NF-κB in Cellular Senescence and Cancer Treatment

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The NF-κB pathway transcriptionally controls a large set of target genes that play important roles in cell survival, inflammation, and immune responses. While many studies showed anti-tumorigenic and pro-survival role of NF-κB in cancer cells, recent findings postulate that NF-κB participates in a senescence-associated cytokine response, thereby suggesting a tumor restraining role of NF-κB. In this review, we discuss implications of the NF-κB signaling pathway in cancer. Particularly, we emphasize the connection of NF-κB with cellular senescence as a response to chemotherapy, and furthermore, present examples how distinct oncogenic network contexts surrounding NF-κB produce fundamentally different treatment outcomes in aggressive B-cell lymphomas as an example.

INTRODUCTION

The nuclear factor κB (NF-κB) is a transcription factor complex composed of homo- and heterodimers of five members of the Rel family including RelA (p65), RelB, c-Rel, NF-κB1 (p50/ p105), NF-κB2 (p52/p100). More than 25 years after it was first described as a nuclear protein binding to the kappa immunoglobulin light chain enhancer in B cells (Sen and Baltimore, 1986), NF-κB is now known to control a complex signaling network via a long list of target genes in response to a variety of cellular stimuli. However, probably due to the pathway array surrounding NF-κB, ascribing common functions to NF-κB in general biological scenarios has been difficult. In tumor development, NF-κB presents with Janus-like features that may have pro- and anti-tumorigenic implications. Similar opposing functionalities may apply to NF-κB’s role in cancer therapy, thereby making thorough analysis of NF-κB network a prerequisite for targeted treatment strategies related to NF-κB.

NF-κB SIGNALING

Of the five NF-κB family members in mammalian cells, p105 and p100 are precursors, and, after post-translational modifica-

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transcripts, but the actual role of NF-κB as a tumor suppressor or rather a tumor promoter, as well as its potential interference with treatment outcome is controversial (Ben-Neriah and Karin, 2011).

**Pro-oncogenic NF-κB**

Since NF-κB is normally involved in B-cell maturation and activation, deregulation of the NF-κB pathway is a prominent feature of hematological malignancies. Mutations in genes encoding NF-κB subunits, IκB proteins, or upstream regulators were identified in a variety of hematological malignancies (Compagno et al., 2009; Franzoso et al., 1992; Neri et al., 1991). There are now a number of lymphoid malignancies known, where constitutive NF-κB has been implicated as an essential onco-
gegenic lesion. Mutations of multiple genes in receptor complexes (e.g. CD79-ITAM and MyD88), signaling complexes (e.g. IKK, c-Rel) cause deregulation of NF-
κB pathway in human lymphomas (Ngo et al., 2011; Rosenwald et al., 2002; Sun et al., 2004; Zhou et al., 2004). A good example of NF-κB’s essential role in cancer development can be found in human large B-cell lymphoma (DLBCL), which can be divided into at least two subtypes according to their gene-expression profiling: the activated B-cell-like (ABC) subtype and the germinal-center B-cell-like (GCB) subtype. The main signature of the ABC subtype is the constitutive activation of the NF-
κB pathway, which is rarely hyperactivated in GCB-DLBCL. Activating mutations in CARD11 (which encodes Carma-1) have been detected in ABC DLBCL, generating constitutively active CARD11 that associates with the Bcl-10-MALT1 complex (CBM complex; mediates NF-κB signaling between anti-
gen receptor and IKK) without antigenic stimulation, leading to persistent activation of NF-κB (Lenz et al., 2008a; Ngo et al., 2006; Staudt, 2010). A20 is a negative regulator of the NF-
κB pathway, as it prevents excessive activation of NF-κB (Seitz et al., 1998). In a diethylnitrosamine-induced hepatocellular carcinoma (HCC) mouse model, hepatocyte-specific ablation of IKKβ strongly enhanced the development of HCC (Mae-da et al., 2005). In other studies, hepatocyte-specific ablation of IKKβ (NEMO) or TAK1 (TGF-
β-activated kinase 1), both required for the activation of IKK and NF-κB, resulted in spontaneous liver damage, hepatocyte death, and interestingly, release of factors leading to liver fibrosis and development of HCC (Inokuchi et al., 2010; Luedde et al., 2007). In the Eμ-myc transgenic mouse lymphoma model, in which oncogene Myc is overexpressed and drives B-cell lymphoma, NF-κB loss ac-
celerated tumor development by impairing Myc’s apoptotic re-
sponse, adding an example for a tumor suppressive function of NF-κB via its non-canonical pathway (Keller et al., 2010).

**Anti-oncogenic NF-κB**

Although NF-κB transcription factors have an oncogenic role in cancer development and confer drug resistance in cancer ther-
apy in some settings, other studies found NF-κB transcription factors or upstream activators rather to act as tumor suppres-
sors, thereby underscoring the complexity and potential context dependency of NF-κB network-mediated effector functions in both tumor development and therapy.

Concluding to its anti-cancer property, NF-κB has been shown to mediate apoptosis in a variety of cell types (Ryan et al., 2000; Wang et al., 1998). For instance, RelA and c-Rel exert proapoptotic function in T cells, B cells, fibroblasts, neu-
ronal cells, and HeLa cells (Kaltschmidt et al., 2000; Kasibhatla et al., 1999; Martin et al., 2009; Schneider et al., 1999; Sheehy and Schlissel, 1999). There are also hints that NF-κB may modulate the apoptotic response depending on the develop-
mental stage of the immune cells. For example, overexpression of RelA caused a cell-cycle arrest that is followed by apoptosis in the pro-B cell line 220-B, whereas overexpression of RelA in the WEHI 231 immature B-cell line or in the mature B-cell line M12 did not induce apoptosis (Sheehy and Schlissel, 1999).

Importantly, genetically defined mouse models supported the view that NF-κB transcription factors or upstream activators possess tumor suppressor functions. First evidence directly linking NF-κB to tumor suppression came from experiments using epidermal cells. Functional blockade of NF-κB in epider-
mal cells resulted in severe hyperplasia of the skin in transgenic mice expressing dominant negative IκBα mutant, which was reversible upon overexpression of active RelA and p50 sub-
units of NF-κB, suggesting a tumor-suppressive effect of NF-κB (Seitz et al., 1998). In a diethylthiourea-induced hepatocellular carcinoma (HCC) mouse model, hepatocyte-specific ablation of IKKβ strongly enhanced the development of HCC (Mae-
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**NF-κB in Cancer Treatment Response:**

**ANTI-APOPTOSIS**

Given the well-established overlap between failsafe barriers such as apoptosis and senescence in tumor development and ther-
apy, the NF-κB pathway has also been linked to chemores-
sistance in cancer treatment. Activation of NF-κB occurs in
response to DNA damage, the mode by which most conventional chemotherapeutic agents exert their anti-cancer activity. Among the NF-κB target genes activated by DNA damage are bcl-2 (Catz and Johnson, 2001), bcl-xL (Tamatani et al., 1999), COX-2 (Yamamoto et al., 1995), cyclin D1 (Guttridge et al., 1999), survivin (Zhu et al., 2001), and XIAP (Stehlik et al., 1998), which are closely involved in anti-apoptotic, pro-survival functions of NF-κB, thereby operating as candidate mediators of chemoresistance in a wide variety of tumor cells.

Since the downstream targets of NF-κB are involved in apoptosis inhibition and may thereby block the action of many forms of chemotherapy (Baldwin, 2001), it may also contribute to the poor response of the ABC DLBCL to chemotherapy (Alizadeh et al., 2000; Lenz et al., 2008b; Rosenwald et al., 2002). This hypothesis is supported by several in vitro experiments. Introduction of the NF-κB super repressor (a non-degradable mutant of IκBα) resulted in rapid apoptosis and cell-cycle arrest, selectively in ABC-DLBCL cells (Davis et al., 2001). GCB-DLBCL cells were not affected in the same condition, indicating that the constitutive NF-κB activity is specifically required for the survival and proliferation of ABC-DLBCL cells. Strictly speaking, such result shows only the lymphoma’s dependency on hyper-active NF-κB, not the contribution of it to chemoresistance. However, it is non-disputable that NF-κB as the “Achilles’ heel” of ABC DLBCL is a good therapeutic target. Likewise, the IKKβ inhibitors PS-1145 and MLX105 were selectively toxic for ABC-DLBCL cells but not for GCB DLBCL cells. Introduction of an estrogen-inducible RelA fusion protein into ABC-DLBCL restored NF-κB activity even in the presence of IKK inhibition. The active form of RelA also stopped apoptosis induction by the kinase inhibitors PS-1145 and MLX105, demonstrating that the NF-κB inhibition is directly responsible for tumor cell death (Lam et al., 2005).

NF-κB IN CANCER TREATMENT RESPONSE: CELLULAR SENESCENCE

Premature cellular senescence is a terminal cell-cycle arrest that can be induced by oncogenic activation or chemotherapy, involving DNA damage response (DDR) signaling in both set-
Fig. 2. A model of how oncogenic network influences cancer treatment outcome. How two oncogenic moieties (NF-κB and bcl2) are connected in a network at diagnosis - a linear pathway in which NF-κB drives bcl2 expression reminiscent of ABC DLBCL, or in independent parallel pathways reminiscent of GCB DLBCL - influences the cellular outcome and sensitivity to treatment using conventional chemotherapy and/or novel NF-κB inhibitor.

- **ABC DLBCL-reminiscent**: NF-κB high \( \rightarrow \) bcl2 high
  - Chemotherapy (induces NF-κB)
  - NF-κB Inhibitor
  - Decision for Treatment: Apoptotic Defect
  - Possible Cellular Outcome: Reduced
  - Chemosensitivity: Enhanced

- **GCB DLBCL-reminiscent**: NF-κB low \( \rightarrow \) bcl2 low
  - Chemotherapy (induces NF-κB)
  - NF-κB Inhibitor
  - Decision for Treatment: Apoptosis (despite increased NF-κB activity)
  - Possible Cellular Outcome: Intermediate

- **NF-κB high**: NF-κB high \( \rightarrow \) bcl2 high
  - Chemotherapy (induces NF-κB)
  - NF-κB Inhibitor
  - Decision for Treatment: Senescence defect / little apoptosis
  - Possible Cellular Outcome: Rather reduced
  - Chemosensitivity: Reduced

- **NF-κB low**: NF-κB low \( \rightarrow \) bcl2 low
  - Chemotherapy (induces NF-κB)
  - NF-κB Inhibitor
  - Decision for Treatment: Senescence and apoptotic defect
  - Possible Cellular Outcome: Enhanced

- **Genetically defined mouse models**: Recently, genetically defined mouse tumor models were instrumental to demonstrate that TIS indeed extends survival of the tumor-bearing mice after chemotherapy (Schmitt et al., 2002). Importantly, these "ABC-like" lymphomas failed to enter senescence in response to therapy, thereby further contributing to their drug insensitivity, although the underlying senescence-compromising mechanism is not entirely clear. Moreover, mice that relapsed upon chemotherapy had lymphomas with significantly higher NF-κB activity at diagnosis as compared to those achieving cure after a single application of cyclophosphamide, a common chemotherapeutic agent widely used in clinical oncology. Accordingly, we desig-
nated "NF-κB low" mouse lymphomas "GC-like". Subsequent-
ly, we tested these "NF-κB low" lymphomas for their ability to
enter TIS: after blocking apoptosis via retroviral introduction of
bcl2, we observed more senescence in response to therapy if
we genetically increased NF-κB levels. Parallel to mouse work,
transcriptome and clinical data from DLBCL patients were also
analyzed. Importantly - unlike ABC-DLBCL - almost half of the
GCB-DLBCL are known to present with high Bcl2 levels that
are independent of NF-κB activity, because they result from a
t(14;18) translocation, which constitutively drives Bcl2 expres-
sion from an immunoglobulin heavy chain promoter (Huang et
al., 2002; Iqbal et al., 2004), a scenario we recapitulated by
retroviral, hence, NF-κB-independent Bcl2 expression in "GCB-
like" mouse lymphomas. Given the particularly TIS-prone condi-
tion in "GCB-like" mouse lymphomas with high Bcl2 and high
NF-κB levels, we re-applied these determinants to a 233-
DLBCL patient transcriptome data set with known subsequent
clinical courses under standard immunchemotherapy. Indeed,
patients with Bcl2-high and NF-κB-high GCB-DLBCL at diag-
nosis were identified as a clinically relevant subcohort that
achieved a significantly longer (P < 0.005) progression-free
survival than patients with Bcl2-high GCB-DLBCL but low NF-
κB activity. Hence, functional investigations in mice identified an
array of novel stratifiers of response that unveiled the clinically
unexpected finding of a patient subgroup with a particularly
good prognosis despite high-level NF-κB activity. All together,
these data underscore the opposing roles NF-κB plays in can-
cer treatment, dependent on the cellular context, which ac-
counts for fundamentally different clinical outcomes even in the
same cancer entity.

NF-κB IN CANCER TREATMENT RESPONSE:
ONCOGENIC NETWORKS

The data described above highlight how oncogenic networks
and interdependencies, wired up during tumor development,
may regulate the actual functions and even opposing roles of
NF-κB in subsequent responses to therapy. NF-κB and Bcl2
are generally considered as indicators of aggressive tumor
biology and poor outcome, but our mechanistic analyses in a
genetically tractable mouse lymphoma model unveiled a setting
in which these moieties contribute to superior outcome. Our
approach demonstrates that functional understanding of onco-
genic networks - and NF-κB/Bcl2 forms the most simple model
of such a "two-factor network" with a linear connection in one
(ABC-type), but an independent role of both factors in the other
setting (GCB-type) - is required to properly utilize molecular
lesions as biomarkers or even therapeutic targets (Fig. 2).

NF-κB IN CANCER TREATMENT RESPONSE:
SASP AND TUMOR MICROENVIRONMENT

The SASP is composed of pro-inflammatory cytokines such as
IL-1α, IL-1β, IL-6, IL-8, bFGF, TGF-β (in some settings), GM-
CSF, as well as inflammation-related chemokines such as
CXCL-1/2/3/5/7, MIP-1α, or MCP-1 (a.k.a. CCL2). It also
contains factors with, at least in some contexts, anti-proliferative
activity such as IGFBPs or PAI-1, as well as factors like the
MMPs that remodel the extracellular matrix (Acosta et al., 2008;
Kortlever et al., 2006; Kuliman and Pepeer, 2009). Moreover, in
an extend view on the SASP program as a senescence-
associated pro-inflammatory signaling array, this phenotype is
not restricted to secreted factors, but also includes membrane-
bound cell surface molecules serving as ligands and receptors,
for instance TNF receptors, CXCR2 or the IL-6R, thereby creat-
ing potential autocrine loops (e.g. CXCL-1/2/3/5/7 or IL-8 with
CXCRL2) or even, as reported, intra-cellular short cuts (e.g. IL-6
and the IL-6R), and self-amplifying cascades (e.g. NF-κB sig-
naling via TNF-R).

Therefore, the expression of surface-presented receptors
and ligands and the secretion of a plethora of factors by senes-
cent cancer cells may have complex, growth-inhibitory or -
promoting effects on adjacent tumor and surrounding bystander
cells. In particular, factors secreted by senescent cells can also
act on macrophages, neutrophils, and NK cells, thereby pro-
moting immune responses that, on one hand, may ultimately
lead to the clearance of senescent tumor cells (Xue et al.,
2007), but, on the other hand, might also create a microenvi-
ronment that fosters tumor progression (Coppé et al., 2010).
Given our own observation that macrophage-derived TGFb
evokes lymphoma cell senescence in a non-cell-autonomous
fashion (Reimann et al., 2010), SASP-activated macrophages
are likely to contribute to TIS in vivo, or, in turn, NF-κB inactiva-
lation in lymphoma cells might result in an inability to launch TIS
because of the disrupted SASP/macrophage link. Thus, the
outcome of anticancer therapy is not only determined by a
quantitative effect on cancer cells forced to irreversibly exit the
cell cycle but may also depend on novel capabilities acquired
by senescent cells that can impact on their malignant and non-
malignant neighbors in different ways. With further elucidation
of these complex interdependencies that regulate tumor cell
survival, senescence, and immune clearance, we expect non-
genotoxic senescence-inducing agents to become a very prom-
ising perspective to be further exploited in cancer treatment.

NF-κB in Cellular Senescence and Cancer Treatment
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Chemotherapy is still the most important treatment method for
many types of cancer. The possible outcomes of chemothera-
petic treatments reach from necrosis, apoptosis, mitotic catas-
trophe, and autophagy to cellular senescence. Most of the
chemotherapeutic agents used in the clinic are assumed to
exert their anti-tumor effect through the induction of apoptosis.
Accordingly, cancer cells with apoptotic defects would exhibit
chemoresistance. As an example, ABC-subtype DLBCL pre-
sent with an inferior prognosis after chemotherapy, and are
characterized by constitutive activation of the NF-κB pathway,
which appears to confer treatment resistance via an apoptotic
block. In contrast, another subtype of DLBCL with lower NF-κB
activity (i.e. the GCB subtype) is associated with a much better
response to chemotherapy. In vitro analyses demonstrated that
inhibition of NF-κB can sensitize ABC- but not GCB-DLBCL to
apoptotic cell death; therefore, the hyperactive NF-κB pathway
is central to the pathogenesis of ABC DLBCL and serves as a
treatment potential target selectively in this subgroup. Many
trials using specific NF-κB inhibitors or more broad range of
proteasome inhibitors, which suppress NF-κB activation by
preventing the degradation of κB, are ongoing (Lim et al.,
2012; clinicaltrialsfeeds.org). However, such treatment should
be applied with caution. Because, in addition to the concerns of
acquired resistance and impaired immunological functions of
patients after repeated treatments, it may not be even clear
who can benefit from NF-κB inhibition, considering the signifi-
cant role NF-κB plays in cellular senescence. Coming back to
the example of DLBCL, cross-species analysis of mouse lym-
phoma model and patient data revealed a patient cohort of
GCB DLBCL, which seems not to be affected by NF-κB activa-

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tion at first sight, with high Bcl2 level, actually benefit from high NF-κB activity (Fig. 2). Here, cellular senescence induced by chemotherapy and mediated by NF-κB is a key factor for the better treatment outcome. This contrasting impact of NF-κB in the same cancer entity should alert us to the consequence of (simply) adding NF-κB antagonists in cancer treatment regi-
mens. Even so, the SASP factors, many of which controlled by NF-κB, may also contribute to treatment outcome not only by affecting tumor cells themselves, but also by modifying the tumor microenvironment in non-cell-autonomous ways. Consi-
dering that the simple oncogenic network of only two factors (NF-κB and bc20) already create multiple scenarios for possible treatment responses (Fig. 2), one can easily imagine how com-
licated it can be to predict cancer treatment outcomes in indi-
viduals with still unknown factors, emphasizing the importance of understanding the network of genes for the development of personalized medicine designed for individual patient.

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