

Case Report

Aggressive natural killer cell leukemia mimicking interstitial lung diseases with the activation of the mitogen-activated protein kinase pathway

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Abstract: Aggressive natural killer (NK) cell leukemia (ANKL) is a rare form of leukemia with an aggressive clinical course. It commonly involves the peripheral blood, bone marrow, liver, and spleen but rarely involves the lungs. We report a 36 year-old woman who presented with pulmonary lesions we suspected to be interstitial lung disease on an imaging study. A lung biopsy showed extensive lymphoid infiltrate growing along pre-existing alveolar septa without destroying the alveolar spaces. Further workup revealed hepatomegaly, borderline splenomegaly, and multiple lymphadenopathies. Her laboratory tests showed leukocytosis, anemia, thrombocytopenia, abnormal liver enzymes, and elevated lactate dehydrogenase. A bone marrow (BM) aspirate smear revealed many intermediate to large lymphocytes with dispersed chromatin, basophilic cytoplasm, and some azurophilic granules. A BM biopsy showed hypercellularity with interstitial lymphoid infiltrate in a background of trilineage hematopoiesis and histiocytosis with hemophagocytosis. Immunohistochemical studies performed on both the lung and BM biopsies showed the neoplastic cells to be positive for CD2, CD3, CD7, CD56, granzyme B, phosphor-MAPK (pMAPK), EBER (Epstein-Barr Virus-encoded small RNA) by in situ hybridization; they were negative for CD4, CD5, CD8, CD30, LMP1, and phospho-STAT3 (pSTAT3). A flow cytometry analysis of the BM aspirate identified a population of atypical lymphocytes with the NK cell phenotype. Molecular studies were negative for T-cell receptor gene rearrangements, and the neoplastic cells displayed a complex karyotype. The patient responded initially to chemotherapy but died of multiorgan failure two months after the diagnosis. We present a case of ANKL mimicking interstitial lung disease with the activation of MAPK pathway.

Keywords: Aggressive NK cell leukemia, natural killer cell neoplasm, leukemia, Epstein Barr virus, pulmonary lymphoma

Introduction

Aggressive natural killer (NK) cell leukemia (ANKL) is a rare neoplasm of mature NK cells accounting for less than 0.1% of all lymphoid malignancies. It is most prevalent in Asians and affects young to middle-aged adults, with a median age of 40 years. The main presentations include fever, constitutional symptoms, and cytopenia. ANKL has a fulminant clinical course often complicated by multiorgan failure, coagulopathy, and hemophagocytic syndrome. The median survival is about two months [1-4]. ANKL is almost always associated with Epstein-Barr virus (EBV) infection but rarely can be EBV negative. Recent studies have shown that EBV-

negative ANKL and EBV-positive ANKL share the same clinicopathological features [5-8]. ANKL is a systemic disease, commonly involving the blood, bone marrow, liver and spleen, and rarely involving the lungs [3, 9]. Here, we report one case of EBV-positive ANKL mimicking interstitial lung disease with a strong expression of phospho-MAPK (pMAPK).

Case report

A 36 year-old woman presented to an outside hospital with a worsening shortness of breath following treatment for a recently diagnosed pneumonia. She had lost eleven pounds in the previous month. An imaging study suggested

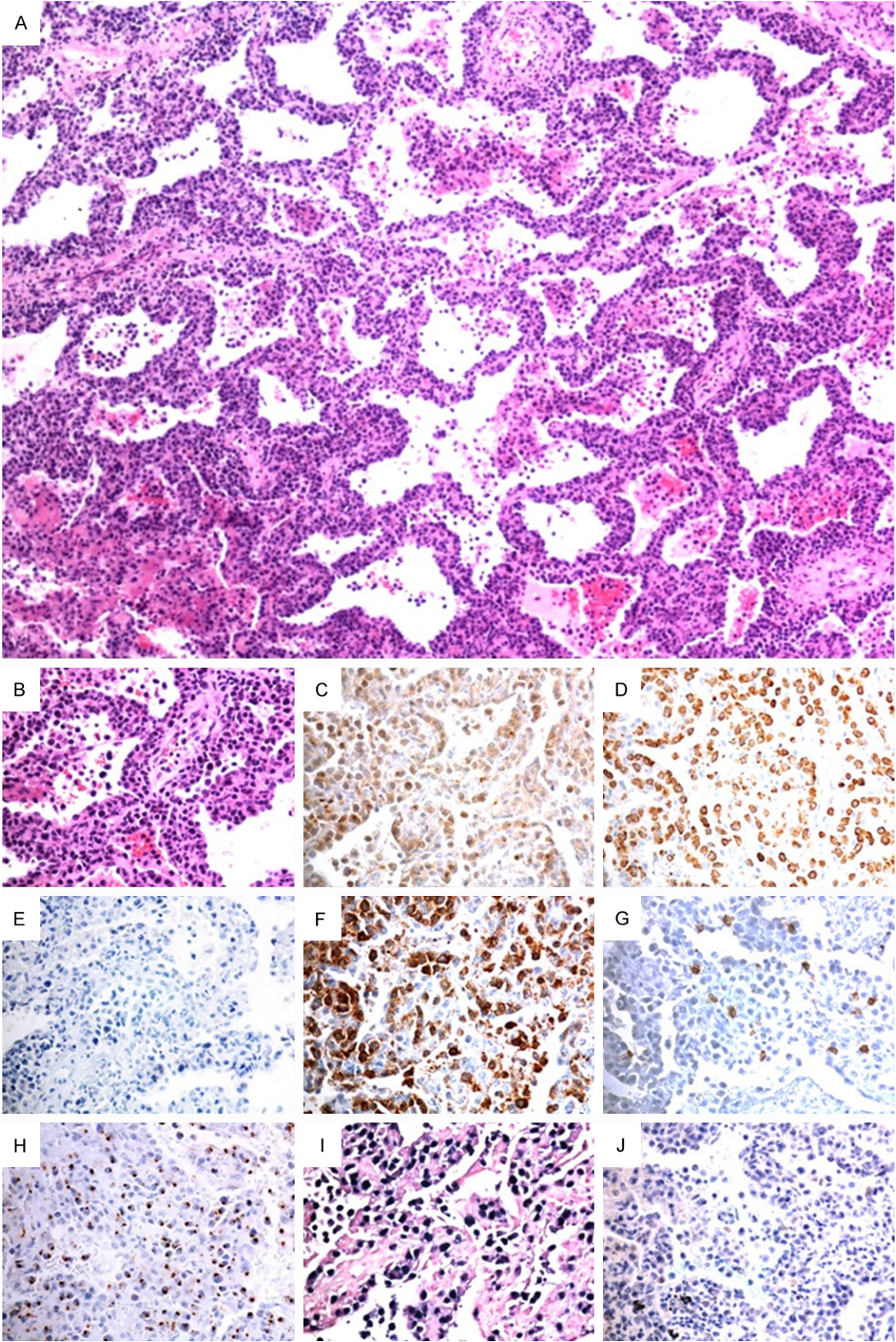


Figure 1. Histological and immunohistochemical features of ANKL in the lungs. Hematoxylin and eosin (HE) stain (A, B). Immunohistochemical stains (CD2 (C), CD3 (D), CD4 (E), CD7 (F), CD8 (G), granzyme B (H), EBER (I, ISH), CD30 (J)).

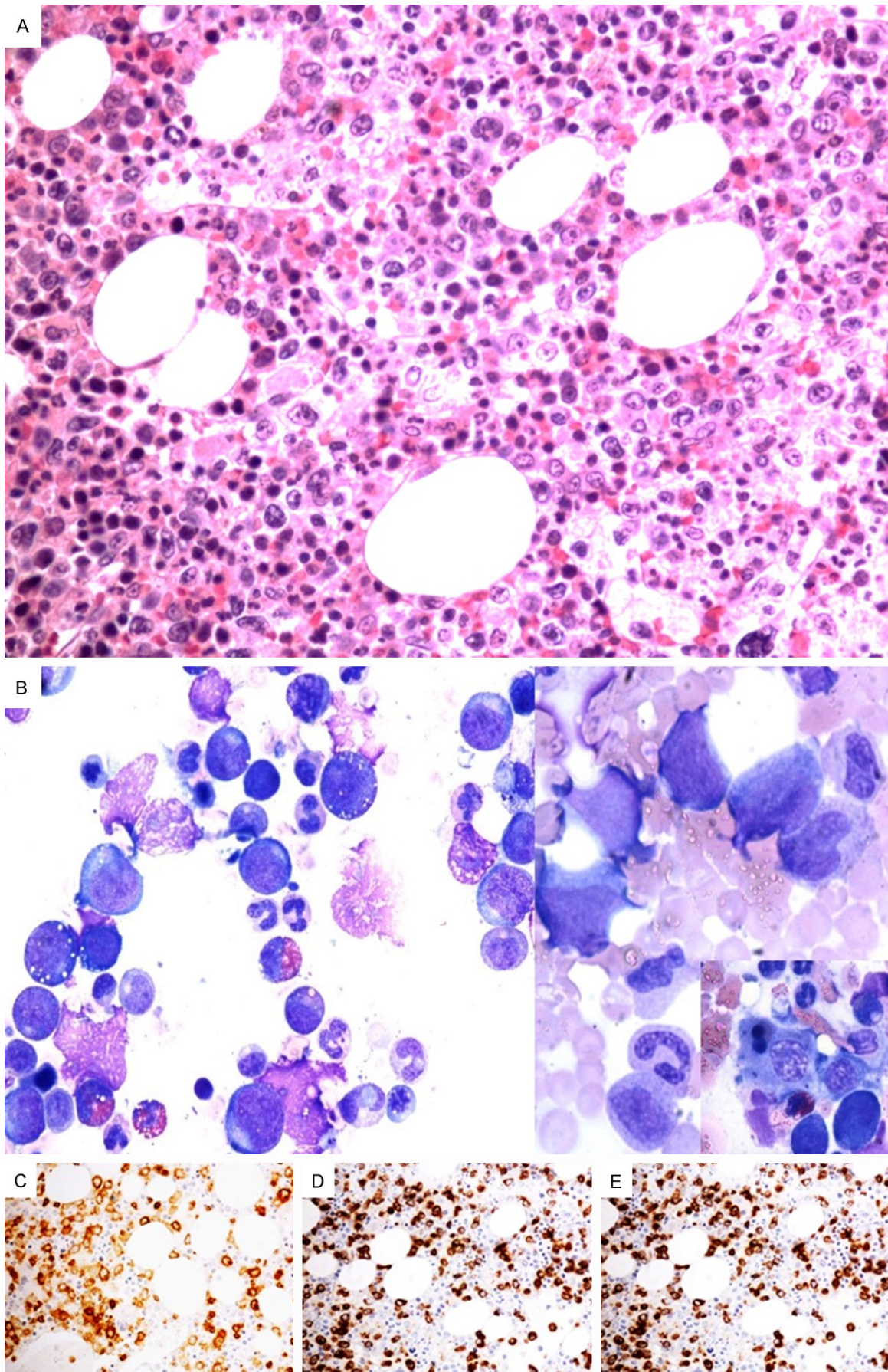
interstitial lung disease and a wedge biopsy of the left lower lobe was performed. The biopsy showed extensive interstitial infiltrate by large atypical cells growing along the preexisting alveolar septa without any destruction of the alveolar structures (**Figure 1A, 1B**). Immunohistochemistry showed that the large atypical cells were positive for CD2 (**Figure 1C**), CD3 (**Figure 1D**), CD7 (**Figure 1F**), granzyme B (**Figure 1H**) and negative for CD4 (**Figure 1E**), CD5, CD8 (**Figure 1G**), CD20, CD30 (**Figure 1J**). In situ hybridization (ISH) for EBER (Epstein-Barr Virus-encoded small RNA) was negative (**Figure 1I**). Based on these findings, a diagnosis of NK/T cell lymphoma was rendered.

The patient was then transferred to our institution for further management. On admission, a complete blood count revealed WBC 21.2 K/ μ L (normal range, 3.8-10.5 K/ μ L), hemoglobin 10.3 g/dL (normal range, 11.5-15.5 g/dL), and platelet 39 K/ μ L (normal range, 150-400 K/ μ L). The serum lactate dehydrogenase (LDH) level was 6660 U/L (normal range, 50-242 U/L). Her liver enzymes were elevated, including alanine transaminase: 941 U/L (normal range, 10-45 U/L), aspartate transaminase: 497 U/L (normal range, 10-40 U/L), and alkaline phosphatase: 419 U/L (normal range, 40-120 K/ μ L). A computer tomography scan revealed hepatomegaly, borderline splenomegaly, multiple lymphadenopathies (mesenteric, mediastinal and retroperitoneal) and no evidence of disease in the upper aerodigestive tract. A bone marrow biopsy was performed for clinical staging. The bone marrow aspirate smear revealed many intermediate to large lymphocytes with round to slightly irregular nuclear contours and dispersed chromatin, basophilic cytoplasm, and some azurophilic granules. There were trilineage hematopoiesis and increased histiocytes (occasional hemophagocytosis) in the background (**Figure 2B**). The bone marrow biopsy showed hypercellularity with extensive interstitial lymphoid infiltrate in a background of trilineage hematopoiesis and prominent histiocytosis with hemophagocytosis (**Figure 2A**). Immunohistochemistry revealed the neoplastic cells to be positive for CD2 (**Figure 2C**), CD3 (**Figure 2D**), CD7 (partial

(**Figure 2E**), granzyme B (**Figure 2F**), CD56 (**Figure 2G**), EBER (**Figure 2H**), pMAPK (nuclear and cytoplasmic) (**Figure 2J**) and negative for CD4, CD5, CD8, LMP-1 (**Figure 2I**), and pSTAT3 (**Figure 2K**). A flow cytometry analysis showed 45% abnormal lymphocytes, and was positive for HLA-DR, CD2, cytoplasmic CD3, CD7 (subset), CD16, and CD56 and negative for surface CD3, CD4, CD5, CD8, CD57, T cell receptor (TCR)-alpha/beta, and TCR-gamma/delta. Molecular studies for TCR gene rearrangement were negative. A chromosomal analysis showed a complex karyotype (76-80,XX,-X,-X,i(1)(q10)x2,-2,-3,-4,-6,-9,-10,-11,-14,-15,-16,-17,-18,-18,+3-4mars [cp3]). Fluorescence in situ hybridization (FISH) studies revealed gains in all the probes tested. The patient received chemotherapy, and her symptoms improved with the recovery of her blood cell counts. However, she developed pancytopenia soon afterwards, and a bone marrow biopsy revealed persistent disease with evidence of clonal evolution (78-80,XX,-X,+i(1)(q10),-3,-4,+5,+7,+7,+12,+add(13)(q32),-14,+add(16)(q24),-18,+19,+del(20)(q1-2q13.3),+21,+22,+1-3mars[cp8]/46,XX[12]). Her conditions rapidly deteriorated, and she died of multiorgan failure two months after the diagnosis.

Discussion

ANKL is a rare form of leukemia with fewer than 200 cases reported in the literature [7]. The commonly involved sites include the peripheral blood, bone marrow, spleen and liver. Pulmonary involvement is very rare, accounting for only 2.7% of ANKL cases [9]. Our patient presented with pulmonary lesions radiologically suggestive of interstitial lung disease. Extranodal NK/T cell lymphoma (ENKTL) shares some clinicopathological features with ANKL, and it is unclear whether ANKL represents a leukemic counterpart of ENKTL. Unlike ENKTL, ANKL is characterized by younger age, systemic disease, fulminant clinical course, lack of disease in the upper aerodigestive tract, frequent CD16 expression by tumor cells, and frequently complex karyotype [3]. By using the comparative genomic hybridization approach, Nakashima et al. [10] identified several copy number varia-



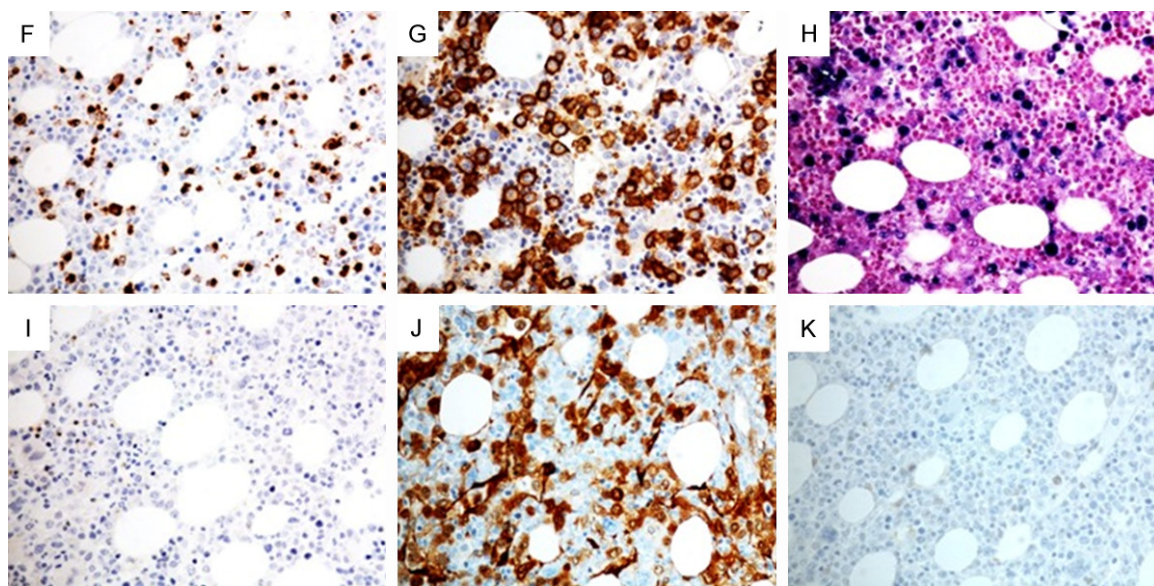


Figure 2. Morphology, histology and immunohistochemistry of ANKL in the bone marrow (BM). HE stain of BM biopsy (A), Wright-Giemsa stain of BM aspirate smear (B). Immunohistochemical stains (CD2 (C), CD3 (D), CD7 (E), granzyme B (F), CD56 (G), EBER (H), LMP1 (I), pMAPK (J), pSTAT3 (K)).

tions characteristic of ANKL, including the gain of 1q23.1-q24.2 and 1q31.3-q44, and the loss of 7p15.1-p22.3 and 17p13.1. Hepatosplenic T-cell lymphoma (HSTL) is also one of the differential diagnoses, given the high frequency of hepatosplenomegaly in ANKL. However, HSTL shows characteristic sinusoidal infiltration pattern in the spleen, liver and bone marrow and is not associated with EBV infection. The lymphoma cells display a T-cell phenotype and rearranged TCR genes.

Our patient presented with fever, weight loss, hepatomegaly, and lymphadenopathy but had no skin lesion or disease in the upper aerodigestive tracts. There was no known history of chronic NK-cell lymphoproliferative disorder. She had two-lineage cytopenias and a markedly elevated serum LDH. Her bone marrow showed extensive interstitial lymphoid infiltrate associated with histiocytosis and hemophagocytosis. The neoplastic cells displayed a NK cell immunophenotype with CD16 expression, a EBER positivity, a complex karyotype, and germline TCRs. The patient's clinical course progressed rapidly, and she died two months after the diagnosis, despite her initial response to chemotherapy. The overall findings support a diagnosis of ANKL.

A strong association between ANKL and EBV infection suggests a possible pathogenetic role

of this virus. As a major oncogenic protein of EBV, LMP1 promotes cell proliferation and apoptosis resistance through the activation of signaling cascades such as NF-kappa B, MAPK, phosphatidylinositol 3-kinase, interferon regulatory factor pathways, and STAT [11, 12]. LMP1 also up-regulates the expression of survivin and PD-L1. Since LMP1 displays a comparatively high immunogenicity for T cells in vivo, LMP1-expressing cells are increasingly targeted by cytotoxic T lymphocytes, which can cause the mutational deletion of LMP-1. However, a lack of LMP-1 expression has been observed in some EBV+ ANKL cases, and EBV can promote the growth of lymphoma cells in the absence of LMP1 expression [13]. These findings indicate that other mechanisms may play a role in their pathogenesis.

A gene expression profiling analysis revealed that the deregulation of JAK-STAT, NF-kB and MAPK signaling pathways was common in NK cell neoplasms. A recent study identified mutations in STAT3 (21%), the RAS-MAPK pathway (21%), DDX3X (29%) and in epigenetic modifiers (50%) in ANKL patients [14]. The STAT5b mutation was also reported in a subset of ANKL [15]. Another study reported 48% of patients carrying mutations in the JAK-STAT pathway. STAT3 and STAT5b mutations are activation mutations that lead to the activation of the JAK-STAT pathway. Other mechanisms resulting in the activa-

tion of JAK-STAT signaling included copy number gains in the 9p24 region containing JAK2 and the 17q21.2 region containing STAT3, STAT5A and STAT5B, gene mutations in negative regulators of STAT3, and the overproduction of IL10. STAT3 activation regulates the MYC transcription programs, leading to MYC overexpression. The JAK/STAT3-MYC biosynthesis axis is critical for the proliferation and survival of ANKL cells [16]. DDX3X is an RNA helicase involved in RNA translation initiation and assembly in the ribosome and spliceosome. The DDX3X mutants showed decreased RNA-unwinding, resulting in a loss of cell-cycle suppression and the transcriptional activation of the NF- κ B and MAPK pathways [17]. Other frequently mutated genes in ANKL included TP53, TET2, CREBBP, MLL2. An enhancer of the zeste homolog 2 (EZH2) was found to be overexpressed in EBV-negative ANKL, which conferred NK/T cell lymphoma cells a growth advantage over the control cells through c-MYC-induced micro RNAs [18].

Currently, there are no effective therapies for ANKL. L-asparaginase-based regimens followed by allogeneic hematopoietic stem cell transplantation has been shown to improve outcomes in ANKL [19, 20]. A drug sensitivity profiling study identified the JAK-STAT pathway as a potential therapeutic target in NK-cell malignancies. Both JAK inhibitors and BCL-2 inhibitors were found to be most selective towards NK cells, but MEK inhibitors were effective only in selective cases [14]. In our case, the neoplastic cells expressed pMAPK but were negative for pSTAT3. PMAPK was previously found to be expressed in a EBV-negative ANKL case by flow cytometry [21]. These findings indicate that ANKL is heterogeneous at the molecular level and the identification of the underlying signaling pathway is important for the selection of the most effective targeted therapy.

Disclosure of conflict of interest

None.

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