Fusion of the Paired Box 3 (PAX3) and Myocardin (MYOCD) Genes in Pediatric Rhabdomyosarcoma

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Abstract. Background/Aim: Fusions of the paired box 3 gene (PAX3 in 2q36) with different partners have been reported in rhabdomyosarcomas and biphenotypic sinonasal sarcomas. We herein report the myocardin (MYOCD on 17p12) gene as a novel PAX3-fusion partner in a pediatric tumor with adverse clinical outcome. Materials and Methods: A rhabdomyosarcoma found in a 10-year-old girl was studied using a range of genetic methodologies. Results: The karyotype of the tumor cells was 48,XX,add(2)(q11),+del(2)(q35),add(3)(q?25),-7,del(8)(p21),-15,add(17)(p11),+20,+der(?)t(?;15) (?;q15),+mar[8]/46,XX[2]. Fluorescence in situ hybridization detected PAX3 rearrangement whereas array comparative genomic hybridization revealed genomic imbalances affecting hundreds of genes, including MYCN, MYC, FOXO3, and the tumor suppressor gene TP53. A PAX3-MYOCD fusion transcript was found by RNA sequencing and confirmed by Sanger sequencing. Conclusion: The investigated rhabdomyosarcoma carried a novel PAX3-MYOCD fusion gene and extensive additional aberrations affecting the allelic balance of many genes, among them TP53 and members of MYC and FOXO families of transcription factors.

Alveolar rhabdomyosarcomas are cytogenetically characterized by the specific chromosome translocations t(2;13)(q36;q14) and t(1;13)(p36;q14) (1-4). The t(2;13)(q36;q14) results in fusion of the paired box 3 (PAX3) gene from 2q36 with the forhead box head O1 gene (FOXO1, also known as FKHR) from 13q14 (5-7), whereas t(1;13)(p36;q14) fuses the paired box 7 (PAX7) gene from 1p36 with FOXO1 (8). The above-mentioned chromosome aberrations and their corresponding fusion genes are found in 80% of alveolar rhabdomyosarcomas (9). In the remaining 20%, fusions of PAX3 with the genes FOXO4 (also known as AFX, in Xq13), nuclear receptor coactivator 1 (NCOAI, in 2p23), nuclear receptor coactivator 2 (NCOA2, in 8q13) or INO80 complex subunit D (INO80D, in 2q33) were found (9-12).

Apart from alveolar rhabdomyosarcomas, PAX3-FOXO1, PAX3-NCOAI, and fusion of PAX3 with the mastermind-like transcriptional coactivator 3 gene (MAML3, from 4q31.1; recombination occurs through a 2q35;4q31-chromosomal translocation) were also detected in biphenotypic sinonasal sarcomas (13-16). Furthermore, a PAX3-NCOA2 fusion was reported in embryonal rhabdomyosarcoma (11, 17).

In the present study, we report the finding in a pediatric rhabdomyosarcoma of a novel fusion of PAX3 with the myocardin (MYOCD) gene which maps to 17p12 and codes for a smooth and cardiac muscle-specific transcriptional coactivator of the serum response factor.

Materials and Methods

Ethics statement. The study was approved by the Regional Ethics Committee (Regional komitét for medisinsk forskningsetikk Sør-Øst, Norge, http://helseforskning.etikkom.no). All patient information has been de-identified.

Case description. The patient was a ten-year-old girl with an advanced stage of rhabdomyosarcoma. The tumor presented as a pelvic mass with spreading to pelvic and abdominal lymph nodes, several pelvic and abdominal viscera, and tumors nodules within the abdominal cavity. Examination of a diagnostic biopsy showed a
malignant, poorly differentiated, round cell tumor with solid and alveolar growth patterns (Figure 1A-C). The tumor cells were loosely arranged in sheets surrounded by fibrous septa (Figure 1A-C). Examination of the surgical specimen revealed a tumor showing little effect of chemotherapy. The histology was heterogeneous but large areas displayed alveolar morphology, solid tumor growth, a spindle cell component, and tumor nests (Figure 1D-F). Immunohistochemistry revealed strong expression of desmin, transcription factor AP-2 beta (TFAP2B, also known as AP-2beta, Figure 1G), and myogenin (MYOG, also known as MYF4, Figure 1H); the latter with positivity in nearly 100% of tumor cells. FISH analysis with separate probes for the PAX3 (2q36.1), PAX7 (1p36.13), and FOXO1 (13q14) genes showed rearrangement of PAX3 whereas PAX7 and FOXO1 were intact.

**G-Banding, karyotyping, and fluorescence in situ hybridization (FISH).** The methodology for cytogenetic investigation of solid tumors was described elsewhere (18). In brief, fresh tissue from a representative area of the tumor was disaggregated mechanically and enzymatically with collagenase II (Worthington, Freehold, NJ, USA). The resulting cells were cultured and harvested using standard techniques. Chromosome preparations were G-banded with Wright’s stain (Stigma Aldrich; St Louis, MO, USA) and examined. Metaphases were analyzed and karyograms prepared using the CytoVision computer assisted karyotyping system (Leica Biosystems, Newcastle, UK). FISH was performed on interphase nuclei using the CytoCell PAX3 breakapart FISH probe (Cytocell, Oxford Gene Technology, Begbroke, Oxfordshire, UK). It consists of a telomeric green 168kb probe and a centromeric red 124kb probe, which are positioned on each side of the PAX3 gene. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems).

**Array comparative genomic hybridization (aCGH) analysis.** Genomic DNA from tumor sample was extracted using the Maxwell RSC Instrument and the Maxwell RSC Tissuel DNA Kit (Promega, Madison, WI, USA) and quantified with the Quantus fluorometer (Promega, Madison, WI, USA). FISH was performed on interphase nuclei using the CytoCell PAX3 breakapart FISH probe (Cytocell, Oxford Gene Technology, Begbroke, Oxfordshire, UK). It consists of a telomeric green 168kb probe and a centromeric red 124kb probe, which are positioned on each side of the PAX3 gene. Fluorescent signals were captured and analyzed using the CytoVision system (Leica Biosystems).

**RNA sequencing.** Total RNA was extracted from frozen tumor tissue adjacent to that used for cytogenetic analysis and histologic examination using the mirNeasy Mini Kit (Qiagen, Hilden, Germany). One μg of total RNA was sent to the Genomics Core Facility at the Norwegian Radium Hospital, Oslo University Hospital for high-throughput paired-end RNA-sequencing. Fusion transcripts were found using the FusionCatcher software (20, 21).

**Reverse transcription (RT) PCR and Sanger sequencing analyses.** The primers used for PCR amplification and Sanger sequencing analysis are given in Table I. The methods for cDNA synthesis, RT-PCR amplification, and Sanger sequencing were described elsewhere (19). For the first, outer PCR amplification, the primer combination PAX3-1352F1/MYOCD-2687R1 was used whereas the primer combination PAX3-1374F1/MYOCD-2664R1 was used for the second, inner PCR. The basic local alignment search tool (BLAST) was used to compare sequences obtained by Sanger sequencing with the NCBI reference sequences NM_181457.4 (PAX3) and NM_153604.3 (MYOCD) (22).

**Results**

G-banding analysis of short-term cultured tumor cells yielded the karyotype 48,XX,add(2)(q11),+del(2)(q35),add(3)(q25),-7,del(8)(p21),-15,add(17)(p11),+20,+der(?)t(1;15) (?;q15),+mar[8]/46.XX[2] (Figure 2).

FISH analysis with a breakapart probe for PAX3 (Figure 3A) showed a green signal (telomeric probe) and two red signals (centromeric probe) in 80 out of 100 examined interphase nuclei (Figure 3B).

aCGH confirmed trisomy for chromosome 20 and showed gains and losses on various parts of chromosomes 2, 3, 6, 7, 8, 9, 10, 17, and 19 (Table II, Figure 4A) which affected hundreds of genes. On chromosome 2, a 1.34Mb size region on 2p24.3-p24.2 (Chr2:15991412-17336196) was found to have seven copies (Table II, Figure 4B). This region contained the MYCN opposite strand/antisense RNA (MYCNOS), MYCN proto-oncogene (MYCN) and a gene with the official name CYFIP related Rac1 interactor A (CYRIA alias FAM49A). The area of the PAX3 gene on 2q36.1 (chr2:223,064,606-223,163,715) showed a complex pattern of gains and losses (Figure 4C). An extra copy was seen for the part of PAX3 between exon 1 and exon 7, whereas the part of the gene between exons 8 and 10 was heterozygously lost (Figure 4C). The red centromeric probe of the PAX3 breakapart FISH probe was found to map to an area which showed gain of one copy (Figure 4C). This might be the explanation for the two red signals seen in FISH-examined interphase nuclei (Figure 3B).

An extra copy was found for each of the genes MYC (paralog to MYCN; on 8q24.21, position chr8:128,748,315-128,753,680) and forkhead box O3 (FOXO3, paralog to FOXO1; on 6q21, position chr6:108,882,069-109,005,971) (Table II).

On chromosome 17 (Figure 4D), within the 17p13.3-p12 region which had lost of one copy, the MAX network transcriptional repressor (MNT) and tumor protein p53 (TP53, on 17p13.1, chr17:7,571,720-7,590,868) genes were mapped (Figure 4D). With regard to MYOCD (on 17p12, position chr17:12,569,207-12,670,651), the number of aCGH probes was inadequate to draw certain conclusions (Figure 4E). In 17p12-p11.1, an extra copy of the FOXO3B gene was seen (chr17:18,570,942-18,585,627).

RNA sequencing analysis using FusionCatcher detected a fusion transcript between PAX3 from 2q36 and MYOCD from
Figure 1. Microscopic examination of the pediatric rhabdomyosarcoma. (A, B, and C) Tumor with alveolar growth pattern showing loosely arranged tumor cells surrounded by fibrous septa. (D) Tumor with spindle cell pattern. (E) Tumor with mixed growth pattern. (F) Tumor in nests. (G) Transcription factor AP-2 beta showed diffuse and strong expression in tumor nuclei. (H) Myogenin showed strong positive staining in nearly 100% of tumor nuclei.
17p12: CAACCCCATGAACCCCACCATTGGCAATGGCCTCTCACCTCAG*CAAATGACCCGGAGTCAGCAGATG

The presence of this PAX3-MYOC fusion transcript was confirmed by RT-PCR together with Sanger sequencing (Figure 5A and 5B). In the PAX3-MYOC fusion transcript, exon 7 of PAX3 (nt 1556 in reference sequence NM_181457.4) was fused in frame with exon 12 of MYOC (nt 2487 in reference sequence NM_153604.3).

Based on the PAX3 reference sequence NM_181457.4/NP_852122.1 and MYOC reference sequence NM_153604.3/NP_705832.1, the PAX3-MYOC fusion transcript would be expected to code for a 600 amino acid residue (aa) chimeric protein composed of the first 390 aa from the PAX3 protein (1-390 from NP_852122.1) and the last 210 aa of MYOC protein containing the transactivation domain of the latter (729-938 from NP_705832.1) (Figure 5C).

Discussion

To the best of our knowledge, this is the first time that a fusion between PAX3 and MYOC is described. Because both genes, PAX3 on 2q36 and MYOC on 17p12, are transcribed from telomere to centromere, a simple balanced translocation should be enough to generate a functional PAX3-MYOC fusion gene on the der(17). However, karyotyping of the tumor cells indicated complex rearrangements and the aberrations seen could not be described more accurately than del(2)(q35) and add(17)(p11). Searching the “Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer” (updated on April 15, 2021), we did not find any rhabdomyosarcoma (alveolar, embryonal, pleomorphic, spindle cell/sclerosing or rhabdomyosarcoma not otherwise specified) carrying a t(2;17)(q36;p12) chromosome aberration nor any tumors with a PAX3-MYOC fusion (23). Among the
237 rhabdomyosarcomas with an abnormal karyotype in the Mitelman Database, ten had cytogenetic aberrations affecting the short arm of chromosome 17 (23): an alveolar rhabdomyosarcoma carried a t(4;17)(q11;p11) (24), three embryonal and two pleomorphic rhabdomyosarcomas showed add(17)(p11) (24-28), a rhabdomyosarcoma not otherwise specified had del(17)(p12) (29), and two embryonal and one pleomorphic rhabdomyosarcoma had aberrations involving band 17p13 (26, 30). Although the possibility of a cryptic rearrangement cannot be excluded, the above-mentioned data

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**Figure 3.** Fluorescence in situ hybridization (FISH) analysis of the pediatric rhabdomyosarcoma using a commercial PAX3 breakapart probe. (A) Diagram showing the proximal (red) and distal (green) parts of the PAX3 breakapart probe. The neighbor gene sphingosine-1-phosphate phosphatase 2 (SGPP2) and the genetic markers D2S102, D2S2599, D2S313, and D2S2300 are also shown. (B) FISH on interphase nucleus with the PAX3 breakapart probe.

| Name: Sequence (5'->3') | Reference sequence: Position | Gene name (gene symbol) | Chromosome band |
|-------------------------|------------------------------|-------------------------|-----------------|
| PAX3-1352F1: TCCCAGCAGCACCAGCTACAG | NM_181457.4: 1352-1373 | Paired box 3 (PAX3) | 2q36 |
| PAX3-1374F1: CCTCAACGGCTTTCCACAGCG | NM_181457.4: 1374-1395 | Paired box 3 (PAX3) | 2q36 |
| MYOCD-2687R1: TCACTGTCGTTGCTCAGGATG | NM_153604.3: 2710-2687 | Myocardin (MYOCD) | 17p12 |
| MYOCD-2664R1: AAGGGGATCTGGTGAGGTCG | NM_153604.3: 2686-2664 | Myocardin (MYOCD) | 17p12 |

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Table I. Primers used for polymerase chain reaction amplification and Sanger sequencing analyses.
Figure 4. Array comparative genomic hybridization (aCGH) analysis of the pediatric rhabdomyosarcoma. (A) Whole genome aCGH showing trisomy for chromosome 20 and gains as well as losses from parts of chromosomes 2, 3, 6, 7, 8, 9, 10, 17, and 19. (B) Regions of chromosome 2 with gains and losses. The positions of the genes MYCNOS, MYCN, CYRIA, and PAX3 are shown. (C) The region around PAX3 showing both gains and losses. The position of the red centromeric and the green telomeric probes of the FISH PAX3 breakapart probe are also shown. The genomic area of PAX3 encompassing exons 1 to 7 is gained whereas the PAX3 area encompassing exons 8 to 10 is heterozygously lost. The FISH red centromeric probe maps to an area which has an extra copy (gain). (D) Regions of chromosome 17 with gain and loss. The positions of the genes MNT, TP53, MYOCD, and FOXO3B are shown. (E) The region around the MYOCD showing loss of a copy at the telomeric area (loss) and gain of a copy near the centromere. The few aCGH probes for MYOCD are inadequate to draw any certain conclusion as to possible copy number change of MYOCD.
contains the transactivation domains of the chimeric PAX3-FR观察er 1, PAX3-FOXO1, and the paired box protein 7 domain (347-390). The C-terminal part of the various 3′-end partner genes transcription factors (5-7, 9-17). All these fusion genes would code for chimeric proteins that have the N-terminal part of PAX3 and the C-terminal transactivation domain of MYOCD (Figure 729).

Table II. Results of array comparative genomic hybridization (aCGH) analysis of the pediatric rhabdomyosarcoma.

| Cytogenetic location | Position on GRCh37/hg19 assembly | Size      | Gain/Loss | Copy number |
|----------------------|----------------------------------|-----------|-----------|-------------|
| 2p25.3-p24.3         | Chr2:280840-15666840             | 15.64Mb   | Gain      | 3           |
| 2p24.3-p24.2         | Chr2:1599142-17336196            | 1.34Mb    | Gain      | 7           |
| 2p24.2-p11.2         | Chr2:17400700-90112889           | 73.11Mb   | Gain      | 3           |
| 2q33.2-q35           | Chr2:204588942-220738051         | 16.14Mb   | Gain      | 9           |
| 2q35                 | Chr2:22073992-221483616          | 745.62Kb  | Loss      | 1           |
| 2q36.1               | Chr2:22512818-222895162          | 1.37Mb    | Gain      | 3           |
| 2q36.1               | Chr2:222928374-223076308         | 147.94Kb  | Loss      | 1           |
| 2q36.1               | Chr2:223084848-223163460         | 78.61Kb   | Gain      | 3           |
| 2q36.1               | Chr2:223176619-223256245         | 79.63Kb   | Loss      | 1           |
| 3q24.2-q27.2         | Chr3:148748271-185798988         | 37.05Mb   | Gain      | 3           |
| 6q21                 | Chr6:108579217-109235546         | 656.33Kb  | Gain      | 3           |
| 7p22.3-p21.3         | Chr7:55649-10831224              | 10.78Mb   | Loss      | 1           |
| 7p21.3-p11.2         | Chr7:10633862-57924753           | 47.29Mb   | Loss      | 1           |
| 7q11.1-q22.1         | Chr7:61300518-102175773          | 40.88Mb   | Loss      | 1           |
| 7q22.1-q36.3         | Chr7:102346891-159006977         | 56.66Mb   | Gain      | 3           |
| 8p23.3-p23.1         | Chr8:31167-11369498              | 11.34Mb   | Loss      | 1           |
| 8p21.2-p21.1         | Chr8:25519631-28783983           | 3.26Mb    | Gain      | 3           |
| 8p12-p11.1           | Chr8:35041928-43210505           | 8.17Mb    | Gain      | 3           |
| 8q13.3               | Chr8:71311272-72740821           | 1.43Mb    | Gain      | 3           |
| 8q21.11-q21.13       | Chr8:74279304-80124748           | 5.85Mb    | Gain      | 3           |
| 8q23.1-q24.11        | Chr8:108369778-117978907         | 9.61Mb    | Gain      | 3           |
| 8q24.13-q24.3        | Chr8:125815021-146238409         | 20.42Mb   | Gain      | 3           |
| 9q33.3-q34.3         | Chr9:127044087-141102523         | 14.06Mb   | Gain      | 3           |
| 10q22.1-q22.2        | Chr10:72766561-75476122          | 2.71Mb    | Gain      | 3           |
| 17p13.3-p12          | Chr17:89086-12580027             | 12.49Mb   | Loss      | 1           |
| 17p12-p11.1          | Chr17:12689977-22219743          | 9.53Mb    | Gain      | 3           |
| 17q11.1-q21.33       | Chr17:25294244-48947156          | 23.65Mb   | Gain      | 3           |
| 19q13.43             | Chr19:57497515-57672410          | 192.9Kb   | Gain      | 3           |
| 20p13-p11.1          | Chr20:72367-26257880             | 26.19Mb   | Gain      | 3           |
| 20q11.21-q13.33      | Chr20:72367-26257880             | 33.5Mb    | Gain      | 3           |

indicate that straightforward two-way aberrations such as t(2;17)(q36;p12) are rare causes of PAX3-MYOCY fusion in rhabdomyosarcomas.

In the PAX3-MYOCY chimeric transcript, the point of fusion in PAX3 was exon 7. The same fusion point was also seen in the transcripts PAX3-FOXO1, PAX3-INOD88, PAX3-NCOA2, the transcript 2 of PAX3-NCOA1, and PAX3-MAML3 (5-7, 9-17). All these fusion genes would code for chimeric transcription factors that have the N-terminal part of PAX3 (aa 1-390 of NP_852122.1). This N-terminal part contains the highly conserved paired box domain (position 34-159) that binds to DNA sequences related to the TCACGG/G motif, followed by the microfibril-associated/pre-mRNA processing region (position 163-286), the homeobox domain (position 222-275), and the paired box protein 7 domain (347-390) (31). The C-terminal part of the various 3′-end partner genes contains the transactivation domains of the chimeric transcription factors (5-7, 9-17).

In vitro studies have shown that the PAX3-FOXO1, PAX3-NCOA1, PAX3-NCOA2, and PAX3-MAML3 chimeric proteins are much stronger transcriptional activators than PAX3 (10, 16, 17, 32-34). Moreover, the PAX3-FOXO1 and PAX3-NCOA2 proteins were found to simultaneously initiate myogenesis and inhibit terminal differentiation, which is why they have been called “pangenes” in tumorigenesis (17, 35).

In a similar manner to what happens with the above-mentioned PAX3-fusion genes, the PAX3-MYOCY fusion is predicted to code for a chimeric transcription factor composed of the N-terminal part, with the DNA binding domain, of PAX3 and the C-terminal transactivation domain of MYOCY (Figure 5C). MYOCY is expressed in heart, aorta, and smooth muscle cell-rich tissues such as the stomach, bladder, small intestine, colon, and uterus (36, 37). It codes for a transcriptional co-activator of receptor response factor with many functional domains, one of which is a transactivation domain at the end of the protein (36, 38, 39). MYOCY regulates the development and differentiation of cardiomyocytes and has been reported to be a master regulator of smooth muscle gene expression (36, 37, 40). The myocardin gene was found highly amplified in non-uterine (41, 42) but down-regulated in uterine leiomyosarcomas (43). Exogenous expression of myocardin in uterine leiomyosarcoma cells resulted in growth arrest and
differentiation to smooth muscle cells (43). Similar results were also found for other sarcoma cells, i.e., MYOCD expression resulted in differentiation and growth inhibition. Repression of MYOCD expression in normal fibroblasts increased their proliferation potential indicating that MYOCD acts as a tumor suppressor (44).

aCGH detected submicroscopic gains and losses from nine chromosomes (Table II, Figure 4A) affecting the copy number status of hundreds of genes. Among them are paralogs of oncogenes MYCN (seven copies), MYC (three copies), as well as the MNT gene (one copy) which codes for a functional antagonist of MYC (45-47). Amplification of MYCN pathway was affected (48-51). Amplification of MYCN is a well-known genetic aberration in alveolar rhabdomyosarcomas and is associated with poor prognosis (52-55). Amplification of MYC was reported in both alveolar and embryonal rhabdomyosarcoma cell lines and tumors (56, 57). Furthermore, only one allele of the tumor suppressor TP53 gene was found in the tumor we examined (Figure 4D).

Mutations of TP53 in alveolar rhabdomyosarcomas carrying the PAX3-FOXO1 fusion are extremely rare but lethal (12, 58, 59). aCGH also detected an extra copy of the FOXO3 gene (6q21) which is a paralog of FOXO1 involved in carcinogenesis (60-63). FOXO3 together with FOXO1 (in 13q14.11), FOX3b (in 17p11.2), FOXO4 (in Xq13.1), and FOXO6 (in 1p34.2) comprise the FOXO family of transcription factors which regulate a plethora of signal pathways; their deregulation plays a key role in cancer (60-63). Both downregulation and overexpression of FOXO3 was reported in cancer and found associated with increased tumor aggressiveness and unfavorable clinical outcome (62). Low expression of FOXO3 was associated with poor prognosis in ovarian cancer, glioma, and clear-cell renal carcinoma (64-66), whereas overexpression of the gene was associated with aggressive phenotype and poor clinical outcome in glioblastoma and hepatocellular carcinoma (67, 68). FOXO3 fusion genes were also reported in leukemias and solid tumors. A t(6;11)(q21;q23) chromosome translocation in leukemia

Figure 5. Molecular genetic analyses of the pediatric rhabdomyosarcoma. (A) Gel electrophoresis showing the amplified PAX3-MYOC DNA fragment with nested PCR using the primer combination PAX3-1374F1/MYOC-2664R1 (lane 1). M: GeneRuler 1 Kb Plus DNA ladder (ThermoFisher Scientific). (B) Partial sequence chromatograms of the cDNA amplified fragment showing the junction position of the PAX3 and MYOC genes (vertical dotted line). In the PAX3-MYOC fusion transcript, exon 7 of PAX3 (nt 1556 in reference sequence NM_181457.4) was fused in frame with exon 12 of MYOC (nt 2487 in reference sequence NM_153604.3). (C) The 600 amino acid (aa) residues of the PAX3-MYOC protein is composed of the first 390 aa (in yellow) from PAX3 (1-390 from NP_852122.1) and the last 210 aa (in green) from MYOC which contains the transactivation domain of MYOC (729-938 from NP_705328.1).
resulted in fusion of the lysine methyltransferase 2A (KMT2A) gene with FOXO3 coding for a KMT2A-FOXO3 chimeric protein (69, 70). In two myoepithelioma-like hyalinizing epithelioid tumors of the hand, fusion of OGT (in Xq13.1, official full name: O-linked N-acetylglucosamine (GlcNAc) transferase) with FOXO3 was reported (71). Recently, the OGT-FOXO1 and OGT-FOXO4 fusion genes were found in tumors with similar pathological features (72, 73) suggesting that fusion of OGT with members of the FOXO family of transcription factors might characterize this type of tumor.

In summary, we present here a pediatric rhabdomyosarcoma carrying a novel PAX3-MYOD fusion gene and extensive genomic imbalances which affect the allelic balance of many genes, among them members of the MYC and FOXO families of transcription factors, as well as the tumor suppressor gene TP53. The result was lethal in the described case.

Conflicts of Interest

The Authors declare that they have no conflicts of interest with regard to this study.

Authors’ Contributions

IP designed and supervised the research, performed molecular genetic experiments and bioinformatics analysis, and wrote the article. LG performed cytogenetic analysis. KA performed pathological examination. FM evaluated the cytogenetic data. SH evaluated the molecular genetic experiments and bioinformatics analysis, and wrote the article. All authors read and approved the final manuscript.

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