Heterogeneity in the Clinical Phenotype of TP53 Mutations in Breast Cancer Patients

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ABSTRACT

TP53 mutation is a strong independent marker for survival in breast cancer with some heterogeneity in the clinical phenotype of various types of mutations. Based on 315 patients with breast carcinoma, we suggest a new model for the differentiation of TP53 mutations. Although TP53 mutation in general was associated with aggressive tumor/patient characteristics, missense mutations outside any conserved or structural domain did not affect the clinical outcome (risk of disseminated disease and death). In contrast, patients with missense mutations affecting amino acids directly involved in DNA or zinc binding displayed a very aggressive clinical phenotype. Null mutations (including missense mutations disrupting the tetramerization domain) and the remaining missense mutations displayed an intermediate aggressive clinical phenotype. When patients with primary early breast cancer were divided into three groups (wild-type together with missense mutations outside structural/conserved domains, null mutations and missense mutations with intermediate clinical phenotype, and very aggressive missense mutations), disease-specific survival rates were 89%, 58%, and 35% (5-year actuarial values, \( P < 0.0001 \)), respectively. In a Cox proportional hazards analysis, separation of TP53 mutations according to these criteria eliminated the prognostic importance of all investigated classical factors except nodal status.

INTRODUCTION

In patients with primary breast cancer, somatic mutation in the p53 gene (TP53) is a strong, independent marker for survival (reviewed in Ref. 1). The p53 protein is a multifunctional protein (reviewed in 2) containing five domains (I–V) that are highly conserved throughout evolution from invertebrates like the squid to vertebrates like humans (3). These conserved domains more or less colocalize with the structural domains identified in the X-ray crystallographic study by Cho et al. (4).

Because different missense mutations (i.e., mutations that cause amino acid substitutions) have different effects on the structure and function of the protein (4), attempts have been made to correlate clinical outcome with the location of the mutation. Thus, Bergh et al. (5) found that mutations in the conserved domains II and V were associated with significantly worse prognosis. Others have compared mutations affecting different structural domains of p53. Børresen-Dale et al. (6) and Gentile et al. (7) have shown that patients with missense mutations in the parts of the protein involving zinc binding have a particularly poor prognosis. Kucera et al. (8) could not confirm the importance of the zinc-binding domain, and finally, Berns et al. (9) found the poorest prognosis associated with missense mutations affecting amino acids directly involved in DNA contact. Although it is still unclear which mutations are associated with the worst prognosis, these studies have demonstrated a heterogeneity in the clinical phenotype of TP53 mutations in breast cancer. Whereas the aggressive phenotype of certain mutations has been seen, it has also been speculated that there might be other missense mutations that display a clinically “silent” phenotype (10).

We have previously evaluated 294 patients with primary early breast carcinoma for mutations in TP53 (11). Among these patients, 69 (23%) carry a sporadic TP53 mutation that is significantly associated with increased risk of distant metastasis, disease-free survival, and overall survival in both node-negative and node-positive patients. Also, in a Cox proportional hazards analysis, TP53 mutation independently predicts the occurrence of distant metastasis and death (overall survival), with relative risks of 2.4 (95% confidence interval, 1.5–3.9) and 2.7 (95% confidence interval, 1.7–4.2), respectively (11).

In the present study, tumor characteristics and clinical outcome of these 294 patients are related to the presence, type, and location of TP53 mutations. Also, we present data on 21 additional patients identified within the same period but presenting with either disseminated or bilateral disease or with other neoplastic disease at the time of diagnosis. We demonstrate that a prediction of biochemical phenotype or aggressiveness of different TP53 mutations correlates with the clinical phenotype (risk of disseminated disease and death).

MATERIALS AND METHODS

Patients and Tumor Samples. Tumor material was collected between August 1992 and January 1994 from 315 pa-
DYE Terminator Cycle Sequencing Kit (Amersham, Cleveland, OH) or with the BigDye

exon 6; and 40–80% for exon 5, respectively. The gels were run and wild-type alleles. The gradient ranges were 35–75% for the previous approach (13), the gradient ranges were narrowed and adapted to each exon to increase the separation between mutant and wild-type alleles. The gradient ranges were 35–75% for exons 2, 3, 4, 7, 8, and 10; 20–60% for exon 9; 25–65% for exon 6; and 40–80% for exon 5, respectively. The gels were run at 160 V for 5 h in 1× TAE buffer at a constant temperature of 58°C. After electrophoresis, gels were stained in 1× TAE buffer containing 2 μg/ml ethidium bromide and analyzed under UV transillumination. Each sample was analyzed once by DGGE.

DNA Sequencing. Mutant heteroduplex or homoduplex bands were excised and reamplified as described previously (13). Sequencing of PCR products was performed either with 3P-end-labeled primers using the ThermoPrime Cycle Sequencing Kit (Amersham, Cleveland, OH) or with the BigDye

the abbreviation used is: DGGE, denaturing gradient gel electrophoresis.
expected to produce a functional protein and will be referred to as null mutations.

In Fig. 1, the location and number of the mutations are illustrated and compared with the domain structure of p53. Six mutations were identified in the tetramerization domain. Four of these were identical nonsense mutations, Q331X. The remaining two mutations consisted of a small in-frame deletion and insertion, respectively. In Fig. 1, these two mutations are classified as missense mutations. However, because missense mutations in the tetramerization domain can destabilize the protein structure without being dominant negative (15, 16), these two in-frame deletions and insertions are also expected to produce a nonfunctional protein and will be included in the null mutations along with the nonsense and frameshift mutations. With this adjustment, the material includes 47 missense mutations (64%) and 27 null mutations (36%). Detailed description of these 74 missense or null mutations and of 1 silent mutation is given in Table 1, together with the clinical details of the patients.

**Grouping of TP53 Mutations.** To differentiate between different biochemical phenotypes, the 74 TP53 mutations were divided into the following four groups (Table 2): (a) the first group contained nine missense mutations located outside any of the conserved domains (domains II–V) and/or structural domains important for DNA or zinc binding; (b) the second group contained all 27 null mutations (including the two small in-frame deletion and insertion mutations in the tetramerization domain); (c) the third group contained 23 missense mutations that were located inside one of the conserved and/or structural domains but did not affect any of the amino acids directly involved in DNA contact or zinc binding (see above); and (d) the fourth group contained the remaining 15 missense mutations affecting one of the amino acids directly involved in DNA contact or zinc binding.

**Relationship with Clinical Parameters and Outcome.** When correlated with classical prognostic factors in breast cancer, tumors with a TP53 mutation were associated with a more aggressive phenotype than tumors that were wild-type for TP53 (Table 2). Thus, if the various TP53 mutations were combined, the frequency of a mutation was significantly higher in tumors characterized by positive lymph nodes [TP53 mutation was seen in 43 of 145 (30%) lymph node-positive tumors versus 31 of 170 (18%) lymph node-negative tumors; \( P = 0.02 \)], large tumor size [TP53 mutation was seen in 47 of 164 (29%) tumors \( \geq 21 \) mm versus 26 of 145 (18%) tumors \(< 21 \) mm; \( P = 0.03 \)], negative estrogen receptor status [TP53 mutation was seen in 35 of 86 (41%) receptor-negative tumors versus 39 of 229 (17%) receptor-positive tumors; \( P < 0.001 \)], and, in ductal carcinomas, high grade of anaplasia [TP53 mutation was seen in 33 of 75 (44%) grade 3 tumors, 32 of 103 (31%) grade 2 tumors, and 1 of 63 (2%) grade 1 tumors; \( P < 0.001 \)]. Only one patient with a grade 1 ductal carcinoma carried a TP53 mutation. The mutation was R280K, which changed an amino acid directly involved in DNA binding. The patient relapsed after 21 months and died 2 years after diagnosis (11); the grading of the sample has not been reevaluated. The general association with an aggressive phenotype seemed to be more or less similar for the four different mutation types (not tested).

Analysis of the outcome for the 294 patients with primary unilateral disease who had no evidence of distant metastasis at the time of diagnosis showed distinct clinical phenotypes associated with the proposed grouping of TP53 mutations (Fig. 2). Thus, 60 months after treatment, disease-specific survival for patients with mutations affecting amino acids directly involved in DNA or zinc binding was only 35% compared with 89% for patients without mutations. Patients with null mutations or with any of the remaining missense mutations within the conserved/structural domains had an intermediate survival of 67% and 48%, respectively. Finally, the disease-specific survival for patients with missense mutations outside the domains was 89%. Similar patterns are found for two other end points, freedom from distant metastasis and overall survival (data not shown).

Although the patients in Fig. 2 are divided into five groups according to the mutational status, the similarity in clinical outcome for some of these groups makes it possible to reduce it to
Table 1  
TP53 mutations in 74 of 315 patients with breast cancer (294 patients with primary cancer, 8 patients with distant metastasis, 6 patients with bilateral disease, and 7 patients with other neoplastic disease at time of diagnosis, respectively)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Exon</th>
<th>Structural change</th>
<th>Domain</th>
<th>Domain status</th>
<th>Size (mm)</th>
<th>ER status</th>
<th>Histology</th>
<th>Grade</th>
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<td></td>
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<td></td>
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<td>Neg</td>
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<tr>
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<td>S2-S2β</td>
<td>1–3</td>
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<td>L130F</td>
<td>S2-S2β</td>
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<td>20</td>
<td>Neg</td>
<td>Duct</td>
<td>2</td>
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<td>c.396G&gt;C</td>
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<td>K132N</td>
<td>S2-S2β</td>
<td>Neg</td>
<td>26</td>
<td>Pos</td>
<td>Duct</td>
<td>3</td>
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<td>V143A</td>
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<td>Neg</td>
<td>15</td>
<td>Neg</td>
<td>Duct</td>
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<td>Duct</td>
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<td>Duct</td>
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<td>Neg</td>
<td>Duct</td>
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<td>R175H</td>
<td>L2</td>
<td>Neg</td>
<td>18</td>
<td>Pos</td>
<td>Duct</td>
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<td>L2(Zn)</td>
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<td>Pos</td>
<td>Duct</td>
<td>2</td>
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<td>Duct</td>
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<td>H193R</td>
<td>L2</td>
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<td>18</td>
<td>Neg</td>
<td>Duct</td>
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<td>L2</td>
<td>&gt;3</td>
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<td>15</td>
<td>Pos</td>
<td>Duct</td>
<td>2</td>
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<tr>
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<td>6</td>
<td>S215I</td>
<td>Outside</td>
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<td>15</td>
<td>Neg</td>
<td>Duct</td>
<td>3</td>
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<tr>
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<td>Y220C</td>
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<td>Neg</td>
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<td>6</td>
<td>E224D</td>
<td>Outside</td>
<td>Neg</td>
<td>25</td>
<td>Neg</td>
<td>Duct</td>
<td>3</td>
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<tr>
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<td>7</td>
<td>M237I</td>
<td>L3</td>
<td>&gt;3</td>
<td>35</td>
<td>Neg</td>
<td>Duct</td>
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<td>C238Y</td>
<td>L3(Zn)</td>
<td>&gt;3</td>
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<td>Pos</td>
<td>Duct</td>
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<td>C242Y</td>
<td>L3(Zn)</td>
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<td>G245S</td>
<td>L3</td>
<td>1–3</td>
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<td>Pos</td>
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<td>G245S</td>
<td>L3</td>
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<td>R248Q</td>
<td>L3(DNA)</td>
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<td>R248Q</td>
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<td>L3(DNA)</td>
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<td>L3(DNA)</td>
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<td>I255F</td>
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<td>G266E</td>
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<td>1–3</td>
<td>18</td>
<td>Pos</td>
<td>Duct</td>
<td>3</td>
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<tr>
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<td>R273C</td>
<td>S10β(DNA)</td>
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<td>90</td>
<td>Neg</td>
<td>Duct</td>
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<tr>
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<td>C275Y</td>
<td>S10β</td>
<td>1–3</td>
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<td>S10β</td>
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<td>R280K</td>
<td>H2(DNA)</td>
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<td>Duct</td>
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<td>8</td>
<td>R282W</td>
<td>H2</td>
<td>Neg</td>
<td>18</td>
<td>Pos</td>
<td>Duct</td>
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<tr>
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<td>H2</td>
<td>1–3</td>
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<td>Duct</td>
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<td>Silent mutation</td>
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<td>Q192X</td>
<td>Null</td>
<td>&gt;3</td>
<td>16</td>
<td>Neg</td>
<td>Duct</td>
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<td>c.586C&gt;T</td>
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<td>R196X</td>
<td>Null</td>
<td>&gt;3</td>
<td>60</td>
<td>Pos</td>
<td>Lob</td>
<td></td>
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<td>c.592G&gt;T</td>
<td>6</td>
<td>E198X</td>
<td>Null</td>
<td>Neg</td>
<td>20</td>
<td>Pos</td>
<td>Duct</td>
<td>2</td>
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<tr>
<td>c.615T&gt;G</td>
<td>6</td>
<td>Y205X</td>
<td>Null</td>
<td>&gt;3</td>
<td>36</td>
<td>Neg</td>
<td>Duct</td>
<td>3</td>
</tr>
<tr>
<td>c.637C&gt;T</td>
<td>6</td>
<td>R213X</td>
<td>Null</td>
<td>&gt;3</td>
<td>22</td>
<td>Neg</td>
<td>Duct</td>
<td>3</td>
</tr>
<tr>
<td>c.637C&gt;T</td>
<td>6</td>
<td>R213X</td>
<td>Null</td>
<td>Neg</td>
<td>36</td>
<td>Pos</td>
<td>Duct</td>
<td>2</td>
</tr>
<tr>
<td>c.642-643delTA</td>
<td>6</td>
<td>H214delX220</td>
<td>Null</td>
<td>Neg</td>
<td>0</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.654delG</td>
<td>6</td>
<td>Y220delX246</td>
<td>Null</td>
<td>&gt;3</td>
<td>20</td>
<td>Neg</td>
<td>Duct</td>
<td>2</td>
</tr>
<tr>
<td>c.681-682insT</td>
<td>7</td>
<td>D228X</td>
<td>Null</td>
<td>&gt;3</td>
<td>40</td>
<td>Neg</td>
<td>Duct</td>
<td>3</td>
</tr>
</tbody>
</table>
three groups as shown in Fig. 3: (a) WT + Neutral, patients with wild-type tumors together with missense mutations outside structural/conserved domains; (b) Very aggressive, patients with missense mutations affecting amino acids directly involved in DNA or zinc binding; and (c) Aggressive, patients with all other mutations. Using this division, the TP53 mutational pattern was a predictor for poor outcome with rates of freedom from distant metastasis of 76%, 48%, and 32% (P < 0.0001, Fig. 3A); disease-specific survival rates of 89%, 58%, and 35% (P < 0.0001, Fig. 3B); and overall survival rates of 85%, 55%, and 31% (P < 0.0001, Fig. 3C), respectively, for WT + neutral, aggressive, and highly aggressive, respectively.

A Cox proportional hazards analysis including the parameters of TP53 mutations specified as “any mutation” or separated as “aggressive” and “very aggressive” mutations, menopausal status, tumor size, positive lymph nodes, histopathology, grade of anaplasia, and estrogen receptor status was performed. In the final model, using risk of distant metastasis as the end point, the only independent prognostic parameters found were the incidence of positive lymph nodes [relative risk, 2.01 (95% confidence interval, 1.28–3.18)], aggressive mutation [relative risk, 2.30 (95% confidence interval, 1.40–3.78)], and very aggressive mutation [relative risk, 3.78 (95% confidence interval, 1.78–8.06)]. Using disease-specific survival as the end point yielded similar results, with relative risks of 2.32 (95% confidence interval, 1.38–3.91) for incidence of positive lymph nodes and 2.43 (95% confidence interval, 1.39–4.23) for incidence of aggressive mutation, and 6.15 (95% confidence interval, 2.91–13.00) for incidence of very aggressive mutation, respectively. The same parameters were also of independent significance for the overall survival outcome [incidence of positive lymph nodes (relative risk, 2.56; 95% confidence interval, 1.57–4.07), aggressive mutation (relative risk, 2.01; 95% confidence interval, 1.20–3.37), and very aggressive mutation (relative risk, 5.30; 95% confidence interval, 2.64–10.64), respectively]. In addition, the older postmenopausal patients also showed an independent risk for this end point with a relative risk of 2.04 (95% confidence interval, 1.18–3.56). No other parameters were found to express independent significance.

### DISCUSSION

Although biased by the choice of methods (17), the majority of published missense mutations in TP53 are located within or near the structural domains important for DNA binding and coordination of the zinc molecule (18). With an unbiased screening of all coding exons, the same pattern is observed here (Fig. 1).

Previous attempts to link missense mutations in specific domains or residues within this central part of the p53 molecule to clinical outcome for breast cancer patients have yielded contradictory results. Thus, it is not clear whether particularly poor prognosis is correlated with missense mutations in the conserved domains II and V, the L2 and L3 domains, or those amino acids directly involved in DNA contact (5–9).

Here, we suggest a new model for the differentiation of TP53 mutations. Null mutations are considered as one group and include traditional nonsense and frameshift mutations. Also, we suggest that missense mutations that are predicted to disrupt the function of the tetramerization domain be classified as null mutations. In the present study, the only two in-frame deletions/insertions identified (Table 1) affect amino acids shown to be essential for tetramerization (15, 16). Missense mutations are divided into three groups: (a) a small group containing amino acids directly involved in DNA or zinc binding; (b) a large group containing the remaining missense mutations within the structural/conserved domains (as depicted in Fig. 1); and (c) a small group containing all mutations affecting amino acids outside these domains.

When correlated with tumor/patient characteristics like tu-
mor size, estrogen receptor status, nodal status, and (for ductal carcinomas) grade of anaplasia, all four groups of mutations are associated with a more aggressive phenotype than tumors from patients without TP53 mutation (Table 2). As a point of interest, it has been suggested that medullary carcinoma is a distinct genetic subtype with a very high frequency of TP53 mutations of up to 100% in typical medullary breast carcinoma (19). The medullary carcinomas in the present study have not been subtyped, but the finding that seven of eight are wild-type for TP53 indicates that this correlation might be less strict.

Although all types of TP53 mutations are associated with an increase in the aggressiveness of the tumor/patient characteristics, different mutations might still be associated with different effects on the ability of the tumor to metastasize and/or the ability to respond to radiation or chemotherapeutic therapy. When TP53 mutation status is correlated with either freedom from distant metastasis, disease-specific survival, or overall survival, a strong association is indeed observed between the different groups of mutations and clinical outcome (Fig. 3). Thus, optimal separation of prognosis, disease progression, and

### Table 2 Patients and tumor characteristics in 315 breast cancer patients

<table>
<thead>
<tr>
<th>p53 status</th>
<th>All</th>
<th>WT* (and silent)</th>
<th>Missense outside domains</th>
<th>Null</th>
<th>Missense inside domains</th>
<th>Missense direct aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>315</td>
<td>241 (77%)</td>
<td>9 (3%)</td>
<td>27 (9%)</td>
<td>23 (7%)</td>
<td>15 (5%)</td>
</tr>
<tr>
<td>Primary, unilateral</td>
<td>294</td>
<td>225 (77%)</td>
<td>9 (3%)</td>
<td>25 (9%)</td>
<td>22 (8%)</td>
<td>13 (4%)</td>
</tr>
<tr>
<td>With distant metastasis</td>
<td>8</td>
<td>5 (63%)</td>
<td>0</td>
<td>0</td>
<td>1 (13%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>6</td>
<td>5 (83%)</td>
<td>0</td>
<td>1 (13%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other neoplastic disease</td>
<td>7</td>
<td>6 (86%)</td>
<td>0</td>
<td>1 (14%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>91</td>
<td>69 (76%)</td>
<td>2 (2%)</td>
<td>8 (9%)</td>
<td>6 (7%)</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>224</td>
<td>172 (77%)</td>
<td>7 (3%)</td>
<td>19 (9%)</td>
<td>17 (8%)</td>
<td>9 (4%)</td>
</tr>
<tr>
<td>Tumor size (6 unknown)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;21 mm</td>
<td>145</td>
<td>119 (82%)</td>
<td>4 (3%)</td>
<td>9 (6%)</td>
<td>11 (8%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>21–50 mm</td>
<td>144</td>
<td>103 (72%)</td>
<td>5 (3%)</td>
<td>16 (11%)</td>
<td>10 (7%)</td>
<td>10 (7%)</td>
</tr>
<tr>
<td>&gt;50 mm</td>
<td>20</td>
<td>14 (70%)</td>
<td>0</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Positive nodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>170</td>
<td>139 (82%)</td>
<td>6 (4%)</td>
<td>12 (7%)</td>
<td>9 (5%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>Yes</td>
<td>145</td>
<td>102 (70%)</td>
<td>2 (2%)</td>
<td>15 (10%)</td>
<td>14 (10%)</td>
<td>11 (8%)</td>
</tr>
<tr>
<td>Histopathology (WHO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ductal</td>
<td>249</td>
<td>183 (74%)</td>
<td>9 (4%)</td>
<td>23 (9%)</td>
<td>22 (9%)</td>
<td>12 (5%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>37</td>
<td>33 (89%)</td>
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<td>3 (8%)</td>
<td>0</td>
<td>1 (3%)</td>
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<tr>
<td>Medullar</td>
<td>9</td>
<td>7 (78%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (22%)</td>
</tr>
<tr>
<td>Other</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplasia (ductal carcinoma only)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>62</td>
<td>61 (98%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>103</td>
<td>71 (69%)</td>
<td>4 (4%)</td>
<td>9 (9%)</td>
<td>13 (13%)</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>74</td>
<td>42 (57%)</td>
<td>5 (7%)</td>
<td>13 (18%)</td>
<td>9 (12%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td>Not determined</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Estrogen receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>86</td>
<td>51 (59%)</td>
<td>6 (7%)</td>
<td>14 (16%)</td>
<td>8 (9%)</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>Positive (&gt;10 fmol)</td>
<td>229</td>
<td>190 (83%)</td>
<td>3 (1%)</td>
<td>13 (6%)</td>
<td>15 (7%)</td>
<td>8 (4%)</td>
</tr>
</tbody>
</table>

*WT, wild type; aa, amino acid.
survival based on TP53 status is obtained by grouping patients into three groups: (a) wild-type + neutral, patients with wild-type tumors together with missense mutations outside structural/conserved domains; (b) very aggressive, patients with missense mutations affecting amino acids directly involved in DNA or zinc binding; and (c) aggressive, patients with the remaining missense mutations and all null mutations. Dividing patients according to other parameters such as the presence of missense...
mutations in L2/L3 or those affecting DNA binding is also prognostic for the clinical outcome, but not to the same extent as shown in Figs. 2 and 3 (data not shown).

The very poor prognosis for patients with missense mutations affecting amino acids directly involved in DNA or zinc binding could reflect dominant positive effects [gain of function (20)] or a particularly strong dominant negative phenotype (21) of these mutations. Among the rest of the missense mutations within the structural/conserved domains, a high percentage might have a dominant negative phenotype. Whether loss of p53 function in general leads to a less aggressive phenotype than dominant positive mutations or whether the intermediate clinical phenotype reflects a heterogeneity within this group of patients is not clear. Null mutations are also associated with an intermediate aggressive phenotype. It is possible that reduction of p53 dosage in itself is sufficient to promote some tumor progression (22), but without information of the retention of the wild-type allele, the intermediate phenotype in this group could also reflect a patient heterogeneity.

The final group of mutations includes the missense mutations outside any structural or conserved domains. Although they occur in only 9 of 69 mutations found in patients with primary unilateral disease and no evidence of distant metastasis (13% of tumors with mutations or 3% of the patients), they appear to define a novel class of TP53 mutations in breast cancer. Although they are associated with aggressive clinical parameters like large size, negative estrogen receptor status, positive nodal status, and the absence of low-grade ductal carcinomas, these mutations do not seem to confer a worse prognosis on the patients as compared to patients without TP53 mutations. Recently, a detailed analysis of mutations selected in BRCA-associated tumors has identified a class of mutations that is frequent in these tumors but rare in sporadic tumors not associated with mutations in BRCA1 or BRCA2 (23). These mutations have lost the ability to suppress transformation and are likely to be selected on that basis; however, they still retain a biochemical phenotype close to that of the normal protein. Thus, to a certain degree, these mutant proteins can still transactivate downstream targets, suppress growth, and induce apoptosis (23). The majority of these BRCA-associated mutations are indeed located outside structural/conserved domains, thus providing a possible link between a novel biochemical phenotype and the lack of effect on clinical outcome for breast cancer patients seen in the present study. In this respect, it might be relevant to note that although BRCA-associated tumors contain a very high frequency of TP53 mutations compared to sporadic tumors without BRCA mutations (24), survival for these patients is apparently not significantly different (reviewed in Ref. 25). Interestingly, in a recent analysis of cases of childhood adrenocortical carcinoma unselected for a family history of cancer, 80% of the children carried a germ-line mutation in TP53 (26). Adrenocortical carcinoma, together with bone and soft tissue sarcomas and breast and brain tumors, is part the Li-Fraumeni syndrome associated with germ-line mutations in TP53 (27, 28). The identified missense mutations were all located outside structural/conserved domains, and several carriers unaffected in their 40s and 50s were identified, demonstrating a low-penetrance phenotype of these mutations (26).

When stratified according to the biochemical phenotype, TP53 mutations together with nodal status contained all of the significant prognostic information (with the addition of postmenopausal status for the end point of overall survival). However, with the number of patients included in this study, it is not possible to clarify whether TP53 mutation in breast cancer is a prognostic marker for distant metastasis or whether it predicts response to specific types of therapy, e.g., adjuvant chemotherapy. The observation that three of eight patients with distant metastasis already present at the time of diagnosis carried a mutation and that two of these three mutations are of the very aggressive type (Table 1) suggests that TP53 mutation can carry prognostic information for metastatic capability. As a predictive marker, this study lacks the power to detect a potential difference in outcome from specific types of therapy. To obtain a sufficient number of patients, a large national study has recently been initiated by The Danish Breast Cancer Cooperation Group.

In conclusion, we suggest a new model for the prediction of the clinical phenotype of different TP53 mutations in patients with primary breast cancer and predict two interesting groups of patients. First, if mutational analysis of the p53 gene is included in any clinical decision-making for breast cancer patients, it is important to realize that mutations might be identified that will have no effect on prognosis and/or response to therapy. A second important group of patients identified in this study is the group of patients with missense mutations affecting amino acids that are directly involved in DNA or zinc binding (the very aggressive group). These patients are at a very high risk of developing distant metastasis within 5 years after treatment. Finally, the results presented here demonstrate the necessity of including at least exons 4–10 in any analysis of TP53 mutations in breast cancer. Although only 1 of the 47 missense mutations occurred outside exons 5–8, 7 of 27 (26%) of the nonsense mutations, frameshift mutations, or in-frame deletions/insertions were found outside these exons, including four Q331X, which might be a novel hot spot in breast cancer.

REFERENCES


Heterogeneity in the Clinical Phenotype of TP53 Mutations in Breast Cancer Patients

Jan Alsner, Mette Yilmaz, Per Guldberg, et al.

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