Allelic loss at chromosome 11q13 alters FGF3 gene expression in a human breast cancer progression model

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Abstract. Identification of markers with the potential to predict tumorigenic behavior is important in breast cancer, due to the variability in clinical disease progression. Genetic alterations during neoplastic progression may appear as changes in total DNA content, single genes, or gene expression. Oncogenic alterations are thought to be prognostic indices for patients with breast cancer. Breast cancer deregulation can occur in the normal cellular process and can be measured by microsatellite instability (MSI)/loss of heterozygosity (LOH). Chromosome 11 is unique in this respect, as three regions of MSI/LOH have been identified (11p15-p15.5, 11q13-q13.3 and 11q23-q24). There are many important families of genes, such as FGF, CCND1, FADD, BAD and GAD2, that are located on chromosome 11 and these play a crucial role in breast cancer progression. Among them, different members of the fibroblast growth factor (FGF) family of genes are clustered around human chromosome 11q13 amplicon, which are constantly altering during breast cancer progression. Therefore, in this study, locus 11q13 and FGF3 gene (11q13) function were investigated in a radiation and estrogen breast cancer model induced by high-LET (α-particle) radiation and estrogen exposure. To assess the effect of ionizing radiation and estrogen at chromosome 11q13 loci and the subsequent role of FGF3 gene expression, various microsatellite markers were chosen in this region, and allelic loses (~20-45%) were identified by PCR-SSCP analysis. Results showed an increase in FGF3 protein expression and a 6- to 8-fold change in gene expression of FGF3 and associated genes. These deregulations could be utilized as an appropriate target for therapeutic intervention in breast cancer.

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Introduction

Molecular analysis of human and experimental animal cancer models has established that they arise as a result of the deregulation of intricate mechanisms that control cell growth and differentiation. A major theme emerging from such studies is that mutations of both oncogenes and tumor-suppressor genes are required for malignancy, both being necessary for cell transformation and the latter for the expression of the malignant phenotype (1). According to Knudson's 'two hit' hypothesis, many types of human cancers are thought to develop by genetic alterations of putative tumor-suppressor genes that require a biphasic process to eliminate both alleles. Most frequently one of these two events involves the loss of one allele due to chromosomal deletion (2). This allelic alteration may occur either by microsatellite instability (MSI) or loss of heterozygosity (LOH) (3).

It is unclear whether allelic imbalance is the cause or the result of carcinogenesis, but it is probably the most common genetic factor associated with cancer. Identifying markers that have the potential to predict tumorigenic behavior is important in breast cancer due to the variability in clinical disease progression (4). Genetic alterations during neoplastic progression may appear as changes in total DNA content, single genes, or gene expression (5). Oncogenic alterations are thought to be prognostic indices for patients with breast cancer. During the multistage process of mammary carcinogenesis, stepwise accumulation of genetic changes causes uncontrolled growth, disruption of normal glandular architecture, and invasion of epithelial cells into the adjacent stroma, which ultimately leads to the subversion of orderly epithelial tissue organization. This subversion is a hallmark of malignancy and plays a crucial role in tumor progression (6). It also produces frequent allelic losses at various chromosomal regions, such as 1p, 3p, 6q, 8p, 11, 13q, 16q, 17 and 18q, associated with breast cancer (7-9).

Chromosome 11 is unique in this context, as at least three separate regions of LOH/MSI have been identified (11p15-p15.5, 11q13-q13.3 and 11q23-q24), pointing to a potentially complicated role of this chromosome in breast carcinogenesis (10,11). Cytogenetic studies and microcell-mediated transfer of human chromosome 11 into tumor cell lines have provided additional evidence of the presence of tumor-suppressor genes on chromosome 11 in melanoma, breast cancer and cervical

cancer (12,13). There are many important families of genes, such as FGF, CCND1, FADD, BAD and GAD2, that are located on chromosome 11 and play a crucial role in breast cancer progression (14,15). Among them, different members of the fibroblast growth factor (FGF) family of genes are clustered around the human chromosome 11q13 amplicon, commonly altered during breast cancer progression (16). Currently, it is well established that activation of various proto-oncogenes, such as c-MYC, c-ERBB-2/NEU and FGF3/INT2, could trigger uncontrolled cell growth and cancer development, but among them FGF3/INT2 gene amplification is found to be a better independent prognostic indicator of human breast cancer (17).

We previously reported chromosomal alterations along 11q23-q24 loci following radiation and estrogen treatment (18) but there is no report available concerning the chromosomal locus 11q13 and alteration of FGF3 gene (11q13) expression. Therefore, to assess the effect of ionizing radiation and estrogen at chromosome 11q13 loci and the subsequent role of FGF3 gene expression, we utilized a human breast cancer model derived from irradiated, transformed and tumorigenic MCF-10F cell lines treated with different doses of high-LET (α-particle) radiation and estrogen exposure (19).

Materials and methods

Cell lines. The recently established radiation-induced breast carcinogenic model based on the MCF-10F cell line was cultured and used in this study as presently described (19,20). From such a model, the following cell lines were used as control: MCF-10F cell line (passage 40); MCF-10F cell line treated with 17β-estradiol [estrogen (E); 10^{-8} M; Sigma Chemical Co., St. Louis, MO, USA], named Estrogen (19). The experimental cell lines used in this study were as follows: MCF-10F cell line irradiated with a double dose of 60 cGy of α particles, namely 60 cGy/60 cGy (Alpha3), which was anchorage-independent but non-tumorigenic in nude mice (19); MCF-10F cell line subjected to a double dose of 60 cGy of α particles and treated with estrogen before each radiation exposure, named 60 cGy+E/60 cGy + E (Alpha 5), which was anchorage-independent and produced tumors in nude/SCID mouse and after injection gave rise to Tumor2. Phenotypic characteristics of these cell lines and their genetic alterations including differentially expressed genes and expression of various proteins have been previously described (21-24).

DNA isolation. Cell cultures were treated with 1 ml of lysis buffer [100 mM NaCl, 20 mM Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 0.5% sodium dodecyl sulfate] with 200 mg/ml of proteinase K and RNase (100 μg/ml), and incubated overnight at 37˚C with constant gentle agitation (25). Then, they were purified and dissolved in TE buffer following standard procedures (26).

Selection of markers for microsatellite polymorphism. Four polymorphic dinucleotide $(CA)_n$ repeat microsatellite markers from chromosome 11q13-q13.3 were selected (Research Genetics, Huntsville, AL, USA). They were selected on the basis of their maximum heterozygosity (>0.70) and their location near mapped, known tumor-suppressor genes, oncogenes or other cancer-related genes (Table IA). The sequences of microsatellite oligonucleotide primers were obtained from the GDB database (http://www.ncbi.nlm.nih.gov/tools/primer-blast) (Table IB). We also tested D2S123 (2p16, 0.77, dinucleotide, 197-227 bp), a CA repeat marker linked to the *HMSH2* gene, mapped at 2p16, where LOH is rarely encountered (data not shown).

PCR-single strand conformation polymorphism analysis. PCR-single strand conformation polymorphism (SSCP) analysis was carried out in a volume of 30 μl containing 50-100 ng of genomic DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 200 μM of each dNTP, 0.8 μM of each primer (Research Genetics), and 0.75 units of AmpliTaq polymerase (Perkin-Elmer Corp., Foster City, CA, USA) (27). One of the primers was 5'-end-labeled with [γ-^{32}p] ATP at 3000 Ci/mmol (Amersham Pharmacia Biotech., Skokie, IL, USA) by T_4-polynucleotide kinase (Amersham Life Science, Arlington Heights, IL, USA). After a 5-min pre-incubation period at 94˚C, DNA was amplified for 35 cycles consisting of 45 sec at 94˚C, 45 sec at 55˚C, and 1 min at 72˚C, followed by a 7-min final extension at 72˚C using the GeneAmp® PCR System 2400 (Perkin Elmer/Applied Biosystems, Foster City, CA, USA). PCR products were processed by diluting 1:1 in denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol FF, and 0.05% bromophenol blue); denaturing at 95˚C for 5 min and then frozen at 4˚C. Two microliters of the aliquot was loaded and electrophoresed on 6% polyacrylamide gels containing 8.3 M urea for 2-3 h at 40 W. The gel was fixed in 10% methanol-10% acetic acid, dried and exposed to Kodak X-omat-AR film (Eastman Kodak Co., Rochester, NY, USA) at -70˚C with an intensifying screen for 12-16 h. PCR reaction was always repeated 2-3 times with different adjacent passages of cells to get consistent results.

Assessment of allelic losses. MSI/LOH were screened by PCR amplification of microsatellite markers. MSI was defined as a shift of a specific allelic band or a change (increase or decrease) in the broadness of a specific allelic band in the autoradiogram, whereas LOH was defined as a total loss (complete deletion) or a 50% or more reduction (in signal density) in one of the heterozygous alleles in the autoradiogram. It was first scored by visual inspection of the autoradiogram, and then band intensity was quantified in a densitometric scanner (model 300A) by Image Quant (ver. 3.3; both from Molecular Dynamics). Optical density range of 0.01 to 4.0 was chosen in OD units, whereas spatial resolution was selected at 100 points/cm in both directions (x and y). Resolution (signal) was selected at 4096 levels (12-bit) of optical density.

Determination of protein expression by immunofluorescence technique. Exponentially growing cells were plated on a glass chamber slide (Nunc Inc., Naperville, IL, USA) as previously described (28), at a density of $1x10^4$ cells/ml of growth medium. Three independent biological experiments were performed. FGF3 protein expression was detected using the primary antibody (sc-135; in a 1:500 dilution from the original stock concentration; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Rhodamine-conjugated secondary antibody was from Jackson ImmunoResearch Lab., West Grove, PA, USA.

Table I. Characteristics of selected repeat markers $(CA)_n$ and sequence of sense and antisense primers of microsatellite markers and other important genes located on chromosome 11q13-q13.3.

A, Characteristics of selected repeat markers $(CA)_n$ on chromosome 11q13-q13.3

Chromosomal locus	Map position[a]	Maximum heterozygosity	Type of sequence	Size range [base pairs (bp)]
D11S2179	11q13-q13.3	0.792	Dinucleotide	123-133
FGF3	11q13	0.853	Dinucleotide	198-220
INT2	11q13	0.788	Dinucleotide	364-379
PYGM(CA)	11q13.1	0.761	Dinucleotide	152-160

B, Sequence of sense and antisense primers of microsatellite markers and other important genes located at chromosome 11q13-q13.3

Chromosomal locus	Primer sequence sense (5'→3')/antisense (5'→3')	Important genes within these marker regions
D11S2179	TAGGCAATACAGCAAGACCCTG/ GCACTGGAATACGATTCTAGCAC	bad, Sfg in breast cancer
FGF3	ATTTCCAGAGCCAGCTCAAA/ CTTTAATGTTGTGATGACACAAAGC	ccnd1, fadd, bad, gad2
INT2	TCTGCCTCCTGGGTTCAAG/ AGGAAAGACAAGGTTGTAGG	ccnd1, int2, fgfr
PYGM(CA)	CTAGCAGAGTCCACCTACTG/ GCTGTCAGGTAGCAACTGAC	gad2 in breast cancer, Tsg

[a]Precise location of the markers on the respective chromosomal arms.

Slides were mounted using Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). Cells were examined using Zeiss Axiovert 100 TV microscope (Carl Zeiss, Thornwood, NY, USA) using a 40X 11.3 NA objective lens equipped with a laser scanning confocal attachment (LSM 410; Carl Zeiss, Thornwood, NY, USA). Staining intensity and fluorescent (argon/krypton laser, 488 nm) images of the cells were generated and quantified as previously described (19,24,28). A semi-quantitative estimation based on relative staining intensity of protein expression was determined for the parental, non-tumorigenic and tumorigenic cell lines. The number of immunoreactive cells (30 cells/field) was counted in 5 randomly selected microscopic fields per sample. Standard error of the mean values are shown in the representative figures. Statistical analysis was carried out with the F-test (randomized block) and comparisons between groups with the Bonferroni t-test with $P<0.05$ considered to indicate a statistically significant difference (29).

Fluorescent-labeled probe preparation for microarray analysis. Poly(A) mRNA from normal, radiation- and estrogen-treated breast cancer cell lines was isolated using QIA-direct mRNA isolation kit (Qiagen). Fluorescent-labeled cDNA was prepared from 1 μg of each of these poly(A) mRNAs using oligo dT-primed polymerization and Superscript II reverse transcriptase kit (Life Technologies), in the presence of either Cy3- or Cy5-labeled dCTP following the usual

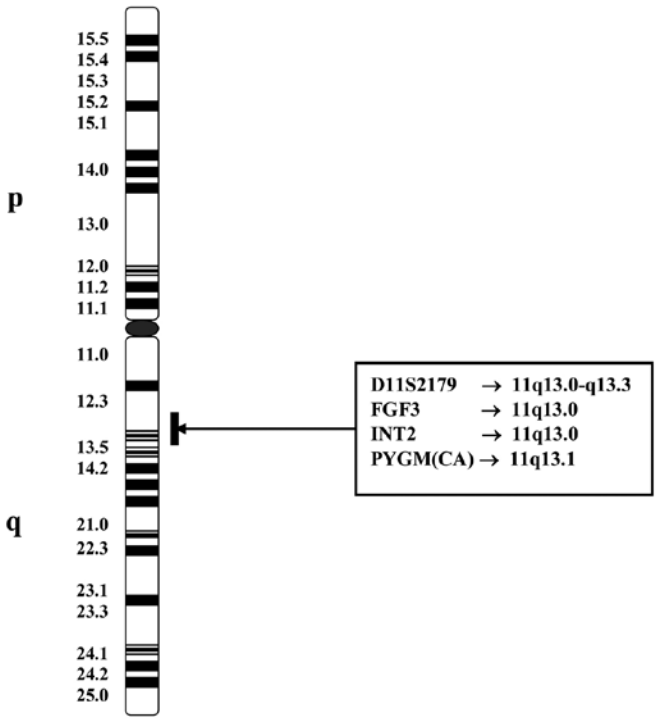

Figure 1. Map of chromosome 11 showing the putative positions of the $(CA)_n$ repeat microsatellite markers used in this study. Bold black vertical lines indicate regions of possible map positions of the markers.

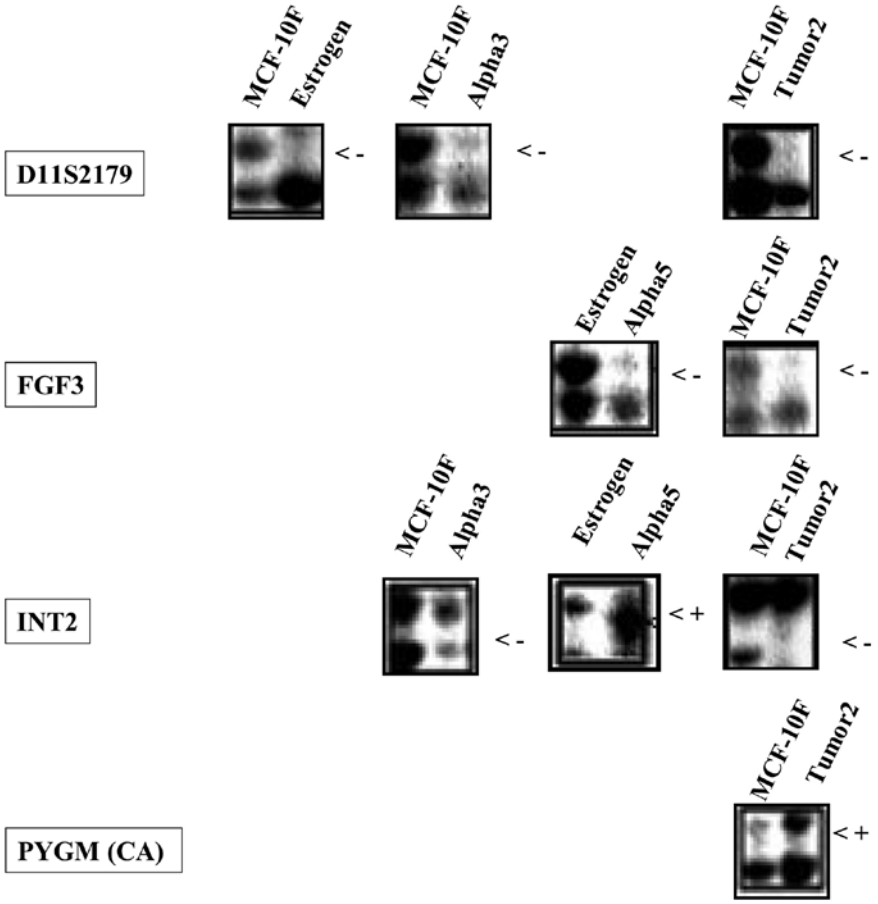

Figure 2. Occurrence of microsatellite instability (<+) and loss of heterozygosity (<-) screened at the respective loci of $(CA)_n$ repeat markers of chromosome 11q in irradiated, tumorigenic and tumor cell lines.

procedure as described in http://cmgm.stanford.edu/pbrown/protocols.html. The appropriate Cy3- and Cy5-labeled probes were pooled and hybridized to a microarray in glass coverslips for 16 h at 65˚C and then washed with high stringency for analysis.

Analysis of gene expression by Affymetrix HG-U133A Plus 2.0 GeneChip microarray. The breast cancer model (Alpha model) containing the i) MCF-10F, ii) Estrogen iii) Alpha3, iv) Alpha5 and v) Tumor2 cell lines was used to analyze gene expression by Affymetrix U133A oligonucleotide microarray (Affymetrix, Santa Clara, CA, USA), which contains 14,500 genes. Arrays were quantitatively analyzed for gene expression using the Affymetrix GeneChip® operating software (GCOS) with dual global scaling option in a Genes@Work software platform of discovery algorithm SPLASH (structural pattern localization analysis by sequential histograms) with a false discovery rate of 0.05 (30,31).

Results

A study of allelic losses and altered gene expression in the human breast Alpha model was analyzed in this study. Identification of allelic losses at the specific chromosomal region of 11q13-q13.3 using a total of four microsatellite markers from chromosome 11q13 was used to assess the allelic alterations in an established breast cancer model. Fig. 1 corresponds to the map of chromosome 11 showing the putative positions of the $(CA)_n$ repeat microsatellite markers used in this study. Bold black vertical lines indicate regions of possible map positions of the markers. The different degrees of allelic imbalance were expressed in the form of MSI or LOH. This research also focused on the differential gene expression of FGF3 and associated genes at locus 11q13. Table IA documents the characteristics of selected repeat markers $(CA)_n$ on chromosome 11q13-q13.3 and Table IB documents the sequence of sense and antisense primers of the microsatellite markers and other important genes located at chromosome 11q13-q13.3.

Fig. 2 shows the frequency of MSI and LOH at the respective loci of $(CA)_n$ repeat markers of chromosome 11q in irradiated, tumorigenic and tumor cell lines. It was found that alterations were more pronounced in cell lines exposed to double doses of radiation, as well as those in which estrogen was added and in the tumor cell line Tumor2 compared to control MCF-10F cell lines. These changes were directly correlated with the phenotypic characteristics of the cell lines as they progressed through different stages of transformation to become tumorigenic.

The presence of MSI and LOH screened at the respective loci of $(CA)_n$ repeat markers of chromosome 11q13-q13.3 in irradiated, tumorigenic and tumor cell lines is shown in Table IIA. The MCF-10F cell line treated with estrogen (Estrogen) was altered in the form of LOH at locus 11q13.3 (D11S2179) when compared with the control MCF-10F cells.

Table II. Allelic imbalance and fold change and pair-wise analysis of FGF3 and associated genes in the human breast cancer cell lines.

A, Allelic imbalance in the radiated and estrogen-treated human breast cell lines as detected using different microsatellite markers on chromosome 11q13-q13.3

		Cell lines				
Markers	Map position	MCF-10F	Estrogen	Alpha3	Alpha5	Tumor2
D11S2179	11q13-q13.3	△	□	□	◇	□
FGF3	11q13	△	△	△	□	□
INT-2	11q13	△	△	□	○	□
PYGM(CA)	11q13.1	△	△	△	△	○

B, Fold change and pair-wise analysis of differential expression of FGF3 and associated genes in human breast cell lines identified by Affymetrix HG-U133A Plus 2.0 GeneChip microarray

		Cell lines					
Gene	Genebank	MCF10F/ Estrogen	MCF10F/ Alpha3	Estrogen/ Alpha5	Alpha3/ Alpha5	Alpha5/ Tumor2	Alpha3/ Tumor2
Fibroblast growth factor binding protein1	NM_005130	-1.2 (↓)	-21.1 (↓)	-9.2 (↓)	2.0 (↑)	4.2 (↑)	8.3 (↑)
Fibroblast growth factor 2 (basic)	M27968	-1.3 (↓)	4.4 (↑)	3.0 (↑)	-2.0 (↓)	-2.8 (↓)	-5.5 (↓)
Fibroblast growth factor 2 (basic)	NM_002006	1.9 (↑)	5.8 (↑)	3.1 (↑)	1.0 (↑)	-3.4 (↓)	-3.3 (↓)
Fibroblast growth factor 3	NC_000011.9	-1.5 (↓)	4.8 (↑)	3.6 (↑)	-1.8 (↓)	-2.3 (↓)	-5.8 (↓)

△, Retention of heterozygosity; □, loss of heterozygosity (< -); ○, microsatellite instability (< +); ◇, inconclusive. ↑, upregulation; ↓, downregulation.

The same locus was also altered in the form of LOH in the Alpha3 and Tumor2 cell lines with respect to the control MCF-10F. Similarly, the presence of LOH was also identified at locus 11q13 (FGF3) in the Alpha5 and Tumor2 cell lines when compared with the Estrogen and MCF-10F cell lines. The locus 11q13 (INT-2) also showed both LOH and MSI in the different irradiated and tumorigenic cell lines when compared to the control MCF-10F and Estrogen cell lines; Alpha3 and Tumor2 cell lines showed LOH, and MSI was observed in the Alpha5 cell line at this specific locus. Similarly, MSI was noted at locus 11q13.1 [PYGM(CA)] only in the Tumor2 cell line when compared with the MCF-10F cell line.

Fig. 3A shows a histogram representing the average and standard error of FGF3 protein expression in the MCF-10F, Estrogen, Alpha3, Alpha5 and Tumor2 cell lines as determined by immunofluorescence staining by confocal microscopy. Representative images of FGF3 protein expression in the MCF-10F, Estrogen, Alpha3, Alpha5 and Tumor2 cell lines are shown in Fig. 3B. The results revealed higher protein expression in the Alpha5 and Tumor2 cell lines when compared with the control MCF-10F cells.

Analysis of gene expression by microarray showed gene expression of FGF3 (11q13) and associated genes such as FGFBP1 and FGF2 in cell lines of the established Alpha model as shown in Table IIB. Fold change and pair-wise analysis of the differential expression of FGF3 and associated genes in the human breast cell lines were identified by microarray. Results of the pair-wise comparison of the cell lines examined for the expression of FGF3 and other associated genes were studied in pairs of cell lines as follows: MCF-10F/Estrogen, MCF-10F/Alpha3, Estrogen/Alpha5, Alpha3/Alpha5, Alpha5/Tumor2 and Alpha3/Tumor2. Results indicated that the pair-wise comparison did not reveal a significant alteration in FGFBP1 gene expression between the MCF-10F/Estrogen and Alpha3/Alpha5 cell lines, whereas an ~21-, 9-, 4- and 8-fold alteration in the MCF-10F/Alpha3, Estrogen/Alpha5, Alpha5/Tumor2 and Alpha3/Tumor2 combinations, respectively, was noted. Similarly, between the MCF-10F/Alpha3 and Alpha3/Tumor2 combinations an ~6- and 5-fold change in FGF2 gene expression, respectively, was noted. Finally, combinations of MCF-10F/Alpha3 and Alpha3/Tumor2 cell lines revealed a 5- and 3-fold alteration in FGF3 gene expression,

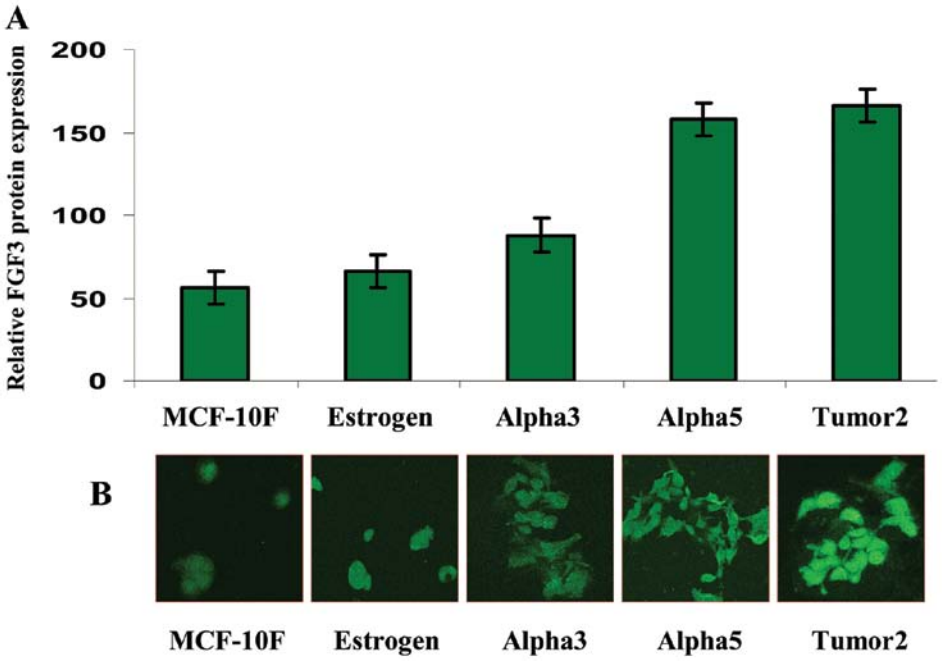

Figure 3. (A) Histogram shows the average and standard error of FGF3 protein expression in the MCF-10F, Estrogen, Alpha3, Alpha5 and Tumor2 cell lines as determined by immunofluorescence staining and quantified using confocal microscopy and a computer program, which provides the area and the intensity of the staining as described in the text. The primary antibody used was a mouse monoclonal antibody. (B) Representative images of FGF3 protein expression in the MCF-10F, Estrogen, Alpha3, Alpha5 and Tumor2 cell lines as determined by immunofluorescence staining.

respectively, whereas there were no significant alterations in the other combinations with respect to this particular gene.

Discussion

The carcinogenic progression of breast tissues is a complex multi-stage process involving various morphological and genetic alterations including activation of oncogenes and loss or inactivation of tumor-suppressor genes. Thus, tumor cells may have altered genes related to their cell cycle (19,21). An array of genetic anomalies during tumor progression increases the probability of random rearrangements, which favor chromosomal disintegration that leads to LOH, also favoring mitotic recombination, which leads to MSI (22,32).

Our previous study indicated that the combined treatment of ionizing radiation and estrogen yielded different stages in a malignantly transformed breast cancer cell model, which we called the Alpha model system (19). Utilizing this model system, a progressive degree of allelic alterations at 11q13-q13.3 and differential expression of FGF3 and associated genes were detected in the parental, non-tumorigenic and malignantly transformed cell lines originally derived from the parental MCF-10A cell line (20).

Specific microsatellite markers belonging to this particular region were selected on the basis of their role in cell-cycle regulation, DNA replication, DNA repair, or signal transduction of gene proteins (33,34). Therefore, allelic alterations were more pronounced and deleterious when MCF-10F cell lines were exposed to double doses of radiation and treated with estrogen in comparison to the cell lines that were treated with only double doses of radiation without estrogen.

It is now well established that estrogen may play a dual role in affecting breast cancer risk (35). It may serve as a pre-initiator, initiator and promoter of breast cancer by DNA damage and mutations in cells or may reduce breast cancer risk during pregnancy, pre-pubertal period and childhood (36,37). Therefore, these results indicate the importance of estrogen in breast tumor progression. Moreover, studies from other laboratories have already placed various putative tumor-suppressor genes in this larger overlapping area (38-40), which is consistent with our present observation. Again, microcell-mediated chromosome transfer of an intact copy of chromosome 11 into tumorigenic HeLa cells has provided additional support for the presence of a tumor-suppressor gene in this chromosomal region (11,41).

LOH/MSI in this region have been identified in several esophageal and laryngeal squamous cell carcinomas, human renal cell carcinoma, prostate and ovarian cancers as well (42-45). There is also an increasing body of evidence indicating the existence of various driver genes in this region. They show genetic and epigenetic alterations in cancer or cancer-predisposing syndromes (39). 11q13 amplification has also been reported in the local recurrence of human primary breast cancer (46).

Identification of numerous LOH/MSI in the same region (11q13-q13.3 loci) by various independent laboratories has supported the importance of this region in breast cancer. Although, the precise mechanism of the high rate of LOH/MSI in this particular region is not known, it is evident from different observations that more than one tumor-suppressor gene reside in this region, which also highlights the relevance and usefulness of this model. Their altered imprinting may lead to tumorigenesis by involving a gene activation hypothesis (47).

Notably there are many important families of genes such as FGF, CCND1, FADD, BAD and GAD2 located around 11q13-

q13.3 with a crucial role in breast cancer progression (14,15). Yet, among them, different members of the FGF family of genes are most important as their amplification is found to be a better independent prognostic indicator of human breast cancer (17,48). In addition, INT-2/FGF3 gene amplications were found to be good indicators of prognosis, potentially in premenopausal patients, and also in lymph node-positive and steroid receptor-negative patients (17). Int-2/FGF3 amplification and progesterone receptor status together proved to be the only independent variable predictive of metastasis-free survival (17). Again, progression in MCF-7 breast cancer cell tumorigenicity also showed the amplification of FGF3 and FGF-4 genes (49). Along with amplification of the FGF family of genes, the fibroblast growth factor receptor (FGFR) cascade also plays crucial roles in tumor cell proliferation, angiogenesis, migration and survival. Accumulating evidence suggests that in some tumor types, FGFRs are bona fide oncogenes to which cancer cells are addicted. Since FGFR inhibition can reduce proliferation and induce cell death in a variety of *in vitro* and *in vivo* tumor models harboring FGFR aberrations, a growing number of research groups have selected FGFRs as targets for anticancer drug development (50).

In can be concluded that characterization of this specific locus and alteration of the FGF3 family of genes at this locus is important. Moreover, evaluation of this gene(s) could be used as an additional parameter to identify appropriate target(s) for therapeutic intervention that contribute to radiation-induced breast carcinogenesis. This has broad implications in diagnosing the clinical and pathological aspects of breast cancer, a heterogeneous disease.

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