Frequent Met Oncogene Amplification in a Brca1/Trp53 Mouse Model of Mammary Tumorigenesis

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Abstract

In a screen for gene copy number alterations in mouse mammary tumors initiated by loss of the Brca1 and Trp53 genes, we observed that the majority (11 of 15; 73%) had high-level amplification of wild-type Met, encoding a growth factor receptor implicated in tumor progression. Met amplification was localized to unstable double minute chromosomes and was uniquely found in mouse breast tumors driven by loss of Brca1 and Trp53. Whereas analogous MET amplification was not found in human breast cancers, the identification of a dominant somatic genetic lesion in the Brca1/Trp53 mouse model suggests that recurrent secondary hits may also exist in BRCA1-initiated human breast cancer. (Cancer Res 2006; 66(7): 3452-5)

Introduction

Cancer arises when a cell sustains an initial genetic lesion, followed by the accumulation of multiple genetic hits during tumor progression. Mouse breast cancer models typically rely on initiation through tissue-specific overexpression or ablation of a particular gene, but the additional loci affected during progression to malignancy are unknown. Secondary genetic events are thought to be particularly critical in cancer models triggered by global genomic instability, such as those linked to inactivation of the BRCA1 breast cancer susceptibility gene. To identify such secondary genetic events, we made use of genome-wide screen for copy number alterations in primary tumors arising in mice with inactivation of both Brca1 and Trp53 genes. The functional interaction between these two tumor suppressors is supported by several lines of evidence. Homozygous inactivation of Brca1 in the mouse is embryonic lethal, with cells displaying pronounced genomic instability followed by apoptosis (1, 2). This developmental defect is partially rescued by inactivation of Trp53 (1), suggesting that loss of the p53-mediated DNA damage checkpoint allows cells to tolerate inactivation of Brca1. The collaboration between loss of Brca1 and Trp53 is also evident in tumor development both in mouse models of mammary carcinogenesis, wherein inactivation of both genes dramatically enhances tumorogenesis, and in human tumors, wherein somatic TP53 mutations are virtually universal in BRCA1-linked familial breast cancer (3, 4). Collectively, these observations support the role of BRCA1 and p53 as genomic “caretakers,” the inactivation of which leads to genomic instability and the emergence of secondary genetic hits that drive tumorigenesis. In this study, we report characterization of secondary genetic lesions emerging during tumor progression in a mouse mammary tumorigenesis model initiated by Brca1 and Trp53 loss. Remarkably, a single recurrent event, amplification of the Met oncogene, is present in 73% of primary tumors analyzed.

Materials and Methods

Experimental mice. Mouse mammary tumors derived from a heterozygous Trp53 deletion with a tissue-specific deletion of Brca1 (Brca1+/-/Trp53-/-; MMTV-Cre) have been previously described (3). Mice harboring MMTV-driven Erbb2 and c-Myc oncogenes have been previously described (5, 6).

Microarray screening. All tumors were histologically analyzed to be >90% pure. DNA from was extracted from matching pairs of tumor and liver (normal control) of the same animal and processed for microarray analysis as described before (7).

Cell lines. Tumor-derived cell lines were established as previously described (5). Low-passage cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, and 50 units/mL penicillin/streptomycin and maintained at 5% CO2 at 37°C. Alternatively, cells were grown in 2% FBS with 40 ng/mL hepatocyte growth factor (R&D Systems, Minneapolis, MN).

Fluorescent in situ hybridization (FISH). All tumors were stained with an antibody against Brca1. BAC RP23-173p9, containing the full-length Met gene, was used as a probe and BAC RP23-137A12, mapping to 663 region, was used as a control probe. Fluorescent in situ hybridization (FISH) was done as previously described (8).

Quantitative real-time PCR. The sequences of the PCR primer pairs and fluorogenic MGB probes (all listed from 3′ to 5′) used for DNA copy number analyses were Mm.Met_F, TTCCAACCCCTTGTGATGCGA; Mm.Met_R, GCTACTTGAAGGCCAAATCCTATAA; Mm.Edem_F, GTTTCCACACACCTTTGATGCT; Mm.Edem_R, GTCAGGAGGAACACCTGTCTTCA; and fluorogenic MGB probes (all listed from 5′ to 3′) were Mm.Edem_probe, VIC-CCCACTGCAGGTGAA-MGBNFQ; Mm.Edem_R, GTCAGGAGGAACACCTGTCTTCA; and Mm.Edem_probe, VIC-CCCAGTGCAAGTGA-MGBNFQ.

All samples were done in triplicates and the relative Met copy number was derived by standardizing the input DNA to the control signal (Edem1), a gene on chromosome 6 that is genomically stable based on the microarray analysis of all 15 tumors.

Immunohistochemistry. Immunohistochemistry was done using Vectastain ABC Kit from Vector Laboratories (Burlingame, CA). The Met antibody (SP260; Santa Cruz Biotechnology, Santa Cruz, CA) was used at a 1:100 dilution. In negative controls, the anti-Met antibody was omitted from the staining procedure.

Mutational screening. See Supplementary data.
Results and Discussion

Mouse mammary tumors derived from a heterozygous \( \text{Trp53}^{-/-} \) deletion with a tissue-specific deletion of \( \text{Brca1} \) (\( \text{Brca1}^{-/-/+} \) \( \text{MMTV-Cre} \)) were subjected to a whole-genome survey using long oligonucleotide microarrays. The use of coding sequence markers for genomic copy number analysis has proved highly effective in identifying gene-centered amplifications and deletions (7). Remarkably, a single recurrent abnormality was evident in 11 of 15 (73%) tumors (i.e., high-level amplification of a locus on chromosome 6; Fig. 1A). Other changes also corresponded to genomic aberrations known to occur in human cancers but occurred only as single events in our analysis (Table 1). The minimal amplicon on chromosome 6 contained only two full-length genes, the oncogene \( \text{Met} \) and the \( \text{Capza2} \) gene, encoding the F-actin capping protein (muscle Z-line) α-2, which is involved in regulation of actin barbed-end dynamics (Fig. 1B). Ten- to fifty-fold amplification of \( \text{Met} \) was confirmed using quantitative real-time PCR (Fig. 1C). Consistent with the presence of DNA amplification, the primary tumors expressed high levels of Met protein (Fig. 2).

\( \text{MET} \) encodes a transmembrane tyrosine kinase receptor for hepatocyte growth factor (scatter factor), which transduces signals implicated in proliferation, migration, and morphogenesis (9, 10). Ectopic expression of \( \text{MET} \), as well as hepatocyte growth factor, confers a tumorigenic and metastatic phenotype in cancer-derived cell lines (11, 12), and activating mutations have been reported in both sporadic and inherited forms of renal papillary carcinomas (13). Mutations in \( \text{MET} \) are rare in breast cancer (14, 15) but tumors with high protein expression are more common and seem to have a worse clinical prognosis (16, 17). Furthermore, increased hepatocyte growth factor/MET signaling can serve as an initiating event for tumorigenesis as mice overexpressing either hepatocyte growth factor or mutant Met in mammary epithelium develop breast tumors (18–20).

We analyzed the coding sequence of \( \text{Met} \) from all 15 mammary tumors of \( \text{Brca1}/\text{Trp53} \) mice: none had \( \text{Met} \) mutations (data not shown), indicating that overexpression of the wild-type receptor in

Table 1. Summary of genomic aberrations identified in the \( \text{Brca1}/\text{Trp53} \) tumors

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Genomic aberration (chromosome, Mb position)</th>
<th>Syntenic human region</th>
<th>Gene candidate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX3</td>
<td>AMP (chr.6:15.7-21.1)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td>CX4</td>
<td>AMP (chr.6:15.2-17.9)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td></td>
<td>AMP (chr.9:7.8-8.6)</td>
<td>11q22</td>
<td>?</td>
</tr>
<tr>
<td>CX5</td>
<td>AMP (chr.10:28.6-33.2)</td>
<td>6q22</td>
<td>?</td>
</tr>
<tr>
<td>CX6</td>
<td>AMP (chr.10:15.4-21.1)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td>CX7</td>
<td>AMP (chr.17.3-18.9)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td>CX9</td>
<td>AMP (chr.13.6-21.1)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td>CX10</td>
<td>AMP (chr.15.2-18.9)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td>CX12</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CX13</td>
<td>AMP (chr.6:15.7-17.9)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td>CX16</td>
<td>DEL (chr.12:106.6-106.9)</td>
<td>4q32</td>
<td>?</td>
</tr>
<tr>
<td>CX18</td>
<td>AMP (chr.6:13.6-18.4)</td>
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<td>( \text{Met} )</td>
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<td>CX20</td>
<td>DEL (chr.7:29.3-29.3)</td>
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<td>?</td>
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<tr>
<td>CX21</td>
<td>AMP (chr.6:17.2-18.9)</td>
<td>7q31</td>
<td>( \text{Met} )</td>
</tr>
<tr>
<td></td>
<td>AMP (chr.6:22.0-23.3)</td>
<td>7q31</td>
<td>( \text{Wnt16} )</td>
</tr>
<tr>
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<td>AMP (chr.15:60.8-63.9)</td>
<td>8q24</td>
<td>( \text{c-Myc} )</td>
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<td>( \text{Met} )</td>
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<tr>
<td>CX27</td>
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</tr>
</tbody>
</table>

Abbreviations: AMP, amplification; DEL, deletion.
tumors with gene amplification may be sufficient to enhance tumorigenesis. Remarkably, in five of five cases, the establishment of cultured cell lines from these primary mammary tumors was accompanied by rapid loss of amplified \textit{Met} gene copies (Fig. 2B), presumably reflecting the absence of selection pressure under \textit{in vitro} culture conditions. Lowering the serum concentration and adding exogenous Met ligand, hepatocyte growth factor, in an attempt to reconstitute dependence of tumor cells on Met signaling, did not result in retention of the amplified \textit{Met} copies \textit{in vitro}.

Consistent with the rapid loss of the amplified \textit{Met} genes, FISH analysis of the primary tumors (Fig. 2C) and of early-passage cells in culture (Fig. 2D) showed the amplified genes to be associated with extrachromosomal double minute elements. These inherently unstable genomic elements are presumably maintained only in the presence of \textit{in vivo} selection pressure within the breast microenvironment.

Amplification of Met was not observed in other common mouse models of mammary carcinogenesis, including MMTV-driven \textit{Erbb2} and \textit{c-Myc} (data not shown; refs. 5, 6), suggesting that the initiating \textit{Brca1}/\textit{Trp53} lesion in our mouse tumor model may dictate subsequent secondary genetic lesions. In humans, overexpression of \textit{MET} has been reported in many epithelial cancers but gene amplification in breast cancer has not been systematically analyzed. We therefore first tested a panel of 100 primary sporadic human breast cancers for alterations in \textit{MET} gene copy number. No high-level \textit{MET} amplification analogous to that seen in the mouse model was observed although \(\sim 10\%\) of these sporadic cases had a greater than diploid gene complement (three to six copies). This modest increase may be due either to low-level specific \textit{MET} gene duplication or to nonspecific aneuploidy (data not shown). We also did not detect significant \textit{MET} amplification in human breast cancers arising in the context of genomic instability syndromes [i.e., \textit{BRCA1}- and \textit{BRCA2}-linked familial breast cancer (0 of 9 cases) and \textit{TP53} mutant Li-Fraumeni syndrome (0 of 13 cases)]. \textit{MET} amplification, therefore, is not a common characteristic of human breast cancer.

The striking difference between mouse and human in the frequency of \textit{MET} amplification in \textit{BRCA1} and \textit{TP53}-driven breast cancers may have a number of explanations. Whereas we have observed several recurrent areas of amplification breakpoints in the mouse tumors, we have not identified any unique structural genomic features, such as repetitive sequences, which might favor amplification of the \textit{Met} locus. However, genomic sequences that facilitate gene amplification in the setting of \textit{Brca1} and \textit{Trp53} inactivation are poorly understood and it is possible that the mouse \textit{Met} locus is more prone to undergo amplification than the corresponding human locus. More likely, amplification of \textit{Met} may serve as a key genetic lesion in mouse mammary tumorigenesis, but not in human breast cancer. Evidence supporting this notion is derived from the ability of...
transgenic hepatocyte growth factor and/or Met overexpression to initiate mouse mammary tumorigenesis (18–20), and the absence of MET amplification or mutation, even as a late event in human sporadic breast cancers. Whatever the explanation for the distinct role of MET amplification in mouse and human breast cancer, our results suggest caution in extrapolating genetic pathways from mouse to man, even based on carefully designed conditional knockout models. Nonetheless, our finding that a small amplicon, harboring a tumor promoting growth factor receptor, constitutes a frequent secondary genetic event in Brca1/Trp53 mouse tumors raises the possibility that similar genomic screens might identify an equivalent recurrent genetic lesion driving cellular proliferation in human BRCA1-associated breast cancers.

Acknowledgments
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References
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