CANCER GENOMICS & PROTEOMICS *18*: 723-734 (2021)
doi:10.21873/cgp.20293

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 forkhead box 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 (*NCOA1*, 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-NCOA1*, 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 komité 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 tumorous nodules within the abdominal cavity. Examination of a diagnostic biopsy showed a

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Key Words: Pediatric, rhabdomyosarcoma, chromosome translocation, *PAX3, MYOCD, PAX3-MYOCD* fusion gene.

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 (Sigma 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 and the QuantiFluor ONE dsDNA System (Promega). Promega's human genomic female DNA was used as reference DNA. aCGH was performed using CytoSure array products (Oxford Gene Technology) as previously described (19). Thus, the CytoSure Genomic DNA Labelling Kit was used for labelling of 1 μg of each of tumor and reference DNA, and the CytoSure Cancer +SNP array was used for hybridization. The slides were scanned in an Agilent scanner using the Agilent Feature Extraction Software (version 10.7.3.1). Data were analyzed with the CytoSure Interpret analysis software (version 4.9.40). Annotations were based on human genome build 19.

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)(q?25),-7,del(8)(p21),-15,add(17)(p11),+20,+der(?)t(?;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 loss 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

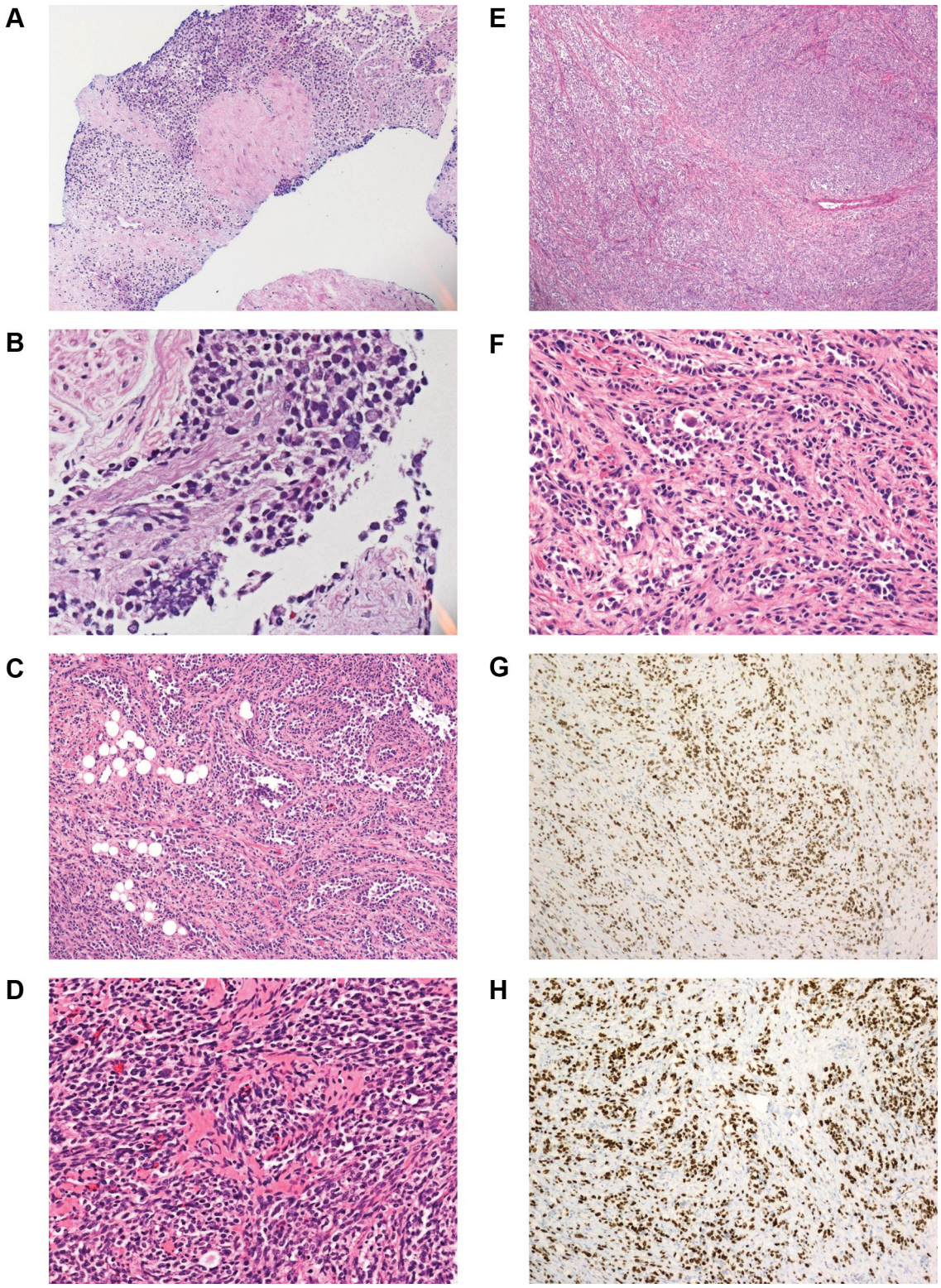

Figure 1. *Microscopic examination of the pediatric rhabdomyosarcoma. (A, B, and C) Tumor with alveolar growth pattern showing loosely arranged tumor cells surrounded by fibrous septa. (D) Tumor with spindle cell pattern. (E) Tumor with mixed growth pattern. (F) Tumor in nests. (G) Transcription factor AP-2 beta showed diffuse and strong expression in tumor nuclei. (H) Myogenin showed strong positive staining in nearly 100% of tumor nuclei.*

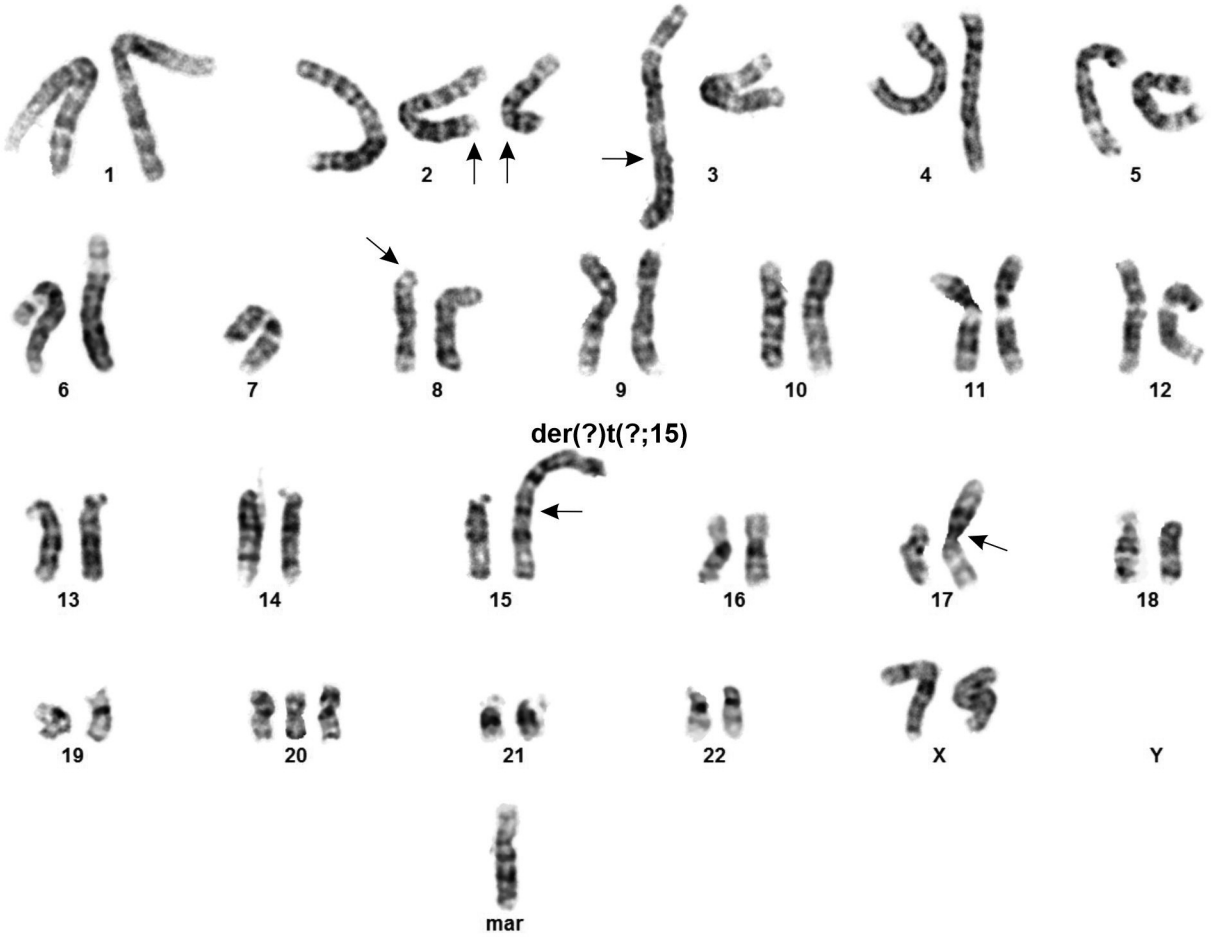

Figure 2. *Cytogenetic examination of the pediatric rhabdomyosarcoma. Representative karyogram showing the abnormal karyotype 48,add(2)(q11),+del(2)(q35),add(3)(q?25),-7,del(8)(p21),-15,add(17)(p11),+20,+der(?)t(?;15)(?;q15),+mar.*

17p12: CAACCCCATGAACCCCACCATTGGCAATGGC CTCTCACCTCAG*CAAATGACCCGGAGTCAGCAGATG GATGAACTCCTGGACGTGC. The presence of this *PAX3-MYOCD* fusion transcript was confirmed by RT-PCR together with Sanger sequencing (Figure 5A and 5B). In the *PAX3-MYOCD* fusion transcript, exon 7 of *PAX3* (nt 1556 in reference sequence NM_181457.4) was fused in frame with exon 12 of *MYOCD* (nt 2487 in reference sequence NM_153604.3).

Based on the *PAX3* reference sequence NM_181457.4/ NP_852122.1 and *MYOCD* reference sequence NM_153604.3/ NP_705832.1, the *PAX3-MYOCD* 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 MYOCD 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 *MYOCD* is described. Because both genes, *PAX3* on 2q36 and *MYOCD* on 17p12, are transcribed from telomere to centromere, a simple balanced translocation should be enough to generate a functional *PAX3-MYOCD* 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-MYOCD* fusion (23). Among the

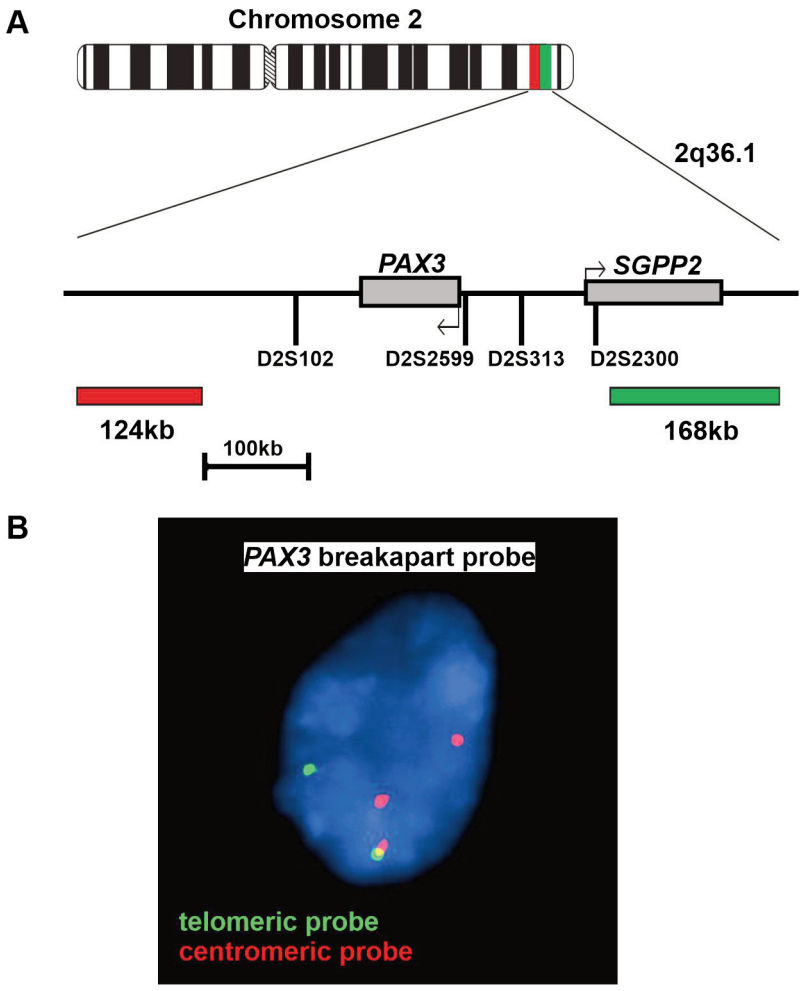

Figure 3. *Fluorescence in situ hybridization (FISH) analysis of the pediatric rhabdomyosarcoma using a commercial PAX3 breakapart probe. (A) Diagram showing the proximal (red) and distal (green) parts of the PAX3 breakapart probe. The neighbor gene sphingosine-1-phosphate phosphatase 2 (SGPP2) and the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

Table I. *Primers used for polymerase chain reaction amplification and Sanger sequencing analyses.*

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

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

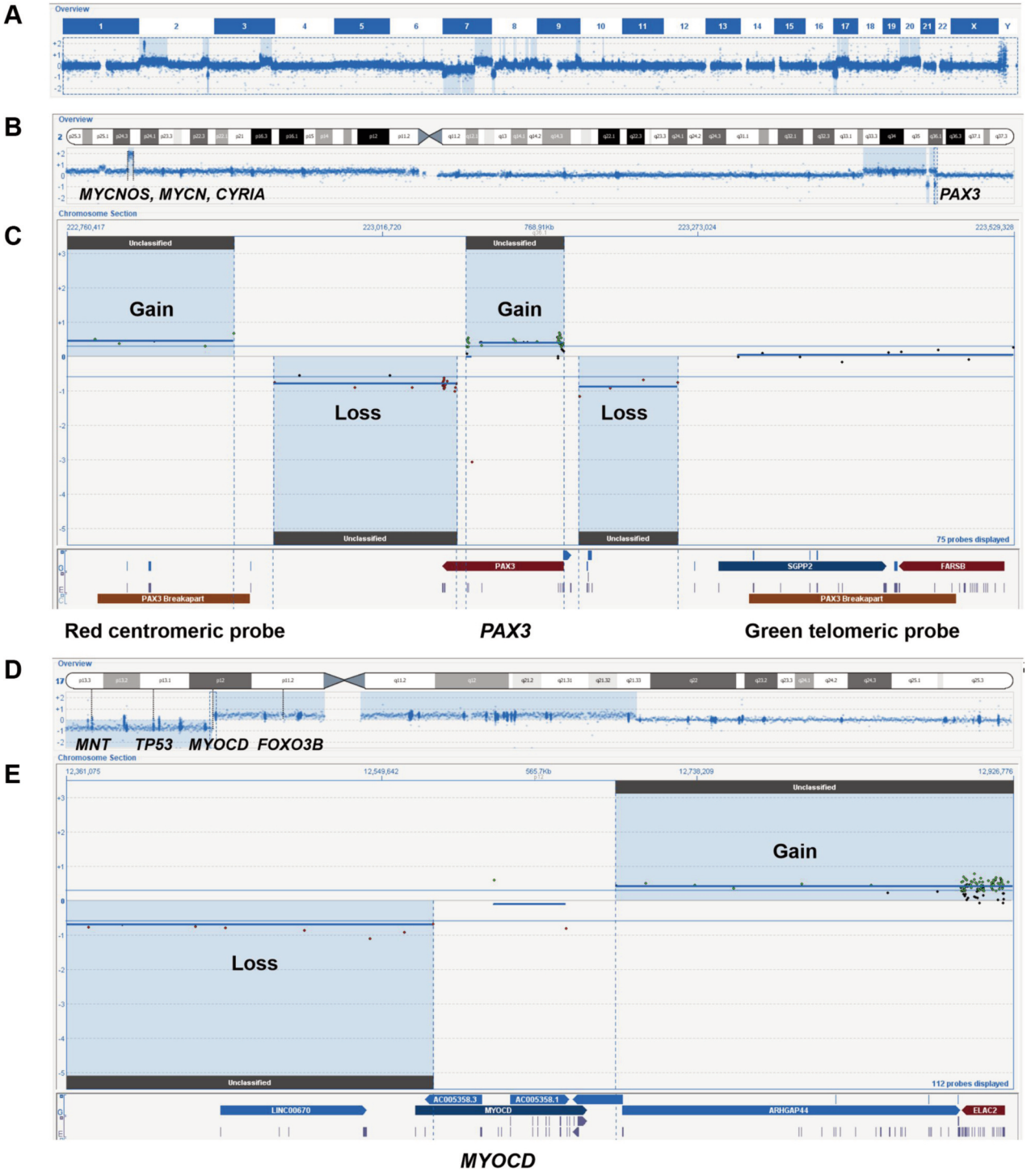

Figure 4. *Array comparative genomic hybridization (aCGH) analysis of the pediatric rhabdomyosarcoma. (A) Whole genome aCGH showing trisomy for chromosome 20 and gains as well as losses from parts of chromosomes 2, 3, 6, 7, 8, 9, 10, 17, and 19. (B) Regions of chromosome 2 with gains and losses. The 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.*

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	Chr2:28080-15666840	15.64Mb	Gain	3
2p24.3-p24.2	Chr2:15991412-17336196	1.34Mb	Gain	7
2p24.2-p11.2	Chr2:17400700-90112889	73.11Mb	Gain	3
2q33.2-q35	Chr2:204598942-220738051	16.14Mb	Gain	3
2q35	Chr2:220737992-221483616	745.62Kb	Loss	1
2q36.1	Chr2:221521686-222895162	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-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	Chr17:12689797-22219743	9.53Mb	Gain	3
17q11.1-q21.33	Chr17:25294244-48947156	23.65Mb	Gain	3
19q13.43	Chr19:57479515-57672410	192.9Kb	Gain	3
20p13-p11.1	Chr20:72367-26257880	26.19Mb	Gain	3
20q11.21-q13.33	Chr20:29453459-62952205	33.5Mb	Gain	3

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

In the *PAX3-MYOCD* 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 TCACGC/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-MYOCD* 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 MYOCD (Figure 5C). *MYOCD* 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 serum response factor with many functional domains, one of which is a transactivation domain at the end of the protein (36, 38, 39). *MYOCD* 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

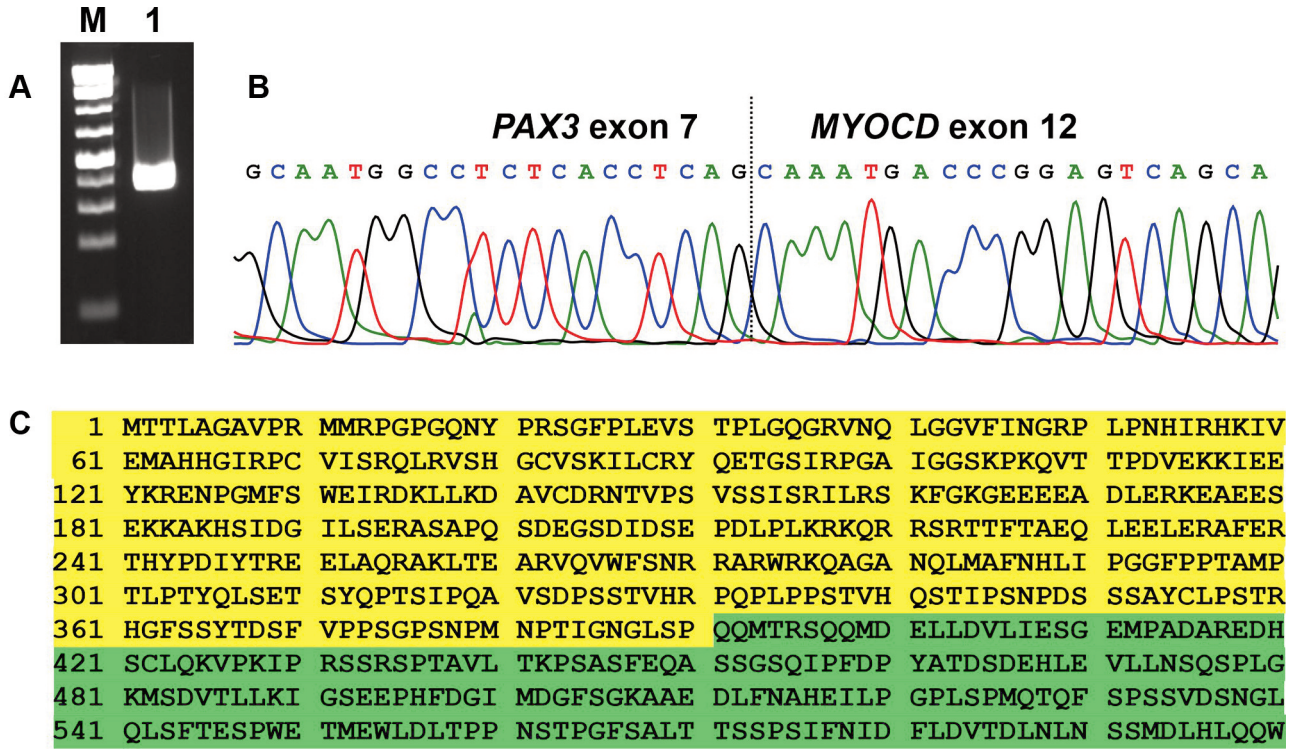

Figure 5. *Molecular genetic analyses of the pediatric rhabdomyosarcoma. (A) Gel electrophoresis showing the amplified PAX3-MYOCD cDNA fragment with nested PCR using the primer combination PAX3-1374F1/MYOCD-2664R1 (lane 1). M: GeneRuler 1 Kb Plus DNA ladder (ThermoFisher Scientific). (B) Partial sequence chromatograms of the cDNA amplified fragment showing the junction position of the PAX3 and MYOCD genes (vertical dotted line). In the PAX3-MYOCD fusion transcript, exon 7 of PAX3 (nt 1556 in reference sequence NM_181457.4) was fused in frame with exon 12 of MYOCD (nt 2487 in reference sequence NM_153604.3). (C) The 600 amino acid (aa) residues of the PAX3-MYOCD 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 MYOCD which contains the transactivation domain of MYOCD (729-938 from NP_705832.1).*

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). Thus, the MYC/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

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-MYOCD* 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 molecular genetic experiments and evaluated the data. ML-I performed pathological examination. ST performed pathological examination. FM evaluated the cytogenetic data. SH evaluated the cytogenetic data, assisted with experimental design, and helped write the article. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by grants from Radiumhospitalets Legater.

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Received July 5, 2021
Revised September 19, 2021
Accepted September 20, 2021