Abstract. Background/Aim: Angiolipoma is a rare benign soft tissue tumor composed of mature adipocytes and blood vessels. Genetic information on angiolipomas is scarce. With the single exception of one tumor which carried a t(X;2)(p22;p12), all angiolipomas hitherto investigated cytogenetically had normal karyotypes. Materials and Methods: G-banding chromosome analysis was performed on three short-term cultured angiolipomas. Fluorescence in situ hybridization (FISH) analysis using a commercially available RB1 deletion probe was also done. Results: All three angiolipomas had abnormal karyotypes with loss or structural rearrangement of chromosome 13. The first tumor had the karyotype 46,XY,-6,del(13)(q14),+mar[cps5], the second had 44–45,XY[1;10](p21–22;q24;q24),-13[cps5], and the third karyotype was 43,XX,t(13;22;17)(q12;q13; q22–23)[14]. FISH analysis showed heterozygous and homozygous deletion of the RB1 probe in case 2 and 3, respectively. FISH analysis failed in case 1. Conclusion: Chromosome 13 was consistently involved in all three angiolipomas.

Angiolipoma is a rare benign soft tissue tumor composed of mature adipocytes and blood vessels (1). Though first described by Bowen in 1912 (2), it was established as a distinct entity in 1960 by Howard and Helwig (3). Angiolipomas typically arise in the subcutaneous tissue of the extremities and trunk of young adults (1) and present as tender to painful single or multiple subcutaneous nodules. On gross pathology, angiolipoma is a circumscribed, yellow-red nodular mass with high fat content and blood vessel proliferation. Microscopically, the tumor consists of mature adipocytes and clusters of small vessels, predominantly in the periphery, and often with fibrin microthrombi in the vessel lumen. The relative proportion of adipose tissue and vessels varies from case to case but mostly the adipose tissue predominates (1).

Genetic information on angiolipomas is scarce (1). With the single exception of one tumor which had the abnormal karyotype 46,Y,t(X;2)(p22;p12), all angiolipomas investigated so far had normal karyotypes (4-6).

We present, here, cytogenetic data on three angiolipomas, all of which turned out to have abnormal karyotypes with involvement of chromosome 13.

Materials and Methods

Ethics statement. The study was approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (REK Sør-Øst: http://helseforskning.etikkom.no) and written informed consent was obtained from the patients. The ethics committee’s approval included a review of the consent procedure. All patient information has been anonymized.

Patients and tumors. Angiolipomas from three patients were studied. Data concerning patients’ gender and age as well as tumor location and size are shown in Table I. Figure 1 shows a microscopic picture after hematoxylin and eosin staining of the angiolipoma of case 1. The pathologic findings were similar in cases 2 and 3.

G-banding, karyotyping, and fluorescence in situ hybridization (FISH). Fresh tissue from a representative area of the tumors was received and analyzed cytogenetically as part of our diagnostic routine. The samples were disaggregated mechanically and enzymatically with collagenase II ( Worthington, Freehold, NJ, USA). The resulting cells were cultured and harvested using standard techniques (7). 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). The karyotypes were described according to the International System for Human Cytogenetic Nomenclature (8).

Interphase and metaphase FISH analyses were performed in order to detect deletion of the \textit{RB1} locus in 13q14.2. The \textit{RB1} deletion probe, purchased from Cytocell (http://www.cytocell.com/), consisted of a 318kb red probe spanning the \textit{RB1} gene locus and a 13qter probe in green acting as a control for chromosome 13. Fluorescent signals were captured and analyzed using the CytoVision system from Leica Biosystems (http://www.leicabiosystems.com/clinical-microscopy-surgery-radiology/cytogenetics/).

**Results**

All 3 angiolipomas had abnormal karyotypes with loss or structural rearrangement of chromosome 13 (Table I). In case 1, there was a del(13)(q14) together with loss of chromosome 6 and a marker chromosome (Figure 2). In case 2, an entire chromosome 13 was lost accompanied by a three-way

<table>
<thead>
<tr>
<th>Cases</th>
<th>Gender/Age</th>
<th>Location of the tumor</th>
<th>Size (cm)</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/57</td>
<td>Right shoulder</td>
<td>15x11x4</td>
<td>46,XY,-6,del(13)(q14),+mar[cp5]/46,XY[16]</td>
</tr>
<tr>
<td>2</td>
<td>M/54</td>
<td>Right foot</td>
<td>8x4.5x2</td>
<td>44<del>45,XY(t;1;10;15)(p21</del>22;q24;q24),-13[cp5]/46,XY[10]</td>
</tr>
<tr>
<td>3</td>
<td>F/28</td>
<td>Left shoulder/back</td>
<td>8.5x4.5</td>
<td>46,XX,t(13;22;17)(q12;q13;q22~23)[14]</td>
</tr>
</tbody>
</table>

Figure 1. \textit{H&E}-stained histological image of an angiolipoma (x10; case 1) with characteristic small vessels predominately in the periphery, focally with small thrombi.

Figure 2. Karyotype of angiolipoma (case 1) showing del(13)(q14), loss of chromosome 6, and a marker chromosome (mar). Arrow indicates breakpoint.
translocation t(1;10;15)(p21~22;q24;q24) (Figure 3A). Case 3 had a three-way translocation, t(13;22;17)(q12;q13;q22~23), as the sole cytogenetic abnormality (Figure 4).

In case 2, FISH analysis showed heterozygous deletion of the RB1 probe in 50 out of 100 investigated interphase nuclei (Figure 3B). In case 3, FISH analysis showed homozygous deletion of RB1 in 98 out of 100 interphase nuclei (Figure 4B). The same result, i.e., homozygous deletion of the RB1 probe, was also seen in metaphase spreads (Figure 4B). FISH analysis failed for case 1.

Figure 3. Cytogenetic analysis of the angiolipoma of case 2. A) Karyotype showing loss of chromosome 13 and the three-way translocation t(1;10;15)(p21~22;q24;q24). Arrows indicate breakpoints. B) Interphase FISH analysis demonstrating heterozygous deletion of RB1.
Discussion

The present study shows consistent involvement of chromosome 13 in all three angiolipomas studied. The tumors of cases 1 and 3 arose in the shoulder and had structural aberrations of chromosome 13 affecting the q12-q14 bands. In the third tumor (case 2), which was located in the foot, one entire chromosome 13 was lost. In the tumors of cases 1 and 2, the loss of material from chromosome 13 was accompanied by other chromosome aberrations whereas the tumor of case 3 had a three-way translocation as the sole cytogenetic abnormality.

Our results are at odds with previously published data. Mandahl et al. (5) reported that in one patient with five subcutaneous angiolipomas, four of 19 metaphases of one tumor showed t(X;2)(p22;p12) as the sole anomaly whereas the four remaining tumors were karyotypically normal. Sciot et al. (6) reported that all 20 subcutaneous angiolipomas occurring in 10 patients showed a normal karyotype based on analysis of a minimum of 20 metaphases. The authors took the normal karyotype to be indicative of a non-neoplastic nature of the lesions, thus supporting the clinicopathologic impression of angiolipomas as being reactive or hamartomatous. The findings of the present study, that acquired clonal chromosome abnormalities exist in cells cultured from three tumors from three different patients, confirm that angiolipomas are neoplastic in nature. Furthermore, the consistent involvement of chromosome 13, with structural aberrations affecting bands 13q12-14 in cases 1 and 3 and deletion of the RB1 locus found by FISH, suggest a (cyto)genetic similarity between angiolipoma and spindle cell lipoma, cellular angiofibroma, and extramammary myofibroblastoma (4, 9-13). Most of the 28 karyotypically abnormal spindle cell lipomas reported in the cytogenetic literature had loss of material from 13q together with other chromosome abnormalities (http://cgap.nci.nih.gov/Chromosomes/Mitelman, Database last updated on August 11, 2016), with bands 13q12-21 or 13q12-22 being the most commonly lost (4, 9, 10). Similarly, G-banding analysis has shown loss of 13q material to be typical of cellular angiofibromas (14).

The deletions of the RB1 locus found by FISH in cases 2 (heterozygous deletion) and 3 (homozygous deletion) add evidence pointing in the same direction: Mono- or bi-allelic deletions of RB1 were found also in spindle cell lipomas, cellular angiofibromas, and extramammary myofibroblastomas (4, 9, 11-15). At the protein level, immunohistochemical staining for RB1 showed that nuclear RB1 expression was deficient in all examined spindle cell lipomas, pleomorphic lipomas, and cellular angiofibromas, and in nearly 90% (17 of 19) of mammary-type myofibroblastomas (16). The protein encoded by the RB1 gene regulates transcription and is a negative regulator of cell proliferation (17). At present, its exact role, if any, in the development of the above-mentioned tumors, angiolipomas included, remains unknown.

In conclusion, chromosome 13 was consistently involved in all three angiolipomas.

Conflicts of Interest

The Authors declare that they have no competing interests.

References


Panagopoulos et al: Involvement of Chromosome 13 in Angiolipoma

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