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Nfkb1 suppresses DNA alkylation-induced tumor formation

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Abbreviations: AML, acute myelogenous leukemia; IR, ionizing radiation; MEF, mouse embryonic fibroblast; MNU, N-methyl-N-nitrosourea; RS, replication stress; t-MN, therapy-related myeloid neoplasia; TMZ, temozolomide.

NF-κB proteins play a complex role in modulating carcinogenesis following DNA damage. Previous work identified p50/NF-κB1 as a necessary factor in the cytotoxic response to alkylation damage. Recently, these findings were extended to demonstrate that in the setting of alkylation damage, this NF-κB subunit acts as a haploinsufficient tumor suppressor that prevents hematologic malignancy formation.

The NF-κB family is composed of 5 subunit proteins that mediate transcriptional activity as dimers. Whereas p65, c-rel, and relB are translated in their mature form, NF-κB1 (p105) and NF-κB2 (p100) are precursor proteins that are proteolytically processed to form p50 and p52, respectively. Although these subunits can compensate for each other when one is depleted,[1,2] each subunit has autonomous actions.[3] NF-κB proteins play an important role in carcinogenesis, and both oncogenic and tumor suppressive actions have been described.[4] However, targeted deletion of each protein has not revealed a direct tumor suppressive role for any subunit.[3]

DNA damage induced either by endogenous/environmental sources or by chemotherapeutic agents is an important cause of carcinogenesis. Although damage repair is the primary mechanism for maintenance of genomic integrity in the setting of DNA damage, cytotoxic pathways also play an important role in eliminating damaged cells. Propagation of DNA damage results in mutations that can eventually lead to tumor formation. A particularly devastating example of this is therapy-related myeloid neoplasia (t-MN).[5] This heterogeneous disease occurs after treatment of an initial cancer with cytotoxic agents and has become a significant clinical problem as cancer survivorship has improved. More than 75% of patients that develop t-MN have received an alkylating agent for the management of their primary tumor.[6]

In examining the role of NF-κB signaling in the response to DNA damage, we previously reported that the p50/NF-κB1 subunit is necessary for cytotoxicity induced by alkylating agents such as temozolomide (TMZ) and N-methyl-N-nitrosourea (MNU), but not that induced by ionizing radiation (IR).[2] Loss of p50/NF-κB1 modulates cytotoxicity by rendering cells tolerant of the induced damage without affecting damage repair. Thus, cells with reduced p50 continue to survive despite having elevated levels of damage (**Fig. 1A**). Given that damage accumulation is mutagenic, it was hypothesized that NF-κB1 acts to maintain genomic stability in the setting of DNA alkylation damage.

To study this hypothesis, we first examined the induction of mutation following treatment with a DNA damaging agent.[7] Loss of Nfkb1 in mouse embryonic fibroblasts (MEFs) led to increased generation of mutations in response to TMZ but not IR, suggesting that Nfkb1 acts to maintain genome stability. Subsequently, to examine whether this subunit actually mediates tumor suppression we examined whether deletion of *Nfkb1* modulates alkylator-induced tumor formation using an animal carcinogenesis model. Remarkably, at 1 y, significantly more thymic lymphomas were induced by MNU in $Nfkb1^{-/-}$ than $Nfkb1^{+/+}$ animals ($P < 0.0001$). Moreover, this differential tumor formation was evident at multiple MNU concentrations regardless of whether the animals were on a mixed (B6/129) or pure (C57BL/6) background. Interestingly, consistent with the lack of effect of Nfkb1 loss on IR-induced mutation induction or cytotoxicity,[2] no significant difference in tumor formation rate was noted between $Nfkb1^{-/-}$ and $Nfkb1^{+/+}$ animals following whole-body IR. Together, these findings indicated that Nfkb1 protects mice against alkylation-induced, but not IR-induced, tumor formation and suggested that this subunit acts as a pathway-specific tumor suppressor.

Heterozygous animals were noted to have a tumor induction rate intermediate

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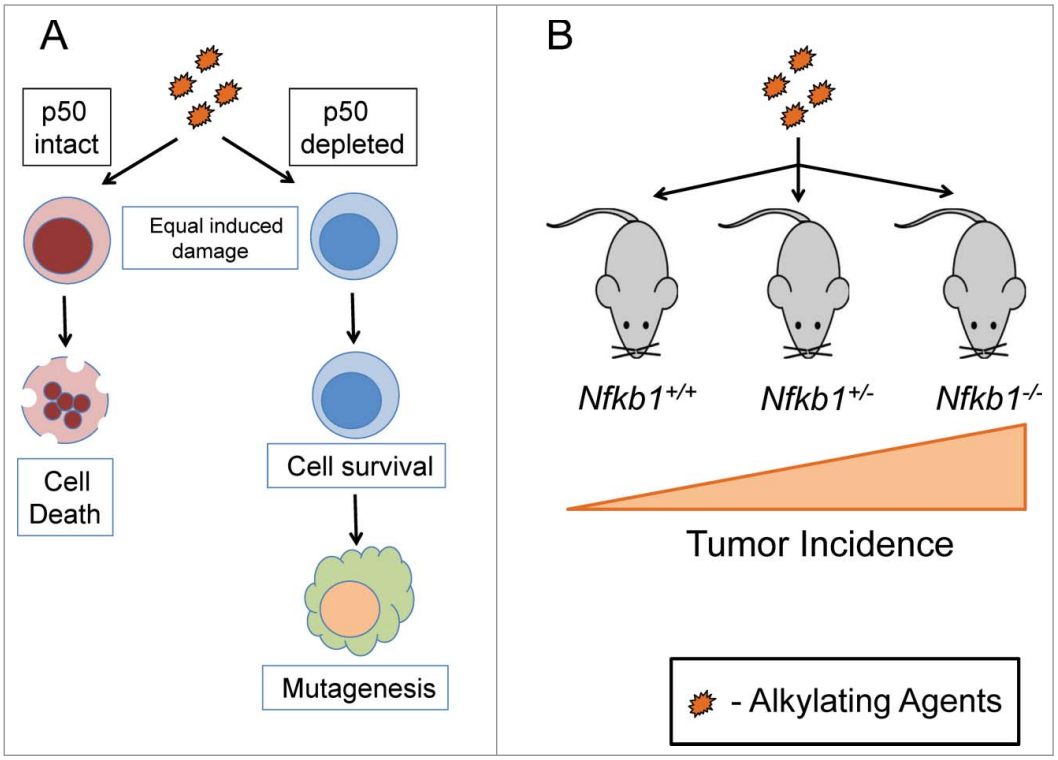

Figure 1. Nfkb1 maintains genome stability in response to DNA alkylation damage. (**A**) p50 (NF-κB1) mediates cytotoxicity in response to DNA damage. Loss of this subunit results in survival of cells with elevated levels of damage, leading to increased mutagenesis. (**B**). Alkylator-induced tumor formation is suppressed by Nfkb1 in a haploinsufficient manner.

between that of *Nfkb1*$^{-/-}$ and *Nfkb1*$^{+/+}$ mice (**Fig. 1B**). Examination of tumor tissue from animals of all 3 genotypes demonstrated that p105 and p50 were present at both the gene and protein level in the tumors from *Nfkb1*$^{+/-}$ animals. In addition, the ability of p50 from these tumors to dimerize and bind DNA was confirmed using gel shift analysis. These findings indicated that tumors from heterozygous mice retain functional p50, supporting the hypothesis that Nfkb1 mediates tumor suppression in a haploinsufficient manner.

Having examined NF-κB1 in mice, we were interested in whether this subunit mediates tumor suppression in humans. Therefore, we examined *NFKB1* mRNA expression in a series of published databases of human hematological malignancies. Almost invariably, it was apparent that tumor tissue has substantially lower *NFKB1* expression than control tissue, an observation not seen with the p65 subunit. In addition, using next-generation RNA sequencing, we examined *NFKB1* expression in t-MN samples from our institution and noted significantly lower expression compared to that in patients with *de novo* AML, which occurs without a history of prior alkylator treatment. The mRNA expression was reflected by similar changes at the protein level.

In summary, our work demonstrates that NF-κB1 is a tumor suppressor that maintains genome stability in response to DNA damage. Moreover, the human malignancy data support the contention that this subunit mediates tumor suppression in humans. It is evident that this tumor suppressive effect is damage-type specific from the observation that Nfkb1 does not prevent tumor formation in response to IR or other carcinogens.[8] In fact, we recently noted that p50/NF-κB1 mediates the cytotoxic response specifically to any agent, or process, that induces replication stress (unpublished data, BY).

Our work shows that Nfkb1 acts in a haploinsufficient manner, suggesting that reduced expression is sufficient to attenuate tumor suppression. This observation is relevant to humans because, although *NFKB1* is rarely deleted or mutated in human tumors, reduced expression is common. Low *NFKB1* expression may be a normal variant found in the population,[9] or may be a secondary response to the expression of an oncogene such as myc, tal1, bcl-6, or lmo1 (for citations see ref. 7). From a mechanistic standpoint, a decrease in p50 results in compensation by p52.[1] However, although p52 can cross-compensate for p50 in certain respects it cannot functionally compensate for p50 in the cytotoxic response to DNA alkylation.[2] We propose that this deficiency in the damage response results in survival of injured cells, ultimately leading to genome instability. Given the lack of increased basal tumor formation with loss of Nfkb1,[10] we propose that this subunit is a low penetrance cancer susceptibility gene that acts in combination with other factors to determine the overall response to genomic insults.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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