Epigenetic aberrations in natural killer/T-cell lymphoma: diagnostic, prognostic and therapeutic implications

Can Küçük, Junli Wang, Ying Xiang and Hua You

Abstract: Natural killer/T-cell lymphoma (NKTCL) is an aggressive malignancy that usually presents in the upper aerodigestive tract. This malignancy shows substantial geographic variability in incidence, and is characterized by Epstein-Barr virus (EBV) infections. Epigenetic aberrations may dysregulate the expression of genes involved in different hallmarks of cancer. A growing body of evidence underscores the importance of epigenetic aberrations in the pathogenesis of NKTCL. Promoter hypermethylation is a common epigenetic mechanism for the inactivation of tumour suppressor genes. Several epigenetically silenced tumour suppressor candidates (e.g. PRDM1, BIM) were identified in this aggressive cancer using locus-specific and genome-wide promoter methylation analyses. Importantly, genes involved in epigenetic modifications were identified to be mutated (e.g. KMT2D) or methylated (e.g. TET2) in NKTCL patients, which may contribute to pathogenesis through global alterations in chromatin states. Cancer-associated microRNAs, some of which are expressed by EBV, and long noncoding RNAs have been observed to be dysregulated in NKTCL. This review focuses on studies investigating epigenetic aberrations in NKTCL to bolster our overall understanding of the role of these abnormalities in disease pathobiology. We also discuss the potential of these epigenetic aberrations to improve diagnosis and prognosis as well as reveal novel targets of therapy for NKTCL.

Keywords: biomarker, epigenetics, histone modifications, NKTCL, noncoding RNAs, promoter hypermethylation

Received: 17 July 2019; revised manuscript accepted: 19 December 2019.

Introduction
Natural killer/T-cell lymphoma (NKTCL) constitutes approximately 10% of peripheral T-cell lymphomas.1 The incidence of this rare type of lymphoma is much higher in East Asia as well as in Central and South America compared with the rest of the world.2 NKTCLs are characterized by infection with the Epstein Barr Virus (EBV), which may have a causal role in pathogenesis.3 An EBV-encoded gene, LMP1, has been shown to upregulate PD-L1 expression in NK-cell lines, and high serum expression of PD-L1 is associated with poor prognosis and low response to treatment in NKTCLs,4 suggesting a role for EBV in immune evasion of NKTCL tumour cells. EBV frequently integrates into NKTCL genomes, and one of these integrations leads to disruption of the host NHEJ1 gene, suggesting a unique means of EBV-induced pathogenesis.5 The frequent expression of P-glycoprotein, which is encoded by the MDR1 gene, may be responsible for the chemotherapy resistance observed in NKTCL patients.6,7 EBV-encoded LMP1 oncoprotein promotes cell cycle progression and inhibits apoptosis via activation of the NFκB pathway or PI3K/ AKT pathway.8,9 Most NKTCLs are of NK-cell origin and usually occur in the nasal and upper aerodigestive tract.10 Many studies have focussed on genetic alterations to identify dysregulated tumour suppressor genes or oncogenes in these lymphomas.11–15 However, accumulating evidence suggests that epigenetic aberrations are at
least as common and critical as genetic abnormalities in the pathogenesis of NKTCLs.

Epigenetics focusses on the heritable modifications in cellular chromatin that modify the expression of genes in the absence of any change in DNA sequence. Epigenetic events may include histone modifications, promoter-associated CpG island hypermethylation, nucleosome remodelling and regulation with noncoding RNAs (e.g., miRNA, IncRNA). Epigenetic abnormalities are known to play critical roles in carcinogenesis. Indeed, epigenetic aberrations are implicated in regulating a variety of different ‘hallmarks’ of cancer. In the following sections, we will discuss different epigenetic aberrations that drive the tumourigenesis of NKTCL. Moreover, we will focus on the epigenetic aberrations associated with the diagnosis, prognosis and chemotherapy resistance of NKTCLs.

Epigenetically silenced tumour suppressor genes in NKTCL

Promoter regions of many tumour suppressor genes contain CpG islands that are hypermethylated during tumourigenesis. Promoter CpG hypermethylation transcriptionally silences genes through recruitment of histone-modifying enzymes such as histone deacetylases (HDACs), which in turn generate repressive chromatin states. Hypermethylation of promoter-associated CpG islands is a common mechanism for downregulation of tumour suppressor genes in several types of cancers, such as colon cancer and multiple myeloma. A number of tumour suppressor gene candidates were found by different research groups to be epigenetically silenced through promoter-associated CpG island hypermethylation in NKTCL tumours by using locus-specific methodologies such as bisulfite sequencing and methylation-specific PCR (MSP). In a previous study, Siu and colleagues evaluated five putative tumour suppressors (i.e. P73, hMLH1, p16, p15, and RARβ) for their promoter methylation status. They reported that these genes have promoter hypermethylation in a significant fraction of the NKTCL patients, with P73 being the most frequently (94%) methylated gene. However, apart from P73, the methylation analysis was based only on MSP, which provides qualitative information on a limited number of CpG dinucleotides evaluated. P73 has significant amino acid similarity to p53, and it induces apoptosis in a manner similar to p53 when overexpressed in an osteosarcoma cell line. It would be interesting to evaluate the frequency of transcriptional silencing of P73 in NKTCL samples and to address whether ectopic p73 inhibits proliferation or induces apoptosis in p73-nonexpressing NK-cell lines. In another study, Ying and colleagues performed comprehensive epigenetic analyses on DLC1 (ARHGAP7) in NKTCLs and other lymphoma types, which revealed aberrant methylation in 77% (34/44) of NKTCL patients. Of note, decitabine (5-aza-2'-deoxycytidine) treatment increased DLC1 transcription in malignant NK-cell lines with epigenetic silencing of DLC1. This gene is known to encode a RhoGAP domain-containing protein with high sequence homology to rat p122RhoGAP, which is a GTPase-activating protein that catalyses the conversion of the active GTP-bound RhoA protein into its inactive GDP-bound form. Given that RAS-mediated transformation involves active RhoA signalling, epigenetic silencing of DLC1 may lead to constitutively active signalling of the RAS signalling pathway in NKTCLs. Using similar approaches, another study showed promoter methylation of PCDH10 (protocadherin 10) causing silencing in 100% (4/4) of malignant NK-cell lines and 20% (2/10) of NKTCL patients evaluated. However, the functional role of PCDH10 in NKTCL pathogenesis has not yet been elucidated. Wang and colleagues reported promoter CpG methylation-mediated silencing of DLEC1 in 67% (2/3) of NK-cell lines and 75% (6/8) of NKTCL patients. The functional consequences of DLEC1 silencing for the development of NKTCL have not been addressed, but its ectopic expression induced G1 cell-cycle arrest and inhibited colony formation in hepatocellular carcinoma cell lines that also have epigenetically silenced DLEC1.

Using locus-specific methods, previous reports showed epigenetic silencing of DAPK1 and PTPN6 (SHPI) in certain malignant NK-cell lines, findings that were confirmed in NKTCL patients in a subsequent genome-wide study. DAPK1, a pro-apoptotic serine/threonine kinase, is a transcriptional target of p53. It was shown to suppress oncogene-induced transformation by activating the p19ARF/p53-dependent apoptotic checkpoint; however, its functional role as a tumour suppressor has not yet been addressed in NKTCLs. Interestingly, TET2, a hydroxylase catalyzing enzymatic steps towards demethylation of 5-methylcytosines in DNA, was identified to be epigenetically silenced due to promoter hypermethylation. Recently, recurrent
methylation and transcriptional silencing of TET1 in a variety of carcinomas and lymphomas, including NKTCL patients and NK-cell lines, were discovered via epigenomic analyses with MeDIP-chip. Interestingly, reintroduction of TET1 into TET1-silenced carcinoma cell lines inhibited colony formation and restored the transcription of epigenetically silenced tumour suppressor genes (e.g. SLIT2, ZNF382 and HOXA9). A total of 95 epigenetically silenced genes were identified in NKTCL patients and NK-cell lines by integrative analyses of genome-wide promoter methylation and gene expression profiling. Based on in silico pathway analyses, most of these genes may have tumour suppressive function, but further studies need to be performed to address those with genuine tumour suppressor function. Given the lack of IDH2 mutations in NKTCL tumours, epigenetic silencing or genetic inactivation of TET1 or TET2 may be responsible for promoter hypermethylation of several tumour suppressor genes observed in NKTCLs.

For some tumour suppressor genes, genetic mechanisms have been reported to cooperate with epigenetic mechanisms during transcriptional silencing in NKTCL patients. Three studies reported promoter hypermethylation-mediated silencing of PRDM1 in NKTCL patients as well as NK-cell lines. Loss-of-function mutations of PRDM1 are rarely observed in NKTCL patients; however, functional studies performed in vitro and ex vivo characterized PRDM1 as a bona fide tumour suppressor gene deleted or epigenetically silenced in NKTCL patients and NK-cell lines. In another study, receptor-type tyrosine-protein phosphatase k (PTPRK) was shown to be transcriptionally downregulated through monoallelic deletion and promoter hypermethylation in NKTCL patients. Restoration of PTPRK expression inhibited the JAK-STAT3 pathway through dephosphorylation of phospho-STAT3 Tyr705. Importantly, ectopic expression of PTPRK inhibited carcinogenesis in malignant NK-cell lines by inhibiting tumour cell growth, invasion, and metastasis. HACE1 was also reported to be transcriptionally downregulated through monoallelic deletions and CpG island hypermethylation; however, its role in NKTCL pathogenesis is still not clear, although its re-expression in a HACE1-null NK-cell line induced G2/M cell cycle arrest and apoptosis. Frequent concomitant epigenetic silencing of CADMI, a stress-responsive tumour suppressor, and its interaction partner DAL-1 was observed in NKTCLs. Further analyses revealed that CADMI expression may be lost due to locus deletion in NKTCL patients. This interesting study showed a correlation between the methylation of the CADMI and DAL-1 genes in NKTCL tumours. As these two genes play roles in cell–cell interactions and cell motility, their silencing may promote invasion and metastasis of neoplastic NK cells. Table 1 lists the pathologically and clinically significant cancer-associated genes silenced through promoter hypermethylation in NKTCLs.

**Aberrations of epigenetic regulatory genes in NKTCL**

Dysregulated expression or mutations of epigenetic regulatory genes may have dramatic consequences in the epigenomic landscape of tumours that may result in altered expression of several oncogenes or tumour suppressor genes. Interestingly, recent NGS-based studies revealed somatic mutations in genes regulating the epigenetic landscape, including ARID1A, ASXL3, CREBBP, KMT2D (MLL2), KDM6A, EP300 and TET2 in NKTCL cases, underscoring the significance of DNA methylation, post-translational modifications of histones and remodelling of chromatin. EP300 is a histone acetyl transferase (HAT) that regulates transcription by modulating chromatin structure, and it acts as a tumour suppressor with loss-of-function mutations in tumours. Of note, p53 activity can be regulated by EP300-mediated acetylation in response to DNA damage. Therefore, the genetic aberrations of EP300 may have pleiotropic biological effects on NKTCL, which may involve aberrations in the DNA damage response pathway. Similar to EP300, CREBBP is a histone acetyl transferase, and both can regulate the transcription of distinct or common genes in B cells. Apart from KDM6A, which was observed to be mutated in an EBV-negative NKTCL patient, these mutated genes were implicated in driving B-cell lymphomagenesis. For instance, inactivation of CREBBP was shown to promote HDAC3-dependent lymphomas or to cooperate with BCL2 overexpression in the promotion of B-cell lymphoma in mice. Another study showed that the histone lysine transferase KMT2D represses B-cell lymphoma development by sustaining a gene expression programme. Of significance, FL/DLBCL-associated KMT2D loss-of-function mutations decreased global H3K4 methylation in germinal-centre (GC) B cells in vivo. Consistent with the
role of TET2 in CpG demethylations, TET2-mutated DLBCL patients showed genome-wide alterations in DNA methylations in promoter-associated CpG islands of tumour suppressor genes.55

There are few reports available in the literature on the genetic changes in epigenetic-regulatory genes that lead to global changes in the DNA methylation landscape of NKTCL tumours. Gao and colleagues recently reported the presence of TET2 as well as KMT2D mutations in NKTCL patients, where mutated TET2 or KMT2D was significantly associated with poor prognosis.47 Considering that loss of expression of these genes was also associated with shorter overall survival of NKTCL patients, it is possible to speculate that these clinically relevant mutations are loss-of-function mutations. Given the epigenomic changes associated with TET2 mutations in DLBCL patients,55 it is possible that several tumour suppressors observed to be epigenetically silenced in NKTCLs may be a consequence of genetic or epigenetic inactivation of TET2. Notably, this type of relationship has already been established for a variety of different cancer types, including NKTCL with the TET1 gene, whose methylation-mediated silencing resulted in promoter methylation and transcriptional silencing of tumour suppressors such as PCDH7 and TCF4 in nasopharyngeal

| Aberrant gene | Functional evidence as a tumour suppressor in NKTCL | Relationship to NKTCL | Reference |
|---------------|---------------------------------------------------|-----------------------|-----------|
| ASNS          | N.A.³                                              | Predictive biomarker of asparaginase-based chemotherapy | Küçük³⁰; Li⁴² |
| BIM1          | Reconstitution of its expression induced apoptosis in NK-cell lines. | Silenced pro-apoptotic gene Potential predictive biomarker of chemotherapy | Küçük³⁰ |
| CADM          | N.A.                                              | Candidate tumour suppressor | Fu⁴¹ |
| DAL1          | N.A.                                              | Candidate tumour suppressor | |
| DAPK1         | N.A.                                              | Silenced pro-apoptotic gene | Röhrs²⁸; Küçük³⁰ |
| DLC1          | N.A.                                              | Candidate tumour suppressor | Ying²³ |
| DLEC1         | N.A.                                              | Candidate tumour suppressor | Wang²⁶ |
| HACE1         | Reconstitution of its expression led to G2/M cell cycle arrest and apoptosis in an NK-cell line. | Candidate tumour suppressor | Küçük⁴⁰ |
| PTPN6 (SHP1)  | N.A.                                              | Candidate tumour suppressor Inhibitor of NK-cell activation | Oka²⁹; Küçük³⁰ |
| PTPRK         | Its ectopic expression inhibited cell growth, and reduced invasion of NKTCL cells | Inhibitor of JAK-STAT3 pathway | Chen³⁹ |
| P73           | N.A.                                              | Candidate tumour suppressor | Siu²¹; Jost²² |
| SOCS6         | Reconstitution of its expression induced apoptosis, and decreased STAT3 phosphorylation in NK-cell lines. | Candidate tumour suppressor Inhibitor of JAK-STAT pathway | Küçük³⁰ |
| TET1          | N.A.                                              | Candidate tumour suppressor DNA CpG demethylase | Li³³ |
| TET2          | N.A.                                              | Candidate tumour suppressor DNA CpG demethylase | Küçük³⁰ |

³Ectopic expression of ASNS did not decrease cell growth in ASNS-nonexpressing NK cell lines.

ASNS: asparagine synthetase; N.A.: not available; NK: natural killer; NKTCL: natural killer/T-cell lymphoma.
carcinoma cells. However, the functional relationship between TET2 or TET1 inactivation and the NKTCL epigenome has not yet been established.

One of the important observations in the epigenomic profile of NKTCL patients is the global hypomethylation observed in genomic locations distal to the promoters. It has long been known that global hypomethylation may lead to genomic instability and tumour formation in vivo. For instance, DNA hypomethylation may contribute to deletions of genomic loci which is also frequently observed in NK-cell malignancies. Moreover, DNA hypomethylation in retrotransposons may cause reactivation of these genes and translocation to other genomic loci that can dysregulate the expression of cancer-related genes. The consequence(s) of this global hypomethylation pattern has not been studied yet in NKTCLs; therefore, it would be interesting to investigate whether global hypomethylation promotes genetic alterations contributing to NKTCL pathogenesis.

EZH2 mediates its oncogenic functions as a part of the polycomb repressive complex 2 (PRC2) by methylating histones and generating H3K27me3 repressive marks. No mutations of EZH2 have been observed in NKTCL patients. However, Yan and colleagues showed that EZH2 is overexpressed in NKTCLs, and its overexpression confers a growth advantage to primary NK cells and NKTCL cell lines independent of its histone methyltransferase activity. Another report showed that phosphorylation of EZH2 by JAK3 in NKTCL results in dissociation of EZH2 from polycomb repressive complex 2 (PRC2), which may lead to global changes in H3K27me3 histone marks near promoters. In this model, the noncanonical oncogenic functions of EZH2 may, at least in part, be related to its role as a transcriptional activator. Table 2 lists the dysregulated epigenetic regulator genes and describes their relationship to epigenetic changes and NKTCL pathogenesis.

Noncoding RNAs in NKTCL pathogenesis
Noncoding RNAs include long or short RNAs that regulate the expression of other genes through a variety of different mechanisms. Among these noncoding RNAs, microRNAs (miRNAs) have been associated with the pathogenesis of many diseases. miRNAs are short (~22nt) noncoding RNAs that inhibit the expression of target genes by inhibiting their translation or promoting transcript degradation. They have been observed to be dysregulated in several cancer types, such as breast and prostate cancer. Like protein-coding genes, miRNAs may promote or suppress carcinogenesis if their expression levels are dysregulated.

miR-155 is an oncogenic miRNA that was shown to be overexpressed and promote lymphomagenesis of diffuse large B-cell lymphoma and anaplastic large-cell lymphoma (ALCL). Several independent studies revealed overexpression of mir-155 in NKTCLs. miR-155 was shown to promote NK-cell effector functions such as IFNγ production in vivo. Intriguingly, mir-155 transgenic mice showed increased cell number and enhanced survival of NK cells, which may be due to activation of AKT and ERK pathways. These studies in B-cell lymphomas and NK cells, together with the observation that Eμ-miR155 transgenic mice develop high-grade B-cell lymphoma, suggest that miR-155 may be a highly potent driver of NKTCL. Ng and colleagues investigated the genome-wide profiles of miRNAs in formalin-fixed paraffin embedded tissues and malignant NK-cell lines, where they observed dysregulated miRNAs. In addition, they showed that ectopic expression of miR-101, miR-26a, miR26b, miR-28-5 and miR-363, which were downregulated in NK lymphoma tumours, reduced the growth of an NK-cell line. However, further studies are required to elucidate the role of these potential tumour suppressor miRNAs in NKTCL pathogenesis. In another study, Paik and colleagues observed epigenetic downregulation of mir-146a in NK-cell lines and formalin-fixed, paraffin-embedded NKTCL tumour samples. Reconstitution of its expression inhibited proliferation and induced apoptosis, at least in part due to inhibition of the nuclear factor κB (NFκB) signalling pathway by targeting TRAF6. Liang and colleagues showed that PRDM1 is a direct target of miR-223 in malignant NK cells, which suggests that a variety of mechanisms are responsible for the transcriptional silencing of this tumour suppressor gene.

Some studies have focused on the role of EBV-encoded miRNAs and NKTCL pathogenesis. One of these studies showed that an EBV-expressed miRNA (i.e. BART9 miRNA) is overexpressed in two NKTCL cell lines, and it promotes cellular growth by upregulating LMP1, an oncogene encoded by EBV. Of note, Ma and colleagues observed that an EBV-encoded
miRNA, EBV-miR-BHRF1-2, inhibits PRDM1 by targeting its 3' UTR region in lymphoblastoid cell lines, suggesting an epigenetic mechanism of PRDM1 silencing during EBV+ lymphomagenesis. It would be interesting to address whether PRDM1 protein expression is inhibited by EBV-encoded miRNAs in NKTCL patients with detectable PRDM1 transcript expression.

Recently, Peng and colleagues investigated the genomic and transcriptomic landscape of EBV in NKTCL patients, which revealed transcriptional dysregulation of EBV-encoded BART miRNAs due to its locus deletion in the EBV genome. Long-noncoding RNAs (lncRNAs) are functional transcripts longer than 200 nucleotides. LncRNAs can modulate the expression of genes using mechanisms more diverse than those of miRNAs. Like miRNAs, lncRNAs play pivotal roles in different hallmarks (resisting cell death, replicative immortality etc.) of cancer. Very few studies have been performed on the role of lncRNAs in NKTCL pathogenesis. Among these, the most comprehensive study is the one performed by Baytak and colleagues that reported a whole-transcriptome analysis of NKTCL patients. This study revealed that 166 lncRNAs and 66 lncRNAs were significantly overexpressed and underexpressed in NKTCL patients, respectively, compared with resting and activated primary NK cells. ZFAS1 was one of the overexpressed lncRNAs identified in this study. Interestingly, the genes whose expression positively or negatively correlated with that of ZFAS1 in normal and malignant NK samples were enriched in biological processes (e.g. stabilization of p53, regulation of apoptosis) or signalling pathways (NFκB or WNT signalling) critical to activation or neoplastic transformation of NK cells. Intriguingly, lncRNAs overexpressed and underexpressed in NKTCL patients are associated with pathways or biological processes that play a role in the activation of NK cells. These in silico analyses suggest that ZFAS1 or other dysregulated lncRNAs may regulate NK-cell function as well as tumorigenesis. Another study reported overexpression of the lncRNA MALAT1 in NK- and T-cell lymphoma tumour samples and cell lines. Of note, high expression levels of components of the polycomb repressive complex 1 (i.e. BMI1) and PRC2

| Table 2. Aberrant epigenetic regulatory genes and their relationship to epigenetic changes and NKTCL pathogenesis. |
|---|
| Aberrant gene | Gene function and aberration | Relationship to NKTCL | Reference |
| ARID1A | ✓ Chromatin remodelling gene ✓ A missense mutation | Unknown | Jiang⁴⁴; Choi⁴⁵ |
| ASXL3 | ✓ Polycomb group protein ✓ Mutated | Unknown | Jiang⁴⁴ |
| CREBBP | ✓ Histone acetyl transferase ✓ Missense mutation | Unknown | Küçük¹⁵ |
| EP300 | ✓ Histone acetyltransferase ✓ Frame-shift or missense mutations | Unknown | Küçük¹⁵ |
| EZH2 | ✓ Histone methyltransferase ✓ Overexpressed | Promotes NK-cell growth independent of histone methyltransferase activity | Yan⁶⁰ |
| KDM6A [UTX] | ✓ Histone demethylase specific for H3K27 ✓ Mutated | Unknown | Tsuyama⁴⁶ |
| KMT2D [MLL2] | ✓ Lysine methyltransferase ✓ Missense mutations | Unknown | Jiang⁴⁴; Küçük¹⁵; Choi¹⁵ |
| TET1 | ✓ DNA CpG demethylase ✓ Promoter hypermethylation | Unknown | Li³³ |
| TET2 | ✓ DNA CpG demethylase ✓ Promoter hypermethylation | Unknown | Küçük¹⁵ |

NKTCL, natural killer/T-cell lymphoma.
(i.e. EZH2, SUZ12, and EED) were also reported in NKTCL patients, which raised the possibility of the involvement of MALAT1 and PRC proteins in the same signalling pathway. In support of this possibility, MALAT1 expression was observed to be positively correlated with the expression of PRC1 and PRC2 genes in NKTCL patients. MALAT1 was shown to interact directly with PRC2 components (i.e. EZH2 and SUZ12) in a T-cell lymphoma line. Despite a lack of direct physical interaction, MALAT1 probably indirectly induces the expression of BMI, a member of the PRC1 complex, through H3K27me3 histone marks. Overexpressed MALAT1 interacts with the polycomb repressive complex, suggesting that MALAT1 may promote the generation of H3K27me3, thereby repressing certain target genes. Cancer-associated noncoding RNAs with pathological and biological significance in NKTCL are shown in Table 3.

EBV-induced alterations in NKTCL epigenomes

EBV-associated NKTCLs are characterized by a latent stage of infection, which was reported to be associated with promoter methylation-mediated silencing of the two immediate-early (IE) genes, BZLF1 and BRLF1, in EBV. A number of studies have reported causal relationships between EBV infection and epigenetic aberrations in the host cells of B-cell lymphomas or certain carcinomas. Importantly, some of the EBV-encoded proteins are known to epigenetically silence tumour suppressor genes in EBV-associated tumours. For instance, an EBV-encoded oncoprotein, LMP1 (latent membrane protein 1), was reported to upregulate expression of DNA methyl transferases 1, 3a and 3b (i.e. DNMT1, DNMT3A and DNMT3B) in EBV+ nasopharyngeal carcinoma cells, which in turn epigenetically silenced E-cadherin. Another study showed that DNMT1 was upregulated by LMP2A via phosphorylation of STAT3, which led to promoter methylation-mediated silencing of the pro-apoptotic BIM gene in B-cell lines, a variety of different oncoproteins encoded by EBV may be involved in silencing tumour suppressor genes in EBV-infected host cells. Zhao and colleagues reported that EBV-encoded LMP2A-mediated upregulation of DNMT3B resulted in global changes in the epigenomic landscape through promoter hypermethylation of hundreds of genes in EBV+ gastric cancer cells. Having extrapolated the causal relationships between EBV infection and subsequent epigenomic changes such as promoter hypermethylation in a variety of different EBV-infected cell types, Li and colleagues proposed a model for EBV-induced epigenetic pathogenesis in which EBV-encoded oncoproteins (e.g. LMP1, LMP2A) or EBV-encoded miRNAs or lncRNAs modulate the host cell epigenetic machinery, which leads to methylation-mediated silencing of tumour suppressors, thereby promoting malignant transformation. However, functional studies on the role of EBV-encoded oncoproteins in modulating NK-cell machinery are still quite scarce. Of significance, a recent report showed that EBV genomes isolated from NKTCL patients form distinct clusters when analysed along with other EBV-infected tumours based on phylogenetic analyses. This observation suggests that the possibility that EBV-induced epigenetic pathogenesis may have characteristics unique to NKTCLs. EBV-encoded miRNAs may promote the development of NKTCL by targeting key cancer-associated genes. One study showed that BART9 miRNA, which is encoded by EBV, leads to increased expression of EBV-encoded LMP1, which in turn promotes proliferation of NKTCL cells.

Diagnostic, prognostic and therapeutic implications of epigenetic aberrations

Several studies have investigated the relationship between epigenetic aberrations and clinical characteristics in NKTCL patients. Chen and colleagues identified PTPRK, a negative regulator of STAT3 signalling, as a bona fide tumour suppressor silenced through the cooperation of genetic and epigenetic mechanisms. Importantly, PTPRK promoter methylation was significantly correlated with advanced disease stage and the number of extranodal sites involved in NKTCL patients. This study also showed that NKTCL patients treated with the SMILE regimen showed poorer overall survival when their tumours had PTPRK promoter methylation. In another study, EZH2 overexpression was reported to have significant clinical consequences. Overexpressed EZH2 was associated with advanced disease stage, poorer overall survival and a high proliferation index. Gao and colleagues recently reported that NKTCL patients with mutations or loss-of-protein expression in KMT2D or TET2 had significantly poorer overall survival, which suggests that alterations in the epigenomic landscape may
be indirectly responsible for the prognostic differences. However, the authors did not address this causal relationship.

Some studies have revealed relationships between miRNA expression and clinical characteristics in NKTCL patients. Paik and colleagues showed that miR-146a expression level is an independent prognostic factor for NKTCL patients. They further showed that NKTCL patients with low miR146a expression have significantly poorer prognosis compared with those with high miR146a expression. These findings suggest that miR146a methylation or expression level can potentially be used as prognostic factors. Another clinically significant finding of this study is the increased chemosensitivity observed in malignant NK-cell lines with ectopic miR146a expression, which suggests that it may be a good target of therapy for chemoresistant NKTCL patients. Huang and colleagues reported two EBV-encoded miRNAs, mir-BART20-5p and mir-BART8, were associated with disease progression in NKTCL. miRNA expression profile analyses and qPCR showed elevated levels of expression of mir-BART20-5p, mir-BART7-3p, mir-BART13-3p and mir-BART1-5p in the sera of NKTCL patients, which may have diagnostic value. Moreover, high miR-BART2-5p level in NKTCL patient sera was associated with disease progression and poor prognosis, suggesting it as a potential biomarker for predicting the risk of the disease.

There are few reports available on the clinical significance of lncRNAs in NKTCL patients. A recent study by Zhu and colleagues showed that high SNHG12 lncRNA expression was associated with the clinical grade of NKTCL patients. In vitro manipulation of SNHG12 expression level in malignant NK-cell lines revealed that high SNHG12 expression conferred cisplatin resistance to NKTCL cells. Of note, SNHG12 over-expression increased P-glycoprotein expression in an NK-cell line, suggesting that this lncRNA may be responsible for the multi-drug resistance observed in NKTCL patients. Another study investigated whether MALAT1 expression in NKTCLs can predict prognosis in NKTCL patients. There was a trend for poorer survival in the high-MALAT1-expression group, but it was not statistically significant. As the mentioned study involved a low number of NKTCL patient samples, future studies with larger cohorts are needed to address whether MALAT1

### Table 3. Cancer-associated miRNAs and lncRNAs dysregulated in NKTCL.

| Aberrant noncoding RNA | Epigenetic aberration | Relationship to NKTCL | Reference |
|------------------------|-----------------------|-----------------------|-----------|
| **BART9**              | Overexpressed EBV-encoded miRNA | Promotes NK-cell growth, upregulates LMP1 | Ramakrishnan80 |
| **miR-155**            | Overexpressed miRNA | Promotes NK-cell survival, induces AKT and ERK pathways | Yamanaka93; Zhang70; Baytak71 |
| miR-101, miR-26a, miR26b, miR-28-5, and miR-363 | Underexpressed miRNA | Candidate tumour suppressor, reduces growth of a malignant NK-cell line | Ng75 |
| **miR-221**            | Overexpressed miRNA | Noninvasive diagnostic and prognostic biomarker | Guo97 |
| **miR-223**            | Overexpressed miRNA | Targets and downregulates PRDM1 in NKTCL cells | Liang77 |
| **MALAT1**             | Upregulated IncRNA | Oncogene, prognostic biomarker | Kim85 |
| **SNHG12**             | Overexpressed IncRNA | Oncogene, prognostic biomarker, predictive biomarker of chemoresistance | Zhu88 |
| **ZFAS1**              | Upregulated IncRNA | Candidate oncogene, regulator of apoptosis and cell cycle | Baytak71 |

miRNA, microRNA; lncRNA, long noncoding RNA; NK, natural killer; NKTCL, natural killer/T-cell lymphoma.
expression can be used as a prognostic marker for NKTCL patients. Interestingly, expression of one of the targets of MALAT1, BMI, showed prognostic value in NKTCL patients.86

One of the interesting questions from a clinical point of view would be to transfer the epigenetic knowledge on NKTCLs to routine clinical practice, in particular for disease monitoring. To address whether previously described promoter CpG island methylations can be used for disease monitoring, Siu and colleagues evaluated the promoter methylation status in NKTCL patients of a panel of genes (i.e. p15, p16, p73, hMLH1, RARb) with methylation-specific PCR (MSP) that were previously shown to be hypermethylated in NKTCLs.101 The concordance between MSP and histological evaluation results was quite high, suggesting that MSP may be useful to monitor minimal residual disease and relapse after treatment in NKTCL patients.

Several studies have shown that circulating miRNAs derived from tumour tissues are stable and detectable in serum; therefore, they can potentially be used as noninvasive biomarkers for cancer diagnosis or prognosis.102–105 Very few studies have investigated the potential of plasma/serum miRNAs as biomarkers in NKTCLs. qPCR-based analyses revealed miR-221 as a potential diagnostic and prognostic biomarker for NKTCL.87 Zhang and colleagues observed significantly higher miR-155 levels in the serum of NKTCL patients compared with the levels in healthy individuals.70 More importantly, serum miR-155 levels were higher in patients with stable or progressive disease compared with those in partial or complete remission.70

Two distinct studies reported an inverse relationship between ASNS (asparagine synthetase) expression level and response to asparaginase-based chemotherapy in NKTCL cell lines and patient samples.30,42 ASNS expression was shown to be downregulated due to promoter hypermethylation, and there was a very high correlation between ASNS mRNA level and survival of malignant NK-cell lines treated with asparaginase.30 Given that asparaginase-based therapies are currently the most effective therapies against NKTCL and a subset of patients are resistant to these therapies,106 assessment of ASNS promoter methylation levels of NKTCL tumours may be used as a predictive biomarker for NKTCL patients who are likely to respond to asparaginase-based chemotherapeutic regimens.

BCL2L11 (BIM) may also be responsible for chemotherapy resistance in NKTCL patients, as reintroduction of BIM into two BIM-silenced NK-cell lines increased apoptosis after chemotherapy treatment.30 Whether BIM promoter methylation level can be used as a predictive biomarker of chemotherapy resistance requires further investigation in NKTCL patients. Indeed, re-expression of BIM or other epigenetically silenced tumour suppressor genes may provide therapeutic benefit by inhibiting the growth of NKTCL tumours.

Epigenetic control is involved in all hallmarks of tumour development107; hence, epigenetic drugs may be effective strategies for therapy. The epigenetic regulatory genes identified to be mutated (i.e. ARID1A, ASXL3, CREBBP, EP300, KMT2D) or methylated (i.e. TET1, TET2) may be of therapeutic value. Given that loss of function or loss of expression of TET genes may be responsible for promoter methylation of several tumour suppressors in NKTCLs,30 DNA methyltransferase (DNMT) inhibitors may be useful for restoring the expression of epigenetically silenced tumour suppressors. Indeed, azacitidine showed efficacy in the treatment of myelodysplastic syndromes.108 However, due to a lack of specificity, DNMT inhibitors may lead to serious side effects owing to increased expression of oncogenes or induction of genomic instability.109 Similarly, HDAC inhibitors may be an effective therapeutic option for NKTCL patients when they are used in appropriate doses with correct timing to have fewer side effects.107 However, functional experiments need to be performed in NKTCLs to show the specific effects of mutations or promoter methylations of epigenetic regulatory genes before proceeding with clinical trials targeting these epigenetic aberrations.

Conclusions and future perspectives
Accumulating evidence suggests that a variety of different epigenetic aberrations have biological or clinicopathological significance in NKTCL. However, it seems that our current knowledge on NKTCL epigenetics is just the tip of the iceberg, and future mechanistic studies are needed to elucidate the extent of regulation of chromatin states, especially in the presence of somatic mutations in epigenetic genes. These abnormalities are not
only useful for better understanding of the biology of NKTCLs but also may potentially be translated into routine clinical practice as diagnostic, prognostic, or predictive biomarkers.

Author contributions
C.K., J.W., Y.X. and H.Y. searched and critically evaluated the literature related to the topic and wrote the manuscript. C.K. and H.Y. financially supported the study.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and publication of this article: This study was supported by the Young Scientists Award Program of the Turkish Academy of Sciences (TÜBA GEBIP 2017) (C.K.). The research activity in the Hua You laboratory is supported by Guangzhou Medical University. Hua You is supported by the National Natural Science Foundation of China (81911530169, 81903088, 81850410547, 81670180, and 81711540047), Venture and Innovation Support Program for Chongqing Overseas Returnees (cx2019051), and the Beijing Nova Program of the Beijing Municipal Science and Technology Commission (Z171100001117091). YX is sponsored by the National Natural Science Foundation of Chongqing, China (cstc2019jcyj-msxmX0793).

Conflict of interest statement
The authors declare that there is no conflict of interest.

References
1. Foss FM, Zinzani PL, Vose JM, et al. Peripheral T-cell lymphoma. Blood 2011; 117: 6756–6767.
2. Aozasa K and Zaki MA. Epidemiology and pathogenesis of nasal NK/T-cell lymphoma: a mini-review. ScientificWorldJournal 2011; 11: 422–428.
3. Vockerodt M, Yap LF, Shannon-Lowe C, et al. The Epstein-Barr virus and the pathogenesis of lymphoma. J Pathol 2015; 235: 312–322.
4. Bi XW, Wang H, Zhang WW, et al. PD-L1 is upregulated by EBV-driven LMP1 through NF-xB pathway and correlates with poor prognosis in natural killer/T-cell lymphoma. J Hematol Oncol 2016; 9: 109.
5. Peng RJ, Han BW, Cai QQ, et al. Genomic and transcriptomic landscapes of Epstein-Barr virus in extranodal natural killer T-cell lymphoma. Leukemia 2019; 33: 1451–1462.
6. Yamaguchi M, Kita K, Miwa H, et al. Frequent expression of P-glycoprotein/MDR1 by nasal T-cell lymphoma cells. Cancer 1995; 76: 2351–2356.
7. Pastan I and Gottesman M. Multiple-drug resistance in human cancer. New Engl J Med 1987; 316: 1388–1393.
8. Sun L, Zhao Y, Shi H, et al. LMP1 promotes nasal NK/T-cell lymphoma cell function by eIF4E via NF-xB pathway. Oncol Rep 2015; 34: 3264–3271.
9. Sun L, Zhao Y, Shi H, et al. LMP-1 induces survivin expression to inhibit cell apoptosis through the NF-xB and PI3K/Akt signaling pathways in nasal NK/T-cell lymphoma. Oncol Rep 2015; 33: 2253–2260.
10. Tse E and Kwong YL. How I treat NK/T-cell lymphomas. Blood 2013; 121: 4997–5005.
11. Li T, Hongyo T, Syaiufudin M, et al. Mutations of the P53 gene in nasal NK/T-cell lymphoma. Lab Invest 2000; 80: 493–499.
12. Takakuwa T, Dong Z, Nakatsuka S, et al. Frequent mutations of Fas gene in nasal NK/T cell lymphoma. Oncogene 2002; 21: 4702–4705.
13. Dobashi A, Tsuyama N, Asaka R, et al. Frequent BCOR aberrations in extranodal NK/T-cell lymphoma, nasal type. Genes Chromosomes Cancer 2016; 55: 460–471.
14. Koo GC, Tan SY, Tang T, et al. Janus kinase 3-activating mutations identified in natural killer/T-cell lymphoma. Cancer Discov 2012; 2: 591–597.
15. Küçük C, Jiang B, Hu X, et al. Activating mutations of STAT5B and STAT3 in lymphomas derived from γδ-T or NK cells. Nat Commun 2015; 6: 6025.
16. Flavahan WA, Gaskell E and Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. Science 2017; 357: eaal2380.
17. Jones PA and Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002; 3: 415–428.
18. Jones PL, Veenstra GJ, Wade PA, et al. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 1998; 19: 187–191.
19. Esteller M, Sparks A, Toyota M, et al. Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. Cancer Res 2000; 60: 4366–4371.
20. Chim CS, Fung TK, Cheung WC, et al. SOCS1 and SHP1 hypermethylation in multiple myeloma: implications for epigenetic activation of the Jak/STAT pathway. Blood 2004; 103: 4630–4635.

21. Siu LL, Chan JK, Wong KF, et al. Specific patterns of gene methylation in natural killer cell lymphomas: P73 is consistently involved. Am J Pathol 2002; 160: 59–66.

22. Jost CA, Marin MC and Kaelin WG Jr. P73 is a simian [correction of human] P53-related protein that can induce apoptosis. Nature 1997; 389: 191–194.

23. Ying J, Li H, Murray P, et al. Tumor-specific methylation of the 8p22 tumor suppressor gene DLEC1 is an epigenetic biomarker for Hodgkin, nasal NK/T-cell and other types of lymphomas. Epigenetics 2007; 2: 15–21.

24. Etienne-Manneville S and Hall A. Rho GTPases in cell biology. Nature 2002; 420: 629–635.

25. Ying J, Gao Z, Li H, et al. Frequent epigenetic silencing of protocadherin 10 by methylation in multiple haematologic malignancies. Br J Haematol 2007; 136: 829–832.

26. Wang Z, Li L, Su X, et al. Epigenetic silencing of the 3p22 tumor suppressor DLEC1 by promoter CpG methylation in non-Hodgkin and Hodgkin lymphomas. J Transl Med 2012; 10: 209.

27. Qiu GH, Salto-Tellez M, Ross JA, et al. The tumor suppressor gene DLEC1 is frequently silenced by DNA methylation in hepatocellular carcinoma and induces G1 arrest in cell cycle. J Hepatol 2008; 48: 433–441.

28. Röhrs S, Romani J, Zaborski M, et al. Hypermethylation of death-associated protein kinase 1 differentiates natural killer cell lines from cell lines derived from T-acute lymphoblastic leukemia. Leukemia 2009; 23: 1174–1176.

29. Oka T, Ouchida M, Koyama M, et al. Gene silencing of the tyrosine phosphatase SHP1 gene by aberrant methylation in leukemias/lymphomas. Cancer Res 2002; 62: 6390–6394.

30. Küçük C, Hu X, Jiang B, et al. Global promoter methylation analysis reveals novel candidate tumor suppressor genes in natural killer cell lymphoma. Clin Cancer Res 2015; 21: 1699–1711.

31. Martoriat A, Doumont G, Alcalay M, et al. Dapkl1, encoding an activator of a p19ARF-p53-mediated apoptotic checkpoint, is a transcription target of p53. Oncogene 2005; 24: 1461–1466.

32. Ko M, Huang Y, Jankowska AM, et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 2010; 468: 839–843.

33. Li L, Li C, Mao H, et al. Epigenetic inactivation of the CpG demethylase TET1 as a DNA methylation feedback loop in human cancers. Scientific Rep 2016; 6: 26591.

34. Cairns RA, Iqbal J, Lemonnier F, et al. IDH2 mutations are frequent in angioimmunoblastic T-cell lymphoma. Blood 2012; 119: 1901–1903.

35. Iqbal J, Kucuk C, Deleuwe RJ, et al. Genomic analyses reveal global functional alterations that promote tumor growth and novel tumor suppressor genes in natural killer-cell malignancies. Leukemia 2009; 23: 1139–1151.

36. Kucuk C, Iqbal J, Hu X, et al. PRDM1 is a tumor suppressor gene in natural killer cell malignancies. Proc Natl Acad Sci USA 2011; 108: 20119–20124.

37. Zhang Z, Liang L, Li D, et al. Hypermethylation of PRDM1/BLIMP-1 promoter in extranodal NK/T-cell lymphoma, nasal type: an evidence of predominant role in its downregulation. Hematol Oncol 2017; 35: 645–654.

38. Karube K, Nakagawa M, Tsuzuki S, et al. Identification of FOXO3 and PRDM1 as tumor-suppressor gene candidates in NK-cell neoplasms by genomic and functional analyses. Blood 2011; 118: 3195–3204.

39. Chen YW, Guo T, Shen L, et al. Receptor-type tyrosine-protein phosphatase ε directly targets STAT3 activation for tumor suppression in nasal NK/T-cell lymphoma. Blood 2015; 125: 1589–1600.

40. Küçük C, Hu X, Iqbal J, et al. HACE1 is a tumor suppressor gene candidate in natural killer cell neoplasms. Am J Pathol 2013; 182: 49–55.

41. Fu L, Gao Z, Zhang X, et al. Frequent concomitant epigenetic silencing of the stress-responsive tumor suppressor gene CADM1, and its interacting partner DAL-1 in nasal NK/T-cell lymphoma. Int J Cancer 2009; 124: 1572–1578.

42. Li Y, Zhang X, Hu T, et al. Asparagine synthetase expression and its potential prognostic value in patients with NK/T cell lymphoma. Oncol Rep 2014; 32: 853–859.

43. Plass C, Pfister SM, Lindroth AM, et al. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. Nat Rev Genet 2013; 14: 765–780.

44. Jiang L, Gu ZH, Yan ZX, et al. Exome sequencing identifies somatic mutations of...
DDX3X in natural killer/T-cell lymphoma. *Nat Genet* 2015; 47: 1061–1066.

45. Choi S, Go JH, Kim EK, et al. Mutational analysis of extranodal NK/T-cell lymphoma using targeted sequencing with a comprehensive cancer panel. *Genomics Inform* 2016; 14: 78–84.

46. Tsuyama N, Asaka R, Dobashi A, et al. Epstein-Barr virus-negative extranodal ‘true’ natural killer-cell lymphoma harbouring a KDM6A mutation. *Hematol Oncol* 2018; 36: 328–335.

47. Gao LM, Zhao S, Zhang WY, et al. Epigenetic changes in natural killer/T-cell lymphoma. *Nat Genet* 2015; 47: 1061–1066.

48. Gayther SA, Batley SJ, Linger L, et al. Mutations truncating the EP300 acetylase in human cancers. *Nat Genet* 2000; 24: 300–303.

49. Grossman SR. P300/CBP/p53 interaction and regulation of the p53 response. *Eur J Biochem* 2001; 268: 2773–2778.

50. Meyer SN, Scuoppo C, Vlasevska S, et al. Unique and shared epigenetic programs of the CREBBP and EP300 acetyltransferases in germinal center B cells reveal targetable dependencies in lymphoma. *Immunity* 2019; 51: 535–547.e9.

51. Jiang Y, Ortega-Molina A, Geng H, et al. Crebbp inactivation promotes the development of HDAC3-dependent lymphomas. *Cancer Discov* 2017; 7: 38–53.

52. Garcia-Ramirez I, Tadros S, Gonzalez-Herrero I, et al. CREBBP loss cooperates with BCL2 overexpression to promote lymphoma in mice. *Blood* 2017; 129: 2645–2656.

53. Ortega-Molina A, Boss IW, Canela A, et al. The histone lysine methyltransferase KMT2D sustains a gene expression program that represses B cell lymphoma development. *Nat Med* 2015; 21: 1199–1208.

54. Zhang J, Dominguez-Sola D, Hussein S, et al. Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. *Nat Med* 2015; 21: 1190–1198.

55. Asmar F, Punj V, Christensen J, et al. Genomewide profiling identifies a DNA methylation signature that associates with TET2 mutations in diffuse large B-cell lymphoma. *Haematologica* 2013; 98: 1912–1920.

56. Eden A, Gaudet F, Waghmare A, et al. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 2003; 300: 455.

57. Chen RZ, Pettersson U, Beard C, et al. DNA hypomethylation leads to elevated mutation rates. *Nature* 1998; 395: 89–93.

58. Rodriguez-Paredes M and Esteller M. Cancer epigenetics reaches mainstream oncology. *Nat Med* 2011; 17: 330–339.

59. Simon JA and Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat Res* 2008; 647: 21–29.

60. Yan J, Ng SB, Tay JL, et al. EZH2 overexpression in natural killer/T-cell lymphoma confers growth advantage independently of histone methyltransferase activity. *Blood* 2013; 121: 4512–4520.

61. Yan J, Li B, Lin B, et al. EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. *Blood* 2016; 128: 948–958.

62. Lu M, Zhang Q, Deng M, et al. An analysis of human microrna and disease associations. *PLoS One* 2008; 3: e3420.

63. Tan JR, Koo YX, Kaur P, et al. Micrornas in stroke pathogenesis. *Curr Med Mol Med* 2011; 11: 76–92.

64. Bartel DP. Micrornas: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116: 281–297.

65. Tavazoie SF, Alarcon C, Oskarsson T, et al. Endogenous human micrornas that suppress breast cancer metastasis. *Nature* 2008; 451: 147–152.

66. Shi XB, Xue L, Yang J, et al. An androgen-regulated mirna suppresses BAK1 expression and induces androgen-independent growth of prostate cancer cells. *Proc Natl Acad Sci USA* 2007; 104: 19983–19988.

67. Huang X, Shen Y, Liu M, et al. Quantitative proteomics reveals that miR-155 regulates the PI3K-AKT pathway in diffuse large B-cell lymphoma. *Am J Pathol* 2012; 181: 26–33.

68. Merkel O, Hamacher F, Griessl R, et al. Oncogenic role of miR-155 in anaplastic large cell lymphoma lacking the t(2;5) translocation. *J Pathol* 2015; 236: 454–546.

69. Yamanaka Y, Tagawa H, Takahashi N, et al. Aberrant overexpression of micrornas activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. *Blood* 2009; 114: 3265–3275.

70. Zhang X, Ji W, Huang R, et al. Microrna-155 is a potential molecular marker of natural killer/T-cell lymphoma. *Oncotarget* 2016; 7: 53808–53819.
71. Baytak E, Gong Q, Akman B, et al. Whole transcriptome analysis reveals dysregulated oncogenic IncRNAs in natural killer/T-cell lymphoma and establishes MIR155HG as a target of PRDM1. *Tumor Biol* 2017; 39: 1010428317701648.

72. Trotta R, Chen L, Ciarlariello D, et al. miR-155 regulates IFN-γ production in natural killer cells. *Blood* 2012; 119: 3478–3485.

73. Trotta R, Chen L, Costinean S, et al. Overexpression of miR-155 causes expansion, arrest in terminal differentiation and functional activation of mouse natural killer cells. *Blood* 2013; 121: 3126–3134.

74. Costinean S, Zanesi N, Pekarsky Y, et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci USA* 2006; 103: 7024–7029.

75. Ng SB, Yan J, Huang G, et al. Dysregulated microRNAs affect pathways and targets of biologic relevance in nasal-type natural killer/T-cell lymphoma. *Blood* 2011; 118: 4919–4929.

76. Paik JH, Jang JY, Jeon YK, et al. MicroRNA-146a downregulates NFκB activity via targeting TRAF6 and functions as a tumor suppressor having strong prognostic implications in NK/T cell lymphoma. *Clin Cancer Res* 2011; 17: 4761–4771.

77. Liang L, Nong L, Zhang S, et al. The downregulation of PRDM1/BLIMP-1 is associated with aberrant expression of miR-223 in extranodal NK/T-cell lymphoma, nasal type. *J Exp Clin Cancer Res* 2014; 33: 7.

78. Navari M, Etebari M, Ibrahimi M, et al. Pathobiologic roles of Epstein-Barr virus-encoded micrornas in human lymphomas. *Int J Mol Sci* 2018; 19: pii: E1168.

79. Mei M and Zhang M. Non-coding RNAs in natural killer/T-cell lymphoma. *Front Oncol* 2019; 9: 515.

80. Ramakrishnan R, Donahue H, Garcia D, et al. Epstein-Barr virus BART9 miRNA modulates LMP1 levels and affects growth rate of nasal NK T cell lymphomas. *PLoS One* 2011; 6: e27271.

81. Ma J, Nie K, Redmond D, et al. EBV-miR-BHRRF1-2 targets PRDM1/BLIMP1: potential role in EBV lymphomagenesis. *Leukemia* 2016; 30: 594–604.

82. Mercer TR, Dinger ME and Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009; 10: 155–159.

83. Wang KC and Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell* 2011; 43: 904–914.

84. Schmitt AM and Chang HY. Long noncoding RNAs in cancer pathways. *Cancer cell* 2016; 29: 452–463.

85. Kim SH, Kim SH, Yang WI, et al. Association of the long non-coding RNA MALAT1 with the polycomb repressive complex pathway in T and NK cell lymphoma. *Oncotarget* 2017; 8: 31305–31317.

86. Kim SH, Yang WI, Min YH, et al. The role of the polycomb repressive complex pathway in T and NK cell lymphoma: biological and prognostic implications. *Tumor Biol* 2016; 37: 2037–2047.

87. Guo HQ, Huang GL, Guo CC, et al. Diagnostic and prognostic value of circulating miR-221 for extranodal natural killer/T-cell lymphoma. *Dis Markers* 2010; 29: 251–258.

88. Zhu L, Zhang X, Fu X, et al. c-Myc mediated upregulation of long noncoding RNA SNHG12 regulates proliferation and drug sensitivity in natural killer/T-cell lymphoma. *J Cell Biochem* 2019; 120: 12628–12637.

89. Li L, Su X, Choi GC, et al. Methylation profiling of Epstein-Barr virus immediate-early gene promoters, BZLF1 and BRLF1 in tumors of epithelial, NK- and B-cell origins. *BMC Cancer* 2012; 12: 125.

90. Leonard S, Wei W, Anderton J, et al. Epigenetic and transcriptional changes which follow Epstein-Barr virus infection of germinal center B cells and their relevance to the pathogenesis of Hodgkin’s lymphoma. *J Virol* 2011; 85: 9568–9577.

91. Ghosh Roy S, Robertson ES and Saha A. Epigenetic impact on EBV associated B-cell lymphomagenesis. *Biomolecules* 2016; 6: pii: E46.

92. Nishikawa J, Iizasa H, Yoshiyama H, et al. The role of epigenetic regulation in Epstein-Barr virus-associated gastric cancer. *Int J Mol Sci* 2017; 18: pii: E1606.

93. Tsai CN, Tsai CL, Tse KP, et al. The Epstein-Barr virus oncogene product, latent membrane protein 1, induces the downregulation of E-cadherin gene expression via activation of DNA methyltransferases. *Proc Natl Acad Sci USA* 2002; 99: 10084–10089.

94. Hino R, Uozaki H, Murakami N, et al. Activation of DNA methyltransferase 1 by EBV latent membrane protein 2A leads to promoter
hypermethylation of PTEN gene in gastric carcinoma. *Cancer Res* 2009; 69: 2766–2774.

95. Paschos K, Parker GA, Watanatanasup E, et al. Bim promoter directly targeted by EBNA3C in polycomb-mediated repression by EBV. *Nucleic Acids Res* 2012; 40: 7233–7246.

96. Zhao J, Liang Q, Cheung KF, et al. Genome-wide identification of Epstein-Barr virus-driven promoter methylation profiles of human genes in gastric cancer cells. *Cancer* 2013; 119: 304–312.

97. Li L, Ma BBY and Chan ATC, et al. Epstein-Barr virus-induced epigenetic pathogenesis of viral-associated lymphoepithelioma-like carcinomas and natural killer/T-cell lymphomas. *Pathogens* 2018; 7: pii: E63.

98. Liu J, Liang L, Huang S, et al. Aberrant differential expression of EZH2 and H3K27ME3 in extranodal NK/T-cell lymphoma, nasal type, is associated with disease progression and prognosis. *Hum Pathol* 2019; 83: 166–176.

99. Huang WT and Lin CW. EBV-encoded miR-BART20-5p and miR-BART8 inhibit the IFN-γ-STAT1 pathway associated with disease progression in nasal NK-cell lymphoma. *Am J Pathol* 2014; 184: 1185–1197.

100. Komabayashi Y, Kishibe K, Nagato T, et al. Circulating Epstein-Barr virus-encoded miRNAs as potential biomarkers for nasal natural killer/T-cell lymphoma. *Hematol Oncol* 2017; 35: 655–663.

101. Siu LL, Chan JK, Wong KP, et al. Aberrant promoter CPG methylation as a molecular marker for disease monitoring in natural killer cell lymphomas. *Br J Haematol* 2003; 122: 70–77.

102. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; 105: 10513–10518.

103. Rabinowits G, Gercel-Taylor C, Day JM, et al. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin Lung Cancer* 2009; 10: 42–46.

104. Madhavan D, Zucknick M, Wallwiener M, et al. Circulating mirnas as surrogate markers for circulating tumor cells and prognostic markers in metastatic breast cancer. *Clin Cancer Res* 2012; 18: 5972–5982.

105. Cheng H, Zhang L, Cogdell DE, et al. Circulating plasma miR-141 is a novel biomarker for metastatic colon cancer and predicts poor prognosis. *PLoS One* 2011; 6: e17745.

106. Jaccard A, Gachard N, Marin B, et al. Efficacy of l-asparaginase with methotrexate and dexamethasone (aspmetdex regimen) in patients with refractory or relapsing extranodal NK/T-cell lymphoma, a phase 2 study. *Blood* 2011; 117: 1834–1839.

107. Azad N, Zahnow CA, Rudin CM, et al. The future of epigenetic therapy in solid tumours—lessons from the past. *Nat Rev Clin Oncol* 2013; 10: 256–266.

108. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. *J Clin Oncol* 2002; 20: 2429–2440.

109. Kelly TK, De Carvalho DD and Jones PA. Epigenetic modifications as therapeutic targets. *Nat Biotechnol* 2010; 28: 1069–1078.