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Review

NF-κB deregulation in Hodgkin lymphoma

Marc A. Weniger, Ralf Küppers *

Institute of Cell Biology (Cancer Research), Medical Faculty, University of Duisburg-Essen, Virchowstr. 173, 45122 Essen, Germany

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ABSTRACT

Hodgkin and Reed/Sternberg (HRS) cells in classical Hodgkin lymphoma (HL) show constitutive activity of both the canonical and non-canonical NF-κB signaling pathways. The central pathogenetic role of this activity is indicated from studies with HL cell lines, which undergo apoptosis upon NF-κB inhibition. Multiple factors contribute to the strong NF-κB activity of HRS cells. This includes interaction with other cells in the lymphoma microenvironment through CD30, CD40, BCMA and other receptors, but also recurrent somatic genetic lesions in various factors of the NF-κB pathway, including destructive mutations in negative regulators of NF-κB signaling (e.g. *TNFAIP3*, *NFKBIA*), and copy number gains of genes encoding positive regulators (e.g. *REL*, *MAP3K14*). In Epstein-Barr virus-positive cases of classical HL, the virus-encoded latent membrane protein 1 causes NF-κB activation by mimicking an active CD40 receptor. NF-κB activity is also seen in the tumor cells of the rare nodular lymphocyte predominant form of HL, but the causes for this activity are largely unclear.

Contents

1. Introduction

Hodgkin lymphoma (HL) is a B cell-derived lymphoid malignancy that is subdivided into a classical form, which accounts for about 95% of cases, and nodular lymphocyte predominant HL (NLPHL) [1]. The tumor cells are called Hodgkin and Reed/Sternberg (HRS) cells in classical HL (cHL) and lymphocyte predominant (LP) cells in NLPHL. HRS cells are presumably derived from pre-apoptotic germinal center (GC) B cells, whereas LP cells stem from positively selected germinal center B cells [2]. Both types of tumor cells typically account for only about one or few% of cells in the lymphoma tissue [1], indicating that microenvironmental interactions play a major role in the pathophysiology of the disease. Because cHL is more frequent than NLPHL, and as several cell lines have been established from HRS cells of cHL, but only a single one from LP cells, we know considerably more about the pathogenesis of cHL than about NLPHL. HRS cells show deregulated activation of numerous signaling pathways, including the JAK/STAT, MAPK/ERK, NOTCH1, and PI3K/AKT pathways [3].

A further prominent deregulated pathway in HRS cells is the NF-κB pathway. In normal GC B cells, NF-κB is only transiently activated when B cells expressing a high affinity B cell receptor (BCR) are positively selected by interaction with T helper cells and signaling through the BCR [4,5]. However, HRS cells show constitutive activity of NF-κB, which was first recognized over 20 years ago [6,7]. Since then, numerous insights have been obtained about

* Corresponding author.
E-mail address: ralf.kueppers@uk-essen.de (R. Küppers).

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the reasons and consequences of the constitutive NF-κB activation in HRS cells.

2. The NF-κB signaling pathways

The NF-κB transcription factor family is composed of five members, namely REL, RELA (p65), RELB, p50 (NF-κB1), and p52 (NF-κB2) [8]. These factors can function as homo- or heterodimers and regulate hundreds of target genes involved in numerous cellular processes, including cell survival, proliferation, cell adhesion, and differentiation [8]. Two signaling pathways are distinguished that lead to activation of particular NF-κB factors, the canonical (or classical) and the non-canonical (or alternative) pathway (Fig. 1) [8]. REL, RELA and p50 are factors of the canonical pathway, whereas p52 and RELB dimers are the principal NF-κB factors of the non-canonical pathway.

In B cells, the canonical pathway is typically activated by stimulation of cell surface receptors such as CD30, CD40, and RANK, which are members of the tumor necrosis factor (TNF) receptor (TNFR) superfamily, the BCR, and Toll-like receptors (TLRs). The various cell surface receptors that activate NF-κB signaling use a variety of adaptor molecules and signaling components that mediate signaling [8]. The TNFR family members, for example, are associated with TNFR-associated factors (TRAFs), which upon activation of the receptors induce activation of the IκB kinase (IKK) complex. This complex is composed of three proteins, the catalytic subunits IKKα and IKKβ, and the regulatory factor IKKγ (also called NEMO) [8]. Upon activation, the IKK complex phosphorylates IκBs, the central negative regulators of the canonical pathway. The most important IκB family member in B cells is IκBα, encoded by the *NFKBIA* gene, but IκBβ and IκBε also contribute to NF-κB regulation. IκBs bind to the NF-κB dimers in the cytosol and prevent them from translocating into the nucleus. Phosphorylation of IκBs by the IKK complex marks the factors for ubiquitination, which then leads to their degradation by the proteasome. Once the IκBs are degraded, the nuclear localization signal of the NF-κB dimers becomes functional so that NF-κB factors can enter the nucleus and bind to κB binding sites in promoters and/or enhancers of their target genes.

Additional negative regulators of the canonical NF-κB pathway are A20, encoded by the *TNFAIP3* gene, and CYLD. These function as deubiquitinating enzymes, removing activating K63-linked ubiquitin chains from their target proteins [8]. In addition, A20 adds K48-ubiquitin chains to its target proteins, inducing their proteasomal degradation [8]. The targets are mainly upstream of the IKK complex in the signaling cascade or components of the IKK complex itself, and include the afore mentioned TRAFs, but also other regulators, including RIP [8].

The non-canonical pathway is triggered by several receptors, including CD40, the lymphotoxin receptor, and TLRs [8]. Receptor signaling leads to activation of the NF-κB inducing kinase (NIK; also called MAP3K14). NIK phosphorylates and thereby activates IKKα dimers. Activated IKKα subsequently phosphorylates p100 in p100-RELB dimers. This leads to a partial degradation of p100 to p52. The p52-RELB dimer is now capable of translocating into the nucleus and stimulating transcription of its target genes. A main negative regulator of the non-canonical pathway is TRAF3, which induces degradation of NIK [8]. In the absence of stimulation of the non-canonical NF-κB pathway, NIK levels are very low, because of TRAF3 activity. Upon stimulation of non-canonical NF-κB signaling, TRAF3 is degraded, causing accumulation of NIK, which then becomes active and mediates further signaling.

Distinct NF-κB dimers have different DNA binding sites, causing distinct, albeit partly overlapping genes to be regulated by the various NF-κB dimers. As already indicated some receptors trigger both NF-κB pathways, and there is additional cross-talk between the pathways at various levels [8].

3. NF-κB activity in HRS cells

HRS cells express all five NF-κB factors [6,9–17]. The dimer composition and activity of these factors were mainly studied in HL cell lines, as the rarity of the HRS cells in the HL microenvironment impairs most molecular biological assays with biopsy specimens. In HL cell lines, nuclear REL, p50 and p65 were identified [6], demonstrating activity of the canonical NF-κB pathway. p50-p65 and p50-REL heterodimers and p50-p50 homodimers were detected [6,9–11]. The immunohistochemical demonstration of nuclear REL and p65 revealed constitutive activation of these factors also in primary HRS cells [12–15]. Moreover, gene expression profiling of primary HRS cells showed a strong NF-κB signature [18], further validating a constitutive NF-κB activity also in primary HRS cells. Although these earlier investigations failed to detect activity of the non-canonical NF-κB pathway, later studies showed that also p52-RelB complexes can be found, indicating activity of the non-canonical NF-κB pathway in HRS cells as well [16,17,19]. This is supported by the detection of NIK protein in HL cell lines and primary HRS cells, as NIK is typically undetectable in cells without activation of non-canonical NF-κB signaling [20,21].

Activity of the canonical NF-κB in HL cell lines with wild-type IκBα is linked to high turnover of this NF-κB inhibitor by phosphorylation and proteasomal degradation. This degradation is mediated by the IKK complex, which is constitutively active in HL cell lines [9]. The constitutive activity of the IKK complex indicates activation of IKK through signaling from upstream signaling factors (further discussed below). The relevance of IκBα degradation for NF-κB activity in IκBα-proficient HRS cells is evident from the observation that inhibition of its proteasomal degradation causes a reduced NF-κB activity [9].

Importantly, inhibition of both canonical and non-canonical NF-κB activity in HL cell lines is toxic for these cells, causing reduced proliferation and increased apoptosis. This demonstrates the essential role of constitutive activation of both NF-κB pathways for HRS cell survival [12,14,20,22].

BCL3 is an unusual member of the IκB family, that instead of inhibiting NF-κB activation as the other family members can promote NF-κB activity by binding to p50 homodimers and increasing their activity [8]. In HL cell lines and primary HRS cells, elevated levels of nuclear BCL3 were detected, and the existence of $(p50)_2$-BCL3 complexes was confirmed for HL cell lines [10]. Thus, is it likely that high level expression of BCL3 further increases canonical NF-κB activity in HRS cells.

We have some insight into the genes regulated by NF-κB activity in HRS cells from gene expression studies with cHL cell lines in which an IκB superrepressor inhibited canonical NF-κB activity [22,23], or in which distinct NF-κB factors were silenced [19]. A recent comprehensive analysis revealed that the canonical and non-canonical NF-κB dimers regulate common as well as distinct gene sets [19]. Most target genes are upregulated by NF-κB, but the expression of a fraction of target genes is also inhibited by NF-κB [19]. NF-κB activity contributes to the strong TRAF1, CD40 and CD86 expression by HRS cells. High level of TRAF1 and CD40 expression likely further increases NF-κB activity in a positive feedback loop. NF-κB activity also promotes HRS cell proliferation by inducing cyclin D2 expression and inhibits apoptosis by upregulation of anti-apoptotic factors, including A1, c-IAP2 and BCL-XL. Notably, the non-canonical NF-κB pathway seems to be most essential for HRS cell survival and inhibits both the intrinsic and extrinsic apoptosis pathways, whereas p50-RelA is mainly involved in inhibiting the intrinsic apoptosis pathway [19]. Fur-

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