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CASE REPORT

Langerhans cell sarcoma following marginal zone lymphoma: expanding the knowledge on mature B cell plasticity

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Abstract The concept of unidirectional differentiation of the haematopoietic stem cell has been challenged after recent findings that human B cell progenitors and even mature B cells can be reprogrammed into histiocytic/dendritic cells by altering expression of lineage-associated transcription factors. The conversion of mature B cell lymphomas to Langerhans cell neoplasms is not well documented. Three previous reports have described clonally related follicular lymphoma and Langerhans cell tumours, whereas no case has been published of clonally related marginal zone lymphoma and Langerhans cell sarcoma. We describe the case of a 77-year-old patient who developed a Langerhans cell sarcoma and 6 years later a nodal marginal zone lymphoma. Mutation status examination showed 100 % gene identity to the germline sequence, suggesting direct trans-differentiation or dedifferentiation of the nodal marginal zone lymphoma to the Langerhans cell sarcoma rather than a common progenitor. We found inactivation of paired box 5 (*PAX-5*) in the lymphoma cells by methylation, along with duplication of part of the long arm of chromosomes 16 and 17 in the sarcoma cells. The absence of PAX-5 could have triggered B cells to differentiate into macrophages and dendritic cells. On the other hand, chromosomal imbalances might have activated genes involved in myeloid lineage maturation, transcription activation and oncogenesis. We hypothesize that this occurred because of previous therapies for nodal marginal zone lymphoma. Better understanding of this phenomenon may help in unravelling the molecular interplay between transcription factors during haematopoietic lineage commitment and may expand the spectrum of clonally related mature B cell neoplasms and Langerhans cell tumours.

Keywords Langerhans cell sarcoma · Marginal zone lymphoma · Trans-differentiation · GeneScan · Array comparative genomic hybridization

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Introduction

The World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues is mainly based on the lineage and stage of differentiation of the tumour cells corresponding to that of normal haematolymphoid differentiation [1]. The pluripotent stem cell typically undergoes restriction of its developmental potential, thus transforming into multipotent progenitor cells, which, in turn, give rise to even more developmentally restricted progenitor cells or terminally differentiated functional cells [2]. Although the classical scheme of haematopoiesis envisages ordered unidirectional maturation of pluripotent stem cells into lineage-committed cells, with distinct morphologic, immunophenotypic and functional characteristics and a sealed and irreversible fate, the degree of commitment has come into question [3]. The concept of unidirectional differentiation has been challenged after recent findings that lineage plasticity occurs in the haematopoietic system, allowing a mature lymphocyte to be reprogrammed to a different lineage [4]. Not only human B cell progenitors [5–10] but even mature B cells

can be reprogrammed into histiocytic/dendritic cells by altering expression of lineage-associated transcription factors [3, 4, 11–17]. Reprogrammed cells demonstrate both phenotypic and functional features of macrophages. However, how one haematopoietic cell lineage can differentiate to another remains to be elucidated. Trans-differentiation as well as evolution from a common progenitor have been proposed to explain this extraordinary phenotypic conversion [14, 16–20]. In the trans-differentiation model, mature neoplastic B cells would differentiate directly into a phenotypically different haematolymphoid lineage via altered transcriptional regulation controlled by genetic or epigenetic mechanisms [15]. Activation of the transcription program related to the myeloid-macrophage lineage (i.e. over-expression of the Ets-family transcription factor PU.1 and CCAAT/enhancer-binding proteins C/EBP α and β) and silencing of B cell transcription factors (i.e. paired box 5 (PAX-5) downregulation) are essential in this process [21]. In this model, the transformed neoplasm partially retains the immunophenotype and expression signature as well as the same immunoglobulin (Ig) heavy-chain gene rearrangement (*IGH*) of the primay tumour [14, 15, 22]. In the dedifferentiation model, the neoplastic mature B cell regresses to the pluripotent progenitor stage and then regains the capability of differentiation along a different route. Again, PAX-5 is thought to exert a key function, since decreased expression of PAX-5 drives dedifferentiation of the B cell into an uncommitted precursor, and primary and derived neoplasm share common genetic alterations and the same *IGH* rearrangement [4, 22, 23]. A third mechanism for cross-lineage transformation might be the presence of a common progenitor in bone marrow, able of myeloid and lymphoid differentiation [5, 18, 19]. Older literature reports the anecdotal association of histiocytic and dendritic cell neoplasms with most precursor and mature B cell lymphomas and considers this phenomenon as a coincidence or as a reaction of the immune system to the treatment [8, 24–28]. Recently, evidence of a clonal relationship between precursor or mature B cell lymphoma and histiocytic and dendritic cell sarcoma has been provided [3–7, 9–18, 21]. However, the conversion of a mature B cell neoplasm (i.e. follicular lymphoma (FL), small lymphocytic lymphoma (SLL) and marginal zone lymphoma (MZL)) to a Langerhans cell neoplasm (i.e. Langerhans cell hystiocytosis (LCH) and sarcoma (LCS)) is not well documented. In only three reports, the association of FL with Langerhans cell neoplasms (LCH in two cases and LCS in one case) sharing an identical *IGH* rearrangement has been described [17, 29]. We have found no published case of MZL in combination with LCS nor cases with in-depth molecular analysis of a possible clonal relationship between the two components. A previously reported case of concurrent MZL and LCH in a thyroid gland with Hashimoto's disease proposed the association as an unusual form of host response to the tumour [25]. We provide convincing evidence for a clonal relationship and possible direct trans-differentiation of the MZL clone into the LCS one.

Clinical history

A 77-year-old male with unremarkable past medical history presented to our hospital with progressively decreased energy and night sweats. Laboratory tests revealed severe anaemia and computed tomography showed diffusely enlarged lymph nodes on both sides of the diaphragm. A left supraclavicular lymph node excision was performed, and the morphologic features and immunophenotyping led to a diagnosis of nodal marginal zone lymphoma (NMZL). A staging bone marrow biopsy showed 10 % lymphomatous involvement consistent with a marginal zone origin. The patient received six cycles of chemotherapy (rituximab *plus* cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP)), and a complete remission was achieved, confirmed by clinical, radiological and laboratory findings. Six years later, the patient presented with a rapidly enlarging cervical lymphadenopathy of 2-month duration. Imaging studies were suspicious for a relapse, and histological evaluation of the excised lymph node resulted in the diagnosis of Langerhans cell sarcoma associated to MZL. Two months later, the patient died due to progressive deterioration of his general condition.

Materials and methods

Histologic and immunohistochemical studies

Lymph node specimens and bone marrow core biopsy were fixed in buffered formalin. The bone marrow biopsy was rapidly decalcified after fixation. All samples were processed according to standard procedures, embedded in paraffin, sectioned and stained with Giemsa and haematoxylin and eosin (H&E). Paraffin sections (4 μm) were immunostained with antibodies against CD20, CD2, CD3, CD4, CD5, CD8, CD21, CD23, CD30, CD10, Bcl-2, Bcl-6, CD79A, PAX5, cyclin-D1, IgM, IgD, S100, CD1a, CD68, ALK-1, Langerin, PU.1, and Ki-67 on Bond-III machine with DAB (Dako, Milan, Italy) as chromogen. To assure specificity, positive and negative control slides for each antibody were included.

Laser capture microdissection

To compare the clonal relationship between the MZL cells and the cells of LCS, the respective neoplastic populations were isolated from tissue sections using immuno-guided laser capture microdissection (LCM). Sections immunostained for PAX-5 (identifying lymphoma cells) and S-100 (identifying sarcoma cells) were microdissected using a PixCell IIe

microscope (Arcturus Engineering, MGW, Florence, Italy) as previously described [30].

GeneScan analysis of clonality and sequence analysis

Genomic DNA (gDNA) was isolated from formalin-fixed, paraffin-embedded tissue (FFPE) using a MagCore Automated Nucleic Acid Extractor (MagCore HP16Plus: RBC Bioscience) and the MagCore Genomic DNA FFPE One-Step Kit following the manufacturer's instructions, providing a total amount of 20 ng of DNA. For PCR amplification of the *IGHV* repertoire, a modified BIOMED-2 protocol [31] was used with an increase of the number of cycles to 40. GeneScan analysis for detection of clonal V(D)J rearrangements was performed as previously described [31] using BIOMED-2 VHFR2-JH primers with a fluorescence JH consensus primer labeled at the 5′ end with FAM and resolved using capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (Applied Biosystem). Raw data were analyzed with GeneMapper v4.0 software (Applied Biosystem). Then, the *IGVH* repertoire was amplified for sequencing analysis using the same conditions as described above but with unlabeled JH consensus primer. PCR products were separated by agarose gel electrophoresis, and bands representing clonal V(D)J rearrangements were cut out and purified with the QIAGEN DNA purification columns (QIAGEN). Purified PCR products were sequenced in both directions with the ABI PRISM BigDye Terminator v1.1 sequencing kit (Applied Biosystem) in ABI PRISM 310 Genetic Analyzer and analyzed with the software Sequencing Analysis 2.1.

To determine the corresponding germline segments and the number and location of somatic mutations, sequence data were analyzed using the IMGT database and IMGT/V-QUEST tools (International ImMunoGeneTics database (IMGT), Marie-Paule Lefranc, Montpellier, France; http://imgt.cines.fr:8104).

To avoid misidentification of mutations when IGHV FR2 consensus primers were used, nucleotide substitutions in the obtained sequences were evaluated from codon 49 in FR2-IMGT. The downstream end of the analyzed V region corresponds to the 5′ end of the germline CDR3-JH as defined by IMGT/Junctional Analysis. Control gene primer sets for quality assessment of DNA were applied according to the BIOMED-2 protocol [31]. Monoclonal gene rearrangements were identified as prominent, single-sized amplification products.

Methylation-specific PCR assay and array-comparative genomic hybridization

Methylation assay was performed as previously described [32], and a positive (CpGenome Universal Methylated DNA, Chemicon International) and a negative control (blood lymphocytes from cancer-free nonsmokers previously demonstrated to be non-methylated) were used for all the PCR amplifications.

Array-CGH was performed using 500 ng of genomic DNA extracted from multiple sections of representative blocks and using the Agilent 4×44 K platform following manufacturer's protocol. Graphical visualization of the results was provided by the Genomic Workbench software v. 6.5.0.18, and the aberrations were called by the ADM1 algorithm [18]. Genes residing in the chromosomes of interest were analyzed by "Atlas of genetics and cytogenetics in oncology and haematology" (http://atlasgeneticsoncology.org/) and gene browser (http://genome.ucsc.edu/).

Results

Histologic and immunophenotypic findings

Histologic evaluation of the first sample showed a lymph node completely effaced by a population of neoplastic cells with a marginal zone pattern of infiltration. The residual follicles were partially expanded or regressed and appeared colonized by neoplastic cells (Fig. 1a). The surrounding cells slightly varied in size, from small centrocyte-like cells with irregular nuclei, condensed chromatin, and scant cytoplasm, to monocytoid cells with a rim of pale cytoplasm. Intermingled blast cells were rare (Fig. 1b). The neoplastic population was positive for CD20 (Fig. 1c), PAX-5 (Fig. 1d) and IgM, and negative for CD5, CD23, CD10, BCL-6 and cyclin D1. CD21 immunostain highlighted follicular dendritic cells in the regressed follicle centre (Fig. 1e). The proliferative index (Ki-67) was about 20 % (Fig. 1f). The bone marrow biopsy showed multiple lymphoid aggregates and infiltration of the interstitium by small- to medium-sized CD20-positive lymphocytes consistent with involvement by MZL. Histologic evaluation of the second lymph node specimen showed the coexistence of diffusely proliferating lymphocytes of variable size, predominantly small/intermediate (Fig. 2a) with few admixed larger cells, characterized by eosinophilic, relatively abundant cytoplasm and pleomorphic, grooved, folded nuclei with fine chromatin and prominent nucleoli (Fig. 2b inset). The small/intermediate lymphocytes had clumped nuclear chromatin, and the larger cells had vesicular nuclei. Medium to large monocytoid cells with ample cytoplasm were also present. Morphologically and immunophenotypically (CD20 and PAX-5 positive and PU.1 negative), the smaller cells represented a MZL. The larger cells, which occupied and greatly expanded interfollicular zones, resembled a sarcoma. They were positive for vimentine, CD68 (Fig. 2c), S-100 (Fig. 2d), CD1a (Fig. 2e), langerin (Fig. 2f) and PU.1 and negative for CD10, CD2, CD3, CD4, CD8, CD-30 and ALK-1. CD20 (Fig. 2g), CD79A and PAX-5 (Fig. 2h) highlighted the double population, with MZL cells being

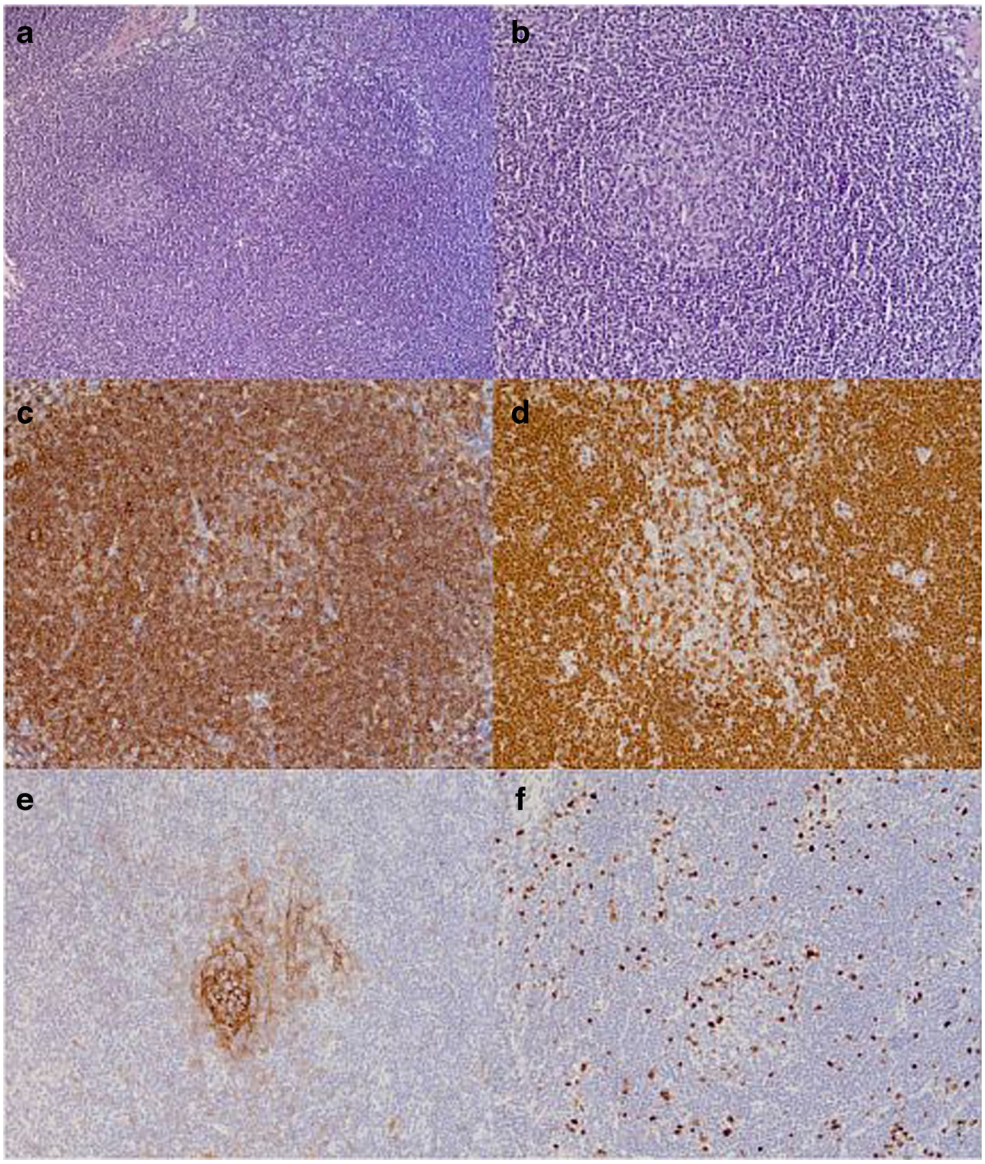

Fig. 1 Histological and immunophenotypical findings of the nodal marginal zone lymphoma in the first specimen. **a** The lymph node was completely effaced by a population of neoplastic cells having a marginal zone pattern of infiltration with partially expanded and regressed follicle, showing neoplastic colonization. **b** The neoplastic cells ranged from small centrocyte-like cells to "monocytoid" cells intermingled with few blast cells. **c** CD20 positivity. **d** PAX-5 stain. **e** CD21 highlights follicular dendritic cells in the regressed follicle center. **f** The proliferative index (Ki-67) was about 20 %. [**a–b** Haematoxylin and eosin (H&E). **a** Original magnification (OM): 5×; **b–f**, OM: 10×]

positive and the LCS cells negative. Ki-67 stain labelled 90 % of neoplastic cells. A few neutrophils and eosinophils were also present.

Clonality analysis

To assess the clonal origin of both neoplasms, we investigated the status of the *IGH* rearrangements with DNA extracted from the first and the second lymph node specimens. On the tissue from the relapse, LCM was performed to clearly separate the MZL cells from the LCS cells. Monoclonal FR21GHV-PCR products of the same size (GeneScan Analysis) and identical IGHV-D-J rearrangement (IGHV3-30-3*01, IGHJR*22*01) were detectable in both neoplasms (Fig. 3a–c). A contamination of IGH-PCR and GeneScan analysis by residual blast cells or by B lymphocytes can be excluded as the analysis was carried out on LCM specimens, and the detection limit of the PCR assay requires at least 1 % of clonal B cells in the sample to generate a dominant PCR product [5]. Sequence analysis identified 100 % gene identity to the germline sequence; therefore, both neoplasms showed an unmutated rearrangement.

Methylation analysis

To determine if alteration in expression of *PAX-5* might be implicated in dedifferentiation or trans-differentiation of MZL cells, we performed methylation analysis of *PAX-5* α and β promoters on microdissected cells. The presence of bands of 166 and 124 bp in lane 1 (LCS) and 2 (positive control) indicates the aberrant methylation of *PAX5* α and β genes (Fig. 3d).

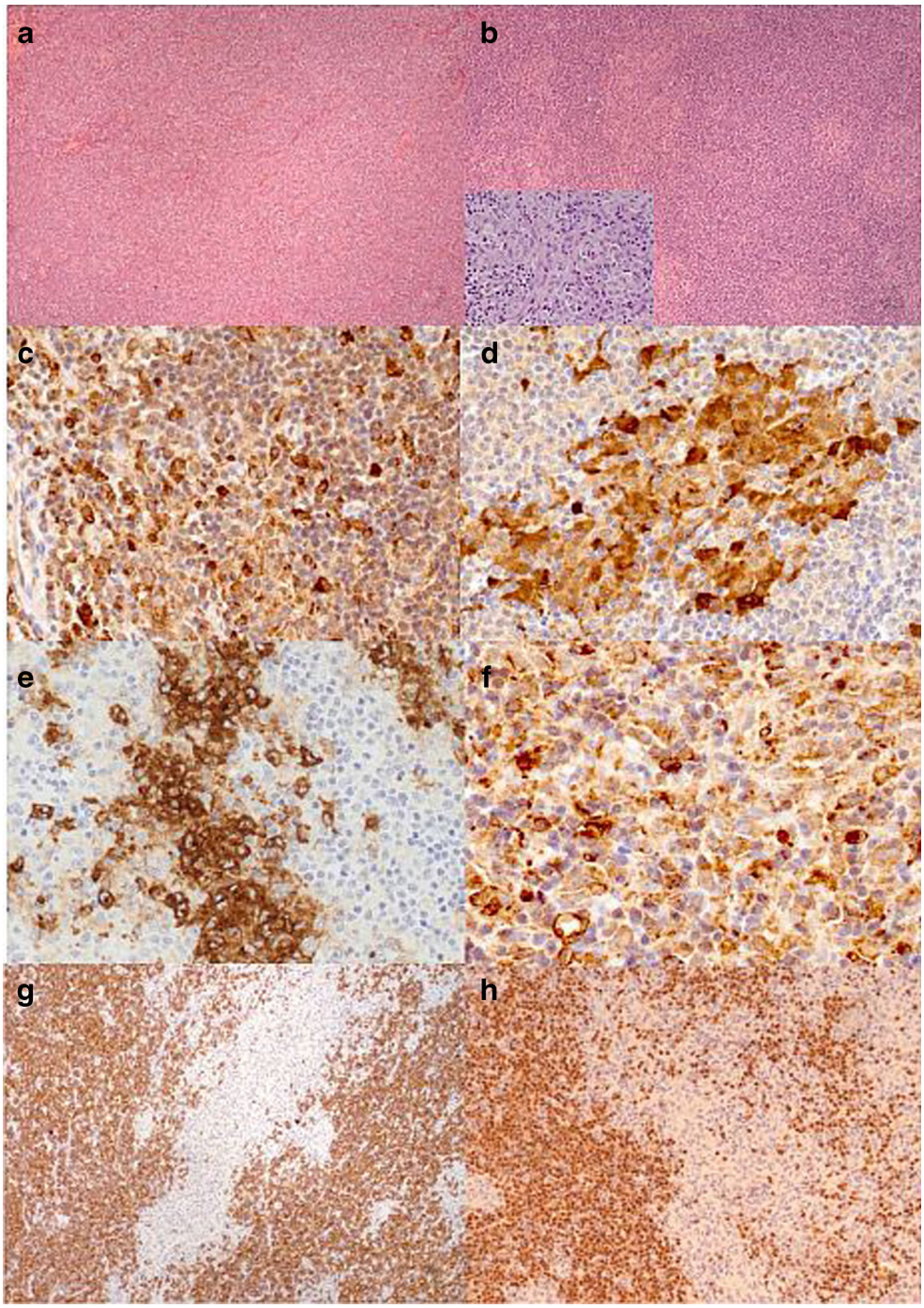

Fig. 2 Histological and immunophenotypical findings of combined marginal zone lymphoma and Langerhans cell sarcoma in the second specimen. **a** A diffuse proliferation of lymphocytes morphologically resembling a MZL is shown. **b** Large cells with eosinophil, abundant cytoplasm and pleomorphic, grooved, folded nuclei with prominent nucleoli (*inset*), consistent with a LCS diagnosis are intermingled with a lymphoid population histologically concordant with a MZL. **c** CD68, **d** S-100, **e** CD1a and **f** langerin positivity in LCS. **g** CD20 and **h** PAX-5 highlighted the double population, with MZL cells being positive and the LCS cells negative. (**a–b** H&E. **a–b** OM 5×. **b** Inset: OM 20×. **c–h** OM 20×. *MZL* marginal zone lymphoma, *LCS* Langerhans cell sarcoma)

Genome alterations associated with transformation from MZL to LCS

To further delineate the mechanisms of transformation of MZL to LCS, high-density aCGH was carried out to identify additional genome copy gains or losses in LCS compared with MZL on microdissected cells of both neoplasms. Both tumours showed deletion in the long arm of the chromosome 11 (11q14. 1q 24.1) and mosaic trisomy of the chromosome 12, suggesting a common origin for both neoplasms (Fig. 4a). Vice versa, LCS showed novel duplications in the long arms of chromosomes 16 and 17 in respect to MZL, which suggested accumulation of additional genetic alterations underlying the second tumour (Fig. 4b). Interestingly, some genes whose dysregulation may be relevant for transformation map in these genetic *loci* (Table 1). Among them, *CBF*, *NOD2*, *NFAT5*, *NFATC3* and *ETV4* may have an impact in the transformation of MZL to LCS.

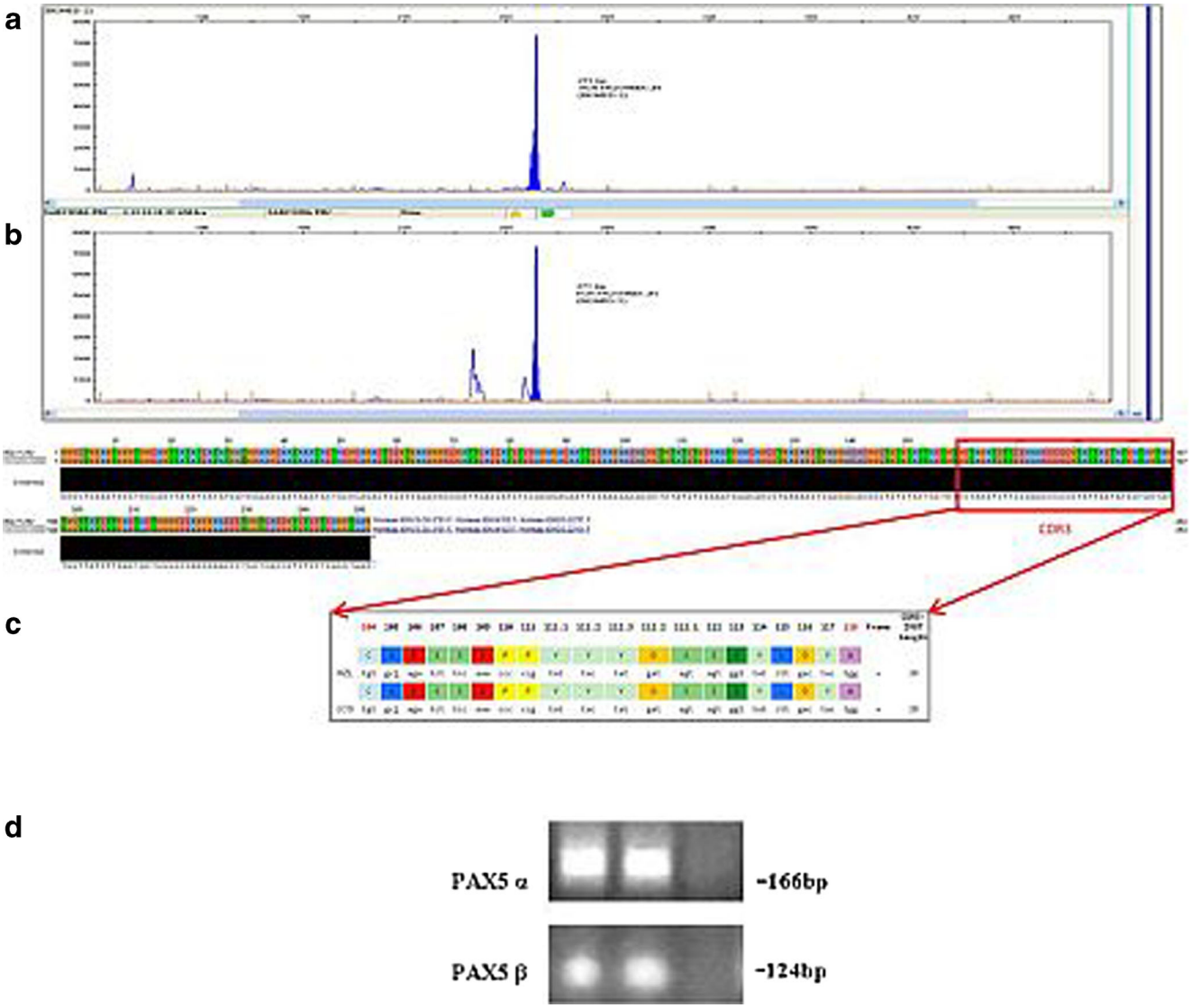

Fig. 3 Immunoglobulin clonality profile, sequencing analysis of IGHV-D-J rearrangements and methylation assay in marginal zone lymphoma and Langerhans cell sarcoma samples. GeneScan profiles showed identical size *IGH* gene rearrangements products in **a** MZL and **b** LCS samples. **c** MZL and LCS were characterized by the same IGHV-D-J rearrangement sequence (IGHV3-30-3*01, IGHJ4*02, IGHD3-22*01). **d** The presence of bands of 166 and 124 bp in lane 1 (LCS) and 2 (positive control) indicates the aberrant methylation of the *PAX5*-α and -β genes

Discussion

Dendritic cells are part of the haematopoietic system and are derived from CD34-positive haematopoietic progenitor cells [5]. They are a heterogeneous group of immune accessory cells with a pivotal role in the onset of innate immunity and peripheral tolerance [5]. There are four types of dendritic cells in the lymph node, each with distinct structural, functional and morphological features: follicular, interdigitating, Langerhans histiocytic/fibroblastic cells and plasmacytoid dendritic cells [5]. Langerhans cell sarcoma is a very rare high-grade neoplasm with overtly malignant cytologic features (pleomorphic nuclei with prominent nucleoli and nuclear grooves) and the Langerhans cell phenotype (S100, CD1a and langerin positive) [33]. Although Langerhans cells generally are believed to be myeloid, they differ from other dendritic cells in their ontogeny, anatomic distribution, phenotype and functional characteristics [17]. Previous studies have suggested a link to T lymphocyte progenitors; in fact, transformation of T lymphoblastic neoplasms to Langerhans cell tumours has been reported [14]. In contrast, a solid link between Langerhans cells and B cells has not been established. However, *IGH* rearrangements have been reported in sporadic cases of LCH [20]. Here, we describe the asynchronous occurrence of LCS 6 years after a diagnosis of MZL. The MZL and LCS cells harboured an identical *IGH* rearrangement, which excludes a merely coincidental occurrence of the two malignancies or a reactive process. Moreover, in both tumour types, a 100 % gene identity with

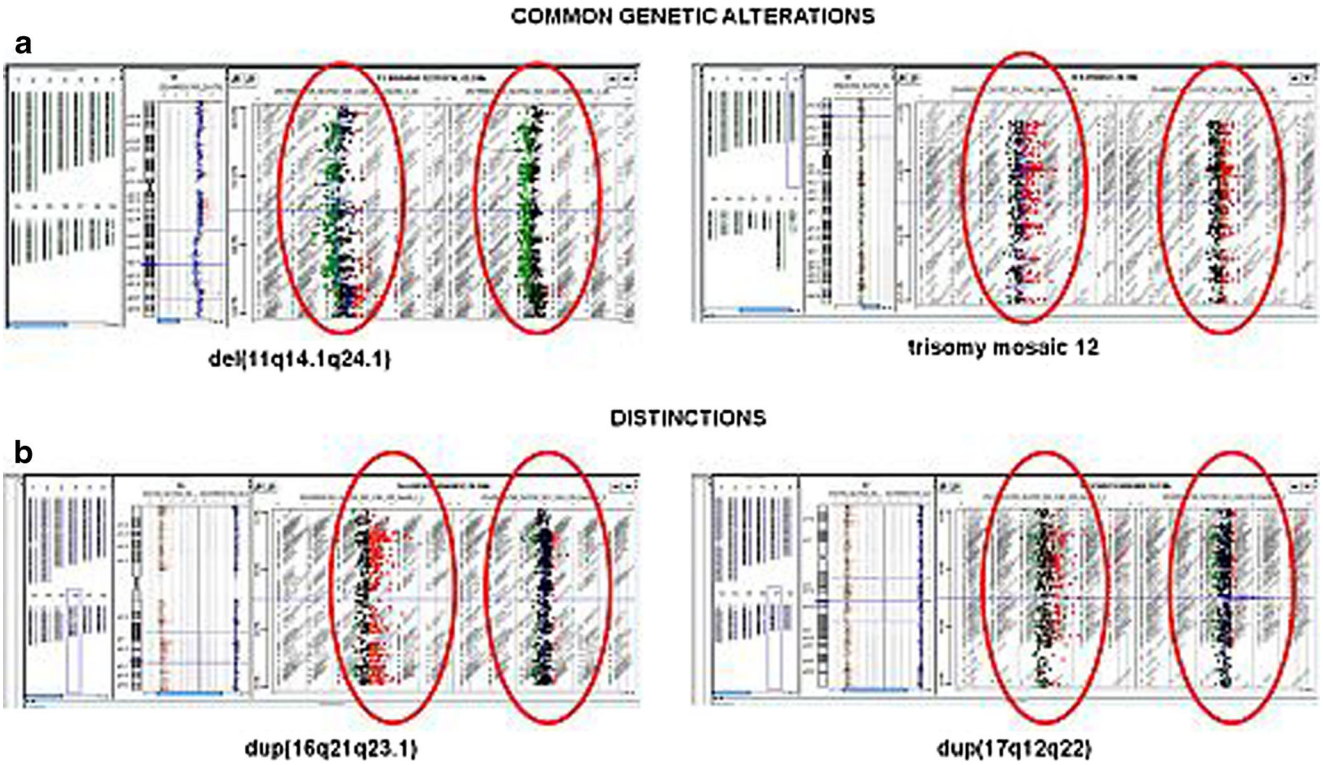

Fig. 4 Genome alterations associated with transformation from MZL to LCS. **a** MZL and LCS showed a common deletion in the long arm of the chromosome 11 (11q14. 1q 24.1) and a mosaic trisomy of the chromosome 12. **b** Duplications in part of the long arms of chromosomes 16 and 17 were observed in LCS in respect to with MZL

Table 1 Function, site and location of genes

Annotated gene	Location (base pairs)		Site	Function
	Start	Stop		
CBFB	67,063,050	67,134,958	16q22.1	Transcription factor which regulates the expression of myeloid and T cell-specific genes and cooperates with various tissue-specific factors to activate these lineage-restricted transcriptions
CTCF	67,596,310	67,673,088	16q21–q22.3	Transcriptional activation of *MYC* and p53
E2F4	67,226,068	67,232,821	16q21–q22	Regulation of the cell cycle by inducing a number of genes required for DNA synthesis and for cell cycle progression, including DNA polymerase α, cyclin-A, cyclin-E, *MYC* and cdc2
NFAT5	69,599,869	69,738,569	16q22.1	Member of the nuclear factors of activated T cells (NFAT) family of transcription factors. Proteins belonging to this family play a central role in inducible gene transcription during the immune response.
NFATC3	68,119,269	68,263,162	16q22.1	Regulation of gene expression in T cells and immature thymocytes
NOD2	50,733,261	50,766,988	16q21	The protein is activated in some types of immune system cells (including monocytes, macrophages and dendritic cells) and is able to induce the nuclear factor-kappa-B pathway
AATF	35,306,175	35,414,171	17q12	Cell growth
CDC6	38,444,146	364,594B	17q21.2	DNA replication
DDX5	62,494,374	62,502,484	17q21	Transcription regulation
ETV4	41,605,211	41,623,800	17q21.31	Transcription regulation by binding to the Ets-binding site in the promoter of its target genes
STAT 3	40,465,343	40,540,513	17q21.2	Transcription regulation

the germline sequence was observed. A previous study reported that about 30–50 % of splenic MZL show unmutated IgVH sequence and an adverse clinical course [34]. Our findings suggest that also NMZL might derive from an unmutated B cell precursor. The unmutated gene rearrangement in LCS cells seems at first glance to be in keeping with the possibility of direct trans-differentiation or dedifferentiation of NMZL to LCS, rather than a common progenitor.

We found PAX-5 expression to be present in the MZL component but absent in the LCS, the latter due to aberrant methylation and subsequent silencing of the gene. Inactivation of *PAX-5* in committed and mature B cells of MZL therefore seems to be the pathogenetic mechanism in our case. In fact, PAX-5 is essential for maintaining the identity and function of mature B cells during late lymphopoiesis [19]. In the absence of PAX-5, B cells show an amazing degree of plasticity and can differentiate in vitro, after stimulation with appropriate cytokines, into macrophages, dendritic cells, granulocytes, NK cells, and T cells by downregulating the transcription of B cell-specific genes and activating expression of non-B cell genes [32]. Accordingly, the lack of expression of PAX-5 together with the over-expression of PU.1 might have led to reprogramming from the lymphoid to the dendritic cell phenotype in our patient.

Array-CGH of both neoplasms showed the same deletion of long arm of chromosome 11 [del (11q14. 1q 24.1)] and mosaic trisomy of the chromosome 12, again confirming a common origin. In addition, duplications of part of 16q (16q21q23.1) and 17q (17q12q22) were identified as main additional alterations in the LCS compared with the MZL sample. It is interesting that these two regions contain genes involved in myeloid lineage maturation, transcriptional activation and oncogenesis. This is in keeping with a critical role of alterations of genes involved in macrophage and/or dendritic cell differentiation (*CBF*, *NOD*, *ETV4*, *PU.1*, *C/EBP* family), as well as in B cell differentiation (*PAX-5*) and in lineage switch and trans-differentiation. Increased activity of transcription factors (NFAT family members NFκB and STAT3) with pluripotent functions in the immune system as well as of genes regulating cell cycle progression (*E2F4*, *CTCF*, *AATF*, *CDC6*, *DDX5*) may provide additional oncogenic stimuli necessary for complete transformation of MZL to LCS. *CBF* and *NOD2* map to the 16q region. *CBF* regulates the expression of myeloid cell-specific genes and cooperates with various tissue-specific factors to activate this lineage-restricted transcription [35]. *NOD2* is active in monocytes, macrophages and dendritic cells in which it turns on the nuclear factor-kappa-B *(NFκB)* pathway [36]. The 16q region also contains two members of the NFAT family: *NFAT5* and *NFATC3*. *NFAT* and NFκB are related proteins that belong to the REL superfamily of transcription factors, which have crucial roles in activation, proliferation and apoptosis of cells in the immune system [11]. Constitutive activation of NFκB and NFAT transcription factors is important for proliferation and survival of aggressive lymphoma cells through regulation of the B lymphocyte stimulator (BLyS) pathway [37]. BLyS can also promote dendritic cell activation and maturation [38]. The *ETV4* gene maps to the 17q region and encodes a protein that binds to the promoter of the two master regulators of myelopoiesis (PU.1 and C/EPB α and β) and induces their transcription. They induce trans-differentiation of committed B cells into non-B cells and synergize to reprogram committed B cells into macrophages [19]. Why this transcriptional reprogramming occurred in our case is an unanswered question. Conceivably, selection pressure exerted by rituximab or other previous therapies might have favoured trans-differentiation. After therapy resting, resistant sub-clones may persist and transform mostly to diffuse large B cell lymphoma. However, they can occasionally evade the conventional pathway and commit toward a different haematolymphoid neoplastic lineage [17]. The lack of an intermediate step does not allow us to draw a final conclusion on whether direct trans-differentiation occurred or, alternatively, dedifferentiation into an uncommitted precursor cell which subsequently developed a Langerhans cell phenotype.

In conclusion, our case provides additional genotypic evidence of a clonal relationship between MZL and LCS occurring in the same patient. Both neoplasms harboured the same mutated *IGH* gene rearrangement. Clinical and molecular observations strongly favour a transformation scenario over that of a common progenitor. MZL followed by LCS was associated with a deteriorating clinical condition. Also, the additional genome changes in the long arms of chromosomes 16 and 17 in LCS cells are consistent with disease progression. Better understanding of the phenomenon of trans-differentiation may help in unravelling the molecular interplay between transcription factors during haematopoietic lineage commitment and the potential effect that therapy might elicit in favouring transformation.

Conflict of interest The authors declare that they have no conflict of interest.

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