The Biology Behind

From Microarray to Bedside: Targeting NF-κB for Therapy of Lymphomas

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INTRODUCTION

Progress in biomedical science often comes from the confluence of different avenues of investigation arriving at shared understandings and pointing to new directions. This is nowhere more apparent than in the development of molecular oncology, the application of molecular approaches to the study, treatment, and prevention of cancer. The confluence of viral oncogenesis and cancer genetics ultimately led to the development of imatinib and the pursuit of other targeted therapies. The confluence of understandings of the immune response and lymphoid malignancies have similarly opened new avenues. Recently, the application of sophisticated genomic and large-scale mRNA expression analysis to the study of lymphomas has fostered the development of new disease classifications that take advantage of evolving concepts in immunology. In this issue of Clinical Cancer Research, Lam et al. have extended studies from the Staudt laboratory to address the hypothesis that the NF-κB pathway, originally identified as a key signaling pathway in the immune and inflammatory responses, could serve as a molecular signature of an aggressive form of human diffuse large B-cell lymphoma (DLBCL), and moreover, as a therapeutic target (1). These studies provide a satisfying validation of the notion that molecular profiling of tumor tissues may aid not only in diagnosis, but also in the identification of new molecular targets for therapy.

THE NF-κB PATHWAY

The origins of the Lam et al. work come from early studies of B-cell gene regulation. NF-κB was originally identified as a nuclear factor that regulated expression of the κ immunoglobulin gene in B lymphocytes (reviewed in ref. 2). Whereas B lymphocytes had constitutive NF-κB nuclear DNA binding activity, other human cells had latent NF-κB activity in their cytoplasm. Stimuli, such as mitogenic activation, led to the liberation of NF-κB dimers from an inhibitor, and thus allowed its subsequent nuclear translocation. Nuclear NF-κB was then able to activate transcription of target genes in non–B cells, as well as in B cells, and NF-κB activation was found to be a particularly important component of T-cell activation and the innate immune response.

Molecular cloning led to the identification of five genes encoding the mammalian NF-κB/Rel proteins, whose products form a variety of homodimers and heterodimers (reviewed in ref. 3). These NF-κB/Rel proteins contain similar NH2-terminal Rel homology domains responsible for DNA binding, dimerization, and nuclear localization; however, they differ in their COOH-terminal segments, defining two subfamilies. The NF-κB family encodes protein products synthesized as long precursors (p105 NF-κB-1 and p100 NF-κB-2) that are processed to mature p50 NF-κB-1 and p52 NF-κB-2 forms. These proteins contribute to DNA binding specificity and to transcriptional regulation, although they themselves do not have transcriptional activation domains. The Rel proteins, RelA, RelB, and c-Rel, have COOH-terminal transcriptional activation domains, and dimers containing these proteins can activate the transcription of target genes. c-Rel was originally identified as the cellular proto-oncogene for v-Rel, an oncogene that induces acute lymphomas in avian species.

With the molecular characterization of the NF-κB proteins and their regulators, the now familiar classic pathway of NF-κB activation was elucidated and is shown in Fig. 1 (reviewed in refs. 4, 5). The classic NF-κB dimer, p50 NF-κB-1, and p65 RelA, is retained in the cytoplasm through binding to an inhibitor, IκBα. This block the efficient nuclear translocation of NF-κB and shuttles nuclear NF-κB dimers back into the cytoplasm, resulting in cytoplasmic accumulation of NF-κB. NF-κB nuclear accumulation and activation of target genes occurs as a result of the proteasomal degradation of IκBα. This is a regulated event signaled by phosphorylation of key serine residues in IκBα followed by ubiquitination of specific lysines, leading to the generation of a substrate for proteasomal recognition and degradation. Phosphorylation of IκBα is done by the IκB kinase (IKK) complex, and ubiquitination is carried out by the βTRCP-SCF E3 ubiquitin ligase complex. In the classic NF-κB pathway, the IKK complex consists of a dimer of enzymatically active subunits, IKKα and IKKβ, as well as a regulatory subunit referred to as IKKγ (reviewed in refs. 5, 6). Regulation of IKK activity is critical for activation of NF-κB, and a large variety of proximal signaling events have been

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shown to activate IKK. Activating stimuli in immune cells include T-cell and B-cell receptor signaling, innate immune receptors, and receptors for a variety of cytokines and growth factors (3). The proximal mechanisms by which these stimuli activate NF-κB vary; however, they converge in IKK activation. Nuclear NF-κB is subjected to additional levels of regulation; the direct phosphorylation of serines in the transactivation domain of RelA contributes to NF-κB activation of target genes through recruitment of transcriptional coactivators such as p300/CBP (reviewed in ref. 5). The IKKs themselves may also translocate to the nucleus and have direct effects on histone phosphorylation (6).

Additional mechanisms of NF-κB regulation also occur. Recent studies implicate protein kinase C–, paracaspase–, and Bcl-10–associated regulation of IKK, possibly through IKK ubiquitination as an important level of control in lymphoid cells (7, 8). A second major pathway of NF-κB regulation, referred to as the “noncanonical pathway” has been recently characterized (reviewed in ref. 9). Briefly, in this pathway, the p100 NF-κB-2 precursor functions as an IκB molecule, which is phosphorylated by IKKα, ubiquitinated, and processed by the proteasome to p52 NF-κB-2, in response to a specific set of extracellular signals. Dimers of p52 with RelB mediate the noncanonical pathway.

The nature and characteristics of cellular genes whose expression is activated by NF-κB is an area of intense interest. Whereas the detailed sets of genes induced by NF-κB vary from cell to cell, overall, NF-κB serves as a cellular response to damage and an organismal response to threat, leading to the production of genes regulating cell survival, proliferation, and interactions with other cells and stroma. Thus, NF-κB targets encompass genes involved in the activation of B and T cells such as those involved their proliferation (such as c-myc and D-cyclins), inhibition of apoptosis (Bcl-2, Bfl1/A1, and IAP’s), and immune signaling and intercellular communication (MHC, cytokines, chemokines, and cellular adhesion molecules; reviewed in ref. 10). In lymphoid cells, inappropriate NF-κB activation therefore results in continuous stimulation of pro-proliferative, activation signals, and inhibition of cell death.

The association of v-Rel with avian lymphomas, as well as the association of NF-κB with cellular proliferation, and resistance to apoptosis, prompted a search for the possible involvement of NF-κB in human cancer (11–13). NF-κB activation of either theclassic or the “noncanonical” pathway is a common feature of many lymphoid malignancies (reviewed in refs. 11–14). Constitutive NF-κB activation was identified in lymphoid cells transformed by oncogenic viruses such as human T-cell lymphotropic virus-I and Epstein-Barr virus. Constitutive activation of the classic NF-κB pathway has also been shown DLBCLs (see below), Hodgkin lymphoma, mucosa-associated lymphoid tissue lymphomas, mantle cell lymphomas, human T-cell lymphotrophic virus-I–associated T-cell lymphomas, and multiple myeloma. Activation of the noncanonical pathway has been observed in cutaneous T-cell lymphomas and B-cell lymphomas.

MOLECULAR ANALYSIS OF DLBCL

The second theme underlying the Lam et al. work derives from the efforts of the Staudt laboratory to understand the cellular origins of human lymphomas through the study of patterns of gene expression. DLBCL is the most common type of non-Hodgkin lymphoma, attaining a prevalence of 30% to 40% (15) among the ~54,000 cases of non-Hodgkin lymphoma diagnosed each year in the United States (16). As a category in the new WHO classification of lymphomas (17), it subsumes several diagnoses from the older Working Formulation, including diffuse mixed and diffuse large cell lymphoma and immunoblastic lymphoma. Morphologically, the large lymphoid cells in the most common variant of DLBCL resemble centroblasts, featuring scanty cytoplasm and large, vesicular nuclei that usually contain several small nucleoli adherent to the
nuclear membrane (ref. 18; Fig. 2). The immunophenotype of DLBCL includes positivity for surface immunoglobulin, CD45, and B cell–associated antigens (CD19, CD20, CD22, and CD79). Smaller subsets express BCL2, CD10, and CD5 (17). The most common molecular genetic lesions include abnormalities of the BCL6 and BCL2 genes (19).

Although DLBCL is an aggressive malignancy, with contemporary chemotherapy 40% to 50% of patients achieve a long-term, disease-free survival (15, 20). Nonetheless, despite common histologic and immunophenotypic patterns, DLBCL is a heterogeneous disease clinically, and a significant number of patients are refractory to therapy. Whereas clinical variables are of some utility in prognostication (21), there is clearly a need for additional, more sophisticated prognostic indicators.

With the advent of DNA microarray techniques and gene expression profiling, it became possible to discover gene expression signatures that identified subtypes of DLBCL (reviewed in ref. 22). In a landmark paper, Alizadeh et al. used cDNA microarray analysis in order to develop gene expression profiles of DLBCL (23). These investigators employed unsupervised computational approaches based on hierarchical clustering to identify subgroups of DLBCL that might provide information on the biology and prognosis of this heterogeneous disease. These studies identified at least two significant subtypes of DLBCL whose gene expression profiles defined populations with distinct B-cell differentiation, resembling either germinal center B cells or activated B cells. They further showed that whereas the overall 5-year survival was 52%, at 5 years, 76% of patients with germinal center-like DLBCL (GC DLBCL) were alive, but only 16% of patients with activated B cell–like lymphomas (ABC DLBCL) had survived.

A larger follow-up study by Rosenwald et al. (24) of 240 patients with DLBCL confirmed the unique prognostic features of the GC DLBCL as compared with the ABC DLBCL. A systematic statistical analysis was used to identify gene expression signatures that would be more strongly predictive of clinical outcome. Based on these studies, a group of 17 genes representative of different molecular signatures was developed to predict overall survival after chemotherapy. Importantly, many of the prognostic gene expression signatures corresponded to groups of genes reflecting fundamental biological characteristics. Not surprisingly, one signature consisted of genes characteristic of germinal B cells thus defining the GC DLBCL subgroup. ABC DLBCL was characterized by low-level expression of the germinal center signature, but expressed higher levels of genes associated with cell proliferation. ABC DLBCL also exhibited enhanced expression of a number of NF-κB target genes, such as c-Myc, Bcl-XL, Bcl-2, c-FLIP, and cyclin D2, suggesting that these tumors have constitutive activation of the NF-κB pathway (14).

Studies in the Shipp laboratory also examined gene expression signatures associated with the response of DLBCL to standard cyclophosphamide-Adriamycin-vincristine-prednisone–based chemotherapy (25). These investigators assessed gene expression using oligonucleotide microarrays and employed a supervised learning approach to identify genes whose expression levels were predictive of prognosis. The highest predictive value was generated by a 13-gene model, which separated patients with a 5-year overall survival of 54% into two groups with 70% and 12%, 5-year survivals. Again, expression of NF-κB target genes such as the antiapoptotic gene Bfl1/A1 was associated with poor prognosis. Another indicator of poor prognosis was protein kinase-Cα2, a potential activator of the NF-κB pathway in the malignant cells.

Recently, both the Staudt and Shipp laboratories have characterized patterns of gene expression characteristic of a
clinically distinct form of DLBCL, primary mediastinal B-cell lymphoma (26, 27). Whereas histologically similar to other DLBCLs, this disease has a distinct immunophenotype and a classic clinical picture, often presenting as bulky mediastinal disease in young women. Gene expression in primary mediastinal B-cell lymphomas was distinct from the ABC and GC DLBCLs but resembled that observed in Reed-Sternberg cells isolated from classic Hodgkin lymphomas, suggesting common biological features. Of particular note, was the high level of expression of a large number of NF-κB target genes in primary mediastinal B-cell lymphoma DLBCL.

NF-κB AS A TARGET FOR DLBCL

Activation of the NF-κB pathway, with increased expression of pro-proliferative and antiapoptotic genes, emerged from the molecular profiling studies as a characteristic of aggressive subtypes of DLBCL. This raised the question of whether the growth and survival of the ABC DLBCL cells was in fact dependent on NF-κB. Alternatively, constitutive NF-κB activation could reflect the fact that ABC DLBCL arose from a precursor cell characterized by NF-κB activation, but that NF-κB activation itself was not critical. Davis et al. showed that cell lines derived from ABC DLBCL, but not GC DLBCL, exhibited constitutive activation of the IKKs and NF-κB (14). Introduction of either a “super-repressor” IκBα (a nondegradable IκB) or a dominant-negative, inactive form of IκBα led to G1 phase growth arrest and apoptosis of the ABC but not GC DLBCL cells, demonstrating that NF-κB activation played a critical role in the oncogenic phenotype of ABC DLBCL. This provided validation of NF-κB activation as a potential therapeutic target for ABC DLBCL.

Given the intense interest in NF-κB inhibitors as anti-inflammatory agents, pharmaceutical companies have been actively investigating NF-κB inhibitors, focusing particularly on inhibitors of the IKKs (28, 29). In their paper in this issue of Clinical Cancer Research, Lam et al. employed two β-carboline derivatives being developed by Millennium Pharmaceuticals as inhibitors of IKK (30). Both compounds showed highly specific inhibition of IKK activity, inhibited NF-κB activity in cell-based assays, and induced cell cycle arrest and apoptosis of ABC DLBCL-derived cell lines at low micromolar concentrations. Inhibition of GC DLBCL cell lines required significantly higher drug concentrations, consistent with a dependence of the ABC, but not the GC DLBCL cell lines on NF-κB-mediated transcription. Toxicity of normal human lymphocytes was not observed at the active concentrations for the lymphoma cells. Thus, this study provides a proof of principle of the possible utility of small molecule IKK inhibitors as cytotoxic agents for the treatment of ABC DLBCLs.

These studies support the concept that NF-κB inhibition may be a viable therapeutic target for poor prognosis DLBCL. Clearly, extensive additional work is needed to realize this possibility. Obviously, appropriate phase I and higher phase clinical trials with IKK inhibitors represent the next steps of this evaluation. A number of other small molecule IKK inhibitors are under development by pharmaceutical companies (reviewed in ref. 28) and will also be of interest in ABC DLBCL. Issues of toxicity are always important for any new agent and target, and IKK inhibitors are no exception. Given the critical role that NF-κB plays in immune responses, it is likely that profound IKK inhibition would be associated with at least some degree of immunosuppression. Another area of potential concern could be hepatotoxicity. IKK knockout mice exhibit apoptosis of hepatocytes, which leads to embryonic death due to the loss of a NF-κB–mediated protective response against physiologic secretion of tumor necrosis factor-α during development (31). It is possible that proinflammatory triggers in the adult might induce hepatic tumor necrosis factor-α secretion, which could lead to hepatotoxicity in the presence of IKK inhibitors. Nonetheless, the poor prognosis of ABC DLBCL certainly warrants further investigation of the potential therapeutic activity and therapeutic index of IKK inhibitors.

The NF-κB pathway offers multiple additional targets for intervention (Fig. 1). These have been extensively reviewed recently (29, 32, 33). Inhibition of proteasomal degradation of IκBα will sequester NF-κB dimers in the cytoplasm. In this regard, bortezomib, the proteasome inhibitor approved for use in refractory multiple myeloma, inhibits NF-κB (34); however, its effects are not specific, as the proteasome is responsible for the degradation of the majority of cell proteins. In fact, recent studies have suggested that the effects of bortezomib are mediated in large part through mitochondrial-dependent apoptosis that is most likely independent of NF-κB (35). More specific NF-κB inhibition could potentially be obtained through the development of ubiquitination inhibitors, such as inhibitors of the E3 ligase complex responsible for IκBα ubiquitination (36). Another approach would be to target the upstream activators of IKK that are responsible for constitutive activation in ABC DLBCL cells. Unfortunately, the specific cellular pathways responsible for IKK activation in ABC DLBCL remain unknown. An intriguing candidate would be PKC-β, shown to be required for B-cell receptor signal–induced NF-κB activation (37, 38) and observed to be up-regulated in poor prognosis DLBCL (25). Small-molecule inhibitors of PKC-δ induced apoptosis of B-lymphoma cells in culture (38). The Btk tyrosine kinase is also required for B-cell receptor–induced activation of NF-κB and could represent an additional target. Additional levels of NF-κB regulation exist in the nucleus, such as phosphorylation of RelA, and the kinases responsible for this level of regulation could also be targets for inhibition. Interestingly, corticosteroids inhibit NF-κB–mediated transcription (29, 39) contributing to their anti-inflammatory effects. This raises the cautionary possibility that NF-κB inhibition may not further improve DLBCL survival, as prednisone is an important component of current DLBCL regimens.

Given the frequent constitutive activation of NF-κB in many different varieties of lymphoid malignancies, ABC DLBCL is unlikely to be the only target for IKK inhibitors. Lam et al. showed inhibitory effects of PS-1145 on the growth of primary mediastinal B-cell lymphoma cells and cells transformed by human T-cell lymphotrophic virus-1, both characterized by high constitutive NF-κB levels. Similarly, Epstein-Barr virus–associated malignancies, multiple myeloma (40), certain mucosa-associated lymphoid tissue lymphomas, and Hodgkin lymphoma would also be attractive potential targets.

Most importantly, the work by Lam et al. stands as a paradigm for moving from large-scale analysis of gene expression in human cancer to the identification and validation of therapeutic
targets. Gene expression analysis of DLBCL has offered at least two additional interesting targets whose expression is increased in poor prognosis disease, PKC-β and the phosphodiesterase, PDE4B (21). PKC inhibitors have been long-studied in laboratory settings and have been shown in vitro to induce apoptosis in B-cell lymphoma cells (38). In fact, the PKC-β inhibitor, enzastaurine (Eli Lilly and Company LY317615) is currently in Phase 2 trials for DLBCL (www.clinicaltrials.gov). Recent work has shown that inhibition of PDE4B sensitizes DLBCL cells to cyclic AMP–induced apoptosis (41). As PDE4 inhibitors are currently in clinical trials for respiratory disorders, their potential utility in DLBCL is also of considerable interest. Looking beyond DLBCL, the thoughtful analysis of gene expression data derived from other human cancers may similarly suggest new targets for therapy, thus fulfilling the promise of molecular oncology and the cancer genomics revolution.

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REFERENCES
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