Multiple Myeloma: Lusting for NF-κB

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Multiple myeloma (MM) is a late-stage B cell malignancy that has received much attention recently because of its therapeutic susceptibility to proteasome inhibitors. Two papers in this issue of *Cancer Cell* show that primary MM samples and MM cell lines frequently have mutations in genes encoding regulators and effectors of NF-κB signaling, and that these mutations lead to chronic NF-κB target gene expression, which is required for the viability of these MM tumor cells. These results reveal the molecular basis for constitutive NF-κB activity in many MMs and further validate the NF-κB signaling pathway as an appropriate target for MM therapy.

Tumor cells are relentless opportunists, willing to take advantage of every imaginable means to sustain their survival. The NF-κB signaling pathway is chronically active in a variety of tumor cell types, and this sustained activity leads to the pathological expression of NF-κB target genes, including ones involved in cell proliferation, cell survival, cell adhesion, and inflammation.

Multiple myeloma (MM) is a relatively common, progressive hematological malignancy, which is characterized by the proliferation of mature, antibody-secreting plasma B cells in the bone marrow. Notable characteristics of MM include the contribution of growth factors from stromal cells in the bone marrow microenvironment to tumor cell survival and growth and an abundance of chromosomal abnormalities in most MM cells. In spite of a variety of current MM therapies, clinical outcome is often poor, variable, and unpredictable. As such, much effort has recently been devoted to using genetic and gene expression microarray approaches to define MM molecular subtypes and how molecular subtypes might be used to direct and predict susceptibility to various treatment regimens (Carrasco et al., 2006; Shaughnessy et al., 2007; Mulligan et al., 2007).

NF-κB defines a family of transcription factor dimers (made up of combinations of p50, p52, c-Rel, p65/RelA, and RelB); such dimers are inactive in the cytoplasm in most normal cells, due to the interaction of NF-κB dimers with IκB inhibitors. Many extracellular signals, usually acting through membrane-bound receptors and a series of adaptor and modulator proteins, activate NF-κB, that is, induce the nuclear translocation of NF-κB as a consequence of proteasome-mediated degradation of IκB. Proteasome-mediated degradation of IκB is promoted by stimulus-provoked phosphorylation of IκB by an IκB kinase (IKK) complex. Two major pathways lead to activation of NF-κB: a classical (or canonical) pathway and an alternative (or noncanonical) pathway (Figure 1). These two pathways, however, show much interplay and overlap: many signals activate both NF-κB pathways, many of the same cytoplasmic effector proteins (adaptors, kinases) are used in both pathways, and many target genes are activated by both pathways. For the most part, the classical pathway is defined by activation of p50-p65 complexes upon degradation of an associated IκB, and the alternative pathway is characterized by processing of an inactive p100-RelB dimer to active p52-RelB through degradation of C-terminal IκB-like sequences of NF-κB2 p100 (the precursor to p52). Both NF-κB pathways can be effectively blocked by proteasome inhibitors, which interrupt NF-κB signaling by inhibiting degradation of IκB proteins.

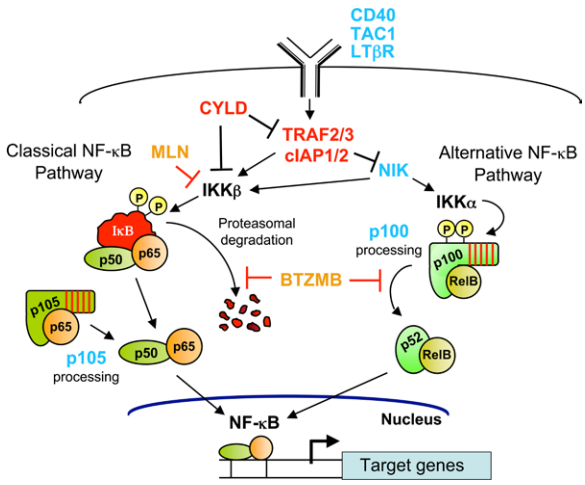

Figure 1. NF-κB Signaling Pathway Mutations in Multiple Myeloma

Shown are the classical and alternative NF-κB signal transduction pathways that lead to activation of nuclear NF-κB DNA binding and target gene expression. Arrows indicate activating steps; bars indicate inhibitory steps. Negative regulators of NF-κB signaling that undergo loss-of-function mutations in MM are designated in red font. Positive regulators and effectors of NF-κB signaling that undergo gain-of-function mutations in MM are in blue font. In both cases, these mutations lead to constitutive nuclear NF-κB activity and increased target gene expression in MM. BTZMB (bortezomib; proteasome inhibitor) and MLN (MLN120b; IKKβ inhibitor) are small molecules that inhibit both NF-κB signaling and MM cell growth. See text for further details.

Several studies have shown that many MM cell lines have constitutively nuclear NF-κB activity and that such NF-κB-positive MM cell lines are sensitive to growth inhibition by blockers of NF-κB signaling (e.g., Hideshima et al., 2006; Jourdan et al., 2007)). Indeed, the proteasome inhibitor bortezomib has shown considerable clinical efficacy toward MM, which is likely due in large part to bortezomib's anti-NF-κB signaling activity (Richardson et al., 2007). However, the molecular basis for constitutive NF-κB activity in MM was largely unknown, and as such, it was not known how one might predict sensitivity of MM patients to anti-NF-κB therapy. In two papers in this issue of *Cancer Cell* (Annunziata et al., 2007; Keats et al., 2007), it is shown that many MM cell lines and primary tumor cells have mutations in genes encoding positive and negative regulators of NF-κB signaling, and these mutations cause these MMs to have constitutive nuclear NF-κB activity, which is required for their survival.

The current papers use a variety of genomic (comparative genomic hybridization, direct gene sequencing) and gene expression studies (microarrays) to identify NF-κB-activating mutations in 15%–20% of several hundred MM cell lines and patient samples. It is noteworthy that extreme variations in individual RNA levels by microarray expression profiling could be used to predict genetic aberrations: that is, extremely low mRNA expression could predict gene deletion/mutation, and high expression sometimes pointed to gene amplification or translocation.

Genes in MM that contained gain-of-function mutations (such as amplifications, translocations, point mutations) for NF-κB signaling included ones encoding receptors known to activate NF-κB (CD40, LTβR, and TAC1), NIK (NF-κB-inducing kinase), NF-κB1 p50/p105, and NF-κB2 p52/p100. For CD40, LTβR, and TAC1, receptor overexpression may be sufficient to activate the NF-κB pathway or might enhance the sensitivity of MM cells to factors in the tumor microenvironment. Overexpression of NIK or NF-κB1 p105 directly leads to constitutive activation of NF-κB. In the case of *NFKB2*, a deletion of sequences in the p100 IκB-like domain promotes processing of p100 to p52 and activation of the alternative NF-κB pathway.

Mutations that induce chronic NF-κB activation by loss of function (e.g., by deletions, point mutations, and/or loss of heterozygosity) occur in negative regulators of NF-κB signaling, including genes encoding TRAF2, TRAF3, cIAP1/2, and CYLD. In both studies, the most commonly found NF-κB-activating mutations in MM were ones that inactivated *TRAF3*. In some of the MM cell lines, inactivation of the TRAF and cIAP genes/proteins causes stabilization of NIK protein, which consequently should lead to chronic NF-κB activation. However, in some TRAF3-deficient MM cell lines, there may be NIK-independent pathways to NF-κB activation. CYLD is a deubiquitinating enzyme that negatively regulates NF-κB signaling at several levels.

Both types of mutations (gain and loss of function) were shown to correlate with chronic activation of NF-κB in MM, as judged by increased nuclear NF-κB DNA-binding activity and NF-κB target gene expression. It is likely that both the classical and alternative NF-κB pathways are activated by NF-κB pathway mutations in MM. However, given that TRAF2 and TRAF3 are generally thought to be positive regulators of the classical NF-κB pathway, but negative regulators of the alternative NF-κB pathway (Xia and Chen, 2005), and NIK activity is usually associated with activation of the alternative NF-κB pathway, the alternative NF-κB pathway may be more important for MM pathology. Nevertheless, it is clear that small-molecule inhibitors of IKKβ (which is specific for the classical NF-κB pathway) can inhibit MM cell growth (Hideshima et al., 2006; Annunziata et al., 2007; Jourdan et al., 2007); moreover, knockdown of IKKα (required for alternative NF-κB signaling in many systems) did not inhibit MM cells with activating mutations in NIK (Annunziata et al., 2007). A possible explanation for these paradoxes is that gross alteration in expression or activity of NF-κB regulatory molecules like NIK or TRAFs (as might occur by amplification or deletion) may lead to misregulation of an NF-κB pathway that they do not ordinarily affect.

The importance of NF-κB-activating mutations for MM cell growth was shown in two ways: knockdown of NIK protein levels in MM cell lines with NIK amplification or re-expression of TRAF3 in TRAF3-deficient MM cell lines was toxic for cell growth/viability. Although NF-κB-activating mutations were identified in about 20% of MM cell lines/tumors, Annunziata et al. found that more than 60% of MM cell lines are sensitive to growth inhibition by a small-molecule inhibitor of IKKβ. This result suggests either that many MM tumors have epigenetic means to activate NF-κB or that several other types of NF-κB-activating mutations remain to be identified in MM samples. Indeed, a somatic tumor-specific mutation in *RELA* that alters p65's DNA-binding activity was reported in one MM case (Trecca et al., 1997). It is interesting that many genes encoding regulators and effectors of the NF-κB pathway are themselves NF-κB target genes, providing a possible feed-forward mechanism for sustaining NF-κB activity.

These two studies also showed that normal plasma B cells and cells from the pre-MM state (monoclonal gammopathy of undetermined significance [MGUS]) have high expression of the same NF-κB target genes seen in MM. In normal plasma B cells, the NF-κB activity is induced by growth factors produced in the bone marrow microenvironment. Thus, these studies suggest that one step in MM development is the progression from a microenvironment-dependent NF-κB-activated state to an NF-κB-activated state with reduced or abolished dependence on the microenvironment, such as can occur by some of the described NF-κB-activating mutations. It is not clear how other common genetic alterations in MM, such as *FGFR3* translocation or

CYCLIND dysregulation (an NF-κB target gene), correlate or cooperate with genetic activation of NF-κB.

By using NF-κB target gene expression profiles to identify MM patients likely to have *TRAF3* deletions among cohorts of patients treated with dexamethasone versus bortezomib, Keats et al. found that only 2/20 patients with *TRAF3* inactivation responded to dexamethasone, whereas 17/19 patients among the *TRAF3* inactivation group responded to bortezomib. These results suggest that constitutive activation of the NF-κB pathway through *TRAF3* inactivation is correlated with dexamethosone resistance and bortezomib sensitivity. Although the exact molecular basis for the differential sensitivity of individual MM patients to bortezomib versus dexamethasone is not clear, it appears that *TRAF3* status, which can be assessed fairly simply by conventional molecular techniques, should dictate treatment with proteasome inhibitors.

These two studies add to the growing body of evidence demonstrating that mutations in NF-κB pathway genes are rather common occurrences in a variety of malignancies (Courtois and Gilmore, 2006). Why certain NF-κB-activating gene mutations appear to occur only in some types of tumors is not clear. Nevertheless, the current studies take us one step closer to understanding the molecular underpinnings of MM and toward designing and designating more effective therapies for individuals with MM.

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Life, Death, BH3 Profiles, and the Salmon Mousse

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New drugs that neutralize the antiapoptotic members of the Bcl-2 family hold promise for rational cancer therapies, both alone and in combination with other agents. An understanding of how and why such agents may trigger apoptosis on their own, and how resistance to these drugs can occur, depends on the complexity of the Bcl-2 family interactions that control mitochondrial outer membrane permeabilization (MOMP). By extracting mitochondria from tumor cells and exposing them to peptides corresponding to the regulatory BH3-only proteins, MOMP predicts not only which cells will undergo apoptosis in response to Bcl-2 antagonists, but also why other cells may be resistant.

As any poet or philosopher can tell us, it is in the contemplation of death that we gain insight into life. Indeed, it was in their seminal *The Meaning of Life* that Monty Python had a dinner guest challenge the figure of Death to explain how their entire party had somehow all died at the same time, to which Death ominously replied, "The salmon mousse." In much the same way, if perhaps less ethereally, the study of the principles of cell death