BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **235,** 123–129 (1997)
ARTICLE NO. RC976744

# Relaxation of Imprinting of Human Insulin-like Growth Factor II Gene, *IGF2,* in Sporadic Breast Carcinomas

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Received May 6, 1997

**Breast cancer is the most frequent malignancy in women and genetically heterogeneous, and a variety of genetic lesions have been identified that tend to accumulate during the disease progress. In breast cancer, loss of heterozygosity (LOH) has been described in the critical regions of chromosomes 11p15 and 11q22-23. Genomic imprinting is defined as gamete specific modification causing differential expression of the two alleles of a gene, in somatic cells. Human insulin like growth factor II gene (IGF2), located on chromosome 11p15, the same region on which LOH frequently occurred in breast cancer, has been recently identified as a genomic imprinting gene expressing preferentially paternal allele. To determine whether loss of *IGF2* imprinting was common in breast cancer we studied 30 patients with sporadic breast carcinoma. A new strategy for detecting intragenic *Apa* I polymorphism in the exon of *IGF2* was used to examine allele-specific expression in the breast cancer specimens by reverse-transcription polymerase chain reaction (RT-PCR). Forty percent (12/30) of the breast cancer samples were identified as heterozygous for *IGF2* and studied further. Nine of the 12 heterozygous patients showed biallelic expression of *IGF2* by cDNA-PCR, indicating relaxation of normal imprinting at this chromosomal locus. Conclusively, aberrant imprinting of *IGF2* in 30% of the breast cancer patients tested provides strong evidence that pathological loss or relaxation of *IGF2* imprinting plays an important role in either tumorigenesis or cytokine dysregulation for breast cancer cells.** 

Breast cancer is the most frequent malignancy in women, with a cumulative lifetime breast cancer risk in an unselected population that has been estimated to be about 10∼12% (1). Although the vast majority of cases appears to be sporadic, hereditary factors may account for about 5∼10% of all cases (2). Breast cancer presents as a herogeneous and progressive disease, the increasing severity of which is thought to result from the accumulation of alteration in multiple genes regulating cell growth and proliferation (3). By analogy with several other forms of cancer, many of these mutations might be expected to occur in a disease stage- or grade-specific manner. One type of genetic alteration common to many tumor types is loss of heterozygosity (LOH), which often seems to unmask recessive mutations in tumor suppressor loci (4). Among the genetic regions commonly undergoing LOH in barest tumor is chromosome 11, in particular regions 11p15 and 11p13 (5). Complete or partial LOH has proven indicative inactivation of tumor suppressor gene residing in that particular chromosomal region. Clustering of high-frequency LOH for the short arm of chromosome 11 has been observed in adult cancers of breast, ovary, bladder, testicles, lung, and cortex as well as in embryonal tumors such as rhabdomyosarcoma, Wilms' tumor, and hepatoblastoma (4). Microcell-mediated chromosome transfer of human chromosome 11 to cells of the MCG-7 breast cancer cell line inhibited tumorigenicity. Also, these microcell-mediated chromosome transfer studies indicated that two regions could affect the tumorigenic behavior of MCF-7 cells (6). This suggests the presence of either a cluster suppressor genes or a single pleiotropic gene in this region (7). The region presently indicated by LOH data appears to lie between 11p15 (from HBB to 11pcen), with involvement of LOH approximately 30% of the sporadic breast tumors analyzed (8).

The human *IGF2* gene is located on chromosome 11p15, the same region on which LOH frequently occurred in breast cancer, just downstream of the insulin gene, and spans 30-kb. It consists of nine exons, of which, exons 7, 8, and 5′ end of exon 9 code for the *IGF2* precursor protein. Exons 1, 4, 5, and 6 are each

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Abbreviations used: IGF2, huamn insulin-like growth factor II gene; LOH, loss of heterozygosite; LOI, loss of imprinting; RT-PCR, reverse-transcriptase polymerase chain reaction; gDNA-PCR, genomic DNA-PCR; cDNA-PCR, complimentary DNA-PCR.



0006-291X/97 $25.00

preceded by a distinct promoter (P1∼P4) (9, 10). Multiple transcripts are synthesized as a result of alternate promoter usage and the splicing of a unique 5′-untranslated region to common coding exons. Expression of the *IGF2* gene is regulated in a development-dependent and tissue-specific manner. Promoter 1 is active only in adult liver, while P2, P3, and P4 are active in most fetal tissues. There are a few adult tissues that express low amounts of *IGF2* transcripts from promoters P2, P3, and P4 (11, 12). Enhanced levels of P3 and P4 promoters driven *IGF2* mRNA has also been detected in many tumors of different origins, which suggested a role for *IGF2* in autocrine or paracrine growth stimulation. High levels of RNA from P3 and P4 promoters were also detected in some tumor-derived cell lines (13, 14). Moreover, *IGF2* transgenic mice developed a diverse spectrum of tumor at higher frequency after 18 months of age than the control mice. This suggests that *IGF2* may function as a tumor progression factor in mice via autocrine and endocrine mechanisms (15).

Genomic imprinting in mammals refers to an epigenetic modification of a gene or chromosome that can affect its expression in a stable, heritable fashion. These modifications appear to contribute to the regulation of a variety of processes during normal development and differentiation (16). Recently, both *IGF2* and *H19* have been shown to be imprinted in humans, with reversal expression of the paternal *IGF2* (17, 18) and maternal *H19* alleles (17, 19). Loss of imprinting (LOI) of *IGF2* and/or *H19* has been reported in a subset of Wilms' tumors and rhabdomysarcoma (17, 18). The occurrence of LOI of *IGF2* would theoretically double the gene dosage with a possibility of doubling the transcription rate. In our recent reports, biallelic *IGF2* expression undergoes in an overgrowth disease, Beckwith-Wiedemann syndrome (BWS) (20) and acute myeloid leukemia (21), suggesting that LOI of *IGF2* in various human tumors attest to the critical role of imprinting for regulating *IGF2* gene dosage during fetal/embryogenesis (16).

The mechanisms by which the gene is imprinted remain unknown. Two proposed mechanisms involving alteration of imprinting processing system have been suggested to play a role during development and tumorigenesis (16). In order to investigate imprinting status of *IGF2* in breast cancer, we have employed reverse transcriptase (RT)-PCR based allele-specific expression adjacent to a common *Apa* I polymorphism within *IGF2* cDNA-PCR products to directly assay expression profiles in 30 patients with sporadic breast tumors and 3 normal breast tissues, revealing that relaxation of normal *IGF2* imprinting occurred in 30% (9/30) of the breast carcinomas studied.

## MATERIAL AND METHODS

*Patient materials.* Primary tumor samples were obtained from 30 randomly selected breast cancer patients from Sunnbrook Health Science Centre following informed consent according to University of Toronto guidelines. Treatment of the disease was initiated only after surgical removal of the primary tumor. Once surgically removed the tumor tissue was either used immediately to prepare RNA and genomic DNA (gDNA) or immediately snap frozen in liquid nitrogen and stored at −70°C until further use. Three normal controls of breast tissues included into this study were obtained from the routine breast biopsies that were diagnosed without defined disease.

*Nucleic acid preparation and cDNA synthesis.* gDNA was prepared by treating breast carcinomas or normal breast tissues with 0.5 mg/mL proteinase K in 1% SDS, followed by phenol/chloroform extraction and ethanol precipitation. Total RNA was extracted from fresh samples of treating breast carcinomas or normal breast tissues by a guanidinium thiocyanate-based method (23). 2 $\mu$g of total RNA was treated with 5 units of RNase-free/DNAse (Promega Corp., Madison, WI) at 37°C for 30 min and then added to 40 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM DTT, 2 mM $MgCl_2$, 10 pM of random hexamer, 2 mM of each deoxynucleotide triphosphate, 1 $\mu$L (40 units) of RNasin ribonuclease inhibitor (Promega Corp., Madison, WI), and 1 $\mu$l (100 units) of Moloney murine leukemia virus reverse transcriptase (M-MuLV-RT) (Gibco-BRL, Burlington, ON) in a volume of 20 $\mu$L and incubated at 37°C for 45 min.

*PCR oligonucleotides.* Allele specific gene expression was examined using an *Apa* I polymorphism of *IGF2* (Genbank X03562/M14118) (24). The strategy used was based on published methods (25) and is shown schematically in Fig. 1. Briefly, *IGF2* gDNA was amplified using primers B (5′-CTT GGA CTT TGA AGT CAA ATT GG-3′) and C (5′-GGT CGT GCC AAT TAC ATT TCA-3′) on exons 9 and 8 respectively, so that any contaminating gDNA would be detected because of the presence of intron 8 between these primers generating a 1.4-kb band. None of the cDNA samples tested resulted in this gDNA specific band, even after extending PCR for additional cycles. *IGF2* cDNA was then amplified using primer A (5′-TCC TGG AGA CGT ACT GTG CTA-3′) and B resulting in a 1.12-kb cDNA-specific product (Fig. 2). Exon-spanning primers specific for human $\beta$2-microglobulin (Genbank M17986/M17987) and $\beta$-actin (Genbank M10277); were used in separate reactions to control for reverse transcription and RNA-PCR efficiency (23).

*PCR amplification.* 5 $\mu$L of cDNA solution or 300 ng of gDNA was added to a 100 $\mu$L final volume of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.4 mM each dNTP, 20 pM each primer and 2.5 units of *Taq* DNA polymerase (Perkin-Elmer Cetus, Rexdale, ON). PCR was performed with an initial denature of 4 min at 94°C, followed by 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min, with a final extension of 10 min at 72°C. Amplified gDNA- and cDNA-PCR products were ethanol precipitated and digested with 10 units of *Hinf* I and then *Apa* I endonucleases. The enzyme-digested PCR products were then separated on a 3% agarose gel containing 0.5 mg/mL ethidium bromide. Reaction conditions of $\beta$2-microglobulin and $\beta$-actin amplification were 30 cycle of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. To visualize the PCR product, 10 $\mu$L of the reaction was electrophoresed in a 2% agarose gel containing 0.5 mg/mL ethidium bromide. $\phi$X174/*Hae* III DNA size markers (Gibco-BRL, Burlington, ON) were used as molecular weight standards.

*Evaluation criteria for RT-PCR.* Samples were considered positive for *IGF2* cDNA-PCR expression if a single band of appropriate size (292-bp) could be observed upon a 2% of agarose ethidium bromide gel, beside human $\beta$2-microglobulin (300-bp) and $\beta$-actin (500-bp) bands derived from separate reactions, was visible upon gel electrophoresis and ethidium bromide.

## RESULTS

*Monoallelic expression of IGF2 in normal breast tissues.* An established allelic-specific expression RT-

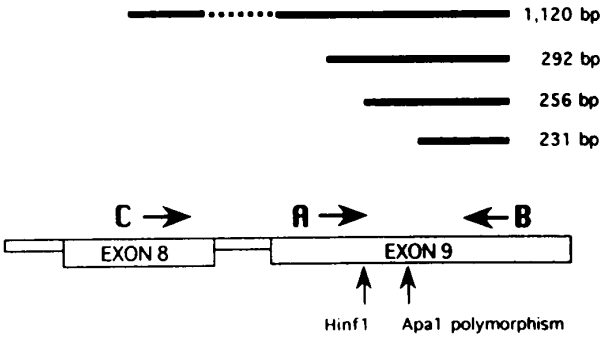


**FIG. 1.** Strategy for examination of allele expression of *IGF2*. Allele expression was identified using an *Apa* I polymorphism in exon 9 of the *IGF2* gene, essentially as described by Davies (25). Primers A and B amplify a 292-bp product from cDNA, which yield a 256-bp band after digestion with *Hinf* I. Primers B and C were used to amplify a 1120-bp band from DNA, which yield a 256-bp band after digestion with *Hinf* I. Different primers were used to amplify cDNA as primers B and C span an intron, allowing gDNA and cDNA derived products to be distinguished by agarose gel electrophoresis and to exclude any gDNA contamination of cDNA preparation. Digestion of the 256-bp band with *Apa* I will yield a 231-bp if the *Apa* I polymorphism is present. Expressed allele can be identified in heterozygous individuals by comparison of the cDNA *Apa* I digestion pattern with the gDNA pattern.

PCR assay for *IGF2* (Fig. 1, 2) was used to determine whether this gene was transcribed from one allele in normal breast tissues (25). The gDNA from the three normal controls of breast tissues was amplified by gDNA-PCR, digested with *Hinf* I-*Apa* I and one heterozygotes was identified as suitable for *IGF2* cDNA-PCR. The normal breast control RNA demonstrated monoallelic expression of *IGF2* (Fig. 3) indicating that differentiated normal breast tissues retain physical imprinting.

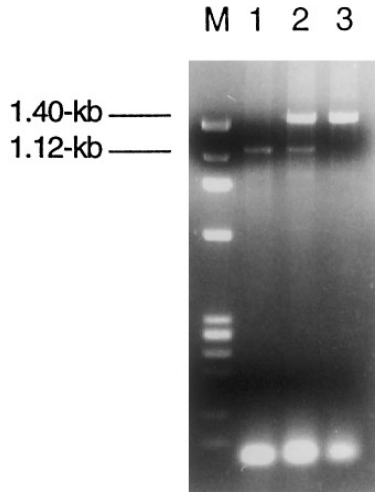


**FIG. 2.** Non-digestion PCR pattern of allele expression of *IGF2*. cDNA- and gDNA-PCR products of *IGF2* were shown in lane 1 (1.12-kb) and lane 3 (1.40-kb), respectively. cDNA preparation contaminated with gDNA is shown in lane 2. Lane 4 is a $\phi$X174/*Hae* III DNA size marker.

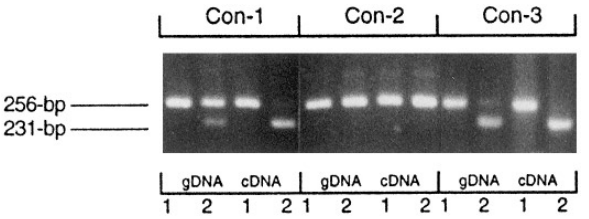


**FIG. 3.** Analysis of allele-specific expression of *IGF2* in 3 normal breast tissue controls by PCR assay. Three gene-specific primers, *IGF2*-A, -B, and -C, were used to amplify gDNA fragment *IGF2* and mRNA from RNA isolated from 3 specimens of normal breast tissues. The heterozygous genotype of *IGF2* is identified with a noticeable 3:1 ratio of staining intensity for the fragment of 292 bp in comparison to the 236-bp fragment. This is thought to occur because heteroduplex DNA is uncut by *Apa* I (24, 25). Lane 1 indicates gDNA- or cDNA-PCR products of *IGF2* digested with *Hinf* I only. Lane 2 indicates gDNA- or cDNA-PCR products of *IGF2* digested with *Hinf* I and *Apa* I. Relative positions of 256- and 231-bp bands are indicated on the left.

*Loss imprinting of IGF2 in primary sporadic breast carcinomas.* gDNA from 30 unselected primary sporadic breast cancer patients diagnosed with infiltrating duct carcinoma was amplified by gDNA-PCR and digested with *Hinf* I-*Apa* I to identify the presence of the previously described polymorphism in *IGF2* gene. 40% (12/30) of the breast cancer samples were found to be heterozygous for the *Apa* I polymorphism and could be used for the *IGF2* allelic-specific transcription assay. Total RNA (already treated by DNase I) was extracted from 12 informative samples and cDNA was synthesized using M-MLV reverse transcriptase. Results of the *IGF2* cDNA-PCR of breast carcinomas are shown in Fig. 4 and Table 1. In each breast cancer specimen, digestion of gDNA-PCR products with *Hinf* I and *Apa* I yields bands of 256- and 231-bp, indicating heterozygosity for the *Apa* I polymorphism (Fig. 1, 2). When PCR product derived from cDNA is digested with *Hinf* I and *Apa* I, both 256- and 231-bp bands are clearly visible in 9 of the 12 of heterozygous cases, indicating expression of both alleles and relaxation of normal imprinting of *IGF2* in 9 informative breast cancer patients tested in our experiments.

## DISCUSSION

Breast cancer is a significant worldwide public health problem, as well as an excellent model system for examining many aspects of tumor biology and genetics. The progression from breast epithelium to matastatic breast cancer is a complex, multistep process. Initially, genetic alterations are thought to confer a growth advantage to individual cells by decreasing tumor-suppressor gene activity or increasing oncogene

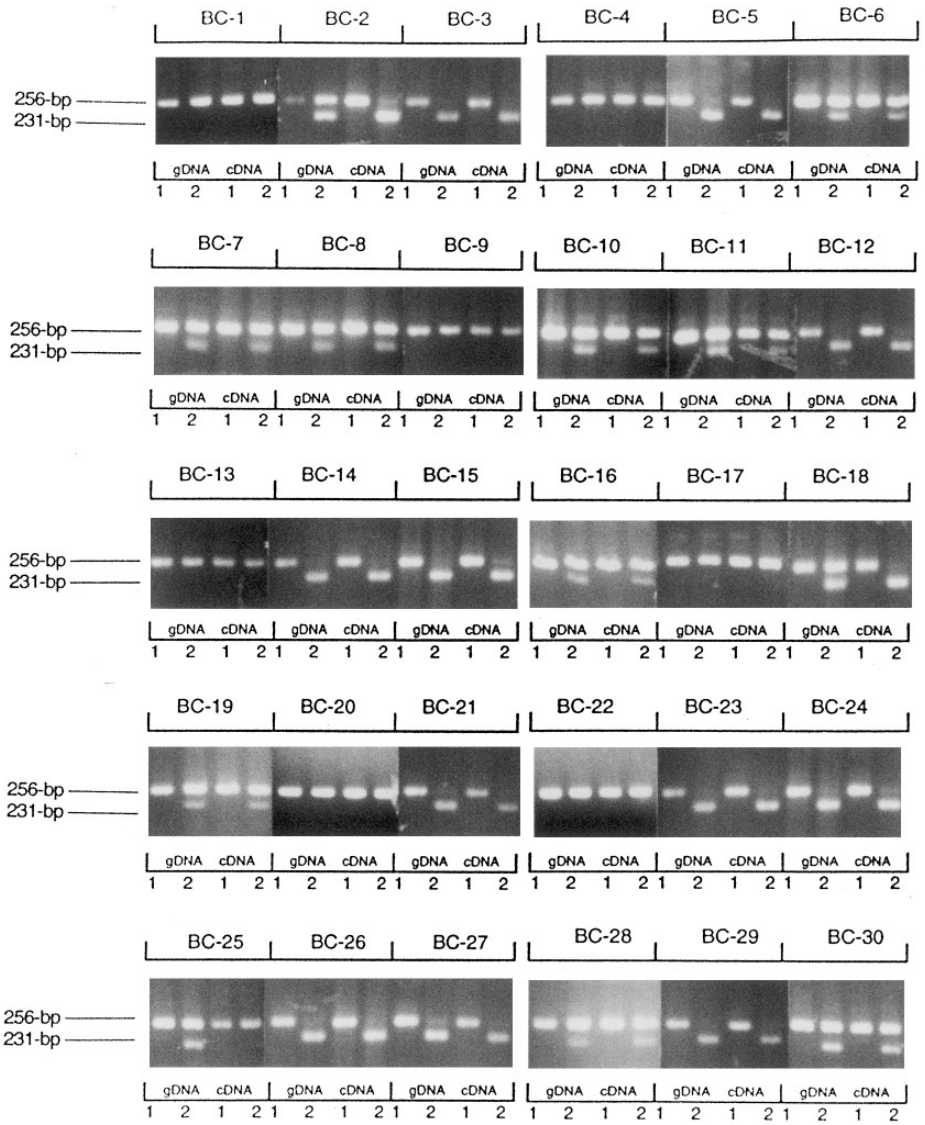


**FIG. 4.** Analysis of allele-specific expression of *IGF2* in 30 breast carcinoma patients by PCR assay. The same PCR assay described above was used to amplify DNA fragment *IGF2* and mRNA from total RNA isolated from 30 breast cancer samples. Lane 1 indicates gDNA- or cDNA-PCR products of *IGF2* digested with *Hinf* I only. Lane 2 indicates gDNA- or cDNA-PCR products of *IGF2* digested with both *Hinf* I and *Apa* I. Relative positions of 256- and 231-bp bands are indicated on the left.

activity, or in both. Further alteration result in the development of cell clones that have the ability to invade adjacent point tissue, establish metastatic deposits, and evade immune surveillance. At some point in this process, these malignant cell clones also lose the normal ability to respond to hormonal growth regulatory signals (26). Mutations in tumor-suppressor genes are believed to play a central role in the development of breast and other cancers. LOH at a specific chromosomal location in tumors is widely interpreted as a marker for the absence of a functional tumor-suppressor gene within the region of loss. Nonrandom deletions, as detected by LOH on chromosome 11p15, has been identified in a number of common human cancers, including carcinoma of the breast, which supports the presence of one or more tumor suppressor genes in this region (27, 28). Our data that relaxation of *IGF2* imprinting was observed in sporadic breast cancer, suggests that this chromosomal region may also be involved in human tumorigenesis. In the present study, we examined allelic expression of the *IGF2* gene in a random population of breast cancer patients, using an *Apa* I transcribed polymorphism in exon 9 of the *IGF2* gene (24, 25). Of the informative cases, the normal imprinting profile was shown as monoallelic expression of *IGF2*. Importantly, of the breast carcinoma specimen examined, 9/30 (30%) malignant breast lesion demonstrated biallelic profiles. Considering that "one-copy"

**TABLE 1**

Histo-Pathological Diagnosis, Clinical Stages, and Allele-Specific Expression of Human IGF2 Gene in Sporadic Breast Cancer Samples

| Breast cancer sample No. | Histological diagnosis[a] | Clinical stages[b] | $\beta$2-Microglobulin[c] | $\beta$-Actin[d] | Genotyping[e] | Allele-specific expression[f] |
|---|---|---|---|---|---|---|
| 1 | Infiltrating duct carcinoma/metaplasia | Grade III | + | + | a, a | a, a |
| 2 | Infiltrating duct carcinoma | Grade I | + | + | a, b | b, b |
| 3 | Infiltrating duct carcinoma | Grade II | + | + | b, b | b, b |
| 4 | Infiltrating duct/lobular carcinoma | Grade III | + | + | a, a | a, a |
| 5 | Infiltrating duct carcinoma | Grade II | + | + | b, b | b, b |
| 6 | Infiltrating duct carcinoma | Grade I | + | + | a, b | a, b |
| 7 | Infiltrating duct carcinoma | Grade III | + | + | a, b | a, b |
| 8 | Infiltrating duct carcinoma | Grade II | + | + | a, b | a, b |
| 9 | Infiltrating duct carcinoma | Grade III | + | + | a, a | a, a |
| 10 | Infiltrating duct carcinoma/matastasis | Grade III | + | + | a, b | a, b |
| 11 | Infiltrating duct carcinoma | Grade I | + | + | a, b | a, b |
| 12 | Infiltrating duct carcinoma | Grade I | + | + | b, b | b, b |
| 13 | Infiltrating duct carcinoma | Grade II | + | + | a, a | a, a |
| 14 | Tubular carcinoma | Grade I | + | + | b, b | b, b |
| 15 | Infiltrating duct carcinoma | Grade II | + | + | b, b | b, b |
| 16 | Infiltrating duct carcinoma | Grade II | + | + | a, b | a, b |
| 17 | Tubular carcinoma | Grade I | + | + | a, a | a, a |
| 18 | Infiltrating duct carcinoma | Grade II | + | + | a, b | b, b |
| 19 | Infiltrating duct carcinoma | Grade I | + | + | a, b | a, b |
| 20 | Infiltrating duct carcinoma | Grade I | + | + | a, a | a, a |
| 21 | Infiltrating duct carcinoma/matastasis | Grade III | + | + | b, b | b, b |
| 22 | Infiltrating duct carcinoma | Grade III | + | + | a, a | a, a |
| 23 | Infiltrating duct carcinoma | Grade II | + | + | b, b | b, b |
| 24 | Infiltrating duct carcinoma | Grade III | + | + | b, b | b, b |
| 25 | Infiltrating duct carcinoma | Grade I | + | + | a, b | a, a |
| 26 | Infiltrating duct/mammary carcinoma | Grade II | + | + | b, b | b, b |
| 27 | Infiltrating duct carcinoma | Grade III | + | + | b, b | b, b |
| 28 | Infiltrating duct carcinoma | Grade II | + | + | a, b | a, b |
| 29 | Infiltrating duct carcinoma | Grade I | + | + | b, b | b, b |
| 30 | Infiltrating duct carcinoma/matastasis | Grade III | + | + | a, b | a, b |

[a] Histo-pathological diagnosis based on analysis of histology-pathology in the breast cancer patients tested in this study.
[b] The grade assessed using the Bloom and Ruchardson grading system (45).
[c/d] Detection of RT-PCR product by agarose gel electrophoresis/ethidium bromide staining.
[e,f] Genotyping (gDNA-PCR) and allele-specific expression (cDNA-PCR) of IGF2 gene identified by agarose gel electrophoresis/ethidium bromide staining after IGF2 PCR products digested with *Hinf I* or *Hinf I + Apa I*.

dosage of imprinted gene is normal, altered gene dosage or disruption of the molecular system involved in the correct identification and maintenance of the imprint could have deleterious effects on cellular function. Because *IGF2* is an autocrine growth factor, it has been proposed that LOI of *IGF2* leads to overexpression of *IGF2* and tumorigenesis. Our observation solely indicated that altered expression of *IGF2* imprinting is present in double dosage in the breast cancer.

In humans, the majority of fetal tissues express *IGF2*, but significant circulating levels of *IGF2* (300∼500 $\mu$g/ml) are also detectable in the serum of adults (29). The human *IGF2* gene is transcribed at least four promoters a developmental and tissue specific fashion (30). Accordingly, the promoter P1 which may be unique to humans, is transcriptionally active in postnatal liver and in the fetal choroid plexus/leptomenings (31). Promoters P2∼P4 are transcriptionally active in many embryonic/fetal and adult tissues, including liver but an overall down regulation is evident after birth suggesting a role for *IGF2* in prenatal development. In another recent study, we clearly demonstrated that biallelic *IGF2* transcription was identified only in human adult liver tissue, providing a supportive notion that stage dependent relaxation of the gene imprinting may be a normal development event that facilitates proper organ maturation and function (32). Moreover, in transgenic mouse model, SV40 large T-antigen induced hepatocellular carcinoma display a reactivation of *IGF2* expression, which was interpreted as being an important event in the proliferation of the tumor (33). In another transgenic model, SV40 Tag induced tumor in the islet of Langerhans showed focal activation of the *IGF2* gene which correlated to proliferation of the tumor, however, interfering with *IGF2* transcription reduced tumor proliferation *in vivo* and

inducing apoptosis (34). Thus, the *IGF2* peptide has been suggested to be an important factor in both embryonic/fetal growth as well as in certain types of tumors in both human and mouse.

As to the role of *IGF2* in breast development, and its possible role in tumorigenesis, evidence shows that *IGF2* is mitogenic for breast tumor epithelial cells (35) and may function both in an autocrine and paracrine fashion in a proposed model of stromal/epithelial interactions (36). Direct evidence that biallelic expression may contribute functionally in the early stage of tumorigenesis come from studies examining *IGF2* allelic expression in an oncogene-driven multistage mouse model (RIPI-Tag2) of islet cell carcinogenesis (34, 37). In the early stage of this model, the "proliferative switch" resulting in hyperproliferation and subsequent tumor development required co-activation of both *IGF2* alleles, with each additively contributing to hyperproliferation, tumor growth and resultant tumor volume. In contrast, examination of tumor cell growth in RiPI-Tag2 mice with disrupted *IGF2* function showed a reduction in tumor cell growth, reduced malignancy and a significant increase in the number of apoptotic bodies (34). In this last regard, *IGF2* may play a role by prolonging cellular survival by inhibiting apoptosis (34). Certainly in cultured cells, its absence in programmed cell death (38) and chromosome 11p15 regions could affect the tumorigenic behavior of MCF-7 cells (6). These functional studies of *IGF2* have provided insights into the regulatory mechanisms which are responsible for tumorigenesis and tumor progression, cytokine dysregulation of *IGF2* is also a significant feature. The results presented here suggests that LOI of *IGF2* is a early and common epigenetic event in tumorigenesis of the breast carcinomas. The finding that 30% of breast carcinomas studied loss *IGF2* impairing is interesting because not only was LOI of *IGF2* observed in late stages but also in the early stages of the breast carcinoma diagnosed with histo-pathology. It seems possible that imprinting of *IGF2* in potentiation for both generation of breast cancer clones and rapid growth of breast cancer cells increases the possibility of *IGF2* undergoing LOI in the early stages of breast cancer.

Genomic imprinting is the phenomenon of gamete specific modification of two alleles of a gene in somatic cells, leading to differential allelic expression (16). In normal individuals, only the paternal allele of the human *IGF2* locus at 11p15 is expressed and the maternal allele is silent (16). Recently we have shown that constitutional LOI of *IGF2* in BWS patients (20) and acute myeloid leukemia (21). Moreover, disruption of allele-specific expression of *IGF2* has been reported for a number types of human neoplasms, including Wilms' tumors (67-77%) (17, 18), embryonal rhabdomyosarcomas (86%) (17), and adult lung carcinomas (47%) (39). LOI of *IGF2* and *H19* has been also reported in choriocarcinomas (40), testicular germ cell tumors (41), and Ewing's sarcoma (42). Furthermore, when LOI of *H19* was found in Wilms' tumors it was associated with significant down-regulation of *IGF2* expression as predicted by the enhanced competition model which involves inverse regulation of *H19* and *IGF2* during normal development (18, 19). This finding led to the proposal that *IGF2* epigenetic mechanisms may inactivate *H19* which may in turn be functioning as a tumor suppressor gene. In addition, altered *IGF2* expression may act a "second signal" in oncogene-induced tumorigenesis (37). The coincidental activation of *IGF2* at the late stage of the tumor model suggested that *IGF2* may be providing a "second signal" favouring progression to a malignant phenotype. The increased expression of *IGF2* is due to either biallelic expression or other transcriptional events, elevated levels of *IGF2* have been reported in several tumors, suggesting that it could hypothetically, have served as a progression signal.

Although the precise mechanism of LOI of *IGF2* is unknown, undermethylation of the methylated CpG base pairs in genomic DNA has been proposed as one possible pathway (43, 44). Otherwise, two proposed mechanisms may be responsible for this biallelic expression, either LOI, resulting in an active maternal copy in addition to an active paternal allele, or paternal *IGF2* heterodisomy, resulting in two active paternal *IGF2* copies (16). In summary, we have examined allele-specific expression of *IGF2* in 30 patients with sporadic breast carcinoma, and identified LOI of *IGF2* was undergone in 9 of the 12 informative specimen, demonstrating that biallelic expression of *IGF2* happened in 30% of the breast carcinomas tested, the later is the same frequency as that of LOH occurred on 11p15 in sporadic breast cancer (8). More importantly, this study involved a relative large number of breast cancer; to our knowledge, this finding provide the first evidence of molecular genetic alteration, namely, relaxation of imprinting of *IGF2* in breast cancer. Conclusively, genomic imprinting of *IGF2* appears to be directly or indirectly involved in tumorigenosis, LOI of *IGF2* may play an important role in either the early stage of tumorigenesis or cytokine dysregulation for breast carcinoma cells.

## ACKNOWLEDGMENTS

This work was funded by the Medical Research Council of Canada, the National Cancer Institute of Canada, and the Cancer Research Society of Canada.

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