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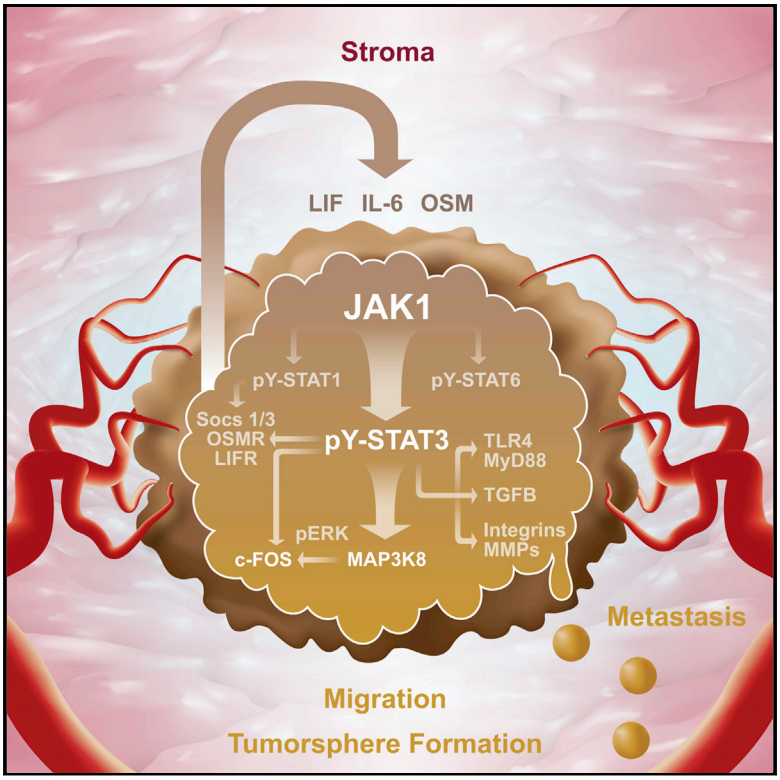
Janus Kinase 1 Plays a Critical Role in Mammary Cancer Progression

Graphical Abstract



Authors

Barbara L. Wehde, Patrick D. Rädler, Hridaya Shrestha, Stevi J. Johnson, Aleata A. Triplett, Kay-Uwe Wagner

Correspondence

wagnerk@karmanos.org

In Brief

Wehde et al. demonstrate that JAK1 is the pivotal kinase that controls cytokine-mediated activation of three STAT proteins in ERBB2-driven mammary cancer cells. They provide experimental evidence that deficiency in JAK1/STAT3 signaling and consequential downregulation of oncogenic targets inhibit migration, tumorsphere formation, and metastatic dissemination of mammary cancer cells.

Highlights

- Loss of JAK1 prevents metastasis in an ERBB2-induced mammary cancer model
- JAK1 is the pivotal kinase mediating oncogenic STAT activation in ERBB2-transformed cells
- JAK1 signaling has pleiotropic effects on pathways associated with cancer progression
- STAT3, FOS, and MAP3K8 are JAK1 targets that promote migration and tumorsphere formation

Wehde et al., 2018, Cell Reports *25*, 2192–2207
November 20, 2018
https://doi.org/10.1016/j.celrep.2018.10.063

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Janus Kinase 1 Plays a Critical Role in Mammary Cancer Progression

Barbara L. Wehde,[1,4] Patrick D. Rädler,[1,2] Hridaya Shrestha,[2] Stevi J. Johnson,[2] Aleata A. Triplett,[1] and Kay-Uwe Wagner[1,2,3,5,*]

[1]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 985950 Nebraska Medical Center, Omaha, NE 68198-5950, USA
[2]Department of Oncology, Wayne State University School of Medicine, Detroit, MI, USA
[3]Tumor Biology Program, Barbara Ann Karmanos Cancer Institute, 4100 John R Street, EL01TM, Detroit, MI 48201, USA
[4]Present address: Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
[5]Lead Contact
*Correspondence: wagnerk@karmanos.org
https://doi.org/10.1016/j.celrep.2018.10.063

SUMMARY

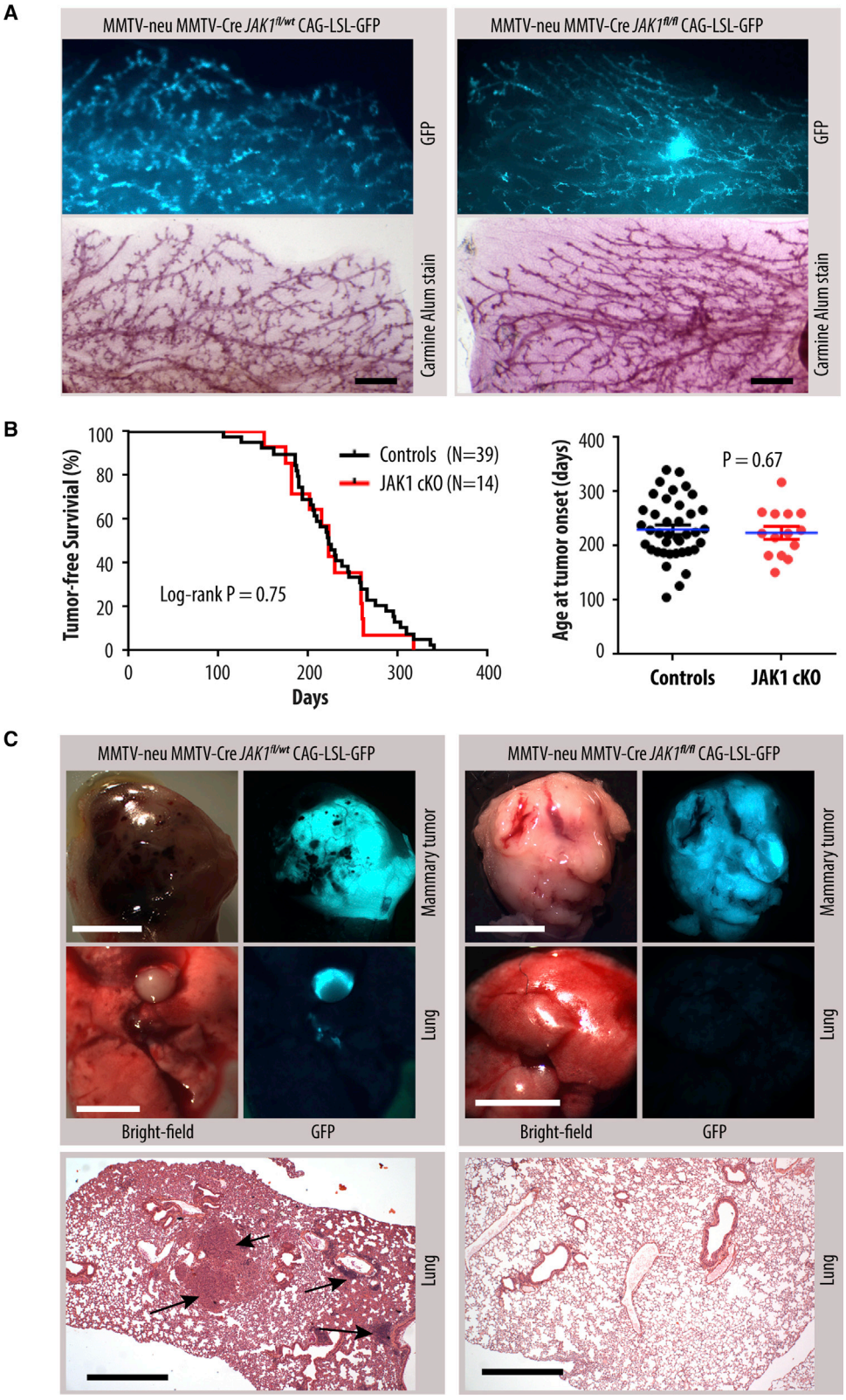
Janus kinases (JAKs) and their downstream STAT proteins play key roles in cytokine signaling, tissue homeostasis, and cancer development. Using a breast cancer model that conditionally lacks Janus kinase 1, we show here that JAK1 is essential for IL-6-class inflammatory cytokine signaling and plays a critical role in metastatic cancer progression. JAK1 is indispensable for the oncogenic activation of STAT1, STAT3, and STAT6 in ERBB2-expressing cancer cells, suggesting that ERBB2 receptor tyrosine kinase complexes do not directly activate these STAT proteins *in vivo*. A genome-wide gene expression analysis revealed that JAK1 signaling has pleiotropic effects on several pathways associated with cancer progression. We established that FOS and MAP3K8 are targets of JAK1/STAT3 signaling, which promotes tumorsphere formation and cell migration. The results highlight the significance of JAK1 as a rational therapeutic target to block IL-6-class cytokines, which are master regulators of cancer-associated inflammation.

INTRODUCTION

Interleukin-6 (IL-6)-class cytokines (e.g., IL-6, leukemia inhibitory factor [LIF], oncostatin-M [OSM], and IL-11) are master regulators of inflammation and play key roles in neoplastic progression. These cytokines signal through specific ligand-receptor complexes that share the glycoprotein 130 (gp130) signal-transducing subunit, which activates Janus tyrosine kinases (JAKs) and downstream signal transducers and activators of transcription (STATs) (Heinrich et al., 1998). In the normal mammary epithelium, IL-6-class cytokine signaling through the gp130/JAK/STAT pathway is tightly regulated in a spatiotemporal manner. Although IL-6-class cytokines play pivotal roles in the activation of STAT3 throughout mammogenesis, indispensable functions of STAT3 and its upstream ligand-receptor complexes are confined to postlactational involution (Chapman et al., 1999; Humphreys et al., 2002; Kritikou et al., 2003; Zhao et al., 2004). Here, the lack of STAT3 activation results in extended survival of differentiated epithelial cells and decelerated remodeling of the entire gland.

In contrast to the normal mammary epithelium, many breast cancer cells exhibit constitutive tyrosine phosphorylation of STAT3, which is indicative of a pro-inflammatory tumor environment (Garcia et al., 2001). The activation of STAT3 is enhanced by SRC and receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR) and ERBB2 (Nam et al., 2013; Ren and Schaefer, 2002; Zhang et al., 2000). More recent evidence suggests that the hyper-phosphorylation of STAT3 is likely the result of elevated and cancer cell-specific production of IL-6 and downstream activation of gp130/JAK complexes in response to RTK signaling (Berishaj et al., 2007). In preneoplastic and transformed epithelial cells, the pro-apoptotic functions of STAT3 can be effectively neutralized by sustained activation of survival factors; in particular, STAT5, phosphatidylinositol 3-kinase (PI3K) and AKT1 (for references, see Rädler et al., 2017). Under these disease-specific conditions, constitutively active STAT3 may still promote other facets of tissue remodeling within a malignant tumor, such as the enzymatically controlled detachment of cancer cells from the basement membrane. This phenomenon might explain why STAT3 plays a significant role in the progression of certain breast cancer subtypes, specifically ERBB2-overexpressing mammary tumors *in vivo* (Ranger et al., 2009).

Although the importance of IL-6-class cytokines and STAT3 has been established, the biological roles of individual JAKs that couple inflammatory cytokines to STAT3 are less defined. Although it has been suggested that JAK1 and JAK2 are both capable of activating STAT3 in response to IL-6 in diverse cell culture models, we observed, in various genetically engineered mammary tumor models, that conditional deletion of the *Jak2* gene had no effect on the oncogenic activation of STAT3 *in vitro* or *in vivo* (Sakamoto et al., 2009, 2010). Our team recently developed JAK1 conditional knockout mice, and the specific deletion of this Janus kinase from embryonic fibroblasts and the epithelium of the developing mammary gland revealed that JAK1 has non-redundant functions for the activation of STAT1,



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STAT3, and STAT6 (Sakamoto et al., 2016b). Despite its significance for the activation of several STAT proteins, the phenotypic abnormalities associated with JAK1 deficiency in the mammary gland closely resemble that of a STAT3 knockout. On the molecular level, we demonstrated that loss of JAK1 uncouples the IL-6-class ligands from their downstream effector STAT3 in the mammary epithelium as well as in fibroblasts.

Here we provide experimental evidence that JAK1 is the pivotal kinase that mediates the persistent oncogenic activation of STAT3 in mammary cancer cells that are driven by ERBB2 receptor tyrosine kinase signaling. Although JAK1 is not strictly required for ERBB2-induced tumor onset, cancer cell-specific loss of this kinase reduces the migratory and tumorsphere-forming capabilities of mammary cancer cells *in vitro* as well as their engraftment and metastatic dissemination *in vivo*. The transcriptome analysis revealed that JAK1 is crucial for the expression of genes that mediate inflammation and epithelial remodeling. Re-expression of hyperactive STAT3 or its downstream targets c-FOS and MAP3K8 was sufficient to partially restore tumorsphere formation and enhance the migration of JAK1-deficient cancer cells. The collective results of this study illuminate the significance of targeting JAK1 to block the oncogenic activation of STAT3 and to prevent malignant progression.

RESULTS

JAK1 Promotes the Metastatic Dissemination of EBBR2-Induced Mammary Cancer Cells

To investigate the role of Janus kinase 1 in ERBB2-associated mammary cancer initiation, we generated a cohort of 18 mammary-specific JAK1 knockout females that overexpress wild-type ERBB2 (*MMTV*-neu *MMTV-Cre* $Jak1^{fl/fl}$). These experimental mice and 39 littermate controls (*MMTV*-neu $Jak1^{fl/fl}$ *or MMTV*-neu *MMTV-Cre* $Jak1^{fl/wt}$) were bred at least twice to adjust for the known effects of parity on tumor latency in this cancer model. Additionally, we kept a cohort of 15 multiparous JAK1 mammary-specific knockouts without the MMTV-neu transgene (*MMTV-Cre* $Jak1^{fl/fl}$) for a period of up to 18 months to control for possible tumor susceptibility functions of JAK1. The absence of macro- and microscopic mammary neoplasms in these control mice (data not shown) suggested that, unlike deficiency in STAT1 (Chan et al., 2012; Schneckenleithner et al., 2011), lack of JAK1 in the mammary epithelium is insufficient to trigger neoplastic transformation. Most experimental females carrying the MMTV-neu oncogene in the absence or presence of endogenous JAK1 also carried a Cre/lox reporter transgene (CAG-LSL-GFP), which was being used to monitor the expression and functionality of the Cre recombinase on the single-cell level. The abundant presence of GFP-positive cells throughout the mammary ducts of *MMTV*-neu *MMTV-Cre* $Jak1^{fl/fl}$ females suggested that the expression of oncogenic ERBB2 did not cause any negative selection of epithelial cells that conditionally lack JAK1 (Figure 1A). Moreover, the frequent occurrence of small, GFP-expressing lesions in these tissues indicated that deficiency in JAK1 did not prevent the initiation of ERBB2-associated mammary neoplasms. As illustrated in the Kaplan-Meier survival plot (Figure 1B), all JAK1 conditional knockout mice developed palpable mammary tumors at a median latency that was similar to their wild-type littermate controls (231 and 238 days, respectively). Also, the average period from detection of a palpable neoplasm to the maximal allowed size of a tumor (1.5 cm in diameter, as mandated by the institutional animal study protocol) was identical in both cohorts (21.9 ± 2.6 days in the controls and 21.2 ± 2.6 days in JAK1-deficient females, $p = 0.8$), suggesting that the growth of ERBB2-induced mammary cancers did not require JAK1 expression. The vast majority of tumors from JAK1 knockout mice and their controls, which carried the Cre/lox reporter, were strongly GFP-positive (Figure 1C). The absence of JAK1 protein in these cancer tissues was further validated by immunoblot (Figure 2A). The collective findings of the phenotypic analysis of JAK1-deficient mice suggested that signaling through JAK1 is not essential for the onset of ERBB2-induced mammary carcinogenesis and the growth of primary tumors. It is important to note that, unlike mammary-specific STAT1 knockout mice (Klover et al., 2010), deletion of *Jak1* had no notable effect on the tumor burden. Given the indispensable role of JAK1 for STAT1 activation (see below), this observation may support the idea that the tumor-suppressive function of STAT1 does not rely on its tyrosine phosphorylation.

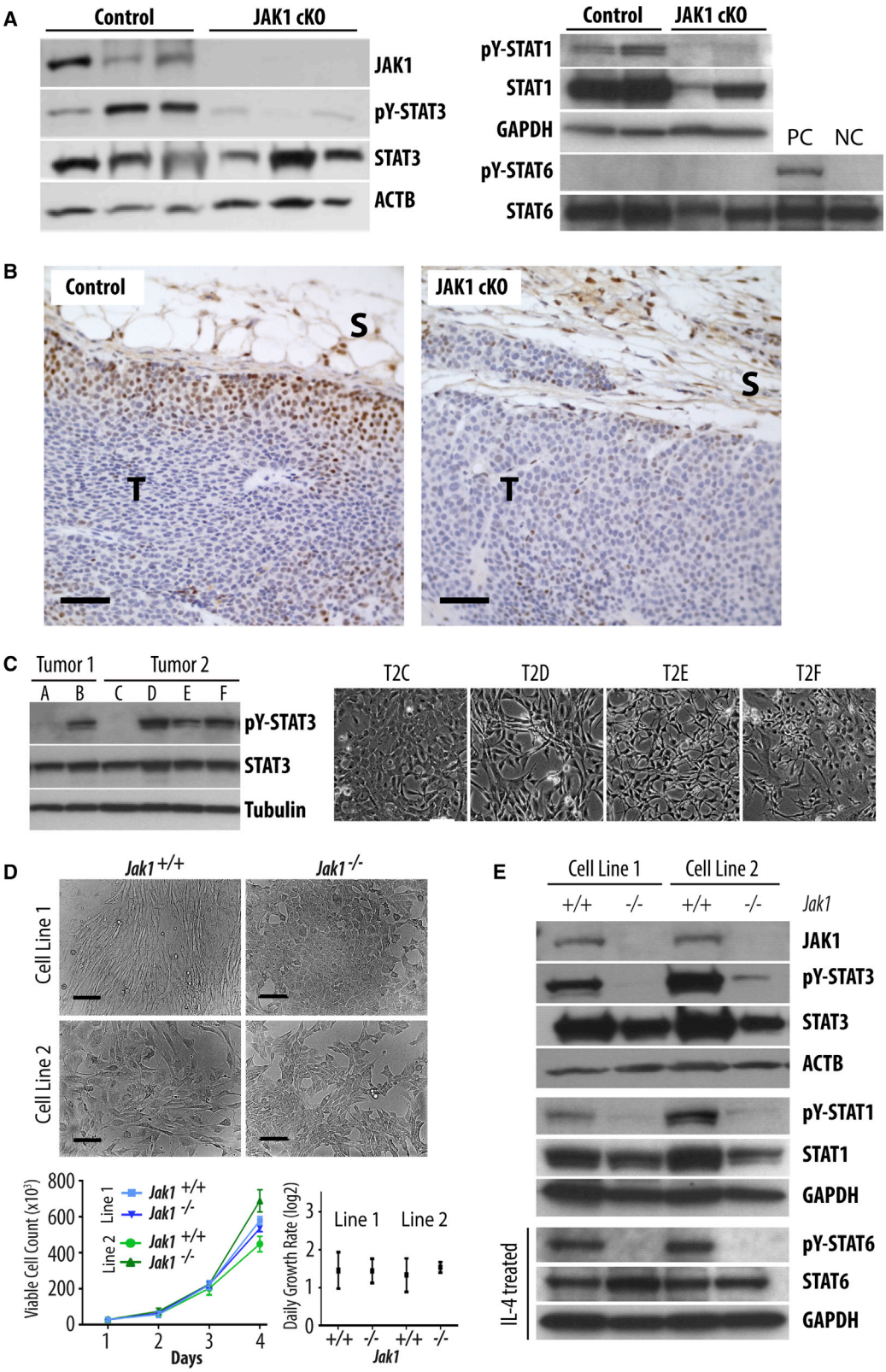
Histologic analysis of JAK1-deficient mammary tumors and their JAK1-expressing controls did not reveal any other major morphological differences (Figure S1). The most striking dissimilarity between JAK1-deficient mammary cancers and controls, however, was their significantly reduced propensity to metastasize. A detailed necropsy and histological analysis of organs from 14 tumor-bearing, JAK1-deficient females and 16 controls revealed that more than half (9 of 16) of the JAK1 wild-type controls exhibited pulmonary metastases. In contrast, we did not detect any GFP-positive, JAK1-deficient tumor cells colonizing the lungs in any of the JAK1 conditional knockout mice at the time of necropsy (Figure 1C). These observations suggest that, despite a dispensable function of JAK1 in tumor initiation, this Janus kinase plays a crucial role in the malignant progression and metastatic dissemination of ERBB2-induced mammary cancer cells.

Figure 1. JAK1 Is Not Essential for ERBB2-Induced Mammary Tumor Initiation but Plays a Critical Role in Malignant Progression and Metastasis

(A) Top: GFP fluorescence images of unfixed mammary glands from a multiparous MMTV-neu female with a mammary epithelium-specific deletion of JAK1 (MMTV-neu MMTV-Cre $Jak1^{fl/fl}$ CAG-LSL-GFP) and an age-matched control female expressing JAK1 (MMTV-neu MMTV-Cre $Jak1^{fl/wt}$ CAG-LSL-GFP). Bottom: the same glands following fixation and carmine alum staining. Scale bars, 1 mm.

(B) Kaplan-Meier survival plot and mean age of tumor onset; means ± SD are presented as bars. No significant differences were found.

(C) GFP and corresponding bright-field images of ERBB2-induced mammary tumors and lungs of a JAK1 conditional knockout and control. Scale bars, 5 mm. Bottom: H&E-stained histologic sections of lung tissues of tumor-bearing mice. Arrows indicate metastatic lesions in the wild-type controls. Scale bars, 1 cm.



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JAK1 Controls the Constitutive Activation of STAT3 and STAT1 and the Ligand-Induced Phosphorylation of STAT6 in ERBB2-Induced Mammary Cancer Cells

We performed immunoblot analyses on ERBB2-induced mammary tumor tissues of JAK1 conditional knockout mice and their littermate controls to assess whether deficiency in JAK1 affected the cancer cell-specific activation of downstream STAT proteins. In contrast to the conditional deletion of *Jak2*, which had no discernable effect on the activation of STAT3 (Sakamoto et al., 2009), the knockout of JAK1 was sufficient to significantly lower the persistent tyrosine phosphorylation of this STAT protein (Figure 2A). This finding was validated using immunostaining of histologic sections of the corresponding tumors (Figure 2B). Expression of nuclear pY-STAT3 was particularly high in cancer cells at the invasive front and in the proximity of blood vessels of JAK1-expressing tumors. These spatial patterns of STAT3 staining were absent in JAK1-deficient tumors. The nuclear localization of active STAT3 in tumor-associated stromal cells was not affected by cancer cell-specific deletion of JAK1 and served as an internal staining control. Similar to its effects on STAT3, JAK1 deficiency resulted in a loss of STAT1 activation (Figure 2A). Although STAT6 protein was present in all mammary tumors, it was not tyrosine-phosphorylated in MMTV-neu-induced tumors regardless of JAK1 expression.

To examine whether deletion of the *Jak1* gene in neoplastic cells (i.e., following tumor onset) causes a block in STAT activation, we derived cancer cell lines from primary mammary tumors of two *MMTV*-neu-expressing control females (Figure 2C). Comparison of the activation of STAT3 in morphologically distinct cellular lineages of a single tumor revealed that the expression of active STAT3 was particularly high in sub-populations of ERBB2-induced mammary cancer cells, which are more spindle-shaped in appearance (e.g., cell lines T2C to T2F). We did not observe any significant differences in proliferation rates among cancer cell lineages from the same tumor despite dissimilar levels of STAT3 activation (data not shown). Using retroviral expression of Cre recombinase in established *MMTV*-neu *Jak1*$^{fl/fl}$ tumor cell lines, we generated two isogenic cell line pairs with and without JAK1 (*Jak1*$^{+/+}$, *Jak1*$^{-/-}$). Although complete loss of this kinase had no significant effect on cell proliferation, we observed that JAK1-deficient cells assumed a more cobblestone-like appearance (Figure 2D). Lack of JAK1 led to complete or significantly reduced tyrosine phosphorylation of STAT3 as well as STAT1 (Figure 2E). Like mammary tumors *in vivo*, cultured ERBB2-overexpressing cancer cells did not exhibit active STAT6 at steady state. In response to IL-4 treatment, however, JAK1-expressing cells showed pronounced phosphorylation of STAT6, which was completely blocked by the lack of JAK1 (Figure 2E, bottom). The collective results of this line of investigation clearly demonstrate that, identical to the normal mammary epithelium, JAK1 is essential for constitutive activation of STAT3 and STAT1 as well as IL-4-inducible phosphorylation of STAT6 in mammary cancer cells. This suggests that the nonredundant role of JAK1 for activation of these three STAT proteins is retained in cancer cells that were transformed by persistent ERBB2 signaling. Therefore, oncogenic signals of ERBB2 receptor complexes and their downstream effectors, such as SRC, do not mediate a direct or JAK1-independent activation of STAT3.

Deletion of the *Jak1* Gene Affects the Orthotopic Engraftment and Metastatic Dissemination of Mammary Cancer Cells

To study the biological effects of loss of function of JAK1 in cancer cells and their ability to form secondary mammary tumors, we constitutively expressed a lentivirus-based luciferase reporter in two isogenic cancer cell lines with and without JAK1 (Figure 3A, left) and transplanted 1×10^6 tumor cells orthotopically into the collateral #4 mammary glands of athymic nude mice (Figure 3A, right). In contrast to JAK2-deficient cancer cells, which did not show any noticeable differences in tumor development (Sakamoto et al., 2009), deletion of the *Jak1* gene had a profound effect on the engraftment and subsequent growth of secondary mammary cancers (Figure 3B). Although JAK1-deficient cancer cells were detectable at the sites of engraftment (Figure 3A, right), they exhibited significantly decelerated numeric expansion, and they did not metastasize (Figure 3C). The majority of JAK1-expressing control cells contained nuclear pY-STAT3 in primary and metastatic lesions (Figure 3D), whereas the knockout cells were devoid of active STAT3 (Figure 3E). As mandated by the institutional animal study protocol, all control females with JAK1-expressing tumors had to be euthanized when tumors reached a size of approximately 1.5 cm in diameter (i.e., around 6 weeks after transplantation). We kept several experimental mice with JAK1-deficient tumor cells over a prolonged period of 3 to 6 months and observed that a subset of females developed palpable neoplasms. These recipient mice did not show any pulmonary metastases. Using immunoblot, we validated that these tumors had retained the significantly lower levels of active STAT3 that were observed in the cancer cell lines (Figure 3F). This suggested that the

Figure 2. JAK1 Is the Pivotal Tyrosine Kinase that Controls the Activation of STAT3, STAT1, and STAT6 in ERBB2-Induced Mammary Cancer Cells

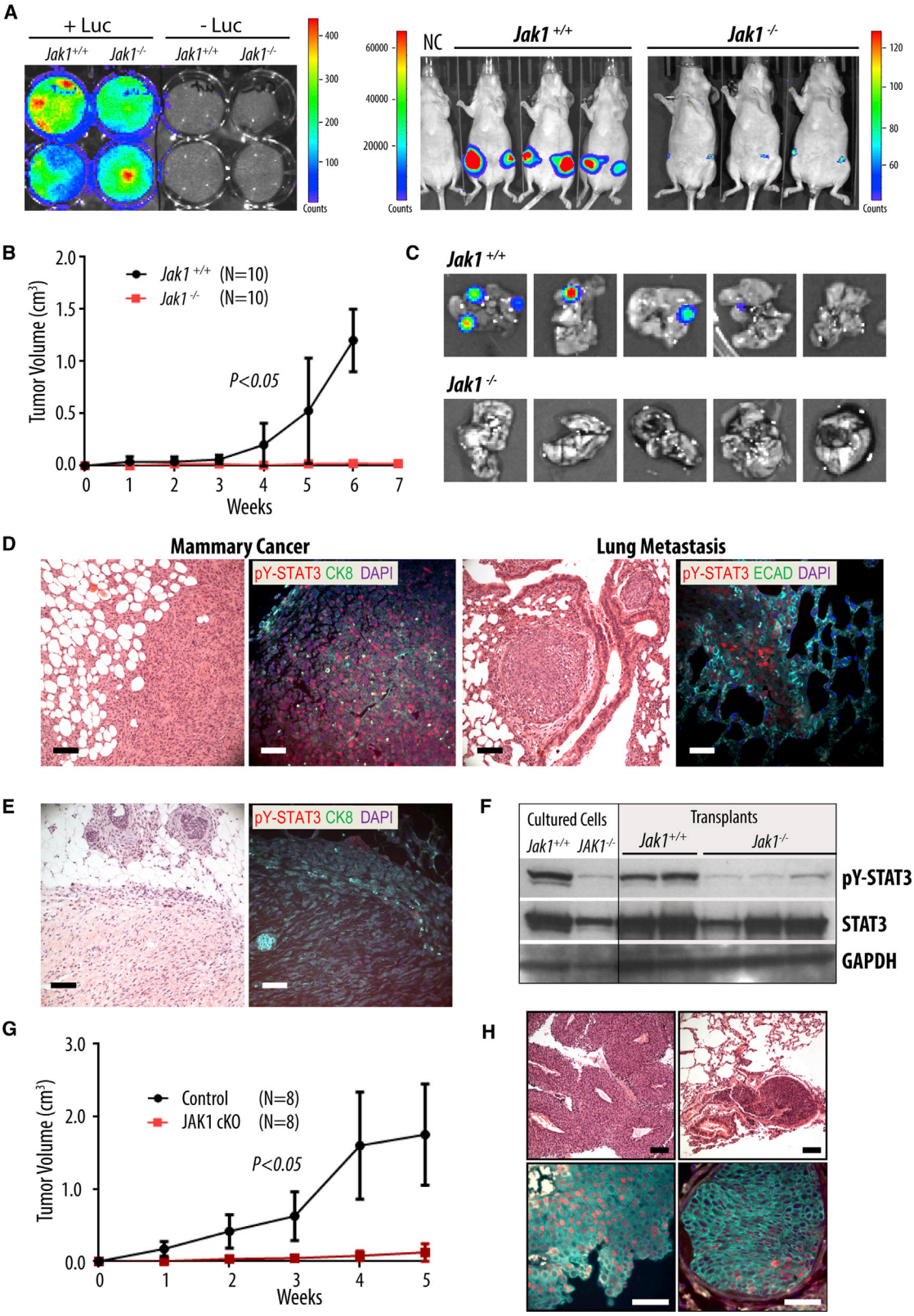
(A) Immunoblot analysis to assess the expression of JAK1 as well as tyrosine-phosphorylated and total levels of STAT3, STAT1, and STAT6 in mammary tumor tissues from JAK1 conditional knockout mice (JAK1 cKO) and wild-type controls.

(B) Immunohistochemical staining of active STAT3 in primary tumors with and without JAK1. Scale bars, 50 μm.

(C) Immunoblot analysis to assess the oncogenic activation of STAT3 in MMTV-neu-induced cancer cell lines expressing endogenous JAK1. Right: bright-field images of the cell lines from tumor 2. Scale bar, 100 μm.

(D) Bright-field images (scale bars, 100 μm), viable cell counts, and daily growth rates following Cre-mediated deletion of the *Jak1* gene in two cancer cell lines (*Jak1*$^{-/-}$) and their isogenic wild-type controls (*Jak1*$^{+/+}$). The results shown are from 3 replicates per cell line and displayed as mean viable cell count ± SEM and mean daily growth rate (log2-transformed) ± SD. No significant differences between any of the cell lines were found by one-way ANOVA and Tukey's multiple comparisons test.

(E) Immunoblot analysis to determine the expression and activation of STAT3 and STAT1 as well as the IL-4-induced activation of STAT6 in the two isogenic cancer cell lines.



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protracted growth of these lesions was not fueled by any mechanisms that mediate a compensatory activation of STAT3 in the absence of JAK1. To exclude the possibility that these collective findings were a consequence of maintaining and manipulating cancer cells *ex vivo*, we also performed a transplantation experiment with mammary tumor fragments (approximately 1 mm^3 in size) that were isolated from JAK1 conditional knockout mice and their wild-type controls. Even under these experimental conditions, JAK1-deficient tumor tissues exhibited significantly decelerated engraftment and growth of secondary tumors (Figure 3G). Only the JAK1-expressing control tissues showed strong activation of STAT3 and were capable of forming metastatic lesions (Figure 3H).

JAK1 Deficiency Impedes Tumorsphere Formation and Migration of ERBB2-Induced Mammary Cancer Cells

The observed differences between JAK1 wild-type and knockout cells in their ability to form secondary tumors and to metastasize may imply that the lack of signaling through this particular Janus kinase alters the biological behavior and neoplastic properties of tumor-initiating cells. In support of this notion, we observed reduced expression of the mammary progenitor marker CD61 (Integrin beta 3, *Itgb3*) by RNA sequencing, as described below. Using flow cytometry, we confirmed an earlier report by Vaillant et al. (2008) that the majority (>80%) of MMTV-neu-induced mammary cancer cells expressing wild-type JAK1 were positive for CD61. Following deletion of the *Jak1* gene, however, we observed a 40%–75% reduction in the number of CD61-expressing cells (Figure 4A). The flow cytometric analysis also revealed that, despite variations in the quantity of CD24-positive cells in the parental JAK1-expressing lines, their JAK1-deficient descendants exhibited an 18% to 30% increase in the number of cells that express high levels of CD24 (Figure S2A). This cellular transition was also evident by the levels of *Itgb3* mRNA expression in both isogenic cell lines with and without JAK1 (Figure S2B). The collective results suggest that JAK1 deficiency promotes changes in the cellular identity and selection of cancer cells with $CD24^{high}$, luminal characteristics, which might explain the more cobblestone-like appearance of the JAK1 knockout cells (Figure 2D).

A reduction in CD61 and an increase in CD24 expression in JAK1-deficient cells might signify potential changes in the neoplastic properties of cancer stem cells. A higher expression of CD24 has been demonstrated previously to correlate with a limited repopulation capacity of normal mammary epithelial progenitors and tumor-initiating and invasive characteristics of cancer stem cells (Sheridan et al., 2006; Sleeman et al., 2006). In support of this premise, we observed a statistically significant reduction in the number of JAK1 knockout tumorspheres when both isogenic cell lines were grown for 9 days under low-attachment conditions with minimal growth factor supplementation (Figure 4B). The size of JAK1-deficient tumorspheres was noticeably smaller compared with the isogenic wild-type controls. In addition to their impaired tumorsphere-forming ability, JAK1-deficient mammary cancer cells also exhibited significantly reduced competence to migrate in Transwell assays (Figure 4C). The collective results of this line of investigation suggested that cytokine signaling through JAK1 plays a pivotal role in controlling the migratory and tumorsphere-forming capabilities of mammary cancer cells; i.e., cellular programs that are associated with cancer progression and metastasis.

Knockdown of JAK1 Blocks the Activation of STAT1/3/6 in Human Breast Cancer Cells and Significantly Reduces Their Migratory and Tumorsphere-Forming Capabilities

We cloned two previously validated short hairpin RNAs targeting the expression of human *JAK1* into the pLKO-TetON lentiviral vector, which permits a doxycycline (Dox)-controlled knockdown of target genes. To assess the biological significance of JAK1 and its effect on STAT activation in ERBB2-positive human breast cancer cells, we infected MDA-MB-453 cells with the lentiviral control and JAK1 short hairpin RNA (shRNA) vectors, followed by selection with puromycin. The specificity of the Dox-inducible targeted knockdown of *JAK1* transcripts was validated by RNA sequencing (Figure S3A). The mRNA levels of JAK2 and TYK2 were not altered by Dox or the JAK1 shRNA, and, as expected, JAK3 was not expressed in these cells. Identical to the conditional deletion of the *Jak1* gene in normal mammary epithelial cells and in ERBB2-transformed mammary cancer cells in genetically engineered mice, knockdown of JAK1 in human MDA-MB-453 cells blocked the activation of STAT1, STAT3, and STAT6 (Figure S3B). Identical results were obtained with the second shRNA vector (data not shown). Lack of JAK1 expression also significantly lowered the migratory and tumorsphere-forming capabilities of ERBB2-positive human breast cancer cells (Figures S3C and S3D). As described in the Discussion, these collective findings regarding the biological roles and indispensable functions of JAK1 for the activation of

Figure 3. Conditional Deletion of *Jak1* after Tumor Onset in Neoplastic Mammary Cancer Cells Prevents Tumor Progression and Metastasis

(A) Bioluminescence imaging of JAK1-deficient mammary cancer cells ($Jak1^{-/-}$) and their isogenic controls expressing JAK1 ($Jak1^{+/+}$) prior to transplantation *in vitro* (left) and following orthotopic engraftment and secondary tumor formation (6 weeks) in athymic nude mice (right; NC, negative control).

(B) Mammary tumor growth curves.

(C) Imaging of lung tissues of mammary tumor-bearing recipient mice that were orthotopically engrafted with JAK1-deficient mammary cancer cells and their controls.

(D and E) H&E-stained histologic sections and immunostaining of active STAT3 in JAK1-expressing primary and metastatic tumors (D) and JAK1-deficient cancer cells at the site of engraftment (E). Scale bars, 100 μm (left) and 50 μm (right).

(F) Activation of STAT3 in JAK1-deficient neoplasms in comparison with wild-type bulk tumors and the parental cancer cells prior to transplantation.

(G) Secondary tumor growth in athymic nude recipient mice that were orthotopically engrafted with ERBB2-induced mammary cancer tissue fragments from JAK1 cKO females and JAK1-expressing controls.

(H) Histologic sections and active STAT3 in primary and metastatic tumors of recipients that were engrafted with JAK1-expressing tumor tissues.

The tumor volumes in (B) and (D) were measured by calipers and calculated as ($width^2$ × length)/2 and are displayed as mean ± SEM. $p < 0.05$ was considered significant.

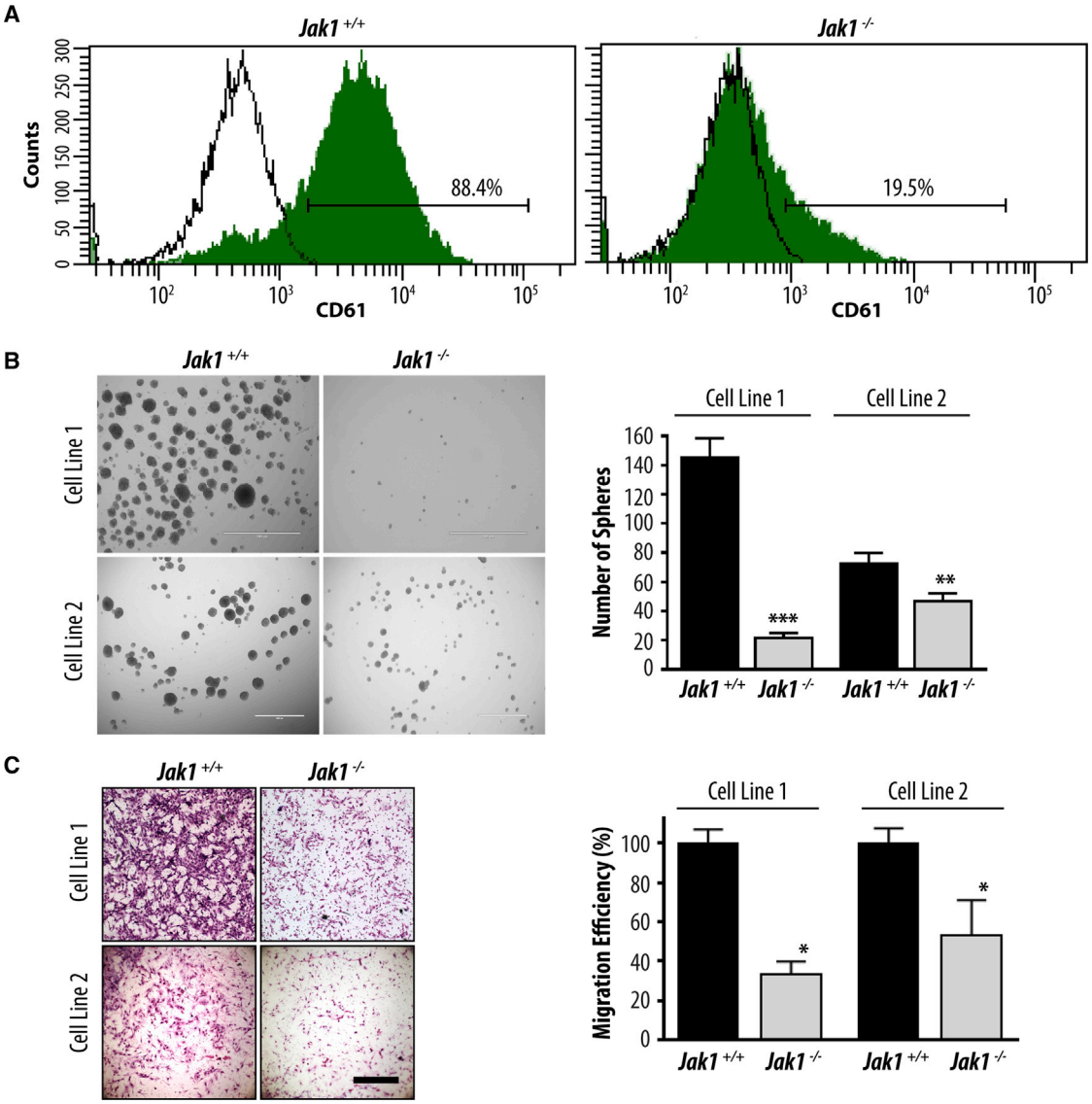


Figure 4. Loss of JAK1 Inhibits Mammary Tumorsphere Formation and Cancer Cell Migration

(A) Flow cytometry histograms showing the expression of CD61 in isogenic mammary cancer cells with JAK1 (*Jak1*$^{+/+}$) and without JAK1 (*Jak1*$^{-/-}$).

(B) Bright-field images (left; scale bars, 1 mm) and quantification (right) of tumorspheres in cell lines lacking JAK1 and their isogenic JAK1-expressing controls. Results are displayed as mean ± SEM from 3 replicate experiments quantifying the number of tumorspheres in the representative field of view.

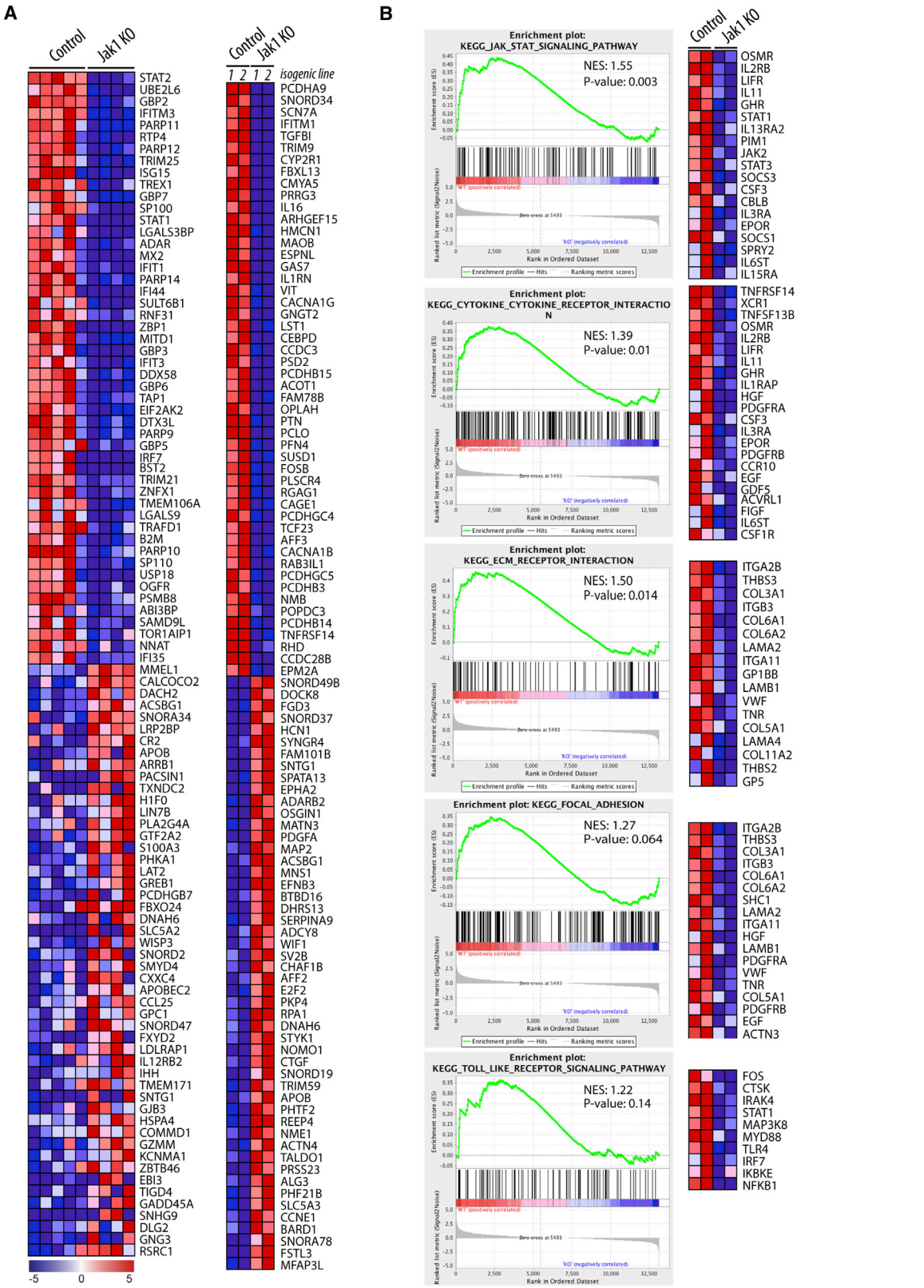
(C) Bright-field images (left; scale bar, 100 μm) and quantification (right) of H&E-stained cells that migrated after 24 hr in a Transwell assay. Results are shown as mean ± SEM from 3 replicate experiments quantifying the number of cells per field of view.

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, illustrating significant differences between means.

particular STAT proteins were validated further in diverse cancer cell lines, suggesting broad applicability of JAK1 as a therapeutic target to ablate the oncogenic activation of STAT3 in human cancer.

JAK1 Deficiency Lowers the Expression of STAT3 Targets and Genes that Facilitate Mammary Epithelial Remodeling

To gain insight into the mechanisms by which JAK1 signaling may contribute to cancer progression, we performed a genome-wide RNA sequencing (RNA-seq) analysis. We first compared the gene expression profiles of four JAK1-deficient mouse mammary cancer cell lines and five wild-type controls (Figure 5A). These datasets included those obtained from the two isogenic JAK1 knockout lines and their wild-type complements (Figure 5A, right). Comprehensive analysis of the expression profiles across all cell lines revealed that 185 genes were downregulated by 2-fold or higher, and 56 genes were upregulated in the JAK1 knockout. A direct comparison of the gene expression levels between the two isogenic cell line pairs yielded



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a greater number of genes (308) that exhibited statistically significant downregulation in the absence of the *Jak1* gene, whereas 245 genes were upregulated. As expected, many of the deregulated genes were known downstream targets of cytokine receptors and effectors of JAK/STAT signaling pathways (Figure 5B). The most consistently downregulated genes were those that we and others had previously identified as STAT3 targets, such as the receptors for OSM and LIF (*Osmr* and *Lifr*), *Socs3*, *Bcl3*, and the *Stat3* gene itself. This analysis also revealed coherent deregulated expression of genes we recently discovered to be targets of JAK1/STAT3 signaling during postlactational remodeling of the normal mammary epithelium (Sakamoto et al., 2016b). These include mediators of transforming growth factor β (TGF-β) signaling (*Tgfbi* and *Tgfbr3*) as well as *c-Fos*, *Map3k8*, *Myd88*, *Irak4*, and *Nfkb1*, which are known to play roles in inflammation and IL-1 and Toll-like receptor (TLR) signaling. JAK1 knockout cells also exhibited consistent downregulation of genes that function in extracellular matrix-receptor interaction, matrix remodeling, and focal adhesion (Figure 5B). In addition to downregulation of Integrin Beta 3/CD61 (*Itgb3*), as shown earlier (Figure 4A), JAK1 deficiency resulted in reduced expression of the Integrin subunit Alpha 11 (*Itga11*; data not shown) as well as Integrin Alpha 2b/CD41 (*Itga2b*) and matrix metallopeptidase 14 and 11 (*Mmp14* and *Mmp11*) (Figure 6A).

The deregulated expression of selected direct or indirect targets of JAK1 signaling was validated by qRT-PCR, Western bot (Figures 6B and 6D), flow cytometry (Figure 4A; Figure S2), and immunostaining on mammary tumors (Figure 6E) and cultured cancer cells (Figure 6C; Figure S4). The collective results of this line of investigation revealed that, similar to postlactational remodeling of the normal mammary epithelium, JAK1 deficiency led to decoupling of IL-6-class inflammatory cytokines from their downstream effectors, which included STAT3-mediated expression of their receptors (i.e., OSM receptor [OSMR] and LIF receptor [LIFR]) and negative feedback regulators (e.g., SOCS3). Additionally, JAK1 signaling is crucial for the expression of known oncogenes, in particular c-FOS, BCL3, and MAP3K8. The lower expression of MAP3K8 (Figures 6B and 6C) coincided with reduced phosphorylation of extracellular signal-regulated kinase (ERK) (Figure 6D; Figure S4) and lack of nuclear accumulation of c-FOS (Figures 6E). In addition to the signal transducers shown in Figure 6, we carried out immunostaining experiments to validate additional putative JAK1 targets (Figure S4). These confirmatory results demonstrated that JAK1 is crucial for the expression of known mediators of inflammation as well as key nodes for TGF-β, IL-1, and TLR signaling. Similar to the results from JAK1-deficient human breast cancer cells (Figure S3B), conditional deletion of the *Jak1* gene in mouse mammary cancer cells had no effect on the protein expression of JAK2. This suggests that the changes in JAK1 target gene expression are not an indirect consequence of co-inhibition of JAK2.

Exogenous Expression of Hyperactive STAT3, c-FOS, or MAP3K8 Increases the Tumorsphere-Forming and Migratory Capabilities of JAK1-Deficient Mammary Cancer Cells

Based on our transcriptome analyses, it is evident that signaling through JAK1 had a pleiotropic effect on several molecular pathways that are suggested to regulate cellular programs of cancer progression. Although it is unlikely that only one of these pathways controls all cellular programs of the metastatic cascade, we conducted experiments to assess the potential biological significance of *bona fide* oncogenes whose expression was consistently downregulated in response to deletion of the *Jak1* gene. Specifically, we choose to investigate MAP3K8 and c-FOS because they are important signaling nodes of IL-1 and TLR as well as nuclear factor κB (NF-κB) activation; i.e., three of the deregulated key pathways in the gene set enrichment analysis (GSEA) of this study. As part of a genetic rescue study, we used lentiviral gene transfer to re-express both oncogenes in JAK1-deficient mammary cancer cells and confirmed by immunostaining that exogenous expression of c-FOS or MAP3K did not lead to reactivation of STAT3 (Figures 7A and 7C). Although upregulation of either c-FOS or MAP3K8 was sufficient to increase the migration of cancer cells compared with JAK1-deficient controls, re-expression of these putative JAK1 targets had a more pronounced effect on reinstating the tumorsphere-forming ability of JAK1 knockout cells (Figures 7B and 7D). In addition to the significant increase in the numbers of tumorspheres, we observed that the individual JAK1-deficient spheres expressing c-FOS or MAP3K8 were considerably larger. We also found that re-expression of MAP3K8, but not c-FOS (data not shown), resulted in a noticeable increase in the number of JAK1 knockout cells with low expression of CD24 that was comparable with isogenic JAK1 wild-type controls (Figure S5). Exogenous expression of MAP3K8 and c-FOS was also sufficient to partially restore the orthotopic engraftment and growth of transplanted JAK1 knockout cancer cells (Figure S6A). However, these cancer cells did not readily disseminate to distant sites, and enlarged pulmonary metastatic lesions were only observed in one recipient of MAP3K8-expressing JAK1 knockout cells (Figure S6B). This supports the previously stated notion that JAK1 signaling may control several cellular and molecular programs of the metastatic cascade.

Because STAT1 is a suggested tumor suppressor and STAT6 was not persistently active, we anticipated that STAT3 is the primary downstream effector of JAK1 signaling and crucial for the migratory and tumorsphere-forming characteristics of cancer cells. To experimentally address this idea, we generated JAK1 knockout cancer cells that express a hyperactive mutant of STAT3 (Bromberg et al., 1999). Hyperactive STAT3 possesses two cysteine substitutions within the SH2 domain and is able to spontaneously dimerize. It was therefore not surprising that

Figure 5. JAK1 Deficiency Results in Downregulation of Genes that Function in Cytokine Receptor Signaling, Extracellular Matrix Remodeling, and Focal Adhesion

(A) Heatmaps of the top 50 up- and downregulated genes that exhibit 2-fold or more deregulated expression in JAK1 knockout cells compared with JAK1 wild-type controls. Right: a direct comparison of gene expression between two isogenic cancer cell line pairs with and without JAK1. Scale, log2 fragments per kilobase million (FKPM) value.

(B) Gene set enrichment plots with heatmaps of genes that are selectively downregulated in JAK1-deficient cells.

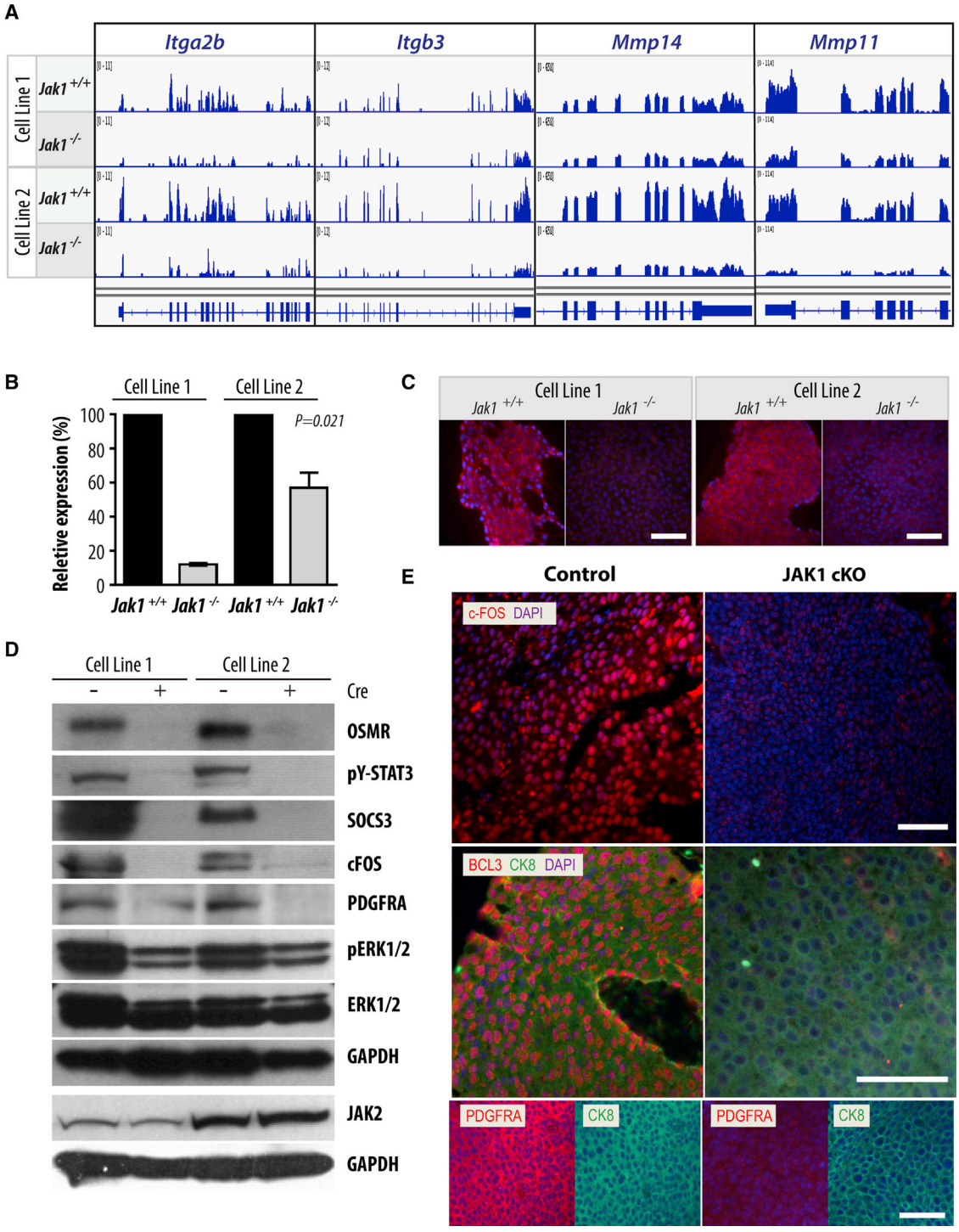

Figure 6. Loss of STAT3 Activation in Response to JAK1 Deficiency Coincides with Reduced Expression of STAT3 Targets, Oncogenes, and Mediators of Mammary Epithelial Remodeling

(A) Histograms of RNA-seq datasets for genes of extracellular matrix singling and remodeling that are differentially expressed in JAK1 knockout cell lines compared with JAK1-expressing isogenic controls.

(B) Validation of the differential expression of *Map3k8* using qRT-PCR. Each sample was examined in triplicate and normalized to *Actin* expression. The results are plotted as relative expression levels of the knockout cells compared with their isogenic wild-type control. Error bars indicate SEM.

(C) Immunofluorescence (IF) staining of MAP3K8 in JAK1 knockout cell lines and controls. Scale bars, 50 μm.

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this mutant was not tyrosine-phosphorylated in JAK1-deficient cancer cells (data not shown). This observation reaffirmed that JAK1 is essential for STAT3 phosphorylation, even in cells with high RTK and SRC signaling. On the other hand, this finding implied that the SRC-dependable, hyperactive functionality of this mutant, as reported by Bromberg et al. (1999), might be restricted in the absence of JAK1. Nonetheless, overexpression of mutant STAT3 was sufficient to elevate the expression of c-FOS and MAP3K (Figure S7A) and to significantly enhance the tumorsphere-forming and migratory capabilities of JAK1 knockout cancer cells (Figures S7B and S7C). These phenotypic changes were accompanied by a reduction in the number of CD24-expressing cancer cells (Figure S7D).

The collective findings of this line of investigation demonstrate that the tumor-promoting roles of inflammatory cytokine signaling through JAK1 are primarily facilitated by downstream activation of STAT3 and its transcriptional targets (Figure 7E). Using genetic rescue experiments, we demonstrated that c-FOS and MAP3K8 are two JAK1/STAT3 target genes that promote the migration and tumor-reinitiating capabilities of mammary cancer cells.

DISCUSSION

Using a JAK1 conditional knockout mouse model, we have recently demonstrated that this Janus kinase is indispensable for the activation of STAT1, STAT3, and STAT6 throughout normal mammary gland development (Sakamoto et al., 2016b). An important finding from this study is that the essential function of JAK1 for tyrosine phosphorylation of these three STAT proteins is conserved in mouse and human mammary cancer cells that are driven by ERBB2 and its associated receptor tyrosine kinase complexes (i.e., ERBB3 and EGFR). STAT3 and STAT1 are persistently activated in various breast cancer subtypes and many other malignancies, where they are suggested to play important roles in neoplastic transformation and cancer progression (Avalle et al., 2012). We demonstrated that constitutive activation of STAT3 and STAT1 can be ablated by conditional deletion of the *Jak1* gene or knockdown of its transcript. Using the same experimental approaches, we have evidence to suggest that the pivotal functions of JAK1 for oncogenic STAT3/1 activation extend to hormone receptor-negative MBA-MD-231 breast cancer cells as well as pancreatic cancer cell lines in both mouse and human (P.D.R. and N. Rajbhandari, unpublished data), which confers broad significance to these findings.

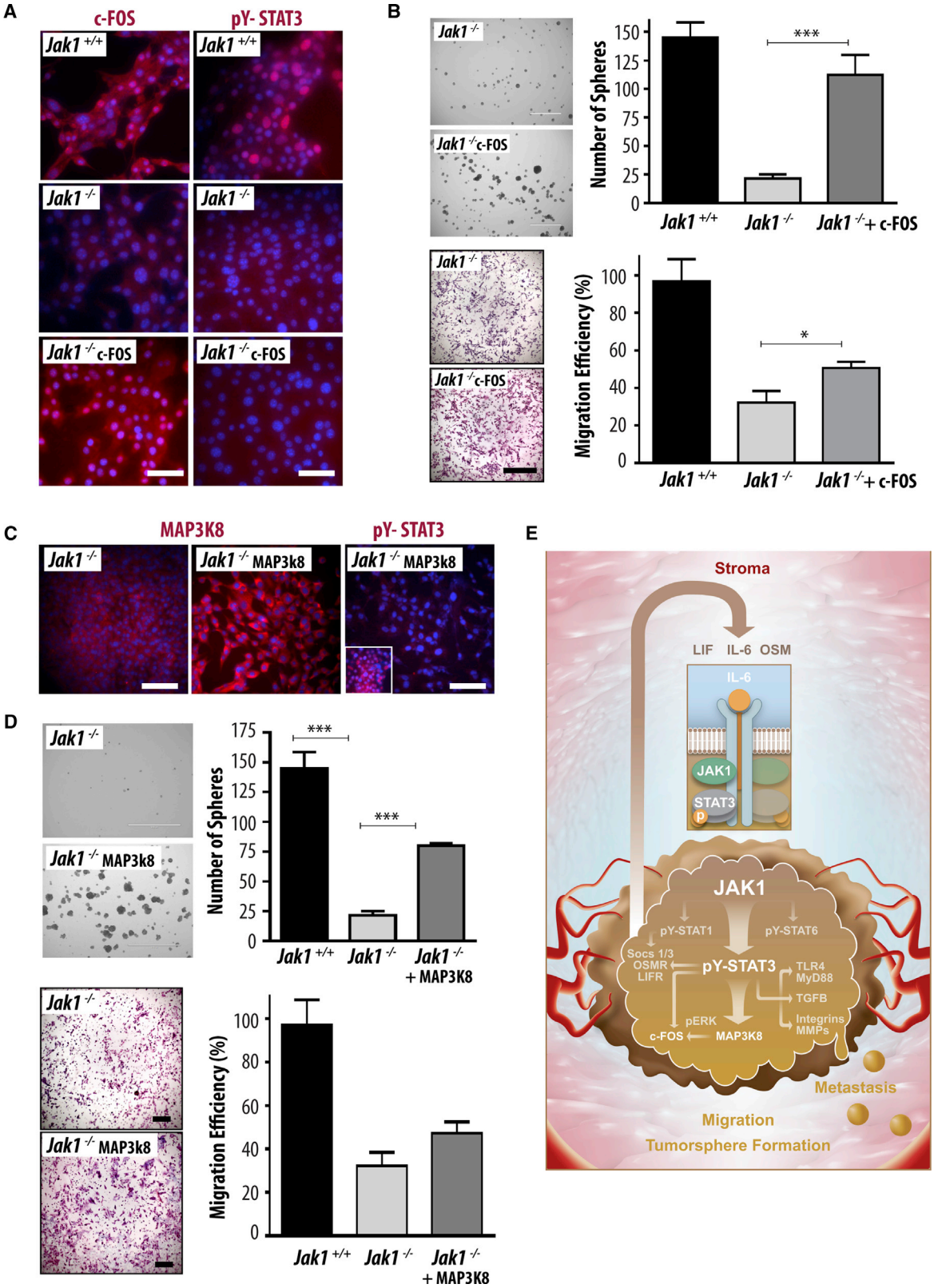
Seminal studies published about two decades ago have established a role for JAK1 as a mediator of IL-6 signaling and downstream activation of STAT3 (Guschin et al., 1995; Lütticken et al., 1994; Rodig et al., 1998). A review of the recent literature in the field of cancer research, however, shows that JAK2 is now being perceived as the primary therapeutic target to block the oncogenic activation of STATs, in particular STAT3 (Rädler et al., 2017). This view appears to have emerged from the experimental use of pharmacological agents that are marketed as "JAK2 inhibitors" despite their known off-target effects on other JAKs. In contrast to this paradigm, our team established almost a decade ago that conditional deletion of JAK2 had no effect on the growth and survival of mammary cancer cells or oncogenic activation of STAT3 (Sakamoto et al., 2009, 2010). In line with these findings, recent work by Balko et al. (2016) confirmed that inhibition of JAK2 alone had no antitumor effects in xenograft models regardless of the amplification status of the *JAK2* locus. This work also validated that the oncogenic activation of STAT3 occurs in a JAK2-independent manner. Therefore, some of the biological effects caused by treatment with the dual JAK1/2 inhibitor ruxolitinib, which include the suggested role of STAT3 in cancer stem cells, are likely due to inhibition of JAK1.

There is a substantial body of literature regarding the role of SRC and RTK signaling in oncogenic activation of STATs in cancer, with a subset of these studies highlighting a cooperative function of JAKs (Ren and Schaefer, 2002; Zhang et al., 2000). We have demonstrated in this study that conditional deletion of JAK1 in ERBB2-driven mammary tumor cells was sufficient to ablate the persistent activation of STAT3/1. Our findings are in agreement with earlier reports that, in contrast to pan-JAK or gp130 blockade, inhibition of SRC or EGFR had no effect on phosphorylation of STAT3 (Berishaj et al., 2007; Gao et al., 2007). The collective results support the concept that the persistent activation of STAT3, and possibly also STAT1, is an indirect consequence of elevated, cancer cell-specific production of cytokines in response to RTK signaling. This principal mechanism highlights the importance of JAK1 as a rational therapeutic target to block the action of IL-6 class cytokines, which are master regulators of cancer-associated inflammation (Grivennikov and Karin, 2011).

In contrast to the pro-survival function of JAK2 and STAT5, which promote the earliest steps of neoplastic transformation (for references, see Rädler et al., 2017), signaling through JAK1 seems to be dispensable for ERBB2-induced mammary cancer initiation. Similar to MMTV-Neu transgenic females with a mammary gland-specific knockout of STAT3 (Ranger et al., 2009), loss of JAK1 had no effect on tumor-free survival but blocked dissemination of ERBB2-driven mammary tumor cells to distant sites. Conditional deletion of the *Jak1* gene impaired the migratory and tumorsphere-forming capabilities of cancer cells in culture as well as their orthotopic engraftment and secondary tumor growth *in vivo*. These biological changes were accompanied by differential expression of cell adhesion molecules that serve as markers for epithelial progenitors and tumor-initiating cells, supporting the concept that cancer stem cells exhibit enhanced invasive properties and an elevated propensity to metastasize (Sheridan et al., 2006). Additionally, our finding that expression of hyperactive STAT3 restored, to a significant extent, the tumorsphere-forming and migratory capabilities of JAK1-deficient cancer cells reinforces the notion that STAT3 activation plays a

(D) Immunoblot analysis of putative JAK1/STAT3 targets and activation of extracellular signal-regulated kinase (ERK). Note that lack of JAK1 had no effect on the expression of JAK2.

(E) IF staining of selected JAK1 targets on histologic sections of MMTV-neu-induced mammary cancer tissues that express (control) or lack JAK1 (JAK1 cKO). Scale bars, 50 μm.



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key role in the biology of breast cancer stem cells (Marotta et al., 2011).

In this study, we performed a transcriptome analysis to gain insight into potential mechanisms by which JAK1 signaling may contribute to cancer cell dissemination and the characteristics of tumor-initiating cells. This line of investigation revealed that JAK1 deficiency caused a significant reduction in the expression of STAT3 target genes and molecular pathways that facilitate postlactational remodeling of the mammary epithelium. Many genes within these pathways have been previously linked to cancer cell metastasis, such as matrix remodeling, focal adhesion, as well as mediators of IL-1, TLR, and TGF-β signaling. Hence, like its essential functions during normal development, the biological effects of JAK1 signaling in cancer progression are pleiotropic, which establishes JAK1 as a central node within oncogenic signaling networks (Figure 7E). Besides validating the deregulated expression of many putative JAK1 targets on the level of the protein, we experimentally assessed the potential biological significance of selected *bona fide* oncogenes (i.e., c-FOS and MAP3K8) that were consistently deregulated in normal and neoplastic JAK1-deficient cells (Sakamoto et al., 2016b). This line of investigation revealed that re-expression of c-FOS and MAP3K8 could rescue, in part, the tumorsphere-forming and migratory capabilities of JAK1 knockout cells as well as enhance their engraftment *in vivo*, suggesting that these tumor susceptibility genes may play a role in cancer progression fueled by inflammatory cytokines. Both oncogenes are likely targets of STAT3 because their expression was restored in JAK1-deficient cells expressing a hyperactive mutant of this STAT protein.

Our collective work on JAK2 and now JAK1 conditional knockout models clearly demonstrates that both Janus kinases have non-redundant roles for the activation of distinct STAT proteins in normal and neoplastic mammary epithelial cells. Although JAK2 is essential for tyrosine phosphorylation of the two STAT5 isoforms (Sakamoto et al., 2009, 2010; Wagner et al., 2004), JAK1 is the pivotal kinase for persistent activation of STAT3 and STAT1 as well as IL-4-induced phosphorylation of STAT6. The discrete molecular functions of JAKs provide unique opportunities for selective inhibition of STAT transcription factors, in particular JAK1-mediated oncogenic activation of STAT3 in response to inflammatory cytokines. As a rational therapeutic target, selective ablation of JAK1 might help to mitigate immunosuppression and other undesirable side effects caused by co-inhibition of JAK2 because of the indispensable roles of the latter kinase in normal hematopoiesis and liver metabolism (Akada et al., 2014; Sos et al., 2011).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and one table and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.10.063.

ACKNOWLEDGMENTS

We are grateful to Karen K. Dulany for the preparation of histologic sections. The authors thank the UNMC Genomics core facility for assistance with next-generation sequencing (NGS) and the UNMC Cell Analysis core facility for flow cytometry. Histologic service was also provided by the core facility of the Karmanos Cancer Institute and Wayne State University (WSU). Lab Rat Design, LLC assisted with design of the graphical abstract. This work was supported, in part, by Public Health Service grants CA117930 and CA202917 (to K.-U.W.). This financial support was also imperative to finance the maintenance of the JAK1 mutant mice. B.L.W. and P.D.R. received

Figure 7. Re-expression of c-FOS or MAP3K8 Rescues the Tumorsphere-Forming and Migratory Capabilities of JAK1-Deficient Mammary Cancer Cells

(A) IF staining of c-FOS and pY-STAT3 in JAK1 knockout cells expressing exogenous c-FOS as well as their isogenic knockout and parental wild-type controls. Scale bars, 50 μm.

(B) Top: bright-field images (left; scale bars, 1 mm) and quantification (right) of JAK1-deficient tumorspheres expressing exogenous c-FOS and their controls. Bottom: bright-field images (left; scale bar, 100 μm) and quantification (right) of H&E-stained c-FOS-expressing cells and controls that migrated after 24 hr in a Transwell assay. The number of tumorspheres and relative number of migrated cells were calculated from representative field of views of 3 replicate experiments and are shown as mean ± SEM.

(C) IF staining of MAP3K8 and pY-STAT3 in JAK1 knockout cells expressing exogenous MAP3K8 as well as their isogenic knockout and parental wild-type controls.

(D) Top: bright-field images (left; scale bars, 1 mm) and quantification (right) of JAK1-deficient tumorspheres expressing exogenous MAP3K8 and their controls. Bottom: bright-field images (left; scale bar, 100 μm) and quantification (right) of H&E-stained MAP3K8 rescue cells and controls that migrated after 24 hr. The number of tumorspheres and relative number of migrated cells were calculated from representative field of views of 3 replicate experiments and are shown as mean ± SEM.

(E) Schematic outline of pleiotropic functions of JAK1 in STAT activation and the control of molecular pathways that promote cancer progression.

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, illustrating significant differences between means.

graduate fellowships through the UNMC Cancer Research Training Program (CA009476). The mammary cancer transcriptome analysis was supported, in part, by the METAvivor Research Foundation. Public Health Service grants CA036727 and CA022453 provided core facility support to UNMC and WSU. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

K.-U.W. formulated the overarching research goals and aims and supervised the research. B.L.W. and P.D.R. conducted the molecular and biological analyses of JAK1 knockout mice and derived mammary tumor cell lines. A.A.T. generated the gene expression libraries, and P.D.R. performed the computational analyses of the RNA-seq data sets. H.S. helped with the cell proliferation assays. S.J.J. assisted with the preparation of histological sections, immunostaining, and flow cytometry. K.-U.W. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: February 20, 2018
Revised: September 18, 2018
Accepted: October 17, 2018
Published: November 20, 2018

REFERENCES

Akada, H., Akada, S., Hutchison, R.E., Sakamoto, K., Wagner, K.U., and Mohi, G. (2014). Critical role of Jak2 in the maintenance and function of adult hematopoietic stem cells. Stem Cells *32*, 1878–1889.

Avalle, L., Pensa, S., Regis, G., Novelli, F., and Poli, V. (2012). STAT1 and STAT3 in tumorigenesis: A matter of balance. JAK-STAT *1*, 65–72.

Balko, J.M., Schwarz, L.J., Luo, N., Estrada, M.V., Giltnane, J.M., Dávila-González, D., Wang, K., Sánchez, V., Dean, P.T., Combs, S.E., et al. (2016). Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. Sci. Transl. Med. *8*, 334ra53.

Berishaj, M., Gao, S.P., Ahmed, S., Leslie, K., Al-Ahmadie, H., Gerald, W.L., Bornmann, W., and Bromberg, J.F. (2007). Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. Breast Cancer Res. *9*, R32.

Bromberg, J.F., Wrzeszczynska, M.H., Devgan, G., Zhao, Y., Pestell, R.G., Albanese, C., and Darnell, J.E., Jr. (1999). Stat3 as an oncogene. Cell *98*, 295–303.

Chan, S.R., Vermi, W., Luo, J., Lucini, L., Rickert, C., Fowler, A.M., Lonardi, S., Arthur, C., Young, L.J., Levy, D.E., et al. (2012). STAT1-deficient mice spontaneously develop estrogen receptor α-positive luminal mammary carcinomas. Breast Cancer Res. *14*, R16.

Chapman, R.S., Lourenco, P.C., Tonner, E., Flint, D.J., Selbert, S., Takeda, K., Akira, S., Clarke, A.R., and Watson, C.J. (1999). Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. Genes Dev. *13*, 2604–2616.

Gao, S.P., Mark, K.G., Leslie, K., Pao, W., Motoi, N., Gerald, W.L., Travis, W.D., Bornmann, W., Veach, D., Clarkson, B., and Bromberg, J.F. (2007). Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J. Clin. Invest. *117*, 3846–3856.

Garcia, R., Bowman, T.L., Niu, G., Yu, H., Minton, S., Muro-Cacho, C.A., Cox, C.E., Falcone, R., Fairclough, R., Parsons, S., et al. (2001). Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene *20*, 2499–2513.

Grivennikov, S.I., and Karin, M. (2011). Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. Ann. Rheum. Dis. *70* (*Suppl 1*), i104–i108.

Guschin, D., Rogers, N., Briscoe, J., Witthuhn, B., Watling, D., Horn, F., Pellegrini, S., Yasukawa, K., Heinrich, P., Stark, G.R., et al. (1995). A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. EMBO J. *14*, 1421–1429.

Guy, C.T., Webster, M.A., Schaller, M., Parsons, T.J., Cardiff, R.D., and Muller, W.J. (1992). Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proc. Natl. Acad. Sci. USA *89*, 10578–10582.

Heinrich, P.C., Behrmann, I., Müller-Newen, G., Schaper, F., and Graeve, L. (1998). Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem. J. *334*, 297–314.

Humphreys, R.C., Bierie, B., Zhao, L., Raz, R., Levy, D., and Hennighausen, L. (2002). Deletion of Stat3 blocks mammary gland involution and extends functional competence of the secretory epithelium in the absence of lactogenic stimuli. Endocrinology *143*, 3641–3650.

Kawamoto, S., Niwa, H., Tashiro, F., Sano, S., Kondoh, G., Takeda, J., Tabayashi, K., and Miyazaki, J. (2000). A novel reporter mouse strain that expresses enhanced green fluorescent protein upon Cre-mediated recombination. FEBS Lett. *470*, 263–268.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. *14*, R36.

Klover, P.J., Muller, W.J., Robinson, G.W., Pfeiffer, R.M., Yamaji, D., and Hennighausen, L. (2010). Loss of STAT1 from mouse mammary epithelium results in an increased Neu-induced tumor burden. Neoplasia *12*, 899–905.

Krempler, A., Henry, M.D., Triplett, A.A., and Wagner, K.U. (2002). Targeted deletion of the Tsg101 gene results in cell cycle arrest at G1/S and p53-independent cell death. J. Biol. Chem. *277*, 43216–43223.

Kritikou, E.A., Sharkey, A., Abell, K., Came, P.J., Anderson, E., Clarkson, R.W., and Watson, C.J. (2003). A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. Development *130*, 3459–3468.

Lütticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., Taga, T., et al. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science *263*, 89–92.

Marotta, L.L., Almendro, V., Marusyk, A., Shipitsin, M., Schemme, J., Walker, S.R., Bloushtain-Qimron, N., Kim, J.J., Choudhury, S.A., Maruyama, R., et al. (2011). The JAK2/STAT3 signaling pathway is required for growth of $CD44^{+}CD24^{-}$ stem cell-like breast cancer cells in human tumors. J. Clin. Invest. *121*, 2723–2735.

Nam, S., Wen, W., Schroeder, A., Herrmann, A., Yu, H., Cheng, X., Merz, K.H., Eisenbrand, G., Li, H., Yuan, Y.C., and Jove, R. (2013). Dual inhibition of Janus and Src family kinases by novel indirubin derivative blocks constitutively-activated Stat3 signaling associated with apoptosis of human pancreatic cancer cells. Mol. Oncol. *7*, 369–378.

Neilson, L.M., Zhu, J., Xie, J., Malabarba, M.G., Sakamoto, K., Wagner, K.U., Kirken, R.A., and Rui, H. (2007). Coactivation of janus tyrosine kinase (Jak)1 positively modulates prolactin-Jak2 signaling in breast cancer: recruitment of ERK and signal transducer and activator of transcription (Stat)3 and enhancement of Akt and Stat5a/b pathways. Mol. Endocrinol. *21*, 2218–2232.

Rädler, P.D., Wehde, B.L., and Wagner, K.U. (2017). Crosstalk between STAT5 activation and PI3K/AKT functions in normal and transformed mammary epithelial cells. Mol. Cell. Endocrinol. *451*, 31–39.

Ranger, J.J., Levy, D.E., Shahalizadeh, S., Hallett, M., and Muller, W.J. (2009). Identification of a Stat3-dependent transcription regulatory network involved in metastatic progression. Cancer Res. *69*, 6823–6830.

Ren, Z., and Schaefer, T.S. (2002). ErbB-2 activates Stat3 alpha in a Src- and JAK2-dependent manner. J. Biol. Chem. *277*, 38486–38493.

Rodig, S.J., Meraz, M.A., White, J.M., Lampe, P.A., Riley, J.K., Arthur, C.D., King, K.L., Sheehan, K.C., Yin, L., Pennica, D., et al. (1998). Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell *93*, 373–383.

Sakamoto, K., Lin, W.C., Triplett, A.A., and Wagner, K.U. (2009). Targeting janus kinase 2 in Her2/neu-expressing mammary cancer: Implications for cancer prevention and therapy. Cancer Res. *69*, 6642–6650.

Sakamoto, K., Triplett, A.A., Schuler, L.A., and Wagner, K.U. (2010). Janus kinase 2 is required for the initiation but not maintenance of prolactin-induced mammary cancer. Oncogene *29*, 5359–5369.

Sakamoto, K., Wehde, B.L., Rädler, P.D., Triplett, A.A., and Wagner, K.U. (2016a). Generation of Janus kinase 1 (JAK1) conditional knockout mice. Genesis *54*, 582–588.

Sakamoto, K., Wehde, B.L., Yoo, K.H., Kim, T., Rajbhandari, N., Shin, H.Y., Triplett, A.A., Rädler, P.D., Schuler, F., Villunger, A., et al. (2016b). Janus Kinase 1 Is Essential for Inflammatory Cytokine Signaling and Mammary Gland Remodeling. Mol. Cell. Biol. *36*, 1673–1690.

Schneckenleithner, C., Bago-Horvath, Z., Dolznig, H., Neugebauer, N., Kollmann, K., Kolbe, T., Decker, T., Kerjaschki, D., Wagner, K.U., Müller, M., et al. (2011). Putting the brakes on mammary tumorigenesis: loss of STAT1 predisposes to intraepithelial neoplasias. Oncotarget *2*, 1043–1054.

Sheridan, C., Kishimoto, H., Fuchs, R.K., Mehrotra, S., Bhat-Nakshatri, P., Turner, C.H., Goulet, R., Jr., Badve, S., and Nakshatri, H. (2006). CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast Cancer Res. *8*, R59.

Sleeman, K.E., Kendrick, H., Ashworth, A., Isacke, C.M., and Smalley, M.J. (2006). CD24 staining of mouse mammary gland cells defines luminal epithelial, myoepithelial/basal and non-epithelial cells. Breast Cancer Res. *8*, R7.

Sos, B.C., Harris, C., Nordstrom, S.M., Tran, J.L., Balázs, M., Caplazi, P., Febbraio, M., Applegate, M.A., Wagner, K.U., and Weiss, E.J. (2011). Abrogation of growth hormone secretion rescues fatty liver in mice with hepatocyte-specific deletion of JAK2. J. Clin. Invest. *121*, 1412–1423.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., and Mesirov, J.P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA *102*, 15545–15550.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. *7*, 562–578.

Vaillant, F., Asselin-Labat, M.L., Shackleton, M., Forrest, N.C., Lindeman, G.J., and Visvader, J.E. (2008). The mammary progenitor marker CD61/beta3 integrin identifies cancer stem cells in mouse models of mammary tumorigenesis. Cancer Res. *68*, 7711–7717.

Wagner, K.U., Wall, R.J., St-Onge, L., Gruss, P., Wynshaw-Boris, A., Garrett, L., Li, M., Furth, P.A., and Hennighausen, L. (1997). Cre-mediated gene deletion in the mammary gland. Nucleic Acids Res. *25*, 4323–4330.

Wagner, K.U., Krempler, A., Triplett, A.A., Qi, Y., George, N.M., Zhu, J., and Rui, H. (2004). Impaired alveologenesis and maintenance of secretory mammary epithelial cells in Jak2 conditional knockout mice. Mol. Cell. Biol. *24*, 5510–5520.

Zhang, Y., Turkson, J., Carter-Su, C., Smithgall, T., Levitzki, A., Kraker, A., Krolewski, J.J., Medveczky, P., and Jove, R. (2000). Activation of Stat3 in v-Src-transformed fibroblasts requires cooperation of Jak1 kinase activity. J. Biol. Chem. *275*, 24935–24944.

Zhao, L., Hart, S., Cheng, J., Melenhorst, J.J., Bierie, B., Ernst, M., Stewart, C., Schaper, F., Heinrich, P.C., Ullrich, A., et al. (2004). Mammary gland remodeling depends on gp130 signaling through Stat3 and MAPK. J. Biol. Chem. *279*, 44093–44100.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal, STAT1	Santa Cruz	Cat# SC-592: RRID:AB_632434
Rabbit polyclonal, pY-STAT1	Origene	Cat# TA309955
Mouse monoclonal, STAT3	Cell Signaling	Cat# 9139S: RRID:AB_331757
Rabbit monoclonal, pY-STAT3	Cell Signaling	Cat# 9145S: RRID:AB_2491009
Rabbit polyclonal, STAT5	Santa Cruz	Cat#SC-836: RRID:AB_632445
Rabbit polyclonal, pY-STAT5	Cell Signaling	Cat# 9351S: RRID:AB_331593
Rabbit polyclonal, STAT6	Santa Cruz	Cat# SC-981: RRID:AB_632450
Rabbit polyclonal, pY-STAT6	Abcam	Cat# Ab54461: RRID:AB_882721
Rabbit monoclonal, GAPDH	Cell Signaling	Cat# 5174: RRID:AB_10622025
Goat polyclonal, b- ACTIN	Santa Cruz	Cat#I-19: RRID:AB_783595
Mouse monoclonal, pTyr	Cell Signaling	Cat# 05-321
Rabbit polyclonal, c-FOS	Abcam	Cat# Ab7963: RRID:AB_306177
Rabbit polyclonal, RUNX1	Origene	Cat# TA309955
Rabbit polyclonal, JAK1	Neilson et al., 2007	N/A
Rabbit monoclonal, JAK2	Cell Signaling	Cat# 3229
Rabbit monoclonal, pERK1/2	Cell Signaling	Cat# 4370: RRID:AB_2315112
Mouse monoclonal, LIFR	Santa Cruz	Cat# SC-515337
Rabbit oligoclonal, MAP3K8	Thermo	Cat# 710377: RRID:AB_2532699
Rabbit polyclonal, MAP3K8	Santa Cruz	Cat# SC-720: RRID:AB_2140669
Rabbit polyclonal, MyD88	Bosterbio	Cat# PA1600
Rabbit polyclonal, ITGA11	MyBioSource	Cat#MBS9414225
Goat polyclonal, OSMR	Santa Cruz	Cat# SC-8494: RRID:AB_2156580
Rabbit polyclonal, BCL3	Biossusa	Cat# Bs-6591R: RRID:AB_11102562
Rabbit monoclonal, TUBULIN	Epitomics	Cat#1878-1
Rabbit polyclonal, TGFB3	Cell Signaling	Cat# 2519S: RRID:AB_10693031
Rabbit polyclonal, PDGFRA	Boster	Cat# PB9771
Rabbit polyclonal, SOCS3	Santa Cruz	Cat# SC-9023: RRID:AB_2193305
Rat monoclonal, PE CD24	BD PharMingen	Cat# 561079- M1/69: RRID:AB_2034001
Hamster monoclonal, APC CD29	BD PharMingen	Cat# ebioHMB1-1: RRID:AB_1210793
Hamster monoclonal, BV421 CD61	BD Biosciences	Cat# 566227: clone 2C9.G2
Biological Samples		
Mouse: Mammary tumors: MMTV-neu MMTV-Cre Jak1 fl/fl	This paper	N/A
Mouse: Mammary tumors: MMTV-neu MMTV-Cre Jak1 fl/+	This paper	N/A
Mouse: Mammary tumors: MMTV-neu Jak1 fl/fl	This paper	N/A
Mouse: Mammary glands: MMTV-Cre Jak1 fl/fl with and without MMTV-neu	This paper	N/A
Mouse: Mammary glands: MMTV-Cre Jak1 fl/+ with and without MMTV-neu	This paper	N/A
Mouse: Lung: MMTV-neu MMTV-Cre Jak1 fl/fl	This paper	N/A
Mouse: Lung: MMTV-neu MMTV-Cre Jak1 fl/+	This paper	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Recombinant mouse IL-4	BD PharMingen	Cat# 550067
XenoLight D-Luciferin - K+ Salt Bioluminescent Substrate	Perkin Elmer	Cat# 122799
Doxycycline Hyclate	Sigma	Cat# D9891-25G
Critical Commercial Assays		
RNeasy Mini Kit	QIAGEN	Cat# 74104
TruSeq RNA sample Prep Kit-v2	Illumina	Cat# RS-122-2001
Super-Script II	Invitrogen	Cat# 18064014
HEMA 3 STAT Pack	Fisher-Scientific	Cat#123-869
Deposited Data		
RNA-Sequencing data	GEO	GSE121064
Experimental Models: Cell Lines		
Mouse: Mammary tumor: MMTV-neu Jak1 fl/fl (47298, line 1)	This paper	N/A
Mouse: Mammary tumor: MMTV-neu Jak1 fl/fl (45555, line 2)	This paper	N/A
Human: MDA-MB-453	ATCC	HTB-131
Experimental Models: Organisms/Strains		
Mouse: Jak1 $^{fl/fl}$ [Jak1tm1Kuw]	Sakamoto et al., 2016a	MGI:5688302
Mouse: MMTV-Cre line A Tg(MMTV-cre)1Mam	Wagner et al., 1997	MGI:2176165
Mouse: MMTV-neu	Guy et al., 1992	MGI:1930204
Mouse: CAG-LSL-GFP	Kawamoto et al., 2000	N/A
Mouse: Athymic, NCr-nu/nu	Charles River	Strain code:553
Oligonucleotides		
Primer: Map3k8 Forward: CTCTGTTTGCTGAGCCTGGA	This paper	N/A
Primer: Map3k8 Reverse: ACGAAACCAGAGCCGATGTT	This paper	N/A
Primer: Actin Forward: TGGATGACGATATCGCTGCGC	This paper	N/A
Primer: Actin Reverse: AAGCTGTAGCCACGCTCGGTC	This paper	N/A
shJak1 (human)	Neilson et al., 2007	TRCN0000003102
		TRCN0000003105
Recombinant DNA		
Tet-pLKO	Addgene	21915
pcDNA3-FLAG-Fos WT	Addgene	8966
pWZL Neo MAP3K8	Addgene	20521
pMXs-Stat3-C	Addgene	13373
pHIV-ZsGreen	Addgene	18121
pHIV-dTomato	Addgene:	21374
pBabe-puro-Cre	This Lab	Krempler et al., 2002
Software and Algorithms		
FastQC	Babraham Bioinformatics	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Cutadapt	Martin, M.. EMBOnet, ISSN 2226-6089. < https://journal.embnet.org/index.php/embnetjournal/article/view/200/479>	https://github.com/marcelm/cutadapt
TopHat2	Kim et al., 2013	https://ccb.jhu.edu/software/tophat/index.shtml
Tuxedo tools, Cufflinks, Cuffmerge, Cuffquant, Cuffdiff, Cuffnorm	Trapnell et al., 2012	http://cole-trapnell-lab.github.io/cufflinks/manual/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Gene Set Enrichment Analysis (GSEA)	Subramanian et al., 2005	http://software.broadinstitute.org/gsea/index.jsp
Prism v6	GraphPad	https://www.graphpad.com/scientific-software/prism/
FACSdiva 8.0.1	BD Biosciences	https://www.bdbiosciences.com/in/instruments/software/facsdiva/features/overview.jsp

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Kay-Uwe Wagner (wagnerk@karmanos.org).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Genetically engineered mice

The JAK1 conditional knockout mice [Jak1^{tm1Kuw}] and MMTV-Cre, line A transgenic mice [Tg(MMTV-cre)1Mam] were generated by our team (Sakamoto et al., 2016a; Wagner et al., 1997). MMTV-neu transgenic mice (Guy et al., 1992) were obtained from the Jackson Laboratory. The CAG-LSL-GFP reporter strain was kindly provided by Dr. Miyazaki (Osaka University) (Kawamoto et al., 2000). All transgenes were carried in the FVB/N genetic background, and the JAK1 conditional knockout allele was crossed back ten times into the FVB/N genetic background. Since this study examined the role of JAK1 in mammary carcinogenesis, only female mice were used as experimental animals. The age of females that were used for specific studies is described in the results section of the manuscript.

For PCR-based genotyping of mice, genomic DNA was extracted from digested tail biopsies of mice (10 mg/ml proteinase K in 1% SDS, 50nM Tris/HCl pH8, 100nM NaCl, and 50 mM EDTA) using an AutoGenprep2000. The primer sets and PCR conditions to genotype the genetically engineered alleles and transgenes are shown in Table S1.

Orthotopic transplantation models and imaging

The procedures for the orthotopic transplantation of mammary cancer tissues and cancer cells into the #4 inguinal mammary glands of 8 to 12 weeks-old female wild-type recipients (Athymic nude mice, NCr strain) were carried out as described (Sakamoto et al., 2009). Expression of luciferase reporters was analyzed using an *in vivo* bioluminescence imaging system (IVIS200). Animals were injected intraperitoneally 5 minutes prior to imaging with luciferin [150mg/kg of body weight, 1mg D-Luciferin in 1mL of 2x PBS, Xenogen, XR-1001]. The IVIS200 bioluminescence imaging machine was equipped with an XGI-8 gas anesthesia system, and isoflurane (Butler Schein, 029404) with a flow rate of 2.5% in O_2 was used. Mice were anesthetized immediately prior to and during imaging. Images were taken at 1 s to 2-minute exposure lengths.

Ethics statement and experimental endpoint

This study was conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal study protocols were approved by the Institutional Animal Care and Use Committee of the Nebraska Medical Center (12-008-03-FC, 09-104-01-FC). The experimental endpoint to assess mammary cancer initiation and growth in individual genetically engineered mice was determined by the maximal size of the tumor regardless of the health or condition of an animal. The maximal allowed tumor size was approximately 1.5 cm in diameter as mandated by the Institutional Animal Care and Use Committee.

METHOD DETAILS

Histologic analysis and immunostaining

Whole mounts were prepared by spreading number 4 mammary glands on glass slides. Tissues were fixed for up to 5h in Carnoy's solution, rehydrated, stained with Carmine Alum overnight, dehydrated and mounted. For histological examination, mammary tumor tissues were fixed overnight at 4°C in 10% buffered formalin (Fisher Scientific Company). Formalin-fixed tumor specimens were embedded in paraffin by standard methods. Sections of 5μm were deparaffinized three times in 100% Histo-Clear (National Diagnostics) and rehydrated in decreasing concentrations of ethanol (100%, 95%, 90%, 70%, 50%, and 30% for 3 minutes each) and washed for 5 min in 1x PBS. Slides were stained with Haemotoxylin and Eosin (H&E) for routine histopathology. For immunostaining, the Antigen Unmasking Solution from Vectastain (H-3300) was used for antigen retrieval. Slides were rinsed for 5 min in 1x PBS and

blocked with 3% BSA for 1h. After rinsing the slides for 5 min, the primary antibodies were added (dilutions varied from 1:250 to 1:1,000, staining conditions can be provided upon request). The incubation of the primary antibodies was performed overnight at 4°C in a moist chamber. The following day, slides were rinsed three times in 1x PBS for 5 minutes. Fluorophore-conjugated secondary antibodies were applied, and slides were incubated in the dark for 1h at room temperature. After washing the slides twice with distilled water, Vectashield DAPI mounting media (Vector, H-1200) and coverslips were applied. Stained slides were examined with an Axio Imager microscope (Carl Zeiss) equipped with a SPOT Flex camera.

Cell culture, deletion or knockdown of JAK1

Mammary cancer cells were derived from enzymatically dissociated primary tumors that originated in MMTV-neu *Jak1*$^{fl/fl}$ females. These cells were cultured in Dulbecco-modified Eagle medium (DMEM/F12) supplemented with insulin, epidermal growth factor, fetal bovine serum, penicillin/streptomycin, and gentamicin. Cells were infected with pBabe-Cre-puro or pBabe-puro and selected with 7μg/ml puromycin to establish conditional knockout cell lines and their isogenic wild-type controls. MDA-MB-453 human breast cancer cells were purchased from American Type Culture Collection (ATCC) with financial support from the Integrative Cancer Biology Program (NCI). The cells were expanded using media and supplements recommended by ATCC. To conditionally knock down JAK1 in human breast cancer cells, two previously validated shJAK1 sequences (TRCN0000003102 and 3105) were cloned into the lentiviral Tet-pLKO vector. Following infection and puromycin selection, cells were treated with 2 mg/mL doxycycline for 24 to 72 hours to downregulate JAK1. To assess the ligand-inducible activation of STAT6, JAK1 knockout cells and their controls were starved in serum-free DMEM for 16 h and then treated with recombinant mouse IL-4 (50 ng/ml) for 15 min at 37°C. Detailed information about the cloning of lentiviral-based vectors (pHIV-dTomato) expressing the rat c-FOS and human MAP3K8 (TLP2/COT) will be provided upon request.

Immunoblot analysis

For the majority of western blots, cell pellets or homogenized tumor tissues were kept on ice for 30 min in complete lysis buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, 0.4 units/ml aprotinin, 1 mM NaF, leupeptin, and 0.1 mM sodium orthovanadate. Whole-cell extracts were resolved by SDS-PAGE and blotted onto polyvinylidene fluoride (PVDF) membranes (Invitrogen). The membranes were blocked for 1 h at room temperature in 5% dry milk in 1xTBST (Tris-buffered saline with 0.05% Tween-20) buffer or in 5% BSA in 1xTBST for phosphotyrosine-specific antibodies. Subsequently, membranes were washed in 1xTBST, incubated with primary antibodies in blocking buffer at 4°C overnight. Next, membranes were washed three times for 5 min in 1xTBST and incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) in blocking buffer. Membranes were washed three times in washing buffer and then for 5 min in 1x TBS (Tris-buffered saline without Tween 20). Protein bands were detected using the ECL chemiluminescence kit for western blot analysis (GE, Amersham, RPN2106) according to the instructions by the manufacturer. Membranes were stripped using 0.2M NaOH for consecutive detection of various proteins.

Tumorsphere and cell migration assays

Three times 10^4 cells were plated into 6-well ultra-low attachment plates containing stem cell media, and tumorspheres were imaged after 9 days using an Evos scanning microscope. Spheres were counted in at least 4 areas of each plate for quantification. For the transwell migration assays, 1 mL of serum-rich media (20% fetal bovine serum, FBS) was added as chemoattractant into a new 24-well plate, and an 8-micron insert was placed in each well (Falcon®, 353097). Single cells from the maintenance culture were resuspended in serum-free media and counted using a hemocytometer. 2.5 x10^4 cells were plated on top of the 8-micron insert and incubated for 24 hours. The following day, the inserts were collected, washed in PBS or distilled water, and consecutively dipped 15 times in HEMA3 fixative. The cytoplasmic and nuclear staining of cells (Wright-Giemsa stain) was carried out using the HEMA 3 STAT Pack (Fisher Scientific, 123-869) according to the manufacture's protocol and washed in distilled water. The membrane inserts were cut out with a razor blade, air-dried, and placed in Permount on microscope slides that were then mounted with a coverslip. The number of migrating cells was determined by counting cells under 200x magnification in three areas of 1 mm^2. Data was presented as means of three replicate experiments.

Flow cytometric analysis

The flow cytometric analysis of the expression of mammary epithelial progenitor markers (CD61, CD24, CD29) was carried out by incubating the cells with primary antibodies against the respective antigen in FACS buffer (1xPBS supplemented with 1% bovine serum albumin and 0.1% sodium azide) for 25 min at 4°C. Subsequently, cells were washed once in ice-cold FACS buffer to remove excess antibodies. PE and APC-conjugated antibodies against CD24, CD29, and CD61 were purchased from Becton Dickinson (see Key Resources Table). The flow cytometric data was acquired on a BD Biosciences LSRII and analyzed using the FACSdiva 8.0.1 software. Fluorescence-activated sorting of GFP-labeled cells was carried out on a FACSVantage DiVa (Becton Dickinson). The purity of sorted populations was consistently greater than 95%.

RNA-Sequencing and quantitative RT-PCR

Total RNA was extracted from JAK1 deficient mammary tumor cell lines and wild-type controls expressing JAK1 using the RNeasy Mini Kit (QIAGEN). The Super-Script II kit from Invitrogen with oligo-dT primers was used to perform the first-strand synthesis according to the manufacture's protocol. Following quality control using a BioAnalyzer 2100, RNA samples were processed using the TruSeq RNA Sample kit and sequenced using a HiSeq2000 sequencer (Illumina). Quality of sequenced reads was determined using FastQC and adaptor portions were trimmed using Cutadapt. The trimmed single-end reads were mapped to the mouse reference genome mm10 using TopHat2. Normalized transcript abundance was estimated using the Tuxedo tools (Cufflinks, Cuffmerge, Cuffquant, Cuffnorm) as described earlier. Differential transcript expression between JAK1 knockout cells and controls was determined by an estimation of FPKM (Fragments Per Kilobase of transcript per Million mapped reads) with Cuffdiff. Genes showing more than a 2-fold up- or downregulation between these samples were selected. The GEO accession number for the sequencing data is GSE121064. A Gene Set Enrichment Analyses (GSEA) was performed to identify groups of genes that are being controlled by JAK1 according to their cellular functions and pathways, and the Broad Institute's Integrative Genomics Viewer (IGV) was used to visualize the expression of individual genes and their exons.

The quantitative detection of *Map3k8* mRNA transcripts was performed by RT-PCR using total RNA from cancer cells. The Super-Script II kit (Invitrogen) with oligo-dT primers was used to perform the first-strand synthesis and the iQ SYBR Green Supermix (Bio-Rad) for the PCR reaction. The quantitative PCRs were carried out in triplicate using a C1000 Thermal Cycler (BioRad System, CFX96-Real Time). The following primer sets were used: *Map3k8* (forward 5′-CTC TGT TTG CTG AGC CTG GA-3′, reverse 5′-ACG AAA CCA GAG CCG ATG TT-3′) and *Actin (*forward TGG ATG ACG ATA TCG CTG CGC, reverse AAG CTG TAG CCA CGC TCG GTC*)*. The expression of *Map3k8* was normalized against *Actin* as internal control using the $2^{-\Delta\Delta Ct}$ method and expressed as arbitrary units.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

All graphic illustrations and statistics were performed with GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA). Unless otherwise indicated in the figure legends, experimental data are shown as mean values ± SEM or mean ± SD. For panels showing the quantification of image data, at least three fields were counted from a minimum of three biological replicates. For statistical analysis, the data was assessed for normality followed by an unpaired Student's t test, Mann-Whitney-Wilcoxon test, or one-way ANOVA and Tukey's multiple comparison test. The tumor-free survival distributions between animals were assessed using the logrank test. P values < 0.05 (*), < 0.01 (**) or 0.001 (***) were considered statistically significant.

DATA AND SOFTWARE AVAILABILITY

The RNA Sequencing data was deposited in the Gene Expression Omnibus (GEO) under accession number GEO: GSE121064.