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

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***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

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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

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least as common and critical as genetic abnormalities in the pathogenesis of NKTCLs.

Epigenetics focusses on the heritable modifications in cellular chromatins 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 hypermethylations, nucleosome remodelling and regulation with noncoding RNAs (e.g. miRNA, lncRNA). Epigenetic abnormalities are known to play critical roles in carcinogenesis. Indeed, epigenetic aberrations are implicated in regulating a variety of different 'hallmarks' of cancer.[16] 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.[17] Promoter CpG hypermethylation transcriptionally silences genes through recruitment of histone-modifying enzymes such as histone deacetylases (HDACs), which in turn generate repressive chromatin states.[18] 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.[19,20] 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.[21] 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.[22] 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,[23] 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.[24] 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.[24] 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.[25] 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.[26] 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.[27]

Using locus-specific methods, previous reports showed epigenetic silencing of *DAPK1* and *PTPN6* (*SHP1*) in certain malignant NK-cell lines, findings that were confirmed in NKTCL patients in a subsequent genome-wide study.[28–30] *DAPK1*, a proapoptotic serine/threonine kinase, is a transcriptional target of p53.[31] 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,[32] was identified to be epigenetically silenced due to promoter hypermethylation.[30] 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.[33] 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*).[33] 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.[30] 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,[34] 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.[35–37] 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.[36,38] 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.[39] *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.[40] Frequent concomitant epigenetic silencing of *CADM1*, a stress-responsive tumour suppressor, and its interaction partner *DAL-1* was observed in NKTCLs.[41] Further analyses revealed that *CADM1* expression may be lost due to locus deletion in NKTCL patients. This interesting study showed a correlation between the methylation of the *CADM1* 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.[41] 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.[43] 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,[15,44–47] 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.[48] Of note, p53 activity can be regulated by EP300-mediated acetylation in response to DNA damage.[49] 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.[50] Apart from *KDM6A*, which was observed to be mutated in an EBV-negative NKTCL patient,[46] 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.[51,52] Another study showed that the histone lysine transferase KMT2D represses B-cell lymphoma development by sustaining a gene expression programme.[53] Of significance, FL/DLBCL-associated KMT2D loss-of-function mutations decreased global H3K4 methylation in germinal-centre (GC) B cells *in vivo*.[54] Consistent with the

Table 1. Pathologically and clinically significant cancer-associated genes epigenetically silenced in NKTCLs.

Aberrant gene	Functional evidence as a tumour suppressor in NKTCL	Relationship to NKTCL	Reference
ASNS	N.A.[a]	Predictive biomarker of asparaginase-based chemotherapy	Küçük[30]; Li[42]
BIM1	Reconstitution of its expression induced apoptosis in NK-cell lines.	Silenced pro-apoptotic gene Potential predictive biomarker of chemotherapy	Küçük[30]
CADM	N.A.	Candidate tumour suppressor	Fu[41]
DAL1	N.A.	Candidate tumour suppressor	
DAPK1	N.A.	Silenced pro-apoptotic gene	Röhrs[28]; Küçük[30]
DLC1	N.A.	Candidate tumour suppressor	Ying[23]
DLEC1	N.A.	Candidate tumour suppressor	Wang[26]
HACE1	Reconstitution of its expression led to G2/M cell cycle arrest and apoptosis in an NK-cell line.	Candidate tumour suppressor	Küçük[40]
PTPN6 (SHP1)	N.A.	Candidate tumour suppressor Inhibitor of NK-cell activation	Oka[29]; Küçük[30]
PTPRK	Its ectopic expression inhibited cell growth, and reduced invasion of NKTCL cells	Inhibitor of JAK-STAT3 pathway	Chen[39]
P73	N.A.	Candidate tumour suppressor	Siu[21]; Jost[22]
SOCS6	Reconstitution of its expression induced apoptosis, and decreased STAT3 phosphorylation in NK-cell lines.	Candidate tumour suppressor Inhibitor of JAK-STAT pathway	Küçük[30]
TET1	N.A.	Candidate tumour suppressor DNA CpG demethylase	Li[33]
TET2	N.A.	Candidate tumour suppressor DNA CpG demethylase	Küçük[30]

[a]Ectopic 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.

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

carcinoma cells.[33] 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.[30] It has long been known that global hypomethylation may lead to genomic instability and tumour formation *in vivo*.[56] For instance, DNA hypomethylation may contribute to deletions of genomic loci,[57] which is also frequently observed in NK-cell malignancies.[35] 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.[58] 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.[59] 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.[60] 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.[61] In this model, the non-canonical 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.[62,63] miRNAs are short (~22 nt) noncoding RNAs that inhibit the expression of target genes by inhibiting their translation or promoting transcript degradation.[64] They have been observed to be dysregulated in several cancer types, such as breast and prostate cancer.[65,66] 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).[67,68] Several independent studies revealed overexpression of mir-155 in NKTCLs.[69–71] miR-155 was shown to promote NK-cell effector functions such as IFNγ production *in vivo*.[72] 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.[73] These studies in B-cell lymphomas and NK cells, together with the observation that Eμ-miR155 transgenic mice develop high-grade B-cell lymphoma,[74] 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.[75] 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.[76] 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.[76] 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.[77]

Some studies have focused on the role of EBV-encoded miRNAs and NKTCL pathogenesis.[78,79] 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.[80] Of note, Ma and colleagues observed that an EBV-encoded

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	Jiang[44]; Choi[45]
ASXL3	✓ Polycomb group protein ✓ Mutated	Unknown	Jiang[44]
CREBBP	✓ Histone acetyl transferase ✓ Missense mutation	Unknown	Küçük[15]
EP300	✓ Histone acetyltransferase ✓ Frame-shift or missense mutations	Unknown	Küçük[15]
EZH2	✓ Histone methyltransferase ✓ Overexpressed	Promotes NK-cell growth independent of histone methyltransferase activity	Yan[60]
KDM6A (UTX)	✓ Histone demethylase specific for H3K27 ✓ Mutated	Unknown	Tsuyama[46]
KMT2D (MLL2)	✓ Lysine methyltransferase ✓ Missense mutations	Unknown	Jiang[44]; Küçük[15]; Choi[45]
TET1	✓ DNA CpG demethylase ✓ Promoter hypermethylation	Unknown	Li[33]
TET2	✓ DNA CpG demethylase ✓ Promoter hypermethylation	Unknown	Küçük[15]

NKTCL, natural killer/T-cell lymphoma.

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.[81] 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.[5]

Long-noncoding RNAs (lncRNAs) are functional transcripts longer than 200 nucleotides.[82] LncRNAs can modulate the expression of genes using mechanisms more diverse than those of miRNAs.[83] Like miRNAs, lncRNAs play pivotal roles in different hallmarks (resisting cell death, replicative immortality etc.) of cancer.[84] 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.[71] 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.[71] 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 tumourigenesis.[71] Another study reported overexpression of the lncRNA MALAT1 in NK- and T-cell lymphoma tumour samples and cell lines.[85] Of note, high expression levels of components of the polycomb repressive complex 1 (i.e. BMI1) and PRC2

(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.[86] In support of this possibility, MALAT1 expression was observed to be positively correlated with the expression of PRC1 and PRC2 genes in NKTCL patients.[85] 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.[85] Overexpressed MALAT1 interacts with the polycomb repressive complex, suggesting that MALAT1 may promote the generation of H3K27me3, thereby repressing certain target genes.[85] 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.[89] 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.[90–92] 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.[93] Another study showed that DNMT1 was upregulated by LMP2A *via* phosphorylation of STAT3, which led to promoter methylation-mediated PTEN silencing in gastric carcinoma.[94] Given that the EBV-encoded EBNA3 gene product is involved in polycomb-mediated repression of the pro-apoptotic *BIM* gene in B-cell lines,[95] 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.[96] 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.[97] 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.[5] 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.[80]

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.[39] Importantly, *PTPRK* promoter methylation was significantly correlated with advanced disease stage and the number of extranodal sites involved in NKTCL patients.[39] 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.[98] Overexpressed EZH2 was associated with advanced disease stage, poorer overall survival and a high proliferation index.[98] Gao and colleagues recently reported that NKTCL patients with mutations or loss-of-protein expression in *KMT2D* or *TET2* had significantly poorer overall survival,[47] which suggests that alterations in the epigenomic landscape may

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*	Ramakrishnan[80]
miR-155	Overexpressed miRNA	Promotes NK-cell survival Induces AKT and ERK pathways	Yamanaka[69]; Zhang[70]; Baytak[71]
miR-101, miR-26a, miR26b, miR-28-5, and miR-363	Underexpressed miRNA	Candidate tumour suppressor Reduces growth of a malignant NK-cell line	Ng[75]
miR-221	Overexpressed miRNA	Noninvasive diagnostic and prognostic biomarker	Guo[87]
miR-223	Overexpressed miRNA	Targets and downregulates PRDM1 in NKTCL cells	Liang[77]
MALAT1	Upregulated lncRNA	Oncogene Prognostic biomarker	Kim[85]
SNHG12	Overexpressed lncRNA	Oncogene Prognostic biomarker Predictive biomarker of chemoresistance	Zhu[88]
ZFAS1	Upregulated lncRNA	Candidate oncogene Regulator of apoptosis and cell cycle	Baytak[71]

miRNA, microRNA; lncRNA, long noncoding RNA; NK, natural killer; NKTCL, natural killer/T-cell lymphoma.

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.[76] They further showed that NKTCL patients with low miR146a expression have significantly poorer prognosis compared with those with high miR146a expression.[76] 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. EBV-encoded miRNAs may be clinically relevant for NKTCL patients. Huang and colleagues reported two EBV-encoded miRNAs, mir-BART20-5p and mir-BART8, were associated with disease progression in NKTCL.[99] miRNA expression profile analyses and qPCR showed elevated levels of expression of miR-BART2-5p, miR-BART7-3p, miR-BART13-3p and miR-BART1-5p in the sera of NKTCL patients, which may have diagnostic value.[100] 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.[100]

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.[88] *In vitro* manipulation of SNHG12 expression level in malignant NK-cell lines revealed that high SNHG12 expression conferred cisplatin resistance to NKTCL cells.[88] Of note, SNHG12 overexpression 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.[85] There was a trend for poorer survival in the high-MALAT1-expression group, but it was not statistically significant.[85] As the mentioned study involved a low number of NKTCL patient samples, future studies with larger cohorts are needed to address whether MALAT1

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 development[107]; 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

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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