberrantly regulate transcription of a common set of target genes involved in the neoplastic development of Ewing's sarcoma and peripheral neuroepithelioma.

In contrast to Ewing's sarcoma in which both chimeric transcripts are expressed from the EWS promoter, the chimeric transcripts in the alveolar rhabdomyosarcoma translocations are expressed from either the PAX3 or PAX7 promoter. Studies of mouse embryogenesis demonstrate that the PAX-3 and PAX-7 genes are expressed with distinct but overlapping developmental patterns (9, 15, 20). In addition to expression in the developing nervous system, both genes are expressed in the developing somites around the time of dermomyotome formation. Whereas PAX-3 expression occurs prior to myoblast migration and formation of the muscleplaque, PAX-7 expression commences a few days later and is maintained during the differentiation of the trunk and limb muscles. The finding of PAX-3 and PAX-7 expression in precursors of the skeletal musculature is consistent with the activity of their promoters in alveolar rhabdomyosarcomas, which differentiate along the striated muscle pathway.

Cytogenetic studies of alveolar rhabdomyosarcomas suggest that the t(2;13) occurs approximately 5 times more frequently than the t(1;13) variant (10, 13). Comparison of clinical parameters has indicated that the t(1;13) variant tends to occur in younger patients and more often presents in the extremities. The elucidation of the molecular basis of the t(1;13) and development of sequence-based assays will now facilitate detection of this translocation in clinical material and more definitive analysis of the clinical significance of this genetic alteration. Furthermore, the expression pattern and transcriptional function of the PAX3 and PAX7 gene products can now be explored to understand the biological and clinical differences between alveolar rhabdomyosarcomas with t(2;13) and t(1;13) translocations.

References

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