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Review

Frequent Mutations in Natural Killer/T Cell Lymphoma

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Key Words
Enktl • High-throughput DNA sequencing • Genetic mutations

Abstract
Extranodal natural killer (NK)/T cell lymphoma (ENKTL-NT or NKTCL), with its aggressive nature and poor prognosis, has been widely studied to discover more effective treatment options. Various somatic gene alterations have been identified by traditional Sanger sequencing. However, recently, novel gene mutations in NKTCL have been revealed by next-generation sequencing (NGS) technology, suggesting the potential for novel targeted therapies. This review discusses recurrent aberrations in NKTCL detected by NGS, which can be categorized into three main groups, specifically, tumor suppressors (TP53, DDX3X, and MGA), the JAK/STAT cascade, and epigenetic modifiers (KMT2D, BCOR, ARID1A, and EP300). Some epigenetic dysregulation and *DDX3X* mutation, which have been rarely identified by traditional sequencing technology, were recently uncovered with high frequencies by NGS. In this review, we summarize the mutational frequencies of various genes in NKTCL. In general, based on our analysis, *BCOR* is the most frequently mutated gene (16.9%), followed by *TP53* (14.7%), and *DDX3X* (13.6%). The characterization of such genes provides new insight into the pathogenesis of this disease and indicates new biomarkers or therapeutic targets.

Introduction

Extranodal NK/T cell lymphoma, nasal type (ENKTL-NT or NKTCL) is a rare but aggressive subtype of non-Hodgkin lymphoma [1], which most commonly affects the upper aerodigestive tract (nasal cavity, nasopharynx, paranasal sinuses, and palate) and sometimes involves extra-nasal tissues such as the skin, gastrointestinal tract, soft tissues, and testis. EB-virus (EBV) infection was found to be present in the vast majority of cases [2]. NKTCL is most prevalent in Asians and the native American population of Mexico, Central America, and South America [3]. Due to its distinct and massive necrotic lesions, it is difficult to obtain suitable specimens; thus, few molecular studies have been performed on this disease [4].

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Although multi-agent chemotherapy and involved-field radiotherapy are used, the survival rate of NKTCL remains poor [5]. Thus, novel treatment options such as targeted therapy are urgently needed, and accordingly, novel genetic aberrations must be identified to facilitate such strategies.

Genome sequencing technology has evolved quickly since the first publication of a DNA sequencing method by Sanger et al [6]. in 1977, which reported an epoch-marking method named Sanger sequencing. It was subsequently termed first-generation sequencing technology and is based on the dideoxy chain termination method, characterizing large clones by low-resolution mapping. In 2005, next-generation sequencing technology (NGS) emerged, which represented another landmark sequencing technology. NGS was based on the traditional Sanger sequencing approach but had undergone revolutionary changes in many aspects. It enables high-resolution sequencing of smaller subclones. With high-throughput sequencing technology, billions of sequencing and detection reactions can be conducted simultaneously, instead of distinct processes; hence, enormous data sets have become available [7]. NGS has been performed for genetic and molecular studies such as identifying mutations and profiling gene expression, and can be used for laboratory research or clinical applications [8]. To date, three main sequencing platforms have been utilized, including Roche/454 FLX, Illumina/Solexa Genome Analyzer, and Applied Biosystems SOLID system [7]. With this efficient method, more reliable results are generated for human genome studies, disease diagnosis, and many other fields; this has significantly promoted human research [9, 10]. Thus, NGS technology, with its advantages of higher throughput and lower cost, compared to those with Sanger sequencing, is being widely used in various fields and is becoming the predominant sequencing technology. As such, studies on genetic alterations in NKTCL have been conducted using NGS, and some prominent mutations have been identified. These are summarized in this review and are classified into three main categories including tumor suppressors, the JAK/STAT cascade, and epigenetic modifiers.

Tumor suppressors

TP53

TP53 is a well-known tumor suppressor gene encoding a protein (p53) with transcriptional activation, DNA binding, and oligomerization domains. p53 performs its function by inducing G1 cell cycle phase arrest in DNA-damaged cells and promotes expression of the *bax* gene, which encodes a protein that induces apoptosis. Other functions include the regulation of DNA repair, apoptosis, senescence, and metabolism [11, 12].

Traditional Sanger sequencing technology revealed very high mutation frequencies of *TP53* in NKTCL, specifically, 63% (17 of 27 cases) in Indonesia [13], 40% (eight of 20 cases) in China [14], 18.9% (six of 32 cases) and 62% (36 of 58 cases) in Japan [15, 16], and 31% (13 of 42 cases) in Korea [15]. These high frequencies were detected using polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) followed by direct sequencing. This method can detect mutant DNA in as little as 3% of the total gene copies in a PCR mixture [17]. Recently, *TP53* mutations in NKTCL

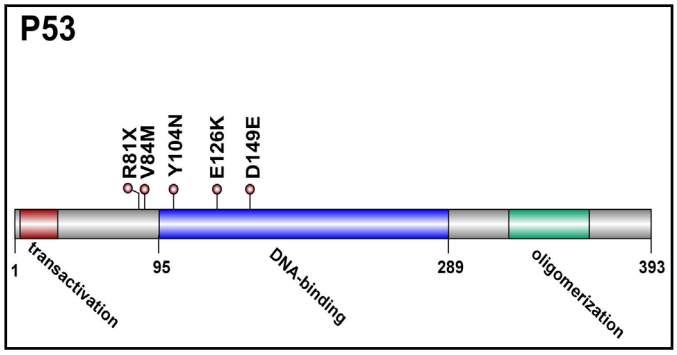

Fig. 1. Locations of TP53 mutations identified by next-generation sequencing in extranodal natural killer (NK)/T cell lymphoma (NKTCL). Graphical view of P53 structure and available data regarding mutation sites.

were also studied by NGS, but the frequencies were relatively lower at 13.3% (14 of 105 cases) among the Chinese population [11], and 16% (four of 25) and 20% (one of five) in Japanese and Korean cohorts [18, 19], respectively. There is thus an apparent discrepancy in reported frequencies between the two methods. It seems that the PCR-SSCP followed by direct sequencing is more sensitive than NGS; however, many other factors including clinical background, diagnostic criteria, and analytical methods might also contribute to this difference.

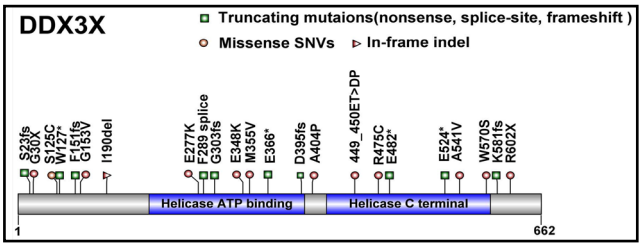

Fig. 2. Locations of DDX3X mutations identified next-generation sequencing in extranodal natural killer (NK)/T cell lymphoma (NKTCL). Square symbols indicate mutations causing truncation of the DDX3X protein; red circles, missense mutations; red triangle, in-frame indel.

Most *TP53* mutations were found to occur at exons 4–8 [15], which comprise the functional domains including the transactivation region, sequence-specific DNA binding region, and proline-rich domain that play critical roles in the transmission of antiproliferative signals (Fig. 1) [20]. G:C to A:T transition was reported as the predominant *TP53* mutation, resulting in the replacement of cytosine with thymine, which is associated with endogenous oxidative or spontaneous deamination of 5-methylcytosine [14, 15, 21]. In addition, *TP53* mutations were thought to be "gain-of-function" mutations [22-24], with the majority of which being missense mutations resulting in the expression of mutant p53 proteins. This type of protein not only exerts a negative effect on wild-type p53 and inhibits its function as a tumor suppressor [25, 26], but also exhibits new oncogenic functions including promoting cell proliferation, the evasion of apoptosis, metabolic changes, migration, invasion, angiogenesis, and metastasis [27-30]. Indeed, clinical studies have demonstrated that *TP53* alterations correlate with advanced stage and poor prognosis in NKTCL [11, 31].

DDX3X

DDX3X is a gene located on the X chromosome encoding a protein of the large DEAD-box protein family. DDX3X has ATP-dependent RNA helicase activity and RNA-independent ATPase activity. It plays important roles in the nucleus by regulating transcription, mRNP assembly, pre-mRNA splicing, and mRNA export, and in the cytoplasm by modulating translation, cellular signaling, and viral replication [32-34]. DDX3X has been reported to be a tumor suppressor in medulloblastoma and its dysregulation is thought to be involved in tumorigenesis [35]. A study of 25 Japanese individuals identified three (12%) DDX3X alterations in NKTCL by traditional Sanger sequencing [18]. However, another larger-scale study [11], comprising 105 samples in China and using NGS, revealed a higher frequency (20%; 21 of 105 cases). Of these 24 alterations, 11 (52%) were nonsense, splice-site, frameshift, and copy-loss mutations, causing truncation of the DDX3X protein, and nine (43%) were missense, single-nucleotide variants located near the RNA-binding site or close to the ATP site (Fig. 2). These alterations have disrupt the RNA-unwinding function of the protein. In addition, cells and tumors with *DDX3X* mutations were found to exhibit higher expression of nuclear RelB and p-ERK as well as upregulation of the NF-κB and MAPK pathways [11], indicating that mutations in DDX3X might result in a loss of function as a negative regulator of NK-cell proliferation [36, 37]. Indeed, proliferation of cells with WT *DDX3X* was found to be significantly suppressed compared to that in cells with *DDX3X* mutations. Further, clinical studies revealed high expression of Ki67 and advanced stage in patients with *DDX3X* mutations [11]. These findings indicate that aberrations in this gene result in loss of RNA-unwinding function and suppression of cell proliferation, thus contributing to the pathogenesis and poor prognosis of NKTCL. It has been reported that DDX3X is an important target for the development of broad-spectrum antiviral agents [38]. In lung cancer, a small molecule inhibitor (RK-33) targeting DDX3X was found to be effective in inducing apoptosis and promoting sensitization

to radiation in DDX3X-overexpressing cell lines [39]. Similarly, targeted therapy for NKTCL based on mutant *DDX3X* is also promising considering its pathological role in this disease. And further studies are recommended.

DDX3X mutations seldom occur simultaneously with *TP53* mutations, indicating that these two genes might be closely related in terms of biological processes. Indeed, recent studies have provided evidence that DDX3X is a target of p53, and that both cooperate in tumor suppressive functions [40, 41]. Loss of DDX3X as a result of p53 inactivation might promote tumor malignancy and lead to poor clinical prognosis in non-small cell lung carcinoma [41]. In NKTCL, based on a large-scale study [11], the prognosis (overall survival and progression-free survival) of patients with mutations in *DDX3X* and *TP53* was much worse than that in individuals without these mutations. Taken together, the high n *DDX3X* and *TP53* mutation frequencies, and the correlation between these alterations and advanced disease stage or poor clinical outcome might explain the aggressive phenotype of NKTCL.

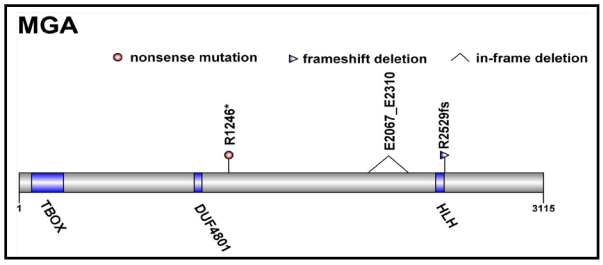

Fig. 3. Structure of MGA and mutation locations in chronic lymphocytic leukemia (CLL). TBOX, T-box DNA binding domain of the T-box family of transcriptional regulators; DUF4801, domain of unknown function; HLH, helix-loop-helix DNA-binding domain. Mutation locations in high risk CLL are shown, of which data are not available in extranodal natural killer (NK)/T cell lymphoma (NKTCL).

MGA

MAX dimerization protein (MGA), was identified as a dual-specificity transcription factor, which contains a T-domain DNA-binding motif. Its function is mediated by heterodimerization with Max. MGA binds Max and inhibits MYC-dependent cell transformation [42]. Further, it is regularly inactivated in high risk chronic lymphocytic leukemia (CLL) [43], lung cancer [44], and colorectal cancers [45]. In a study on high risk CLL, three of 55 cases (5.4%) were identified as harboring *MGA* mutations (Fig. 3), including one in-frame intragenic deletion of 729 bp (c.6201_6929del729; p.E2067_E2310) disrupting the basic motif of MGA, one nonsense substitution (c.C3736T; p.R1246 ∗) and one frameshift deletion (c.7586delG; p.R2529fs ∗ 28) causing truncation of the C-terminal region of the MGA protein [43]. Recently, *MGA* mutations were identified in NKTCL by NGS. Specifically, these were reported in two of 25 cases (8%) in Japan [18], and nine of 105 cases (8.6%) in China [11]. However, the details regarding these mutations are not available, and their underlying significance in NKTCL have not been reported to date. Further studies on MGA mutations in NKTCL are thus recommended.

JAK/STAT cascade

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway is associated with ligands that include diverse cytokines, growth factors, and other related molecules. It transmits signals involved in cell growth/proliferation, differentiation, and evasion of apoptosis, which are critical for normal hematopoiesis and immune responses. Molecule–receptor interactions at the cell surface lead to the activation of JAK family kinases, resulting in the phosphorylation of downstream STAT proteins. Phosphorylated STATs are crucial for the JAK/STAT pathway, and these proteins can move to the nucleus to direct the transcription of different genes involved in normal cellular functions [46]. Deregulation of the JAK/STAT signaling pathway causes persistent activation of JAKs or STATs, which strongly promotes cell survival and proliferation. Mutations involving genes of the JAK/STAT cascade have not only been reported in many hematologic malignancies including T-cell acute lymphoblastic lymphoma/leukemia, cutaneous T-cell lymphoma, mantle cell lymphoma, acute megakaryoblastic leukemia, and myeloproliferative diseases [47-56], but have also

been found in some solid tumors including breast, stomach, and lung cancer [57].

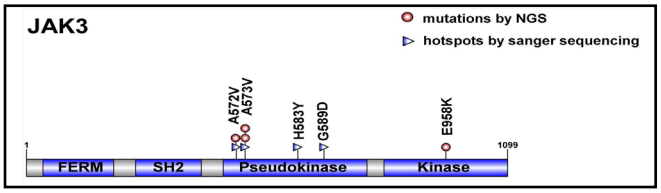

Fig. 4. Locations of JAK3 mutations in extranodal natural killer (NK)/T cell lymphoma (NKTCL). Graphical view of the JAK3 mutations, as detected by NGS, as well as hotspots revealed by traditional Sanger sequencing. Each circle indicates one identified mutation. Triangle symbols indicate hotspots instead of specific cases. JAK3 mutations are clustered at the pseudokinase domain.

JAK3

The *JAK* family comprises protein tyrosine kinases such as *JAK1*, *JAK2*, *JAK3*, and *TYK2*, each of which possesses seven domains (JH1–7). Among them, *JAK1*, *JAK2*, and *TYK2* have been detected in most tissues; however, JAK3 is expressed only in myeloid and lymphoid cells [58]. Mutations in *JAK3* has been reported with varying frequencies since they were first identified by Koo et al. [59]. In their study, whole exome sequencing detected two *JAK3* mutations (JAK3^{A572V} or JAK3^{A573V}) from four cases of NKTCL in Singapore. Sanger sequencing was further performed on 61 additional cases, and in total, 23 of 65 (35.4%) cases were found to be positive for *JAK3* mutations. This result was supported by a study of the French population by Bouchekioua et al [60]. who observed mutated *JAK3* in one of four (25%) NKCL cell lines and four of 19 (21%) clinical samples (three of which were *JAK3*^{A573V}) using NGS. However, research on a Japanese population by Kimura et al [61]. failed to detect *JAK3* mutations in seven NK cell lines and 17 clinical samples of NKTCL or in 10 cases of aggressive NK cell leukemia; however, sufficient information regarding the detection method were not available. Another larger-scale analysis of 105 Chinese individuals using NGS also did not detect *JAK3* mutations [11]. Subsequent studies in Korean also showed low mutation frequencies, specifically 5.1% (2/39) and 7.0% (5/71) [62], in contrast to the aforementioned results. These discrepant proportions might result from differences in ethnicity, diagnostic criteria, and clinical backgrounds; sequencing methods could also a play critical role.

Previous studies suggested that *JAK3*^{A572V} and *JAK3*^{A573V} are hotspot mutations in NKTCL, and these residues are located in the JH2 pseudokinase domain [59, 62]. NKTCL cells with *JAK3* alterations were shown to be more malignant; further, NK-S1 (a NKTCL cell line) cells with a homozygous *JAK3*^{A572V} mutation can proliferate without IL-2 and these cells exhibit constitutive JAK3 phosphorylation and STAT5 expression [59]. Recently, two additional novel activating variants (*JAK3*^{H583Y} and *JAK3*^{G589D}) were detected (Fig. 4). Ba/F3 (a NKTCL cell line) cells transfected with the *JAK3*^{H583Y} and *JAK3*^{G589D} mutations were subsequently shown to proliferate without IL-3 stimulation [62]. Promisingly, treating cells harboring the aforementioned mutations with JAK3 inhibitors has proven to be efficient. Tofacitinib (CP-690550), which was shown to inhibit the growth of adult T-cell lymphoma/leukemia (ATLL) cells and ATLL xenograft tumors [63], and which also is used as a monotherapy for rheumatoid arthritis [64], proved to be useful for inhibiting STAT5 phosphorylation and reducing cell viability in *JAK3*-mutant NKTCL cell lines [59, 60].

STAT3

There are seven known mammalian *STAT* family members (*STAT1*, 2, 3, 4, 5a, 5b, and 6), among which, STAT3 and STAT5 have been most studied in NKTCL. Six functional domains in STATs have been identified to date, including the N-terminal, DNA-binding, Src homology 3 (SH3)-like, Src homology 2 (SH2), transactivation, and C-terminal domains.

Mutations in *STAT3* occur at a higher rate than those in *JAK3*. A study of 105 Chinese individuals revealed 11 *STAT3* mutations (10.4%), which were clustered at the SH2 domain [11]. Another study of a Chinese population revealed an similar frequency of 12% (three of 25) [65]. This frequency was found to be higher in Korea (26.4%, nine of 34) [66] and slightly lower in Japan (8%, two of 25) [18]. *STAT3* mutations were found to occur at several

different sites (Fig. 5) [18, 66]. Previous studies revealed mutational hot spots in large granular lymphocytic leukemia, including Y640F in 13 (17%) cases, D661V in seven (9%), D661Y in seven (9%), and N647I in three (4%) [67]. Interestingly, all mutations were located in the SH2 domain, which is critical for STAT activation [59]. Mutations in *JAK3* and *STAT3* result in persistent phosphorylation of STAT3 (P-STAT3), which is a critical process required for the JAK-STAT signaling. The activation of Stat3 can be mediated by extrinsic or intrinsic cues. EBV, which is intimately-associated with NKTCL, was thought to be one such extrinsic activator of STAT signaling [68]. The novel fusion gene *LEP*, which produces a protein product that was found to activate JAK2/3 and STAT3 by stimulating tyrosine phosphorylation [69], was identified in one of 34 Koreans. The SH2B adaptor protein 3 (SH2B3) encodes a negative regulator of cytokine signaling, the loss of which also intrinsically increases STAT3 phosphorylation [70]. Additionally, PIK3R3, a downstream element of the JAK/STAT cascade that mediates cell proliferation, was found to be upregulated in all ENKL samples with *STAT3* mutation [71]. All these factors, together with mutations in *JAK3* and *STAT3*, or each independently, might play crucial roles in the initiation of NKTCL. In addition, *STAT3* was found to be mutated at a much higher frequency in NK (50%) and γδ-T cell lines (67%) than in NKTCL (5.9%) [65], indicating a more critical role of the JAK-STAT3 pathway in cell survival independent of stromal components. High expression of p-Stat3 was also reported to be associated with poor clinical outcome in diffuse large B cell lymphoma [72], but not in NKTCL [62].

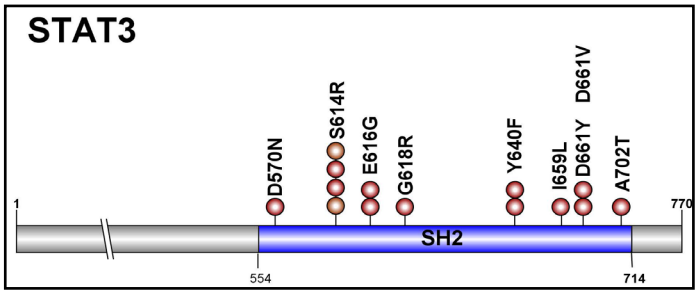

Fig. 5. Locations of STAT3 mutations identified by next-generation sequencing in extranodal natural killer (NK)/T cell lymphoma (NKTCL). Each circle symbol indicates one identified mutation. All mutations are clustered at the SH2 domain (other domains of the protein are not shown in this figure).

Accordingly, significant efforts have been made to explore novel therapies targeting the JAK-STAT pathway. In addition to the aforementioned JAK3 inhibitors, STAT3 inhibitors have also been studied. For example, Static can effectively inhibit the proliferation of YT (a NKTCL cell line) cells harboring $STAT3^{Y640F}$ mutations and SNK6 (a NKTCL cell line) cells with $STAT3^{D661Y}$ mutations, but is less effective for cells expressing wide-type *STAT3* [62]; WP1066 (a novel selective STAT3 inhibitor) was found to inhibit proliferation and induce apoptosis in SNK6 cells through downregulation of STAT3 signaling [73]. In addition, AZD1480, a selective JAK1/2 inhibitor [74], was shown to inhibit the growth of KAI3, YT, and NKYS cell lines harboring STAT3 mutations [65].

Epigenetic modifiers

MLL2

The *MLL2* gene, also termed *KMT2D* or *MLL4*, encodes a histone methyltransferase that methylates the Lys-4 position of histone H3 (H3K4)—a modification associated with transcriptionally active chromatin [75]. MLL2 is widely expressed in human tissues and is important for growth during the embryonic phrase. This protein contains two clusters of plant homeotic domains (PHDs), in addition to the N-terminal domain and an enzymatically active C-terminal SET domain (Fig. 6). The second cluster of PHDs recognizes H4 tails and might methylate the MLL2-catalyzed nucleosome. The C-terminal SET domain is important for the maintenance of H3K4 methyltransferase activity and MLL2 protein stability [76]. MLL2 plays critical roles in regulating cell development, differentiation, and metabolism; it also

functions as a tumor suppressor [77]. Frequent mutations in *MLL2* have been observed in various diseases including gastric adenocarcinoma, medulloblastoma, congenital heart disease, lymphoma [78, 79], and particularly in B cell non-Hodgkin lymphomas (B-NHL). Approximately 30% of diffuse large B cell lymphomas [77, 80, 81] and ~90% of follicular lymphomas [82] were found to harbor *MLL2* mutations, which account for more than 70% of B-NHLs. The majority of these mutations were identified as nonsense mutations, frameshift-inducing insertions, and deletions that cause truncated proteins lacking part or all of the C-terminal domain [77, 81, 82]. This type of alteration leads to an abnormally short, non-functional histone methyltransferase that has lost its function as a tumor suppressor [19]. Missense mutations impairing MLL2 methyltransferase activity were also identified. Moreover, loss of MLL2 was found to cooperate with Bcl2 during lymphomagenesis [83]. Recently, reports of alterations in *MLL2* in NKTCL based on NGS have emerged. Seven mutations were revealed in 105 cases (6.7%) among a Chinese population [11]. In Korea, the frequency was found to be higher, with specific incidences of six in 34 (17.6%) [66] and four in five (80%) [19]. Both nonsense and missense mutations were identified, but little is known about their functional roles in NKTCL. Considering its mechanism underlying B-NHL tumorigenesis and the identification of recurrent alterations in NKTCL, it is rational to speculate that MLL2 may play critical roles in the pathogenesis or progression of NKTCL. However, further studies are needed to verify this speculation and explore the underlying mechanism.

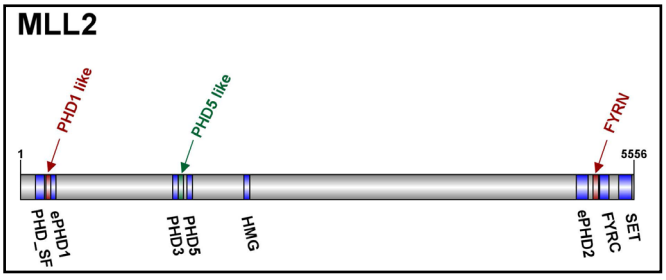

Fig. 6. Structure of MLL2. PHD_SF, PHD finger superfamily; PHD1-like, PHD finger 1 found in KMT2C and KMT2D; ePHD1, extended PHD finger 1; PHD5-like, PHD finger 5 found in KMT2C and PHD finger 4 found in KMT2D; HMG, high mobility group; ePHD2, extended PHD finger 2; FYRN, F/Y-rich N-terminus; FYRC, C-terminal region. Green and red colors are used to distinguish the closed domains. Mutation details of MLL2 based on next-generation sequencing of extranodal natural killer (NK)/T cell lymphoma (NKTCL) are not available in the present data set.

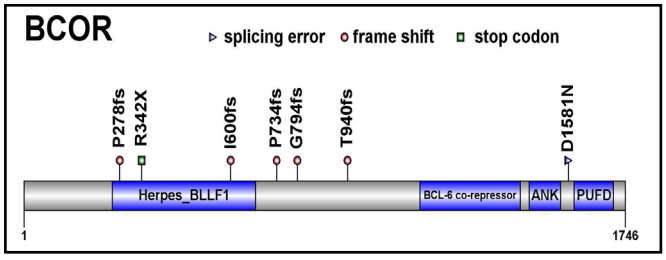

Fig. 7. Locations of BCOR mutations identified by next-generation sequencing in extranodal natural killer (NK)/T cell lymphoma (NKTCL). ANK, ankyrin repeat; PUFD, PCGF Ub-like fold discriminator.

BCOR

Another frequently mutated epigenetic modifier in NKTCL is *BCOR*, which encodes a protein that interacts with and functions as a co-repressor of BCL6 [84]. BCOR interacts with BCL6 via the POZ domain and plays a critical role in the formation of germinal centers and apoptosis. Some histone deacetylases (HDAC1, HDAC3, and HDACB/5) have been reported to interact with BCOR. Further, various malignancies are associated with *BCOR* mutations, such as uterine endometrial carcinoma [85], lung adenocarcinoma [86], melanoma [87], and colorectal adenocarcinoma [88]. In addition, mutations have been reported in hematologic malignancies such as acute myeloid leukemia (AML) [89], myelodysplastic syndromes [90], and NKTCL[18, 71]. In NKTCL, as detected by NGS, the reported frequency of this mutation among the Japanese population is 32% (eight of 25 cases) [18], which is higher than that reported in the Korean population, specifically 20.6% (seven of 34 cases) [66] and 25%

(one of five cases) [19]. *BCOR* alterations tend to emerge more frequently in malignancies associated with EBV infections and often occur in the form of loss-of-function mutations in exon 4 (Fig. 7) [66]. In addition, EBV infection has been reported to cause NKTCL via various epigenetic regulatory mechanisms [91]. Thus, it can be speculated that EBV infection together with *BCOR* mutations can lead to NKTCL through an epigenetic mechanism. However, no significant relations have been found between this aberration and clinical features to date [91]. Larger-scale studies are needed to verify this result.

Epigenetic dysregulation is an emerging component of cancer genomics, which represents a new category that has been discovered by NGS technology. Apart from *MLL2* and *BCOR*, other mutations in epigenetic modifiers including *ARID1A*, *EP300*, *ASXL3*, and *MLL3* have been identified in NKTCL. *ARID1A* (AT-rich interaction domain 1A) encodes a protein of the SWI/SNF family, the large ATP-dependent chromatin remodeling complex. Mutations in *ARID1A* have been reported in intrahepatic cholangiocarcinomas, gastric cancer, and ovarian carcinoma [92-96]. In NKTCL, this gene was found to be mutated in six of 130 patients (4.6%) in China, three of 39 (7.7%) cases in Korea, and one of 25 (4%) individuals in Japan. *EP300* encodes a histone acetyltransferase that is important for transcriptional regulation, cell proliferation, and differentiation. *EP300* mutations in NKTCL were detected in seven of 130 (5.4%) cases in China but were not identified in other regions. In total, epigenetic aberrations were identified in 29 of 130 (22.3%), 21 of 39 (53.8%), and 13 of 25 (52%) patients in China, Korea, and Japan, respectively. Furthermore, frequent mutations in *MLL2* and *BCOR*, both of which belong to the same gene ontology (GO) group, namely "chromatin modification", tend to occur exclusively in NKTCL. Considering all of the findings discussed, epigenetic dysregulation might be another important pathogenic mechanism associated with NKTCL.

Other gene mutations

Through NGS, other genes have been found to be mutated in NKTCL at a relatively lower frequency, such as *MSN*, *NARS*, *FAT4*, *CHPF2*, and *MGAM*. The MSN protein belongs to the ERM family (ezrin, moesin, and radixin), functioning as a cross-linker between plasma membranes and actin-based cytoskeletons. It plays critical roles in signal transduction, cell movement, and recognition between cells. MSN has been suggested to modulate hepatitis C virus infection [97]. However, its significance in NKTCL has rarely been reported. *NARS* mutations, shown to be predictive of poor prognosis in AML [98], were not determined to be associated with ENKTL prognosis [18].

Mutations in genes encoding K-Ras, c-Kit, β-catenin, Bak, and Fas have been widely studied by Sanger sequencing [14, 15, 99, 100], but seldom by NGS; thus, they are not discussed in this review.

Conclusion

Targeted therapies focusing on frequently mutated genes that are involved in tumorigenesis have been conducted for several malignancies, and these have greatly improved the prognosis of these diseases. Researchers have made great efforts to explore potential therapeutic targets in NKTCL. Many of genetic alterations have been detected and some frequently mutated genes are considered promising molecular candidates for targeted therapy, as discussed previously herein. In addition to some well-known tumor suppressors, mutations in epigenetic modifiers have become attractive targets in recent years. Emerging studies have demonstrated that some epigenetic modifiers can function as tumor suppressors in specific malignancies, such as BCOR in T/B lymphocyte malignancies [101-103], MLL2 in melanoma and follicular lymphoma [104, 105], MLL3 in acute myeloid leukemia [106], ARID1A in gynecological cancers (mammary, ovarian, and uterine cancers) [107-110], and EP300 in epithelial cancers (colon, breast, and ovarian carcinomas) [111, 112]. Mutations in tumor suppressors including some epigenetic modifiers result in the impaired expression

Table 1. Recurrent Genetic Alterations and Their Effects on Clinical Outcome

Categories	Genes involved	Notes	Clinical outcome
Tumor suppressors	TP53	DNA damage response; "gain-of-function" mutation.	poor
	DDX3X	Target of TP53; loss of function mutation.	poor
	MGA	Little is known in NKTCL	No available data
JAK/STAT	JAK3	Mutations at JH2 domain; sensitive to JAK inhibitor.	No significant evidence
	STAT3	Mutations at SH2 domain; sensitive to JAK/STAT inhibitors.	No significant evidence
	STAT5B	Mutations at SH2 domain sensitive to JAK inhibitor.	No available data
Epigenetic modifiers	MLL2	Histone methyltransferase activity; Tumor suppressor; loss of function mutation.	No available data
	BCOR	Tumor suppressor; loss-of-function mutation.	No significant evidence
	ARID1A	Chromatin remodeling; Tumor suppressor.	No available data

Table 2. Distribution of Mutations Detected by NGS in ENKTCL. WES: whole exome sequencing; TS: targeted sequencing; RS: RNA sequencing. [a]cases of mutation; [b]frequency of mutation; [c]cases of category; [d]frequency of category; [e]genes not involved in targeted sequencing.

	Genes involved	total	Cases sequenced		China [11, 65]			Korea [19, 66]			Japan [18]			Singapore [59]		
total		198			130			39			25			4		
WES		43			26			13			0			4		
TS		131			80			26			25			0		
RS		31			24			7			0			0		
	TP53	26	177	14.7%	16[a]	12.3%[b]		5	12.8%		4	16.0%		1	25.0%	
tumor suppressors	DDX3X	24	177	13.6%	22	16.9%	47[c] 36.2%[d]	0[e]	0.0%[e]	5 12.8%	0	0.0%	6 24.0%	2	50.0%	3 75.0%
	MGA	11	177	6.2%	9	6.9%		0[e]	0.0%[e]		2	8.0%		0	0.0%	
	JAK3	6	118	5.1%	0[e]	0.0%[e]		2	5.1%		2	8.0%		2	50.0%	
JAK/STAT cascade	STAT3	25	198	12.6%	14	10.8%		9	23.1%		2	8.0%		0	0.0%	
	STAT5B	3	177	1.7%	3	2.3%	17 13.1%	0[e]	0.0%[e]	13 33.30%	0	0.0%	7 28.0%	0	0.0%	3 75.0%
	JAK1	5	118	4.2%	0[e]	0.0%[e]		2	5.1%		2	8.0%		1	25.0%	
	MLL2	19	198	9.6%	8	6.2%		10	25.6%		1	4.0%		0	0.0%	
	BCOR	20	118	16.9%	4[e]	8%(4/50)[e]		8	20.5%		8	32.0%		0	0.0%	
	ARID1A	10	198	5.1%	6	4.6%		3	7.7%		1	4.0%		0	0.0%	
epigenetic modifiers	EP300	7	177	4.0%	7	5.4%	29 22.3%	0[e]	0.0%[e]	21 53.80%	0	0.0%	13 52.0%	0	0.0%	0 0.0%
	ASXL3	4	152	2.6%	4	3.1%		0[e]	0.0%[e]		-	-		0	0.0%	
	MLL3	3	97	3.1%	0[e]	0.0%[e]		0[e]	0.0%[e]		3	12.0%		0	0.0%	
	MSN	9	152	5.9%	9	6.9%		0[e]	0.0%[e]		-	-		0	0.0%	
	FAT4	4	97	4.1%	2[e]	4%(2/50)[e]		0[e]	0.0%[e]		2	8.0%		0	0.0%	
	NARS	5	177	2.8%	2	1.5%		0[e]	0.0%[e]		2	8.0%		1	25.0%	
others	CHPF2	4	152	2.6%	4	3.0%	29 22.3%	0[e]	0.0%[e]	2 5.10%	-	-	7 28.0%	0	0.0%	1 25.0%
	MGAM	4	152	2.6%	4	3.0%		0[e]	0.0%[e]		-	-		0	0.0%	
	IL6R	2	93	2.2%	0[e]	0.0%[e]		2	5.1%		-	-		0	0.0%	
	MIR17HG	2	97	2.1%	0[e]	0.0%[e]		0[e]	0.0%[e]		2	8.0%		0	0.0%	

of functional proteins, some of which have been shown to be correlated with a poor NKTCL outcome (Table 1). Alterations in JAK/STAT lead to the constitutive activation of p-STAT3 or p-STAT5, which increases the malignancy of tumor cells. However, no significant evidence has been found regarding its association with the clinical outcome of NKTCL. There are also some recurrent alterations that have not been studied to date. Thus, further research in this area is recommended.

In addition, the frequencies of different mutations detected by NGS in NKTCL vary considerably among different studies and regions (Table 2). To summarize, *BCOR* is the most frequently mutated gene (16.9%), followed by *TP53* (14.7%) and *DDX3X* (13.6%), based on available data to date. Of the three gene classifications, the predominant classes of mutated genes in this disease are tumor suppressors (36.2%) in China and epigenetic modifiers in Korea (53.8%) and Japan (52%). Specifically, the predominant alteration differs greatly depending on geographical region and includes *DDX3X* in China (17%), *MLL2* in Korea (25.6%), *BCOR* in Japan (32%), and *JAK3* and *DDX3X* in Singapore (50%). Even in the same region, frequencies have also been reported to differ [11, 65]. *JAK3*, which was previously reported to be actively mutated by Sanger sequencing, is associated with low aberration frequency based on our NGS analysis. These significant differences might be caused by various factors including ethnicity, clinical backgrounds, diagnostic criteria, and analytical methods. Additional larger-scale studies are needed to verify these results, especially in Singapore. Predominant alterations in each region indicate potential therapeutic targets and might suggest clear directions for further studies. Novel therapies targeting these genetic lesions are promising. Some small molecule inhibitors have been discovered such as tofacitinib (JAK3 inhibitor), AZD1480 (JAK1/2 inhibitor), WP1066 (STAT3 inhibitor),

and RK-33 (DDX3X inhibitor). However, additional clinical or *in vivo* studies on NKTCL are advised.

In summary, advances in sequencing technology have revealed novel genetic alterations in NKTCL, which are known to be genetic drivers. The discoveries discussed herein contribute to the understanding of the pathogenesis of this disease. Based on this information, the implementation of targeted therapies becomes a possibility. Future studies on biomarkers, therapeutic targets, and innovative clinical trial designs will be helpful for the diagnosis, therapy, and prognosis of NKTCL.

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Disclosure Statement

The authors declare no conflicts of interest regarding this manuscript.

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