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Role of JNK in a Trp53-Dependent Mouse Model of Breast Cancer

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Abstract

The cJun NH2-terminal kinase (JNK) group of signaling enzymes are activated by cytokines/growth factors and also by exposure to environmental stress [1]. Targets of the JNK pathway include members of the activator protein 1 (AP1) group of transcription factors (e.g. c-Jun, JunB, and JunD). JNK is therefore a major regulatory mechanism of AP1-dependent gene expression [1]. In addition, JNK can regulate many cytoplasmic and nuclear processes [2]. These studies have implicated the JNK signaling pathway in the regulation of cell growth and cell death [1]. Dysregulation of the JNK pathway may therefore contribute to the development of cancer [3].

The role of JNK in cancer has been studied using mouse models that are JNK-deficient. Two genes (Jnk1 and Jnk2) encode isoforms of JNK that are ubiquitously expressed [1]. Jnk1−/− mice and Jnk2−/− mice are viable, but compound mutant Jnk1−/−/Jnk2−/− mice exhibit an early embryonic lethal phenotype [1]. Studies using Jnk1−/− mice and Jnk2−/− mice indicate that JNK may have isoform-dependent effects on cancer. Thus, Bcr-Abl-induced lymphoma [4] and carcinogen-induced hepatocellular carcinoma [5] are suppressed in Jnk1−/− mice. Moreover, carcinogen-induced skin cancer is suppressed in Jnk2−/− mice [6]. Similarly, important roles for JNK2 have been identified in studies of human glioblastoma, prostate cancer, and lung carcinoma cell lines [7–10]. Together, these data confirm that both JNK1 and JNK2 can play roles in tumor development.

The purpose of this study was to test the requirement of JNK1 and JNK2 in a model of mammary carcinoma. Somatic mutation of the human p53 gene (TP53) is common in sporadic breast cancer [11]. Furthermore, mammary carcinoma is the most common form of cancer in women with heritable mutations in TP53 (Li-Fraumeni syndrome) [12]. Initial studies using mouse models demonstrated that Tp53−/− animals develop lymphoma with high frequency and that Tp53−/− animals display a moderately broader tumor spectrum with slower onset of disease [13,14]. Subsequent studies using Tp53−/− mice on a BALB/c strain background demonstrated that, like humans with Li-Fraumeni syndrome, mammary carcinomas were frequently observed, together with some lymphomas and sarcomas [15]. The BALB/c mouse model can therefore be used to examine Tp53-dependent formation of mammary carcinoma.

We report that JNK1 and JNK2 are not required for the development of mammary carcinoma in the Tp53−/− BALB/c mouse model. In contrast, the tumor-free survival of JNK-deficient Tp53−/− mice was significantly reduced compared with control Tp53−/− mice. We conclude that JNK1 and JNK2 can act as suppressors of mammary tumor development.
Materials and Methods

Mice

We have described Jnk1−/− mice [16] and Jnk2−/− mice [17] on a C57BL/6J strain background [18], and mice with Trp53 gene ablation [13] on a BALB/c×C3H strain background [19]. The mice used in this study were backcrossed (ten generations) to the BALB/c strain (Jackson Laboratories) and were housed in a facility accredited by the American Association for Laboratory Animal Care (AALAC). The Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School approved all studies using animals (Docket A-1032).

Genotype analysis

Genotype analysis was performed by PCR using genomic DNA as the template. The wild-type Jnk1 (460 bp) and knockout Jnk1 (390 bp) alleles were identified using the amplimers 5′-CGCCAGTCCAAAATCGAAGATC-3′, 5′-GCCATTCTGGTGAAGGAAATGGTCTC-3′, and 5′-CCAGCTGATCCGCTCACTCATG-3′. The wild-type Jnk2 (400 bp) and knockout Jnk2 (270 bp) alleles were identified using the amplifiers 5′-GGAACGCCGATAGTATCGAGTTACC-3′ and 5′-CCAGCTGATCCGCTCACTCATG-3′. The wild-type Trp53 (390 bp) and knockout Trp53 (300 bp) alleles were identified using the amplimers 5′-CACGCTGATCCGCTCACTCATG-3′ and 5′-CTATCAGGACATGCGTTTG-3′.

Analysis of tissue morphology

Mammary gland development was examined in virgin female mice (8 to 10 weeks of age), lactating mice (1 week postpartum), and mice with mammary gland involution (pups removed at 1 week post-partum). The fourth inguinal mammary gland pair was dissected from each mouse; one gland was analyzed by whole gland preparations of fourth inguinal mammary glands of JNK-deficient and mice with mammary gland involution (pups removed at 1 week post-partum). The fourth inguinal mammary gland pair was dissected from each mouse; one gland was analyzed by whole gland preparations of fourth inguinal mammary glands of JNK-deficient and knockout mice.

Effect of JNK-deficiency on tumor development in Trp53−/− BALB/c mice

We examined the tumor-free survival of Trp53−/− mice, Jnk1−/− Trp53−/− mice, and Jnk2−/− Trp53−/− mice on a BALB/c strain background. The mice rapidly developed cancer and died (Figure 4A). No significant differences in tumor-free survival between control and JNK-deficient mice were detected. Pathological examination of the mice demonstrated, as expected, a high incidence of lymphoma (Figure 4B). The second most frequent type of tumor detected in Trp53−/− mice and Trp53−/− Jnk2−/− mice was hemangiosarcoma (Figure 4B). In contrast, Jnk1−/− Trp53−/− mice displayed fewer hemangiosarcomas and a higher incidence of lymphoma compared with Trp53−/− mice (Figure 4B). These data suggest that JNK1 may influence the tumor spectrum of Trp53−/− mice. Indeed, a low incidence of mammary carcinoma was observed in both male and female Jnk1−/− Trp53−/− mice, but not in Trp53−/− mice or Jnk2−/− Trp53−/− mice (Figure 4B). The presence of mammary carcinoma in Jnk1−/− Trp53−/− mice indicates that JNK may be relevant to breast cancer.

Results

Effect of JNK-deficiency on mammary gland development

We backcrossed Jnk1−/− mice [16] and Jnk2−/− mice [17] to the BALB/c strain background. To test whether JNK-deficiency altered mammary gland development, we examined Jnk1−/− and Jnk2−/− BALB/c mice. No defects were detected in whole mount preparations of fourth inguinal mammary glands of JNK-deficient virgin female mice compared with control mice (Figure 1A). Sections prepared from these mammary glands confirmed that JNK-deficiency did not cause major defects in virgin mammary gland development (Figure 1B).

Pregnancy causes major changes in mammary gland development, including the formation of alveoli. Sections prepared from the fourth inguinal mammary glands of JNK-deficient lactating mice and control lactating mice were similar (Figure 2). Indeed, sections stained for proliferating cell nuclear antigen (PCNA) indicated that JNK-deficiency did not alter epithelial cell proliferation in the lactating mammary gland (Figure 2).

Involution of the lactating mammary gland occurs after weaning pups. We compared sections of the fourth inguinal mammary glands prepared on day 2 and day 3 following weaning. No defects in involution were detected in JNK-deficient mice compared with control mice (Figure 3).

Together, these data demonstrate that JNK1-deficiency and JNK2-deficiency did not cause detected changes in mammary gland development. Similarly, no developmental defects caused by JNK1-deficiency or JNK2-deficiency were detected in Trp53−/− mice.

Effect of JNK-deficiency on tumor development in Trp53−/− BALB/c mice

We performed studies of tumor-free survival of Trp53−/− mice, Jnk1−/− Trp53−/− mice, and Jnk2−/− Trp53−/− mice on a BALB/c strain background. The mice rapidly developed cancer and died (Figure 4A). No significant differences in tumor-free survival between control and JNK-deficient mice were detected. Pathological examination of the mice demonstrated, as expected, a high incidence of lymphoma (Figure 4B). The second most frequent type of tumor detected in Trp53−/− mice and Trp53−/− Jnk2−/− mice was hemangiosarcoma (Figure 4B). In contrast, Jnk1−/− Trp53−/− mice displayed fewer hemangiosarcomas and a higher incidence of lymphoma compared with Trp53−/− mice (Figure 4B). These data suggest that JNK1 may influence the tumor spectrum of Trp53−/− mice. Indeed, a low incidence of mammary carcinoma was observed in both male and female Jnk1−/− Trp53−/− mice, but not in Trp53−/− mice or Jnk2−/− Trp53−/− mice (Figure 4B). The presence of mammary carcinoma in Jnk1−/− Trp53−/− mice indicates that JNK may be relevant to breast cancer.

Effect of JNK-deficiency on tumor development in Trp53−/− BALB/c mice

We performed studies of tumor-free survival of Trp53−/− mice, Jnk1−/− Trp53−/− mice, and Jnk2−/− Trp53−/− mice on a BALB/c strain background. Tumor development in the Trp53−/− mice delayed compared with Trp53−/− mice (Figure 5A). Interestingly, JNK1-deficiency (p = 0.026) and JNK2-deficiency (p = 0.012) caused significantly shortened tumor-free survival compared with control Trp53−/− BALB/c mice (Figure 5A). Pathological analysis demonstrated that mammary carcinoma was the most common type of tumor detected. Mammary carcinomas were detected in 43% of control mice, 70% of Jnk1−/− mice, and 53% of Jnk2−/− mice (Figure 5B). Analysis of mammary carcinoma-free survival of Trp53−/− mice, Jnk1−/− Trp53−/− mice, and Jnk2−/− Trp53−/− mice demonstrated that JNK1-deficiency (p = 0.018) and JNK2-deficiency (p = 0.039) significantly decreased survival compared with control Trp53−/− mice (Figure 5C). No significant difference in mammary carcinoma-free survival between JNK1-deficient mice and JNK2-deficient mice was detected (Figure 5C).

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The increased mammary carcinoma detected in JNK1-deficient \( \text{Trp53}^{-/-} \) mice was associated with a decreased incidence of hemangiosarcoma (Figure 5B). No hemangiosarcomas were detected in JNK2-deficient \( \text{Trp53}^{-/-} \) mice (Figure 5B). These changes in tumor spectrum may reflect the shortened tumor-free survival of \( \text{Trp53}^{-/-} \) mice (Figure 5A,C).

Together, these data indicate that JNK1 and JNK2 are not required for mammary carcinoma development in the \( \text{Trp53}^{-/-} \) BALB/c mouse model of breast cancer. However, both JNK1 and JNK2 can influence breast cancer development. It appears that JNK can contribute to tumor suppression.

**Discussion**

JNK1 and JNK2 are not required for the development of mammary carcinoma in the \( \text{Trp53} \) BALB/c mouse model

JNK plays a critical role in the development of some forms of cancer [1]. Thus, carcinogen-induced hepatocellular carcinoma [5] and BcrAbl-induced lymphoma [4] are strongly suppressed in \( \text{Jnk}^{1/-} \) mice and carcinogen-induced skin cancer is suppressed in \( \text{Jnk}^{2/-} \) mice [6]. Moreover, studies of glioblastoma, prostate cancer, and lung carcinoma cell lines have identified important roles for JNK2 [7–10]. Together, these data confirm that JNK plays an important role in cancer development.

The results of this study suggest that JNK may play a different role in mammary carcinogenesis because neither JNK1-deficiency nor JNK2-deficiency in the \( \text{Trp53} \) BALB/c mouse model caused a reduction in the incidence of mammary carcinoma. This observation strongly contrasts with the finding that JNK-deficiency can markedly suppress hepatocellular carcinoma, lymphoma, and skin cancer [4–6].

Although JNK1-deficiency and JNK2-deficiency did not suppress mammary carcinogenesis in the \( \text{Trp53} \) BALB/c mouse model, we cannot exclude the possibility that deficiency of both JNK1 plus JNK2 might reduce the formation of mammary carcinoma. Indeed, the \( \text{Jnk}^{1/-} \) and \( \text{Jnk}^{2/-} \) genes may have partially redundant functions [18,20–22]. Studies of compound mutants with disruption of \( \text{Jnk}^{1/-} \) plus \( \text{Jnk}^{2/-} \) are required. The early embryonic lethal phenotype of \( \text{Jnk}^{1/-} \) \( \text{Jnk}^{2/-} \) mice [23] makes such studies difficult. Nevertheless, the effect of compound JNK-deficiency on mammary carcinoma development needs to be tested in future studies.

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**Figure 1. Effect of JNK-deficiency in virgin mice on breast development.**

A) Whole mount preparations of the fourth inguinal mammary gland of 10-week-old female virgin mice were stained with carmine red. Representative images are presented. Scale bar: 5 mm (upper panel); 800 μm (lower panel). B) Sections of the breast tissue were stained with hematoxylin and eosin. Representative images are presented. Scale bar: 100 μm. doi:10.1371/journal.pone.0012469.g001
Figure 2. Effect of JNK-deficiency on breast development during lactation. Sections of the fourth inguinal mammary gland of female mice at day 7 post-partum were examined by staining with hematoxylin and eosin (upper panels). Sections were also stained with an antibody to the proliferation marker PCNA (red) and the DNA stain DAPI (blue) (lower panels). Scale bar: 200 µm (upper panel); 50 µm (lower panel).

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Figure 3. Effect of JNK-deficiency on mammary gland involution. The pups were removed from female mice at day 7 post-partum to induce mammary gland involution. Sections of the fourth inguinal mammary gland were examined at two days or three days post-weaning by staining with hematoxylin and eosin. Scale bar: 200 µm.

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JNK and tumor suppression

The analysis of JNK1-deficiency and JNK2-deficiency in the Trp53−/− BALB/c mouse model of mammary carcinoma development demonstrates that neither JNK1 nor JNK2 is required for breast tumorigenesis (Figures 5). In contrast, the mammary carcinoma-free survival of both Jnk1−/− Jnk2−/−, Jnk1−/− Trp53−/−, and Jnk2−/− Trp53−/− mice was significantly reduced compared with control mice (Figure 5C). These data suggest that JNK may have a tumor suppressor role in breast cancer. This conclusion is consistent with the observation that JNK2-deficiency increases breast cancer in a transgenic mouse model with expression of polyoma virus T antigen [24]. Moreover, human genetic analysis has identified mutations in the JNK signaling pathway in breast cancer that correlate with tumor suppression and metastasis [3]. Specifically, loss-of-function mutations in MKK4, a human gene that encodes an activator of JNK, is mutated at low frequency in human breast cancer [25,26,27,28]. It is likely that JNK1-deficiency and JNK2-deficiency in the mouse may phenocopy the effects of MKK4 gene mutation on breast cancer in humans. The molecular mechanism of tumor suppression by the JNK signaling pathway is unclear, but may be related to a requirement of JNK for genetic stability [24]. Indeed, it has been reported that genes that encode DNA repair enzymes are over-represented as targets of JNK signaling pathway [29]. A role for JNK in the maintenance of genetic stability is also consistent with the finding that a dominant genetic trait in the Trp53−/− BALB/c mouse model of mammary carcinogenesis is loss of heterozygosity at the Trp53 locus [30]. This observation indicates that the formation of mammary carcinoma in JNK-deficient mice may be caused by accelerated loss of heterozygosity of tumor suppressor genes.

**Figure 4. Effect of JNK-deficiency on Trp53−/− mouse survival.** A) Kaplan-Meier analysis of the tumor-free survival of wild-type (WT), Jnk1−/− Jnk2−/−, Trp53−/− Jnk1−/− Trp53−/−, and Jnk2−/− Trp53−/− mice were presented. No statistically significant differences between Trp53−/−, Jnk1−/− Trp53−/−, and Jnk2−/− Trp53−/− mice were detected (Log-rank test, p>0.05). The data represent groups of 44–62 mice. These groups include equal numbers of male and female mice. B) The spectrum of tumors detected in Trp53−/−, Jnk1−/− Trp53−/−, and Jnk2−/− Trp53−/− mice following euthanasia is presented. No statistically significant differences in the tumor profiles between genotypes were detected using Fisher’s exact test.

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Figure 5. Effect of JNK-deficiency on Trp53+/− mouse survival. A) Kaplan-Meier analysis of the tumor-free survival of wild-type (WT), Jnk1−/−, Jnk2−/−, Trp53−/−, Jnk1−/− Trp53+/−, and Jnk2−/− Trp53+/− mice is presented. The survival of Jnk1−/− Trp53−/− mice and Jnk2−/− Trp53−/− mice was reduced compared with Trp53+/− mice (Log-rank test, p = 0.026 and 0.012, respectively). The data represent groups of 14 - 20 female mice. B) The spectrum of tumors detected in Trp53+/−, Jnk1−/− Trp53+/−, and Jnk2−/− Trp53+/− female mice following euthanasia is presented. No statistically significant differences in the tumor profiles between genotypes were detected using Fisher’s exact test. C) Kaplan-Meier analysis of the mammary carcinoma-free survival of Trp53+/−, Jnk1−/− Trp53+/−, and Jnk2−/− Trp53+/− mice is presented. Cohorts of Trp53−/− mice (n = 6), Jnk1−/− Trp53−/− mice (n = 14), and Jnk2−/− Trp53−/− mice (n = 8) with mammary carcinoma were examined. JNK1-deficiency and JNK2-deficiency caused reduced mammary carcinoma-free survival compared with Trp53+/− mice (Log-rank test, p = 0.018 and 0.039, respectively). doi:10.1371/journal.pone.0012469.g005
Conclusions
We tested the hypothesis that JNK1 or JNK2 plays a critical role during breast cancer development. We found that neither JNK1 nor JNK2 is required for mammary carcinoma in the Trp53−/− Balb/c mouse model. Breast tumor-free survival was significantly reduced by JNK1-deficiency or JNK2-deficiency. These data suggest that JNK1 and JNK2 may play a role in mammary carcinoma suppression.

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Author Contributions
Conceived and designed the experiments: CC CRW HKS RJD. Performed the experiments: CC CRW JR DSG. Analyzed the data: CC CRW DSG HKS RJD. Contributed reagents/materials/analysis tools: DJJ. Wrote the paper: CC DII HKS RJD.