Cytogenetic Analysis of a Pseudoangiomatous Pleomorphic/Spindle Cell Lipoma

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Abstract. *Background: Pseudoangiomatous pleomorphic/spindle cell lipoma is a rare subtype of pleomorphic/spindle cell lipoma. Only approximately 20 such tumors have been described. Genetic information on pseudoangiomatous pleomorphic/spindle cell lipoma is restricted to a single case in which deletion of the forkhead box O1 (FOXO1) gene was found, using fluorescence in situ hybridization (FISH). Materials and Methods: G-banding and FISH analyses were performed on a pseudoangiomatous pleomorphic/spindle cell lipoma. Results: G-banding of tumor cells showed complex karyotypic changes including loss of chromosome 13. FISH analysis revealed that the deleted region contained the RB1 gene (13q14.2) and the part of chromosome arm 13q (q14.2-q14.3) in which spans the TRIM13 gene, the two non-coding RNA genes, DLEU1 and DLEU2, and the genetic markers RH44686 and D13S25. Conclusion: Several acquired genomic aberrations were found in the tumor. Among them was loss of chromosome 13 material. Results confirm the (cyto)genetic similarity between pseudoangiomatous pleomorphic/spindle cell lipoma and spindle cell lipomas.*

Pseudoangiomatous pleomorphic/spindle cell lipoma is a rare subtype of pleomorphic/spindle cell lipoma first described by Hawley *et al.* in 1994 in five patients (1). The tumors had ‘the features of typical spindle cell lipoma but, in addition, exhibited irregular and branching spaces with villiform connective tissue projections, giving a striking angiomatoid

appearance’ (1). To date, only 20 patients have been described in the literature with this diagnosis, 15 of whom were males (1-10). The pseudoangiomatous pleomorphic/spindle cell lipomas were mostly found in the neck (seven patients) and shoulders (four patients), but have also been seen in the cheek, chest, chin, elbow, finger, subscapular region, and thumb. Genetic information on pseudoangiomatous pleomorphic/spindle cell lipoma is restricted to one case only (8) in which fluorescence *in situ* hybridization (FISH) with a probe for the forkhead box O1 (FOXO1) gene, which maps to chromosome sub-band 13q14.11, showed a signal pattern indicating monoallelic loss of the gene in 57% of the examined cells.

We present here cytogenetic data on another case of pseudoangiomatous pleomorphic/spindle cell lipoma which showed loss of 13q material.

Case Report

Ethics statement. The study was approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (Regional komité for medisinsk forskningsetikk Sor-Ost, Norge, <http://helseforskning.etikkom.no>, REC no S-07474a). All patient information has been de-identified.

Patient. A 35-year-old man noticed a tumor of his left elbow after a trauma. It seemed to grow over 2 years, when a magnetic resonance imaging investigation measured it at 6 cm in diameter. The subcutaneous tumor contained fatty and myxoid tissues and was surgically removed by marginal excision.

Macroscopically, the encapsulated tumor’s yellow cut surface was partly myxoid, partly fleshy. Microscopically, the appearance was mixed, with mature fat cells intermingled with bands of fibrous, partly myxoid tissue, bundles of ‘ropey’ collagen and small, spindled cells that mostly lacked atypia (Figure 1A-C). In some areas, however, atypically-appearing cells and scattered floret-like

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Figure 1. Histological examination of the pseudoangiomatous pleomorphic/spindle cell lipoma. A: Pseudoangiomatous cavity [$\times 4$ hematoxylin and eosin stain (HE)]. B: Lipoma transition to spindle cell lipoma ($\times 4$ HE). C: Pleomorphic/spindle cell lipoma with floret cells ($\times 10$ HE). D: Immunohistochemical analysis showing positivity for CD34 ($\times 10$). E: Immunohistochemical analysis demonstrating focal CD31-positive cells along the cavities ($\times 20$).

giant cells were seen (Figure 1B-C). Centrally, the tumor had a pseudoangiomatous appearance, with papillary formations surrounding the vascular spaces (Figure 1A). There was no necrosis and the mitotic activity was low (Ki67 index $< 5\%$).

Immunohistochemical analysis showed extensive positivity for CD34 in the fibrous tissue (Figure 1D). The fat cells were S100-positive (Figure 1E). The angiomatous areas were partly CD31-positive, but negative for podoplanin (using D2-40). MDM2 proto-oncogene (MDM2) staining was negative.

The combination of 'ropey' collagen, fibrous tissue with no atypia, and floret-like giant cells fits very well with a spindle cell or pleomorphic lipoma. The rare pseudoangiomatous growth led to the final diagnosis of pseudoangiomatous pleomorphic/spindle cell lipoma.

G-Banding and karyotyping. As part of our diagnostic service, fresh tissue from a representative area of the tumor was received and analyzed cytogenetically after short-term culturing as described elsewhere (11). The karyotype was described according to the International System for Human Cytogenetic Nomenclature 2016 guidelines (12).

FISH analysis. The following three probes were used for FISH analysis. I: Vysis LSI FOXO1 (13q14) Dual Color Break Apart Rearrangement Probe (Abbott Laboratories, Abbott Park, IL, USA). This probe hybridizes to a 1.5 Mbp sequence on 13q14.11 which contains the *FOXO1* gene. II: RB transcriptional corepressor 1 (*RBI*) deletion probe, purchased from Cytocell (Cytocell, Cambridge, UK), was used to detect deletion of the *RBI* locus in 13q14.2. It consists of a 318-kb red probe spanning *RBI* and a 13qter green probe acting as a control for chromosome 13. III: The 13q14.3 deletion probe from Cytocell. This probe is normally used for detection of 13q14 deletion in B-cell chronic lymphocytic leukemia. It spans the tripartite motif containing 13 (*TRIM13*) gene, two non-coding RNA genes, deleted in lymphocytic leukemia 1 (*DLEU1*) and deleted in lymphocytic leukemia 2 (*DLEU2*), and the genetic marker D13S319. It also contains a 13qter subtelomere specific probe (clone 163C9), labeled in green, which allows for identification of chromosome 13 and acts as a control.

Fluorescent signals were captured and analyzed using the CytoVision system from Leica Biosystems (<http://www.leicabiosystems.com/pathology-imaging/cytogenetics/>).

Figure 2. G-Banding analysis of the pseudoangiomatous pleomorphic/spindle cell lipoma. Karyotype showing the chromosomal aberrations of tumor cells: 45,XY,del(1)(p32),add(3)(p13),ins(6;?)(p21;?),-13,der(17;22)(q10;q10),-17,-22,+der(?)t(3;?)(p13;?),+mar1,+mar2[14]. Arrows indicate breakpoints.

Findings. G-Banding analysis of short-term cultured cells from the pseudoangiomatous pleomorphic/spindle cell lipoma yielded the karyotype 45,XY,del(1)(p32),add(3)(p13),ins(6;?)(p21;?),-13,der(17;22)(q10;q10),-17,-22,+der(?)t(3;?)(p13;?),+mar1,+mar2[14] (Figure 2).

Interphase FISH was performed, using commercially available probes, for the *FOXO1*, *RBI*, and *TRIM13* genes, the two *DLEU1* and *DLEU2* non-coding RNA genes, and the genetic markers RH44686 and D13S25 (Figure 3A). All of them are mapped on chromosome band 13q14 to different sub-bands (Figure 3A).

No loss of the *FOXO1* probe (sub-band 13q14.11) was seen in the 115 nuclei examined (Figure 3B). However, FISH showed heterozygous loss of the *RBI* probe (sub-band 13q14.2). In 102 out of 112 (91%) nuclei examined, one red signal (probe spanning *RBI*) and two green signals (13qter probe) were seen (Figure 3C). Similarly, heterozygous loss of

the region spanning *TRIM13*, *DLEU1*, *DLEU2*, and the genetic markers RH44686 and D13S25 was found (sub-bands 13q14.2-q14.3). In 92 out of 98 (94%) interphase nuclei, one red signal (probe spans *TRIM13*, *DLEU1*, *DLEU2*, RH44686, and D13S25) and two green (13qter subtelomere specific probe) signals were observed (Figure 3D).

Discussion

The present study described the first karyotypic analysis of a pseudoangiomatous pleomorphic/spindle cell lipoma. Several clonal aberrations were found, including what at first seemed to be loss of one copy of chromosome 13, and FISH confirmed the heterozygous loss of 13q14 markers. This is in agreement with the cytogenetic pattern seen in most of the 28 karyotypically abnormal spindle cell lipomas reported in the literature, *i.e.* loss of material from 13q together with

Studies of spindle cell lipomas have identified two minimal deleted regions (MDR) in 13q14 (14, 15). In MDR1 (13q14.2), the genes *RBI*, lysophosphatidic acid receptor 6 (*LPAR6*), *RCC1* and BTB domain containing protein 2 (*RCBTB2*), and cysteinyl leukotriene receptor 2 (*CYSLTR2*) are located (chr13:48,868,089-49,294,385). MDR2 lies between the genes fibronectin type III domain containing 3A (*FNDC3A*) and transmembrane phosphoinositide 3-phosphatase and tensin homolog 2 pseudogene 3 (*TPTE2P3*, formerly *LOC220115*). It

is approximately 3600-Mbp long (chr13:49,550,048-53,269,926) and has more than 30 gene loci (among them *DLEU1* and *DLEU2*) as well as the two microRNA genes *miR-15a* and *miR-16-1* (14). Based on the expression of *RBI*, which was not significantly reduced, and the absence of mutations, the authors concluded that there was no decisive support for *RBI* as the main target for chromosomal deletions in spindle cell lipomas. Instead, their data implicated *miR-16-1* as a potential target (14). On the other hand, immunohistochemical staining showed that nuclear *RBI* expression was deficient in all examined spindle cell lipomas, pleomorphic lipomas, and cellular angiofibromas, as well as in 17 out of 19 (89%) mammary-type myofibroblastomas (22). At the moment, the molecular target of chromosome 13 aberrations remains unknown in these as well as most other tumors.

In conclusion, our study showed several acquired chromosomal changes including heterozygous loss of 13q material in pseudoangiomatous pleomorphic/spindle cell lipoma. The deleted region contained the *RBI* gene and the part of chromosome arm 13q (q14.2-q14.3) which spans the *TRIM13* gene, the two non-coding RNA genes, *DLEU1* and *DLEU2*, and the genetic markers RH44686 and D13S25.

Conflicts of Interests

The Authors declare that they have no competing interests.

Acknowledgements

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