

Loss of Heterozygosity Events Impeding Breast Cancer Metastasis Contain the *MTA1* Gene¹

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Abstract

Breast cancer mortality is seldom attributable to the primary tumor, but rather to the presence of systemic (metastatic) disease. Axillary lymph node dissection can identify the presence of metastatic breast cancer cells and serves as a marker for systemic disease. Previous work in our laboratory determined that rates of loss of heterozygosity (LOH) of a 1.6-Mb region of chromosome 14q 31.2 is much higher in axillary lymph node-negative primary breast tumors than in axillary lymph node-positive primary breast tumors (P. O'Connell *et al.*, *J. Natl. Cancer Inst.*, 91: 1391–1397, 1999.). This unusual observation suggests that, whereas the LOH of this region promotes primary breast cancer formation, some gene(s) mapping to this 1.6-Mb region is rate-limiting for breast cancer metastasis. Thus, if primary breast cancers delete this region, their ability to metastasize decreases. To identify this gene(s), we have physically mapped this area of chromosome 14q, confirmed the position of two known genes and 13 other expressed sequence tags into this 1.6-Mb region. One of these, the metastasis-associated 1 (*MTA1*) gene, previously identified as a metastasis-promoting gene (Y. Toh *et al.*, *J. Biol. Chem.*, 269: 22958–22963, 1994.), mapped to the center of our 1.6-Mb target region. Thus, *MTA1* represents a strong candidate for this breast cancer metastasis-promoting gene.

Introduction

One of the strongest prognostic factors for cancer-free survival after treatment of the primary tumor is the presence or absence of local metastatic spread. For women with axillary lymph node-negative breast cancer, 90% survive more than 5 years after diagnosis. This is compared with a 70% 5-year survival for women with axillary lymph node-positive disease, and only a 20% 5-year survival for women with distant metastases (1). The development of the metastatic phenotype of a tumor cell involves a complicated series of events that include detachment of tumor cells from the primary tumor, invasion into and survival in the circulatory and lymphatic systems, extravasation, and induction of angiogenesis and growth at the metastatic site. The development of a genetic test that could predict the metastatic potential of a primary breast tumor would increase the effectiveness of breast cancer treatment.

Previous work in our laboratory involved LOH³ analysis to compare DNA samples from paired normal and breast tumor tissues to examine whether specific genetic changes in primary breast cancer can serve as markers of metastatic potential (2). As expected, increas-

ing rates of LOH were correlated with progressively higher stages of breast cancer (3, 4). Unlike all other 14 markers tested, LOH at marker D14S62 was much lower in metastases than in primary breast tumors. D14S62 LOH proved to be associated with node-negative primary cancers and thus with slower spread to distant sites. Higher resolution LOH studies narrowed this phenomenon to a 1.6-Mb region near marker D14S62 (2). Here we have assembled a physical map and identified a minimum tiling path of three YAC clones that span this region. One of the ESTs that mapped into this region in our study was *MTA1*, a gene previously shown to be highly expressed in both metastatic breast cancer cell lines and metastatic gastrointestinal carcinomas (5, 6).

Materials and Methods

YAC DNA Preparation and Mapping. CEPH YACs were selected for mapping of *MTA1* by screening with D14S62 region markers or on the basis of available mapping information.⁴ Total YAC DNA from each clone was purified as described previously (4). Each YAC clone was confirmed by PCR analysis using oligonucleotide primers for *MTA1* and selected ESTs mapping into the region on the basis of the radiation hybrid mapping of chromosome 14 (Gene Map 1999 and the GDB). The primers used were a *MTA1*-expressed sequence tag (RH78599) designed by the Sanger Center and based on known human genomic chromosome 14 sequence.⁵ The primer sequences were 5'GGTTCGGATTTGGCTTGTTA3', which is contained within a unique sequence of the *MTA1* cDNA; and 5'CGTGGTCTGGACAAGGG3', which is contained in the adjacent genomic sequence of *MTA1*. PCR was performed in a Gene Amp PCR system 9600 (Perkin-Elmer Corp., Norwalk, CT) using ~20 ng of YAC DNA in a volume of 50 μ l in 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s. A 20- μ l volume of each product was electrophoresed in a 1% agarose gel, and PCR products were visualized by ethidium bromide staining.

BAC DNA Preparation and Mapping Analysis. BAC 76E12 was obtained from Research Genetics (Birmingham, AL). Total BAC DNA was purified according to the protocol supplied by the manufacturer. *MTA1* was mapped to BAC 76E12 by PCR analysis using the same oligonucleotide primers for *MTA1* as above. PCR was performed in a Gene Amp PCR system 9600 (Perkin-Elmer Corp.) using ~30 ng of BAC DNA in a volume of 50 μ l in 30 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s. A 10- μ l volume of each product was electrophoresed in a 1% agarose gel, and PCR products were visualized by ethidium bromide staining.

Results

As part of our preliminary mapping studies of the region, we determined all of the ESTs that mapped into the target region according to the radiation hybrid-based NCBI Gene Map. A total of 12 ESTs, but no known genes, map to this 1.6-Mb metastasis-related region. However, we performed additional mapping analysis on the YACs used in the preliminary mapping analysis and confirmed two known genes and 13 ESTs that actually map to these YACs, rather than their location indicated by the radiation hybrid-based NCBI Gene

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³ The abbreviations used are: LOH, loss of heterozygosity; *MTA1*, metastasis-associated 1; EST, expressed sequence tags; NCBI, National Center for Biotechnology Information; GDB, Genome Database; YAC, yeast artificial chromosome; BAC, bacterial artificial chromosome.

⁴ For example, Internet address: <http://www-genome.wi.mit.edu>; <http://www.gdb.org>.

⁵ Internet address: <http://www.sanger.ac.uk>.

Map. Because of these findings, we included the region from marker D14S1066 to the telomere of chromosome 14 because of uncertainties inherent in radiation hybrid mapping to insure that we had complete coverage of the telomeric end of chromosome 14q. Approximately 392 ESTs map into this region, with 63 of these being previously identified genes. Table 1 summarizes the known genes in the region from D14S1066 to the telomere (including *MTA1*) considered to be possible metastasis candidate genes.

MTA1 had been mapped previously using radiation hybrids onto the NCBI Gene Map some distance away from our region of interest near the telomere of chromosome 14q. We had, however, discovered several ESTs thought to map elsewhere on chromosome 14 that were actually mapped to our target region. Because *MTA1* represented a promising candidate gene even though the gene map showed it to be outside our target region, we then tested PCR primers for the *MTA1* gene onto our physical map of the region detected by our LOH studies. We determined *MTA1* mapped onto YACs 859d4 and 765h7 (Fig. 1). These mapping studies were subsequently confirmed when we mapped the gene onto BAC 76E12, which has a completed draft sequence that confirms its mapping onto YAC 859d4. The *MTA1* gene was therefore determined to map onto chromosome 14q in the vicinity of markers D14S62 and D14S51, or ~21 cM proximal to its previously reported location (see Fig. 1). Fig. 2, A and B, summarizes the gel-mapping data for *MTA1*. *MTA1* was mapped onto the overlap-

Table 1 Possible candidate genes in the 14q region of interest

Nineteen known genes were determined to map into the area of interest on chromosome 14. Both the gene name and the GDB no. are given for identification.^a

Gene name	GDB no.
<i>CALM</i> , calmodulin 1	9611304
<i>PRSC1</i> , protease, cysteine, 1	700617
<i>CGHA</i> , chromogranin A	119777
<i>PI</i> , protease inhibitor 1	120289
<i>AACT</i> , α -1-antichymotrypsin	118955
<i>PCI</i> , protein C inhibitor	134739
<i>TCL1A</i> , T-cell leukemia/lymphoma 1A	250785
<i>CCNK</i> , cyclin K	9957298
<i>YY1</i> , YY1 transcription factor	216988
<i>CKB</i> , creatine kinase, brain	120590
<i>AKT1</i> , V-akt murine thymoma viral oncogene	118989
<i>EIF5</i> , eukaryotic translation initiation factor 5	126411
<i>IGHG3</i> , immunoglobulin- γ 3	119339
<i>MTA1</i> , metastasis-associated 1	9955068
<i>MARK3</i> , MAP/microtubule affinity-regulating kinase	9315109
<i>EMAPL</i> , echinoderm microtubule-associated protein	6328385
<i>KNS2</i> , kinesin 2	304673
<i>TRAF3</i> , TNF receptor-associated factor 3	9836800
<i>EEF10</i> , eukaryotic translation elongation factor 10	216099

^a More information on each gene can be found at <http://www.gdb.org/>.

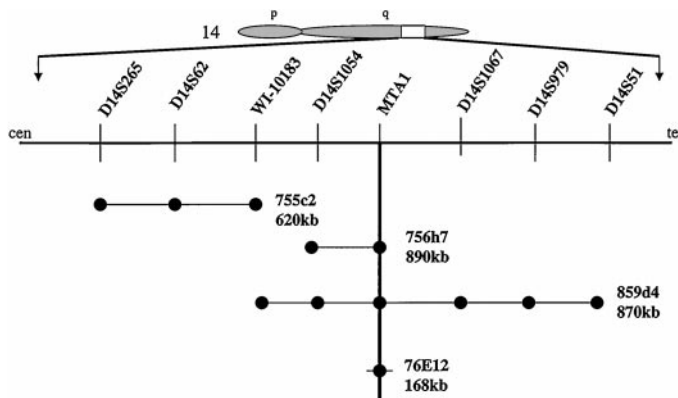


Fig. 1. YAC and BAC mapping of *MTA1*. A 1.6-Mb section of the region of interest was mapped, spanning from marker D14S265 to marker D14S51. The position of *MTA1* is shown relative to other markers on chromosome 14q. *MTA1* was determined to map onto both YAC 756h7 and YAC 859d4 and also to BAC 76E12.

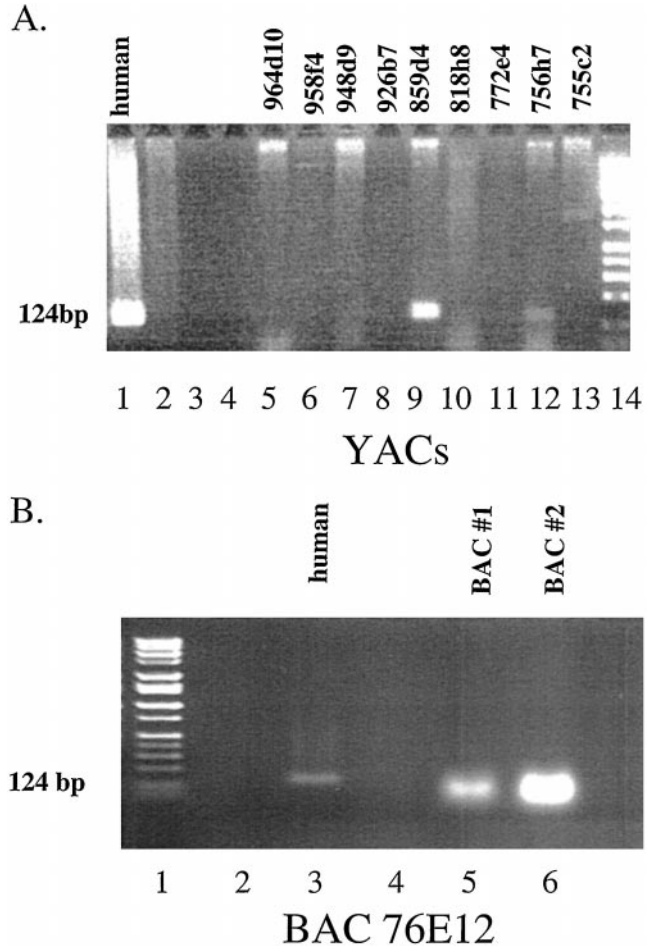


Fig. 2. Gel-mapping data on *MTA1*. A, PCR amplification of *MTA1* primers on YAC clones spanning the metastasis gene target region (band shown in Lanes 1, 9, and 12). Positive control was provided with 25 ng of human genomic DNA shown in Lane 1. B, PCR amplification of *MTA1* primers on BAC clone 76E12. Two positives are shown in Lanes 5 and 6 that represent two different preparations of the same BAC. Twenty-five ng of human genomic DNA was once again used as a positive control (Lane 3). Product size in both experiments was ~124 bp.

ping YAC clones 859d4 and 756h7. Fig. 2B indicates that *MTA1* also maps to BAC 76E12, and its location to this sequenced BAC clone is confirmed by a BLAST search with *MTA1* cDNA sequences.

Discussion

MTA1 was previously identified as a metastasis-promoting gene overexpressed in both rat and human metastatic cell lines (5). The human *MTA1* gene was cloned and sequenced by Nawa *et al.* (7) in 2000. In 1994, the rat gene was cloned and sequenced by the same group (5). The expression of *MTA1* in the human breast cancer cell line MDA-MB-231, a metastatic cell line, was determined to be approximately four times higher than its expression levels in the breast cancer cell line MDA-MB-468, which is nonmetastatic (5). The rat cell lines MTC.4, a benign line that remains phenotypically stable with prolonged passage, and the highly metastatic line MTLn3 were also tested for expression of *mta1* (the rat homologue). The expression level of *mta1* was found to be 4-fold higher in the MTLn3 line than in the MTC.4 line by Northern blotting (8). Different forms of cancer have also been shown to overexpress *MTA1*. Esophageal, colorectal, gastric, and pancreatic carcinomas have all been reported previously to express higher levels of *MTA1* mRNA than paired normal tissues, and this overexpression correlated with the invasiveness or lymph node metastasis of each of the carcinomas (6, 9, 10).

Recently, MTA1 has also been shown to be associated with histone deacetylase activity. Xue *et al.* (11) found that MTA1 was identical to one subunit of the nucleosome remodeling and histone deacetylation complex. This complex contains both ATP-dependent chromatin-remodeling and histone deacetylase activities (12). Interestingly, two homologues of MTA1 have also been discovered. Zhang *et al.* (13, 14) reported that a protein similar to MTA1 was also a component of the nucleosome remodeling and histone deacetylation complex. This gene, since designated *MTA1-L1*, has been cloned and shows significant homology to *MTA1* (15). *MTA1-L1* maps to chromosome 11 on the NCBI Gene Map. An even more distantly related MTA1 homologue, now referred to as *MTA2*, maps to chromosome 2 on the NCBI Gene Map. The MTA1 (and MTA1-L1) proteins are both nuclear proteins containing motifs associated with transcriptional corepressors, gene methylation, and signal transduction (11). All of these observations fit well our model in which LOH of the MTA1 region impedes metastasis. However, because LOH events involve the loss of large segments or entire chromosomes, loss of additional MTA1-region genes (see Table 1) could influence the metastasis phenotype.

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