Gene fusions AHRR-NCOA2, NCOA2-ETV4, ETV4-AHRR, P4HA2-TBCK, and TBCK-P4HA2 resulting from the translocations t(5;8;17)(p15;q13;q21) and t(4;5)(q24;q31) in a soft tissue angiofibroma

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Abstract. We present an angiofibroma of soft tissue with the karyotype 46,XY,t(4;5)(q24;q31),t(5;8;17)(p15;q13;q21)[8]/46,XY,t(1;14)(p31;q32)[2]/46,XY[3]. RNA-sequencing showed that the t(4;5)(q24;q31) resulted in recombination of the genes TBCK on 4q24 and P4HA2 on 5q31 with generation of an in-frame TBCK-P4HA2 and the reciprocal but out-of-frame P4HA2-TBCK fusion transcripts. The putative TBCK-P4HA2 protein would contain the kinase, the rhodanese-like domain, and the Tre-2/Bub2/Cdc16 (TBC) domains of TBCK together with the P4HA2 protein which is a component of the prolyl 4-hydroxylase. The t(5;8;17)(p15;q13;q21) three-way chromosomal translocation targeted AHRR (on 5p15), NCOA2 (on 8q13), and ETV4 (on 17q21) generating the in-frame fusions AHRR-NCOA2 and NCOA2-ETV4 as well as an out-of-frame ETV4-AHRR transcript. In the AHRR-NCOA2 protein, the C-terminal part of AHRR is replaced by the C-terminal part of NCOA2 which contains two activation domains. The NCOA2-ETV4 protein would contain the helix-loop-helix, PAS_9 and PAS_11, CITED domains, the SRC-1 domain of NCOA2 and the ETS DNA-binding domain of ETV4. No fusion gene corresponding to t(1;14)(p31;q32) was found. Our findings indicate that, in spite of the recurrence of AHRR-NCOA2 in angiofibroma of soft tissue, additional genetic events (or fusion genes) might be required for the development of this tumor.

Introduction

Angiofibroma of soft tissue is a recently described benign fibrovascular tumor of unknown cellular origin (1). It arises most commonly in the extremities of middle-aged adults but displays a broad anatomic and age distribution. Microscopically, it is characterized by bland, uniform, probably fibroblastic spindle cell set in an abundant fibromyxoid stroma, with a prominent and highly characteristic vascular pattern composed of innumerable branching, thin-walled blood vessels (1). Cytogenetic knowledge about angiofibroma of soft tissue is based on the analysis of six such tumors of which four showed a balanced t(5;8)(p15;q12) translocation and a fifth tumor showed a three-way t(5;8;8)(p15;q13;p11) (1). Molecular analysis of four tumors carrying the t(5;8)(p15;q12) showed in-frame AHRR-NCOA2 and NCOA2-AHRR tran- script. In the AHRR-NCOA2 protein, the C-terminal part of AHRR is replaced by the C-terminal part of NCOA2 which contains two activation domains. The NCOA2-ETV4 protein would contain the helix-loop-helix, PAS_9 and PAS_11, CITED domains, the SRC-1 domain of NCOA2 and the ETS DNA-binding domain of ETV4. No fusion gene corresponding to t(1;14)(p31;q32) was found. Our findings indicate that, in spite of the recurrence of AHRR-NCOA2 in angiofibroma of soft tissue, additional genetic events (or fusion genes) might be required for the development of this tumor.

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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), and written informed consent was obtained from the patient 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.

Case history. The patient was a 45-year-old male in whom MRI of the abdomen and pelvis showed a 53-mm tumor in the right inguinal region partially surrounding large vessels. The patient had been aware of the lesion for several years. Surgery was performed with removal of the entire tumor including part of the right deep femoral artery with immediate reconstruction of the vessel. The postoperative period was eventless and to date there is no sign of tumor relapse.

The specimen (58x45x45 mm) showed an encapsulated, well-circumscribed tumor with a homogeneous gray/white cut surface. There were no signs of necrosis or bleeding. Routine microscopy showed a tumorous proliferation of small, spindled cells without atypia or mitotic activity (Fig. 1A-C). There were a lot of small, thin-walled blood vessels in the background (Fig. 1A-C). Immunohistochemical examination showed low proliferative activity (MIB1/Ki67 <5%) (Fig. 1D) and the vessels highlighted by the endothelial marker CD34 (Fig. 1E). The clinical setting as well as histopathological features fit well with a diagnosis of angiofibroma of soft tissue (1).

G-banding and karyotyping. Fresh tissue from the tumor was processed for cytogenetic analysis as part of our diagnostic routine. The sample was disaggregated mechanically and enzymatically with collagenase II (Worthington Biochemical Corp., Freehold, NJ, USA). The resulting cells were cultured and harvested using standard techniques. Chromosome preparations were G-banded with Wright stain and examined. The karyotype was written according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013 guidelines (8).

High-throughput paired-end RNA-sequencing. Total RNA was extracted using miRNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Tumor tissue was disrupted and homogenized in QIAzol Lysis Reagent (Qiagen) using a 5-mm stainless steel bead and TissueLyser II (Qiagen). Subsequently, total RNA was purified using QIAcube (Qiagen). The RNA quality was evaluated using the Experion Automated Electrophoresis System (Bio-Rad).
Laboratories, Oslo, Norway). The RNA quality indicator (RQI) was 8.5. Total RNA (3 µg) was sent for high-throughput paired-end RNA-sequencing at the Norwegian Sequencing Centre, Ullevål Hospital (http://www.sequencing.uio.no/). Detailed information about the high-throughput paired-end RNA-sequencing was given elsewhere (9). The software FusionCatcher (10) (https://github.com/ndaniel/fusioncatcher) was used for the discovery of fusion transcripts.

Molecular genetic analyses. The primers used for PCR amplification and Sanger sequencing analyses are listed in Table I. The primer combinations, target fusion transcripts, and results of PCR amplifications are shown in Table II. cDNA was synthesized from 2 µg of total RNA in a 20-µl reaction volume using iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer’s instructions (Bio-Rad laboratories). cDNA was diluted to 100 µl and 2 µl were used as template in subsequent PCR assays. The 25-µl PCR volumes contained 12.5 µl of Premix Taq (Takara Bio Europe SAS, Saint-germain-en-Lay, France), 1 µl of diluted cDNA, and 0.4 µM of each of the forward and reverse primers (Table II). The quality of the cDNA synthesis was examined by amplification of a cDNA fragment of the TBCK gene using the primers TBCK-2558F1 and TBCK-2908R1. The PCRs were run on a C1000 Thermal cycler (Bio-Rad laboratories) with the following cycling for the amplifications: an initial denaturation at 94˚C for 30 sec, 35 cycles of 7 sec at 98˚C, 7 sec at 60˚C, 1 min at 72˚C, and a final extension for 5 min at 72˚C.

The PCR products were analyzed on a QIAxcel Advanced System according to the manufacturer’s instructions (Qiagen). The remaining PCR products were purified using the QIAquick PCR Purification Kit or the QIAquick gel Extraction Kit (both from Qiagen) and direct sequenced using the dyeode procedure with the ABI Prism BigDye Terminator v.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on the Applied Biosystems 3500 Genetic Analyzer sequencing system. The BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/) was used for computer analysis of the sequence data.

Results

Cytogenetic analysis. The G-banding analysis showed that the tumor had two cytogenetically unrelated clones. The first clone, found in eight metaphases, had the t(4;5)(q24;q31) and t(5;8;17)(p15;q13;q21) chromosome aberrations (Fig. 2A). The second, found in two metaphases, had the t(1;14)(p31;q32) abnormality (Fig. 2B). This yielded the following karyotype: 46,XY,t(4;5)(q24;q31),t(5;8;17)(p15;q13;q21),t(1;14)(p31;q32)[2]/46,XY[3].

High-throughput paired-end RNA-sequencing analysis. Using the FusionCatcher software with the FASTQ files obtained

| Name            | Sequence (5’→3’) | Position     | Reference sequence | Gene    |
|-----------------|------------------|--------------|--------------------|---------|
| TBCK-2908R1     | TGGCCTGGATATGGAAGAACTGTCG | 2931-2908    | NM_033115.4        | TBCK    |
| TBCK-2558F1     | CCTGTTGGTGGACATCCGGAATAG  | 2558-2581    | NM_033115.4        | TBCK    |
| P4HA2-785R1     | AGCCAGTGGACCCTGAGCATCAG  | 807-785      | NM_004199.2        | P4HA2   |
| P4HA2-33F1      | CCGCGGGAGGTTTCTGGAAAC | 33-52        | NM_00142598.1      | P4HA2   |
| NCOA2-intr14-R1 | CACCATGTCAGACTGCTGGCTC  | 71106777-71106799 | NC_018919.2 | NCOA2   |
| NCOA2-3364R1    | TCACTCGGAGACTCAGTCGAGG | 3386-3364    | NM_006540.2        | NCOA2   |
| NCOA2-2858F1    | CTGGACCTTTCCCACACATCAGAA | 2858-2881    | NM_006540.2        | NCOA2   |
| ETV4-1496R1     | GGGGCTCTCCTCACAAGTGGGAC | 1517-1496    | NM_001986.2        | ETV4    |
| ETV4-863F1      | TGGGATCAATGCGGGAGCAGATAC | 863-884    | NM_001986.2        | ETV4    |
| AHRR-1932R1     | TGCAGGGTGAAAGGGGTGCA   | 1952-1932    | NM_020731.4        | AHRR    |
| AHRR-1503F1     | AGCAAGGACCTGGGAGATGTC  | 1503-1523    | NM_020731.4        | AHRR    |
| AHRR-1425F1     | TGTGTCAGGGACATTTCAGGAA | 1425-1447    | NM_020731.4        | AHRR    |
| EGF77-353F1     | ACCCCAAAAGCCACATCGTAGCC | 353-375    | NM_016215.4        | EGF7    |
| MCF2L-3271R1    | GCCCACGACCTGTTATATTCCGT | 3293-3271    | NM_024979.4        | MCF2L   |
| CYP1B1-132F1    | TCAAGCTGTTGAGGAAACCTCGA | 132-154     | NM_00104.3         | CYP1B1  |
| CLU-1164R1      | GACCTGGAGGATTCTGCGAC   | 1185-1164    | NM_001831.3        | CLU     |

| Primer combination | Target fusion transcripts | Results    |
|--------------------|--------------------------|------------|
| P4HA2-33F1/TBCK-2908R1 | P4HA2-TBCK          | Positive   |
| TBCK-2558F1/P4HA2-785R1 | TBCK-P4HA2         | Positive   |
| AHRR-1503F1/NCOA2-intr14-R1 | AHRR-NCOA2        | Positive   |
| AHRR-1425F1/NCOA2-3364R1 | AHRR-NCOA2        | Positive   |
| ETV4-863F1/AHRR-1932R1 | ETV4-AHRR         | Positive   |
| NCOA2-2858F1/ETV4-1496R1 | NCOA2-ETV4       | Positive   |
| EGF77-353F1/MCF2L-3271R1 | EGF7-MCF2L       | Negative   |
| CYP1B1-132F1/CLU-1164R1 | CYP1B1-CLU        | Negative   |
from the Norwegian Sequencing Centre, Ullevål Hospital (http://www.sequencing.uio.no/), 39 potential fusions were found: 28 fusions were described as readthrough short-distance fusions and 5 as pseudogenes (Table III). Among the other fusions, the program detected the $P4HA2$ and the reciprocal $TBCK$-$P4HA2$. According to the UCSC Genome Browser on Human, Feb. 2009, (GRCh37/hg19) assembly (http://genome-euro.ucsc.edu/cgi-bin/hgGateway), $P4HA2$ maps on chromosome subband 5q31.1 and $TBCK$ on band 4q24. Thus, the two fusions $P4HA2$-$TBCK$ and the reciprocal $TBCK$-$P4HA2$ most probably were the result of the balanced chromosome translocation t(4;5)(q24;q31). FusionCatcher also detected $AHRR$-$NCOA2$ and $ETV4$-$AHRR$ which correspond to the three-way t(5;8;17)(p15;q13;q21) found in the tumor. The three genes $AHRR$, $NCOA2$, and $ETV4$ map to chromosome subbands 5p15.33, 8q13.3, and 17q21.31, respectively (https://genome.ucsc.edu/). In the three-way t(5;8;17), the moving of 5p15 to 8q13 generated the $AHRR$-$NCOA2$ fusion whereas the translocation of 17q21 to 5p15 generated the $ETV4$-$AHRR$. We assume that the moving of 8q13 to 17q21 would have generated an $NCOA2$-$ETV4$ fusion but no such fusion was, for unknown reasons, detected by FusionCatcher.

Molecular genetic confirmation of fusions. PCR with the primers TBCK-2558F1 and TBCK-2908R1 amplified a cDNA indicating that the synthesized cDNA was of good quality.

**Discussion**

The examined angiofibroma of soft tissue carried the recurrent $AHRR$-$NCOA2$ fusion transcript but lacked the reciprocal $NCOA2$-$AHRR$. This finding supports the initial suggestion that $AHRR$-$NCOA2$ is the pathogenetically significant fusion transcript in tumors carrying a t(5;8)(p15;q12) (2,3). While we were examining the current tumor, a report was published describing 13 cases of angiofibroma of soft tissue with an $AHRR$-$NCOA2$ but with only eight of them carrying the reciprocal $NCOA2$-$AHRR$ (11). Current data therefore agree that the $AHRR$-$NCOA2$ fusion gene is recurrent in angiofibroma of
soft tissue (2,3,11), present case) and indicate that this is the pathogenetically crucial outcome of the t(5;8).

Using FISH on formalin-fixed, paraffin-embedded specimens, Sugita et al (5) found that 16-36% of the tumor cells showed NCOA2 rearrangement. A fairly small proportion of NCOA2 gene rearrangement-positive cells (4-12 split signals per 50 tumor cell nuclei) was recently reported also by Yamada et al (11). The split signals were mostly detected in relatively large, spindle-shaped nuclei, indicating that these were the ones belonging to the neoplastic parenchyma (11).

The present tumor had two cytogenetically unrelated clones: one (eight metaphases) with the translocations t(4;5)(q24;q31) and t(5;8;17)(p15;q13;q21) and another (2 cells) with t(1;14)(p31;q32) as the sole chromosome abnormality. Thus, our data not only are in agreement with previous observations that only a fraction of tumor cells carry the NCOA2 gene rearrangement, but also demonstrate genetic heterogeneity of uncertain pathogenetic significance within the tumor. Although no fusion gene was found corresponding to t(1;14)(p31;q32), this should not lead us to conclude that

| 5'-Partner gene | 3'-Partner gene | Fusion description | Fusion sequence |
|-----------------|-----------------|--------------------|-----------------|
| PCDP1           | TMEM177         | Readthrough         | ATTCCTAGAATGGAAGTCACCAGTGA*Tgaaggggacaaggcagagagttga |
| MIR155HG        | JAM2            | Readthrough         | CAGAGGAGACGGCTCTTGGCAGCTGAG*atcatagcctatgtgttgctcg |
| GOLT1A          | KISS1           | Readthrough         | ATGATCTCCATATCGGAATGCCAGA*ctcaaggaactaggccacctgcc |
| SHIS9A          | U91319.1        | Readthrough         | AAGTAGCCGCTCTTATGAAGACATGCG*tgaagggaggatggatggacttgc |
| VPS45           | PLEKH01         | Readthrough         | GCACCCACGTGCACACAGCAAGGA*ggacacagcaggggagacttgc |
| P4HA2           | TBCK            | Readthrough         | AACCCGGGAGGCTGGATTTGGTTAG*tttggattttggacttgc |
| TBCB            | P4HA2           | Readthrough         | GCATGTGGGAAAAACACAGCAGTAG*acactctctagttgacacttgaggaag |
| ADCK4           | NUMBL           | Readthrough         | TCCAGCTCTCAGTGTGGAGAGAC*aggggcgcagcagctgtgggag |
| ETV4            | AHRR            | Readthrough         | AAGAGTCAGAAGATGACTTGGTGGATG*gggcctgttggatttttggacttgc |
| FOSB            | PPM1N           | Readthrough         | TCCACCCACGGCGCAGCTGCCAG*ggagggagactttcctggtcggcag |
| MFSD7           | ATP5            | Readthrough         | GGGAGGATCCATTGACTGGAACAG*taacattaacctgggagacttgc |
| DYPY19L2        | DYPY19L2P2      | Pseudogene          | TTCTCTATTCTTGGATTAGCCTGAG*ttttggctttttggacttgc |
| DYPY19L2P2      | DYPY19L2        | Pseudogene          | TTCTCTATTCTTGGATTAGCCTGAG*ttttggctttttggacttgc |
| MATR3           | PAIP2           | Readthrough         | CCGGCTCCGCTCGTGGAGAGAG*tttgaagggcagcagcagcagcag |
| LINC00893       | LINC00894       | Antisense           | AGGAAGCAGAAGTGGAGAGATGAG*aggggattttggctttttggacttgc |
| PTPRG           | C3orf14         | Readthrough         | GAGGCTGGTGGATCATCACAGATG*gggaaggctttttggacttgc |
| SICX3           | AC012354.6      | Readthrough         | AGACCCGCGACTCTCATTTCCTCG*Acaaggggattttggacttgc |
| CTBS            | GNG5            | Readthrough         | GCGGGCTCTTTAATATATATATA*ttttggctttttggacttgc |
| CYP1B1          | CLU             | Readthrough         | CGAGTTGGGAGTTAAGGGCTTGGACAG*tttgaagggcagcagcagcagcag |
| ZBTB16          | NNMT            | Readthrough         | CGGAGACCCCTGACGCTCACTTAG*tttgaagggcagcagcagcagcag |
| KB-1507C5.4     | ATP6V1C1        | Readthrough         | TCACTGTTGTAATTCACAAAGAG*taaaggctgttggacttgc |
| PPP1R21         | STON1           | Readthrough         | TGAACACAAATGATTCGACTGAA*ttttggactttttggacttgc |
| SUZ12           | SUZ12P          | Pseudogene          | GAAAATCACCAGAACACATCAAGAG*tttgaagggcagcagcagcagcag |
| SUZ12P          | SUZ12           | Pseudogene          | AAATGCAAGTTGATTGAAAAACAGCAGAGTCGATG*gggcctgttggatttttggacttgc |
| TPREM3          | TREML1          | Readthrough         | CTGCTCATCTTCTTCTTCACAG*tttgaagggcagcagcagcagcag |
| TRIM2           | MND1            | Readthrough         | CGACTGAGGAAACACAGGACTGACAG*tttgaagggcagcagcagcagcag |
| AC015977.6      | CIB4            | Readthrough         | GGTCTGGCCAAGGACCTGACGGATG*gggcctgttggatttttggacttgc |
| AHRR            | NCOA2           | GCAAGGTGACTGTCGGCAGCGG*AAGTAGGGTGCAGCAGCAGCAG |
| CHD4            | NOP2            | Readthrough         | GGCCACCCGAATCTACCACACAGCAG*tttgagggcagcagcagcagcag |
| EGFL7           | MCF2L           | Readthrough         | GGGATGACCTTGTTCTCGCAGCAG*tttgaagggcagcagcagcagcag |
| GPR65           | LINC01146       | Readthrough         | AAACACATCACGGAGGAAGGATATAG*ttttggctttttggacttgc |
| HERC3           | FMAM3A-A51      | Readthrough         | AATCTTCAAGTATAAGAAGATCAGCATT*ttttggctttttggacttgc |
| KB-1572G7.2     | AP000347.4      | Readthrough         | ACACCCACTTCTTCTGTTGCCAACAG*ttttggctttttggacttgc |
| LCAT            | PSMB10          | Readthrough         | TGAATAAAGACCTTCCCTTCTTCTACC*ttttggctttttggacttgc |
| LPS1            | NTNS3          | Short-distance      | CCGGCTCTTACTGGGTTCCTCGAG*ttttggctttttggacttgc |
| LTP2P2          | NPC2            | Readthrough         | GATGCCGCCCAATGGCCGCTCGTAG*ttttggctttttggacttgc |
| OSBP2L2         | ADRM1           | Readthrough         | GGTTCAGTGCTGACAGGTACCCAGAG*tttgaagggcagcagcagcagcag |
| PARL            | MAP6D1          | Readthrough         | ACCTGGGAGGAGCTCTTTGGGAAT*tttgaagggcagcagcagcagcag |
| PTP3N22         | RSN1            | Readthrough         | AACTCCAGCTATTCTTGAGTTTG*ttttggctttttggacttgc |

Table III. Fusion transcripts detected using FusionCatcher.

The present tumor had two cytogenetically unrelated clones: one (eight metaphases) with the translocations t(4;5)(q24;q31) and t(5;8;17)(p15;q13;q21) and another (2 cells) with t(1;14)(p31;q32) as the sole chromosome abnormality. Thus, our data not only are in agreement with previous observations that only a fraction of tumor cells carry the NCOA2 gene rearrangement, but also demonstrate genetic heterogeneity of uncertain pathogenetic significance within the tumor. Although no fusion gene was found corresponding to t(1;14)(p31;q32), this should not lead us to conclude that
the translocation was pathogenetically unimportant. The t(1;14)(p13;q32) chromosome aberration may exert its influence through a position effect causing deregulation of a gene in the proximity of the breakpoints. Alternatively, the current methodology may be unable to detect a fusion gene as has been demonstrated (9).

So far, three types of \textit{AHRR}-\textit{NCOA2} fusion transcripts have been described: in the first type, exon 9 of \textit{AHRR} is joined with exon 16 of \textit{NCOA2}, the second type shows exon 10 of \textit{AHRR} being joined to exon 14 of \textit{NCOA2}, and in the third type there is an insertion of an intronic sequence from the \textit{NCOA2} gene between exon 9 of \textit{AHRR} and exon 14 of \textit{NCOA2} (2,11). In the present angiofibroma of soft tissue, two novel fusion transcripts were found with different fusion positions from those previously described: a fusion transcript in which nt 1670 (sequence with accession no. NM_020731) from exon 12 of the \textit{AHRR} gene was fused with a sequence from intron 14 of \textit{NCOA2} and a transcript in which nt 1553 (also from exon 12) of \textit{AHRR} was fused to exon 15 of \textit{NCOA2} (sequence with accession no. NM_006540.2). The resulting putative \textit{AHRR}-\textit{NCOA2} protein would be similar to those reported (2) in as much as the C-terminal part of \textit{AHRR} is putative \textit{AHRR}-\textit{NCOA2} protein would be similar to those previously described: a fusion transcript (2,11). In the present angiofibroma of soft tissue, the \textit{AHRR} gene was fused with a sequence from intron 14 of \textit{NCOA2} and a transcript in which nt 1533 would contain the ETS DNA-binding domain of \textit{ETV4} (Fig. 4).

\textit{ETV4} was reported to contribute the 3'-part of the oncogenic protein in the subset of Ewing's sarcomas characterized by a t(17;22)(q12;q12) translocation (22,23). The EWSR1-\textit{ETV4} protein, in which the N-terminal part of EWSR1 is fused to the ETS DNA-binding domain of \textit{ETV4}, has an oncogenic potential similar to that of the EWS1-FL1, EWS1-ERG, EWS1-FEV, and EWSR1-ETV1 fusion proteins which may also be found in Ewing's sarcoma (24). The \textit{ETV4} gene was also described as the 3'-partner in fusion genes found in prostate carcinoma (25-27). \textit{ETV4} was found to fuse with the \textit{TMPrss2}, \textit{KLK2}, \textit{Canti1}, and \textit{DDX5} (25-27). All these fusions genes, \textit{TMPrss2}-\textit{ETV4}, \textit{KLK2-ETV4}, \textit{Canti1-ETV4}, and \textit{DDX5-ETV4}, contain (like the present \textit{NCOA2-ETV4}) the part of \textit{ETV4} coding for the ETS DNA-binding domain.

The chromosome translocation t(4;5)(q14;p11) generated the \textit{P4HA2-TBCK} and \textit{TBCK-P4HA2} fusion transcripts. \textit{P4HA2-TBCK} does not encode any functional protein, whereas \textit{TBCK-P4HA2} encodes a chimeric 1,355-amino acid protein. \textit{TBCK-P4HA2} would contain the first 794 out of 830 amino acids of the \textit{TBCK} protein (accession no. NP_149106.2), 6 amino acids from the untranslated region of exon 2 of \textit{P4HA2} (accession no. NM_004199.2), and the entire 535 amino acid-\textit{P4HA2} protein (NP_004190.1). The function of this putative chimeric protein is difficult to predict since it would contain both the protein kinase domain, the Rhodanese-like domain, and the Tre-2/Bub2/Cdc16 (TBC) domain of \textit{TBCK} together with the \textit{P4HA2} protein which is a component of the prolly 4-hydroxylase. The \textit{TBCK} protein is thought to play a role in actin organization, cell growth, and cell proliferation by regulating the mammalian target of the rapamycin (mTOR) signaling pathway. This protein may also be involved in the transcriptional regulation of the components of the mTOR complex (http://www.ncbi.nlm.nih.gov/gene/93627). Depletion of \textit{TBCK} significantly inhibits cell proliferation, reduces cell size, and disrupts the organization of actin but not microtubule. Knockdown of \textit{TBCK} induces a significant decrease in the protein levels of components of mTOR complex (mTORC), and suppresses the activity of mTOR signaling, but not the MAPK or PDK1/Akt pathway (28).

The protein encoded by the \textit{P4HA2} gene is one of several different types of a subunit of the prolly 4-hydroxylase and provides the major part of the catalytic site of the active enzyme (http://www.ncbi.nlm.nih.gov/gene/8974). In collagen and related proteins, prolly 4-hydroxylase catalyzes the formation of 4-hydroxyproline that is essential to the proper three-dimen-sional folding of newly synthesized procollagen chains. In breast cancer, \textit{P4HA2} was shown to promote progression and metastasis by regulating collagen deposition (29). In squamous cell carcinoma of the oral cavity, \textit{P4HA2} was identified as a metastasis associated protein (30).

In spite of the now repeatedly documented recurrence of \textit{AHRR}-\textit{NCOA2} in angiofibroma of soft tissue [present
our findings indicate that also additional genetic events, some of which lead to fusion genes, may be important in tumor development. Worthy of mention is that of the eight hitherto cytogenetically reported tumors, including the present case, three had three-way translocations (1-3). What lies behind this highly unusual feature is unknown. Obviously, more such tumors must be studied cytogenetically and molecularly before all important aspects of their pathogenesis are laid bare.

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