CANCER GENOMICS & PROTEOMICS *20*: 556-566 (2023)
doi:10.21873/cgp.20405

Pathogenetic Dichotomy in Angioleiomyoma

IOANNIS PANAGOPOULOS[1], KRISTIN ANDERSEN[1], MARTA BRUNETTI[1], LUDMILA GORUNOVA[1], ILYÁ KOSTOLOMOV[2], WANJA KILDAL[3], HANNE REGINE HOGNESTAD[4], INGVILD LOBMAIER[4], FRANCESCA MICCI[1] and SVERRE HEIM[1]

[1]*Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;*
[2]*Section for Applied Informatics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;*
[3]*Section for Interphase Genetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;*
[4]*Department of Pathology, Oslo University Hospital, Oslo, Norway*

Abstract. *Background/Aim: Angioleiomyoma is a benign tumor, occurs at any age, and arises most frequently in the lower extremities. Genetic information on angioleiomyomas is restricted to six reported abnormal karyotypes, losses in chromosome 22 and gains in Xq found by comparative genomic hybridization, and mutation analysis of notch receptor 2 (NOTCH2), NOTCH3, platelet-derived growth factor receptor beta (PDGFRB), and mediator complex subunit 12 (MED12) in a few tumors. Herein, we report the genetic findings in another three angioleiomyomas. Materials and Methods: The tumors were examined using G-banding and karyotyping, RNA sequencing, reverse transcription-polymerase chain reaction, Sanger sequencing, and expression analysis. Results: The first tumor carried a t(4;5)(p12;q32) translocation resulting in fusion of the cardiac mesoderm enhancer-associated non-coding RNA (CARMN in 5q32) with the TXK tyrosine kinase gene (TXK in 4p12) leading to overexpression of TXK. To our knowledge, this is the first time that a recurrent chromosome translocation and its resulting fusion gene have been described in angioleiomyomas. The second tumor carried a four-way translocation, t(X;3;4;16)(q22;p11;q11;p13) which fused the myosin heavy chain 11 gene (MYH11 in 16p13) with intergenic sequences from Xq22 that mapped a few kilobase pairs distal to the insulin receptor substrate 4 gene (IRS4), resulting in enhanced IRS4 expression. The third angioleiomyoma carried another rearrangement of chromosome band Xq22, t(X;9)(q22;q32), as the sole cytogenetic aberration, but no material was available for further molecular investigation. Conclusion: Our data, together with previously reported abnormal karyotypes in angioleiomyomas, show the presence of two recurrent genetic pathways in this tumor type: The first is characterized by presence of the translocation t(4;5)(p12;q32), which generates a CARMN::TXK chimera. The second is recurrent rearrangement of Xq22 resulting in overexpression of IRS4.*

Correspondence to: Ioannis Panagopoulos, Section for Cancer Cytogenetics, Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Montebello, PO Box 4954 Nydalen, NO-0424 Oslo, Norway. Tel.: +47 22782362, e-mail: ioannis.panagopoulos@rr-research.no

Key Words: Angioleiomyoma, recurrent genetic pathway, t(4;5)(p12;q32), t(X;3;4;16)(q22;p11;q11;p13), t(X;9), *CARMN::TXK* chimeric gene, chromosome band Xq22, *IRS4* overexpression.

Angioleiomyoma, also known as angiomyoma, vascular leiomyoma or dermal angioma, is a benign tumor believed to arise from the smooth muscle layer (*tunica media*) of subcutaneous blood vessels (1). In the World Health Organization classification of soft-tissue and bone tumors, published in 2020, angioleiomyoma is considered a distinct tumor entity which, together with glomus tumor and myopericytoma, form the group of pericytic (perivascular) tumors (2). Angioleiomyoma accounts for 5% of all benign soft tumors, may occur at any age with a peak incidence between the fourth and sixth decade of life, and may arise anywhere but most frequently in the lower extremities (3). Fewer than 50 angioleiomyomas of the knee have been reported to date (4).

Angioleiomyomas are typically firm, well-circumscribed nodules in which well-differentiated, perivascular smooth-muscle cells are arranged around numerous vascular channels (2). The tumor cells are consistently positive for smooth

Table I. *Angioleiomyomas reported with chromosomal abnormalities.*

Sex/age, years	Location	Karyotype	Reference
F/29	Trunk	46,XX,del(6)(p21p23),del(21)(q21)[12]/46,XX[7]	Heim *et. al.*, 1986 (7)
M/73	Trunk	46~47,XY,-13,+1~2mar[20]/46,XY[2]	Nilbert *et. al.*, 1989 (8)
F/58	Finger	46X,t(X;10)(q22;q23.2)[15]/46,XX[9]	Sonobe, *et al.*, 1996 (9)
F/41	Uterus	46,X,t(X;11)(p11.4;p15)[7]/46,idem,inv(2)(p15q13),t(5;20)(q13;q13.2)[10]	Hennig *et. al.*, 1999 (10)
M/24	Thigh	46,XY,del(6)(q13q23),add(8)(q24),del(19)(q11),+mar	Welborn *et. al.*, 2010 (11)
F/63	Ankle	45,XX,t(4;5)(p12;q33),der(13;15)(q10;q10)	Welborn *et. al.*, 2010 (11)

F: Female; M: male.

Table II. *Clinicopathological and genetic data of the study cases of angioleiomyoma.*

Case	Sex/age, years	Location	Material for genetic analysis	Karyotype
1	M/57	Knee	Resection	46,XY,t(4;5)(p12;q32)[11]/46,idem,der (10)t(3;10)(q11;p15)[2]/46,XY[4]
2	M/57	Foot	Resection	46,Y,t(X;3;4;16)(q22;p11;q11;p13)[7]/ 46,idem,t(1;5)(p36;q11)[3]/46,idem,t(6;15) (q15;q23~24),t(7;13)(q22;q34)[3]
3	M/42	Ankle	Needle biopsy	46,Y,t(X;9)(q22;q32)[13]

F: Female; M: male.

muscle actin (encoded by *ACTA2* on 10q23.31) and the calcium-binding protein calponin 1 (encoded by the *CNN1* gene on 19p13.2). They are variably positive for H-caldesmon, desmin, and vimentin but negative for pre-melanosome protein (also known as HMB45) and estrogen receptor (5, 6).

Genetic information on angioleiomyomas is limited. Abnormal karyotypes have been reported for six angioleiomyomas (Table I) (7-11). Recurrent losses from chromosome 22 and recurrent gains from Xq were found by comparative genomic hybridization (12). Rearrangement of the notch receptor 2 gene (*NOTCH2*) was detected in a single case (13). No mutations in exons 12 and 14 of the platelet-derived growth factor receptor beta gene (*PDGFRB*) were found in nine examined angioleiomyomas (14). A recent study described mutations of *PDGFRB* and *NOTCH3* in 3/11 and 4/11 examined angioleiomyomas, respectively (15). In other studies, no mutations in exon 2 of the mediator complex subunit 12 gene (*MED12*) were found in 18 angioleiomyomas examined (16, 17).

Herein, we report the cytogenetic and molecular genetic findings in three angioleiomyomas and discuss the possible pathogenetic pathways of angioleiomyoma tumorigenesis against the background of these data as well as previously published cases.

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). Written informed consent was obtained from the patients to publication of the case details. The ethics committee's approval included a review of the consent procedure. All patient information has been de-identified.

Tumor description. The patients were three males aged 57, 57, and 42 years (Table II). The tumors were located in the knee, foot, and ankle, respectively. In two cases, resected tumors were used for genetic examinations whereas material from a needle biopsy was used in the third case.

G-Banding and karyotyping. Samples were received and analyzed cytogenetically as part of our diagnostic routine. They were disaggregated mechanically and enzymatically with collagenase II (Worthington, Freehold, NJ, USA). The resulting cells were cultured, harvested, and processed for cytogenetic examination using standard techniques (18, 19). Chromosome preparations were G-banded with Wright's stain (Sigma-Aldrich, St Louis, MO, USA) and examined (18, 19). Metaphases were analyzed and karyograms prepared using a CytoVision computer-assisted karyotyping system (Leica Biosystems, Newcastle upon Tyne, UK). The karyotypes were described according to the International System for Human Cytogenomics Nomenclature (20).

RNA sequencing. Total RNA was extracted from frozen material of the two resected specimens (Table II) adjacent to that used for cytogenetic analysis and histological examination using miRNeasy Mini Kit (Qiagen, Hilden, Germany) and QiaCube automated purification system (Qiagen). The RNA concentration was measured with a QIAxpert microfluidic UV/VIS spectrophotometer (Qiagen). One microgram of total RNA was sent to the Genomics Core Facility (http://genomics.no/oslo/) at the Norwegian Radium Hospital, Oslo University Hospital for high-throughput paired-end RNA-sequencing. The software FusionCatcher and deFuse were

Table III. *Designation, sequence (5´–>3´), and position in reference sequences of the forward (F) and reverse (R) primers used for direct sequencing, i.e. polymerase chain reaction amplification and Sanger sequencing analyses. The forward primers had the M13 forward primer sequence TGTAAAACGACGGCCAGT at their 5'-end. The reverse primers had the M13 reverse primer sequence CAGGAAACAGCTATGACC at their 5'-end.*

Designation: Sequence (5'–>3')	Reference sequence: Position
CARMN-351F1: AAGAACTCTGGAGAAGCAGCCTCC	NR_105059.1: 351-374
CARMN-329F1: GCCATGCTGATGTCAGAGAAGCA	NR_105059.1: 329-351
TXK-130R1: TCGCTTCTGCACTGAACAGCAAC	NM_003328.3: 152-130
TXK-138R1: TCTCATTTGTCGCTTCTGCACTGA	NM_003328.3: 161-138
Seq3-Xq22-F1: AACTATTCGGTGAAATTTCCAAGCC	AL732308.7: 36754-36778
Seq3-16p13-R1: GACCTCCTCCAGGGTAGACAGATTG	NG_009299.1: 38590-38566
Seq3-Xq22-F2: GTGAAATTTCCAAGCCCATTTCTTC	AL732308.7: 36763-36787
Seq3-16p13-R2: TCCAGGGTAGACAGATTGCACAGAA	NG_009299.1: 38583-38559

CARMN: Cardiac mesoderm enhancer-associated non-coding RNA; TXK: TXK tyrosine kinase; 16p13: chromosome band 16p13; Xq22: chromosome band Xq22.

used for detection of fusion transcripts (21-23). For quantification of RNA sequencing data (expression analysis), the program Kallisto was used (24). Quantification was calculated as transcripts per million (TPM) which is a measurement of the proportion of transcripts in the pool of RNA (24). Expression analysis was based on Ensembl release 95 (January 2019).

Reverse transcription-polymerase chain reaction (RT-PCR) and Sanger sequencing analyses. The primers used are described in Table III. One microgram of total RNA was reverse-transcribed using an iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). cDNA corresponding to 20 ng total RNA was used as template in the PCR assay. The quality of the cDNA synthesis was assessed by amplification of a cDNA fragment from the ABL proto-oncogene 1, non-receptor tyrosine kinase (*ABL1*) gene using the primer combination ABL1-91F1/ABL1-404R1 (25). A BigDye Direct Cycle Sequencing Kit was used to perform both PCR and cycle (Sanger) sequencing according to the company's recommendations (ThermoFisher Scientific, Waltham, MA, USA). In case 1, the primer combinations were CARMN-329F1/TXK-130R1 and CARMN-351F1/TXK-138R1; in case 2, the primer combinations were Seq3-Xq22-F1/Seq3-16p13-R1 and Seq3-Xq22-F2/Seq3-16p13-R2.

Sequencing was run on an Applied Biosystems SeqStudio Genetic Analyzer system (ThermoFisher Scientific). The Basic Local Alignment Search Tool (BLAST) was used to compare the sequences obtained by Sanger sequencing with the National Center for Biotechnology Information (NCBI) reference sequences (26). In case 1, the reference sequences were NM_003328.3 for TXK tyrosine kinase (*TXK*) and NR_105059.1 for cardiac mesoderm enhancer-associated non-coding RNA (*CARMN*), which corresponds to transcript variant 1 of the long non-coding RNA.

The sequences obtained by Sanger sequencing in case 2 were compared with sequences from chromosome bands 16p13 and Xq22. On band 16p13, the sequences were NG_009299.1 which is the reference sequence for myosin heavy chain 11 (*MYH11* maps on 16p13.11), and AF001548.1 which is the complete sequence of BAC clone CIT987SK-A-815A9 (on 16p13.11) and contains most of the *MYH11* gene sequence. On band Xq22, the comparison was made with the sequence NC_000023.11, which is chromosome X primary assembly, BX322556.5 (complete DNA sequence of YAC

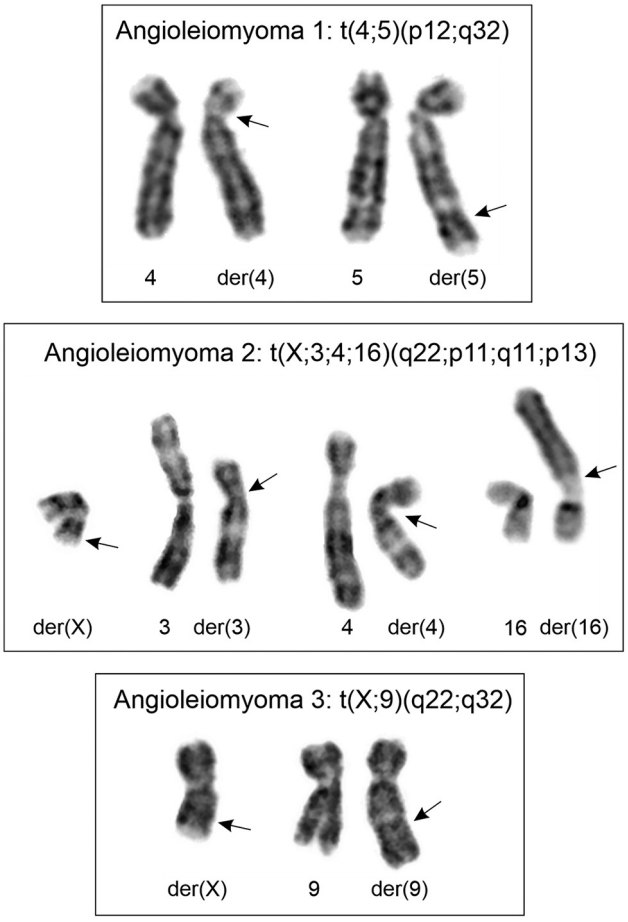

Figure 1. *Cytogenetic analysis of the three angioleiomyomas. Partial karyotypes showing the derivative (der) chromosomes together with the corresponding normal chromosome homologs. For angioleiomyoma 1, der(4) and der(5) together with chromosomes 4 and 5 are shown. For angioleiomyoma 2, der(X), der(3), der(4), and der(16) together with chromosomes 3, 4, and 16 are shown. For angioleiomyoma 3, der(X), der(9), and the normal chromosome 9 are shown. Breakpoint positions are indicated by arrows.*

Table IV. *The cardiac mesoderm enhancer-associated non-coding RNA–TXK tyrosine kinase (CARMN::TXK) fusion transcripts detected in angioleiomyoma of case 1 carrying t(4;5)(p12;q32) after analysis of RNA sequencing data with the FusionCatcher program. Exons are based on the reference sequences NR_105059.1 for CARMN and NM_003328.3 for TXK.*

CARMN (5´-end)	*TXK* (3´-end)	Spanning reads	Fusion sequence
Exon 3	Exon 2	27	GGACCAGAGGAGGGCCAGCAGCAGGCAGCCCGGAGACAGAACT::ATAACACCATCCAGTCGGTTTTCTGTTGCTGCTGTTGCTGTTC
Exon 1	Exon 2	16	TCCAGGCCCCAAGGAGCCACACCTGGACCAGACCCCAGGAAAG::ATAACACCATCCAGTCGGTTTTCTGTTGCTGCTGTTGCTGTTC
Intron 3	Exon 2	14	AATGACCAAGAAACTGGGCCTGAGGATGACAAGAAGCAGAATGGGCCTTG::ATAACACCATCCAGTCGGTTTTCTGTTGCTGCTGTTGCTGTTCAGTGCAG
Exon 3	Exon 3	6	GGACCAGAGGAGGGCCAGCAGCAGGCAGCCCGGAGACAGAACT::ACAAATGAGAACACAGATAAGCCTGAGCACAGATGAAGAGCTT
Exon 3	Intron 1	4	CCTCCTGGGACCAGAGGAGGGCCAGCAGCAGGCAGCCCGGAGACAGAACT::CTTCTGTTAATTTGATTTGATGGTAAGAATCACCTGTTGTCTACTGTATA
Exon 3	Exon 6	4	GGACCAGAGGAGGGCCAGCAGCAGGCAGCCCGGAGACAGAACT::GTGGTACCATAGAAACATTACCAGAAATCAGGCAGAACATCTA
Exon 1	Exon 3	3	TCCAGGCCCCAAGGAGCCACACCTGGACCAGACCCCAGGAAAG::ACAAATGAGAACACAGATAAGCCTGAGCACAGATGAAGAGCTT
Exon 3	Exon 10	3	GGACCAGAGGAGGGCCAGCAGCAGGCAGCCCGGAGACAGAACT::AAAAGTGGGAGATAGATCCATCTGAGTTGGCTTTTATAAAGGA

clone ICI-4AC12), AL732308.7 (complete DNA sequence of BAC clone RP11-199F23), and AL928646.3 (complete DNA sequence of BAC clone CTD-2328D6).

In addition, the BLAST-like alignment tool (BLAT) and the human genome browser at UCSC were used to map the sequences obtained by Sanger sequencing, on the Human GRCh37/hg19 assembly (27, 28).

Results

G-Banding and karyotyping. G-Banding analysis of angioleiomyoma 1 detected two related clones which had the translocation t(4;5)(p12;q32) in common. The karyotype was 46,XY,t(4;5)(p12;q32)[11]/46,idem,der(10)t(3;10)(q11;p15)[2]/46,XY[4]. The t(4;5)(p12;q32) translocation is shown in Figure 1. In angioleiomyoma 2, G-banding analysis detected three related clones which had a four-way translocation, t(X;3;4;16)(q22;p11;q11;p13), in common. The karyotype describing the primary aberration, as well as clonal evolution, was 46,Y,t(X;3;4;16)(q22;p11;q11;p13)[7]/46,idem,t(1;5)(p36;q11)[3]/46,idem,t(6;15)(q15;q23~24),t(7;13)(q22;q34)[3]. The t(X;3;4;16)(q22;p11;q11;p13) translocation is shown in Figure 1. In angioleiomyoma 3, a single translocation between chromosome bands Xq22 and 9q32 was seen in all examined metaphases, yielding the karyotype 46,Y,t(X;9)(q22;q32)[13] (Figure 1). Thus, cases 2 and 3 had rearrangement of chromosome band Xq22 in common.

RNA sequencing, RT-PCR, and Sanger sequencing analyses. *Case 1:* Analysis of the fastq files of RNA sequencing data with the FusionCatcher program detected eight *CARMN::TXK* chimeric transcripts (Table IV). RT-PCR together with Sanger sequencing of the PCR products confirmed the presence of a chimeric transcript in which exon 3 of *CARMN* from 5q32 (nt 419 in reference sequence NR_105059.1) was fused to exon 2 of *TXK* from 4p12 (nt 97 in Reference sequence NM_003328.3). No other fusion transcripts were examined (Figure 2).

Case 2: Analysis of fastq files of the RNA sequencing data with the FusionCatcher program did not detect fusion transcripts compatible with the observed t(X;3;4;16)(q22;p11;q11;p13) (data not shown). However, analysis of RNA sequencing data with the deFuse algorithm detected six transcripts in which sequences of the *MYH11* gene from 16p13 were fused with sequences which mapped on chromosome band Xq22 distal but close to the insulin receptor substrate 4 (*IRS4*) gene (Table V and Figure 3). RT-PCR together with Sanger sequencing of the PCR products for the chimeric transcript designated in Table V as Seq 3 confirmed its presence in the tumor cells (Figure 3). No other fusion transcripts were examined.

Expression analysis. Transcripts from the genes *ACTA2*, *MYH11*, *IRS4* and *TXK* were quantified using the program Kallisto. The results for the transcripts with the highest expression are shown in Table VI. As expected, *ACTA2* expression was high in both angioleiomyomas studied. Both also expressed *MYH11* but the transcripts differed. In angioleiomyoma with t(4;5)(p12;q32), the transcript ENST00000576790.7 had the highest expression. This transcript corresponds to the transcript variant SM2A of *MYH11* (NCBI reference sequence: NM_022844.3). In

Table V. *Chimeric transcripts in angioleiomyoma of case 2 with t(X;3;4;16)(q22;p11;q11;p13) in which sequences of the myosin heavy chain 11 gene (MYH11) gene from 16p13 fused with sequences mapped on chromosome band Xq22 close, but distal to, the insulin receptor substrate 4 (IRS4) gene. Chimeric transcripts were detected with the deFuse algorithm.*

Chimeric transcript	Spanning reads	Fusion sequence
Seq 1	11	AGAACTGCAGACAAGCAGGACAGGAGGGGCTCCCTGGAGCTGGCCCCGTGCAGCCC
		TGAGTTCACTCACCTTGAAGCATGCTCCGGTAGGCCGTGTCTGCGATGGCGTAGATGT
		GAGGCGGCATCTCGTGCCTCTTCTTGCCCTTGTACATGTCGACGATCTTCTCCGAGTAG
		ATGGGCAGGTGTTTATAGGGGTTGACCACCACGCAGAAGAGGCCAGAGTACGTCTGC
		AGACAGAGAACCCAGCTTACTTCCAGACCTCCTCCAGGGTAGACAGATTGCACAGAA
		GCACTCAA::GTTCCTGCTGGATCCTTGCTGGTGCCATCTGGTATCATTAGGTGTGGAAT
		GGAGATACCAGACCCACAAGATGAAAAGTTTTTCTCCTGACCTGTTCCCCCATCACTG
		GATGCCTGCCAGTGGGCTTCCCACTATTATTTCTGCTTTTGCCTCTAGGGGAGGAAAGT
		ACCTGCATGGACCACCTTCTGACACTAGGTGGAGGGGC
Seq 2	5	GTCGTGGCTACTTGGCCAGAAAGGCTTTTGCCAAGAGGCAGCAGCAGCTGACCGCCA
		TGAAGGTGATTCAGAGGAACTGCGCCGCCTACCTCAAGCTGCGGAACTGGCAGTGGT
		GGAGGCTTTTCACCAAAGTGAAGCCACTGCTGCAGGTGACACGGCAGGAGGAGGAG
		ATGCAGGCCAAGGAGGATGAACTGCAGAAGACCAAGGAGCGGCAGCAGAAGGCAGA
		GAATGAGCTTAAGGAGCTGGAACAGAAGCA::TCGCGAAGGCCCGCGGCGGGTGTTGA
		TGCGATGTGATTTCTGCCCAGTGCTCTGAATGTCAAAGTGAAGAAATTCAATGAAGCG
		CGGGTAAACGGCAGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCATCT
		AATTAGTGAGGCGCATGAATGGATGAACGAGATTCCCACTGTCCCTACCTACTATCCAG
		CGAAACCACAGCCAAGGGAACGGACTTGGCGGAATCAGCGGGGAAAGAAGACTCTG
		TTGAGCTTGACTCTAGTCTGGCACGGTGAAGAGACGTGAGAGGTGTAGAATAAGTGG
		GAGGCCCCCGGCGCCCCCCGGTGTCCCCGCGAGGGGTCCAGGGCGGGGTCTGCCGG
		CCCTGCGGGCCGCCGGTGAAATACCACTACTCTGATCGTTTTTTCACTGACCCGGT
Seq 3	42	AGTAACTCCTGGTCCCTCGCAGTGCCTGCCAAACTCCACATTCCTGGCAAGAACTGCA
		GACAAGCAGGACAGGAGGGGCTCCCTGGAGCTGGCCCCGTGCAGCCCTGAGTTCAC
		TCACCTTGAAGCATGCTCCGGTAGGCCGTGTCTGCGATGGCGTAGATGTGAGGCGGC
		ATCTCGTGCCTCTTCTTGCCCTTGTACATGTCGACGATCTTCTCCGAGTAGATGGGCAG
		GTGTTTATAGGGGTTGACCACCACGCAGAAGAGGCCAGAGTACGTCTGCAGACAGAG
		AACCCAGCTTACTTCCAGACCTCCTCCAGGGTAGACAGATTGCACAGAAGCACTCAA
		TATTCTCATGTTAGAGATGGGGAAACTGGAGCTTGGAGTCTTAGTGGAAACCCTGGGC
		CTCACTGATATTCCAG::CATCTATCACTTTCTGGTAGAAGTGTGAATATGCCAGAAACC
		AGCCAAAATGTCAACAAGAGAAGAAGAAATGGGCTTGGAAATTTCACCGAATAGTTA
		ATCAGTTGTGAGTAATTGTTAATGGAAATAGTTA
Seq 4	5	GAAGGAGGAGGAGCTGCAGGCGGCCCTGGCCAGGCTTGACGATGAAATCGCTCAGA
		AGAACAATGCCCTGAAGAAGATCCGGGAGCTGGAGGGCCACATCTCAGACCTCCAGG
		AGGACCTGGACTCAGAGCGGGCCGCCAGGAACAAGGCTGAAAAGCAGAAGCGAGAC
		CTCGGCGAGGAGCTGGAGGCCCTAAAGACAGAGC::TTCGCGAAGGCCCGCGGCGGGT
		GTTGATGCGATGTGATTTCTGCCCAGTGCTCTGAATGTCAAAGTGAAGAAATTCAATGA
		AGCGCGGGTAAACGGCAGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCAT
		CTAATTAGTGAGGCGCATGAATGGATGAACGAGATTCCCACTGTCCCTACCTACTATCCA
		GCGAAACCACAGCCAAGGGAACGGACTTGGCGGAATCAGCGGGGAAAGAAGACTCTGT
		TGAGCTTGACTCTAGTCTGGCACGGTGAAGAGACGTGAGAGGTGTAGAATAAGTGGGA
		GGCCCCCGGCGCCCCCCGGTGTCCCCGCGAGGGGTCCAGGGCGGGGTCTGCCGGCCCTGC
		GGGCCGCCGGTGAAATACCACTACTCTGATCGTTTTTTCACTGACCCGGTGAGGCGGGGGG
Seq 5	5	GGCAAGAACTGCAGACAAGCAGGACAGGAGGGGCTCCCTGGAGCTGGCCCCGTGCAGCC
		CTGAGTTCACTCACCTTGAAGCATGCTCCGGTAGGCCGTGTCTGCGATGGCGTAGATGTGA
		GGCGGCATCTCGTGCCTCTTCTTGCCCTTGTACATGTCGACGATCTTCTCCGAGTAGATGG
		GCA::AGAAAGAACGTATGTTGAAATATTGAGGGCGGGTTCCCCCAATATGCTTCCCCTTCTG
		CCATGATTGTAAGTTTCCTGAGGCTTCCCCAGCCACGCAGAACTGTGAGTCAATTAAACTT
		CCTTTGTTTATAATTTACCCAGTCTCAGGTAGTATCTTTATAGCAGTGTGAAAATGAACTAA
		TAACTAATAACATTAGCTATTTTCTCCTTCTTCTCCTCCTTCTTTTAGGTTAGTCTTGCCAG
		GAGTCTGTCAATTCTTTTCATATTTTCAAATAATCCACTTTTGTCTATAC
Seq 6	22	CCTCGCAGTGCCTGCCAAACTCCACATTCCTGGCAAGAACTGCAGACAAGCAGGACAGGA
		GGGGCTCCCTGGAGCTGGCCCCGTGCAGCCCTGAGTTCACTCACCTTGAAGCATGCTCCGG
		TAGGCCGTGTCTGCGATGGCGTAGATGTGAGGCGGCATCTCGTGCCTCTTCTTGCCCTTGTA
		CATGTCGACGATCTTCTCCGAGTAGATGGGCAGGTGTTTATAGGGGTTGACCACCACGCAGAA
		GAGGCCAGAGTACGTCTGCAGACAGAGAACCCAGCTTACTTCCAGACCTCCTCCAGGGTAGA
		CAGATTGCACAGAAGCACTCAATATTCTCATGTTAGAGATGGGGAAACTGGAGCTTGGAGT
		CTTAGTGGAAACCCTGGGCCTCACTGATATTCCAG::AGGAAGCCATTAAGGAGAATCTTTAA
		TCCATATGAAATTTGAAGTACCAGAAGCATTCAATCATAAACAGTTAAACAGGTAAGTTCTA
		GACCTGATGGTAAATAATATACACATCAGGTGCCACTTACTCAGATATTCTGAAGAAACTCA
		AAGGTGAAATGGTGGTATAACAGCTTCAAGTCATGAACTGTTCCCGGAAACAGAACTAGAA
		GATTTTCAAGATGACTAAAATCCACCAACAAAAACCTGGACCAGAAAAAC

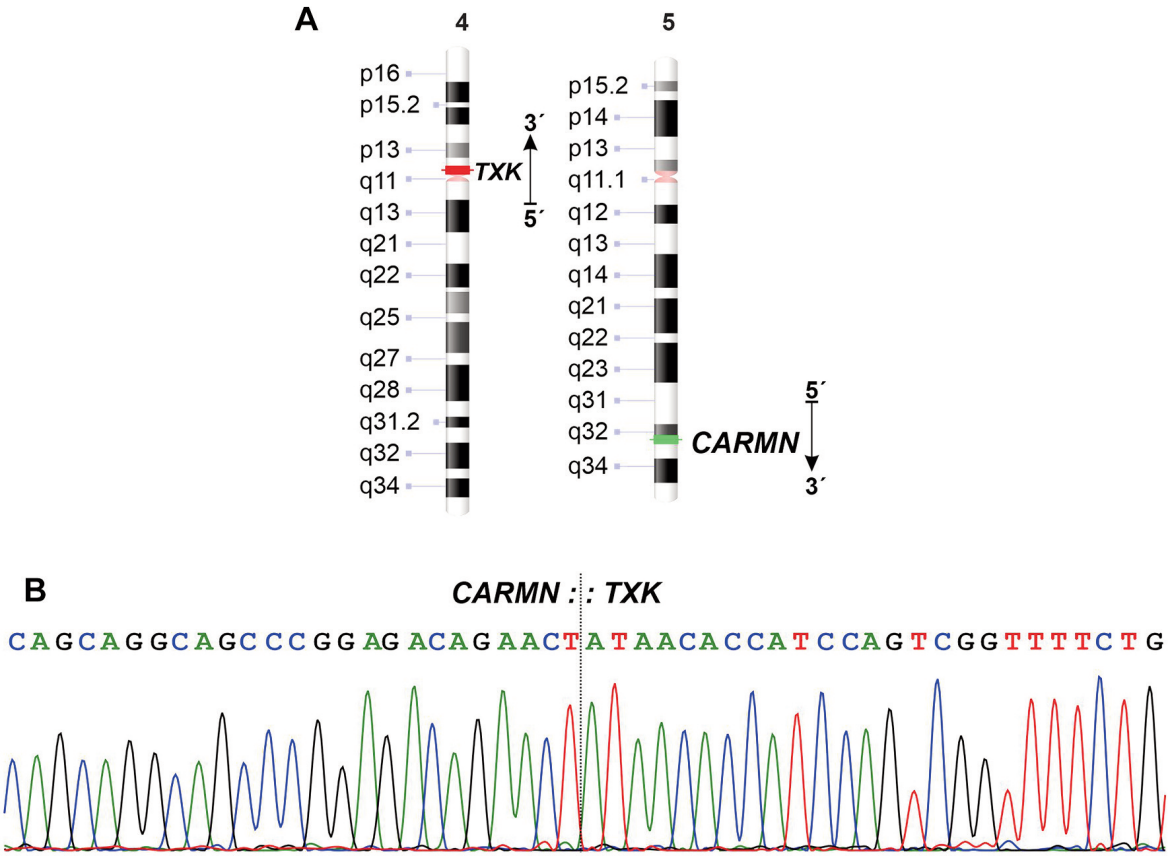

Figure 2. *Molecular genetic examination of the case 1 angioleiomyoma carrying a t(4;5)(p12;q32). A: The positions and transcription orientations (5´->3´) of the tyrosine kinase gene (TXK) and the cardiac mesoderm enhancer-associated non-coding RNA (CARMN) are shown on ideograms of chromosomes 4 and 5, respectively. B: Partial sequence chromatogram showing the junction position in exons 3 of CARMN and 2 of TXK.*

angioleiomyoma with t(X;3;4;16), the transcript ENST00000300036.6 had the highest expression. This transcript corresponds to the transcript variant SM1A of *MYH11* (NCBI reference sequence: NM_002474.3) (Table VI). The *TXK* gene was significantly expressed in the tumor with t(4;5)(p12;q32) but expression was negligible in the tumor with t(X;3;4;16). In contrast, expression of *IRS4* was negligible in the former but high in the latter.

Discussion

In 2010, Welborn and co-workers reported an angioleiomyoma with a t(4;5)(p12;q33) chromosomal aberration (11) (Table I). We herein present an angioleiomyoma with a t(4;5)(p12;q32) chromosomal translocation proving that a t(4;5)(p12;q32-33) translocation is recurrent in these tumors. We also show that the translocation results in a *CARMN::TXK* chimeric gene. To the best of our knowledge, this is the first time that a recurrent chromosomal translocation and its resulting fusion gene have been described in angioleiomyomas.

CARMN is a smooth muscle-specific long noncoding RNA located on chromosome band 5q32 and is transcribed from centromere to telomere (29). *CARMN* is a super enhancer-associated RNA important in differentiation and homeostasis of cardiac precursor cells (30, 31). It is evolutionarily conserved (29-31) and has many alternatively spliced isoforms. These are involved in cardiac cell specification and precursor cell lineage commitment (29-33). The noncoding *CARMN* physically interacts with various proteins affecting cellular biology. The interaction of *CARMN* with proteins SUZ12 and EZH2, which are subunits of the polycomb repressive complex 2, indicates a suppressive role during cardiac differentiation (30). Interaction of *CARMN* with myocardin helps preserve the contractile phenotype of vascular smooth muscle cells (29). Interaction of *CARMN* with the serum response factor regulates the plasticity of vascular smooth muscle cell and contributes to preventing the development of atherosclerosis (34). In the neoplastic context, fusions of *CARMN* with *NOTCH2* were reported in glomus tumors (13, 35-38).

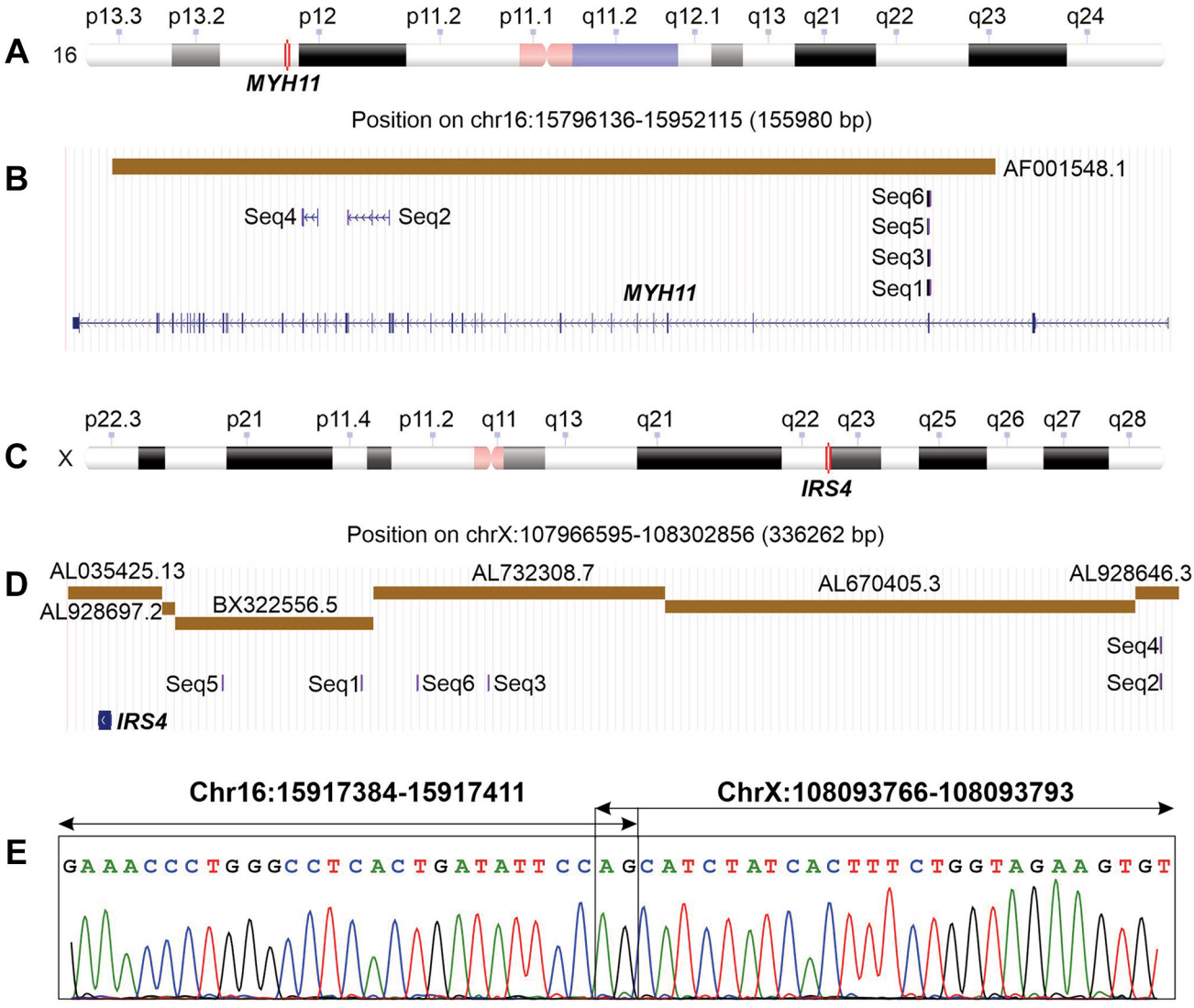

Figure 3. *Molecular genetic examination of the case 2 angioleiomyoma carrying the four-way translocation t(X;3;4;16)(q22;p11;q11;p13). A: Position of the myosin heavy chain 11 gene (MYH11) on chromosome 16. B: Alignment of the six fusion transcripts (Seq 1 to Seq 6) from Table V on chromosome 16 using the BLAT algorithm showing that part of the sequences aligned in the MYH11 locus. C: Position of the insulin receptor substrate 4 gene (IRS4) on chromosome X. D: Alignment of the six fusion transcripts (Seq 1 to Seq 6) from Table V on chromosome X showing that part of the fusion transcripts aligned on Xq22 close and distal to the IRS4 locus. E: Partial sequence chromatogram showing the junction position of the fusion sequence 3 (Seq 3 in Table V) found with the deFuse algorithm.*

The *TXK* gene is located on chromosome band 4p12, is transcribed from centromere to telomere, and codes for a cytoplasmic protein tyrosine kinase which is a member of the TEC subfamily of non-receptor protein-tyrosine kinases (39-42). The genes encoding proteins within the TEC subfamily of non-receptor protein-tyrosine kinases include *TEC* in 4p12, interleukin 2-inducible T-cell kinase (*ITK*) in 5q31-32, BMX non-receptor tyrosine kinase (*BMX*) in Xp22.2, and the Bruton tyrosine kinase gene (*BTK*) in Xq22.1. All the members of the TEC subfamily, *TXK*, *TEC*, *ITK*, *BMX* and *BTK*, are selectively expressed in hematopoietic cells (39-43). *TXK* expression is highly restricted to the Th1/Th0 subset of T-cells (39, 40, 44). The TEX protein regulates expression of the interferon gamma gene (*IFNG* in 12q15) in Th1 cells (40, 44, 45).

The t(4;5)(p12;q32) chromosomal translocation in angioleiomyoma places the coding region of *TXK* under the control of the *CARMN* promoter. In smooth muscle cells, this promoter is normally highly active, causing high-level expression of the gene. Thus, the result of t(4;5)(p12;q32) is

Table VI. *Expression analysis of the genes actin alpha 2, smooth muscle (ACTA2), myosin heavy chain 11 (MYH11), insulin receptor substrate 4 (IRS4), and TXK tyrosine kinase (TXK) in two angioleiomyomas using the program Kallisto: Angioleiomyoma 1 with a t(4;5)(p14;q32) and angioleiomyoma 2 with t(X;3;4;16)(q22;p11;q11;p13). Quantification was calculated as transcripts per million (TPM). Only the transcripts coding for protein are reported. Expression was based on Ensembl 96: April 2019.*

Gene	Transcript	Expression (TPM)	
		Angioleiomyoma 1 t(4;5)	Angioleiomyoma 2 t(X;3;4;16)
ACTA2	ENST00000224784.10	7093.51	8514.48
ACTA2	ENST00000458159.5	61.9219	4.4482
MYH11	ENST00000576790.7	359.253	<0.001
MYH11	ENST00000300036.6	30.7248	759.511
MYH11	ENST00000396324.7	25.1201	<0.001
MYH11	ENST00000452625.7	<0.001	50.1152
TXK	ENST00000506073.1	813.023	0.962064
TXK	ENST00000264316.9	418.419	0.751982
TXK	ENST00000507351.1	139.464	0.0806992
IRS4	ENST00000372129.3	0.018286	294.86

activation of *TXK* and deregulation of its downstream targets. Because both *CARMN* and *TXK* are transcribed from centromere to telomere, the chimeric *CARMN::TXK* gene is predicted to be on the der(5)t(4;5)(p12;q32) chromosome.

The tumors of cases 2 and 3 carried karyotypes in which chromosome band Xq22 was rearranged: Case 2 had a t(X;3;4;16)(q22;p11;q11;p13) whereas case 3 had a t(X;9)(q22;q32). Taking into consideration the previously reported angioleiomyoma with a t(X;10)(q22;q23.2) (9), we conclude that rearrangement of band Xq22 is a recurrent genetic event in angioleiomyoma tumorigenesis. In case 2, where material was available for molecular investigation, we showed that the complex translocation t(X;3;4;16)(q22;p11;q11;p13) resulted in fusion of highly expressed *MYH11* from 16p13 with intergenic sequences from Xq22, generating six chimeric, truncated *MYH11::Xq22* transcripts (Table IV). The Xq22 intergenic sequences map a few kilobase pairs distal to *IRS4*. Thus, the t(X;3;4;16) translocation brings the highly expressed *MYH11* into the close vicinity of *IRS4,* causing high expression of *IRS4*. We hypothesize, indeed believe, that in angioleiomyomas with Xq22 rearrangements [t(X;9)(q22;q32), namely in case 3 of the present report and the tumor carrying t(X;10)(q22;q23.2) reported by Sonobe *et al*. (9)], the salient molecular consequence of the translocations is an increased expression of *IRS4*.

Rearrangements of Xq22 resulting in increased expression of *IRS4* were previously reported in subungual exostosis through the translocation t(X;6)(q22;q13~14) (46, 47), and in a pediatric T-cell acute lymphoblastic leukemia (T-ALL) with t(X;7)(q22;q34) (48). In subungual exostosis, the t(X;6)(q22;q13~14) resulted in rearrangement of collagen type XII alpha 1 chain (*COL12A1* from 6q13~14) whose 5′ end was moved to Xq22, close to the *IRS4* locus (46, 47). The molecular consequence was overexpression of *IRS4*. In T-ALL, t(X;7)(q22;q34) brought *IRS4* under the transcriptional control of T-cell receptor beta locus (from 7q34) regulatory elements (48). Rearrangements of Xq22/*IRS4* and 14q11.2/T-cell receptor alpha-delta locus (*TCRAD)* were also found in a T-ALL carrying the translocation t(X;14)(q22;q11.2) (49). In a pan-cancer study, *IRS4* was overexpressed in 10 different tumor types due to regulatory elements, such as enhancers being rearranged and juxtaposed near to *IRS4* (50, 51). In all the above-mentioned studies, the breakpoint region in Xq22 was within the same area from which Xq22 intergenic sequences were fused to *MYH11* in angioleiomyoma, generating *MYH11::Xq22* chimeric transcripts.

The *IRS4* gene is expressed at very low levels in many normal adult human tissues but highly so in skeletal muscle cells (52, 53) (https://www.ncbi.nlm.nih.gov/gene/8471). The IRS4 protein was found to control the phosphoinositide 3-kinase (PI3K) signaling pathway (50, 51, 54). Overexpression of *IRS4* has been reported in lung cancer (55), hepatocellular carcinoma (56), and colorectal cancer (57). Because overexpression of *IRS4* in human cancer results in deregulation of the PI3K pathway, *IRS4* is a potential therapeutic target (50, 51, 54).

Conclusion

Despite the low number of angioleiomyomas that have been genetically analyzed, a pathogenetic dichotomy can already be discerned: a recurrent t(4;5)(p12;q32) chromosomal translocation resulting in a *CARMN::TXK* chimeric gene in one subset of angioleiomyomas, whereas rearrangement of chromosome band Xq22 resulting in overexpression of the *IRS4* gene is seen in another. Whether other nonrandom

patterns of aberration reflecting alternative pathogenetic pathways exist in this tumor type can only be established when more tumors are examined using appropriate genetic methodologies.

Conflicts of Interest

The Authors declare that they have no potential conflicts of interest.

Authors' Contributions

IP designed and supervised the research, performed molecular genetic experiments and bioinformatics analysis, and wrote the article. KA performed molecular genetic experiments and evaluated the data. MB performed molecular genetic experiments and evaluated the data. LG performed cytogenetic analysis. IK performed bioinformatic analysis. WK performed expression analysis. HRH and IL performed the pathological examination. FM evaluated data. SH evaluated data, assisted with experimental design, and helped write the article. All Authors read and approved the final article.

References

1 Hwang JW, Ahn JM, Kang HS, Suh JS, Kim SM, Seo JW: Vascular leiomyoma of an extremity: MR imaging-pathology correlation. Am J Roentgenol 171(4): 981-985, 1998. DOI: 10.2214/ajr.171.4.9762979

2 WHO Classification of Tumours Editorial Board: Soft tissue and bone tumours. Fifth edition. Lyon, France, International Agency for Research on Cancer, 2020.

3 Ramesh P, Annapureddy S, Khan F, Sutaria P: Angioleiomyoma: a clinical, pathological and radiological review. Int J Clin Pract 58(6): 587-591, 2004. DOI: 10.1111/j.1368-5031.2004.00085.x

4 Montemurro N, Ortenzi V, Naccarato GA, Perrini P: Angioleiomyoma of the knee: An uncommon cause of leg pain. A systematic review of the literature. Interdiscipl Neurosurg 22: 100877, 2020. DOI: 10.1016/j.inat.2020.100877

5 Matsuyama A, Hisaoka M, Hashimoto H: Angioleiomyoma: a clinicopathologic and immunohistochemical reappraisal with special reference to the correlation with myopericytoma. Hum Pathol 38(4): 645-651, 2007. DOI: 10.1016/j.humpath.2006.10.012

6 Mocellin S: Angioleiomyoma. In: Soft Tissue Tumors: A Practical and Comprehensive Guide to Sarcomas and Benign Neoplasms. Mocellin S (ed.). Cham, Switzerland, Springer International Publishing, pp. 85-86, 2021.

7 Heim S, Mandahl N, Kristoffersson U, Mitelman F, Rööser B, Rydholm A, Willén H: Structural chromosome aberrations in a case of angioleiomyoma. Cancer Genet Cytogenet 20(3-4): 325-330, 1986. DOI: 10.1016/0165-4608(86)90091-9

8 Nilbert M, Mandahl N, Heim S, Rydholm A, Willén H, Mitelman F: Cytogenetic abnormalities in an angioleiomyoma. Cancer Genet Cytogenet 37(1): 61-64, 1989. DOI: 10.1016/0165-4608(89)90075-7

9 Sonobe H, Ohtsuki Y, Mizobuchi H, Toda M, Shimizu K: An angiomyoma with t(X;10)(q22;q23.2). Cancer Genet Cytogenet 90(1): 54-56, 1996. DOI: 10.1016/0165-4608(96)00070-2

10 Hennig Y, Caselitz J, Stern C, Bartnitzke S, Bullerdiek J: Karyotype evolution in a case of uterine angioleiomyoma. Cancer Genet Cytogenet 108(1): 79-80, 1999. DOI: 10.1016/s0165-4608(98)00123-x

11 Welborn J, Fenner S, Parks R: Angioleiomyoma: a benign tumor with karyotypic aberrations. Cancer Genet Cytogenet 199(2): 147-148, 2010. DOI: 10.1016/j.cancergencyto.2010.01.022

12 Nishio J, Iwasaki H, Ohjimi Y, Ishiguro M, Kobayashi K, Nabeshima K, Naito M, Kikuchi M: Chromosomal imbalances in angioleiomyomas by comparative genomic hybridization. Int J Mol Med 13(1): 13-16, 2004.

13 Mosquera JM, Sboner A, Zhang L, Chen CL, Sung YS, Chen HW, Agaram NP, Briskin D, Basha BM, Singer S, Rubin MA, Tuschl T, Antonescu CR: Novel *MIR143-NOTCH* fusions in benign and malignant glomus tumors. Genes Chromosomes Cancer 52(11): 1075-1087, 2013. DOI: 10.1002/gcc.22102

14 Agaimy A, Bieg M, Michal M, Geddert H, Märkl B, Seitz J, Moskalev EA, Schlesner M, Metzler M, Hartmann A, Wiemann S, Michal M, Mentzel T, Haller F: Recurrent somatic *PDGFRB* mutations in sporadic infantile/solitary adult myofibromas but not in angioleiomyomas and myopericytomas. Am J Surg Pathol 41(2): 195-203, 2017. DOI: 10.1097/pas.0000000000000752

15 Iwamura R, Komatsu K, Kusano M, Kubo C, Inaba Y, Shiba E, Nawata A, Tajiri R, Matsuyama A, Matoba H, Koga K, Takeda M, Itami H, Hisaoka M: *PDGFRB* and *NOTCH3* mutations are detectable in a wider range of pericytic tumors, including myopericytomas, angioleiomyomas, glomus tumors, and their combined tumors. Modern Pathology 36(3): 100070, 2023. DOI: 10.1016/j.modpat.2022.100070

16 de Graaff MA, Cleton-Jansen A, Szuhai K, Bovée JV: Mediator complex subunit 12 exon 2 mutation analysis in different subtypes of smooth muscle tumors confirms genetic heterogeneity. Human Pathology 44(8): 1597-1604, 2013. DOI: 10.1016/j.humpath.2013.01.006

17 Matsubara A, Sekine S, Yoshida M, Yoshida A, Taniguchi H, Kushima R, Tsuda H, Kanai Y: Prevalence of *MED12* mutations in uterine and extrauterine smooth muscle tumours. Histopathology 62(4): 657-661, 2013. DOI: 10.1111/his.12039

18 Polito P, Dal Cin P, Debiec-Rychter M, Hagemeijer A: Human solid tumors: Cytogenetic techniques. Methods Mol Biol 220: 135-150, 2003. DOI: 10.1385/1-59259-363-1:135

19 Lukeis R, Suter M: Cytogenetics of solid tumours. Methods Mol Biol 730: 173-187, 2011. DOI: 10.1007/978-1-61779-074-4_13

20 McGowan-Jordan J, Hastings RJ, Moore S: ISCN2020: an international system for human cytogenomic nomenclature. Basel, Switzerland, Karger, pp. 164, 2020.

21 Kangaspeska S, Hultsch S, Edgren H, Nicorici D, Murumägi A, Kallioniemi O: Reanalysis of RNA-sequencing data reveals several additional fusion genes with multiple isoforms. PLoS One 7(10): e48745, 2012. DOI: 10.1371/journal.pone.0048745

22 Nicorici D, Satalan H, Edgren H, Kangaspeska S, Murumagi A, Kallioniemi O, Virtanen S, Kikku O: FusionCatcher - a tool for finding somatic fusion genes in paired-end RNA-sequencing data. bioRxiv, 2014. DOI: 10.1101/011650

23 McPherson A, Hormozdiari F, Zayed A, Giuliany R, Ha G, Sun MG, Griffith M, Heravi Moussavi A, Senz J, Melnyk N, Pacheco M, Marra MA, Hirst M, Nielsen TO, Sahinalp SC, Huntsman D, Shah SP: deFuse: an algorithm for gene fusion discovery in tumor RNA-Seq data. PLoS Comput Biol 7(5): e1001138, 2011. DOI: 10.1371/journal.pcbi.1001138

24 Bray NL, Pimentel H, Melsted P, Pachter L: Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol 34(5): 525-527, 2016. DOI: 10.1038/nbt.3519

25 Torkildsen S, Brunetti M, Gorunova L, Spetalen S, Beiske K, Heim S, Panagopoulos I: Rearrangement of the chromatin organizer special AT-rich binding protein 1 gene, *SATB1*, resulting from a t(3;5)(p24;q14) chromosomal translocation in acute myeloid leukemia. Anticancer Res 37(2): 693-698, 2017. DOI: 10.21873/anticanres.11365

26 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J Mol Biol 215(3): 403-410, 1990. DOI: 10.1016/S0022-2836(05)80360-2

27 Kent WJ: BLAT—the BLAST-like alignment tool. Genome Res 12(4): 656-664, 2002. DOI: 10.1101/gr.229202

28 Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D: The human genome browser at UCSC. Genome Res 12(6): 996-1006, 2002. DOI: 10.1101/gr.229102

29 Dong K, Shen J, He X, Hu G, Wang L, Osman I, Bunting KM, Dixon-Melvin R, Zheng Z, Xin H, Xiang M, Vazdarjanova A, Fulton DJR, Zhou J: *CARMN* is an evolutionarily conserved smooth muscle cell-specific LncRNA that maintains contractile phenotype by binding myocardin. Circulation 144(23): 1856-1875, 2021. DOI: 10.1161/CIRCULATIONAHA.121.055949

30 Ounzain S, Micheletti R, Arnan C, Plaisance I, Cecchi D, Schroen B, Reverter F, Alexanian M, Gonzales C, Ng SY, Bussotti G, Pezzuto I, Notredame C, Heymans S, Guigó R, Johnson R, Pedrazzini T: *CARMEN*, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. J Mol Cell Cardiol 89: 98-112, 2015. DOI: 10.1016/j.yjmcc.2015.09.016

31 Ounzain S, Pedrazzini T: Super-enhancer lncs to cardiovascular development and disease. Biochim Biophys Acta 1863(7): 1953-1960, 2016. DOI: 10.1016/j.bbamcr.2015.11.026

32 Plaisance I, Perruchoud S, Fernandez-Tenorio M, Gonzales C, Ounzain S, Ruchat P, Nemir M, Niggli E, Pedrazzini T: Cardiomyocyte lineage specification in adult human cardiac precursor cells *via* modulation of enhancer-associated long noncoding RNA expression. JACC Basic Transl Sci 1(6): 472-493, 2016. DOI: 10.1016/j.jacbts.2016.06.008

33 Plaisance I, Chouvardas P, Sun Y, Nemir M, Aghagolzadeh P, Aminfar F, Shen S, Shim WJ, Rochais F, Johnson R, Palpant N, Pedrazzini T: A transposable element into the human long noncoding RNA *CARMEN* is a switch for cardiac precursor cell specification. Cardiovasc Res 119(6): 1361-1376, 2023. DOI: 10.1093/cvr/cvac191

34 Ni H, Haemmig S, Deng Y, Chen J, Simion V, Yang D, Sukhova G, Shvartz E, Wara AKMK, Cheng HS, Pérez-Cremades D, Assa C, Sausen G, Zhuang R, Dai Q, Feinberg MW: A smooth muscle cell-enriched long noncoding RNA regulates cell plasticity and atherosclerosis by interacting with serum response factor. Arterioscler Thromb Vasc Biol 41(9): 2399-2416, 2021. DOI: 10.1161/ATVBAHA.120.315911

35 Girard N, Marin C, Hélias-Rodzewicz Z, Villa C, Julié C, de Lajarte-Thirouard AS, de Beauce SM, Lagorce-Pages C, Renaud F, Cazals-Hatem D, Guedj N, Cros J, Raffin-Sanson ML, Selves J, Terris B, Fléjou JF, Garchon HJ, Coindre JM, Emile JF: *CARMN-NOTCH2* fusion transcript drives high *NOTCH2* expression in glomus tumors of the upper digestive tract. Genes Chromosomes Cancer 60(11): 723-732, 2021. DOI: 10.1002/gcc.22981

36 Larsen N, Pavlidakey P, Harada S, Prieto-Granada CN: Cutaneous malignant glomus tumor with an *MIR143(CARMN)::NOTCH2* fusion. J Cutan Pathol 50(2): 101-103, 2022. DOI: 10.1111/cup.14351

37 Miao R, Bui MM, Walko C, Mullinax JE, Brohl AS: A malignant glomus tumor of the liver harboring *MIR143-NOTCH2* rearrangement: from diagnosis to management. Cureus 14(10): e30718, 2022. DOI: 10.7759/cureus.30718

38 Papke DJ, Sholl LM, Doyle LA, Fletcher CD, Hornick JL: Gastroesophageal glomus tumors. Am J Surg Pathol 46(10): 1436-1446, 2022. DOI: 10.1097/pas.0000000000001925

39 Haire RN, Ohta Y, Lewis JE, Fu SM, Kroisel P, Litman GW: *TXK*, a novel human tyrosine kinase expressed in T cells shares sequence identity with *Tec* family kinases and maps to 4p12. Hum Mol Genet 3(6): 897-901, 1994. DOI: 10.1093/hmg/3.6.897

40 Kashiwakura J, Suzuki N, Nagafuchi H, Takeno M, Takeba Y, Shimoyama Y, Sakane T: Txk, a nonreceptor tyrosine kinase of the Tec family, is expressed in T helper type 1 cells and regulates interferon gamma production in human T lymphocytes. J Exp Med 190(8): 1147-1154, 1999. DOI: 10.1084/jem.190.8.1147

41 Smith CE, Islam TC, Mattsson PT, Mohamed AJ, Nore BF, Vihinen M: The Tec family of cytoplasmic tyrosine kinases: mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. Bioessays 23(5): 436-446, 2001. DOI: 10.1002/bies.1062

42 Yin Z, Zou Y, Wang D, Huang X, Xiong S, Cao L, Zhang Y, Sun Y, Zhang N: Regulation of the Tec family of non-receptor tyrosine kinases in cardiovascular disease. Cell Death Discov 8(1): 119, 2022. DOI: 10.1038/s41420-022-00927-4

43 Hu Q, Davidson D, Schwartzberg PL, Macchiarini F, Lenardo MJ, Bluestone JA, Matis LA: Identification of Rlk, a novel protein tyrosine kinase with predominant expression in the T cell lineage. J Biol Chem 270(4): 1928-1934, 1995. DOI: 10.1074/jbc.270.4.1928

44 Takeba Y, Nagafuchi H, Takeno M, Kashiwakura J, Suzuki N: Txk, a member of nonreceptor tyrosine kinase of tec family, acts as a Th1 cell-specific transcription factor and regulates IFN-γ gene transcription. J Immunol 168(5): 2365-2370, 2002. DOI: 10.4049/jimmunol.168.5.2365

45 Maruyama T, Nara K, Yoshikawa H, Suzuki N: Txk, a member of the non-receptor tyrosine kinase of the Tec family, forms a complex with poly(ADP-ribose) polymerase 1 and elongation factor 1alpha and regulates interferon-gamma gene transcription in Th1 cells. Clin Exp Immunol 147(1): 164-175, 2007. DOI: 10.1111/j.1365-2249.2006.03249.x

46 Storlazzi CT, Wozniak A, Panagopoulos I, Sciot R, Mandahl N, Mertens F, Debiec-Rychter M: Rearrangement of the *COL12A1* and *COL4A5* genes in subungual exostosis: molecular cytogenetic delineation of the tumor-specific translocationt(X;6)(q13-14;q22). Int J Cancer 118(8): 1972-1976, 2006. DOI: 10.1002/ijc.21586

47 Mertens F, Möller E, Mandahl N, Picci P, Perez-Atayde AR, Samson I, Sciot R, Debiec-Rychter M: The t(X;6) in subungual exostosis results in transcriptional deregulation of the gene for insulin receptor substrate 4. Int J Cancer 128(2): 487-491, 2011. DOI: 10.1002/ijc.25353

48 Karrman K, Kjeldsen E, Lassen C, Isaksson M, Davidsson J, Andersson A, Hasle H, Fioretos T, Johansson B: The t(X;7)(q22;q34) in paediatric T-cell acute lymphoblastic

leukaemia results in overexpression of the insulin receptor substrate 4 gene through illegitimate recombination with the T-cell receptor beta locus. Br J Haematol 144(4): 546-551, 2009. DOI: 10.1111/j.1365-2141.2008.07453.x

49 Kang DH, Kim SH, Jun JW, Lee YW, Shin HB, Ahn JY, Hong DS, Lee YK, Jeon BR: Simultaneous translocation of both *TCR* Loci (14q11) with rare partner loci (Xq22 and 12p13) in a case of T-lymphoblastic leukemia. Ann Lab Med 32(3): 220-224, 2012. DOI: 10.3343/alm.2012.32.3.220

50 Ikink GJ, Hilkens J: Insulin receptor substrate 4 (IRS4) is a constitutive active oncogenic driver collaborating with HER2 and causing therapeutic resistance. Mol Cell Oncol 4(2): e1279722, 2017. DOI: 10.1080/23723556.2017.1279722

51 Weischenfeldt J, Dubash T, Drainas AP, Mardin BR, Chen Y, Stütz AM, Waszak SM, Bosco G, Halvorsen AR, Raeder B, Efthymiopoulos T, Erkek S, Siegl C, Brenner H, Brustugun OT, Dieter SM, Northcott PA, Petersen I, Pfister SM, Schneider M, Solberg SK, Thunissen E, Weichert W, Zichner T, Thomas R, Peifer M, Helland A, Ball CR, Jechlinger M, Sotillo R, Glimm H, Korbel JO: Pan-cancer analysis of somatic copy-number alterations implicates *IRS4* and *IGF2* in enhancer hijacking. Nat Genet 49(1): 65-74, 2017. DOI: 10.1038/ng.3722

52 Sesti G, Federici M, Hribal ML, Lauro D, Sbraccia P, Lauro R: Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. FASEB J 15(12): 2099-2111, 2001. DOI: 10.1096/fj.01-0009rev

53 Schreyer S, Ledwig D, Rakatzi I, Klöting I, Eckel J: Insulin receptor substrate-4 is expressed in muscle tissue without acting as a substrate for the insulin receptor. Endocrinology 144(4): 1211-1218, 2003. DOI: 10.1210/en.2002-220723

54 Hoxhaj G, Dissanayake K, MacKintosh C: Effect of IRS4 levels on PI 3-kinase signalling. PLoS One 8(9): e73327, 2013. DOI: 10.1371/journal.pone.0073327

55 Hao P, Huang Y, Peng J, Yu J, Guo X, Bao F, Dian Z, An S, Xu T: IRS4 promotes the progression of non-small cell lung cancer and confers resistance to EGFR-TKI through the activation of PI3K/Akt and Ras-MAPK pathways. Exp Cell Res 403(2): 112615, 2021. DOI: 10.1016/j.yexcr.2021.112615

56 Guijarro LG, Sanmartin-Salinas P, Pérez-Cuevas E, Toledo-Lobo MV, Monserrat J, Zoullas S, Sáez MA, Álvarez-Mon MA, Bujan J, Noguerales-Fraguas F, Arilla-Ferreiro E, Álvarez-Mon M, Ortega MA: Possible role of IRS-4 in the origin of multifocal hepatocellular carcinoma. Cancers (Basel) 13(11): 2560, 2021. DOI: 10.3390/cancers13112560

57 Sanmartín-Salinas P, Toledo-Lobo MV, Noguerales-Fraguas F, Fernández-Contreras M, Guijarro LG: Overexpression of insulin receptor substrate-4 is correlated with clinical staging in colorectal cancer patients. J Mol Histol 49(1): 39-49, 2018. DOI: 10.1007/s10735-017-9745-0

Received June 1, 2023
Revised July 22, 2023
Accepted July 24, 2023