Cellular and Molecular Life Sciences

REVIEW

NF-κB pathways in hematological malignancies

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Abstract The nuclear factor κB or NF-κB transcription factor family plays a key role in several cellular functions, i.e. inflammation, apoptosis, cell survival, proliferation, angiogenesis, and innate and acquired immunity. The constitutive activation of NF-κB is typical of most malignancies and plays a major role in tumorigenesis. In this review, we describe NF-κB and its two pathways: the canonical pathway (RelA/p50) and the non-canonical pathway (RelB/p50 or RelB/p52). We then consider the role of the NF-κB subunits in the development and functional activity of B cells, T cells, macrophages and dendritic cells, which are the targets of hematological malignancies. The relevance of the two pathways is described in normal B and T cells and in hematological malignancies, acute and chronic leukemias (ALL, AML, CLL, CML), B lymphomas (DLBCLs, Hodgkin's lymphoma), T lymphomas (ATLL, ALCL) and multiple myeloma. We describe the interaction of NF-κB with the apoptotic pathways induced by TRAIL and the transcription factor p53. Finally, we discuss therapeutic anti-tumoral approaches as mono-therapies or combination therapies aimed to block NF-κB activity and to induce apoptosis (PARAs and Nutlin-3).

Introduction

The hallmarks of cancer are the distinctive and complementary capabilities of malignant cells to enable tumor growth and metastasis. They include sustenance of proliferative signaling, evasion of growth suppression, resistance to cell death, ability of replicative immortality, induction of angiogenesis, activation of invasion and metastasis, reprogramming of energy metabolism and evasion of immune destruction [1]. As well, the inflammatory process has proved to be an emerging enabling hallmark for cancer progression [2]. Very interestingly, the nuclear factor-κB (NF-κB) is able to induce most of these cellular alterations. There are several mechanisms that uncouple NF-κB from its normal regulatory processes and lead to its constitutive activation. Viral oncoproteins, i.e. Tax or v-Rel, have been shown to activate NF-κB and induce rapidly progressing lymphomas and leukemias. Cancer-related chromosomal translocations, deletion or mutations can disrupt genes encoding for the components of NF-κB signaling, i.e. MALT1, Bcl10, CARD11, TRAF3, NF-κB2, resulting in constitutive NF-κB activation. Constitutively activated NF-κB have been associated with several aspects of tumorigenesis, including promoting cancer cell proliferation, preventing apoptosis, increasing angiogenesis, cancer-related inflammation and metastatic potential [3, 4].

Before considering the role of the NF-κB pathways in hematological malignancies, we will introduce the pathways and the functions relevant to NF-κB activity in normal cells, with a particular attention to leukocytes, which represent the cellular targets of hematological malignancies.

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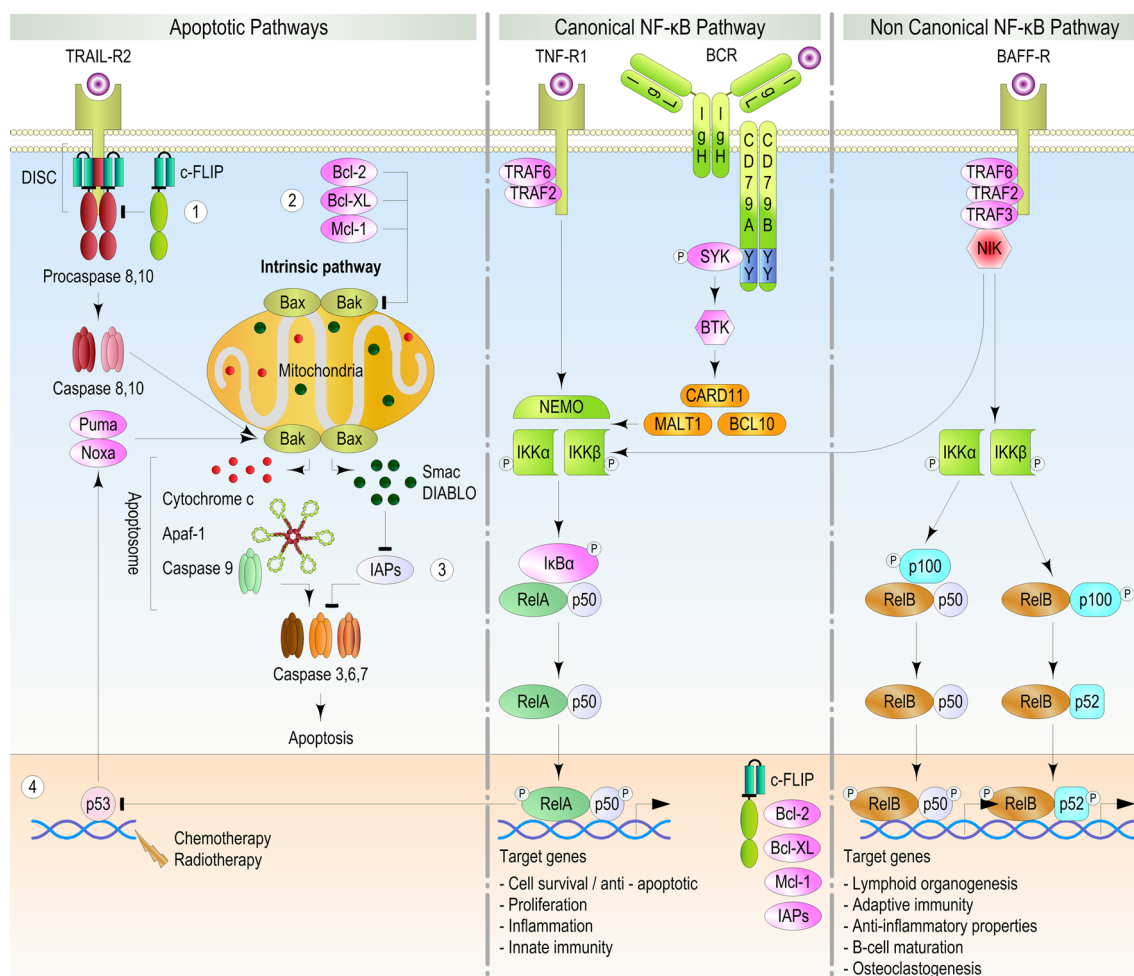Department of Life Sciences, University of Trieste, Trieste, Italy

Fig. 1 The canonical and non-canonical pathways of NF-κB and their interaction with the apoptotic pathways. The canonical and non-canonical NF-κB signaling pathways are represented in the *center* and to the *right* of the figure. The canonical pathway is activated through the TNF receptors' family, TLR4 and the antigen receptors BCR and TCR, while the non-canonical pathway is activated through a limited number of receptors, such as BAFF-R, CD40, RANK, CD30, and LTβ-R. The apoptotic pathways are summarized to the *left* of the figure in the extrinsic pathway (mediated by TRAIL) and the intrinsic pathway (mediated by p53). *Arrows* indicate activating steps, and *bars* indicate inhibitory steps. *Numbers in circles* indicate the steps of the apoptotic pathways inhibited by molecules that are induced by NF-κB activation (c-FLIP, Bcl-2 family's molecules, IAPs). This is the case of ① procaspase 8 and 10, whose activation is inhibited by cFLIP, ② the pro-apoptotic molecules Bak/Bax inhibited by the pro-survival Bcl2, Bcl-X$_L$, Mcl-1, ③ the effector caspases 3, 6, 7 targeted by the inhibitors of apoptosis (*IAPs*) family proteins, ④ the transcription factor p53 inhibited directly or indirectly by NF-κB

The two NF-κB pathways

The nuclear factor κB or NF-κB transcription factor family counts five subunits: RelA or p65, RelB, c-Rel or Rel, p50 (that with its precursor p105 is collectively known as NF-κB1) and p52 (that with its precursor p100 is collectively known as NF-κB2). NF-κB subunits act as dimers to allow a functional NF-κB activation. All subunits share an ~300 residues long homologous element, referred to as the Rel homology region (RHR), which is responsible for dimerization, inhibitor binding, nuclear localization and DNA binding. These NF-κB proteins can form homodimeric and heterodimeric combinations, with the exception of RelB, which does not share the common functional properties of the NF-κB family and seems not to form a stable, detectable homodimer. RelB has evolved into a specialized NF-κB subunit with unique amino acids optimized for selective formation of heterodimers with p50 and p52 [5, 6].

NF-κB signaling has been rationalized in two independent, yet interlinked, signaling pathways (Fig. 1).

The canonical or classical pathway, which is the most widely known NF-κB pathway, is mediated by the action of the RelA/p50 subunits (Fig. 1, center). In resting conditions, RelA/p50 dimers reside in the cytoplasm complexed with the physiological inhibitor of NF-κB α (IκBα), but upon cell activation, IκBα is degraded by

ubiquitin-mediated proteasome activity. The complex RelA/p50 can translocate into the nucleus, and, in its active form, starts the transcription of target genes. The canonical pathway is activated by the IκB-kinase (IKK) complex, which is composed by IKKα or IKK1, IKKβ or IKK2, and IKKγ or NEMO, and that, in turn, phosphorylates IκBα and causes its degradation.

The non-canonical or alternative pathway is dependent on the activation of the RelB subunit associated with p50 or p52 (Fig. 1, right). Differently from the canonical one, this pathway is activated by a more restricted number of ligands, such as the B cell-activating factor belonging to the TNF family (BAFF), CD40L, lymphotoxin β (LTβ), receptor activator nuclear factor-κB ligand (RANKL) or CD30L through the NF-κB-inducing kinase (NIK), which activates IKKα and, in turn, causes the degradation of p100, an IκB-like molecule which is the precursor of the p52 subunit. When cells are at rest, RelB is retained in the cytoplasm by p100 in the complexes RelB/p100 or RelB/p50/p100 and, upon p100 degradation, the dimers RelB/p50 and RelB/p52 [7] are released, translocate into the nucleus and start the transcription of target genes.

TNF-R-associated factors (TRAFs) are key intermediates in both the canonical and non-canonical NF-κB signaling pathways and act upstream of IKK or NIK activation, respectively. TRAF2 and TRAF3, in particular, seem to have distinct signaling functions and activities. While the recruitment of TRAF2 and TRAF6 is required for the activation of the canonical NF-κB pathway, TRAF3 does not seem to directly contribute to it, but rather to act as a negative regulator of TRAF2 and TRAF6 action, and seems to have a major role in the non-canonical pathway [8].

NF-κB subunits: functional roles by KO mice analysis

NF-κB was first discovered in 1986 in activated B cells for its property of interacting with the enhancer sequence of the immunoglobulin κ light-chain gene [9], stressing its relevance in B lymphocyte development. During the 1990s, the new technology of transgenic mice overexpressing exogenous copies of NF-κB or IκB genes, and of mice homozygous for null mutations [knock-out (KO) mice] in NF-κB, IκB or IKK genes, has provided invaluable models for elucidating the physiological functions and the regulation of the components of the two NF-κB pathways [10] (see Table 1). These approaches have shown that the different NF-κB subunits exert a relevant role in the development or functionality of mature lymphoid or myeloid cells. It was very soon evident that p50 and RelA are ubiquitously expressed in all cells, while the expression of RelB and p52 is restricted mostly to selected areas of hematopoietic organs and lymph nodes, i.e. thymic medulla, germinal centers (GCs), and marginal zone (MZ) of the spleen. Moreover, RelB expression seems to be relevant in dendritic cells (DC) and B cells [11–13]. c-Rel expression is restricted to lymphocytes, monocytic, granulocytic, and erythroid cells in mouse fetal and adult hematopoietic organs [14, 15].

Functionally, KO mice for RelA are characterized by embryonic lethality due to fetal hepatocyte apoptosis, while double KO mice for RelA and p50 show an earlier onset of embryonic death [16] (Table 1). These findings indicate that the dimer RelA/p50 has an important anti-apoptotic/prosurvival role, since its absence causes rapid cell death [17]. RelA acquires importance upon cell activation, since cell proliferation and functionality is impaired in RelA$^{-/-}$ cells [18]. Moreover, RelA/p50 seems to be important in dendritic cell development from bone marrow precursors [19].

The overall picture for RelB KO mice is clearly different. Up to 50 % of mice die by 3 months from birth for multiple pathological lesions and defects in acquired and innate immunity, while double KO mice for RelB and p50 show a markedly increased inflammatory phenotype in both severity and extent of organ involvement, leading to premature death within 3–4 weeks after birth [20]. If RelA seems to have a general anti-apoptotic and proliferative role, RelB seems to mediate mostly anti-inflammatory responses and to affect the functionality of the cells involved in the immune response. In B cells, RelB is necessary for the normal production of Ag-specific IgG in response to T cell-dependent and T cell-independent stimulation [21], and in natural killer (NK) and T cells, RelB is required for the development of innate and adaptive responses [22] (Table 1). Moreover, in RelB$^{-/-}$ mice, T cell functions are impaired with T cell inflammatory infiltrates in various organs, and T cell-dependent myeloid hyperplasia and splenomegaly due to extramedullary hematopoiesis [23, 24]. The absence of certain thymic and splenic dendritic cell (DC) populations [25] and the defective T cell-macrophage interaction in RelB$^{-/-}$ mice [21] seem to indicate that the immune defects could be due to the functional impairment in DC. In double KO mice for RelB and p50, the functionality of myeloid and lymphoid cells is further impaired if compared to RelB$^{-/-}$ mice: B cells are absent from inflammatory infiltrates and their development and number are reduced, while myeloid hyperplasia increase and the thymus is atrophic [20].

c-Rel subunit is essential for a variety of functions in hematopoietic cells, although it is dispensable for the differentiation of hematopoietic precursors. Upon cell activation, c-Rel is required for T cell and B cell proliferation, isotype switching, cytokine and cell-surface activation markers production and for regulating gene expression and immune effector functions in different mature macrophage populations [14, 15]. Recently, c-Rel has been found to be

Table 1 Role of the components of the canonical and non-canonical NF-κB pathway in the differentiation and activation of B cells, T cells, dendritic cells (DC) and macrophages

Subunit/s	B cells	T cells	Dendritic cells / Macrophages	Mouse phenotype
RelA	Differentiation: dispensable. Upon activation: required for B cell proliferation and isotype switching[18]	Differentiation: dispensable. Upon activation: required for T cell proliferation[18]	TNF stimulation: anti-apoptotic role[17]	Lethality: embryonic (days E15 and E16). Cause: fetal hepatocyte apoptosis
NF-κB1 /p50	Survival of non-activated B cells[29], B cell activation[30]: required			Mice develop normally. Hematopoiesis not affected. Defect in the immune system
RelA NF-κB1 /p50			DC differentiation: relevant (DC have 90% tendency to die[19]	Lethality: embryonic (days E13). Cause: fetal liver apoptosis[16]
RelA c-Rel	Differentiation: dispensable. Survival of T2 and mature B cells: required[28]	Differentiation: dispensable[27]	Erythrocyte and monocyte differentiation: required	Lethality: embryonic (days E13 ± E13.5). Cause: liver degeneration. Multiple hematopoietic cell defects. No B cells in lymph nodes[27]
IκBα	Inhibition of constitutive nuclear p50/RelA and p50/p50: required	Inhibition of constitutive nuclear p50/RelA and p50/p50: required		Lethality: 7 ± 10 days. Cause: severe inflammatory dermitis and granulocytosis[32,33]
IKKβ				Lethality: embryonic (days E12.5 and E14.5)[34,35]. Cause: fetal hepatocyte apoptosis
c-Rel	Differentiation: dispensable Upon activation: required for B cell proliferation, isotype switching, cytokine and immune molecules' production	Differentiation: dispensable. Upon activation, required for T cell proliferation, cytokine and immune molecules' production[14,15]. Generation of nTreg precursors: required[26]	Macrophage differentiation: dispensable	Mice are viable. Hematopoiesis is not affected. Defect in the immune system
c-Rel NF-κB1	Differentiation: dispensable	Upon activation: required for proliferation	Differentiation: dispensable. Upon DC activation: required[19]	Immune defects in the double mutants are more severe than in single mutants
Components of the non-canonical pathway				
RelB	T cell-dependent and -independent activation: required for normal production of Ag-specific IgG[21]	Development of NK cells and adaptive T cell responses: required[22].	Thymic and splenic DC development: required[25]. T cell-macrophage interaction and cytotoxic T cell response: required[21]	Lethality: 3 months (<50% mice). Cause: multiple pathological lesions, defects in acquired and innate immunity[22]. Characteristics: impaired GC, MZ structures, follicular DC networks upon Ag challenge in the spleen, impaired production of ELC, BLC, SLC[13]
NF-κB2 /p52	B cell proliferation and normal antibody production: dispensable[12]	T cell proliferation: dispensable	DC and macrophages APC functions: required	Mice develop normally. Disruption of splenic and lymph node architecture (no perifollicular MZ and B follicular areas)[11,12]
NF-κB1 NF-κB2	Development of T1 B cells: required			Growth retardation and craniofacial abnormalities (bone thickening, osteopetrosis, lack of teeth)[31]
RelB p50	B cell development and number: required.			Lethality: 3-4 weeks. Cause: severe inflammation of numerous organs. Myeloid hyperplasia, thymic atrophy, no B cells in inflammatory infiltrates[20]
RelB NF-κB2				Lethality: 3 months (50%)
RelB NF-κB1 NF-κB2				Lethality: soon after birth
IKKα	B cell maturation, Ab production: required			Ikkα -/-. Lethality: post-natal. Cause: multiple morphological defects[37-39]. IkkαAA. Mice are viable. Defects in lymphoid organogenesis and GC formation[40,41]
Aly/Aly	Upon activation: required for B cell proliferation.	Upon activation: required for IL-2 production	DC development and functions: dispensable	Aly/aly. No lymph nodes and PP, impaired Ab production, immune responses, no MZ B cells in the spleen[36]

Data are gathered from single and double KO mice for NF-κB subunits or components of the NF-κB pathways. Cells with a gray background indicate that the NF-κB signaling components are required for cell differentiation

Germinal centers (*GC*), marginal zones (*MZ*), Peyer's patches (*PP*), natural regulatory T cells (*nTreg*)

important for generating normal numbers of nTreg precursors and for the efficient induction, but not maintenance, of forkhead box P3 (Foxp3) + nTregs [19, 26–28].

Mice lacking the p50 subunit show multiple defects of immunity, since their B cells fail to proliferate in response to mitogens and elevated cell death [29] and are defective in basal and specific antibody production [30]. On the contrary, mice lacking the p52 subunit show an abnormal architecture of spleen and lymph nodes since peri-follicular marginal zones and B follicular areas are absent and the defect seems to be related to an altered functionality of dendritic cells and macrophages rather than B and T cells' [11, 12]. Double KO mice for p50 and p52 exhibit, soon after birth, growth retardation and craniofacial abnormalities, since they fail to generate mature osteoclasts and B cells, which are blocked at T1 stage [31].

KO mice for IκBα, the negative regulator of the RelA/p50 complex, show, as expected, sustained NF-κB cellular activation and die 7–10 days postnatally, affected by a severe widespread inflammatory phenotype, indicating a major irreplaceable function of IκBα in the termination of the NF-κB response [32, 33]. KO mice for the kinase IKKβ are characterized by embryonic death due to liver degeneration and apoptosis, a phenotype remarkably similar to RelA and p50 double KO mice [34, 35] (Table 1). IKKβ is indeed the kinase of the IKK complex that is required for the activation of the canonical RelA/p50 pathway.

Much of the knowledge gathered about the non-canonical pathway comes from studies on alymphoplasia (aly) mice, a natural strain with a mutant NIK gene [36], or on mice genetically modified for IKKα, the IKKα KO mice or the IKKα^{AA} knock-in mice, in which the two serines in the activating phosphorylation sites of IKKα are replaced by alanines. IKKα KO mice die postnatally and show severe impairment of multiple morphogenetic events, including limb and skeletal patterning, and proliferation and differentiation of epidermal keratinocytes [37, 38], while IKKα^{AA} mice are viable, but show defects in lymphoid organogenesis and GC formation [39]. IKKα is required for B cell maturation and the formation of secondary lymphoid organs, and induces the production of homeostatic chemokines, i.e. SLC, BLC, ELC, SDF1, and cytokines, i.e. BAFF, relevant in lymphoid organogenesis and adaptive immune reactions [40, 41].

Further in vivo and in vitro studies clarified that the two NF-κB pathways regulate several different functions that depend on the cell type and the stimulus applied [42]. The RelA/p50 canonical pathway was shown to promote inflammation, cell proliferation, cell survival through the production of several inhibitors of the apoptotic signaling, and to contribute to angiogenesis, tumor promotion and metastasis. The non-canonical pathway, instead, regulates lymphoid development and organization, adaptive immunity and has an anti-inflammatory activity [43, 44].

Receptors of the non-canonical pathway

Both pathways play an important role in the functionality and development of myeloid and lymphoid cells, but the privileged localization of the RelB and p52 subunits in the lymphoid organs, where lymphocytes, macrophages, and dendritic cells reside and interact, seems to indicate an important involvement of the non-canonical pathway in lymphoid functions. Moreover, several members of the TNF receptors superfamily, such as LTβ-R, BAFF-R, CD40, RANK, have a major role in the activation of the non-canonical pathway, and mediate relevant functions in lymphocyte development and activation that stress the importance of this pathway in such activities. LTβ-R is expressed in lymphoid stromal and epithelial cells, and binds to two different ligands, lymphotoxin and LIGHT, both being primarily expressed in lymphocytes. The major function of LTβR is to mediate the development and maintenance of peripheral lymphoid organs. BAFF-R is predominantly expressed in B cells, and plays an important role in mediating the survival and maturation of peripheral B cells.

CD40 is a TNFR member that is expressed on various cell types, including B cells, DC, monocytes, endothelial cells, and neurons. A major function of CD40 signaling is to regulate B cell activation and differentiation events, including proliferation and survival of activated B cells, germinal center (GC) formation, and antibody isotype switching. Another major function of CD40 is to mediate DC maturation and antigen presentation. CD30 expression on normal cells is restricted to activated T and B lymphocytes and eosinophils, and its cross-linking activates pleiotropic signals depending on the cell type and agents used. In malignant cells, CD30 is highly expressed in Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL), making it a relatively specific target for immunotherapy [45]. RANK is expressed in osteoclast precursors, DC and activated B cells, and primarily regulates osteoclastogenesis. It also has a role in DC survival and lymphoid organogenesis [46]. TNF-R2 is expressed by almost all cells, but differently from the TNF-R1, which is the main pro-inflammatory TNF receptor, its functions are not well known. In T cells, TNF-R2, but not TNF-R1, has been shown to induce the activation of the non-canonical pathway [47]. Since the two NF-κB pathways play a very important role in the development, survival and functional activation of lymphocytes, DC and macrophages, it is not unexpected that these pathways, when defective or over-activated, are the culprits of several hematological malignancies.

Antigen receptors and NF-κB

The B cell receptor (BCR) is the organizing principle of B cell biology, playing an obligatory role in B cell development, survival, antigen-driven clonal selection, and humoral immunity. The active, antigen-dependent BCR signaling pathway activates NF-κB, and is started by BCRs' antigen binding and clustering on the cell membrane [48].

In this pathway, the BCR consists of the antigen-binding IgH and IgL chains that are non-covalently coupled to the CD79A (Ig-α) and CD79B (Ig-β) subunits, which regulate BCR surface expression, internalization, and trafficking (Fig. 1, center). Upon antigen encounter and BCR clustering, CD79A and CD79B activate a variety of downstream signaling pathways. Each of these subunits has an immunoreceptor tyrosine-based activation motif (ITAM) consisting of two tyrosines in a conserved amino acid setting. BCR aggregation promotes tyrosine phosphorylation of these ITAMs by Src-family kinases, principally LYN, FYN, and BLK. Dually phosphorylated ITAMs recruit the kinase SYK, whose activation initiates a signaling cascade that engages the signaling pathways NF-κB, phosphoinositide 3-kinase (PI3-K), nuclear factor of activated T cells (NF-AT) and mitogen-activated protein kinase (MAP-K), ultimately leading to cell survival and proliferation. In the signaling cascade that finally triggers the NF-κB pathways, SYK interacts with the Bruton tyrosine kinase (BTK) that, in turn, activates the phospholipase Cγ2 (PLCγ2). PLCγ2 then catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] into diacylglycerol (DAG) and inositol triphosphate (IP3), resulting in increased intracellular calcium levels. The combination of DAG and increased intracellular calcium activates protein kinase Cβ (PKCβ), which in turn phosphorylates many substrates, including caspase recruitment domain-containing protein 11 (CARD11), which is a domain of the adaptor protein CARMA1. Once activated, CARD11 move to the plasma membrane and binds BCL10 and the mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) to form the complex termed the CBM complex, which, together with the transforming growth factor (TGF) β-activated kinase 1 (TAK1), is finally responsible for the activation of the IKK complex and the canonical NF-κB pathway. This pathway is characteristic of GC B cells and initiates the germinal center response towards selection and terminal differentiation of GC B cells. On the contrary, there is a 'tonic' BCR signaling typical of mature B cells that relies solely on PI3-K signaling, does not need BCR aggregation and is required for B cell survival. Such tonic signaling is likely to be antigen-independent as it affects all B cells, regardless of their IgH and IgL V regions.

In T lymphocytes, the primary activating signal is the recognition of suitable peptide–MHC ligands on the surface of antigen presenting cells (APC). The binding of MHC/antigen complexes by the T cell receptor (TCR)/CD3 orchestrates the intracellular events that depend on protein tyrosine phosphorylation. The signaling downstream of the TCR is similar to that of the BCR. Following the activation of the TCR, the Src family kinase LCK is recruited to the TCR complex via its association with CD4 or CD8 coreceptors, and phosphorylates the conserved ITAMs of the TCR, leading to the recruitment of ZAP-70, a 70 kDa protein tyrosine kinase of the SYK family. In T cells, the protein kinase Cθ (PKCθ), and not PKCβ, acts on CARD11 and allows the recruitment of the CBM complex to the immunological synapse (IS), and the subsequent activation of the IKK complex and of the canonical NF-κB pathway. Signals from other cell surface receptors, in particular CD28, cooperate with TCR signaling to produce full T cell activation [49].

Both BCR and TCR activate the canonical NF-κB pathway and the functions that are modulated by this pathway. Other receptors are required for the development or the functionality of B and T cells, and often activate both the canonical pathway and the non-canonical pathway. This is the case for BAFF-R, CD40, RANK, CD30 and LTβ-R. While BAFF-R and LTβ-R mediate primarily the non-canonical NF-κB pathway, CD40 and RANK have a role in the canonical NF-κB pathway, too. This unique feature of BAFFR is primarily due to its possession of an atypical TRAF-binding sequence, which interacts with TRAF3 and allows the triggering of the non-canonical NF-κB signaling [50].

NF-κB in lymphoid and myeloid development

Hematological malignancies are characterized by altered signaling of the molecules belonging to the intracellular signaling pathways activated by the antigen receptors on B cells (BCR) or T cells (TCR), several growth factor receptors, or by receptors accessory to immune functions. These malignancies derive from a precursor cell that can be at different stages of development and can rely more or less strongly on NF-κB signaling [51, 52].

B cells

Prepro-B cells are the earliest recognized B-cell precursors in the bone marrow, and become pro-B cells as soon as they begin to rearrange genes that encode the variable regions of the immunoglobulin μ (Igμ) heavy chain. When they begin to also recombine the variable regions of the light chains [V(D)J recombination] and start to assemble the BCR, B cells become small pre-B cells. This process is mediated by the recombination activating gene 1 (RAG1) and recombination activating gene 2 (RAG2). The canonical NF-κB is

more active in pre-B cells if compared to pro-B cells, promotes the transition to small pre-B cells [53], and together with the non-canonical pathway, controls the generation of λ-chain B cells [54]. These cells, called immature B cells, strongly interact with self-antigens and are either eliminated by apoptosis (negative selection), or undergo further rearrangement at the Igκ or Igλ loci to modify their specificity [55]. When they move to the spleen, immature B cells die by apoptosis in response to BCR crosslinking, as a checkout for potential self-reactivity [56]. During the transition from immature to mature B cells, the response to BCR interaction changes from cell death to cell proliferation, since B cells become responsive to the survival cytokine BAFF.

Immature B cells become transitional B cells through stages T1 and T2, and develop either into mature follicular (FO) B cells, which can leave and enter the circulation, and are the major source of T-dependent immunity and B cell memory, or into non-circulating marginal zone (MZ) B cells that can mount T-dependent and T-independent immune responses. At this stage, BCR cross-linking (1) induces the activation of the classical pathway of NF-κB and of c-Rel [57] with production of anti-apoptotic proteins, (2) upregulates the expression of *Nfkb2* gene resulting in the production of the p100 protein, and (3) induces the survival signaling mediated by BAFF [58], which, in turn, mediates the activation of the non-canonical NF-κB pathway.

NF-κB activity seems to have a major role in MZ B cells, since the number of these cells is reduced in several of NF-κB subunit's single gene deficiencies, including *Nfkb1* and *RelB*, but not *c-Rel* [59]. Mature B cells, when stimulated by a non-self (foreign) antigen and costimulated by antigen specific T helper cells, migrate into the B cell follicles of secondary lymphoid organs, such as lymph nodes, spleen and Peyer's patches, and start a productive immune response. At this stage, a new microenvironmental niche, known as the germinal center (GC) of the secondary lymphoid tissues, is formed and comprises B cells, now developed into germinal center B cells (GC B cells), T follicular helper (TfH) cells and follicular dendritic cells (fDC). GC B cells undergo affinity maturation and clonal expansion, are large, highly dividing cells, and the IgH of their BCR switches to IgG, IgA or IgE [60]. The antigen trapped on fDC is seized by the GC B cells and presented to the TfH cells, producing an immunological synapse that activates the B cell. During the germinal center reaction, at least two distinct DNA modifications—(1) somatic hypermutation (SHM) and (2) class switch recombination (CSR)—occur that are mediated by the B cell specific enzyme activation-induced cytidine deaminase (AID). GC B cells that recognize, through their BCRs, the antigen with high affinity, are positively selected to differentiate terminally into antibody-secreting plasma cells or memory B cells.

We can conclude that both the canonical and non-canonical NF-κB pathway subunits are required for the functional activation of leukocytes, while the non-canonical pathway, in the form of the subunits p50, RelB/p50 and the kinase IKKα, has a privileged role in B cell generation and maintenance (see Table 1). The receptors BCR and BAFF-R are essential for mature B cell survival, since mice that carry genetic mutations in either one of these genes lack mature B cells [57].

T cells and myeloid cells

T cell development takes place in the thymus, where CD4⁻CD8⁻ double negative (DN) thymocytes progress towards CD4⁺CD8⁺ double positive (DP) thymocytes, and finally to the mature CD4⁺ or CD8⁺ single positive T cells, which exit the thymus and enter the circulation. All thymocytes have some constitutive NF-κB activity, which is particularly high in the DN phase, when the pre-T cell receptor (pre-TCR) is assembled. Still, it is not clear whether NF-κB is essential for DN thymocytes' survival or only contributes to it. In DP thymocytes, TCR seems to activate NF-κB to pro-apoptotic functions in negative selection and to anti-apoptotic/pro-survival activities in positive selection [61]. As for B cells, in mature peripheral CD4⁺ or CD8⁺ T cells, TCR activates NF-κB to pro-survival signaling, most probably via the canonical pathway, given the involvement of IKKβ and IκBα. NF-κB seems to have a role in the generation and suppressive functionality of thymic T regulatory cells [62]. In particular, the NF-κB subunit c-Rel binds to the conserved non-coding sequence 3 (CNS3) of the Foxp3 locus and plays a major role in thymic and peripheral FoxP3 expression [63]. Moreover, in vitro studies have shown that NF-κB, in the form of c-Rel, RelA or p50 subunits, also plays a role in the development of effector T cells [64].

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) are among the growth factors responsible for the differentiation of a myeloid progenitor into granulocytes and/or macrophages. The NF-κB subunits c-Rel and RelA have been shown to be involved in the development of macrophages, differently from granulocytes [27]. NF-κB subunits p50, RelA and RelB seem to contribute to the differentiation of DC from progenitors [19, 25] (Table 1), while the NF-κB canonical pathway [65], but not the non-canonical RelB/p50 [66], seems to drive DC functional activation.

Hematological malignancies

Hematological malignancies comprise a collection of heterogeneous diseases, all originating from cells of the bone

marrow and the lymphatic system. The overall prevalence of hematological cancers is increasing, and they represent the fifth most common cancer group. Hematological malignancies include leukemias, lymphomas, plasma cell neoplasms, myeloproliferative neoplasms, histiocytic tumor, and dendritic cell neoplasms.

Hematological malignancies are classified according to several parameters, such as cell morphology and immunephenotype, together with genetic, molecular and clinical features [67]. More recently, genomic studies including candidate gene sequencing, copy number profiling and whole-genome/exome sequencing have led to the identification of recurrent mutations and amplifications/deletions in a spectrum of hematologic malignancies that have been shown to be prognostic of the clinical outcome. The disease entities are stratified according to their cell lineage (myeloid, lymphoid, and histiocytic/dendritic neoplasms) [68, 69] and their derivation from precursor to mature lymphoid cells, as for leukemias, lymphomas or malignancies of plasma cells [70, 71].

Leukemias

Leukemias are generally characterized by the accumulation of immature myeloid or lymphoid cells in the bone marrow, accompanied by the presence of large numbers of abnormal or immature leukocytes in the peripheral blood. While acute leukemias are clinically defined by a more rapid disease timing, which progress over weeks to months, ultimately culminating in bone marrow failure, chronic leukemias have a less aggressive presentation. Depending on the originating cell lineage, acute and chronic leukemia can be divided into acute lymphoid or myeloid leukemia (ALL or AML) and chronic lymphoid or myeloid leukemia (CLL or CML).

ALL is the predominant pediatric leukemia, while AML is the most predominant adult acute leukemia. AML is characterized by the accumulation of immature myeloid cells (erythrocytes, granulocytes, monocytes, platelets) in the bone marrow. The major subgroups of ALL include the precursor B and T lymphoblastic leukemias (pre B-ALL and pre T-ALL) with about 85 % of ALLs being pre-B cell tumors that typically manifest as childhood acute leukemias with extensive bone marrow and variable peripheral blood involvement [72].

Among chronic leukemias, CML is a rare disease and was the first malignancy for which a recurrent chromosomal translocation was identified, due to the rearrangement of the ABL1 proto-oncogene with the BCR gene. Whereas normal ABL is a weak tyrosine kinase, the BCR–ABL1 fusion gene encodes a chimeric protein with markedly enhanced tyrosine kinase (TK) activity. Moreover, chronic leukemias include chronic myeloproliferative neoplasms or disorders (MPDs), which are clonal disorders of haemopoiesis characterized by excessive production of terminally differentiated myeloid cells (i.e. the granulocytic, erythroid and megakaryocytic series). Myelodysplastic syndromes (MDSs) show ineffective myelopoiesis, with both excessive and at the same time ineffective cell proliferation [73].

Lymphomas and plasma cell neoplasms

Lymphomas are a heterogeneous group of disorders characterized by the malignant proliferation of mature B or T cells, and generally present as solid tumors of the lymphoid system, particularly localized in lymph nodes, spleen, thymus and bone marrow. A few lymphomas can be localized at extranodal sites, such as skin, stomach, intestine or brain. The most common form of lymphoma is the diffuse large B cell lymphoma (DLBCL), which accounts for 30–40 % of newly diagnosed lymphomas. The molecular characterization of these tumors allowed DLBCL to be divided into three histologically indistinguishable molecular subtypes that differ in the expression of thousands of genes and apparently arise from B cells that are at separate stages of differentiation and activation. Germinal center B cell like (GCB) DLBCLs express genes typical of normal germinal center B cells, while activated B cell like (ABC) DLBCLs are characterized by genes found in B cells activated via the BCR. Primary mediastinal large B cell lymphomas typically express genes of mature B cells [74]. This classification has a prognostic relevance, since ABC DLBCL is associated to a less favorable outcome as compared to GCB DLBCL.

Other B lymphomas include Hodgkin's lymphomas, Burkitt's lymphoma, follicular lymphoma and marginal zone B cell lymphomas (MZL), which include the extranodal marginal zone B cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), nodal MZL and primary splenic MZL. MALT lymphoma represents the most common extra-nodal lymphoma. Many cases of MALT lymphoma arise at sites affected by chronic inflammation and can be successfully treated by eradicating the source of inflammation. However, the concomitant insurgence of particular chromosomal abnormalities can promote disease progression.

Plasma cell or terminally differentiated B cell neoplasms have in common the expansion of a single clone of Ag-secreting plasma cells and a resultant increase in serum level of a single homogeneous Ig or its fragments. Among these, multiple myeloma (MM) is the most important and the most common (10 % of hematological malignancies), and is characterized by multiple tumorous masses of neoplastic plasma cells scattered throughout the skeletal system [75, 76]. It is noteworthy that, together with the clonal

proliferation of malignant plasma cells, the bone marrow microenvironment of tumor cells has a pivotal role in the pathogenesis of multiple myeloma.

Mature T cell and natural killer (NK) cell neoplasms are less numerous than mature B cell neoplasms, and among them we can consider adult T cell leukemia/lymphoma (ATLL), anaplastic large cell lymphomas (ALCL) or extranodal lymphomas, i.e. the enteropathy-associated T cell lymphoma, primary cutaneous T cell lymphomas [77].

BCR and the pathogenesis of hematological malignancies

BCR activation is utilized not only by normal B cells, but also by neoplastic B cells for survival and expansion. This knowledge comes from gene expression profiling and functional analyses of BCR activation and signaling, as well as from the clinical efficacy of kinase inhibitors that interfere with BCR signaling.

The processes of V(D)J recombination in pre-B cells or of somatic hypermutation (SHM) and class switch recombination (CSR) in the germinal center reaction represent critical steps that might predispose to malignancy. For example, during V(D)J recombination, translocations t(14;18) or t(11;14) can arise and cause the dysregulation of the genes BCL2 or cyclin D1, which are juxtaposed to the IgH locus, and are characteristic of follicular lymphoma or mantle cell lymphoma, respectively [78]. Activation-induced cytidine deaminase (AID), which operates during SHM and CSR, can mutate other genes in addition to Ig genes, as is the case of BCL6 or c-MYC. Deregulation of BCL6, which is a master regulator of germinal center B differentiation, characterizes most of DLBCLs, while translocation of the c-MYC gene is found in Burkitt's lymphoma. MALT lymphomas are associated with t(1;14) and t(11;18), where the t(1;14) moves the IgG promoter upstream of the gene that encodes BCL10, and results in overexpression of a truncated BCL10 protein and constitutive NF-κB activation [79, 80].

Genomic investigations of ABC DLBCL have provided clear genetic evidence that BCR signaling is central to the pathogenesis of human lymphomas [48] and depends on the activation of the NF-κB pathway. The B cell receptors in ABC DLBCL, similarly to BCRs in antigen-stimulated normal B cells, form prominent clusters in the plasma membrane. For these properties, the term 'chronic active BCR signaling' is used to describe the constitutive BCR activity in ABC DLBCL and several other lymphomas. On the contrary, Burkitt's lymphoma is characterized by the tonic BCR signaling and mainly depends on the activation of the PI3-K signaling pathway, and, together with GCB DLBCL, has been demonstrated to be scarcely dependent on NF-κB activity.

NF-κB pathways in hematological malignancies

The two NF-κB pathways seem to exert a specific role in the development of B and T cells or myeloid cells, with a major involvement of the non-canonical pathway in B cell development. Once matured, B cells and T cells exert their functional activities, probably through the combined activation of the two pathways, to allow the optimal cellular performance; on one side, the non-canonical pathway, which is more immune centered, and on the other side, the canonical pathway, which drives pro-survival and inflammatory activities.

The notion that the constitutive activation of NF-κB in cancer cells regulates the survival and pathogenesis of several malignancies is well acknowledged [81, 82]. This can happen since NF-κB regulates the expression of pro-inflammatory cytokines, chemokines, growth factors, cell cycle regulators, anti-apoptotic molecules, adhesion molecules and matrix proteases. On the contrary, the individual contribution of the two NF-κB pathways in tumorigenesis is not so well appreciated. Here, we present an update of the current knowledge on the specific involvement of the canonical and non-canonical pathways in hematological malignancies (see Table 2).

AML

The myelodysplastic syndrome (MDS) comprises a group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis leading to blood cytopenias and by a high risk of progression to acute myeloid leukemia (AML). In samples from 57 patients with MDS, activation of RelA was associated with blast counts, apoptosis suppression, and disease progression. Inhibition of the canonical pathway via siRNA, anti-RelA or Bortezomib, a drug that inhibits the proteasomal degradation of IκBα and p100 (see "Therapeutic approaches" for more details), induced rapid apoptosis of bone marrow cells [83]. A similar study on AML patients confirmed that the canonical pathway of NF-κB is constitutively active in AML cells in the form of RelA/p50 and p50/p50 complexes [84]. Death-associated protein kinase 1 (DAPK1) is a tumor suppressor and its expression is epigenetically suppressed in several tumors. In AML, the FMS-like tyrosine kinase 3/internal tandem duplication (Flt3/ITD) initiates the activation of a non-canonical NF-κB signaling pathway mediated by RelB/p52. It has been demonstrated that RelB/p52 is able to inhibit the tumor suppressor DAPK1 and promotes tumor development [85].

In murine erythroleukemia cells, the dimer RelB/p50, and not RelB or p50 alone, is induced by cAMP-dependent protein kinases and causes the upregulation of the transcription factor c-MYB, which is necessary for the proliferation

Table 2 The NF-κB canonical and non-canonical complexes are mostly tumorigenic in hematological malignancies

	RelB/p50	RelB/p52	RelB	RelA/p50	p50/p50
Acute myeloid leukemia (AML)		Activator: Flt3ITD. Inhibits: tumor repressor DAPK1, c.l.[85]		Constitutive activation (11 patients)[84] Bortezomib and anti-RelA siRNA induce apoptosis, c.l.[83]	Constitutive activation (11 patients)[84]
Erythroleukemia	Activator: cAMP. Induces: c-myb and inhibits cell differentiation, mouse model[86,87]		-		-
Chronic myeloid leukemia (CML)				Activator: c. a. BCR-ABL. Induces: RelA constitutive activation (8 patients)[88]	
Acute lymphoblastic leukemia (ALL)		Activator: c.a. Notch1, mouse models[90]		Constitutive activation (c-ALL, pre-B-ALL, pro-B-ALL, B-ALL and T-ALL), 39 patients[89] Activator: c a. Notch1, mouse models[90]	Constitutive activation (39 patients)[89]
Chronic lymphoblastic leukemia (CLL)			Constitutive activation of RelB and RelA. Sustain: cell survival and sensitivity to proteasome inhibitor (67 patients)[92] Activator: CD40L. Induces: Survivin and inhibits apoptosis (5 patients)[93]	Activator: STAT3 (15 patients)[91]	
Adult T leukemia/ lymphoma (ATLL)	Activator: HTLV-1 transforming protein Tax, mouse model[97]	Activator: HTLV-1 transforming protein Tax. Induces: p100 degradation[95] Activator: rearranged NF-κB2/p100 gene, c.l.[96]		Constitutive activation (7 patients and cell lines)[94] Activator: HTLV-1 transforming protein Tax. Induces: IKKβ and RelA activation[95]	Constitutive activation (7 patients and c.l.)[94]
DLBCL			Activator: overexpressed RelB. Inhibits: canonical pathway (RelA), Bcl-X$_L$ production and reduces cell survival, c.l.[99]	Activator: Constitutive activation of IKK2. Induces: development of lymphoma when BLIMP1, mouse model[100]	
Hodgkin's lymphoma (HL)		Activator: Notch1, c.l.[104] Activator: CD30 through TRAF1, c.l.[103] Activator: CD30, EBV-negative c.l. [102] Activator: CD30, c.l.[105]	Activator: CD30, EBV-negative c.l.[102] Activator: stable NIK, c.l.[101]	Activator: CD30, EBV-negative c.l.[102] Activator: CD30, c.l.[105] Activator: TRAF1. Induces: anti-apoptotic effects, c.l.[103]	Inducer: Bcl-3, c.l.[106]
Anaplastic large-cell lymphomas (ALCL)		Activator: CD30, c.l.[45,105]		Activator: CD30. Induces: cell cycle arrest, c.l.[45,105] Activator: TNF, c.l.[106]	Inducer: Bcl-3, c.l.[106]
Burkitt's lymphoma			Constitutive low activation, c.l.	Activator: IKKβ. Induces: growth inhibition and apoptosis, c.l.[107]	
Multiple myeloma (MM)	Activator: fibronectin adhesion, c.l.[112]	Activators: NFKB2, NIK, c.l.[108] Activators: mutated NFκB2, NIK, c.l.[110] Activators: TRAF2, TRAF3, CYLD, cIAP1/cIAP2, NFκB1, NFκB2, CD40, LTβR, TACI (155 patients)[109]	Constitutive activation in 40% primary MM. Promotes: cIAP2, Bcl-X$_L$, Bcl-2 and cell survival (52 patients)[111]	Constitutive activation, c.l.[108] Activator: mutated CYLD, NFκB1, TACI, NIK, c.l.[110]	

The triggers of the NFκB subunits' constitutive activation (if known) and the action mediated by the activated subunits are reported for several malignancies. Cells in gray indicate the few malignancies in which either the canonical or non-canonical pathway induces anti-tumorigenic activities. Data are presented in the RelB column when there is no mention in the paper of the subunit interacting with RelB. Data are from cell lines (*c.l.*), patients' material or mouse models. Constitutively active (c.a.)

of hematopoietic precursor cells, and in turn, inhibits cell differentiation [86, 87].

CML

In CML, the deregulated BCR-ABL tyrosine kinase has been demonstrated to induce NF-κB activation. In particular, constitutive activation of RelA-containing dimers has been measured in peripheral blood blasts from CML patients, indicating a role of the canonical NF-κB pathway in this malignancy [88]. We cannot exclude a contribution of the non-canonical pathway, since its activation has not yet been verified.

ALL

In a large population of patients with either B-ALL and T-ALL, the vast majority presents a constitutive activation of the canonical NF-κB pathway in the form of the RelA/p50 and p50/p50 complexes. It seems therefore that this constitutive activation of NF-κB serves as an important switch that ensures survival of ALL cells either by blocking apoptosis or by enhancing their proliferation [89]. T-ALL is characterized by activating mutations of Notch1. When constitutively active, Notch1 positively regulates the expression of the NF-κB subunits RelB and NF-κB2, and activates the IKK complex, causing the activation of the non-canonical and canonical NF-κB pathways. Target genes overexpressed by NF-κB activation are BCL2A1, CD83, CCR7, CCL5, and TRAF1, which are regulated by both the canonical and the non-canonical pathways [90].

CLL

In chronic lymphocytic leukemia (CLL), NF-κB has been found to be activated, to a variable degree, regardless of the disease stage or treatment status. In particular, RelA-binding complexes have been demonstrated to be constitutively active in peripheral blood samples of CLL patients, and their activation seems to be dependent on the action of the transcription activator STAT3 (signal transducer and activator of transcription-3) [91]. More recently, a role for RelB has been determined, together with RelA, by studying B cells isolated from bone marrow aspirates of CLL patients. RelB activity seems not only to sustain tumor cell survival, but also to enhance cell sensitivity to proteasome inhibitor [92]. In another study, RelB activation appeared to suppress apoptosis in CLL cell lines by inducing the anti-apoptotic gene Survivin [93]. More generally, the canonical and non-canonical pathways seem to cooperate to CLL progression.

ATLL

Human T cell leukemia virus type I (HTLV-I) is an oncogenic retrovirus that is etiologically associated with adult T cell leukemia/lymphoma (ATLL). NF-κB is constitutively activated in this cancer in the form of RelA/p50 and p50/p50 complexes [94] and can be activated by Tax, the transforming protein of HTLV-1, that directly interacts with the IKK complex or with IKKα and stimulates the processing of p100 to p52. Tax is thought therefore to activate both the canonical and the non-canonical pathways [95]. There are, anyway, alternative mechanisms independent of Tax that lead to NF-κB activation in this neoplasm. As for other lymphoid malignancies, in ATLL, a mutated form of the NF-κB2 gene has been described, and seems to interact with RelA and RelB and sustain the constitutive activation of NF-κB, playing a role in ATLL pathogenesis [96]. Another study performed on Tax-expressing mice demonstrated that NF-κB constitutive activation is dependent on RelB/p50 complex and follows the inactivation of the pro-apoptotic p53, indicating that decline in p53 function could be the first stage in the onset of ATLL [97].

DLBCL

Most genetic abnormalities in the diffuse large B cell lymphomas, except for the GCB DLBCL, result in the activation of the classical NF-κB pathway, with only two examples of mutations that are predictive of the activation of the non-canonical NF-κB pathway: (1) the structural alterations of the NF-κB2 gene and (2) the inactivation of TRAF3. By the way, it has not been demonstrated that their dysfunction will lead to the constitutive activation of the non-canonical pathway, rather than the canonical one [98].

ABC DLBCL, the most aggressive subtype of DLBCL, is strongly associated with the constitutive activation of the canonical NF-κB pathway. A recent paper has described a negative regulatory role of RelB in the activation of ABC DLBCL cell lines. RelB would be cleaved by the adapter Malt1 of the CBM complex and then undergo proteasome-dependent degradation within a time frame that correlates with persistent nuclear translocation of NF-κB. In this way, RelB has been demonstrated to negatively regulate the activation of the canonical NF-κB, measured as RelA and c-Rel subunits, and therefore, its degradation by Malt1 could induce enhanced activation of the canonical pathway [99]. Other key features of this lymphoma are the genetic alterations that interfere with terminal B cell differentiation, as the inactivating mutations of the B lymphocyte induced maturation protein 1 (BLIMP1), a key regulator of plasma cell differentiation. It seems indeed that the canonical NF-κB pathway, as an oncogenic event, and the BLIMP1, as

a tumor suppressor, interplay in the pathogenesis of ABC DLBCL [100].

Hodgkin's lymphoma and ALCL

Hodgkin's lymphoma can be divided into several subtypes, including the classical Hodgkin lymphoma (cHL), which is characterized by a low frequency of malignant Hodgkin and Reed–Sternberg (HRS) cells that derive from mature B cells embedded in a reactive background of non-neoplastic cells, with the majority of the tumor composed of various inflammatory cell types, including B cells, T cells, macrophages and granulocytes. This unique inflammatory milieu provides abundant cytokines that can activate NF-κB. Anaplastic large cell lymphomas (ALCL) are unique lymphomas originating from cytotoxic T cells. They are defined by the proliferation of predominantly large lymphoid cells, and the cytotoxic molecules perforin and granzyme B.

ALCLs share a number of molecular aberrations with Hodgkin's lymphoma. Interestingly, the neoplastic HRS cells of cHL show a global downregulation of the B cell-specific gene expression program, whereas the T cell-derived ALCLs have lost most of the expression of T cell-specific genes, supporting the view of a biologic analogy between both entities. Both lymphomas are characterized by the constitutive activation of the canonical and non-canonical NF-κB pathway and by the overexpression of the Notch1 and CD30 receptors, the last being overexpressed in multiple myeloma (MM), and adult T cell leukemia (ATLL), too.

In HL, it has been found that the maintenance of the HL phenotype strongly depends on the non-canonical NF-κB pathway, since the subunit RelB and the activating kinase NIK play a unique role in HL survival, differently from several DLBCLs [101]. HL is often associated with Epstein–Barr virus (EBV), and the EBV-encoded latent membrane protein-1 (LMP-1) has been demonstrated to activate the canonical and non-canonical NF-κB signaling pathways. Very interestingly, NF-κB constitutive activation has been observed in EBV-negative HL cell lines, and CD30 could be responsible for its activation [102]. CD30 seems to activate the non-canonical pathway through the action of TRAF1, which is typically overexpressed in this malignancy [103]. Notch1, too, seems to play a role in the activation of the non-canonical pathway in HL, since it induces the processing of the NF-κB2 gene product p100 into its active form p52. Moreover, Notch1 inhibition has been demonstrated to reduce NF-κB target genes and to impair the survival of HRS cells [104].

Also in ALCL, CD30 is among the receptors responsible for NF-κB activation and mediates several functions, i.e.

cell proliferation, but also cell cycle arrest and apoptosis. It is very interesting that only in ALCL, and not in HL, CD30 is able to mediate anti-proliferative effects. In particular, a prolonged stimulation of CD30 has been demonstrated to induce cell cycle arrest through the activation of the canonical pathway (RelA) [105], depending on specific intracellular domains of CD30 [45].

Finally, in both ALCL and HL, the dimer p50/p50 seems to be constitutively active and would be induced by the overexpression of Bcl3, a protein that shares structural features with IκBα proteins. Bcl3 has an anti-apoptotic effect in B and T lymphocytes and is tumorigenic in several malignancies [106].

Burkytt's lymphoma

Human Burkitt's lymphoma (BL) is a highly aggressive lymphoma, with the transcriptional deregulation of MYC due to chromosomal translocation being the pivotal event in lymphomagenesis. This lymphoma is characterized by a very low basal NF-κB activity consisting of RelB-containing heterodimers. Upon IKKβ activation, NF-κB is induced in the form of RelA and p50-containing complexes, and a reduced cell proliferation is observed together with enhanced apoptosis. MYC is able to induce the activation of the p53 pathway, and therefore apoptosis as a safeguard mechanism against malignant transformation. It seems that MYC overexpression sensitizes cells to NF-κB-induced apoptosis, and that persistent inactivity of NF-κB signaling is a prerequisite for MYC-mediated tumorigenesis [107].

Multiple myeloma

The activation of both the canonical and non-canonical NF-κB pathway is a clear hallmark of multiple myeloma and the driving force for its pathogenesis. The genetic screening of 155 MM samples reported mutations in ten genes involved in NF-κB signaling that cause the inactivation of TRAF2, TRAF3, cylindromatosis (CYLD), cellular inhibitor of apoptosis (cIAP)1/cIAP2 and the activation of NF-κB1, NF-κB2, CD40, LTBR, transmembrane activator and calcium-modulator, and cytophilin ligand interactor (TACI) [82, 108, 109]. More recently, it has been described that NF-κB2 mutation selectively activates the non-canonical NF-κB pathway, whereas CYLD, NF-κB1, and TACI mutations selectively activate the canonical pathway. Moreover, mutations affecting NIK expression, such as mutations in NIK, TRAF2, TRAF3, cIAP1, cIAP2, and CD40 genes, seem to activate primarily the non-canonical pathway. NIK overexpression and TRAF3 inactivation are two very common mechanisms in MM pathogenesis, even if their preferential involvement in the canonical or non-canonical NF-κB is still debated [108, 110].

Recently, Baud et al. described further the important role of the non-canonical pathway in the pathogenesis of MM. RelB was found to be constitutively active in nearly 40 % of the newly diagnosed MM patients studied, to induce the expression of the anti-apoptotic genes cIAP2, Bcl-X_L and Bcl-2, and to promote cell survival [111].

The tumor microenvironment is extremely relevant to MM pathogenesis, growth and drug resistance. Adhesion of MM cells to fibronectin induces the activation of the complex RelB/p50 of NF-κB and expression of the antiapoptotic cIAP2, indicating non cell-autonomous pro-tumoral activities from the microenvironment cells [112].

Microenvironment relevance in tumorigenesis

The tumor microenvironment is constituted by a varied society of cells (fibroblasts, endothelial cells, smooth muscle cells, granulocytes, B, T and NK lymphocytes, mast cells, macrophages and dendritic cells), and by the extracellular matrix (ECM) that surrounds cancer cells. Non-cell-autonomous, or micro-environmental, factors play an important role in tumor development, and therefore have been considered as important therapeutic targets for cancer treatment. Moreover, it has been demonstrated that gene expression profiling of the microenvironment is a good predictive tool of the clinical course of the malignancy [113].

There are several examples of non-autonomous relevance in tumorigenesis. In Hodgkin's lymphoma, the scarce HRS cells are embedded in a majority of inflammatory cells, and by expressing high level of several TNF receptor family members, can be chronically stimulated by the pro-inflammatory cytokines produced by the microenvironment. A recent work on an in vivo model of human T-ALL showed the unique role of the non-canonical NF-κB pathway (RelB subunit), which was activated in the stromal compartment and exerted a non-autonomous tumorigenic effect on leukemic cells [114]. CLL is characterized by the accumulation of mature, monoclonal B lymphocytes in peripheral blood, bone marrow, lymph nodes and other secondary lymphoid organs. It has been demonstrated that the lymph node microenvironment promotes B cell receptor signaling, NF-κB activation, and tumor proliferation [115]. Another study demonstrated that, by co-culturing CCL bone marrow-derived B cells and bone marrow-derived stromal cells, the presence of high RelB activity, together with RelA activity, conferred survival advantages to CLL BM B cells [92]. Finally, in multiple myeloma, the activation of NF-κB in bone marrow stromal cells (BMSCs) also contributes to MM pathogenesis, since several NF-κB-dependent factors produced by BMSCs lead to MM growth and prevent cell apoptosis [82].

From this overview on the canonical and non-canonical pathways in hematological malignancies, we can summarize that in most neoplasms, from B and T leukemias to B and T lymphomas and multiple myeloma, both pathways are activated. The canonical pathway has generally a major role in tumorigenesis, as for ABC DLBCL, ALL, CML, AML, and MM (Table 2), but its relevance is clearly tumor and context-dependent. There are some exceptions to be considered: in ALCL (Table 2), the canonical pathway has the ability to induce growth arrest and apoptosis depending on CD30 stimulation, and in Burkitt's lymphoma, a cancer with a low NF-κB activity, the canonical pathway, when activated, seems to mediate apoptosis and to counteract the oncogenic action of MYC.

Several evidences point to the relevant role of the non-canonical pathway in the pathogenesis of Hodgkin's lymphoma, probably due to its peculiar features, a deregulated BCR signaling, the overexpression of the receptors able to activate the non-canonical pathway, and the great majority of inflammatory cells surrounding the HRS cells that can chronically stimulate cancer cells. Very interestingly, in ABC DLBCL, RelB has been shown to counteract the activity of the canonical pathway and to induce a final anti-tumorigenic and pro-apoptotic activity. An inhibitory action of RelB on the canonical pathway or on genes expressed by the canonical pathway has been described in several cell types [116, 117], and recently, RelB has been shown to inhibit cell proliferation and tumor growth through the activation of the transcription factor p53 [118]. In some malignancies, RelB activation could be a mechanism to control excessive canonical pathway activation. Whether RelB is a tumor suppressor or tumor promoter depends most likely on several factors, such as the tumor type and the surrounding microenvironment.

NF-κB signaling and apoptotic pathways

The cellular commitment to survival or cell death is fundamental to the benefit of the organism, and errors in this process can lead to autoimmunity or cancer. One of the main functions of NF-κB is the blockade of cell death through the transcriptional induction of genes encoding anti-apoptotic and antioxidant proteins. The apoptotic pathway is controlled by two distinct, yet interlinked, signaling pathways. Extrinsic apoptosis is induced by the interaction of the pro-apoptotic ligands—tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), TNF or Fas ligand—to their specific receptors on infected or damaged cells. Intrinsic apoptosis can be triggered by several intracellular stress conditions, including DNA damage and oxidative stress, which can activate the transcription factor p53. NF-κB has been demonstrated to counteract apoptosis by regulating critical steps along the apoptotic pathways [119] (see Fig. 1, left).

TRAIL activates the extrinsic apoptotic signaling pathway through TRAIL-R1 or TRAIL-R2, leading to the activation of caspase-8 and -9 and the assembly of the death-inducing signaling complex (DISC). Then, DISC activates Bid, which interacts with Bax and Bak and starts the intrinsic apoptotic pathway, or directly activates caspase-3, -6 and -7, inducing apoptosis. NF-κB activation induces the expression of several anti-apoptotic components of TRAIL signaling. Examples are (1) c-FLIP, which inhibits the activation of the caspases 8/10 (Fig. 1, ①); (2) the pro-survival Bcl-2 proteins (Bcl-2, Bcl-X$_L$, Mcl-1), which inhibit the action of the pro-apoptotic (Bax, Bak) components (Fig. 1, ②); (3) inhibitor of apoptosis (IAP) proteins (c-IAP1, c-IAP2, X-IAP, survivin), which inhibit the caspases involved in the effector phase of apoptosis (Fig. 1, ③).

The p53 tumor suppressor and transcription factor is one of the first lines of defense against the effects of genotoxic damage, oncogene activation, metabolic changes and hypoxia [120]. p53 induces the activation of the intrinsic apoptotic pathway, as previously described [121]. Briefly, p53 upregulates NOXA and PUMA, two proteins of the Bcl-2 family that interact with Bax and Bak at the mitochondrial site and start the apoptotic cascade with the formation of the signaling complex apoptosome, and the final activation of the effector caspases-3, -6 and -7 that induce cell apoptosis. The amount of p53 protein in cells is regulated by HDM2 (known as MDM2 in mice), which functions as an E3 ubiquitin ligase and induces p53 proteolysis with the final inhibition of the apoptotic pathway. Expression of HDM2 and the consequent downregulation of p53 can be induced by IKK and by NF-κB activation. NF-κB interacts with p53 in different ways, and it seems to act mainly as an antagonist to p53 transactivation (Fig. 1, ④). The canonical pathway seems to have a major role: IKKβ was shown to induce NF-κB activation, HDM2 expression and the subsequent p53 downregulation [122], while RelA was able to antagonize p53 activation through the sequestration of the p300 and CREB binding protein (CBP) co-activators [123]. Also, the non-canonical pathway has been demonstrated to have an antagonistic role to p53, since IKKα, through the phosphorylation of CBP, switches the binding preference of CBP from p53 to NF-κB [124]. Recent evidence reports that the canonical and non-canonical NF-κB pathways can, under some circumstances, mediate the pro-apoptotic effect, growth arrest and the inhibition of cancer development. In particular, RelA was able to stabilize p53 and cooperate with the pro-apoptotic signaling (via TRAIL-R2 induction) [125, 126], and RelB could inhibit cell proliferation and tumor growth through p53 transactivation [127]. NF-κB2 is aberrantly expressed in many tumor types and has been implicated as a regulator of cell proliferation. In particular, p52 was shown to cooperate with p53 to regulate p53 target genes such as PUMA, TRAIL-R2 by associating directly to

the p53-regulated promoters [128]. Very interestingly, p53 has a non-autonomous effect, since it has a pro-tumorigenic role when its pathway is deregulated in stromal cells [129], or, when expressed in stromal fibroblasts, p53 is able to attenuate cancer cell migration and invasion [130].

Therapeutic approaches

Given their relevance in cancer pathogenesis and progression, NF-κB and its signaling components are considered a good therapeutic target for the treatment of malignancies [131].

To this purpose, numerous small inhibitory peptides have been designed to target specific patterns of the molecules most relevant to NF-κB activation, i.e. IKKs kinases, the proteasome 26S, or, more recently, the NF-κB subunits, and block their activity. Bortezomib is an inhibitor of the proteasome 26S, which is responsible for the degradation of IκBα and the processing of p100. Bortezomib has been approved for the treatment of newly diagnosed multiple myelomas, relapsed/refractory multiple myelomas, and mantle cell lymphomas. The anti-cancer mechanisms of Bortezomib, described in preclinical studies, include the upregulation of pro-apoptotic proteins (i.e. Noxa, IκBα), the inhibition of NF-κB and the suppression of several anti-apoptotic proteins (Bcl-X$_L$, Bcl-2). Other compounds selectively target and inhibit the catalytic subunits of the IKKs, preventing IκBα from degradation and the canonical NF-κB subunits from nuclear translocation. The main problem with the current targeting of kinase activity or proteasome degradation is specificity: the blocked mechanisms share their activity with other signaling pathways, and therefore, an effect on a pathway different from NF-κB can be observed. By using innovative technologies with an increased specificity to the target, such as siRNA, aptamers or combination of the two [132], potential toxic effects could be avoided. As we recently described, the optimization of these drugs with increased specificity towards NF-κB subunits is ongoing [42].

TRAIL has emerged as a promising candidate for cancer therapy by virtue of its ability to trigger extrinsic apoptosis in various types of cancer cells without significant toxicity toward normal cells. The description and rationale for TRAIL-based therapies have been extensively treated in a recent review [133], and have shown to be relevant for the treatment of hematological malignancies, too [134, 135]. Recombinant TRAIL molecules or agonistic antibodies targeting TRAIL receptors, termed pro-apoptotic receptor agonists (PARAs), have been shown to activate the apoptotic pathway that is often inhibited in cancer cells, due, among other factors, to p53 inactivation. A major limitation to the clinical benefit derived from cancer drug therapies, and from TRAIL-based therapies as well, is drug

resistance. Mechanisms of TRAIL resistance have been extensively studied in the last decade, and it seems that NF-κB activation, which is generally deregulated in cancer, has a major role in it.

Another important anti-neoplastic strategy relies on the possibility to activate the p53 pathway by disrupting the protein interaction between p53 and MDM2. The Nutlins were identified as the first potent and selective small-molecule MDM2 antagonists able to inhibit the p53/MDM2 interaction, leading to stabilization of p53 and activation of the p53 pathway [136]. Nutlins are able to induce apoptotic cell death when added to primary leukemic cell cultures, but also to promote leukemic maturation [137], and paradoxically activate anti-apoptotic genes [138]. This suggests a preferential use of Nutlins in synergistic effect when used in combination with the chemotherapeutic drugs for the treatment of hematological malignancies. Nutlins also display non-cell autonomous biological activities, such as inhibition of osteoprotegerin [139], a soluble inhibitor of TRAIL [140], and stromal derived factor-1 (SDF-1) expression by primary endothelial cells or stromal fibroblasts, respectively [130], or induction of T cell proliferation by dendritic cells [141].

Combination therapies

In hematological malignancies, there are several evidences of the antagonism between the apoptotic pathway driven by TRAIL or p53, and the pro-survival NF-κB pathway. Whatever the nature of the anti-tumoral drug considered, the activation of the NF-κB pathway can confer resistance to the treatment.

In follicular lymphoma, resistance to TRAIL-based therapies is promoted by CD40L through the activation of NF-κB and the subsequent upregulation of c-FLIP and Bcl-X$_L$ [142]. In CLL, constitutively activated Notch 1 signaling is involved in the activation of NF-κB and in the increased expression of c-IAP2 and X-IAP, which eventually cause survival and apoptosis resistance [143]. Drug resistance can also be driven in a non-autonomous fashion by stromal cells, as in CLL, where stromal cells of the bone marrow express CD40L and show NF-κB activity [144, 145]; or in MM, due to increased synthesis of c-FLIP by the NF-κB-activated stromal microenvironment [146].

Combined regimens of therapeutics inhibiting NF-κB activation on one side and specifically activating the pro-apoptotic pathway have shown promising results, both in preclinical models and in clinical trials. This is the case of hematological malignancies, too. Nutlin-3, by stabilizing p53, enhances Bortezomib-mediated mitochondrial apoptosis in p53-mutated mantle cell lymphomas [147], or potentiates the cytotoxic effects of Bortezomib in multiple myelomas [148], even overcoming non-autonomous protumorigenic effects of surrounding stromal cells [149].

Bortezomib, on the other side, cooperatively enhances TRAIL-mediated apoptosis in AML [150], CLL, MM [146], and Burkitt's lymphoma via upregulation of TRAIL and its death receptors [151]. Failure in overcoming TRAIL resistance in TRAIL-based therapy could be due to the fact that, along with the apoptotic extrinsic pathway, TRAIL is able to induce non-apoptotic functions on several malignancies [152]; a fact that has been ascribed to the activation of NF-κB and PI3-K/AKT [153]. Therefore, the final choice of the combination therapy to consider strongly depends on the particular malignancy that is targeted.

Conclusions

The anti-apoptotic role of the canonical NF-κB pathway is a common feature of all malignancies, independent of the cell of origin. Hematological malignancies can be considered differently from other malignancies in terms of NF-κB pathways, since (1) the components of the non-canonical pathway are preferentially expressed in lymphoid organs where the development and activation of lymphoid and myeloid cells take place; and (2) the receptors that mediate the non-canonical pathway are strongly involved in the processes of immune response and lymphocyte survival. For these reasons, hematological malignancies appear to be a preferential site for the action of the non-canonical NF-κB signaling. In this regard, it is interesting that in Hodgkin's lymphoma, characterized by unpaired BCR activity and enhanced expression of "non-canonical receptors", the non-canonical pathway seems to have a relevant role, while in ABC DLBCL, which is strongly dependent on the BCR-dependent activation of the canonical pathway, RelB activity antagonizes the activation of the canonical pathway.

More research is needed to better define the relative role of the two NF-κB pathways, and in particular, in what cancers the non-canonical pathway cooperate or inhibit the activation of the canonical pathway. In this way, new therapies targeting specifically either or both NF-κB pathways could be developed. The combined therapeutic approach, obtained by blocking NF-κB and inducing the apoptotic response, will possibly obtain a most favorable outcome.

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