Aggressive Mature Natural Killer Cell Neoplasms: from Disease Biology to Disease Manifestations

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Abstract

Nature killer (NK)/T cell lymphoma, nasal type, and aggressive NK-cell leukemia are rare tumors with higher prevalence in Asia, Central and South America, which are etiologically related to the Epstein Barr virus (EBV). Proteins encoded by EBV genes and non-coding viral RNAs expressed on the infected cells are involved in immune deregulation and cell transformation and lymphomagenesis occur as a consequence of multiple oncogenic events. Complex chromosomal abnormalities are frequent and loss of chromosomes 6q, 11q, 13q, and 17p are recurrent aberrations. In accordance, many genes are differentially expressed, often due to gene deletion, mutation or methylation. These include, among others, tumor suppressor genes and oncogenes, as well as genes involved in cell signal transducer pathways, cell survival and apoptosis, cell cycle, cell motility and cell adhesion, as well as in cell communication through cytokine networks. Consequently many biochemical pathways are affected in NK-cell neoplasms, which could contribute to cancer development and progression, as well as to disease manifestations. This review focuses on the molecular and biochemical mechanisms by which EBV induces NK-cell lymphomagenesis, disrupting genes and molecules involved in crucial biological processes. Improving the knowledge in this subject will help to better understand the disease biology and clinical manifestations and to develop new treatment approaches for the NK-cell malignancies.

Keywords: NK-cell neoplasms; NK/T-cell lymphoma; Nasal-type; Aggressive NK-cell leukemia; Extranodal lymphomas; Epstein Barr virus; Oncogenesis; Lymphomagenesis; Angioinvasion; Vascular destruction; Hemophagocytic syndrome

Abbreviations: AIM1: Absent in Melanoma-1; ANKCL: Aggressive NK-cell Leukemia; ATG5: Autophagy related 5; ATM: Ataxia Telangiectasia Mutated; ATR: ATM Rad3-related; AURKA: Aurora kinase A; BBC3: BCL2-Binding Component 3; BIM: Bcl-2-like Protein 11; BIRKS: Baculoviral Inhibitor of apoptosis Repeat-containing 5; BLIMP1: B-lymphocyte-Induced Maturation Protein 1; BM: Bone Marrow; BTK: STK15 or Aurora kinase A (AURKA); CAM: Cell Adhesion Molecules; CCL2: C-C Motif Chemokine Ligand Type 2 (MCP-1); CCL3: C-C Motif Chemokine Ligand Type 3 (MIP-1alpha); CCL4: C-C Motif Chemokine Ligand type 4 (MIP-1beta); CCL5: C-C motif chemokine ligand type 5 (RANTES); CCL8: C-C motif chemokine ligand type 8 (MCP-2); CCL18: C-C Motif Chemokine Ligand Type 18 (PARC); CCL19: C-C Motif Chemokine Ligand Type 19 (ELC); CCNA2: Cyclin-A2; CCNR: C-C Motif Chemokine Receptor Type 5 (CD195); CD11b: Integrin alpha M Complement Receptor Type 3; CD49b: Integrin alpha 2 alpha 2 Subunit of VLA-2 Receptor; CD49d: Integrin alpha 4 alpha 4 Subunit of VLA-4 Receptor; CDK: Cyclin-Dependent Kinases; CGH: Comparative Genomic Hybridization; CHK: Checkpoint Protein Kinases; C-KIT: Cellular homolog of the feline sarcoma viral oncogene v-KIT; CLPD-NK: Chronic Lymphoproliferative Disorders of NK-cells; CPP32: Apoptosis-Induced Cysteine Protease 32; CTL: Cytotoxic T lymphocytes; CXCL1: C-X-C Motif Chemokine Ligand Type 1 (IL-8); CXCL9: C-X-C Motif Chemokine Ligand Type 9 (MIG); CXCL10: C-X-C Motif Chemokine Ligand Type 10 (IP-10); CXCL11: C-X-C Motif Chemokine Ligand Type 11 (IP-10); CXCL12: C-X-C Motif Chemokine Ligand Type 12 (SDF-1); CXCR1: C-X-C Motif Chemokine Ligand Receptor Type 1 (CD181); CXCR3: C-X-C Motif Chemokine Ligand Receptor Type 3 (CD183); CYR61: Cysteine-rich Angiogenic Inducer 61; DEFB: Defensin Beta; EBV: Epstein-Barr Virus; ELC: EB11 Ligand Chemokine (CCL18); Fasl: Fas Ligand (CD95L); FGF: Fibroblast Growth Factor; FLIP: FLICE-like Inhibitory Protein; FOX: Forkhead Family of Transcription Factors; FOXO: Forkhead Transcription Factors of the O class; HACE1: HECT Domain and Ankyrin Repeat Containing E3 Ubiquitin Protein Ligase 1; HS: Hemophagocytic Syndrome; ICAM-1: Intercellular Adhesion Molecule 1 (CD54); IFN: Interferon; IGF: Insulin-like Growth Factor; IGIF: Interferon-Gamma Inducing Factor (IL-18); IL: Interleukin; IL18BP: IL-18 Binding Protein; IL-6R: Interleukin 6 Receptor; IP-10: IFN-Gamma-Inducible Protein 10 (CXCL10); Jak: Janus kinase; K-RAS: v-Ki-ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog; LACE1: Lactation Elevated 1; LFA: Leukocyte Function Adhesion Molecule; LFA-1: Lymphocyte Function Molecule Type 3 (CD11a); LFA-3: Lymphocyte Function Molecule Type 3 (CD58); LMP: EBV-Encoded Latent Membrane Protein; MCP-1: Monocyte Chemotactic Protein-1 (CCL2); MCP-2: Monocyte Chemotactic Protein-1 (CCL8); MIB1: Mindbomb E3 Ubiquitin Protein Ligase 1 also known as K67; MIG: Monokine Induced by Gamma Interferon (CXCL9); Monokine Induced by IFN-Gamma (CCL9); MIP: Macrophage Inflammatory Protein; MIP-1alpha: Macrophage inflammatory protein-1 alpha (CCL3); MIP-1beta: Macrophage Inflammatory Protein (CCL4); MMP: Matrix Metalloproteinases; Mymi: Myelocyteumatosis Viral Oncogene Homolog; NFkB: Nuclear Factor kB; NK: Natural Killer; NKTL: NK/T Cell Lymphoma; NOTCH: Notch Homolog translocation-associated; PAR: Plasminogen Activator Receptor; PARC: Pulmonary and Activation-Regulated PARC (CCL18); v-erbA: v-erbA; v-erbB: v-erbB; v-raf: v-raf; v-src: v-src

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PDGF: Platelet-Derived Growth Factor; PDGFR: Platelet-Derived Growth Factor Receptor; PDGFRα: Platelet-Derived Growth Factor Receptor Alpha; PI3K: Phosphatidylinositol 3 Kinase; PI9: Protease Inhibitor Type 9; PKB: Protein Kinase B; POPDC3: Popeye Domain Containing 3; PRDM1: PR Domain Zinc Finger Protein 1; PREP: Prolyl Endopeptidase; PTEN: Phosphatase and Tensin Homolog; PUMA: p53 Upregulated Modulator of Apoptosis; RNA: Ribonucleic acid; SDF-1: Stromal Cell-Derived Factor-1 (CXCL12); STAT: Signal Transducers and Activators of Transcription; STK15: also known as BTAK or Aurora Kinase A (AURKA); TGF: Transforming Growth Factor; TH: T Helper; TH1: T Helper Type 1; TH2: T Helper Type 2; TIA-1: T-cell-Restricted Intracellular Antigen; TNF: Tumor Necrosis Factor; TNEAIP3: Tumor Necrosis Factor Alpha-Induced Protein 3 also known as A20; TNFR: Tumor Necrosis Factor Receptor; TP53: Tumor Protein p53; TP73: Tumor Protein p73; TRAF: TNF Receptor Associated Factor; uPAR: Urokinase-Type Plasminogen Activator Receptor; VCAM-1: Vascular Cell Adhesion Molecule Type 1 (CD106); VEGF: Vascular Endothelial Growth Factor; vIL-10: viral homologue of Interleukin 10; WHO: World Health Organization.

Introduction

Natural killer (NK) cell neoplasms are rare diseases that are much more prevalent in Central and South America and Eastern countries for reasons that are not completely clear. They comprise a limited spectrum of Epstein Barr virus (EBV)-related neoplasms [1-21], two of which were recognized by the World Health Organization (WHO) classification as distinct entities [22]: extranodal NK/T Cell Lymphomas, Nasal Type (NKTCL) [23] and Aggressive NK-Cell Leukemia (ANKCL) [24]. The histological hallmark of these aggressive tumors is a polymorphic neoplastic infiltrate with extensive angiotropism, vascular destruction and tissue necrosis.

There are two variants of NKTCL, the nasal and extranasal forms [1,10,14,25]. The nasal form primarily affects the upper aerodigestive tract, although dissemination may occur in advanced disease stages; the extranasal variant is frequently disseminated at the time of the diagnosis, most patients having multiple organs and tissues involved. Bone Marrow (BM) involvement at the diagnosis is uncommon [4,26], whereas the Hemophagocytic Syndrome (HS) is frequent, especially in patients with advanced disease [27].

Aggressive NK-cell leukemia is very rare and has a fulminating clinical course [2,28-32]. Patients often present with systemic symptoms, BM involvement, pancytopenia, hepatosplenomegaly and abnormal liver function, and frequently develop HS, multiorgan failure and disseminated intravascular coagulation [33-39].

The oncogenic properties of the EBV have been recognized for almost fifty years, when the virus was discovered inside cultured Burkitt’s lymphoma cells [40]. In Europe and North America, the primary neoplasms associated with EBV are B-cell lymphomas and nasopharyngeal carcinomas, probably reflecting the primary targets of EBV infection, which are B cells and tonsillar epithelial cells [41,42]. NK-cell lymphomagenesis results from multiple oncogenic events occurring in EBV-infected NK-cells, which involve proteins encoded by EBV genes and non-coding viral RNAs expressed on the infected cells. The oncogenic events include deletion, mutation or methylation of genes involved in cell signal transducer pathways, survival and apoptosis, cycle progression and division, motility and adhesion, as well as in cytokine networks.

We have recently reviewed the epidemiological and diagnostic features of the NKTCL and ANKCL [18]. Herein, we review the molecular and biochemical mechanism involved in oncogenesis and disease manifestations of these aggressive NK-cell neoplasms.

Mechanisms involved in NK-cell oncogenesis

Although the pathogenesis of the NK-cell tumors remains poorly understood, some insights have been gained in the recent years concerning the mechanisms involved in oncogenesis and disease progression [43-45]. In accordance, disruptions of molecular mechanisms and cell signaling pathways involved in NK-cell maturation may lead to the development of NK cell malignancies [43]. Multiple genes involved in crucial biological processes are differentially expressed in NK-cell neoplasms, often due to deletions, mutations or abnormal methylation patterns (Table 1). However, in NK-cell tumors, where necrosis usually associates with tissue infiltration by mesenchymal cells and reactive inflammatory cells, it is often difficult to directly attribute the molecular alterations to the tumor cells without confirming it with biochemical or immunohistochemical studies.

Chromosomal and genomic abnormalities

Despite the difficulties in obtaining representative NKTL biopsy samples, several cytogenetic, genetic and genomic studies, using Comparative Genomic Hybridization (CGH) and loss of heterozygosity techniques, were performed [45-53]. Although genetic abnormalities specific for NKTCL and ANKL have not yet been identified, complex chromosomal abnormalities occur in a large fraction of cases, abnormalities of the chromosome 6 being the most frequent [46]. Cytogenetic abnormalities are seen in most patients and include pseudodiploidy in about half, hyperdiploidy in about one third, and hypodiploidy in about a tenth of cases [46]. Frequent cytogenetic aberrations are loss of chromosome 6q, 11q, 13q, and 17p and a common deletion of 6q in the target area 6q21-25 was identified [46,52]. An array-based CCG analysis revealed clear genetic differences between ANKCL and extranodal NKTCL, suggesting that these are two distinct diseases [53]. In accordance, recurrent aberrancies in NKTCL are gain of 2q, and losses of 6q16.1-q27, 11q22.3-q23.3, 15p14.1-p14.3, 5q34-q35.3, 1p36.23-p36.33, 2p16.1-p16.3, 4q12, and 4q31.3-q32.1; whereas those recurrently found in ANKCL are gain of 1q and losses of 7p15.1-p22.3 and 17p13.1 [53]. Most of the implicated genes have not been identified so far.

Tumor suppressor genes and oncogenes

Two 6q21 regions are frequently deleted in NKTCL [54]. One of these regions includes POPDC3 (Popeye Domain Containing 3), PREP (Prolyl Endopeptidase), PRDM1 (PR Domain Zinc Finger Protein 1, also known as BLIMP1, B-lymphocyte-induced Maturation Protein), AIM1 (Absent in Melanoma-1) and ATG5 (Autophagy related 5), whereas the other region includes LACE1 (Lactation Elevated 1) and FOXO3 (forkhead transcription factors of the O class Type 3) genes. Most of these genes are down-regulated in NK-cell neoplasms and are therefore considered as possible tumor-suppressor genes, whereas other act as oncogenes.

Tumor suppressor genes: FOXO3 (6q21) and PRDM1 (6q21) were identified as tumor suppressor genes having a potentially critical role in the biology of NKTCL and ANKCL as re-expression of PRDM1 and FOXO3 genes in NK-cell lines suppress NK-cell proliferation [55,56]. FOXO are class O forkhead family of transcription factors (FOX), which share the ability to be inhibited and translocated out of the nucleus by phosphorylation by proteins such as AKT/PKB (Protein
PRDM1 is a repressive transcription factor that is essential for the terminal B-cell differentiation and also plays a pivotal role in the negative regulation of NK-cell activation, by suppressing the release of Interferon (IFN)-gamma, and Tumor Necrosis Factor (TNF)-alpha, suggesting a secondary event rather than a triggering mechanism [82].

TP53 gene mutations have been associated with more advanced disease, of 100 cases of nasal NKTCL, nearly half had TP53 mutations [81]. TP53 has also been described in a significant number of NKTCL cases [45,74-84]. PRDM1 is a repressive transcription factor that codifies for p53, a protein that causes cells with damaged DNA to arrest at the G1 phase of cell cycle are frequently inactivated in tumor NK-cells by a combination of deletion and hypermethylation [45,67-69].

TNFAIP3 (6q23) was identified as a gene whose expression is induced by TNF-alpha, which codifies for a zinc finger protein and ubiquitin-editing enzyme, with both ubiquitin ligase and deubiquitinase activities; this protein has been shown to inhibit NFkB (nuclear factor-kB) activation and TNF-mediated apoptosis and is involved in the cytokine-mediated inflammatory and immune responses [70,71]. The HACE1 gene (6q21) codifies for an E3 ubiquitin-protein ligase involved in the terminal B-cell differentiation and also plays a pivotal role in the negative regulation of NK-cell activation, by suppressing the release of IFN-γ and TNF-alpha, which codifies for a zinc finger protein and ubiquitin-editing enzyme, with both ubiquitin ligase and deubiquitinase activities; this protein has been shown to inhibit NFkB (nuclear factor-kB) activation and TNF-mediated apoptosis and is involved in the cytokine-mediated inflammatory and immune responses [70,71].

Table 1: Major molecular and biochemical mechanisms potentially involved in aggressive mature NK-cell neoplasms.

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feline sarcoma viral oncogene v-kit), MYC (v-my c myelocytomatosis viral oncogene homolog), MAFB (V-maf musculoaponeurotic fibrosarcoma oncogene homolog), K-RAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) and β-catenin oncogenes have been described in a significant number of NKTCL cases [45,74,81,85-92].

The C-KIT proto-oncogene (4q11-q12) encodes a receptor tyrosine kinase (CD117) which is involved in normal hematopoiesis via interaction with the c-KIT ligand. Previous studies in nasal NKTCL performed in Asia revealed a variable frequency of cases with C-KIT gene mutations, depending on the country [81,85].

The MYC genes (8q24.21) encode for nuclear phosphoproteins (c-MYC, MYCN, and MYCL) that act as transcription factors to regulate expression of genes involved in cell cycle progression that codify for cyclins, such as CCNA2 (cyclin A2), and CDK (cyclin dependent kinases), such as CDKN1A and CDKN2B. Besides regulating genes involved in cell proliferation, they also control the complex networks of micro-RNAs and apoptosis mediators [86,87]. Although MYC expression appears to be up-regulated in NKTCL lymphoma cells, mutations, amplifications, and translocations of this oncogene have not yet been found [74].

Other studies have suggested that the inactivation of the cell cycle regulatory genes by DNA methylation could also contribute to tumorigenesis [88].

The MAFB gene (20q11.2-q13.1) codifies for MAFB, a transcription factor that plays an important role in hematopoiesis by repressing erythroid-specific genes in myeloid cells. Up-regulation of this oncogene was observed in NKTCL but also in T-cell and B-cell lymphomas [45,89].

The Ras and beta-catenin genes are implicated in various non-hematological cancers, but also in some hematological malignancies.

RAS is a family of related small proteins with GTPase activity, involved in signal transduction. Mutations in specific codons of one of the three RAS genes, H-RAS, K-RAS, and N-RAS, giving rise to constitutively active RAS proteins, are found in a variety of non-hematological tumors, but also in myeloid leukemia [90].

β-Catenin, an integral structural component of cadherin-based adherent junctions, exerts a crucial role in a multitude of biological processes. More specifically, β-catenin interacts with different transcription factors, being the key nuclear effector of canonical WNT signaling in the nucleus. Mutations in the β-catenin gene (3p21-3p22) resulting in imbalance in the structural and signaling properties of β-catenin have been implicated in non-hematological cancers, metastasis and angiogenesis [91]. Moreover, constitutive activation of the WNT/β-catenin pathway was observed in some hematological malignancies, such as chronic lymphocytic leukemia and mantle cell lymphoma [92].

Mutations of K-RAS and beta-catenin genes were examined in nasal NKTCL from Korea and Japan, using PCR-SSCP followed by direct sequencing. K-RAS and beta-catenin mutations were found in higher incidence in Japan as compared with Korea [81].

DNA repair

Cellular responses to DNA damage are mediated by a number of protein kinases, including ATM (Ataxia Telangiectasia Mutated), ATR (ATM And Rad3-Related) and CHK (Checkpoint Protein Kinases), which play an important role in DNA repair and chromosomal stability; these two kinase signaling cascades, the ATM-CHK2 and ATR-CHK1 pathways, are activated by DNA double-strand breaks and single-stranded DNA, respectively [93]. Alterations in the ATR gene resulting in an abnormal response to DNA single-strand break repair were also found in NKTCL, suggesting a role in lymphomagenesis [94].

Cell signaling transducing pathways

The STAT factors (Signal Transducers and Activators of Transcription), are transcription factors activated in response to cytokines or growth factors [95]. They act by a mechanism that requires tyrosine phosphorylation of the STAT proteins, as a result of their association with surface receptors having intrinsic tyrosine kinase activity, or through recruitment of members of the Jak (Janus kinase) family to activated surface receptors (Jak/STAT pathways) [95]. Constitutive activation of STAT factors, such as STAT3, has been shown to play a role in oncogenesis in a large variety of tumors, including NKTCL, this protein being localized in the nucleus of the tumor cells in the majority of the cases [96,97].

Other possible involved signaling pathways include those of the NOTCH (Notch homolog, translocation-associated), the NFκB (nuclear factor κB), and the WNT/β-catenin [45,98-101].

The NOTCH signal cascade comprises the NOTCH transmembrane protein and its ligands, as well as intracellular proteins transmitting the NOTCH signal to the nucleus [98]. The NOTCH signaling pathway leads to activation of the PI3K (phosphatidylinositol 3 kinase)/PKB and down-modulation of PTEN expression. PI3K/AKT was found to be activated in NKTCL, and nuclear expression of phosphorylated-AKT was observed in the nucleus of the tumor cells in most NKTCL samples [45].

The NFκB, a master regulator that controls the expression of a number of genes, is also known to be activated in EBV-infected cells, through LMP-1 (EBV-encoded latent membrane protein type 1) and/or TRAF (TNF receptor associated factor) signaling [100]. Signaling by the transcription factor NFκB involves its release from its inhibitor IκB, followed by its translocation into the nucleus. RelA, the protein constituting the most abundant form of NFκB, is detected in the nucleus of the neoplastic NK-cells, supporting the activation of this pathway in NKTCL. In addition it has been disclosed that TNFAIP3 (TNF-α-induced protein gene), an inhibitor of NFκB, is down-regulated in NKTCL. Surprisingly, the NFκB pathway genes were not included in the NKTCL gene signature in another recent study [101].

Concerning the activation of the WNT/β-catenin signaling pathway, mediated by WNT proteins, a group of secreted lipid-modified signaling proteins, nuclear expression of β-catenin was not observed in the tumor NK-cells, making its significance ambiguous [45].

Cell survival and apoptosis

The disequilibrium between pro-apoptotic and anti-apoptotic signals probably contributes to the survival of the neoplastic NK-cells [74,102,103]. In fact, although Fas (CD95) and Fas ligand (FasL/CD95L) are frequently expressed in NKTCL cells, mutations of the FAS gene are observed in about half of cases, most likely inducing resistance to apoptosis [102,103]. These mutations are often frameshift mutations arising in the death domain, leading to Fas proteins that are unable to transduce the apoptotic signal [102,103]. More recently, survivin (BIRC5, baculovirus inhibitor of apoptosis repeat-containing 5), an inhibitor of apoptosis frequently involved in tumor oncogenesis, was found to be overexpressed in NKTCL [74].
Cell division, chromosome segregation and cytokines

Aurora kinase A (AURKA, also called STK15 and BTAK), is a member of the Aurora/Pi3 family of mitotically regulated serine/threonine kinases, which are centrosome-associated and play an important role as regulators of chromosome segregation and cytokinesis, a process that ensure that each daughter cell receives the full genetic material [104,105]. Previous studies showed that AURKA is overexpressed in NK-cell neoplasms, being involved in the induction of centrosome amplification–distribution abnormalities [101]. In addition, overexpression of AURKA leads to increased degradation of p53, causing down-regulation of checkpoint pathways [106]. All these abnormalities can lead to centrosome amplification, chromosome instability, aneuploidy and propagation of genetic abnormalities, thereby inducing oncogenic transformation.

Angiogenesis

Angiogenesis is regulated by the coordinated action of various proteins with pro- and anti-angiogenic functions [107]. Pro-angiogenic factors include VEGF (Vascular Endothelial Growth Factor), FGF (Fibroblast Growth Factor), PDGF (Platelet-Derived Growth Factor), IGF (Insulin-Like Growth Factor), TGF (Transforming Growth Factor), CYR61 (Cysteine-Rich, Angiogenic Inducer 61), angiopoietins, and chemokines; anti-angiogenic factors include thrombospondin-1, angiostatin, and endostatin. Matrix metalloproteinases display a dual role in vascular development. NOTCH signaling affects remodeling of the primary vascular network into functionally and morphologically distinct arteries, veins, and capillaries.

Over-expression of the mRNAs transcribing for CYR61, a secreted protein that promotes the adhesion to endothelial cells, and VEGF, a signaling protein involved in both vasculogenesis and angiogenesis, is found in the vast majority of NKTCL cases by real-time quantitative PCR; expression of the CYR61 and VEGF proteins is detected in lymphoma cells by immunohistochemistry [108]. The expression of VEGF mRNA in NKTCL seems to be far more frequent than that of FGF mRNA, and CD44 expression is also detected in a considerable proportion of cases using immunohistochemistry studies, although these markers can not predict the angiogenesis potentiality of the NK-cells [109]. Previous studies that combined gene expression profiling and array-based CGH analyses showed that NKTCL overexpress several genes related to vascular biology, including the PDGFR (Platelet-Derived Growth Factor Receptor) alpha gene (PDGFRα) [45]. These results were also confirmed by immunohistochemistry studies, suggesting that PDGFR is involved in the disruption of the angiogenic pathways observed in NK-cell neoplasms [110].

Cytokine networks

Cytokines, such as IL-9, IL-10, and IP-10 (IFN-gamma-inducible protein-10, CXCL10), have also been implicated in the pathogenesis of the NK-cell neoplasms [8]. Cytokine production may occur due to the effect of EBV-oncogenic proteins in the lymphoma cells and often act in an autocrine manner, taking an important part in the neoplastic cell proliferation and invasion. BCRF1, an open reading frame of EBV, codifies for a viral protein that exhibits extensive functional homologies with human IL-10, a pleiotropic cytokine with immunosuppressive properties. Previous studies showed that human IL-10 is frequently expressed on NKTCL tumor cells, whereas the expression of viral IL-10 (vIL-10) seems to be variable [111,112]. In addition, the neoplastic NK-cells often express IL-9 and IL-9 receptors suggesting an autocrine loop [113]. Other genes codifying for cytokines and cytokine receptors that map to regions with recurrent aberrations include the IL-6 receptor (IL6R) (1q21.3) and the TNF receptor (TNFRSF21) (6p12.3) genes [45].

Mechanisms underlying disease manifestations

NK-cell neoplasms are recognized by their ability to invade the blood vessels and to destroy the tissues, but also to induce an uncontrolled macrophage activation that culminates with the development of the HS. Such disease manifestations are probably related to the chemotaxis and adhesion properties of the neoplastic NK-cells, to the release of cytotoxic proteins, as well as to their ability to produce proteases and TIl cytokines [114-116] (Figure 1).

Cell motility and tissue invasion

NKTCL tumors were shown to over-express invasion-associated genes and proteins, including those related to proteolysis, cell motility and chemotaxis. These include genes codifying for matrix metalloproteinases and their inhibitors, cathepsins and chemokines, among others [114-129].

Proteolytic enzymes: Tumor invasion and metastasis are facilitated by the up-regulation of various types of proteases, which induce the escape of cancer cells from the primary site, by breaking down the connective tissue and basement membrane, such as metalloproteinases and cathepsins both of which appear to be affected in NKTCL [114-124].

Matrix metalloproteinases (MMP, 1 to 28) are zinc-dependent endopeptidases capable of degrading extracellular matrix proteins, such as collagen and gelatin, being also able to process a number of bioactive molecules and thereby regulating cell growth, migration, invasion and angiogenesis [114,115]. Among them, MMP-2 (Gelatinase A) and -9 (Gelatinase B) have been associated with cancer [116]. The activity of MMP is inhibited by tissue inhibitors of metalloproteinases (TIM, 1 to 4) [117]. The unbalance of the expression of MMP and TIMP may contribute to the extensive necrosis observed in NKTCL, as well as to the tendency of these tumors to disseminate locally [120-123]. For instance, on a study in which the expression of MMP-1, -2, -3, -9, -11, -13 and TIMP-1 and -2 was evaluated by immunohistochemistry studies revealed that NKTCL cells and fibroblasts were positive for MMP-1 and MMP-11 in most of the cases, whereas MMP-2, -3 and -9 were expressed in neoplastic cell in between 30 to 65% of the cases; TIMP-1 was presented mainly in the epithelium and TIMP-2 was poor expressed of the all cases [120]. Another study revealed that most NKTCL were MMP-9+, expression of MMP-2 being also noted in some cases [121]. In addition, nasal NKTCL usually have a strong expression of MMP-9 as compared to nasal non-NKTCL [122]. NKTCL involving the upper aerodigestive tract over-express MMP-2 and MMP-9 genes as compared to non upper aerodigestive tract cases moreover, the MMP-9 expression is accompanied by an increased expression of uPAR (urokinase-type Plasminogen Activator Receptor) and MMP-2 and MMP-9 expression correlates with a poor prognosis [121,123].

In addition to metalloproteinases, well known to be secreted outside cells, there is increasing evidence that cathepsins (CTS), a group of lysosomal proteases that have a key role in cellular protein turnover, may also play an important role in the development and progression of malignant tumors [118,119]. NKTCL cells overexpress CTSB and CTSL, the genes that codify for cathepsins B and L, respectively and reduced expression of cathepsin D in NKTCL seems to be related to autophagic cell death [45,123,124].

Chemokines and chemokine receptors: Chemokines are a group of small (approximately 8 to 14 KD) structurally related molecules, which
are divided into 2 major subfamilies, CXC and CC [125]. They regulate leukocyte trafficking by interacting with a subset of 7-transmembrane, G protein-coupled receptors expressed on the cell surface and play fundamental roles in the development, homeostasis, and function of the immune system; they also have effects on endothelial cells, being involved in angiogenesis or angiostasis. Mature NK cells express a definite repertoire of CXC and CC chemokine receptors, including CXCR1 (CD181), CXCR3 (CD183) and CCR5 (CD195).

NK/T-cell lymphoma cells usually express CXCR3, whose main ligand is CXCL11 (IP9, IFN-gamma inducible protein type 9) [126,127]. Previous studies revealed that ANKL cells are positive for CXCR1 and CCR5, whose major ligands are CXCL8 (interleukin-8, IL-8) and CCL3 (MIP-1alpha, macrophage inflammatory protein type 1 alpha), CCL4 (MIP-1beta) and CCL5 (RANTES, regulated on activation, normal T cell expressed and secreted), respectively [128]. In addition, the serum level of IL-8, MIP-1alpha and MIP-1beta, are significantly elevated in ANKL patients and ANKL cells are positive for IL-8, MIP-1alpha, MIP-1beta and RANTES [129]. Moreover, NKTCL tissues over-express the genes codifying for several cytokines, including CCL2 (monocyte chemotactic protein-1, MCP-1), CCL8 (MCP-2), CCL18 (pulmonary and activation-regulated, PARC), CCL19 (EBI1 ligand chemokine, ELC), CXCL10 (IP10), CXCL12 (stromal cell-derived factor-1, SDF-1), and CXCL9 (monokine induced by gamma interferon, MIG) [45]. Altogether, these data would suggest that the chemokine system plays an important role in determining tissue infiltration by NKTCL and inflammatory cells.

Angioinvasion, angiodestruction and tissue damage

Angioinvasion by the neoplastic cells, angiodestruction and tissue necrosis are characteristic features of NKTCL. Although the exact mechanism is unknown, previous studies revealed that the presence of cytotoxic granule proteins, and the expression of apoptosis related and cell adhesion molecules on the neoplastic NK-cells are relevant factors [129-134].

Cytotoxic molecules and pro-apoptotic proteins: NKTCL overexpress the genes codifying for cytotoxic granule molecules, including granzymes, perforin and cathepsins [45]. In addition, previous studies have shown that NKTCL tumor cells usually express cytotoxic molecules (TIA-1, granzymes and perforin), and that Fas Ligand (FasL) is expressed in the majority of the lymphoma cells, while Fas (CD95) is found both in lymphoma cells and non-neoplastic cells [130]. In addition, ANKL patients have high serum levels of soluble Fasl and ANKL cells express the Fasl protein [129]. Lymphoma cells from cases with angiodestruction also express frequently FasL, Fas, CPP32 (apoptosis-induced cysteine protease 32), the apoptosis-promoting protein BAX, and the Ki67/MIB1 (mindbomb E3 ubiquitin protein ligase 1) nuclear proliferating marker, whereas in cases without angiodestruction, the neoplastic cells are frequently positive for FasL and BAX, and negative for Fas, CPP32, and Ki67/MIB1 [131].

Granzyme B-specific serine protease inhibitors, such as SERPINB9 (also known as protease inhibitor type 9, PI9) protect effector cells from their own cytotoxic activity and may participate in tumor escape and loss of expression of PI9 on NKTCL tumor cells was described as a poor prognostic factor [132,133].
Defensins are small cysteine-rich cationic peptides made by neutrophils with cytotoxic and microbicidal properties, which act by binding to the microbial cell membrane and forming pore-like defects [135]. Defensin beta 1 (DEFB1) is implicated in the resistance of epithelial surfaces to microbial colonization and there is evidence that the DEF1 gene may function as a tumor suppressor gene, its expression being associated with a large number of cancers [136]. The fact that DEF1 was found to be overexpressed in NKTCL, would suggest that this cytotoxic protein may also be involved in tissue damage [123].

Cell adhesion molecules: In that concerning Cell Adhesion Molecules (CAM), the neoplastic NK-cells frequently express the CD2, CD11a (lymphocyte function associated molecule type 1, LFA-1), and CD49d (integrin alpha 4) receptors and their ligands, CD58 (lymphocyte function associated molecule type 3, LFA-3), CD54 (intercellular adhesion molecule type 1, ICAM-1) and CD106 (vascular cell adhesion molecule type 1, VCAM-1). The frequency of CD2, CD54, CD58 and CD106+ cases is higher among NKTCL with angiodestruction, as compared with those without [131]. Finally, the integrin subunits alpha2 (CD49b) and alpha M (CD11b) are expressed at a significantly higher level on lymphoma cells in NKTCL with angioinvasion than in those without [134]. The VCAM-1 gene is overexpressed in NKTCL tissues, in comparison to that observed in normal NK cells [45].

Macrophage stimulation and hemophagocytosis

The Hemophagocytic Syndrome (HS) results from an uncontrolled proliferation and activation of macrophages and usually occurs as a consequence of T- and NK-cell activation, with production of IL-1 cytokines, such as IFN-gamma and TNF-alpha [137]. It may result from genetic defects on cytotoxic T-cells (CTL) and NK-cells or rather may be secondary to infections, autoimmune diseases and malignancy, including NK-, T- or B-cell lymphomas. Previous studies have shown that IL-18, also known as interferon-gamma inducing factor (IGIF), is a potent pro-inflammatory cytokine produced by activated monocytes that act on NK-cells and CTL, enhancing the Th1 response and inducing the production of IFN-gamma, a cytokine that is crucial for monocye/macrophage activation [138]. This effect is balanced by the action of IL-10, a potent anti-inflammatory cytokine, as well as of the IL-18 binding protein (IL-18BP), the natural inhibitor of IL-18 [139]. There is increasing evidence that both cytokines, IL-18 and IFN-gamma, relate to the genesis of the HS. In fact, patients with lymphoma-associated HS, and especially those with NK/T cell neoplasms, usually have high serum levels of IL-18 and IFN-gamma [33-35,140,141]. In addition, patients with HS usually show a severe imbalance between IL-18 and IL-18BP and recent studies in animal models demonstrate that the IL-18BP reduces the severity of the HS, by decreasing hemophagocytosis and reversing organ damage [142,143].

Previous studies have shown that NKTCL, as other entities that frequently associate to HS, such as EBV-associated CNKCL, exhibit a high expression of IFN-gamma and certain chemokines, particularly those induced by IFN-gamma, such as IP-10 (CXCL10), MIG (CXCL9), as well as MIP-1alpha (CCL3) [144-146]. The fact that MIP-1alpha causes macrophage chemotaxis and IFN-gamma promotes macrophage activation, would suggest that these molecules may play an important role in the pathogenesis of the HS, by recruiting and activating the macrophages, which are induced to undergo phagocytosis.

Conclusion

Proteins encoded by EBV genes and non-coding viral RNAs expressed on the infected cells are involved in NK-cell lymphomagenesis and disease progression, which occur as a consequence of multiple EBV-induced oncogenic events. The genes affected include, among others, tumor suppressor genes and oncogenes, as well as genes involved in cell signal transducer pathways, cell survival and apoptosis, cell cycle control and cell division, as well as cell motility, adhesion and signaling through cytokine networks, many of which are known to be involved in a wide variety of human cancers. These complex molecular and biochemical disturbances, not only justify the aggressiveness of the NK-cell neoplasms, but also explain most of the disease manifestations.

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