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Frequent loss of heterozygosity at the interferon regulatory factor-1 gene locus in breast cancer

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Abstract

The interferon regulatory factor-1 (*IRF1*) gene, localized on chromosome 5q31.1, is mutated or rearranged in several cancers including some hematopoietic and gastric cancers. However, whether loss of *IRF1* occurs in sporadic breast cancer is unknown. Loss of 5q12-31 is reported in 11% of sporadic breast cancers, and high-resolution array-CGH studies have shown loss at 5q31.1 in 50% of breast cancers with a mutated *BRCA1* gene. Functionally, overexpression of *IRF1* reduces, and a dominant negative *IRF1* construct increases, tumorigenesis of human breast cancer xenografts. Taken together, these observations indicate that the *IRF1* gene may play a potentially important role as a breast cancer tumor suppressor gene. In this study, we investigated allelic loss of the *IRF1* gene in breast tumor specimens from 52 women with invasive breast cancer using an *IRF1* intragenic dinucleotide polymorphic marker. Thirty-seven cases were informative. LOH at the *IRF1* locus was detected in 32% of these informative cases (12/37). There was a significant association between *IRF1* loss and both older age ($P = 0.0167$) and earlier stage (Stages 1 and 2) ($P = 0.0165$). To assess the association of *IRF1* mRNA expression with clinical outcomes in breast cancer, we studied data from two published gene expression microarray datasets. In breast cancer patients, low *IRF1* mRNA expression is strongly correlated with both risk of recurrence (OR = 3.00; $P = 0.003$; $n = 273$ cases) and risk of death (OR = 4.18; $P = 0.004$; $n = 191$ cases). Our findings strongly imply a tumor suppressor role for the *IRF1* gene in breast cancer.

Keywords

Interferon regulatory factor-1; *IRF1*; Breast cancer; Disease survival; Tumor suppressor gene

Introduction

The interferon regulatory factor-1 (*IRF1*) gene mediates interferon and other cytokine effects and exhibits antitumor activity in vivo and in vitro. *IRF1* can also reverse the oncogenic transformation of cells induced by the overexpression of both *RAS* and *MYC* in mouse models [1]. Since functional roles for *RAS* and *MYC* are established in human breast cancer [2,3], a loss of *IRF1* function might be important in this disease. Functionally, overexpression of *IRF1* reduces [4,5], and a dominant negative *IRF1* construct increases [4], tumorigenesis of human breast cancer xenografts. We and others have identified *IRF1* as a key regulator of breast cancer cell survival [5–8] that can activate a caspase cascade [4] and induce apoptosis [5,6]. More specifically, the proapoptotic effects of *IRF1* include activation/regulation of caspases-1 [9], -3 [4], -7 [4,10], and -8 [4,11]. *IRF1* can also induce apoptosis through both *TP53*-dependent and *TP53*-independent signaling [9,12]. *TP53* is often mutated in breast cancer [13], and many breast tumors initially respond to drugs and hormones through *TP53*-dependent and *TP53*-independent signaling. We have shown that *IRF1* is a key determinant of responsiveness to antiestrogen therapies in breast cancer [6,7].

Whether *IRF1* is a true tumor suppressor gene (TSG) in breast cancer is unknown. Established TSGs often show evidence of homozygous or heterozygous gene loss. For instance, while loss of *BRCA1* function in inherited breast cancers is usually a consequence of gene mutation(s), loss of *BRCA1* expression in sporadic breast cancers is often the result of loss of heterozygosity (LOH) accompanied by hypermethylation of a CpG island in the 5' region close to the transcription start site of the remaining allele [14,15]. *IRF1* has been implicated as a putative TSG in leukemias and preleukemic myelodysplasias, and *IRF1* is either mutated or rearranged in some hematopoietic disorders [16]. *IRF1* was shown to be the true target of frequent deletions (LOH) in esophageal cancer [17] and gastric cancer [18]. *IRF1* is located at 5q31.1, a region shown to be commonly lost in two large studies evaluating breast tumors by chromosomal comparative genomic hybridization (CGH). Deletion of 5q12-31 is detected in 11% of sporadic breast cancers [19] and 5q deletion is seen in 86% of *BRCA1* tumors [20]. More recently, a high-resolution array-CGH study has shown loss at 5q31.1 in 50% of *BRCA1*-positive breast cancers [21]. Whether loss at the *IRF1* locus is the driver in these cancers and whether *IRF1* gene loss occurs in sporadic breast cancers are unknown.

IRF1 is inactivated by a point mutation in gastric cancer, suggesting that the loss of function of *IRF1* may be critical for the development of this disease [18]. When attempting to generate an *IRF1* riboprobe from MCF-7 breast cancer cells mRNA, we found a single nucleotide polymorphism (SNP) in the *IRF1* coding region [22] and a novel *IRF1* splice variant (K. B. Bouker; unpublished observations). The *IRF1* A4396G SNP is more frequent in human breast cancer cell lines than in the general population and is more frequently expressed in African-American than Caucasian subjects [22]. It is not known whether *IRF1* A4396G contributes to the earlier age [23] or higher stage at diagnosis [24] or the lower overall survival rate of African-American compared with non-Hispanic white and Hispanic women [25]. When considered together, these observations strongly suggest that *IRF1* may be a TSG in breast cancer. Since one of the key features of TSGs is somatic loss, we designed this study to determine the incidence of *IRF1* loss in a series of 52 invasive breast tumors. Considering that *IRF1* LOH might be expected to reduce mRNA expression, we also explored whether low *IRF1* mRNA expression is associated with poor clinical outcomes in breast cancer patients.

Methods

We determined *IRF1* loss by LOH in breast tissue specimens from 52 patients with sporadic breast cancer obtained from the tumor bank at the Lombardi Comprehensive Cancer Center (LCCC). In each case, a paraffin block with breast tumor tissues and a second block with normal tissues (skin, negative lymph node, or a normal breast tissue block) were identified. An H&E-stained slide from each block was evaluated by a breast pathologist to confirm the diagnosis and mark the areas with malignant tissue or normal tissue. A 100- μ m consecutive section was obtained from each block, and the tissues of interest were grossly microdissected with a razor blade to isolate malignant cells. Corresponding normal cells from a different block were obtained for each case. DNA was extracted from the tissue using the DNeasy QIAGEN kit according to manufacturer's instructions (QIAGEN Inc. Valencia, California).

To study LOH at the *IRF1* locus, we selected an intragenic, dinucleotide, polymorphic marker (*IRF1* Dinucleotide Repeat, Allele Set GDB: 211036), with a high degree of heterozygosity (74% heterozygosity). The sequences of the oligonucleotide primers were obtained from the Genome Data Base (GDB) (<http://www.gdb.org>): Forward: 5'-ATGGCAGATAGGTCCACCGG-3'/Reverse: 5'-TCATCCTCATCTGTTGTACG-3'. Primers were fluorescently labeled and PCR amplification was performed using a standard protocol. Allele sizes were determined by electrophoresis of PCR products in 6% denaturing polyacrylamide gels and were compared to ROX 500 size standards (Applied Biosystems, Foster City, CA), using an automated sequencer (ABI 377), according to the manufacturer's instructions (Applied Biosystems). Fluorescent signals from the different size alleles were recorded and analyzed using GENOTYPER version 2.1 and GENESCAN version 3.1 software (Applied Biosystems). Following visual examination of computer printouts by two independent observers, LOH was determined mathematically according to the Genotyper User's Manual (Applied Biosystems).

The publicly available ONCOMINE cancer gene expression microarray database [www.oncomine.org; 26] was used to search for relationships between *IRF1* mRNA expression and outcomes in breast cancer clinical studies. Normalized Affymetrix MAS 5.0 gene expression data, originally published by Wang et al. [27] and Desmedt et al. [28], were downloaded from ONCOMINE. Statistical analysis was done using SigmaStat (Systat Software, Inc., San Jose, CA) and S-PLUS (Insightful, Seattle, WA). Median *IRF1* expression values across all samples, and between the top and bottom quartiles of expression, were compared by Mann-Whitney Rank-Sum test, and odds ratios were calculated for the association of low *IRF1* expression with poor outcomes following logistic regression analysis. Statistical significance was defined as $\geq 95\%$ confidence interval, or $\alpha = 0.05$.

Results

In this study, 37 cases (71%) were informative for the *IRF1* dinucleotide marker used for LOH analysis. LOH was detected in 12 of these informative cases (12/37; 32%). Figure 1 shows a representative case with no LOH (Fig. 1a, bottom panel) and another representative case with LOH (Fig. 1b, bottom panel). A significant correlation was found between LOH at the *IRF1* locus and both older age (P value = 0.0167 based on a two sample t -test) and earlier stage (Stages 1 and 2) (P value = 0.0165 based on Fisher's exact test).

The data from two published gene expression microarray datasets were assessed to investigate mRNA expression and clinical outcome [27,28]. In both studies, median *IRF1* mRNA expression, as detected by Affymetrix U133A GeneChips, was significantly reduced in patients with a worse outcome (recurrence, $P = 0.004$; death, $P = 0.021$). Moreover, when these expression values were separated into quartiles and analyzed by logistic regression (Table 1),

low *IRF1* mRNA expression (first compared with fourth quartile) was significantly associated with both recurrence (OR = 3.00, $P = 0.003$) and death (OR = 4.18, $P = 0.004$).

Discussion

In our study, a significant correlation was found between LOH at the *IRF1* locus and both older age ($P = 0.0167$) and earlier stage (Stages 1 and 2) ($P = 0.0165$). An inverse association between *IRF1* protein expression and tumor grade has been reported [29], and *IRF1* protein levels are lower in breast tumors than in adjacent normal cells [30]. However, subcellular location also affects *IRF1* correlation with clinical measures, and these correlations would not be apparent with LOH measurements. For example, cytosolic IRF-1 protein (but not nuclear *IRF1*) is associated with age (similar to LOH findings) and ER expression, whereas nuclear *IRF1* protein expression (but not cytosolic *IRF1*) correlates with PgR expression [31].

Since *IRF1* LOH might be expected to reduce mRNA expression, we explored the association of *IRF1* mRNA with the key measures of recurrence status (recurrent; non-recurrent) and vital status (alive; dead). Using the publicly available ONCOMINE cancer gene expression microarray database [26], we searched for relationships between *IRF1* mRNA expression and outcomes from two breast cancer clinical studies. The Wang et al. study includes 273 women diagnosed with lymph node-negative breast cancer who had not received systemic adjuvant therapy, but whose tumors are representative of a wide range of clinical/pathological features (including age, stage, and tumor size). The original goal of that study was to identify a gene expression signature that could predict recurrence of metastatic breast cancer in women with node-negative disease [27]. The more recent Desmedt et al. study is an independent validation of Wang et al. and includes 191 untreated patients less than 61 years of age with node-negative, T1 and T2 breast cancer [28]. The primary end-point for the study was overall survival, and median follow-up time was 13.6 years. While *IRF1* gene copy number data and protein expression data were not available from these studies, we could assess the potential of *IRF1* to act as a TSG as predicted by the likelihood that low *IRF1* mRNA expression was associated with poor clinical outcomes. We have observed that low *IRF1* mRNA expression is strongly correlated with both risk of recurrence and risk of death. Studies to determine directly the role of *IRF1* LOH in these outcomes are in progress.

IRF1 LOH in breast cancer may reflect haploinsufficiency; over 35 TSGs are known in which haploinsufficiency accounts for their contribution to carcinogenesis (though not necessarily breast carcinogenesis) [32,33]. Reduced IRF1 activity in experimental breast cancer models is functionally associated with increased cell survival, reduced caspase activation, and apoptosis (4–6). While LOH may not be the only contributor to low *IRF1* expression [34,35], the strong association of low *IRF1* mRNA and recurrence status and survival imply an important TSG role for *IRF1* in many sporadic breast cancers.

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Fig. 1. LOH analysis at the *IRF1* locus of two representative breast cancer cases. **a** A case with no LOH. **b** A case with LOH. (In **a**, **b**, *Top panels* shows the analysis performed in normal cells and *bottom panels* the analysis performed in tumor cells.)

IRF1 mRNA expression and clinical outcomes

Table 1

Study	Endpoint	<i>IRF1</i> (median)	<i>IRF1</i> (quartile)	Odds ratio ^a
Wang et al.	No disease (<i>n</i> = 180)	<i>P</i> = 0.004	First = 0.971	OR = 3.00 CI = 1.44-6.27
	Recurrence (<i>n</i> = 93)		Fourth = 0.455	<i>P</i> = 0.003
Desmedt et al.	Alive (<i>n</i> = 135)	<i>P</i> = 0.021	First = 0.905	OR = 4.18 CI = 1.56-11.21
	Dead (<i>n</i> = 56)		Fourth = 0.355	<i>P</i> = 0.004

^a Odds ratios were calculated for the first (highest) versus fourth (lowest) quartiles of expression data, and denote the association of low *IRF1* mRNA expression with poor outcome (recurrence or death); CI = 95% confidence interval