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EBV-positive low-grade follicular lymphoma transforming to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements

To the Editor,

Epstein–Barr virus (EBV) is associated with some types of lymphoma, notably of B-cell lineage, such as Burkitt lymphoma, Hodgkin lymphoma, EBV-positive diffuse large B-cell lymphoma (DLBCL) not otherwise specified (NOS), DLBCL associated with chronic inflammation, plasmablastic lymphoma, primary effusion lymphoma, and immunodeficiency-associated lymphoproliferative disorders.¹ However, the association of follicular lymphoma with EBV has not been described until recently.² Two large cohorts of follicular lymphoma conducted by Mackrides *et al.* found that less than 3% of follicular lymphomas were EBV-positive, and these cases were mostly high grade (grade 3A or 3B) and tended to transform to EBV-positive DLBCL or high-grade follicular lymphoma with poor prognosis. Whether this virus plays a role in lymphomagenesis or participates in a later stage of tumour progression is yet to be determined.^{2,3} We present a rare case of EBV-positive low-grade follicular lymphoma transforming to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (so-called double-hit lymphoma) showing clonal relationship, which has not been previously reported. According to the chronological change in this case, we propose that *BCL2* rearrangement occurred early in the follicular lymphoma, and then EBV infected some lymphoma cells with clonal expansion, forming EBV-positive follicular lymphoma. Subsequently, *MYC* rearrangement in some EBV-negative cells induced transformation to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements.

A 76-year-old man presented with poor appetite and general discomfort for 2 months. He had medical history of DLBCL, Ann Arbor Stage IVB, diagnosed in another hospital 7 years previously. He had received chemotherapy with nine cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) and two cycles of R-ESHAP (rituximab, etoposide, methylprednisolone, high-dose cytarabine, and cisplatin) with disease progression. The positron emission tomography-computed tomography (PET-CT) scan revealed multiple fluorodeoxyglucose (FDG) uptakes in the lymph nodes of bilateral neck, bilateral supraclavicular, mediastinum, bilateral pulmonary hila, upper abdominal, mesentery, abdominal, para-aortic, bilateral iliac and bilateral inguinal areas, compatible with lymphoma involvement. The right inguinal lymph node biopsy showed small to medium-sized lymphocytes in follicular pattern with mostly centrocytes and few scattered centroblasts (Fig. 1A,B). Focal geographic necrosis with nuclear debris and vague granuloma were noted, suggesting EBV infection. No obvious Hodgkin/Reed–Sternberg (HRS)-like cells or angioinvasion were seen. No microorganisms were found by periodic acid–Schiff (PAS) and acid-fast stains. Immunohistochemically, most of these lymphocytes expressed BCL2, BCL6, CD10, CD20 and CD79a (Fig. 1C,D). CD3, CD5, CD30, CD138, cyclin D1, HHV8 LANA1 (human herpes virus 8 latency-associated nuclear antigen 1) and MYC were negative (Fig. 1E). CD21 stain highlighted follicular dendritic cell meshwork, indicating follicular pattern. Ki-67 proliferation index was up to 20% (Fig. 1F). Interestingly, EBV-encoded small RNA (EBER) *in situ* hybridisation (ISH) was positive in more than 75% of the neoplastic lymphocytes in follicular pattern (Fig. 2A). EBV LMP1 was positive, while EBNA2 was negative, indicating latency type II EBV infection. Thus, EBV-positive follicular lymphoma, World Health Organization (WHO) grade 1–2, was diagnosed. Blood tests of EBV antibodies were not available. The bone marrow biopsy showed involvement of follicular lymphoma with characteristic paratrabecular pattern and EBER positivity in about 5% of the lymphocytes. The previous diagnosis of DLBCL in another hospital 7 years ago was revised to follicular lymphoma, WHO grade 1–2, with about 3% of EBER-positive lymphocytes after reviewing the sections. He received further chemotherapy with RICE (rituximab, ifosfamide, carboplatin, and etoposide) regimen.

Several months later, the patient complained of limb weakness. Magnetic resonance imaging (MRI) demonstrated an extradural spinal tumour from T2 to T7. The tumour biopsy exhibited totally different morphology with diffuse infiltrate of monotonous medium-sized atypical lymphocytes showing Burkitt lymphoma-like morphology, brisk mitoses and starry sky pattern (Fig. 1G,H). Immunohistochemically, the lymphoma cells expressed BCL2, CD10, IRF4/MUM1, MYC and PAX5 (Fig. 1I–K), but not BCL6, CD3, CD20, cyclin D1, CD34 or TdT (Fig. 2H). Ki-67 proliferation index was high, up to 80% (Fig. 1L). EBER ISH was totally negative in the lymphoma cells with positive external control in the same slide (Fig. 2G). Fluorescence *in situ* hybridisation (FISH) study confirmed concurrent *MYC* and *BCL2* rearrangements in the tumour cells without *BCL6* rearrangement, indicating double-hit lymphoma (Fig. 2I,J). In comparison, the previous follicular lymphoma carried *BCL2* rearrangement, but no *MYC* rearrangement by FISH study (Fig. 2C,D).

Fig. 1 (A,B) The inguinal lymph node shows follicular lymphoma, WHO grade 1–2, in follicular pattern (H&E), expressing CD20, (C) CD10 and (D) BCL2, but (E) not MYC. (F) Ki-67 proliferation index is up to 20%. (G,H) After disease progression, the spinal tumour shows Burkitt lymphoma-like high-grade morphology (H&E stain), expressing (I) CD10, (J) BCL2 and (K) MYC. (L) Ki-67 proliferation index is high, up to 80%.

Polymerase chain reaction (PCR)-based clonality study for B-cell receptor gene rearrangement proved the same monoclonal results in the double-hit lymphoma and previous follicular lymphoma, using BIOMED2 IGH tube B and IGK tube A reactions (Fig. 2E,F,K,L). We concluded this was an EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements, transformed from EBV-positive follicular lymphoma, WHO grade 1–2. Further bone marrow biopsy showed involvement of high-grade B-cell lymphoma. Unfortunately, the patient died of disease with infection and septic shock 3 months after the diagnosis of high-grade B-cell lymphoma.

EBV is the most common virus infection found in humans, as well as the first human tumour virus to be identified.¹ A variety of tumours, including those of epithelial and lymphoid origins, are known to be associated with EBV. Most EBV-related lymphomas are of B-cell lineage, with a few exceptions, such as extranodal NK/T-cell lymphoma, nasal type, and aggressive NK-cell leukaemia.¹

EBV-positive follicular lymphoma is rare. It has been described in only a handful of papers,^{2–9} but not in the 2017 WHO classification of haematolymphoid tumours. Two large cohorts of follicular lymphoma confirmed that the prevalence of EBV-positive follicular lymphoma was low, which was 2.6% in an unselected cohort of 382 follicular lymphoma patients,² and 2.5% in another group of 488 patients with newly diagnosed follicular lymphoma.³ By their criteria, EBV-positive cases were defined as those with greater than 10 EBER-positive tumour cell nuclei per 0.5 cm², while cases with bystander EBER-positive non-neoplastic lymphocytes were excluded. Their studies observed that EBV-positive

follicular lymphoma tended to be high grade (grade 3A or 3B), had higher CD30 expression, transformed more frequently to DLBCL or high-grade follicular lymphoma, and had a worse prognosis compared to EBV-negative follicular lymphoma. One DLBCL case transformed from EBV-positive follicular lymphoma was still EBV-positive, while EBV status in other transformed cases was unknown due to unavailable tissue. In addition to DLBCL and high-grade follicular lymphoma, a composite follicular lymphoma *in situ* (FLIS) and EBV-positive classic Hodgkin lymphoma has been described by Jegalian *et al.*¹⁰ The clonal relationship was not available in their study, and the role of EBV in the progression of follicular lymphoma is still unclear. Although a couple of early case reports noted that these patients were immunosuppressed,⁵ a subsequent larger series found that most of the patients had no known history of immunodeficiency, which was similar to our case.² The clinical features, morphology and immunophenotype of EBV-positive follicular lymphoma were indistinguishable from those of EBV-negative follicular lymphoma. Mackrides *et al.* suggested that EBER screening should be performed in all high-grade follicular lymphomas.

Herein, we present a rare case of EBV-positive low-grade follicular lymphoma transforming to EBV-negative high-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements showing clonal relationship, which has not been reported previously. The patient was immunocompetent, which was consistent with the findings of the study conducted by Mackrides *et al.*² However, our case showed focal geographic necrosis with nuclear debris and vague granuloma, which were histological features, and hints of EBV

Fig. 2 (A) EBER is positive in most follicular lymphoma cells. (B) Bone marrow biopsy shows lymphoma involvement (H&E). (C) The follicular lymphoma carries *BCL2* rearrangement, but (D) not *MYC* rearrangement by fluorescence *in situ* hybridisation [FISH, Vysis *BCL2* Break Apart FISH Probe and Vysis LSI *MYC* Dual Color Break Apart Rearrangement Probe (Abbott, USA)]. (G) The high-grade lymphoma cells are negative for EBER and (H) TdT. (I,J) FISH shows concurrent *MYC* and *BCL2* rearrangements, indicating double-hit lymphoma. (E,F,K,L) B-cell clonality study proves the same monoclonal results in the follicular lymphoma and double-hit lymphoma.

infection, prompting a test for EBER ISH. EBER was positive in more than 75% of the neoplastic lymphocytes in follicular pattern with co-expression of BCL2, BCL6, CD10 and CD20, fulfilling the criteria of EBV-positive follicular lymphoma. However, unlike other cases,^{2,4,8} our case did not transform to EBV-positive high-grade lymphoma, but rather to EBV-negative high-grade lymphoma. Interestingly, we found that the percentage of EBV-positive neoplastic cells showed dynamic change at different time points and at different anatomical sites. EBER was positive early, but in only a minority of neoplastic cells. The population and proportion of EBV-positive neoplastic cells expanded with time, up to more than 75% of lymphoma cells in the lymph node. At the same time, EBV-positive neoplastic cells accounted for only 5% of the lymphoma cells in bone marrow. Micro-environment factors, chemotherapy and/or immune dysregulation might play a role in selecting EBV-positive cells. Nevertheless, based on the chronological change in this case, we found that *BCL2* was rearranged early in the follicular lymphoma, and then EBV infected some lymphoma cells with clonal expansion, particularly in the lymph node, forming EBV-positive follicular lymphoma. Because EBV was positive early in the previous follicular lymphoma 7 years ago, clonal B-cell expansion driven by EBV reactivation was suggested. However, the EBV-negative lymphoma cells underwent high-grade transformation and clonal expansion after acquiring *MYC* rearrangement later, in addition to the underlying *BCL2* rearrangement, that led to the transformation to EBV-negative double-hit lymphoma and mortality of this patient.

In addition to this case, we also performed EBER *in situ* hybridisation in 54 cases of follicular lymphoma in our archives (from 2012 to 2021), including 42 cases of WHO grade 1–2, 10 cases of grade 3A, and two cases of grade 3B. Of these, 14 cases were associated with DLBCL, while none were associated with double-hit lymphoma. All 54 follicular lymphomas were EBV-negative. Interestingly, one EBV-positive DLBCL case was associated with EBV-negative follicular lymphoma, WHO grade 1–2. The prevalence of EBV-positive follicular lymphoma, WHO grade 1–2, was 2.3% (1/43) in our data. Although our sample size was relatively small, we used the full tissue sections in order to avoid missing cases with partial EBER positivity. Nevertheless, the prognosis of EBV-positive versus EBV-negative low-grade follicular lymphoma is still unclear.

In summary, we present an extremely rare case of EBV-positive low-grade follicular lymphoma transforming to EBV-negative double-hit lymphoma. Despite most reported cases of EBV-positive follicular lymphoma not demonstrating specific morphological features to predict EBV positivity, our case showed geographic necrosis with nuclear debris and vague granuloma, which was unusual for conventional follicular lymphoma, suggesting possible EBV infection. We propose that apart from screening for EBER in high-grade follicular lymphoma, low-grade follicular lymphoma showing geographic necrosis with nuclear debris, vague granuloma, HRS-like cells and/or angioinvasion should also be tested for EBER to select EBV-positive low-grade follicular lymphoma, which tends to transform to higher-grade lymphoma with worse prognosis.

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Cutaneous lymphoplasmacytic lymphoma with *MYD88* L265P mutation, bone marrow involvement, and paraproteinaemia

To the Editor,

We present four cases of cutaneous small B-cell infiltrates classified as cutaneous involvement by lymphoplasmacytic lymphoma (LPL). Three patients had a known history of *MYD88* mutated LPL and presented with cutaneous recurrences, and one patient presented with a primary cutaneous lesion. The clinical presentation included three cases with single erythematous skin nodules on the trunk, the upper extremity (Fig. 1A), and ear (Fig. 1B), and a diffuse papular and macular rash on the trunk in one case (Fig. 1C,D). Polymerase chain reaction (PCR) for *MYD88* L265P mutation was positive in the