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THYROID CARCINOMA-ASSOCIATED GENETIC MUTATIONS ALSO OCCUR IN THYROID LYMPHOMAS

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Abstract

Molecular testing for mutations activating the mitogen-associated protein kinase signaling pathway is being used to help diagnose thyroid carcinomas. However, the prevalence of these mutations in thyroid lymphomas has not been reported. Therefore, we studied the prevalence of *BRAF*, *NRAS*, *HRAS*, and *KRAS* mutations in 33 thyroid lymphomas and correlated the mutational status with the clinical, pathologic, cytogenetic, and immunophenotypic findings. Eleven cases were also tested for *PAX8/PPARγ* translocations. The lymphomas included 25 diffuse large B-cell lymphomas, 6 extranodal marginal zone lymphomas of mucosa associated lymphoid tissue type, and 2 follicular lymphomas. Seventeen diffuse large B-cell lymphomas were germinal center type, 6 non-germinal center type and 2 unclassifiable (Hans algorithm). None of the cases had an associated thyroid carcinoma. Mutations of the *BRAF* gene were identified in 6 (24%) diffuse large B-cell lymphomas (three D594G in germinal center diffuse large B cell lymphomas, two K601N in germinal center diffuse large B cell lymphomas, and one V600E in non-germinal center diffuse large B cell lymphomas) and of the *NRAS* gene in two (8%) non-germinal center diffuse large B-cell lymphomas (Q61K and Q61H). *BRAF* and *NRAS* mutations were not found in any extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type or follicular lymphoma. *HRAS* and *KRAS* mutations were not identified in any of the cases, nor were *PAX8/PPARγ* translocations found. Thus, interpretation of finding a *BRAF* or *NRAS* mutation in the thyroid, particularly in preoperative thyroid aspirates, must take into account the differential diagnosis of a lymphoma. In addition to the diagnostic importance, our data also demonstrate that alteration in the mitogen-associated protein kinase pathway may play a role in the pathogenesis of some large B-cell lymphomas of the thyroid with potential therapeutic implications.

Keywords

thyroid; lymphoma; BRAF; RAS; mutations

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Introduction

Papillary carcinoma is the most common type of thyroid cancer, representing approximately 80% of all malignant thyroid tumors.[1] Primary thyroid lymphomas, on the other hand, are rare and account for 1-5% of all thyroid malignancies and approximately 2% of all malignant extranodal lymphomas.[2-4] Initial diagnosis of the epithelial thyroid neoplasms, and subsequent therapeutic planning, is often based on fine needle aspiration biopsy and its cytological evaluation. In order to increase the diagnostic accuracy of thyroid fine needle aspiration material, molecular testing for a panel of mutations is being more commonly used.[5] Specifically, molecular analyses have focused on a set of somatic alterations of genes in the mitogen-activated protein kinase (MAPK) pathway which are frequently present in carcinomas of the thyroid. These include point mutations of the *BRAF* and *RAS* genes and *RET/PTC* and *PAX8/PPARγ* chromosomal rearrangements.[6-8] V600E mutation in the *BRAF* gene has been identified as the most common genetic event in papillary thyroid carcinoma occurring in 40-45% of cases.[9, 10] This mutation is also found in poorly differentiated and anaplastic thyroid carcinomas that typically have a component of residual well differentiated papillary carcinoma.[11] *RAS* mutations, on the other hand, are seen more commonly in thyroid tumors with a follicular pattern, including follicular carcinoma, follicular adenoma, and follicular variant of papillary carcinoma.[5]

Until the very recent discovery of BRAF V600E mutations in virtually all cases of hairy cell leukemia,[12-16] the presence of these mutations in non-Hodgkin lymphomas had received little attention. The recent studies of *BRAF* mutations in hairy cell leukemia have also included a very large number of B-cell lymphomas and have identified only 2 cases of other chronic lymphoproliferative disorder, not fulfilling the criteria for hairy cell leukemia with *BRAF* V600E mutations. However, the presence of V600E mutation in these 2 chronic lymphoproliferative disorders could not be confirmed on Sanger sequencing.[12-14, 16, 17] In an older study, Lee et al. reported *BRAF* mutations in 6% (4/67) of diffuse large B-cell lymphomas involving various non-thyroid sites. However, all four of these *BRAF* mutations involved codons other than codon 600, where the most common mutation occurs in papillary thyroid carcinoma.[18] Borie et al studied immunodeficiency related non-Hodgkin lymphomas with microsatellite instability and found V600E *BRAF* mutations in 3 of their 9 cases which included one diffuse large B cell lymphoma, one T cell post transplant lymphoproliferative disorder and one primary central nervous system B-cell non-Hodgkin lymphoma.[19] V600E *BRAF* mutations have also been reported in more than half of Langerhans cell histiocytoses,[20] rare T-cell acute lymphoblastic leukemias,[21] and few cases of multiple myeloma.[22] In addition, *NRAS* and *KRAS* mutations have frequently been reported in plasma cell myeloma and plasma cell leukemias,[23, 24] B-cell acute lymphoblastic leukemias[21] and some cutaneous T-cell lymphomas.[25] The frequency of these mutations in lymphomas of the thyroid, where they might cause the greatest diagnostic confusion, is unknown.

We recently encountered a thyroid mass sampled by fine needle aspiration, where a cytological diagnosis of malignant tumor was rendered with a differential diagnosis that included both carcinoma and lymphoma. Molecular analysis was positive for a *BRAF* V600E (c. 1799T>A) mutation, which was thought to support the diag-cell lymphoma with no evidence of a papillary carcinoma in the thyroid gland. This case prompted us to perform

a systematic study of *BRAF* and other common mutations activating the MAPK pathway (*NRAS, KRAS* and *HRAS*) in 33 lymphomas of the thyroid. Eleven cases were also investigated for *PAX8/PPARγ* rearrangement.

Methods

Case selection and review

Thirty-three B-cell lymphomas presenting in the thyroid were obtained from the Department of Pathology at the University of Pittsburgh. The study was approved by the University of Pittsburgh Institutional Review Board. The following clinical features were recorded after review of the de-identified information from electronic medical records: gender, age, clinical presentation, treatment and follow-up.

The routine histologic sections and cytologic preparations, together with all available immunohistochemical stains, flow cytometric data and cytogenetic findings were reviewed (N.A., S.H.S.). When not already available, at least the following immunohistochemical stains for the following antigens were performed: CD20, CD3, kappa, lambda, CD10, BCL6, IRF4/MUM1, Ki-67, Cyclin D1, PAX8 (Table 1). Lymphomas were classified using WHO criteria[26] and Diffuse large B cell lymphoma further characterized using the Hans' algorithm into germinal center (CD10 + or BCL6+ and IRF4/MUM1−) and non-germinal center (CD10−, BCL6− or BCL6+, IRF4/MUM1 +) types.[27] Results of all available classical cytogenetic or fluorescence in situ hybridization studies were also reviewed.

Microdissection & Nucleic Acid Isolation

Histologic sections with the most dense lymphoma infiltration were identified and 5-μm sections cut for *BRAF* and *RAS* mutation analysis. The targeted areas were manually microdissected to collect tumor tissue for DNA isolation. In one case, two different areas representing extranodal marginal zone lymphoma of mucosa associated lymphoid tissue type and diffuse large B cell lymphoma were microdissected separately. Genomic DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. Total nucleic acid was isolated from snap frozen tissue in 11 available cases (8 diffuse large B-cell lymphoma, germinal center type; 2 diffuse large B-cell lymphoma, non-germinal center type and 1 extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type) using the MagNA Lyser and MagNA Pure (Roche) for *PAX8/PPARγ* rearrangement studies.

Detection of point mutations

First, mutational analysis was performed for hotspots typically found in thyroid cancer, i.e. *BRAF* codons 600 and 601, *KRAS* codons 12 and 13, *HRAS* codon 61, and *NRAS* codon 61 using real-time LightCycler PCR followed by fluorescence melting curve analysis as previously described.[6] Briefly, for each gene a pair of oligonucleotide primers flanking the mutation site was designed together with two fluorescent probes with the sensor probe spanning the codon of interest (TIB Molbiol, Berlin, Germany). Amplification was performed for 40 cycles. Post amplification fluorescence melting curve analysis was performed by gradual heating of samples at a rate of 0.1 C/sec from 45 C to 95 C. For each

mutation hot spot, DNA from a tumor or cell line known to carry a specific mutation was used as a positive control and DNA from peripheral blood lymphocytes was used as a wild-type negative control. The sensitivity of mutation detection by melting curve analysis was 10% of cells with a mutant allele in the background of normal cells, as established by serial dilutions of the positive controls. Those samples that revealed no mutations in these hotspots, were further analyzed for mutations in the entire exon 15 of the *BRAF* gene, exon 2 of *NRAS* and *HRAS*, and exon 1 of *KRAS* using Sanger sequencing. Specifically, PCR amplification was performed using 25 ng of DNA and AmpliTaq Gold PCR Master Mix (Applied Biosystems, Inc, Foster City, CA). The PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems).

Detection of PAX8/PPARγ rearrangement by RT-PCR

In those samples where frozen tissue was available, *PAX8/PPARγ* rearrangement was detected by RT-PCR as previously described.[6] Briefly, reverse transcription and PCR amplification were performed in one-step using QuantiTech Probe RT-PCR Kit (Qiagen) and gene-specific primers and probes. The reverse transcription was carried out at 50 C for 30 min, followed by 40-cycle PCR amplification. RNA from a tumor known to carry the *PAX8/PPARγ* rearrangement was used as a positive control. The sensitivity of mutation detection by RT-PCR was 1% of cells carrying the rearrangement in the background of normal cells, as established by serial dilutions of the positive control.

Cytogenetic studies

Classical G-banded cytogenetic karyotypes were obtained using previously described methods.[28] Fluorescence in situ hybridization was performed on interphase nuclei according to the manufacturer's protocol using *IGH/MYC* dual color translocation probe, *IGH* breakapart probe, and *MYC* breakapart probe (Vysis DNA probes, Abbott Molecular Inc., Des Plains, IL). Two hundred nuclei were analyzed for each of the probes. The cut-off value used for false positive signals for the translocation probe was 1% and for the breakapart probe was 3%.[29]

Results

The patients included 28 females and 5 males with a mean age of 65 years (range of 30-94 years). All patients presented with a neck mass and a clinical diagnosis of a thyroid tumor. Biopsies/excisions showed 25 diffuse large B-cell lymphoma with 17 of germinal center, 6 of non-germinal center type and 2 unclassifiable (Hans' algorithm); 6 extranodal marginal zone lymphomas of marginal zone lymphoid tissue, including 3 with extensive plasmacytic differentiation, and 2 follicular lymphomas (one grade 1-2 of 3 and the other grade 3A of 3). None of the cases revealed an associated thyroid carcinoma based on histopathologic and immunohistologic studies. However, in addition to staining of thyroid epithelium, more than 80% of PAX8 positive neoplastic cells were present in 26/33 lymphomas, consistent with a recent study reporting that this antibody cross-reacts with PAX5.[30] The more plasmacytic areas of the tumors were PAX8 negative.

The index patient had a thyroid mass that was rapidly expanding and clinically suspected to be a carcinoma or lymphoma. Fine needle aspiration biopsy of the lesion showed numerous large neoplastic cells with scant to moderate amount of cytoplasm, irregular nuclear contours and conspicuous nucleoli in a background of many small lymphocytes (Figure 1A). A part of the specimen was sent for flow cytometric immunophenotypic evaluation and another for molecular testing. The flow cytometric studies revealed polyclonal small B lymphocytes and admixed T-cells, however, they did not characterize the large atypical cells. Molecular analysis revealed a *BRAF* V600E (c. 1799T>A) mutation (Figure 1B). The case was diagnosed as a malignant neoplasm favoring an anaplastic thyroid carcinoma. The thyroidectomy was performed and pathologic examination revealed a CD20 positive diffuse large B cell lymphoma of non-germinal center type (Figure 1C-E). The thyroid gland was entirely sectioned and submitted for microscopic examination, which revealed no papillary or anaplastic carcinoma. The mutation analysis was repeated on cells isolated from four different areas of lymphoma, all of which confirmed the presence of a *BRAF* V600E mutation. The adjacent normal thyroid tissue revealed wild-type *BRAF*. The patient was subsequently treated with CHOP-R chemoimmunotherapy (cyclophosphamide, Vincristine, Adriamycin and Prednisone with Rituximab) and radiation following his surgery. The patient is alive and had no evidence of disease on PET-CT scan 2 years post-diagnosis.

Molecular analysis of the 33 total thyroid lymphomas revealed 8 (24%) cases positive for one of the studied mutations. All mutations were identified in diffuse large B-cell lymphoma (8/25, 32%) (Table 2). There were 6 *BRAF* mutations including the *BRAF* V600E (c. 1799T>A) mutation found in the diffuse large B-cell lymphoma, non-germinal center index case, three D594G (c.1781A>G) mutations (Figure 2A) and two K601N (Figure 2B) mutations, all in diffuse large B-cell lymphoma, germinal center type. Two of the non-germinal center type diffuse large B-cell lymphoma had *NRAS* mutations, both at codon 61, Q61K and Q61H (Figure 3). There was no statistically significant difference between the frequencies of mutation in germinal center versus non-germinal center diffuse large B cell lymphoma. One case of diffuse large B cell lymphoma also showed a low grade area of extranodal marginal zone lymphoma of mucosa associated lymphoid tissue type which was separately analyzed for the mutations. Both areas (diffuse large B-cell lymphoma and extranodal marginal zone lymphoma of mucosa associated lymphoid tissue type) were negative for mutations. None of the cases showed mutations of *KRAS* codons 12/13 or *HRAS* codon 61(0/33) or *PAX8/PPARγ* rearrangement (0/11).

Classical cytogenetic studies demonstrated complex structural and numerical abnormalities in 7/12 diffuse large B-cell lymphoma cases tested (Table 3). No translocation t(2;3) (q13;p25) that corresponds to *PAX8/PPARγ* rearrangements was observed. No apparent abnormalities were seen in 7q34 (*BRAF*), 1p13.2 (*NRAS*), 12p12.1 (*KRAS*), or 11p15.5 (*HRAS*). Two cases showed *IGH/MYC* translocation by fluorescence in-situ hybridization, one of which also had classical cytogenetics that showed a t(8;14) translocation.

Treatment information was available for 30 cases. Twenty-five patients received definitive surgery (9 surgery alone, 5 surgery with chemotherapy, 8 surgery with radiation, and 3 surgery, radiation and chemotherapy), 4 patients received chemoimmunotherapy alone and 1 patient received chemotherapy and radiation. Chemoimmunotherapy was CHOP-R in all

cases except for one patient who was given a Vanderbilt regimen. Four patients had a past history of lymphoma. Two patients developed diffuse large B cell lymphoma, germinal center type of the thyroid following a prior lymphoma of unspecified type (one renal, one gastric). The other two patients had a prior history of diffuse large B-cell lymphoma, one in the left lobe of thyroid and the other of the mediastinum. Both underwent surgery and received chemoradiation and then presented with an extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type of the right thyroid lobe.

Eleven (11/33, 33%) patients died after their initial diagnosis of thyroid lymphoma. All patients diagnosed with either extranodal marginal zone lymphoma of mucosa associated lymphoid tissue type or follicular lymphoma were alive after a median follow-up of 3.5 years. Four out of eight (50%) patients with diffuse large B-cell lymphoma with mutations and 7/17 (41%) patients with diffuse B cell lymphoma without mutations died. There was no statistically significant difference in overall survival between the two groups (p=0.77) (Figure 4).

Discussion

We report here for the first time the occurrence of *BRAF* and *NRAS* mutations in thyroid lymphomas. None of the cases studied had a coexistent thyroid carcinoma. It is also important to recognized that at least one commonly used antibody for PAX8, which may be helpful in confirming the follicular thyroid cell origin,[31] cross-reacts with PAX5 and thus stains many B-cell lymphomas.[30]

All 8 of these MAPK signaling pathway-activating mutations were identified in diffuse large B-cell lymphoma, with none found in the extranodal marginal zone lymphoma of mucosa associated lymphoid tissue type or follicular lymphoma, although the number of these more indolent lymphomas was relatively small. There was no difference in the incidence of these mutations between germinal center and non-germinal center subgroups of diffuse large B-cell lymphoma. However, these two subgroups have been shown to be different, genetically and clinically, and the germinal center group has been reported to have a better prognosis.[32]

The MAPK cascade controls a major signaling network involved in various cellular functions. The signal is propagated through the RAS-RAF-MEK-ERK proteins into the nucleus regulating cell proliferation, survival, and differentiation.[33] Constitutive activation of the effectors of this signaling pathway plays a critical role in thyroid carcinogenesis. *RET/PTC*, *RAS*, and *BRAF* mutations appear to be mutually exclusive in thyroid carcinomas, suggesting that activation of this pathway at one level is sufficient for carcinogenesis.[10]

Molecular testing for alterations in the MAPK pathway genes is becoming an important part of the routine evaluation of fine needle aspiration samples obtained from thyroid nodules. It is considered reliable and feasible and has been shown to improve the overall accuracy of fine needle aspiration cytology.[6, 34, 35] A prospective evaluation of 470 fine needle aspiration samples showed that molecular testing was particularly informative for cases in the indeterminate cytology category, where molecular testing increased the probability of carcinoma from 40% to close to 100% in cases where mutations were identified.[6] Detection

of *BRAF* mutation was reported to have close to 100% positive predictive value for papillary thyroid carcinoma, and the risk of malignancy is above 80% when *RAS* mutation is detected.[5] The importance of the diagnostic use of molecular markers has been reflected in the revised American Thyroid Association's management guidelines, which recommends the use of the mutational panel for nodules with indeterminate fine needle aspiration cytology to help guide clinical management.[36] In view of the increasing use of molecular testing in thyroid fine needle aspiration samples, the specificity of these mutations becomes an important diagnostic and clinical issue.

The *BRAF* gene encodes a cytoplasmic serine/threonine kinase that is regulated by binding of RAS and propagates signals downstream to MEK kinase along the mitogen-associated protein kinase signaling pathway. Virtually all *BRAF* mutations reported to date are located within the activation segment domain or G-loop, with the most common mutation being V600E (c.1799T>A).[37] V600E *BRAF* mutation is the most common genetic event in papillary thyroid carcinoma, where it is found in 40-45% of cases.[38] It is also found in poorly differentiated and anaplastic thyroid carcinomas but not in medullary carcinoma, follicular tumors or benign hyperplastic nodules.[11]

Although screening of human tumors for *BRAF* mutations has been widely performed, the data concerning the frequency and type of mutations in lymphomas are very limited. Lee et al detected *BRAF* mutations in 4 of 164 (2.4%) non-Hodgkin lymphomas.[18] All four cases were diffuse large B cell lymphoma and no mutations were identified in other non-Hodgkin lymphoma analyzed (3 mantle cell lymphomas, 4 follicular lymphomas, 49 extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type, and 34 T-cell lymphomas). Whereas most *BRAF* mutations in human cancers, including carcinomas of the thyroid, involve V600E, all four *BRAF* mutations reported in this study involved other amino acids. These included one G469A, two G469R, and one D594G (previously reported as G468A, G468R and D593G respectively). V600E *BRAF* mutation has been observed in immunodeficiency related non-Hodgkin lymphoma with microsatellite instability. The *BRAF* mutation in this subset of patients was not restricted to diffuse large B cell lymphoma but was also seen in a T-cell post transplant lymphoproliferative disorder and a primary central nervous system B-cell non-Hodgkin lymphoma.[19]*BRAF* V600E mutations have also been identified in over half of Langerhans cell histiocytoses[20] and in all hairy cell leukemias.[12-16] Recent studies have also identified *BRAF* mutations including V600E and non-V600E mutations in a small number of plasma cell myelomas (9/238 with 4 V600E mutation),[14, 22] and some acute lymphoblastic leukemias (2/3 T-acute lymphoblastic leukemias with 1 V600E and 4/25 B-acute lymphoblastic leukemias with no V600E).[21]*BRAF* mutations other than V600E have also been identified in chronic lymphocytic leukemias[17] and 1 splenic marginal zone lymphoma.[13] In contrast, however, other than the 2 cases of chronic lymphoproliferative disorder described by Arcaini et al[12], several recent large studies of *BRAF* V600E mutations in hairy cell leukemia have not found any other B-cell lymphomas with V600E mutations. [12-16]

In the current study, *BRAF* mutations were found in 6 of the 25 diffuse large B-cell lymphomas. The index case had a V600E *BRAF* mutation, the type typically associated with papillary thyroid carcinoma. The other mutations in the *BRAF* gene that were found (D594G

and K601N) have not been found in thyroid carcinomas. The most commonly identified mutation, present in three cases was D594G (c.1781A>G). It has been previously identified in diffuse large B cell lymphoma,[18] sporadic colorectal adenocarcinoma[39] and malignant melanoma.[40] A different mutation at the same site (D594N) has been reported in a plasma cell myeloma.[14] The K601N mutation has been reported in colorectal adenocarcinoma and plasma cell myeloma.[22,39] A K601E mutation has been identified in one case of splenic marginal zone lymphoma.[13]

RAS proteins are a molecular switch involved in propagating signals along several signaling pathways, including the MAPK and PI3K pathways. Approximately 10-20% of all human tumors have mutated RAS proteins.[41] Activating point mutations of the *RAS* genes occur predominantly in epithelial thyroid neoplasms with a follicular pattern, including follicular adenomas and carcinomas and the follicular variant of papillary carcinoma.[5] They are also seen in poorly differentiated and anaplastic thyroid carcinomas.[42-44]*RAS* gene mutations are reported in a small number of non-Hodgkin lymphoma,[45] cutaneous T-cell lymphomas[25] and in up to about half of plasma cell neoplasms.[23, 24] In this study, we identified *NRAS* mutations in two diffuse large B cell lymphomas, with the overall prevalence of this mutation of 8% in thyroid diffuse large B cell lymphoma. Both mutations (Q61H and Q61K) involved codon 61, which is the most common mutation site in this gene.[46] The Q61K mutation is commonly seen in thyroid follicular carcinoma[46] and in other malignancies such as melanoma.[47] The Q61H mutation has also been reported in some thyroid carcinomas[48] and papillary carcinoma of the breast.[49]

In summary, our results indicate that among primary thyroid tumors, *BRAF* and *NRAS* mutations are not restricted to epithelial neoplasms and may also be seen in thyroid lymphomas, specifically in diffuse large B cell lymphomas. Importantly, mutations of these genes may be identical to those frequently seen in thyroid carcinomas, specifically V600E *BRAF* and Q61K *NRAS* mutations. Hence, when these mutations are detected in thyroid fine needle aspiration samples, the differential diagnosis must also include lymphomas. Additional morphologic and immunophenotypic studies remain critical in this situation to determine the correct diagnosis. Furthermore, our findings highlight the potential importance of the MAPK pathway mutations in the pathogenesis of diffuse large B-cell lymphomas developing in the thyroid gland.

Acknowledgements

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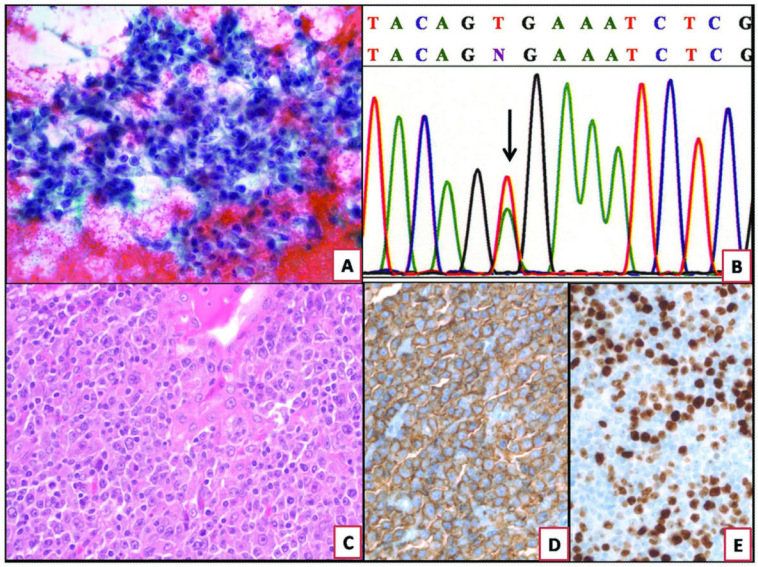

Figure 1.

Diffuse large B-cell lymphoma, Index case. **A**: The fine needle aspiration biopsy demonstrates numerous large round cells with scant cytoplasm and admixed small lymphocytes. Occasional mitosis is seen. (Papanicolaou stain, original magnification 400×) **B**: Sequencing of exon 15 of the *BRAF* gene revealed a 1799T>A, V600E mutation. **C**: The excision demonstrated a diffuse proliferation of large transformed cells (Hematoxylin and eosin). **D**: The neoplastic cells were CD20 positive. **E**: Relatively high Ki-67 proliferative index (C-E, original magnification, 200×).

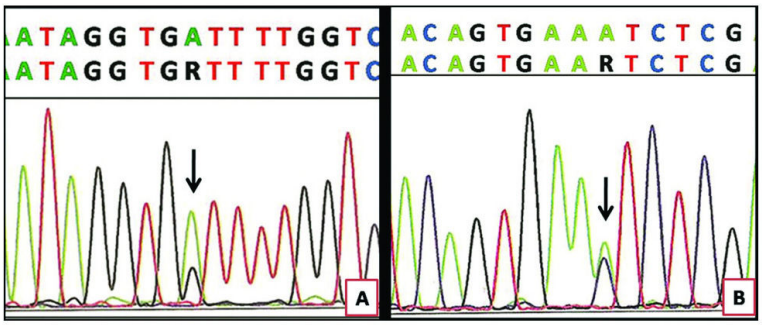

Figure 2.

Sequencing analysis of *BRAF* exon 15. **A**: Diffuse large B-cell lymphoma of germinal center type with D594G (c.1781A>G) mutation, **B**: Diffuse large B-cell lymphoma of germinal center type with K601N (c.1803 A>C)

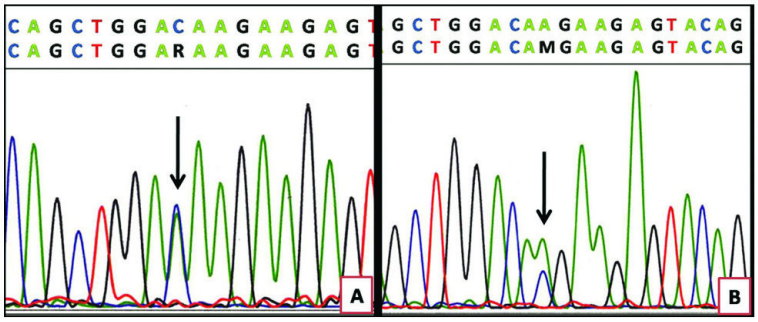

Figure 3.

Sequencing of *NRAS* exon 2. **A**: Diffuse large B-cell lymphoma of germinal center type with Q61K (c.181C>A) mutation. **B**: Diffuse large B-cell lymphoma of germinal center type with Q61H (c.183A>C) mutation.

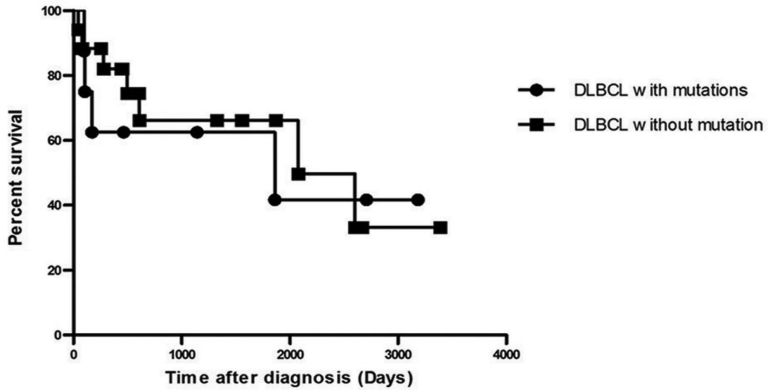

Figure 4.

Overall survival analysis of patients with diffuse large B-cell lymphoma with and without mutations (p=0.77).

Table 1

Clones of antibodies used for the immunohistochemistry

	Clone	Dilution	Vendor	Antigen retrieval
CD20	Clone L26	prediluted	Ventana, Tucson, AZ	CC1 standard, pH 8.0
CD3	Polyclonal rabbit,	1:100	Dako, Carpenteria, CA	CC1 standard, pH 8.0
PAX8	Polyclonal	1:100	Polyclonal from Proteintech Group (PTG), Chicago, IL	CC1 standard, pH 8.0
Bcl6	GI191E/A8	prediluted	Ventana, Tucson, AZ	CC1 standard, pH 8.0
CD10	56C6	prediluted	Ventana, Tucson, AZ	CC1 standard, pH 8.0
MUM1	MUM1p	1:100	Dako, Carpenteria, CA	CC1 standard, pH 8.0
Bcl2	124	prediluted	Ventana, Tucson, AZ	CC1 standard, pH 8.0
Kappa	L1C1	1:400	Labvision, Freemont, CA	CC1 standard, pH 8.0
Lambda	HP6054	1:100	Labvision, Freemont, CA	CC1 standard, pH 8.0
Ki67	MIB-1	1:100	Dako, Carpenteria, CA	CC1 standard, pH 8.0
Cyclin D1	SP4	prediluted	Cell Marque, Rocklin, CA	CC1 standard, pH 8.0

CC1 solution: Ventana, tris based buffer pH 8.0

Table 2

Summary of mutations identified in diffuse large B-cell lymphoma of the thyroid

Mutations identified	n	Lymphoma
BRAF D594G (c.1781A>G)	3	Diffuse large B-cell lymphoma, germinal center type
BRAF K601N (c.1803 A>C)	2	Diffuse large B-cell lymphoma, germinal center type
BRAF V600E (c.1799T>A)	1	Diffuse large B-cell lymphoma, non-germinal center type
NRAS Q61K (c.181C>A)	1	Diffuse large B-cell lymphoma, non-germinal center type
NRAS Q61H(c.183 A>C)	1	Diffuse large B-cell lymphoma, non-germinal center type

Table 3

Cytogenetic abnormalities found in diffuse large B-cell lymphoma of the thyroid and their correlation with mutational status and lymphoma type

Lymphoma type	Mutation	Classical Cytogenetics	FISH
MALT	None	uninformative	NP
DLBCL, GC	None	43-46, X, −X, der(1)del(1)(p34.3)del(1)(q42),del(8)(p21p23),−9,del(10)(q24q26), del(13)(q12q34),der(16)t(16;17)(p13.3;q21),hsr(16)(q13),−17,+1~3mar,+hsr(mar) [cp15]	NP
DLBCL, GC	D594G, BRAF	46, XX	NP
DLBCL, GC	None	NP	*IGH/MYC* +, *BCL2*−
DLBCL, GC	None	46, XX	NP
DLBCL, GC	D594G, BRAF	45,−X,t(X;2)(p22.1;q31) [15]/46,XX[5]	NP
DLBCL, GC	K601N, BRAF	45, X,−Y,t(1;2)(p13;p23)[4]/45,X,−Y[15]	NP
DLBCL, GC	None	46,XX[19]	NP
DLBCL, NGC	NRAS	71-80<3n>,XX,−X,+2,+3, del(6)(q21q25),+7,+8,t(8;22)(q24;q11.2)x2,del(9)(p22), t(11;18)(q23;q12.2),+der(11)t(11;18),+12,add(14)(q32)X2,+20,+21,+22,+2~3mar [cp16]/46,XX[2]	NP
DLBCL, GC	None	45, X,−Y, inv(3)(p11.2q27),add(14)(q32)[cp3]	NP
DLBCL,NGC	None	51,XX,del(1)(p13p22),del(2)(q31q33),+5,add(5)(p14),add(6)(p25), +7,add(7)(q31), t(8;14)(q24;q32),+12,+13,+18[cp19]	*IGH* +, *MYC* +
DLBCL, GC	None	82-85<4n>, XXX,−X,−1,i(1)(q10),add(1)(q21),−2,−4,−4,add(4)(q27),−5,−5,−8,add(9)(q22), del(10)(q22),del(10)(q22),+11,+11,+12,+12,−13,t(14;18)(q32;q21), der(14)t(14;18)(q32;21),−15,−15,−16,−18,+19,−21,+1~10mar[cp6]	NP

DLBCL: Diffuse large B-cell lymphoma; GC: Germinal center type; NGC: Non-germinal center type; FISH: Fluorescence in-situ hybridization; MALT: Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue type; NP: Not performed