The S387Y Mutation of the Transforming Growth Factor-β Receptor Type I Gene Is Uncommon in Metastases of Breast Cancer and Other Common Types of Adenocarcinoma

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Abstract

Recently, mutations of the transforming growth factor-β receptor type I gene have been reported to occur at high frequency in breast cancer metastases, with all mutations being an identical C to A transversion at nucleotide 1160 of the gene (T. Chen et al., Cancer Res., 58: 4805–4810, 1998). This mutation would result in a serine to tyrosine substitution at codon 387 (S387Y) and would reportedly disrupt receptor function. Because this mutation reportedly occurred at high frequency in breast cancer metastases (42%) and much less frequently in primary breast cancer tumors (6%), this would seem to represent a pivotal genetic alteration in breast cancer progression. To further investigate the possible role of this specific genetic alteration in the progression of breast cancer and other forms of adenocarcinoma, we analyzed 20 breast cancer metastases, 15 lung adenocarcinoma metastases, and 13 colorectal cancer metastases for possible mutations at this site. Using both single-strand conformation polymorphism screening and sequencing, we found no mutations of this gene in any of our samples. Our results suggest the S387Y mutation of the transforming growth factor-β receptor type I gene is not common in these types of human cancers.

Introduction

The molecular basis of metastasis is not well understood. Several investigators have found differential expression of specific genes, such as nm23, to be correlated with frequency of metastases for some types of human cancers, including breast cancers (1). Variable expression of nm23 is not always associated with tumor metastases, however, and no single metastasis gene or specific genetic alteration that may be responsible for metastases has yet been identified (2).

According to a recent study, mutations of the TGF-β receptor type I gene (RI) were found in 2 of 31 primary breast cancer tissues and 5 of 12 breast cancer lymph node metastases (3). All mutations reported were identical C to A transversions at nucleotide 1160 of the gene, which would result in a serine to tyrosine substitution at codon 387 (S387Y). In transient transfection assays, this mutation was also found to disrupt TGF-β-dependent effects on gene expression. The differential frequencies reported for this mutation in primary and metastatic breast cancers would suggest that this mutation is closely associated with the development of breast cancer metastases and, therefore, could represent a critical event in breast cancer progression. Because there is very little data regarding genetic alterations that are associated with cancer metastases, we began a survey of adenocarcinoma metastases for possible mutations of RI. Our study included metastases from breast cancers, pulmonary adenocarcinomas, and colorectal adenocarcinomas.

Materials and Methods

Tissue Specimens and Preparation of DNA. Lymph node metastases from 20 breast cancers, 15 pulmonary adenocarcinomas, and 13 colorectal adenocarcinomas were collected from the surgical pathology archives of the Johns Hopkins Hospital and the Johns Hopkins Bayview Medical Center. The metastatic breast cancers included 15 cases of ductal carcinoma, 2 cases of ductal carcinoma with lobular features, 1 case of medullary cancer, 1 case of lobular cancer, and 1 case of mucinous cancer. Tumor cells were microdissected from paraffin sections, as described previously (4), so that all samples consisted of at least 80% tumor cells. Lysates were used directly in PCR reactions.

Mutation Analysis. In the previous study of the RI gene in breast cancers, the entire coding region was screened for mutations, and mutations were found only to occur at a single position, nucleotide 1160. We, therefore, restricted our analysis to this relevant region, designing PCR primers to amplify genomic DNA to include sequences of exon 7 from nucleotide 1129 to nucleotide 1240 (forward: 5’TGTCTGAAAGGAGGTTTCACCC3; reverse: 5’GTCGAGCAAATTTCTCCAGAATA3’). Amplification of genomic DNA with these primers results in the predicted product of 188 nucleotides, including 76 nucleotides of the 5’ intronic sequence.

Single-strand confirmation polymorphism analysis was performed on all samples using a two-step amplification. An initial unlabeled PCR reaction of 10 μl contained each primer at 0.5 μM, 1.5 mM MgCl2, and 0.5 unit of Taq polymerase (Life Technologies, Inc., Gaithersburg, MD). After an initial 4-min denaturation at 95°C, PCR was performed for 33 cycles (95°C for 45 s, 60°C for 45 s, and 72°C for 1 min), followed by a final extension of 3 min at 72°C. A 2-μl aliquot of the product was tested by agarose electrophoresis gel for correct size of the product. The remaining product was then used in a second reaction that included radiolabeled dCTP. This PCR product (2 μl) was then mixed with an equal volume of stop solution containing 20 mM NaOH, denatured for 3 min at 95°C, and electrophoresed on an MDE gel (FMC Bioproducts, Rockland, ME) at 6 W for 9 hs. Because of the previously reported mutations of this gene in breast cancer metastases, we also sequenced the PCR products from each of the 20 breast cancer metastases in our study. We used the T7 Sequenase v2.0 reagents (Amersham, Arlington Heights, IL) according to the manufacturer’s protocol.

Results and Discussion

We observed only wild-type bands in all single-strand confirmation polymorphism reactions for RI from 20 samples of breast cancer metastases, 15 samples of lung cancer metastases, and 13 samples of colon cancer metastases. Because these findings conflicted with the previously reported mutational analysis of breast cancer samples, we performed new PCR reactions of each of our metastatic breast cancer sample and sequenced the reaction products. For all 20 samples, we again found only wild-type sequences of nucleotides 1129–1240. The results of these sequence reactions are represented in Fig. 1.

Our results clearly conflict with those of the previous study in which C to A transversions were seen at nucleotide 1160 of RI in 5 of
Mutations of the TGF-\(\beta\) RI Gene Are Uncommon

12 breast cancer metastases samples (3). It is possible that many of the breast cancer metastases in the previous study were from a unique group of patients, and this could also explain why all tumors had exactly the same mutation. In general, mutations that inactivate tumor suppressor genes affect critical domains but do not always involve a single specific nucleotide change. Arguing against the mutations at nucleotide of RI 1160 being related to a selected group of patients, however, is the reported absence of mutations in corresponding normal cells of the patients with these cancers. The possibility that RI mutations occur only in a particular subtype of breast cancer could also be considered, but the previous study did not specify the histological types of cancers studied. It is probably reasonable to assume that the majority of the samples were ductal cancers (the most common and most aggressive type of breast cancer) and our sample, therefore, probably represented a similar spectrum of breast cancer types as the study in which RI mutations were reported (3).

The RI gene would seem to be a reasonable target for mutations that contribute to the cancer phenotype. TGF-\(\beta\) inhibits the replication of cultured human epithelial cells derived from a number of tissues, including breast (5). In contrast, cancer-derived cell lines, including those of breast origin (6), are usually resistant to TGF-\(\beta\)-regulated growth inhibition and transcriptional response in breast tumor cell lines that have decreased expression of the RI gene or the Smad4 gene (9, 10), and two breast cancer cell lines have mutations of Smad4 in conjunction with loss of the second allele (11, 12). In contrast, no mutations of the Smad1, Smad3, Smad5 or Smad6 genes were found in breast cancer cell lines (13), and the Smad2 and Smad4 genes have been found to be normal in substantial numbers of primary breast cancer samples (14, 15).

Based on these previous studies, therefore, there is substantial evidence that disruption of the TGF-\(\beta\) signaling pathway can play a role in the pathogenesis of human cancers, including breast cancers. Furthermore, an experimentally mutated form of the RI gene diminishes TGF-\(\beta\) signaling in transfected cells, providing a rationale for possible involvement of this specific gene in human carcinogenesis. However, previous surveys of RI gene sequences in human tumors have found no mutations of this gene in any of the nine exons, with the exception of the reported mutations found in breast cancers at nucleotide 1160 in exon 7 (3). Our results suggest that this specific mutation of the RI gene is also uncommon in common forms of adenocarcinoma, including breast cancer.

References

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