Gene Expression and Single Nucleotide Polymorphism Array Analyses of Spindle Cell Lipomas and Conventional Lipomas with 13q14 Deletion

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Spindle cell lipomas (SCL) are circumscribed, usually s.c. tumors that typically occur on the posterior neck, shoulder, and back of middle aged men. Cytogenetically, almost all SCL are characterized by deletions of chromosome arm 13q, often in combination with loss of 16q. Deletions of 13q are seen also in approximately 15% of conventional lipomas. Through single nucleotide polymorphism (SNP) array analyses, we identified two minimal deleted regions (MDR) in 13q14 in SCL. In MDR1, four genes were located, including the tumor suppressor gene *RB1*. MDR1 in SCL overlapped with the MDR detected in conventional lipomas with 13q14 deletion. In MDR2 in SCL there were 34 genes and the two microRNA (miRNA) genes *miR-15a* and *miR-16-1*. Global gene expression analysis was used to study the impact of the deletions on genes mapping to the two SCL-associated MDR. Five genes (*C13orf1*, *DHRS12*, *ATP7B*, *ALG11*, and *VPS36*) in SCL and one gene (*C13orf1*) in conventional lipomas with 13q-deletions were found to be significantly underexpressed compared with control tissues. Quantitative real-time PCR showed that *miR-16-1* was expressed at lower levels in SCL than in the control samples. No mutations were found at sequencing of *RB1*, *miR-15a*, and *miR-16-1*. Our findings further delineate the target region for the 13q deletion in SCL and conventional lipomas and show that the deletions are associated with down-regulated expression of several genes, notably *C13orf1*, which was the only gene to be significantly down-regulated in both tumor types.

INTRODUCTION

Spindle cell lipomas (SCL) are circumscribed, usually s.c. tumors that typically occur on the posterior neck, shoulder, and back of middle aged men (Fletcher et al., 2002). Cytogenetically, almost all SCL are characterized by deletions of chromosome arm 13q, often in combination with loss of 16q (Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, 2011). Deletions of 13q are also seen in 15% of conventional lipomas with karyotypic aberrations (Bartuma et al., 2007). Previous fluorescence in situ hybridization (FISH) studies of lipomatous tumors with 13q-deletions identified a minimal commonly deleted region (MDR) in 13q14 (Dahlén et al., 2003a). The molecular consequences of 13q-deletions in lipomatous tumors are unknown, but the frequent occurrence of this deletion strongly implies a role in the pathogenesis of SCL as well as conventional lipomas. 13q14 is also recurrently deleted in several other human neoplasms, including chronic lymphocytic leukemia (CLL), multiple myeloma (MM), acute lymphoblastic leukemia (ALL), myeloid malignancies, prostate cancer, ovarian cancer, breast cancer, oral cancer, and non-small cell lung cancer (NSCLC) (La Starza et al., 1998; Nupponen and Visakorpi, 2000; Zojer et al., 2000; Schlade-Bartusiak et al., 2005; Zhang et al., 2006; Bandi et al., 2009; Moorman et al., 2010; Palamarchuk et al., 2010). The molecular consequence(s) of the deletions are not extensively examined in all tumors, but in CLL it seems as if the targets are the two microRNA (miRNA) genes *miR-15a* and *miR-16-1*, which share the same seed sequence (Calin et al., 2002). In prostate cancer loss at 13q14 correlates with tumor progression, and loss of *miR-15a* and *miR-16-1* induced cell proliferation and invasion (Bonci

Additional Supporting Information may be found in the online version of this article.

Supported by: The Swedish Cancer Society; The Swedish Research Council; The Royal Physiographic Society in Lund.

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Received 9 February 2011; Accepted 24 March 2011

DOI 10.1002/gcc.20884

Published online 11 May 2011 in Wiley Online Library (wileyonlinelibrary.com).

et al., 2008). *miR-15a* and *miR-16-1* are also located in the MDR identified in MM (Corthals et al., 2010), but their expression levels are not consistent (Roccaro et al., 2009; Corthals et al., 2010).

In this study, we were interested in delineating the MDR in SCL and conventional lipomas with 13q14 deletion, and to study the expression of the genes and/or miRNA genes located in that region. We thus investigated a series of SCL and conventional lipomas with and without 13q-deletions by single nucleotide polymorphism (SNP) array and global gene expression (GGE) analyses. Candidate target genes and miRNA genes were further analyzed by quantitative real-time PCR (qRT-PCR) and sequencing.

MATERIALS AND METHODS

Patients

A total of 49 adipocytic tumors were selected on the basis of their histopathologic diagnosis and/or cytogenetic profile. In brief, the study included 12 SCL, 28 conventional lipomas (including 11 with 13q14-deletion), and nine angiolipomas. Data concerning patient sex and age, tumor location, depth and size, karyotype, and experiments conducted in this and previous publications are shown in Table 1.

Cytogenetic Analyses

Culturing, harvesting, and chromosome banding of the tumor cells were performed as previously described (Mandahl et al., 1988). Karyotypes were described according to ISCN (2009). The karyotypes of 33 samples have been reported before (Table 1; Mandahl et al., 1994a,b, 1988; Dahlén et al., 2003a; Bartuma et al., 2007, 2009).

Metaphase FISH Analyses

Metaphase FISH was performed on 11 cases to study deletions in chromosome band 13q14 and/or cryptic rearrangements of the *HMGA2* locus. Specific probes for the *RB1* gene were used to study deletions of chromosome band 13q14 (Abbott Molecular, Des Plaines). As a control, the BAC RP11-310D8, mapping to 13q34, was used. Cases 8, 10, 16, 21, and 32 were studied previously (Dahlén et al., 2003a). *HMGA2* was studied by BAC clones RP11-299L9 and RP11-427K2 that span the 5′ and 3′ ends, respectively. Seven cases had been analyzed previously (Dahlén et al., 2003a; Bartuma et al., 2007, 2009). Slides were prepared and analyzed and probes labeled as described elsewhere (Dahlén et al., 2003b). When applicable, whole chromosome painting probes were used to ensure that tumor cells were analyzed (Applied Spectral Imaging, Migdal Haemek, Israel).

DNA, RNA, and miRNA Extraction and cDNA Synthesis

Total DNA and RNA from fresh frozen tissue were extracted as previously described (Bartuma et al., 2009). miRNA was extracted from tumor cells using TRIzol® (Invitrogen, Carlsbad), according to the manufacturer's instructions, with the substitution of ethanol for isopropanol. DNA, RNA, and miRNA concentrations were measured with a Nanodrop ND-1000 spectrophotometer (Saveen & Werner AB, Malmö, Sweden). RNA and miRNA quality were assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara). cDNA synthesis was performed as previously described (Bartuma et al., 2009). For miRNA, 10 ng of total RNA were reverse transcribed to cDNA using 50 nM stem-loop RT primers (Assay IDs 000391, 000389, and 000407, Applied Biosystems, Foster City).

SNP Array Analysis

SNP array analysis was conducted on 40 cases: 11 SCL, 23 conventional lipomas, and six angiolipomas. DNA was hybridized to the Illumina HumanCNV370-Quad or Illumina HumanOmni-Quad version 1.0 array (Illumina, San Diego). The position of the SNPs was based on the UCSC hg18/NCBI Build 36 sequence assembly. SNP array analysis was done according to the manufacturer's instructions and data analysis was performed using the GenomeStudio software (Illumina). Imbalances were identified through combining visual inspection with segmentation analysis of normalized data (Staaf et al., 2008a,b). Constitutional copy number polymorphisms were excluded based on comparison with the Database of Genomic Variants (http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg18/) (Iafrate et al., 2004).

GGE Analysis

A total of 47 cases were studied by GGE analysis: 12 SCL, 24 conventional lipomas (including eight with 13q14-deletion), seven angiolipomas,

TABLE 1. Clinical, Cytogenetic and Molecular Genetic Data on 12 Spindle Cell Lipomas, 28 Conventional Lipomas and 9 Angiolipomas

Case[a]	Sex/Age[b]	Site	Depth/Size[c]	Karyotype[d]	13q14[e]	*HMGA2*[f]	GGE[g]	qRT-PCR[h]	Previously published karyotype[i]
Spindle cell lipoma									
1	M/49	Scapula	S/8	44,XY,*add(1)(p35)*,der(2)t(2;6)(q12;q21),-*dup(3)(p26p26)*,*del(3)(p14p14)*,*add(3)(q13)*,der(6)t(6;20)(q11;q11),***del(13)(q14-q14)***,***del(13)(q21q21)***,−16,−20,der(21)t(3;21)(q13;q21)	SNPdel	SNPN qRT-PCRneg	Yes	Yes	5797:120
2	M/76	Upper arm	D/5	46,XY,inv(9)c,**del(13)(q12q21)**	SNPdel	SNPN qRT-PCRneg	Yes	Yes	12980:48
3	M/63	Back	S/8	44–45,XY,***der(2)t(2;13)(q21;q21–22)***,der(6;16)(p10;p10),***del(13)(q13q21)***	SNPdel	SNPN qRT-PCRneg	Yes	Yes	12980:47
4	M/78	Neck	S/4	58,XXY,+Y,−1,−2,−4,−6,−7,+8,−9,−10,−12,**−13**,−14,−16,−17,−22	SNPdel	SNPN qRT-PCRneg	Yes	No	12980:45
5	M/85	Neck	S/3	44,XY,der(2;6)(p10;p10),***del(13)(q13q13)***,***del(13)(q14q34)***, der(14)t(?6;14)(q24;q32),−16	SNPdel	SNPN qRT-PCRneg	Yes	Yes	
6	M/79	?	?/5	45,XY,***der(6)del(6)(q12)t(6;13)(p23;q12)***,-***del(13)(q14q21)***, *del(16)(q21q24)*	SNPdel	SNPN qRT-PCRneg	Yes	Yes	5797:160
7	M/53	Neck	S/?	45,XY,*dup(7)(p21p21)*,inv(9)(p11q12)x2,−10,***del(13q12q14)***,*add(16)(q22)*	SNPdel	SNPN qRT-PCRneg	Yes	Yes	
8	F/53	Lower leg	D/4	46,XX,t(1;6)(p32;p21),***del(13)(q13q33)***	SNPdel FISHdel	SNPN qRT-PCRneg	Yes	Yes	9986:37
9	M/92	Neck	?/20	45,XY,***der(6)t(6;13)(q23;q22),dic(13;16)(q13;q21)***	SNPdel	SNPN qRT-PCRneg	Yes	Yes	
10	M/66	Shoulder	S/15	46,XY,***del(13)(q14q22***	SNPdel FISHdel	SNPN qRT-PCRpos	Yes	Yes	9986:32 12980:44
11	F/81	?	S/2	45,XX,add(2)(p11),t(3;3)(q21;q29),*dup(7)(p14p14)*,-***del(13)(q14q14)***, *del(16)(q11q24)*,*add(17)(p11)*/76–78,idemx2	SNPdel	SNPN	Yes	No	5797:163
12	F/49	Neck	S/8	44,XX,*add(4)(p11)*,*−9*,*add(10)(q22)*,**−13**,−16,add(17)(q11), der(17)t(9;17)(p13;p13),add(22)(q11),+mar/88,idemx2	NA	qRT-PCRneg	Yes	No	5797:162 12980:46
Conventional lipoma with 13q14 deletion									
13	M/49	Arm	S/?	46,XY,**der(6)t(6;13)(p2?3;q3?2),der(13)t(6;13)(p2?3;q13)**	SNPdel	SNPN qRT-PCRneg	Yes	Yes	11858:198 12980:30
14	F/44	Shoulder	S/9	45,XX,*del(6)(q14q23)*,***del(13)(q11q34)***, *der(16)del(16)(p11p12)del(16)(q11)*	SNPdel	SNPN qRT-PCRneg	Yes	Yes	9986:22 11858:195 12980:29
15	M/51	Back	S/3	46,XY,i(6)(p10),***del(13)(q13q33)***/46,idem,t(2;17)(p23;p13)	SNPdel	SNPN qRT-PCRneg	Yes	Yes	11858:192
16	M/49	Arm	S/8	46,XY,**der(6)t(6;13)(p22;q22),*del(13)(q13q31)***	SNPdel	SNPN	Yes	Yes	11858:199

(Continued)

TABLE 1. Clinical, Cytogenetic and Molecular Genetic Data on 12 Spindle Cell Lipomas, 28 Conventional Lipomas and 9 Angiolipomas (Continued)

Case[a]	Sex/Age[b]	Site	Depth/Size[c]	Karyotype[d]	13q14[e]	*HMGA2*[f]	GGE[g]	qRT-PCR[h]	Previously published karyotype[i]
					FISHdel	qRT-PCRneg			9986:10
17	F/30	Shoulder	D/11	46,XX,t(3;12)(q27;q15),***del(13)(q14q14)***	SNPdel	SNPN qRT-PCRpos	Yes	Yes	11858:53 12980:4
18	F/64	Shoulder	D/9.5	45,X,***dic(X;?13)(q11;q11)***,*dup(12)(q14q24)*/44,X,-X,*dup(12)*, ***del(13)(q12q33)***	SNPdel	SNPgain qRT-PCRpos	Yes	Yes	6495:7
19	M/60	Neck	S/5	58,XXY,+X,−1,−2,−3,−6,−9,−10,−11,**−13,−13**,−14,−15,−16,+19,−22	SNPdel	SNPN qRT-PCRpos	No	No	9986:30
20	M/47	Thigh	D/9	46,XY,***del(13)(q13q31***	SNPdel	SNPN qRT-PCRpos	Yes	Yes	11858:191 12980:26
21	M/63	Shoulder	D/13	46,XY,***der(12)t(12;13)(q14;q22)***,*del(12)(q14q14)*, ***der(13)t(12;13)(q14;q13)***	SNPdel FISHdel	SNPdel.c qRT-PCRpos	Yes	Yes	9986:9 11858:159
22	M/75	Neck	S/10	45,XY,*del(10)(p15q21)*,***del(13)(q12q33)***, *del(16)(q12q24)*	SNPdel	SNPN	Outlier	No	
23	M/36	Neck	S/?	46,XY,?t(3;8)(p1?3;q24),add(5)(q3?3),−6,add(10)(q2?2), *del(12)(q14q14)*, ***del(13)(q14q14)***,del(15)(q22),+mar	SNPdel	SNPdel.c SNPdel5′	No	No	
Conventional lipoma without 13q14 deletion									
24	M/49	Back	D/7	46,XY,t(3;12)(q27;q13)	FISHN	FISHS qRT-PCRpos	Yes	No	11858:50 12980:1
25	F/81	Lower arm	D/?	46,XX,t(3;12)(q28;q14)	FISHN	FISHS	Yes	No	
26	F/43	Thigh	D/?	46,XX,t(3;12)(q27;q13)	FISHN	FISHS	Yes	No	
27	F/47	Thorax	S/9	46,XX,*dup(3)(p26p25)*,t(3;12)(q27;q14), *dup(22)(q11q11)*	SNPN	SNPN qRT-PCRpos	Yes	Yes	11858:42 12980:2
28	F/88	Upper arm	D/6	46,XX,t(5;12)(q33;q15)	SNPN	SNPN qRT-PCRpos	Yes	Yes	11858:94 12980:6
29	M/70	Leg	D/12	46,XY,*del(2)(p24p23)*,*dup(2)(q13)*,t(5;12)(q33;q14),-*del(21)(q21)*/46,idem, t(1;6)(p36;p23)	SNPN	SNPN qRT-PCRpos	Yes	Yes	5197:75 11858:97 12980:8
30	M/66	Thigh	D/?	46,XY,t(12;13)(q24;q14)	SNPN	SNPN	Yes	No	
31	F/51	Buttock	D/15	46,XX,t(12;13)(q22;q14)	SNPN	SNPN	Yes	No	9986:1 11858:205
32	F/42	Arm	S/6	46,XX,t(4;15)(p15;q22),t(5;9)(q22;q32), **ins(8;13)(q24;q34q14)**,*del(12)(q14q14)*, add(16)(q13),der(20)t(16;20)(q13;q12)	SNPN FISHN	SNPdel.p FISHN qRT-PCRpos	Yes	Yes	9986:7 11858:201 12980:18
33	F/73	Upper arm	S/10	46,XX,*del(12)(q14q14)*,**ins(13;12)(q22;q21q23),-*del(13)(q31q31)***/45,idem,−21/ 46,XX,**t(13;14)(q14;q32)**	SNPN	SNPdel.c qRT-PCRpos	Yes	Yes	5197:84 11858:206
34	M/58	Shoulder	S/14	45,XY,der(1;15)(q10;q10)	SNPN	SNPN	Yes	No	
35	M/59	Shoulder	D/14	47,XY,t(2;4)(p16;q35),+8	FISHN	FISHN	Yes	No	11858:232

(Continued)

TABLE 1. Clinical, Cytogenetic and Molecular Genetic Data on 12 Spindle Cell Lipomas, 28 Conventional Lipomas and 9 Angiolipomas (Continued)

Case[a]	Sex/Age[b]	Site	Depth/Size[c]	Karyotype[d]	13q14[e]	*HMGA2*[f]	GGE[g]	qRT-PCR[h]	Previously published karyotype[i]
36	M/55	Scapula	?/7	46,XY,t(3;22)(q23;q13)	SNPN	SNPN	No	No	
37	F/37	Back	S/11	46,XX,t(6;19)(q22;p13)	FISHN	FISHN	Yes	No	11858:248
38	M/58	Back	S/3	46,XY,ins(7;10)(q33;q22q26),*del(10)(q21q22)*, *del(12)(q14q14)*	SNPN	SNPdel.c	Yes	No	2480:22 11858:251
39	M/75	Thorax	?/23	46,XY,*del(8)(q12q12)*,*dup(8)(q23q23)*,t(8;10)(q13;q26)	SNPN	SNPN	Yes	No	11858:254
40	M/54	Leg	S/5	46,XY,*del(10)(q21q21)*,t(10;22)(q22;q13),- *del12(q14q14)*,*dup(15)(q21)*/45,idem,−20	SNPN	SNPdel.c qRT-PCRpos	Yes	No	5197:60 11858:258 12980:17
Angiolipoma									
41	M/43	Upper arm	S/4	46,XY	SNPN	SNPN qRT-PCRC	Yes	Yes	12980:38
42	M/49	Trunk	S/?	46,XY	SNPN	SNPN FISHN qRT-PCRC	Yes	Yes	12980:39
43	F/44	?	S/?	46,XX	SNPN	SNPN qRT-PCRC	Yes	Yes	12980:40
44A	F/45	Lower arm	S/?	46,XX	SNPN	NA	No	No	
44B	F/45	Lower arm	S/?		SNPN	NA	No	No	
44C	F/45	Lower arm	S/?		NA	NA	Yes	No	
44D	F/45	Lower arm	S/?		NA	NA	Yes	Yes	
45A	F/50	Lower arm	S/?	46,XX	SNPN	qRT-PCRC	Yes	Yes	
45B	F/50	Lower arm	S/?		SNPN	qRT-PCRC	Yes	Yes	

[a]A, B, C, and D = distinct primary tumors from the same patient.

[b]M = male; F = female; Age in years at diagnosis.

[c]S = superficial; D = deep-seated; size is given as largest diameter in cm.

[d]Karyotypes based on G-band and SNP array results. Aberrations in italics were breakpoints defined by SNP-array and aberrations involving chromosome 13 are highlighted in bold.

[e]Information regarding chromosome band 13q14. SNPN, no gain or loss at 13q14 at SNP array analysis; SNPdel, deletion of 13q14 at SNP array analysis; FISHN, normal signals for 13q14 at FISH analysis; FISHdel, deletion of 13q14 at FISH analysis; NA, not analyzed by FISH or SNP array or sequencing. For complete SNP-data, see Supporting Information Table 2.

[f]Information regarding the *HMGA2* gene. SNPN, no aberration in the *HMGA2* gene at SNP array analysis; SNPgain, gain of exons 1–3 of *HMGA2* at SNP array analysis; SNPdel.c, deletion centromeric to *HMGA2* at SNP array analysis; SNPdel5′, deletion of the 5′ part of *HMGA2*; FISHN, normal signals for the *HMGA2* locus at FISH analysis; FISHS, split signals for the *HMGA2* locus at FISH analysis; qRT-PCRneg, No expression with qRT-PCR of the *HMGA2* gene exons 1–2; qRT-PCRpos, expression of the *HMGA2* gene exons 1–2 at qRT-PCR; qRT-PCRC, used as a control at qRT-PCR; NA, not analysed by SNP, FISH or qRT-PCR. For complete SNP-data see Supporting Information Table 2.

[g]Information regarding GGE analysis. Yes, case analyzed for GGE; No, case not analyzed for GGE. For complete GGE data for chr13:47,883,306–48,187,410 and chr13:48,430,973–52,065,308, see Supporting Information Table 3.

[h]Information regarding qRT-PCR. Yes, case analyzed with qRT-PCR regarding the expression of genes *RB1*, *HMGA1*, *HMGA2* or miRNAs *miR-15-a* and *miR-16–1*; No, not analyzed with qRT-PCR.

[i]Reference number and case number used in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer 2011 (http://cgap.nci.nih.gov/Chromosomes/Mitelman); Conventional RT-PCR data on the expression of full-length and truncated *HMGA2* in cases 31 and 39 have been reported before (Bartuma et al., 2007); Sequencing data, genomic PCR, and RT-PCR regarding the status of the 3′ UTR of *HMGA2* in cases 17, 20, 31, and 39–42 have been reported before (Bartuma et al., 2009); qRT-PCR results regarding the expression of *HMGA2* exons 1–2 and exons 4–5 in cases 1, 3, 4, 10, 12–14, 17, 20, 23, 26–28, 31 and 39–42 have been reported before (Bartuma et al., 2009). FISH results regarding the *HMGA2* and 13q14 status in cases 8, 10, 17, 21, 31, and 34 have been reported before (Dahlén et al., 2003a; Bartuma et al., 2007, 2009).

and three RNA samples from normal human white adipose tissue (WAT) (Ambion's total Human RNA, Austin, Clontech's total Human RNA, Mountain View, and Biochain's total Human RNA, Hayward). The seven angiolipomas and the three WAT samples were used as controls. The analysis was performed using Affymetrix Human Gene 1.0 ST arrays according to the manufacturer's instructions (Affymetrix, Santa Clara). Expression data were normalized, background-corrected, and summarized using the robust multichip average (RMA) algorithm implemented in the Affymetrix Expression Console software version 1.0 (Affymetrix). Case 22 was identified as an outlier and excluded from further analysis.

Statistical Analysis

The two-tailed Mann-Whitney *U* test was used to assess whether genes mapping to MDR1 (nt 47.883–48.187 Mb) and MDR2 (nt 48.430-52.065 Mb) were equally expressed in tumors with deletions and control samples. *P* values were corrected for multiple testing by Bonferroni correction and values less than 0.0013 were considered significant. When comparing expression levels for genes in MDR2, case 17 was excluded (no deletion of MDR2).

qRT-PCR

qRT-PCR was carried out to validate the results of the GGE profiling for the *RB1*, *C13orf1*, and *HMGA1* genes, to provide separate information on the 5′ part of *HMGA2*, and to measure the expression levels of the two miRNA genes (*miR-15a* and *miR-16-1*) located in MDR2. The following TaqMan gene expression assays were used: Hs01078070_m1 (*RB1*), Hs00971794_m1 (*C13orf1*), Hs00600784_g1 (*HMGA1*), Hs00171569_m1 (*HMGA2*), assay IDs 000389 (*hsa-miR-15a*), and 000391 (*hsa-miR-16-1*) (Applied Biosystems). As endogenous controls, *HPRT1* for gene expression and *hsa-miR-26b* for miRNA expression were used [Human HPRT1 (HGPRT) Endogenous Control FAM/MGB Probe, Non-Primer Limited, and Assay ID 000407 *hsa-miR-26b*, Applied Biosystems]. Both endogenous controls showed uniform expression in a test panel of tumors and control tissue (data not shown). qRT-PCR was performed according to the manufacturer's instructions and all reactions were run in triplicate. Calculations were done using the comparative C_T method (i.e., $\Delta\Delta C_T$ method) (Livak and Schmittgen, 2001) using the SDS software 1.3.1 (Applied Biosystems). Total RNA from the three WAT samples and five angiolipomas, cases 41-43, 45A and B, was the calibrator for cDNA control.

Sequence Analysis

Sequencing was performed to search for mutations in the promoter and all 27 exons except exon 14, due to technical problems, of the *RB1* gene and the two miRNA genes *miR-15a* and *miR-16-1*. Cases 1–3, 13, 14, and 16 were analyzed for *RB1* mutation. Cases 1–3, 5, 8, 13, 14, 16, 17, and 20 were analyzed for *miR-15a* and *miR-16-1* mutation. In addition, five conventional lipomas and four angiolipomas were analyzed with regard to the status of *miR-15a* and *miR-16-1* (Table 1). The PCR reactions and analyses were performed as described (Bartuma et al., 2009), using primers listed in Supporting Information Table 1. All PCR-products identified were verified by sequencing, and the corresponding nucleotide sequences were analyzed using SeqScape version 2.6 (Applied Biosystems), and the Chromas software (http://www.technelysium.com.au/chromas.html). Nucleic acid sequences were compared with reference sequences (see Supporting Information Table 1) using the BLAST program (http://www.ncbi.nlm.nih.gov/blast).

RESULTS

Cytogenetics and FISH

Forty-five of the 49 tumors were analyzed by G-banding (Table 1). Metaphase FISH was performed on 11 cases to search for deletion of chromosome band 13q14 and/or cryptic rearrangement of the *HMGA2* locus (Table 1). In brief, FISH confirmed the cytogenetic findings in two SCL (cases 8 and 10) and two conventional lipomas (cases 16 and 21) with 13q14 deletion (Dahlén et al., 2003a). No deletion was seen in six conventional lipomas (cases 24–26, 32, 35, and 37) without cytogenetic signs of 13q14 deletion (Table 1). Normal signals for the *HMGA2* locus were seen in three cases (32, 35, and 37) without 12q-rearrangement at banding analysis, whereas split signals, with the 3′ end being translocated to chromosome 3, was seen in the three cases (24–26) with t(3;12) (Table 1).

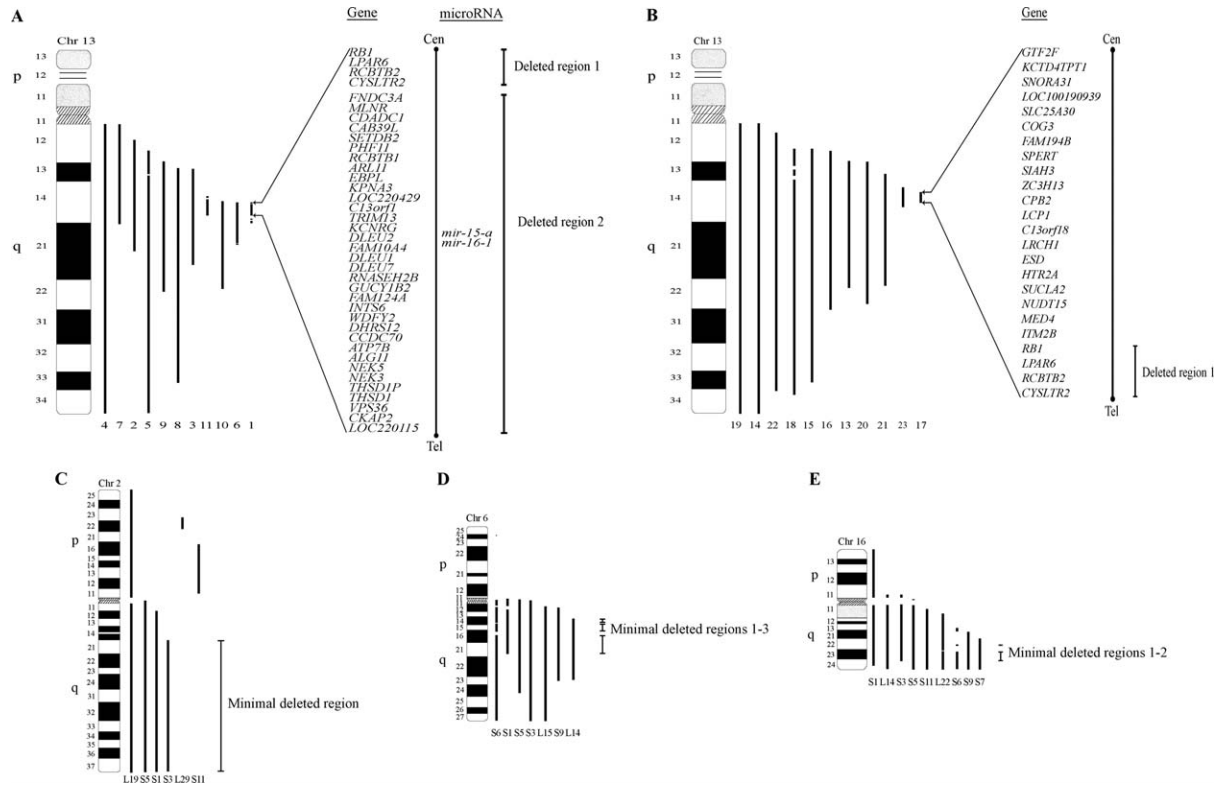

Figure 1. Ideograms showing the copy number losses affecting chromosomes 2, 6, 13, and 16 at SNP array analysis. The chromosome number is indicated above each ideogram. The vertical lines to the right of each chromosome show the extension of the detected deletions. The tumor in which each deletion was identified is indicated below the vertical line; L, onventional lipoma; S, SCL. (A) Chromosome 13 deletions in SCL. The minimal deleted regions (MDRs) were identified through case 1. Deleted regions 1 and 2 are enlarged and genes and miRNA genes located in those regions are shown. (B) Chromosome 13 deletions in conventional lipomas. Case 17 delineated the MDR. The telomeric part of the MDR was identical with MDR1 in SCL. (C) Chromosome 2 deletions in SCL and conventional lipoma. One MDR was identified. (D) Chromosome 6 deletions in SCL and conventional lipoma. Three MDRs were identified. (E) Chromosome 16 deletions in SCL and conventional lipoma. Two MDRs were identified. The exact locations of all identified imbalances are listed in Supporting Information Table 2.

SNP Array Analysis

SNP array analysis was conducted on 40 cases: 11 SCL, 23 conventional lipomas (including 11 with 13q14-deletion), and six angiolipomas. Through case 1, two MDR deleted in all SCL could be identified in chromosome 13: MDR1 (nt 47.883–48.187 Mb) and MDR2 (nt 48.430–52.065 Mb). MDR1 harbors four genes (*RB1*, *LPAR6*, *RCBTB2*, *CYSLTR2*) and MDR2 harbors 34 genes, including *KPNA3*, *C13orf1*, *TRIM13*, *KCNRG*, *DHRS12*, *ATP7B*, *ALG11*, *VPS36* and two miRNA genes (*miR-15a* and *miR-16-1*); see Fig. 1A, Table 2 and Supporting Information Tables 2 and 3 for extension of the deletions and complete gene lists. Eleven of 23 conventional lipomas had a 13q-deletion, with a MDR in chromosome band 13q14 (nt 44.640–48.181 Mb), harboring 24 genes (Fig. 1B). The telomeric part of the MDR in conventional lipomas was identical to MDR1 in SCL (Fig. 1B).

Recurrent deletions were also found in chromosomes 2, 6, and 16. Four cases of SCL as well as two conventional lipomas (one with and one without 13q14 deletion) had deletion of most of the long arm of chromosome 2, with one MDR (nt 130.036–242.415 Mb) encompassing around 540 genes and 18 miRNA genes (Fig. 1C). Five SCL and two conventional lipomas with 13q14 deletion had deletion in chromosome 6, with three MDRs being identified (Fig. 1D): one at 6q14 (nt 80.761–84.992 Mb) with 16 genes, one at 6q14–q16 (nt 85.645–92.698 Mb) with 31 genes and one at 6q16–q21 (nt 95.692–112.123 Mb) with 72 genes and two miRNA genes. Seven SCL and two conventional lipomas with 13q14 deletion had deletion in chromosome 16, with two MDRs (Fig. 1E): one at 16q22 (nt 70.523–71.262 Mb) with seven genes and one at 16q23 (nt 75.697–82.701 Mb) with 33 genes.

Through SNP array we also identified a small deletion centromeric (upstream) to *HMGA2*, which was seen in six conventional lipomas, two of which had 13q14 deletion (Supporting

TABLE 2. Statistical Analysis of the Expression Levels of 38 Genes in the Minimal Deleted Regions 1 and 2 of Chromosome 13[a]

Gene name	SCL vs. Control	SCL vs. Lipoma	Lipoma 13q vs. Control	Lipoma 13q vs. Lipoma	Lipoma vs. Control
RB1	■		■		
LPAR6					
RCBTB2					
CYSLTR2					
FNDC3A			■		■
MLNR					
CDADC1					
CAB39L					
SETDB2					
PHF11					
RCBTB1	■				
ARL11					
EBPL					
KPNA3	■		■		
LOC220429	■				
C13orf1	■				
TRIM13	■		■		
KCNRG	■				■
DLEU1					
FAM10A4					
DLEU2					
DLEU7					
RNASEH2B					
GUCY1B2					
FAM124A					
INTS6					
WDFY2					
DHRS12	■			■	
CCDC70					
ATP7B	■				
ALG11	■		■		
NEK5					
NEK3					
THSD1P					
THSD1					
VPS36	■				
CKAP2					
LOC220115					

[a]The dark lines represent significantly ($P \leq 0.0013$; Bonferroni correction) lower expression levels; SCL, spindle cell lipomas; Control, seven angiolipomas and three white adipose tissue samples; Lipoma, conventional lipomas without 13q-deletion; Lipoma 13q; conventional lipomas with 13q-deletion. For complete statistical data see Supporting Information Table 3.

Information Table 2). A deletion affecting exons 1–3 of *HMGA2* was also seen in one of the conventional lipomas with 13q14 deletion. Gains were also seen; in two conventional lipomas with 13q14 deletion the entire *HMGA1* gene and exons 1-3 of *HMGA2*, respectively, were gained (Supporting Information Table 2).

No copy number aberrations involving chromosomes 2, 6, 12, 13, or 16 could be seen in seven of the conventional lipomas or in any of the angiolipomas. For complete SNP results, see Supporting Information Table 2.

GGE Analysis

The expression levels of all 38 genes mapping to the SCL-associated MDR1 and MDR2 could be investigated in 12 SCL and 7–8 conventional lipomas with cytogenetic or SNP identified deletions of 13q14. Gene expression levels in these tumors were compared with 16 conventional lipomas without 13q14-deletion, and a control group including seven angiolipomas and three WAT. The Mann-Whitney *U* test, with Bonferroni corrected *P* values, identified ten genes with significantly lower expression in SCL compared with

the control group (*RB1*, *RCBTB1*, *KPNA3*, *C13orf1*, *TRIM13*, *KCNRG*, *DHRS12*, *ATP7B*, *ALG11*, and *VPS36*). When compared with conventional lipomas without 13q-deletion, five genes showed significantly lower expression in SCL (*C13orf1*, *DHRS12*, *ATP7B*, *ALG11*, and *VPS36*). In conventional lipomas with 13q14-deletion six genes with significantly lower expression was seen compared with the controls (*RB1*, *FNDC3AN*, *KPNA3*, *C13orf1*, *TRIM13*, and *ALG11*). When compared with conventional lipoma without 13q-deletion, two genes showed significantly lower expression in conventional lipomas with 13q14-deltion (*C13orf1* and *DHRS12*). In summary, one gene (*C13orf1*) with lower expression in all comparisons between SCL and conventional lipomas with 13q14-deletion and two control sets was identified. See Table 2 for a summary of the different comparisons and Supporting Information Table 3 for complete gene expression data.

qRT-PCR

qRT-PCR analyses for *RB1*, *C13orf1*, *HMGA1*, exons 1–2 of *HMGA2*, and miRNA genes *miR-15a* and *miR-16-1* were carried out in 7–9 SCL, 7–8 conventional lipomas with 13q14 deletion and 4–5 conventional lipomas without 13q-deletion. A *P* value was calculated through the Mann-Whitney *U* test using the dC_T values of the tumors compared with the dC_T values of the control samples.

The majority of SCL and conventional lipomas with 13q14-deletion showed lower expression of *RB1*, *C13orf1*, *miR-15a*, and *miR-16-1* when compared with the mean expression levels among the controls (Figs. 2A–2D); the only significant differences, however, were the lower expression levels of *C13orf1* ($P = 0.02$) and *miR-16-1* ($P = 0.03$) in SCL and *C13orf1* ($P = 0.02$) in conventional lipomas with 13q14–deletion. No significant differences were seen for *HMGA1* (Fig. 2E). Aberrant expression ($\log_{10} > 1$) of *HMGA2* was seen in one of seven SCL, in 4/8 conventional lipomas with 13q14 deletion and in all four conventional lipomas without 13q-deletion (Fig. 2F).

Sequencing

No mutation was found in any of the 20 cases analyzed for *miR-15-a* and *miR-16-1* (Table 1). Sequencing of all exons except exon 14 of the *RB1* showed no mutation. In case 16 the primers did not bind specifically to exon 1, so several bands were seen at the RT-PCR analysis and sequencing gave rise to two or more unspecific sequences.

DISCUSSION

In this study, we attempted to delineate the region(s) in chromosome 13 affected by recurrent deletions in SCL and conventional lipoma, and to study potential target genes for the deletions. Although SCL and conventional lipoma are considered distinct entities, morphologically as well as clinically (Fletcher et al., 2002), the distinction is not always easy to make at histopathologic analysis. Thus, it may well be that some of the conventional lipomas studied here actually represent lipoma-like SCL (Fletcher et al., 1996). It should be emphasized, however, that all tumors were diagnosed by experienced soft tissue pathologists, following the criteria outlined in the WHO classification (Fletcher et al., 2002). The potential difficulties of distinguishing SCL from conventional lipoma notwithstanding, it is reasonable first to discuss the findings in the two tumor types separately, before considering the possibility that the targets for the 13q-deletions are the same.

All 11 SCL that could be analyzed by SNP array showed hemizygous deletions affecting 13q, ranging in size from more or less the entire chromosome arm in two cases to deletions of a few megabases in two; all cases shared loss of two discontinuous regions (MDR1 and MDR2) in 13q14. A previous FISH study on various benign or borderline malignant adipocytic tumors with 13q-deletions (Dahlén et al., 2003a), in part including tumors included also in this study, identified a minimally deleted region that overlaps with the MDR2 identified here.

To select potential target genes in MDR1 and MDR2, GGE analysis was performed. As we are not aware of any SCL without 13q-deletion, we instead used RNA from seven angiolipomas, which are benign adipocytic tumors without any known genetic aberrations, and three WATs as control. In addition, 16 conventional lipomas without cytogenetic or SNP signs of 13q-deletion formed a second set for comparisons. When looking at the expression levels for the genes in the MDRs, none of the four genes in MDR1 and only five genes (*C13orf1*, *DHRS12*, *ATP7B*, *ALG11*, and *VPS36*) in MDR2 were found to be expressed at significantly lower levels in SCL both when compared with the control samples

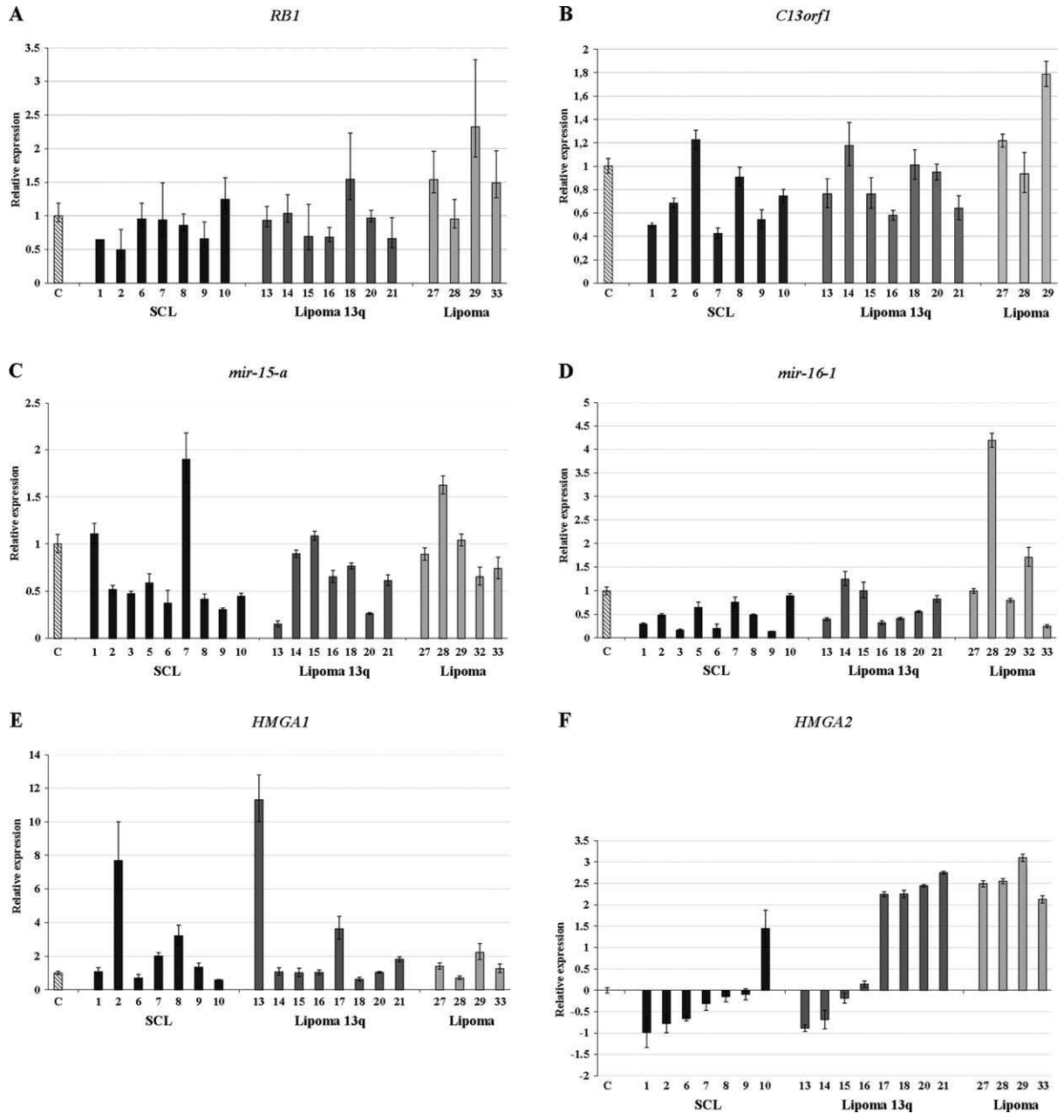

Figure 2. Gene expression levels detected by quantitative real-time PCR in Controls (C), Spindle cell lipomas (SCL), conventional lipomas with 13q-deletion (Lipoma 13q) and conventional lipomas without 13q-deletion (Lipoma). The values for the control group represent the mean expression among three white adipocytic tissue and five angiolipomas. The individual relative expression levels are $\log_2$ values or, for *HMGA2*, $\log_{10}$ values. The case number is indicated under each bar. (A) *RB1*, (B) C13orf1, (C) *miR-15a*, (D) *miR-16-1*, (E) *HMGA1*, and (F) *HMGA2*.

and to conventional lipomas without 13q-deletion (Table 2 and Supporting Information Table 3). Of these five genes, *C13orf1* has previously been identified as deleted (Mabuchi et al., 2001; van Everdink et al., 2003) and down-regulated (Mertens et al., 2002) in CLL; the loss of gene expression was, however, not associated with hypermethylation or mutation of the other allele (Mertens et al., 2002). The function of *C13orf1* is still unknown and the predicted protein sequence revealed no homology with known proteins (Mabuchi et al., 2001). *ALG11* encodes a mannosyltransferase involved in protein glycosylation. Rind et al., (2010) recently described two siblings with a constitutional, homozygous mutation in the *ALG11* gene, leading to a multisystem

metabolic disorder. Interestingly, the older of the two siblings developed fat pads on the breast, neck and temples between four and eight months of age. Both parents, who were obligate carriers, were, however described as healthy. None of the three other genes has been implicated in tumor suppression or in adipocytic differentiation.

RB1, mapping to the MDR1 in SCL, is a well known tumor suppressor gene (Friend et al., 1986; Weinberg, 1995). It showed lower expression levels in SCL compared with controls and conventional lipomas without 13q-deletion, but only the former difference was significant (Table 2 and Supporting Information Table 3). Constitutional mutations of the *RB1* gene confer an increased risk for retinoblastoma, but other tumor types have been implicated (Weinberg, 1995). Of particular interest in this context, Li et al., (1997) showed that lipomas, most of which were solitary and located in the neck or back, occurred more frequently in patients with hereditary retinoblastoma than in patients with sporadic retinoblastoma (3.6% vs. 0.6%); interestingly, 86% of the patients developing lipomas were men. Genuardi et al., (2001) have presented a family with constitutional *RB1* mutation and multiple lipomas, which were preferentially located on the neck, shoulder, face and upper chest, and in 13 of 15 cases affecting male relatives. As SCL are usually located s.c. on the neck, shoulder, and back and preferentially affect men, it is possible that the reported "lipomas" in the retinoblastoma families actually were SCL. In any case, these reports suggest a link between constitutional *RB1* mutations and the development of lipomatous tumors. Also experimental data implicate the *RB1* gene in the development of adipocytic tumors. It has been shown that mouse $Rb1^{-/-}$ fibroblasts fail to differentiate into fat-storing cells (Chen et al., 1996; Hansen et al., 1999; Classon et al., 2000) and that RB1 suppresses peroxisome proliferator-activated receptor γ (*PPARγ*), a key player in adipocytic differentiation (Auwerx et al., 1996; Fajas et al., 2002a,b; Calo et al., 2010). Furthermore, HMGA1 has been shown to inhibit RB1 (Ueda et al., 2007; Esposito et al., 2009). Thus, although the GGE data did not provide compelling evidence for significantly reduced expression of *RB1* in SCL, we considered it worthwhile to perform also qRT-PCR and sequencing of the gene. The expression levels were indeed reduced, but not significantly so (Fig. 2A), and no mutations were seen at sequencing. Despite the clinical and experimental arguments favoring a role for *RB1* in the development of lipomatous tumors, as well as the fact that it mapped to one of the two MDRs, we therefore must conclude that there is no decisive support for *RB1* being the main target for the 13q-deletions in SCL.

miRNA genes are small non-coding RNAs that regulate gene expression by binding to mRNA, leading to its degradation or inhibition of protein synthesis (Meister and Tuschl, 2004). In this way, miRNA genes have an impact on many biological processes, including tumorigenesis. *miR-15a* and *miR-16-1*, mapping to MDR2, have a common seed sequence and show 80% homology (Bonci et al., 2008). There is strong evidence that they act as tumor suppressors in a number of tumor types, including CLL, prostate cancer and NSCLC (Calin et al., 2002; Bonci et al., 2008; Bandi et al., 2009; Klein et al., 2010). It was recently shown that *mir-16-1* regulates the expression of the *HMGA1* gene (Kaddar et al., 2009), implicated in lipoma development through recurrent translocations affecting band 6p21; loss of *miR-16-1* could thus be an alternate way of up-regulating *HMGA1*. Hence, the two miRNA genes as well as *HMGA1* were studied by qRT-PCR. We found significantly reduced expression of *miR-16-1*, but not *miR-15a*, in SCL compared with the control samples (Figs. 2C and 2D). Possibly, the detection of *miR-15a* was influenced by the expression of a homologous gene locus, *miR-15b/miR-16-2*, located at 3q26 (Mourelatos et al., 2002; Zhang et al., 2006). Sequencing did not reveal any mutations in *miR-15a* or *miR-16-1*, which thus far has been detected only in a small fraction of CLL (Calin et al., 2005). Nor was the expression of *HMGA1* higher among SCL than among the controls, or was there any association between expression levels of *HMGA1* and *miR-16-1* in the individual cases (Figs. 2D and 2E). Thus, our data implicate *miR-16-1* as a potential target for the deletions at 13q in SCL, but the pathogenetic mechanism does not seem to involve the *HMGA1* gene.

As transcriptional up-regulation of the *HMGA2* gene, typically through translocations separating the first three exons from the 3′ UTR, has previously been shown to be a very frequent phenomenon in conventional lipomas (Ashar et al., 1995; Schoenmakers et al., 1995; Ligon et al., 2005), we were also interested in studying whether there was any correlation between 13q-deletions and *HMGA2* up-regulation/rearrangement. No cryptic deletions involving the *HMGA2* locus were seen at SNP array analysis, and exons 1–3 were transcriptionally silent (lower expression than in the

controls) in six of seven cases (Table 1, Fig. 2F). *HMGA2* expression is thus of little or no importance in SCL development, separating SCL from most conventional lipomas and atypical lipomas (Bartuma et al., 2009).

We could analyze 11 conventional lipomas with 13q-deletions; in two tumors (cases 17 and 23) the deletion was not detectable at banding analysis. The deletion in one of these two cases delineated a 3.5 Mb MDR in 13q14, hemizygously lost in all 11 tumors. The MDR in the conventional lipomas overlapped with the MDR1 in SCL and hence shared the loss of *RB1*, *LPAR6*, *RCBTB2*, and *CYSLTR2* (Figs. 1A and 1B). It should be noted that the remaining 10 conventional lipomas also showed hemizygous loss of the MDR2 in SCL. Thus, it was reasonable to study the expression of genes mapping to both MDR1 and MDR2 also in the conventional lipomas. Only one gene—*C13orf1*—was expressed at significantly lower levels in conventional lipomas with 13q-deletions compared with the controls and lipomas without 13q-deletion (Fig. 2B; Table 2 and Supplementary Table 3).

The arguments raised for analyzing *RB1* in SCL apply also to conventional lipomas. Although the gene showed lower expression in conventional lipomas with 13q14-deletion than in the controls, the reduced expression could not be verified at qRT-PCR (Fig. 2A). Furthermore, no mutation was found upon sequencing of *RB1*. Thus, our data do not suggest that *RB1* is the target for 13q-deletions in conventional lipomas. Nor was there any significant reduction of *miR-15a* or *miR-16-1* in conventional lipomas with 13q14-deletions.

Whereas SCL did not show any aberrant *HMGA2* expression, the association between 13q-deletion and *HMGA2* seems more complex in conventional lipomas. We have previously shown that some conventional lipomas with 13q-deletion express full-length or truncated *HMGA2* (Bartuma et al., 2009). In this study, four of eight conventional lipomas with 13q-deletion expressed *HMGA2*. One of these (case 17) was identified by G-banding to have a t(3;12), and it is reasonable to assume that the deletion on 13q was a secondary hit. Interestingly, also two of the three other cases with *HMGA2* expression showed genomic alterations of the *HMGA2* region; one with a discontinuous duplication of 12q, leading to gain of exons 1-3 of *HMGA2*, and one with a small deletion centromeric (upstream) to *HMGA2* as well as a 12q14-rearrangement at G-banding. The remaining four conventional lipomas with 13q-deletion that could be analyzed at the expression level did not show aberrant expression or genomic alterations of *HMGA2*. Our results indicate that 13q-deletions in conventional lipomas might occur in two contexts, first, as a secondary hit in lesions with aberrant *HMGA2* expression and, second, as a primary hit in lipoma-like SCL.

The classic approach to identify target genes for neoplasia-associated chromosome deletions is to start by identifying MDRs. Often, however, deletions are fairly large, and the delineation of the MDR(s) depends on only a fraction of the cases. The SNP array analysis performed here provided no exception. In both the SCLs and, in particular, the conventional lipomas the deletions typically extended over several chromosome bands, and the MDRs in each tumor type were based on single cases; hence, it is prudent to acknowledge the possibility that other loci, outside the MDRs, may be of pathogenetic importance in cases with larger deletions. Still, it is of interest to note that not only do the MDRs detected in SCL and conventional lipoma partly overlap, but they also correspond to MDRs identified in several other tumor types characterized by 13q-deletions (La Starza et al., 1998; Nupponen and Visakorpi, 2000; Zojer et al., 2000; Schlade-Bartusiaket al., 2005; Zhang et al., 2006; Bandi et al., 2009; Moorman et al., 2010; Palamarchuk et al., 2010). Bearing in mind that SCL and conventional lipoma share morphological features, we consider it likely that the target gene(s) for the recurrent 13q-deletions is the same in the two tumor types. A further argument for the hypothesis that there are shared pathogenetic mechanisms in SCL and a subset of conventional lipomas is that both tumor types showed recurrent deletions also on other chromosomes, notably chromosomes 2, 6, and 16. It was of interest to note that the 6q- and 16q-deletions only occurred in tumors with simultaneous loss of 13q, whereas 2q-deletions were found also in tumors without 13q-deletions. Thus, as already indicated by cytogenetic analyses (Mandahl et al., 1994b), 13q-, 16q-, and 6q-deletions may cooperate in lipomatous tumorigenesis.

ACKNOWLEDGMENTS

SCIBLU Genomics at Lund University, Lund, Sweden is acknowledged for help with the SNP array and global gene expression experiments. The Department of Pathology, Rikshospitalet,

Oslo, Norway is acknowledged for providing the PCR protocol for sequencing of *RB1*. Helene Jacobsson, biostatistician at the Region Skåne Competence Center for clinical research at Lund University, Lund, Sweden, is acknowledged for help with the statistical analyses.

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