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JNK1 and JNK2 play redundant functions in Myc-induced B cell lymphoma formation

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Dear Editor

The c-Jun amino-terminal kinases (JNKs) belong to the mitogen-activated protein kinase family, and are involved in transducing mitogenic, inflammatory and stress signals in cells through the phosphorylation of transcription factors like c-Jun and ATF-2, which participate in the activation and formation of the activator-protein 1 (AP-1) transcriptional complex.[1] The JNK family consists of the three highly homologous members JNK1, JNK2 and JNK3. Among them, JNK3 is expressed predominantly in the brain whereas both JNK1 and JNK2 are expressed ubiquitously.[2] Combinatorial use of the various JNKs and their upstream kinases are thought to lead to differential regulation of substrate proteins in response to multiple stimuli, thereby establishing signal-specificity. Genetic analyzes using JNK knockout mice that we and others have generated have revealed specific roles for JNK1 and JNK2 in neuronal apoptosis, neuronal microtubules maintenance, T cell activation and apoptosis, acute liver inflammation and failure, regulation of insulin resistance and obesity, bile acid production, osteoclastogenesis, fibroblasts apoptosis and proliferation as well as tumorigenesis (reviewed in Ref. 2). Importantly, we have shown that JNK2 appears to be a negative regulator of fibroblast cellular proliferation in contrast to JNK1.[3] These findings together strongly suggest that the JNKs have both overlapping and distinct functions in different cell types.

Although JNK activity is often noted to be upregulated in cancers,[4] limited mouse model analysis have suggested that JNK1 and JNK2 may have opposing functions in regulating carcinogenesis. For example, 7,12-dimethylbenz(*a*)anthracene/12-*O*-tetradecanoylphorbol-13-acetate-induced skin tumor development was found to be suppressed in *Jnk2*$^{-/-}$ mice but enhanced in *Jnk1*$^{-/-}$ mice.[5,6] In contrast, in a colitis model, loss of either JNK1 or JNK2 led to aggravation of dextran sodium sulfate (DSS)-induced colitis.[7] Besides these, not many studies have evaluated the specific roles of JNK1 or JNK2 in other models of carcinogenesis.

Activation of B cells have been shown to lead to JNK activation,[8] and the leukemogenic oncogene BCR-ABL has been demonstrated to activate the JNK signaling pathway and lead to the increase of AP-1 transcriptional activity.[9] Consistently, the inhibition of c-Jun or JNK prevents BCR-ABL-induced cell transformation *in vitro*.[10] Although this implicates the JNK signaling pathway in transformation by BCR-ABL, the possible role of JNK in this process was unclear until studies using the *Jnk1*$^{-/-}$ mice showed that JNK1 deficiency caused defective transformation of pre-B cells by BCR-ABL *in vitro* and *in vivo*.[11] The JNK1 protein was required for the survival of the transformed cells in the absence of stromal support, demonstrating that JNK1 signals cell survival in transformed B lymphoblasts and suggested that it may contribute to the pathogenesis of some proliferative diseases.[11] By contrast, similar effects were not observed in *Jnk2*$^{-/-}$ mice, highlighting selectivity of the JNKs.

In an attempt to evaluate the specific roles of JNK1 and JNK2 in B cells lymphoma formation in other models, we have utilized the Eμ-Myc-induced B cell lymphoma model.[12] The Eμ-Myc transgenic mice were crossed to JNK1 or JNK2 deficient mice, and the development of tumors were monitored over time. All Myc-transgene expressing mice succumbed to B cell lymphoma over time, and the Kaplan–Meier survival curves of these cohorts are shown in Figure 1*a*. The median survival of Myc-transgenic mice deficient for JNK1 was not significantly different from wild-type (WT) mice (median survival in days—WT *vs.* *Jnk1*$^{-/-}$: 148 *vs.* 101), and there was also no statistically significant difference between the WT and *Jnk2*$^{-/-}$ group (median survival in days—WT *vs.* *Jnk2*$^{-/-}$: 148 *vs.* 115; Fig. 1*a*). Logrank test did not reveal any significant differences between the survival curves of *Jnk1*$^{-/-}$ and WT mice ($p = 0.0739$), or between WT and the *Jnk2*$^{-/-}$ groups ($p = 0.7662$). Analysis between the *Jnk1*$^{-/-}$ and *Jnk2*$^{-/-}$ groups did not also reveal any difference statistically in median survival ($p = 0.0704$; Fig. 1*a*). We further analyzed the survival among the heterozygous and homozygous mice cohorts for the two genotypes, to confirm the lack of any difference. Median survival was again not significantly different between the Myc-transgenic *Jnk1*$^{-/-}$ and *Jnk1*$^{+/-}$ mice (median survival in days—*Jnk1*$^{-/-}$ *vs.* *Jnk1*$^{+/-}$: 101 *vs.* 102; $p = 0.7474$; Fig. 1*b*) and between the Myc-transgenic *Jnk2*$^{-/-}$ and *Jnk2*$^{+/-}$ mice (median survival in days—*Jnk2*$^{-/-}$ *vs.* *Jnk2*$^{+/-}$: 115 *vs.* 130; $p = 0.5525$; Fig. 1*c*). Altogether, these data suggest that lack of either JNK1 or JNK2 does not affect the survivability of the mice expressing the Myc-transgene.

We did not also notice any significant differences in the number or size of lymphomas on the various JNK-deficient backgrounds (data not shown). Analysis of B cell lymphoma

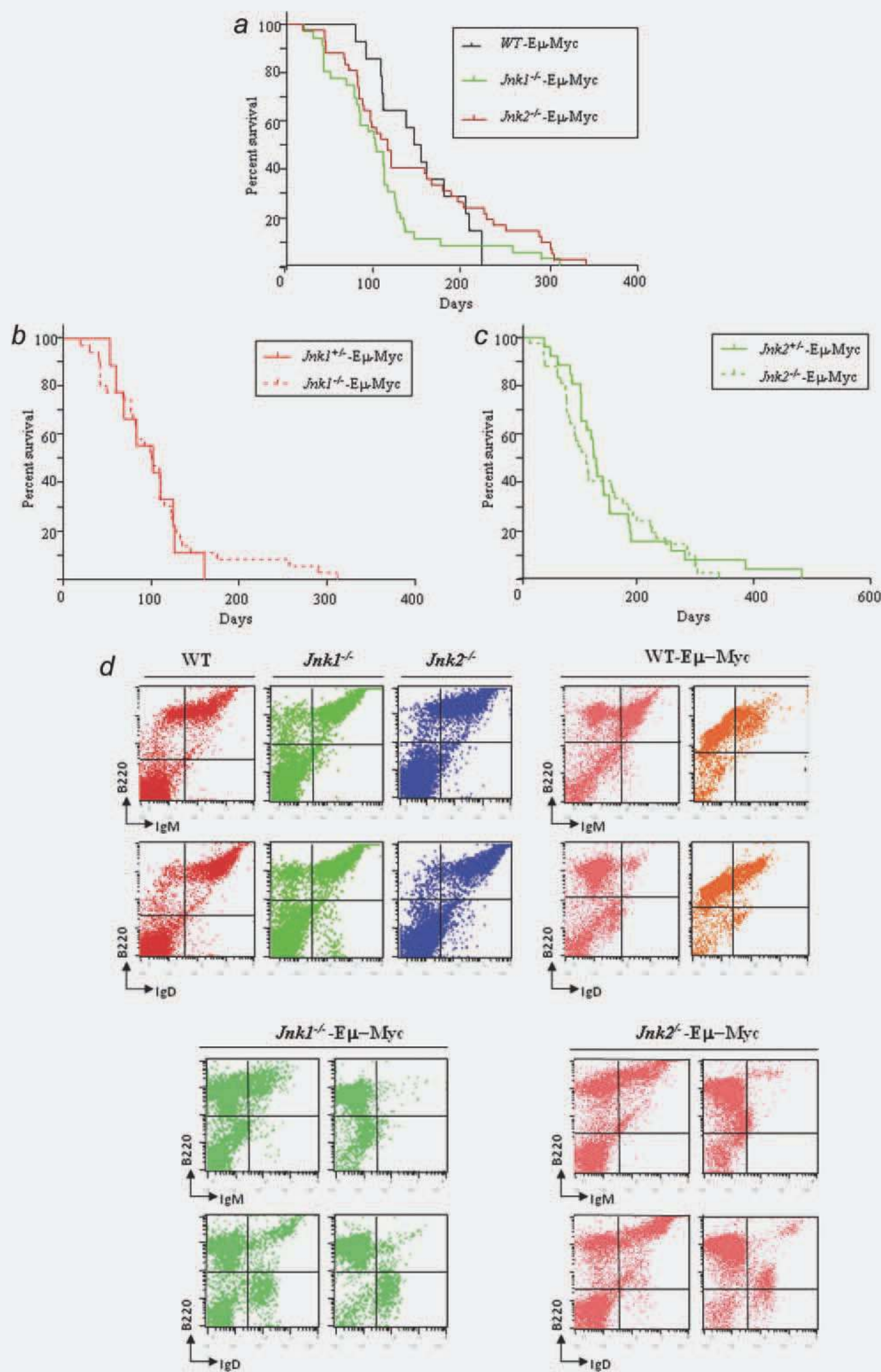

Figure 1. Kaplan–Meier survival curves of Eμ-Myc transgenic mice with differing JNK deficiencies. Eμ-Myc transgenic mice were crossed to either *Jnk1*$^{+/-}$ or *Jnk2*$^{+/-}$ mice and bred further to obtain colonies as shown above. All mice were monitored for tumor development, and survival curves for all mice (*a*), the JNK1 colony (*b*) and JNK2 colony (*c*) are shown. All Myc-transgenic mice developed B cell lymphomas, which were immunophenotyped by staining with B220 and IgM or IgD. Lymph nodes of normal nontransgenic mice were used as controls. Representative data from flow cytometric analysis shows the immunotype of the B cell lymphomas from several Myc-transgenic mice lacking JNK1 (3), JNK2 (2) or WT (3), as well as nontransgenic mice (1 per genotype; (*d*). Logrank (Mantel–Cox) test was performed to determine statistical significance. *p* Values are shown in the text. $n = 14$ (WT), 9 (*Jnk1*$^{+/-}$), 36 (*Jnk1*$^{-/-}$), 26 (*Jnk2*$^{+/-}$) and 42 (*Jnk2*$^{-/-}$). All mice are in the B6/129 background.

phenotype from all these groups of mice did not show any differences in maturation status (Fig. 1*d*). All non-Myc B220$^+$ B cells from lymphnodes were positive for IgM and IgD, whereas lymphomas from Myc-transgenic mice had varying degrees of loss of IgM and IgD in all genotypes, similar to the WT mice (Fig. 1*d*). These data, therefore, indicate that deficiency of either JNK1 or JNK2 does not have any appreciable effect on Myc-induced B cell lymphoma formation, leading us to conclude that although B cell activation induces JNK signaling, and that JNK activity is unregulated in B cell lymphomas,[8,13] absence of either JNK1 or JNK2 is probably compensated for by the other remaining JNK in this model. This, therefore, suggests that JNK1 and JNK2 may play redundant roles, similar to that seen in mature T cells.[14] Moreover, the data also points out that the type of inducing signal, *i.e.* Myc-oncogene *vs.* BCR-ABL oncogene may have determinant effects on the role of JNKs, as the later oncogene has been shown to require JNK1 for efficient B cell transformation, which cannot be compensated by the remaining JNK2 protein.[11] Altogether, these data highlights that the signal and the cell-type context play critical roles on the use of the JNK proteins.

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Yours sincerely,
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