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Review

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Genetics and genomics of extranodal natural killer/T cell lymphoma: from etiology to treatment

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Abstract

Extranodal natural killer/T cell lymphoma (NKTCL) is a heterogenous and unique epidemiological non-Hodgkin's lymphoma, which is strongly associated with Epstein-Barr virus (EBV) infection. Based on the development of various sequencing methods and molecular biology technologies, genome- and transcriptome-wide association studies of NKTCL have provided insight into the etiology and pathogenesis of NKTCL. Comparative genomic hybridization detected variations in tumor suppressor genes such as *PRDM1*, *RUNX3*, and *EZH2*. Whole-exome sequencing identified pathogenic variant such as *DDX3X*, and *TP53*. Signal pathways such as the Janus kinase/signal transduction and activator of transcription pathway and nuclear factor kappaB pathway are frequently abnormal in NKTCL. In addition, programmed death-1, programmed death ligand-1, and the human leukocyte antigen risk alleles are significantly associated with NKTCL pathogenesis. Meanwhile, epigenetics analysis has also exposited changes such as *PTPRK*, *HACE1*, microRNAs, and long non-coding RNAs, which play important role on the development and biology of NKTCL. EBV infection is tightly correlated with NKTCL. Viral genomic alterations and lytic genes of EBV are reported to have pathogenic effects on host cells that contribute to the etiology of NKTCL. We summarize the genomic and genetic alterations during the pathogenesis and development of NKTCL and exhibit the potential therapeutic targets that are worth exploring in future research and clinical trials.

Keywords: Extranodal natural killer/T cell lymphoma, Epstein-Barr virus, genomics, genetics, therapeutic targets

INTRODUCTION

Extranodal natural killer/T cell lymphoma (NKTCL) commonly involves the upper aerodigestive tract such as the nasal cavity and is prevalent in Asia, Mexico, and Latin America[1,2] while rare in the United States and Europe[2,3]. NKTCL was previously called "lethal midline granuloma", of which the pathological features are pleomorphic and atypical cells ranging from medium to large, obvious necrosis, irregular nucleus and cytoplasmic changes, and showing an angiocentric/angiodestructive growth pattern with infiltration of immune inflammatory cells[1,4-7]. Neoplasm cells are positive for CD56, CD2, and CD3ε with the expression of cytotoxic molecules, such as TIA1 and Granzyme B. Epstein-Barr virus (EBV) DNA and non-coding EBV-encoded RNAs could be detected in tumor tissues[8-10], which leads to the correlation of EBV infection and the pathogenesis of NKTCL. In addition, NKTCL tumor cells express P-glycoprotein, which is related to multidrug resistance, resulting in poor response to anthracyclines[11]. Concurrent L-asparaginase-containing regimens with or without radiotherapy are the current standard treatment. The International T-cell Project's report showed great improvement in the survival of NKTCL patients over the past decade[12]. The five-year overall survival rates for stage I, stage II, and stage III-IV are 55%, 42%, and 24%, respectively[12]. However, the treatment for advanced-stage, relapsed, or refractory patients is still challenging[12]. Therefore, there is an urgent need for more effective treatments to improve the survival of NKTCL patients.

Identifying the unique gene expression profile, dysregulated molecules, and signaling pathways of NKTCL provided a new perception for understanding pathogenic mechanisms and potential therapeutic targets[13,14]. Genome-wide association study revealed susceptible genes significantly associated with the higher risk of NKTCL[15,16]. EBV genome and transcriptomics analysis showed that EBV infection might contribute to the pathogenesis of NKTCL by affecting the host cell genome[17]. Exploring the genomics and genetic variations in the tumorigenesis and development of NKTCL from the molecular aspects has vital biological significance for the future clinical diagnosis, treatment, and prognosis of NKTCL. Xiong *et al.*[18] integrated the cell origin, EBV expression pattern, clinical significance and characteristics of alterations in the human genome of NKTCL samples, and divided NKTCL into three molecular subtypes: TSIM subtype [based on variants in the Janus kinase/signal transduction and activator of transcription (JAK/STAT) pathway and *TP53*], MB subtype (based on *MGA* mutation and 1p22.1/*BRDT* loss of heterozygosity), and HEA subtype (based on *HDAC9*, *EP300*, and *ARID1A* mutation)[18]. Disease stratification bridges the pathogenesis of NKTCL with clinical intervention from pathogenesis theory into clinical practice.

THE ALTERATION OF HUMAN GENETICS AND POTENTIAL TREATMENTS

The network of NKTCL pathogenesis has not been totally revealed. However, changes in a variety of genes and signaling pathways have been reported. We try to excavate underlying signatures from reported genetic variations and search the genetic hallmarks increasing the risk of NKTCL, which furnish guidance for mechanism-based anti-tumor therapy.

Tumor suppressor genes and Somatic mutations

Dysfunction of tumor suppressor genes occupies a significant position in the development of hematological malignancies, which has been discussed in leukemia and myeloma[19,20]. Many major candidate genes, such as *PRDM1*, *TP53*, *HACE1*, *FOXO3*, and *ATG5*, are located in the deletion region of chromosome 6q21, which is the most frequently discussed in NKTCL[13,14,21]. There are recurrent somatic mutations in NKTCL as well.

PRDM1

PRDM1 encodes Blimp, a transcription inhibitor of NK cell and T cell differentiation, and regulates the proliferation and maturation of NK cells[22]. As a tumor suppressor gene (TSG) located in the 6q21 region, *PRDM1* is inactivated due to mutation, promoter methylation, or deletion, with a low expression level in NKTCL[23,24]. Inhibition or inactivation of *PRDM1* is supposed to upregulate genes or pathways related to proliferation and cell cycle regulation such as MYC and to downregulate the pro-apoptotic factor BIM[21]. The above indicates that *PRDM1* may mediate malignant transformation of cells and be a potential genetic target. A recent study found that pan-acetyltransferase inhibitor vorinostat might restore *PRDM1* response to IL21 through decreasing BCL6 bound to *PRDM1* in follicular lymphoma (FL) cells[25]. According to the results from vorinostat on nonfunctional CREBBP, FL cells showed a significant increase in *PRDM1* expression after IL21 exposure. The expression of *PRDM1* might be an important response predictor for pan-HDAC inhibitors on FL cells. However, whether agents that restore *PRDM1* exert anti-tumor activity in NKTCL is still to be explored.

RUNX3 and MYC

Runt-related transcription factor 3 (RUNX3) presents a bilateral significance in different tumor backgrounds. It is overexpressed in colorectal cancer and could promote TRAIL-induced apoptosis exerting anti-tumor effect[26]. In cutaneous T-cell lymphoma, the re-expression of *RUNX3* decreases tumor cell survival and induces apoptosis, indicating that *RUNX3* acts as tumor suppressor gene[27].

There are different degrees of expression of *MYC* in different types of lymphoma[28]. Compared with NK cells, *MYC* is highly expressed and activated in NKTCL. The inhibition of its target genes may be a possible mechanism for it playing an important role in the development of NKTCL[29].

Selvarajan *et al.*[30] demonstrated that *RUNX3* is overexpressed and oncogenic in NKTCL. *MYC* is involved in the positive transcriptional regulation of *RUNX3*[30]. JQ1, a small molecule inhibitor, may induce apoptosis of NKTCL cell lines by inhibiting transcription of *MYC* and downregulating *RUNX3*, which indicates that *RUNX3* and *MYC* may be potential therapeutic targets in NKTCL[30]. In addition, homoharringtonine is considered to downregulate the expression of *MYC* via directly binding to NKRF, the inhibitor of nuclear factor kappaB (NF-κB)[31]. Relevant clinical trials have shown that homoharringtonine-based induction regimens can significantly improve the complete remission (CR) rate and progression-free survival of acute myeloid leukemia patients[32]. The potential therapeutic implication of this reagent deserves to be further explored in NKTCL.

EZH2

Enhancer of zeste homolog 2 (*EZH2*) is overexpressed in NKTCL and acts as oncogene in tumor progression[33,34]. Previous studies have shown that *EZH2* undergoes frequently somatic mutations in FL and diffuse large B-cell lymphoma (DLBCL), resulting in dysregulation of epigenetic silence function of methyltransferase[35]. Interestingly, Yan *et al.*[36] considered that *EZH2*, as a transcriptional activator, promoted proliferation through regulation of cyclin D1 expression by a non-canonical pathway. JAK3 kinase inhibitor PF956980 can induce the arrest of *EZH2* phosphorylation and block its non-canonical pathway via limiting the growth advantage of NKTCL cells[36]. A recent study found that MELK (maternal embryonic leucine zipper kinase), which regulates the ubiquitination and turnover of *EZH2*, increased EZH2 S220 phosphorylation and promoted stabilization of EZH2 protein in NKTCL[37]. These studies demonstrate a potential target role of *EZH2*, and a JAK3 inhibitor may be a prospective treatment option. Additionally, a phase II clinical trial (*Clinical Trial* No. NCT01897571) about the EZH2 inhibitor tazemetostat treating refractory/relapsed B-cell non-Hodgkin's lymphoma patients is ongoing. Of note,

results from a phase I clinical trial demonstrated that tazemetostat had a favorable safety profile and anti-tumor activity in refractory NHL and advanced solid tumors, including epithelioid sarcoma[38]. Analogously, EZH2 inhibitors might be worth exploring in NKTCL treatment.

DDX3X

DDX3X belongs to the RNA helicase family, and its gene mutation is involved in the formation of a variety of human tumors[39-41]. According to Jiang *et al.*[42], *DDX3X* is the most common somatic mutation gene (20%, 21/105) in NKTCL. Notably, tumors with *DDX3X* mutation show activation and upregulation of NF-κB and mitogen-activated protein kinase (MAPK) pathways, which also reflects that *DDX3X* mutation has biological significance in NKTCL pathogenic process. However, the actual significance in clinical treatment of NKTCL still needs more studies and trials to confirm.

Other TSGs and Somatic mutations

The tumor suppressor gene *TP53* is considered to be dysfunctional due to somatic mutations[41,43]. Genes normally suppressed by TP53 are upregulated in tumor tissues, which may lead to the progression of NKTCL[29]. FOXO3 is lowly expressed in NKTCL and induces apoptosis and cell cycle arrest of NK cell lines, which is recognized to be of great significance in the pathogenesis of NKTCL[23]. Hexokinase domain component 1 has been proven to be transcriptionally upregulated in the NKTCL cell line. It not only promotes the proliferation of tumor cells but also inhibits EBV replication and P-glycoprotein expression through promoting the overproduction of ROS and DNA damage[44]. Besides, survivin is overexpressed in NKTCL. *In vitro*, terameprocol, a survivin inhibitor, can significantly inhibit the growth of NKTCL cell lines[29].

The alterations of these genes further enrich the molecular network of NKTCL pathogenesis and provide potent targets for anti-tumor therapy. The relevant content is summarized in Table 1. Whether they could be used as valuable clinical factors for diagnosis and treatment needs to be verified in larger-scale experiments.

Oncogenic signaling pathways

JAK/STAT signaling pathway

Targeted capture sequencing observed somatic alteration of the JAK/STAT pathway in 78% (85/109) of NKTCL samples. *STAT3* and *TP53* genes (21%, 23/109) were mutated most frequently, followed by *JAK3*, *JAK1*, and suppressor of cytokine signaling 1 mutations. Furthermore, *STAT3* activation resulting from mutations or abnormal phosphorylation may drive the high expression of programmed death ligand-1 (PD-L1), which may have an influence on immune escape of NKTCL[45]. In another study, *STAT3* is the most common mutant gene of NKTCL (9/34, 26.5%), and all mutations are located in the SRC homology 2 domain that seems to be an underlying effective target region[46]. Apart from mutations, abnormal expression of phosphorylated STAT3 (pSTAT3) at Tyr705 is also recognized as aberrant activation signature[13]. Dysregulated JAK2 mediates the constitutive phosphorylation of STAT3 (Tyr705) facilitating the growth of NKTCL MEC04 cells and Ser727 phosphorylation of activated MAP-Kinase/Erk pathway, both of which have similar oncogenic significance[47]. In addition, receptor-type tyrosine-protein phosphatase κ (PTPRK) binding to pSTAT3 led to the dephosphorylation of pSTAT3, and some NKTCL patients suffer from function deficiency of PTPRK according to the previous formulation[48]. The above suggests that STAT3 is activated through different mechanisms in NKTCL.

STAT3 obtained phosphorylated activation following JAK3 constitutive phosphorylation on tyrosine Tyr980[49]. Whole-exome sequencing detected *JAK3* mutations (A572V and A573V) that exceptionally

Table 1. Key targets and their potential pathogenic mechanisms and therapeutic significance from the alterations TSGs and somatic mutations in NKTCL

The alterations of human genetics	Potential hallmark/signaling pathways	Role in pathogenic mechanism of lymphoma	Ref.	Potential treatment significance	Ref.
TSGs and somatic mutations	PRDM1	Downregulated and promotes cell proliferation and reduces apoptosis	Karube *et al.*[23] Küçük *et al.*[24]	IL21 plus vorinostat upregulates PRDM1 expression in FL and may be explored in NKTCL treatment	Desmots *et al.*[25]
	RUNX3	Overexpressed and oncogenic in NKTCL	Selvarajan *et al.*[30]	JQ1, a molecule inhibitor, may induce apoptosis *in vitro*	Selvarajan *et al.*[30]
	EZH2	Overexpressed and promotes cell proliferation as a transcriptional activator by a non-canonical pathway	Yan *et al.*[36]	JAK3 inhibitor PF956980 blocks the non-canonical pathway of EZH2 and limit cell growth *in vitro* A phase II clinical trial about EZH2 inhibitor tazemetostat treating NHL is ongoing, and it may try to be included in NKTCL therapy	Yan *et al.*[36] Italiano *et al.*[38]
	MYC	Highly expressed and inhibits its target genes	Ng *et al.*[29]	HHT improved the CR and PFS of AML cases in a clinical trial and deserves to be further explored in NKTCL	Jin *et al.*[32]
	DDX3X	*DDX3X* mutation has vital significance in lymphoma pathogenesis	Jiang *et al.*[42]	N/A	N/A
	P53	Dysfunctional and may lead to the progression of NKTCL	Ng et al.[29] Choi *et al.*[43]	N/A	N/A
	FOXO3	Downregulated and induces apoptosis and cell cycle arrest in vitro.	Karube *et al.*[23]	N/A	N/A
	HKDC1	Upregulated, promotes the proliferation of tumor cells, and inhibits EBV replication and P-gp expression.	Chen *et al.*[44]	N/A	N/A
	Survivin	Upregulated and inhibits apoptosis in NKTCL.	Ng *et al.*[29]	Terameprool, as a survivin inhibitor, inhibits the survival of NKTCL cell lines	Ng *et al.*[29]

NKTCL: Extranodal natural killer/T cell lymphoma; TSGs: tumor suppressor genes; PRDM1: PR/SET domain 1; IL21: interleukin 21; RUNX3: runt-related transcription factor 3; FL: follicular lymphoma; EZH2: enhancer of zeste homolog 2; JAK3: Janus kinase 3; MYC: bHLH transcription factor; HHT: homoharringtonine; CR: complete remission; PFS: progression-free survival; AML: acute myeloid leukemia; DDX3X: DEAD-box helicase 3 X-linked; FOXO3: forkhead box O3; HKDC1: hexokinase domain component1; EBV: Epstein-Barr virus; P-gp: P-glycoprotein.

activated the JAK/STAT signaling pathway and had a tumorigenic effect on NKTCL[50]. Moreover, Sim *et al.*[51] identified two novel *JAK3* mutations (H583Y and G589D) on exon 13 with carcinogenic properties. Tofacitinib, a JAK3 inhibitor, could inhibit the growth of mutant NKTCL cell lines. The study also discovered that the malignant growth advantage of STAT3 Y640F and STAT3 D661Y mutant were inhibited by the STAT3 inhibitor Stattic but not affected by Tofacitinib[51]. Besides, the JAK3 inhibitor CP-690550 restrained the growth and invasion of tumor cells *in vivo* and *in vitro*[49,50]. A recent study also uncovered that PRN371, a highly selective inhibitor of JAK3, apparently suppressed proliferation of NKTCL cells with the overexpression of phosphorylated JAK3 and phosphorylated STAT3/5, which showed a more durable inhibitory effect compared to tofacitinib[52].

According to the above studies, the dysregulation of JAK/STAT pathway is highly prevalent in NKTCL and may play an important role in the pathogenesis of the disease through diverse mechanisms. JAK3 and STAT3 are two latent therapeutic targets, and their inhibitors might have promising results in the treatment of NKTCL patients. In the future, the feasibility and safety of the treatment strategy targeting the deregulated JAK/STAT pathway should be further studied.

NF-κB signaling pathway

NF-κB has critical biological implications in the growth, proliferation, differentiation, and regulation of lymphocytes that are regarded as vital pathogenetic factors in lymphomas[53]. Expression of RelA and cRel, two canonical molecules of the NF-κB pathway in NKTCL, suggested abnormal activation of canonical NF-κB pathway[13]. In another study, RelB, a molecule of the alternative NF-κB pathway, was positive in NKTCL tumor tissue. The differential expression of molecules might imply constitutive activation of NF-κB pathway in NKTCL and that it might be involved in the development of the disease through various mechanisms[29]. Interestingly, EBV-encoded latent membrane protein 1 (LMP1) induced aberrant expression of eukaryotic translation initiation factor 4E[54], survivin[55], and PD-L1[56] to participate in NKTCL progression via NK-κB pathway, which further implied that targeting this carcinogenic pathway might have potent clinical value for NKTCL patients.

PDGF signaling pathway

Platelet-derived growth factor alpha (*PDGFRα*) is overexpressed in NKTCL, and imatinib mesylate, a PDGFR inhibitor, has a limited effect on the growth of the PDGFRα + NKTCL cell line, which indicates that the PDGF pathway was involved in the pathogenic process of NKTCL. However, there was still no evidence explaining the dysregulation of PDGFRα completely[13]. Piccaluga *et al.*[57] revealed that activated PDGFRα fostered the proliferation of peripheral T-cell lymphomas, not otherwise specified (PTCL or NOS), cells through autocrine loop.

Other oncogenic signaling pathways

Gene sets analysis also discerned other biological pathways in NKTCL including the MAPK, WNT, AKT, and vascular endothelial growth factor (VEGF) signaling pathways[13]. The NOTCH and aurora kinase A (AURKA) pathways were upregulated in NKTCL. Notch inhibitors, which potently inhibited γ-secretase and Notch processing, and an AURKA inhibitor (MK-8745) both may inhibit the proliferation and cell cycle regulation of NK-lymphoma cell lines[58]. The Akt/mammalian/mechanistic target of rapamycin (mTOR) pathway is abnormally activated in EBV-associated T- and NK-cell lymphoma, and their inhibitors restricted effectually proliferation of cell lines[59]. The excessive expression of phosphatidylinositol 3-kinase PIK3 isoforms, containing PIK3α, PIK3β, PIK3γ, and PIK3δ, indicated the abnormally dysregulated activation of PIK3 pathway in NKTCL[60]. VEGF was overexpressed in cutaneous NKTCL and related to poor prognosis. It also provided a potential basis for disorder of the VEGF pathway in NKTCL[61]. The MYC/MAP3K6 pathway is considered to be a characteristic manifestation of MB subtype NKTCL[18].

Integrating the alterations of human genetics in NKTCL, we simply depict an oncogenic molecular network linking dysregulated genes and signaling pathways in Figure 1. We also summarize the relevant content about oncogenic signaling pathways in Table 2.

Epigenetic variations

PTPRK

PTPRK directly and selectively dephosphorylates the substrate, and loss of phosphatase activity will cause the disruption of cell junctions and enhance invasive characteristics[62]. Because of promoter

Table 2. Vital signaling pathways and their potential oncogenic mechanisms and target significance in NKTCL

The alterations of human genetics	Potential hallmark/signaling pathways	Role in pathogenic mechanism of lymphoma	Ref.	Potential treatment significance	Ref.
Signaling Pathways	JAK/STAT	Aberrant activity via mutations or abnormal phosphorylation	Huang *et al.*[13] Song *et al.*[45] Bouchekioua *et al.*[49]	JAK3 inhibitor: tofacitinib inhibited the growth of cell lines CP-690550 restrained growth and invasion of tumor cells PRN371 apparently inhibited proliferation of NKTCL cells STAT3 inhibitor: stattic inhibited malignant growth advantage *in vitro*	Bouchekioua *et al.*[49] Koo *et al.*[50] Sim *et al.*[51] Nairismägi *et al.*[52]
	NK-κB	Dysregulated and LMP1 induces eIF4E, survivin, and PD-L1 via NK-κB pathway, which may contribute to NKTCL progression	Ng *et al.*[29] Huang *et al.*[13] Sun *et al.*[54] Sun *et al.*[55] Bi *et al.*[56]	NF-κB has critical biological implications in NKTCL and targeting the pathway might bring potent clinical value	N/A
	PGDF	PDGFRα is overexpressed in NKTCL, and the actual oncogenic sense needs to disclose	Huang *et al.*[13]	Imatinib mesylate, a PDGFR inhibitor, has a limit effect on cell growth *in vitro*	Huang *et al.*[13]
	AKT/mTOR	Abnormally activated and promotes cell growth	Kawada *et al.*[59]	mTOR inhibitors: Rapamycin suppressed mTOR activity and limited cell proliferation *in vitro* CCI-779 inhibited tumor growth *in vivo* and *in vitro*	Kawada *et al.*[59]
	MAPK	MYC/MAP3K6 pathway is a characteristic manifestation of MB subtype NKTCL	Xiong *et al.*[18]	N/A	N/A
	AURKA	Upregulated and may accelerate neoplasm cell proliferation	Iqbal *et al.*[58]	AURKA inhibitor MK-8745 inhibits proliferation and cell cycle regulation	Iqbal *et al.*[58]
	NOTCH	Upregulated and plays a role in development of neoplasm	Iqbal *et al.*[58]	NOTCH inhibitors potently inhibit NK-lymphoma cell lines	Iqbal *et al.*[58]

NKTCL: Extranodal natural killer/T cell lymphoma; JAK/STAT: Janus kinase/signal transduction and activator of transcription; JAK3: Janus kinase 3; STAT3: signal transduction and activator of transcription 3; NK-κB: nuclear factor kappaB; LMP1: latent membrane protein 1; eIF4E: eukaryotic translation initiation factor 4E; PDL1: programmed death ligand-1; PGDF: platelet-derived growth factor; PDGFRα: platelet-derived growth factor receptor alpha; PDGFR: platelet-derived growth factor receptor; AKT/mTOR: protein kinase B /mechanistic target of rapamycin; MAPK: mitogen-activated protein kinase; MYC/MAP3K6: bHLH transcription factor /mitogen-activated protein kinase kinase kinase 6; MB: one molecular subtype of extranodal natural killer/T cell lymphoma that was divided based on *MGA* mutation and 1p22.1/*BRDT* loss of heterozygosity; AURKA: aurora kinase A.

hypermethylation (16/27, 59%) or monoallelic gene deletion (8/27, 30%), PTPRK expression is frequently downregulated in NKTCL, which is thought to promote constitutive activation of STAT3 and mediate the inhibition of cells proliferation, invasion, and migration. Similarly, demethylation reagent 5-aza-2'-deoxycytidine-induced PTPRK re-expression confirms the aberrant epigenetic changes of PTPRK in NKTCL[48]. PTPRK may be a potential target of NKTCL epigenetic therapy. Whether its promoter methylation and pSTAT3 level can be used as biomarkers for diagnosis or prognosis of NKTCL needs further exploration.

HACE1

HACE1, the novel E3 ubiquitin ligase mapping to the deletion region of 6q21 chromosome, inhibits cell cycle progression via regulating the degradation of cyclin D1. As a tumor suppressor gene, it has been found

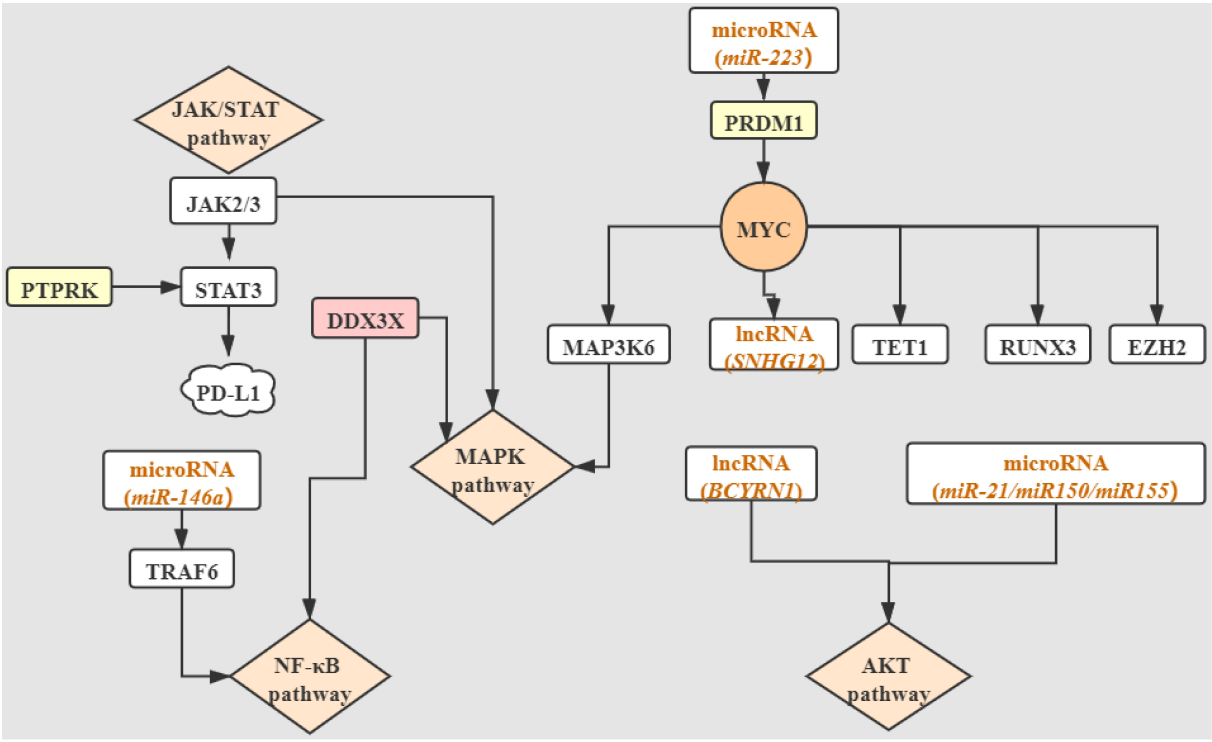

Figure 1. The interrelationships among JAK/STAT, NF-κB, MAPK, and AKT carcinogenic pathways and dysregulated tumor suppressor genes, somatic mutations, and epigenetic mutations. Molecules located at junctions of the non-arrow ends have effects on the part pointed by the arrows. Specific explanations are disclosed in the corresponding sections. JAK/STAT: Janus kinase/signal transduction and activator of transcription; JAK2/3: Janus kinase2/3; STAT3: signal transduction and activator of transcription 3; PTPRK: receptor-type tyrosine-protein phosphatase κ; PDL1: programmed death ligand-1; NK-κB: nuclear factor kappaB; TRAF6: tumor necrosis factor receptor associated factor 6; DDX3X: DEAD-box helicase 3 X-linked; MAPK: mitogen-activated protein kinase; MAP3K6: mitogen-activated protein kinase kinase kinase 6; PRDM1: PR/SET domain 1; MYC: bHLH transcription factor; SNHG12: small nucleolar RNA host gene 12; TET1: methylcytosine dioxygenase ten-eleven translocation 1; RUNX3: runt-related transcription factor 3; EZH2: enhancer of zeste homolog 2; BCYRN1: brain cytoplasmic RNA 1; AKT: protein kinase B.

to be silenced by its CpG methylation in a variety of tumors[63]. Interestingly, another study showed a normal HACE1 protein expression level in the NKTCL cell line but did not observe a similar cell cycle arrest[64]. These inconsistent results indicate that the specific function of HACE1 in NKTCL is not clear, and more studies are needed to discover the real function of HACE1 in NKTCL.

The ten-eleven translocation 1

The expression of the methylcytosine dioxygenase ten-eleven translocation 1 (TET1) maintains DNA hydroxymethylation and prevents the DNA hypermethylation of cells, whose deficiency leads to B-cell lymphoma. Promoter CpG methylation is the main transcriptional silencing mechanism of TET1[65]. However, Poole *et al.*[66] showed that inactivation of MYC relieved the regulation of TET1 resulting from the overexpression of TET1 in T-cell acute lymphatic leukemia acting as a tumor promoting factor. There was continual methylation of TET1 occurring in 83% (10/12) of NKTCL[67]. The functional mechanism of TET1 in the tumorigenesis of NKTCL has not been completely revealed, but it is indeed a potentially meaningful epigenetic marker.

Other epigenetic variations

The lysine [K]-specific methyltransferase 2D (*KMT2D*), also known as *MLL2*, encodes a histone methyltransferase. Mutations of *KMT2D* may cause dysregulation of gene transcription regulation, which

may contribute to the development of NKTCL[43]. Loss of function mutations of *BCOR* are considered highly specific in NKTCL[41]. Another study demonstrated that *BCOR* and *MLL2* were mutated genes followed only by *STAT3*[46]. The two aberrant mutated chromatin-modifying genes may also be significant targets of NKTCL epigenetic therapy. All the content regarding epigenetic variations is summarized in Table 3.

microRNAs and lncRNAs

microRNAs

microRNAs are small non-coding RNAs with a length of 19-24 bp nucleotides that negatively regulate target genes and are associated with the regulation of crucial biological processes such as cell growth, differentiation, and apoptosis[68]. Many studies have revealed the functional significance of microRNAs dysregulation in NKTCL.

Ng *et al.*[69] discovered that miR-342-5p, miR-26b, miR-363, miR-150, miR28-5p, miR-26a, and miR-101 were downregulated in NKTCL tumor tissues and *in vitro*, as compared with normal NK cells. These microRNAs inhibited the growth of NKTCL cell lines *in vitro*. Nevertheless, the expression of miR-155 and miR-378 were upregulated[69]. miR-150 may impact the sensitivity of NKTCL cells to radiotherapy by inhibiting the PI3K/AKT/mTOR pathway and participate in the development of lymphoma[70].

miR-15a is downregulated in NKTCL cell lines and tumor tissues, which may predict poor prognosis and response to treatment[71]. Paik *et al.*[72] proved that the overexpression of miR-146a in NKTCL cells may inhibit NF-κB pathway and thus serve as a feasible tumor suppressor. In addition, Liang *et al.*[73] found that miR-223 targeted the 3'-untranslated region (UTR) of *PRDM1* gene to downregulate its expression at the post-transcriptional level. miR-21 is overexpressed in NKTCL, acting as an oncogenic RNA which mediates apoptotic activity reduction through the abnormally dysregulated PTEN/AKT signaling pathway. Aberrant overexpression of miR-155 may induce activation of SHIP1/AKT pathway and affect the occurrence of NKTCL[74].

lncRNAs

In addition to microRNAs, some long non-coding RNAs (lncRNAs) have also been thought to be involved in the oncogenesis of NKTCL. ZFAS1 suggested genes participate in critical pathways associated with cell growth and tumor transformation, such as NF-κB and WNT signaling pathways[75]. Recently, Wang *et al.*[76] discovered that brain cytoplasmic RNA 1 in NKTCL tissue was significantly higher in contrast to normal NK cells, which may induce autophagy via inhibiting PI3K/AKT/mTOR and p53/mTOR signaling pathways and enhance the resistance to L-asparaginase. lncRNA X-inactive-specific transcript (XIST) is overexpressed in NKTCL, and its downstream target miR-497 could decrease the synthesis of the anti-apoptotic molecule Bcl-w to further regulate XIST-mediated proliferation and migration of NKTCL cells[77]. In addition to these, small nucleolar RNA host gene 12 is upregulated in NKTCL, as a direct transcription target of c-myc, which accelerates the proliferation of tumor cells and may suppress the response to cisplatin[78].

In summary, studies on the connection between non-coding RNAs and NKTCL not only present the possible therapeutic value of non-coding RNA but also provide enlightenment for overcoming the resistance of NKTCL patients. These potential targets and their significance are summarized in Table 4.

Immune-related changes

PD-1 and PD-L1

Programmed death-1 (PD-1) is an immune checkpoint receptor that binds to PD-L1 expressed by neoplasm

Table 3. Summary of key targets, potential pathogenesis mechanisms, and treatment significance in NKTCL epigenetic variations

The alterations of human genetics	Potential hallmark/signaling pathways	Role in pathogenic mechanism of lymphoma	Ref.	Potential treatment significance	Ref.
Epigenetic variations	PTPRK	Downregulated for methylation or deletion and mediates inhibition of cells proliferation, invasion, and migration	Chen *et al.*[48]	PTPRK may be a potential target of NKTCL epigenetic therapy	N/A
	HACE1	Encodes E3 ubiquitin ligase and inhibits cell cycle progression, but silenced due to CpG methylation The specific function of HACE1 is not clear	Zhang *et al.*[63]	N/A	N/A
	TET1	Frequently methylated, and the oncogenic mechanism has not been revealed	Li *et al.*[67]	N/A	N/A
	KMT2D	Mutations may cause dysregulation of gene transcription regulation	Choi *et al.*[43]	N/A	N/A
	BCOR	Loss of function mutations of BCOR are considered highly specific in NKTCL	Dobashi *et al.*[41]	N/A	N/A

NKTCL: Extranodal natural killer/T cell lymphoma; PTPRK: receptor-type tyrosine-protein phosphatase κ; HACE1: HECT domain and ankyrin repeat containing E3 ubiquitin-protein ligase 1; CpG: cytosine-phosphate-guanine; TET1: methylcytosine dioxygenase ten-eleven translocation 1; KMT2D: lysine [K]-specific methyltransferase 2D; BCOR: bcl6 corepressor.

Table 4. Important non-coding RNAs and their potential roles and therapeutic significance associated with NKTCL

The alterations of human genetics	Potential hallmark/signaling pathways	Role in pathogenic mechanism of lymphoma	Ref.	Potential treatment significance	Ref.
microRNAs	miR-15a	Downregulated in NKTCL cell lines	Komabayashi *et al.*[71]	May predict poor response to treatment and survival of NKTCL patients	Komabayashi *et al.*[71]
	miR-21	Upregulated and mediates apoptotic activity reduction	Yamanaka *et al.*[74]	N/A	N/A
	miR-146a	Overexpressed and may limit Bcl-2 expression by inhibiting NF-κB pathway	Paik *et al.*[72]	N/A	N/A
	miR-150	Downregulated in NKTCL tumor tissues and *in vitro*	Ng *et al.*[69]	Overexpression of miR-150 impacts the sensitivity to radiotherapy	Wu *et al.*[70]
	miR-155	Overexpressed and induce aberrant activation of SHIP1/AKT pathway	Yamanaka *et al.*[74]	N/A	N/A
	miR-223	Upregulated and targets and downregulates PRDM1	Liang *et al.*[73]	N/A	N/A
lncRNAs	BCYRN1	Upregulated and may induce autophagy	Wang *et al.*[76]	Aberrant expression of BCYRN1 enhances resistance to L-asparaginase.	Wang *et al.*[76]
	XIST	Overexpressed and mediates proliferation and migration *in vitro*	Liu *et al.*[77]	N/A	N/A
	SNHG12	Upregulated and advances cell proliferation as a direct transcription target of c-myc	Zhu *et al.*[78]	C-myc may suppress the response to cisplatin by promoting SNHG12 expression in NKTCL	Zhu *et al.*[78]

NKTCL: Extranodal natural killer/T cell lymphoma; Bcl-2: B cell leukemia/lymphoma 2; NK-κB: nuclear factor kappaB; SHIP1/AKT: inositol polyphosphate-5-phosphatase D/ protein kinase B; PRDM1: PR/SET domain 1; BCYRN1: brain cytoplasmic RNA 1; XIST: X-inactive-specific transcript; SNHG12: small nucleolar RNA host gene 12.

cells and inhibits the activation, proliferation, and cytokine expression of T cells[79]. The combination of PD-

1 and PD-L1 is one of the most important pathways for tumors to escape immune surveillance[80]. PD-1/PD-L1 blockade in various tumors, such as relapsed/refractory Hodgkin's lymphoma[81], non-small-cell lung cancer[82], melanoma[83], and colorectal cancer[84], has been verified to be a newly favorable treatment option distinctive from traditional chemotherapeutics.

There are also studies on PD-1/PD-L1 blockade therapy applied to NKTCL patients. In the trial of Kwong *et al.*[85], all patients responded for a long time, including two patients with CR, three patients achieving clinical and radiological CR, and the rest with partial remission. Relapse/refractory NKTCL patients who failed previous chemotherapies were treated with pembrolizumab, and the response was favorable. Four out of seven patients responded to the treatment and the adverse effects were tolerable[86]. Whole-genome sequencing on 19 refractory/relapsed NKTCL patients receiving pembrolizumab showed that the structural rearrangement of the *PD-L1* gene (*PD-L1*^{MUT}) disrupting the 3'-UTR was the only gene variation in tumor samples of patient who had response to pembrolizumab in contrast with non-responders. The somatic mutation was detected in 4/7 patients who completely responded to pembrolizumab, but it was not seen in all non-responders. Besides, researchers revealed this mutation was associated with better survival (P = 0.0279)[87]. This may be a predictive response marker of PD-1/PD-L1 blockade therapy for NKTCL. As treatment for PD-L1 blocking therapy, a phase II clinical trial of avelumab was reported that 21 NKTCL patients showed a CR rate of 24% (5/21) and an overall response rate of 38% (8/21). The study also found patients with high PD-L1 expression levels had better treatment response[88].

A meta-analysis including 4174 cases of five types of advanced or metastatic tumors displayed that PD-1/PD-L1 inhibitors are more effective than conventional chemotherapy, and the overall survival (OS) of patients is significantly prolonged, whether PD-L1 positive or negative[89]. Based on the above research reports, PD-1/PD-L1 blockade is a promising molecular-targeted approach. It is necessary to further explore how to improve the therapeutic effect and safety of PD-1/PD-L1 blockade therapy in NKTCL.

HLA risk alleles

A Japanese study containing 25 NKTCL cases and 303 control individuals reported that the frequency of human leukocyte antigen (HLA)-A*0201 in NKTCL patients was significantly lower than the baseline control population[90]. However, genome-wide association study did not observe similar results, probably due to the small sample size. Notably, Li *et al.*[15] identified 51 single-nucleotide polymorphisms (SNPs) associated with NKTCL that are mapped to the MHC region of chromosome 6. rs9277378 (located in HLA-DPB1) exhibits the strongest association with susceptibility of NKTCL [$P = 4.21 \times 10^{-19}$, odds ratio (OR) = 1.84]. Afterwards, Lin *et al.*[16] reported two novel NKTCL risk loci, the IL18RAP region on 2q12.1 (rs13015714; $P = 2.83 \times 10^{-16}$, OR = 1.39) and the HLA-DRB1 region on 6p21.3 (rs9271588; $P = 9.35 \times 10^{-26}$, OR = 1.53). The rs1420106-A variant that is highly correlated with rs13015714 can upregulate the expression of IL18RAP, which may be conducive to the proliferation of tumor cells. In addition, a haplotype association analysis showed that 47F-67I, a component of the antigen binding pocket of HLA-DRB1, was associated with a reduced risk of NKTCL, and 47Y-67L was the opposite to 47F-67I for the genetic risk for NKTCL[16].

Furthermore, a seven-SNP-based classifier was designed based on the seven SNPs that correlated to *WDR27*, *UMAD1*, *TENM2*, *LINC02463*, *KDM4C*, *FGD4*, and *FAM71A*. It had better predictive accuracy than clinicopathological risk variables on the survival of NKTCL patients. The combined application of the seven-SNP-based classifier and clinicopathological risk factor should be more accurate for predicting the prognosis of NKTCL patients[91].

All the above research provides new insights for the tumorigenesis and development of NKTCL and reveals the significance of inflammation and immune regulation through the IL18/IL-18RAP axis and antigen presentation involving HLA-DRB1. The relevant content is summarized in Table 5. The above implicated guiding significance for the risk stratification of NKTCL patients and clinical intervention. It could be combined with other genetic risk factors or prognostic models to help identify high-risk populations for targeted prevention.

THE VARIATIONS OF EBV GENOME

Epstein-Barr virus (EBV) is a widespread human herpes virus that has infected more than 90% of population in a lifetime[92]. EBV infection is believed to be associated with various human cancers such as nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's lymphoma, gastric cancer, DLBCL, NKTCL, *etc*.[92]. The virus expresses six Epstein-Barr nuclear antigens (EBNAs 1, 2, 3A, 3B, and 3C and EBNA leader protein), latent membrane proteins (LMP1 and LMP2), non-coding EBV-encoded RNAs (EBER1 and EBER2), and viral microRNA[93]. The virus presents a type II latent pattern with the episomal form (EBNA1+/LMP-1+ and EBNA-2) in the host body[94,95]. LMP1 is one of the main oncogenes encoded by EBV that is of significance for EBV-mediated B-cell immortalization[96]. EBNA1 makes a difference in virus replication and the maintenance of episomal form in the latent state and promotes the malignant transformation of B cells[97].

Structural variation of EBV genome

There are common intragenic EBV deletions (73-49,847 bp) detected in NKTCL (10/23), EBV-positive DLBCL (10/14), and other malignancies (2/7)[98]. Sanger sequencing revealed that *LMP1* gene contained a 30 bp deletion, which may be related to the poor prognosis of NKTCL patients[99]. Analyzing the EBV genome and transcriptome derived from NKTCL, in addition to the 30 bp deletion in *LMP1*, small deletions in *BARTs*, *EBNA2*, *EBNA3s*, *BLLF1/2*, and other regions were also detected, which disclosed the heterogeneity in EBV cloning in NKTCL patients[17]. Interestingly, this study also clarified an insertion of EBV fragments into the human nonhomologous end-joining 1 (*NHEJ1*) gene region, which may lead to changes in the expression and function of *NHEJ1*. The *NHEJ1* gene is vital in repairing DNA damage and maintaining genome stability[100]. Thus, integration of the EBV genome and human genome might have crucial impact on the pathogenesis and development of NKTCL. The molecular mechanism of this integration affecting NKTCL tumorigenesis and whether the integration indeed contributes to clinical treatment of NKTCL need to be explored in further research.

Lytic genes

There are two infection routes of EBV-infected cells: latent infection and lytic infection[101]. Similar to latent genes, lytic genes also play a crucial role in the promotion of EBV infection and tumorigenesis of NKTCL. Previous studies demonstrated that the lytic genes *BNLF2a* and *BNLF2b* were highly expressed in NKTCL tissues[17], and the expressions of *BARF1*, *BHRF1*, and *BZLF1* were detected in the NKTCL cell line[102]. Besides, the single-nucleotide variations of lytic gene *BALF3* frequently were detected, and overexpression of *BALF3* might drive DNA damage and bring about genomic instability in NKTCL. Intragenic EBV deletions often affect BamHI A rightward transcript (*BART*) microRNA clusters, core genes necessary for lytic DNA replication (*BMRF1*, *BSLF1*, *BALF2*, *BALF5*, *BBLF2*/*BBLF3*, and *BBLF4*) and some genes related to the lytic cycle and latent infection[98]. High-throughput sequence identified that EBV noncoding BART lncRNAs RPMS1 and A73 were strongly expressed in NKTCL and delivered regulatory signals to host cells without triggering specific immunity, which is helpful to retain the latent state of EBV in the host[103]. However, deletions located on (*BART*) microRNA clusters often have an unfavorable effect on EBV-miR-BART6-5p, EBV-miR-BART6-3p, EBV-miR-BART18-5p, and EBV-miR-BART20-5p, which negatively regulated early genes *BZLF1* and *BRLF1* that are thought to upregulate the lysis cycle and promote

Table 5. Crucial immune checkpoint and risk alleles and their potential significance to the pathogenesis and treatment of NKTCL

The alterations of human genetics	Potential hallmark/signaling pathways	Role in pathogenic mechanism of lymphoma	Ref.	Potential treatment significance	Ref.
Immune evasion	PD-1/PD-L1	The combination of PD-1 and PD-L1 is one of the most important pathways for tumors to escape immune surveillance	Iwai *et al.*[80]	PD-1/PD-L1 blockade therapies applied to NKTCL have been developed or are ongoing, and there are a few promising results	Kwong *et al.*[85] Li *et al.*[86] Shen *et al.*[89] Lim *et al.*[87] Kim *et al.*[88]
HLA risk alleles	rs9277378	Exhibits the strong association with susceptibility of NKTCL	Li *et al.*[15]	Susceptible population screening and disease risk stratification	N/A
	rs13015714	Located at the IL18RAP region and involved in inflammation and immune regulation	Lin *et al.*[16]	Identifying high risk population for targeted prevention	N/A
	rs9271588	Located at the HLA-DRB1 region and involved in antigen presentation	Lin *et al.*[16]	Identifying the high-risk population for targeted prevention	N/A

NKTCL: Extranodal natural killer/T cell lymphoma; PD-1/PD-L1: programmed death ligand-1/ programmed death-1; HLA: human leukocyte antigen; IL18RAP: interleukin 18 receptor accessory protein; HLA-DRB1: major histocompatibility complex, class II, DR beta 1.

lymphoma pathogenesis[104-106].

Immune cell therapies targeted EBV

Studies have proved that EBV is closely related to the pathogenesis of NKTCL. While exploring the relevant molecular pathogenic mechanisms, scholars commit to explore the target of EBV for therapy. After 29 high-risk or recurrent cases with EBV+ lymphoma received autologous LMP-cytotoxic T lymphocytes (CTLs) therapy, 27 patients achieved CR[107]. Similarly, allogeneic donor-derived LMP-specific T cells (LMP-Ts), as an ancillary therapy, maintained the clinical response of EBV+ lymphoma patients who had underwent allogeneic bone marrow transplantation, and the two-year OS of 26 patients was 68%[108]. Noticeably, a recent study reported that EBV-specific induced pluripotent stem cell-derived antigen-specific CTLs can prompt enduring and strong antineoplastic activity[109]. The above research implies that specific immune cell therapies targeting EBV-related antigens are potential strategies. In the future, the safety and effectiveness of cell therapy should be further verified in larger multi-center trials which may bring more clinical benefits to NKTCL patients.

CONCLUSION

During the past decade, with the development of gene expression profiling and next-generation sequencing technology, people have expanded the understanding of the functional structure of genes. This provides novel perspectives for exploring the genetic mechanism of NKTCL and opportunities to develop new therapeutic strategies for NKTCL patients. Up to now, PD-1/PD-L1 blockade therapy has undoubtedly been an extremely noteworthy treatment option. As a novel regulator, CMTM6 regulates PD-L1 through endosystemic circulation, and PD-L1 can be specifically reduced due to the deletion of CMTM6. This discovery will be an interesting direction for further uncovering the NKTCL pathogenic mechanism[110]. Dysregulated signaling pathways are of noticeable significance in NKTCL and studies about related inhibitors also brings potential targets for the treatment of NKTCL[45,46,52,58,59]. The intimate relationship between EBV infection and NKTCL has been clearly proposed, and specific immune cell therapies targeting EBV are being investigated. Nevertheless, the interaction mechanism between the EBV genome and the human genome in NKTCL is yet to determined. As the results of various basic and clinical trials have been reported, feasible targets and possible treatments of NKTCL have been proposed and further studied. To

sum up, more accurate biomarkers and predictors for the response to treatment and survival are needed. More effective treatment and the optimal combination of these therapeutic options still need to be explored in the future according to the development of the above progress and further challenges.

DECLARATIONS

Authors' contributions

Conception and design: Peng R, Jiang J
Acquisition of data: Jiang J, Ruan Z, Wang Q, Jiang L
Writing, review, and/or revision of the manuscript: Peng R, Jiang J
Administrative, technical, or material support: Ruan Z, Wang Q
Study supervision: Peng R

Availability of data and materials

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Consent for publication

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