Genetic and epigenetic regulation of human breast cancer progression and metastasis

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Introduction

Breast cancer is the most common malignancy and a major cause of cancer-related deaths among women in the United States and Western Europe (American Cancer Society 1998, Wingo et al. 1998). Most women succumb to breast cancer if their tumors metastasize but cures are more likely if the cancers remain localized (Harris et al. 1992a,b,c, Walker et al. 1997). Thus, a greater understanding of the metastatic process in human breast cancer should translate into substantial improvements in therapeutic outcome for breast cancer patients. Towards that end, we will review and summarize the literature about, and begin to develop a working model for, the genetics of human breast cancer metastasis. There have been great strides in recent years with regard to our overall understanding of metastasis. Yet our apparently straightforward objective — to define cause-effect relationships for genes in breast cancer — was difficult because of four issues. First, many reports fail to distinguish between oncogenesis and progression or invasion and metastasis when reporting data. Secondly, there is a failure, by some, to recognize that breast cancer is not a single disease, but a collection of diseases. This is particularly apparent in the genetics literature. Thirdly, it is difficult to evaluate the relative importance of correlative data, particularly as they relate to mechanistic control of steps in the metastatic cascade. Fourthly, there is a tremendous noise-to-signal ratio for genetics of late-stage, metastatic breast cancers resulting from genotypic instability, phenotypic drift and tumor heterogeneity.

There are several assertions in the literature claiming a role for genes in controlling progression and/or metastasis of breast cancer. Out-of-hand dismissal of some of those claims was possible because the studies lacked necessary controls. For other genes, the data were more preliminary or correlative and, for an extremely small number of genes, functional data demonstrating regulation of breast cancer metastasis were available. The text of this review will focus on the latter; however, we decided that the utility of this article would be maximized if we summarized the known role(s) of individual breast cancer-associated genes, clearly discriminating the genes that regulate oncogenesis from those that control metastasis. The most effective method to accomplish this goal was to create tables that summarize the references providing evidence for a particular role(s) of genes in human breast cancer. Table 1 is designed to be used as a resource. Putative role(s) of individual genes in breast cancer are separated into two categories — oncogenesis and progression/metastasis — where key references are given to substantiate/refute a role. Although we attempted to be thorough and inclusive, the extensive historical literature combined with the rapidly evolving breast cancer genetics field limit the completeness of this review. We apologize to those whose work was not included because of space considerations or whose papers were inadvertently omitted. However, we hope that this review fulfils our fourfold objective: (1) to highlight the genes for which roles in late-stage human breast cancer and/or metastasis have been functionally demonstrated; (2) to distinguish those genes from the more numerous oncogenic or tumor suppressors involved in breast cancer; (3) to evaluate the literature in order to identify needs for the field of breast cancer metastasis research to move to the next level; and (4) to propose a working model for the genetics of human breast cancer progression, focusing on the genes that have demonstrable metastasis-regulatory activity.

Breast cancer is a collection of diseases

Invasive breast cancers are an histologically and biochemically heterogeneous set of diseases. Lesions are typically categorized on the basis of histological appearance, resembling either ductal or lobular components of the healthy breast. Most studies suggest that the majority of tumors arise in the terminal ductal unit of the breast, perhaps in a single type of ‘target’ cell (Goehring & Morabia 1997, Russo & Russo 1997). By far
Table 1 Genes reportedly involved in oncogenesis and/or progression of human breast cancer.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Map location</th>
<th>Presumptive mechanism(s) of action</th>
<th>Identified aberrations in clinical samples or cell cultures</th>
<th>References containing or citing evidence for roles in: oncogenesis clinical stage/grade, tumor progression or metastasis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC / FAP</td>
<td>5q21</td>
<td>Regulate β-catenin; cytoskeletal organization</td>
<td>LOH</td>
<td>(Thompson et al. 1993)</td>
</tr>
<tr>
<td>α-catenin</td>
<td>5q31</td>
<td>Cytoplasmic component of E-cadherin; cytoskeletal organization</td>
<td>Reduced expression: (Glukhova et al. 1995; Rimm et al. 1995)</td>
<td>Inversion: (Glukhova et al. 1995; Rimm et al. 1995)</td>
</tr>
<tr>
<td>bcl-2</td>
<td>18q21</td>
<td>Apoptosis; interacts with c-myc</td>
<td>Overexpression, amplification</td>
<td>Progression: (Olopade et al. 1997; Silvestrini et al. 1994; Zschiesche et al. 1997)</td>
</tr>
<tr>
<td>BrCa1</td>
<td>17q21</td>
<td>DNA repair, genome stability</td>
<td>LOH, mutation</td>
<td>(Casey 1997; Dickson &amp; Lippman 1995; Holt et al. 1996; Rao et al. 1996)</td>
</tr>
</tbody>
</table>

*References containing or citing evidence for roles in oncogenesis clinical stage/grade, tumor progression or metastasis.
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</thead>
<tbody>
<tr>
<td>Thymosin β15</td>
<td></td>
<td>Cytoskeletal organisation, motility</td>
<td>Increased mRNA expression</td>
<td>Progression (Bao et al. 1998)</td>
</tr>
<tr>
<td>BrCa2</td>
<td>13q12-q13</td>
<td></td>
<td>LOH, mutation</td>
<td>(Casey 1997; Cleton-Jansen et al. 1995; Collins et al. 1995; Wooster et al. 1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNA repair, genome stability</td>
<td></td>
<td>(Patel et al. 1998; Sharan et al. 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differentiation</td>
<td></td>
<td>(Ludwig et al. 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell cycle</td>
<td></td>
<td>(Wang et al. 1997)</td>
</tr>
<tr>
<td>BrCa3</td>
<td>8p12-p22</td>
<td>DNA repair</td>
<td>LOH</td>
<td>(Casey 1997; Hoekstra 1997; Lavin &amp; Shiloh 1997; Meyn 1995; Seitz et al. 1997)</td>
</tr>
<tr>
<td>Brush-1</td>
<td>13q12-q13</td>
<td></td>
<td></td>
<td>(Schott et al. 1994)</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>11p13-pter</td>
<td>Proteinase</td>
<td>Overexpression</td>
<td>(Westley &amp; May 1996)</td>
</tr>
<tr>
<td>CD31 (PECAM)</td>
<td>17q23</td>
<td>Angiogenesis (marker)</td>
<td>Increased expression in stromal component</td>
<td>(Charpin et al. 1995; Fox et al. 1997; Martin et al. 1997)</td>
</tr>
<tr>
<td>CD44</td>
<td>11p13</td>
<td>Adhesion</td>
<td>Amplification, Overexpression</td>
<td>(Hofmann et al. 1991; Joensuu et al. 1993)</td>
</tr>
<tr>
<td>Gene</td>
<td>Map location</td>
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<tr>
<td>Cyclin E</td>
<td>ND</td>
<td>Cell cycle</td>
<td>Overexpression</td>
<td>(Bortner &amp; Rosenberg 1997; Gay-Bablin et al. 1996)</td>
</tr>
</tbody>
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</tr>
</thead>
<tbody>
<tr>
<td>DCC</td>
<td>18q21</td>
<td>LOH</td>
<td>(Thompson et al. 1993)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERβ</td>
<td>6q24-q27</td>
<td>Hormone receptor, transcription</td>
<td>Mutation, loss of expression LOH</td>
<td>(Andersen et al. 1994)</td>
<td>Tumor progression: (Estes et al. 1987; Graham et al. 1990; Leygue et al. 1996; Mackay et al. 1988; Magdelénat et al. 1994; Scott et al. 1991; Sheikh et al. 1994; Thompson et al. 1992)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Invasion: (Garcia et al. 1992; Hoelling et al. 1995; Sheikh et al. 1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Metastasis: (Fuqua et al. 1991a; Garcia et al. 1992)</td>
</tr>
<tr>
<td>ERβ</td>
<td>14q22-24</td>
<td>Hormone receptor, transcription</td>
<td>Mutation</td>
<td>(Dotzlaw et al. 1997; Enmark et al. 1997; Kuiper et al. 1996; Leygue et al. 1996a; Vladusic et al. 1998)</td>
<td></td>
</tr>
<tr>
<td>ETS-2</td>
<td></td>
<td>Transcription</td>
<td>Overexpression</td>
<td></td>
<td>Invasion: (Sapi et al. 1998)</td>
</tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fragile histidine triad; genomic stability</td>
<td>(Barnes et al. 1996; Huebner et al. 1997)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hormone receptor, transcription</td>
<td>(Martin et al. 1993; Tonetti &amp; Jordan 1997)</td>
<td></td>
</tr>
<tr>
<td>IGF2R (mannose 6-phosphate receptor)</td>
<td>6q26-q27</td>
<td>Overexpression</td>
<td>Progression: (Chappell et al. 1997)</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>2q13</td>
<td>Cytokine</td>
<td>Increased expression</td>
<td>Progression: (Jin et al. 1997)</td>
</tr>
<tr>
<td>IL-8</td>
<td>4q13</td>
<td>Cytokine</td>
<td>Increased expression</td>
<td>Progression/angiogenesis: (Green et al. 1997)</td>
</tr>
<tr>
<td>int-1</td>
<td>12q13</td>
<td>Amplification</td>
<td>(Meyers et al. 1990)</td>
<td></td>
</tr>
<tr>
<td>int-2/FGF-3</td>
<td>11q13</td>
<td>Growth factor</td>
<td>Amplification, overexpression</td>
<td>(Huebner et al. 1988; Liscia et al. 1989)</td>
</tr>
<tr>
<td>KAI-1 (CD82)</td>
<td>11p11.2</td>
<td>Adhesion</td>
<td>Decreased expression</td>
<td>Progression: (Yang et al. 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transfection/metastasis: (Phillips et al. 1998)</td>
<td></td>
</tr>
<tr>
<td>KiSS-1</td>
<td>1q32</td>
<td>Signal transduction</td>
<td>Decreased expression</td>
<td>Transfection/metastasis: (Lee &amp; Welch 1997b)</td>
</tr>
<tr>
<td>Laminin-5</td>
<td>1q</td>
<td>Adhesion, invasion</td>
<td>Overexpression</td>
<td>Invasion: (Pyke et al. 1995)</td>
</tr>
<tr>
<td>mdm-2</td>
<td>12q13-q14</td>
<td>Inhibit TP53</td>
<td>Overexpression</td>
<td>Progression: (Jiang et al. 1997)</td>
</tr>
<tr>
<td>MMPs / TIMPs</td>
<td>Multiple</td>
<td>Invasion</td>
<td>(Lochter et al. 1997a, b)</td>
<td>Progression: (Tryggvason et al. 1993)</td>
</tr>
</tbody>
</table>
| Angiogenesis        |              |                                    | (Thorgeirsson et al. 1996) }

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<th>clinical stage/grade, tumor progression or metastasis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnSOD (SOD2)</td>
<td>6q25</td>
<td>Reduce oxygen radicals</td>
<td>Decreased expression</td>
<td>(Li et al. 1995)</td>
<td></td>
</tr>
<tr>
<td>MRP-1/CD9</td>
<td>12p13</td>
<td>Differentiation; motility</td>
<td>Loss of expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFκB</td>
<td>4q24</td>
<td>Transcription</td>
<td>Overexpression</td>
<td>(Sovak et al. 1997)</td>
<td></td>
</tr>
<tr>
<td>NME1 Nm23-H1</td>
<td>17q21.3</td>
<td>NDP kinase? Some find that NDPK activity is not associated with metastasis suppression (MacDonald et al. 1993)</td>
<td>Decreased expression, mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NME2 Nm23-H2</td>
<td>17q</td>
<td>NDP kinase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nm23-DR</td>
<td>ND</td>
<td>Differentiation, apoptosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nm23-H4</td>
<td>16p13</td>
<td>NDP kinase</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References containing or citing evidence for roles in:

- Histologic grade: (Hirayama et al. 1991; Yamashita et al. 1993)
- No correlation: (Goodall et al. 1994; Sastre-Garau et al. 1992; Sawan et al. 1994)
- Transfection/metastasis: (Fukuda et al. 1996; Leone et al. 1993)
- Transfection/metastasis: (Fukuda et al. 1996; Kraeft et al. 1996)
- Transfection: (Postel et al. 1993)
- No suppression: (Tokunaga et al. 1993)
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<th>clinical stage/grade, tumor progression or metastasis*</th>
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</thead>
<tbody>
<tr>
<td>PKCα</td>
<td>17q22-q23.2</td>
<td>Signal transduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammaglobin</td>
<td>11q13</td>
<td>Steroid binding?</td>
<td>Overexpression, amplification</td>
<td>(Watson &amp; Reming 1996)</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Map location</td>
<td>Presumptive mechanism(s) of action</td>
<td>Identified aberrations in clinical samples or cell cultures</td>
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</tr>
<tr>
<td>Raf-1</td>
<td>3p25</td>
<td>Signal transduction</td>
<td>Overexpression (measured in cell lines only)</td>
<td></td>
<td>Progression: (Callans et al. 1995)</td>
</tr>
<tr>
<td>Telomerase</td>
<td></td>
<td>Maintain telomere length</td>
<td>Increased activity</td>
<td></td>
<td>Progression: (Hoos et al. 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inhibit angiogenesis</td>
<td>(Castle et al. 1997; Dameron et al. 1994,a,b; Volpert et al. 1995; Weinstat-Saslow et al. 1994)</td>
<td>Transfection/metastasis: (Weinstat-Saslow et al. 1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Induce apoptosis</td>
<td>(Guo et al. 1997)</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Map location</td>
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<tr>
<td>TGF-β1</td>
<td>19q</td>
<td>Growth factor; can promote VEGF, or MMP expression</td>
<td>Increased protein expression, mutation</td>
<td>(Park et al. 1997) Growth inhibitor: (Arteaga et al. 1996; Butta et al. 1992; Mazars et al. 1995; Sun et al. 1994) (Note: conflicting data that TGF-β1 inhibits or promotes progression) Increased invasiveness: (Hildenbrand et al. 1998; Ott et al. 1996; Welch et al. 1989) Progression: (Cardillo et al. 1997) Possible role in metastasis: (Walker et al. 1994)</td>
<td></td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Xp11.23-1p11.4</td>
<td>Inhibitor of MMPs</td>
<td>Increased expression</td>
<td>Progression/invasion: (Yoshiji et al. 1996b)</td>
<td></td>
</tr>
<tr>
<td>TIMP-2</td>
<td>17q</td>
<td>Inhibitor of MMPs</td>
<td>Increased expression</td>
<td>Progression/invasion: (Visscher et al. 1994)</td>
<td></td>
</tr>
<tr>
<td>uPA / tPA PAI-1 / PAI-2</td>
<td>Various</td>
<td>Invasion</td>
<td>Increased expression (proteinases) Decreased expression (inhibitors)</td>
<td>Progression: (Duffy et al. 1996; Foekens et al. 1995; Ishikawa et al. 1996; Sappino et al. 1987)</td>
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<tbody>
<tr>
<td>VHL</td>
<td>3p25-p26</td>
<td>Cell cycle; inhibits VEGF mRNA accumulation; binds to elongin</td>
<td>Mutations</td>
<td>(Beroud et al. 1998)</td>
<td></td>
</tr>
<tr>
<td>WNT</td>
<td>Wnt14 1</td>
<td>Most data are for murine tumors, but possible correlations exist in human breast carcinomas</td>
<td>Wnt1-2 (Dale et al. 1996) Wnt14 and Wnt15 (Bergstein et al. 1997) Wnt10b (Bui et al. 1997)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Progression indicates only that correlations have been seen in clinical and/or experimental systems corresponding with advanced stage or grade. Attributes of later stages of progression for which specific data are correlated are noted. Table 1 contains some data from other models, particularly with regard to mechanism of action. However, most of the data presented are from breast or mammary tumors. LOH, loss of heterozygosity; MMP, matrix metalloproteinase; ND, not determined; VEGF, vascular endothelial growth factor.
the most common type of breast cancer is infiltrating ductal carcinoma. This class of tumors represents nearly three-quarters of all human breast cancers. Infiltrating lobular carcinomas account for 5-10% of breast carcinomas and are often characterized by multicentric tumors in the same or contralateral breast. Both ductal and lobular carcinomas have a predisposition for metastases to draining axillary lymph nodes, but each has differential predisposition for bone or visceral metastasis (Harris et al. 1984, Coleman et al. 1998). The molecular basis for these differences are mostly unknown. There are numerous other special types of invasive breast carcinomas. The most common are medullary, tubular and mucinous carcinomas. Medullary accounts for 5-7% of all breast carcinomas and are frequently well-circumscribed and exhibit lymphocytic infiltration (Fisher et al. 1990). Mucinous (or colloid) carcinomas account for 1-3% of breast carcinomas and are characterized, as their name implies, by accumulation of mucin around the tumor cells. Overall prognosis for mucinous tumors is better than ductal or lobular carcinomas.

Solely on the basis of their clinical behaviors, these are distinct types of breast carcinoma. It is likely that different genes are involved in controlling development and progression of each type. Yet most discussions of breast cancer genetics have not, for the most part, discriminated between each type of carcinoma. This is even more apparent when discussing the genetics of late-stage breast cancer. Since infiltrating ductal carcinomas are the most prevalent breast carcinoma type, most of the published results probably apply to ductal carcinomas, but this is not necessarily a good assumption (Larsson et al. 1990, Afify et al. 1996, Nishizaki et al. 1997, Toikkanen et al. 1997). There is, fortunately, a recent trend towards studying cancer genetics using more refined pathological criteria; however, more effort is required.

Further complications occur because of the use of cell lines which have been maintained in culture or passaged in animals for several years. The cells have probably undergone genotypic and phenotypic drift as well as selection pressures so that resemblance of the cell lines to the original tumor may be minimal. Sadly, although most breast carcinoma cell lines were derived from metastatic lesions, most no longer retain this ability in experimental systems (i.e. metastasis from mammary fat pads in immunocompromised (athymic or severe combined immunodeficiency (SCID) mice). This limitation severely hinders the ability of investigators to assess directly the metastasis-regulatory effects of individual genes. Given these caveats, any generalizations should be viewed with healthy skepticism. Nonetheless, certain patterns emerge and allow us to make a reasonable first approximation for a model of the molecular underpinnings of breast cancer progression and metastasis.

Oncogenesis and tumor progression are linked, but distinct, phenotypes

One area of confusion relates to terminology. Sloppy use of, and dual meanings of, some terms (depending upon one’s specialization) are prevalent in the literature. Of particular relevance to this review are the distinctions between tumorigenesis vs tumor progression and malignant vs metastatic. Tumorigenesis and oncogenesis refer to the ability of cells to proliferate continuously in the absence of persistent stimulation by the triggering agent(s). Tumor progression is the evolution of already tumorigenic cells (populations) towards an increasingly autonomous state (i.e. decreased dependence upon host-derived growth factors and/or increased resistance to negative regulatory molecules). The distinction between oncogenesis and progression is crucial when asking whether a gene is important in controlling steps associated with malignancy, as compared with whether that gene is involved in tumor formation.

The distinctions between malignant and metastatic are more subtle. Attributes of malignant cells include (but are not limited to) less differentiated morphology, less differentiated cytology, level of vascularity, level of necrosis, mitotic index, aneuploidy, nuclear/cytoplasmic ratio. The incontrovertible hallmarks of malignancy are invasion of cells through a basement membrane and/or metastasis. All other characteristics used to label a tumor (and the cells within it) as malignant have exceptions (Pfeifer & Wick 1995). For example, morphologically indolent cells may be behaviorally malignant and vice versa. Clearly, parameters associated with pathological examination are invaluable when estimating the probability for local, regional or distant recurrence in a clinical setting. Nonetheless, subjectivity leads to ambiguity when trying to assign responsibility for a phenotype (i.e. metastasis).

Metastasis is defined as the formation of secondary tumor foci discontinuous from the primary tumor. The metastases can be nearby or at distant sites. Metastases can form following dissemination of cells via the lymphatic system, hematogenous system, coelomic cavities or epithelial cavities. Since they are by far the most common routes for metastatic spread of human breast cancer, lymphatic and hematogenous metastasis will be the focus here. In order to metastasize, cells must complete every step of a complex cascade. Malignant cells invade adjacent tissues and penetrate into the lymphatic and/or circulatory systems. Then tumor cells detach from the primary tumor and disseminate. During transport, cells travel individually or as emboli composed of tumor cells (homotypic) or tumor cells and host cells (heterotypic). At a secondary site, cells or emboli arrest either because of physical limitations (e.g. too large to traverse a capillary
lumen) or by binding to specific molecules in particular organs or tissues. Once there, tumor cells then proliferate either in the vasculature or, after extravasation, into surrounding tissue (Chambers et al. 1995, Koop et al. 1996). To form macroscopic metastases, cells must then recruit a vascular supply (Weinstat-Saslow & Steeg 1994, Ellis & Fidler 1995, Folkman 1995, Kohn & Liotta 1995) and respond appropriately to the tissue’s environmental milieu (Nicolson 1994, Radinsky 1995). Fewer than 0.1% of cells that enter the vasculature survive to form clinically detectable, macroscopic metastases (Fidler 1970, Tarin et al. 1984). At which step(s) of the metastatic cascade circulating tumor cells commonly succumb is debatable (Chambers et al. 1995, Koop et al. 1995, 1996).

In the context of a multistep, multifogenic cascade, it is critical to recognize that the terms invasiveness and adhesion are not equivalent to metastatic propensity. Both invasion and adhesion are necessary, but not sufficient for metastasis. Cells that are efficient at either or both — but which lack the ability to complete any other step of the metastatic cascade — are non-metastatic (Fidler & Radinsky 1990). Therefore, correlations of genetic expression to a particular step in the metastatic cascade may lead to erroneous conclusions.

Taken together, these points emphasize the importance of distinguishing tumor-suppressor and metastasis-suppressor genes. The former predominantly inhibit tumor formation when wild-type expression is restored in a neoplastic cell. By definition, then, metastasis would also be suppressed (since the cells are non-tumorigenic). Metastasis-suppressor genes, on the other hand, block only the ability to form metastases. Restoring expression of a metastasis-suppressor would yield cells which are still tumorigenic, but are no longer metastatic.

At diagnosis, breast carcinomas are typically mixtures of genotypically and phenotypically distinct cells, despite having arisen from a single cell (Welch & Tomasovic 1985, Fuji et al. 1996a,b, Rebeck et al. 1996, Show et al. 1997). One of the earliest detectable changes in transformed cells (anchorage-independent, not contact-inhibited, immortal but not necessarily able to form a tumor in an appropriate host) is a several-fold increase of genomic instability compared with normal cells (Ling et al. 1985, Cheng & Loeb 1993, Tlsty et al. 1993, Tlsty 1997). Karyotypic and genomic instability is present in transformed cells even before they acquire tumorigenic potential (Otto et al. 1989, Tlsty 1990, 1993, Jonczyk et al. 1993). Thus, genomic instability appears to be the driving force by which cells acquire the cumulative genetic defects necessary to be fully tumorigenic. Likewise, the development of heterogeneity, coupled with selective pressures results in continued evolution of the tumor population, usually towards increasing autonomy from the host (Foulds 1954, Heppner 1984, Welch & Tomasovic 1985, Heppner & Miller 1997). Eventually, some subpopulations of cells within the mass are amply self-sufficient that they have the ability to metastasize. This does not imply that metastatic cells do not respond to host-derived growth signals. Rather, it means that they do not necessarily require them. In conclusion, oncogenesis is a prerequisite for metastasis formation. In other words, metastatic cells represent a subset of tumorigenic cells.

One measure of genetic instability is microsatellite instability. Several reports have suggested that microsatellite instability is a useful prognostic indicator for breast cancer (Patel et al. 1994, Yee et al. 1994, Paulson et al. 1996); however, a role in development of metastasis has not been established. Recently, another means for developing genetic instability in non-hereditary nonpolyposis coli colorectal cancers was described (Cahill et al. 1998). Defective segregation machinery results in unequal partitioning of chromosomes in daughter cells, leading to aneuploidy. While it is common for breast carcinomas to be aneuploid, it has not yet been determined whether a similar mechanism is taking place in breast. Regardless of mechanism, genetic instability has practical consequences with regard to our ability to isolate and characterize metastasis-associated genes — key genetic changes are sometimes clouded by background ‘noise’ due to heterogeneity. Techniques such as tissue microdissection are now being utilized to minimize this problem (Zhuang et al. 1995).

Therefore, the ability to establish a role for a given gene in breast cancer metastasis is complicated by a variety of factors. The following discussion will focus on those genes for which genetic manipulation has been utilized to establish a role in controlling metastasis. Largely, the results are based upon experimental systems. Combined with clinical correlations, there is substantial evidence for controlling the metastatic potential of breast carcinoma.

The use of knockout and transgenic mice to study various aspects of breast cancer biology has been increasing in recent years (reviewed in (Thomas & Balkwill 1994, Amundadottir et al. 1996, Clarke 1996, Bennett & Wiseman 1997, Li et al. 1998). The use of such models has focused on tumor development rather than the later stages of tumor progression and metastasis. While improvements are occurring at a rapid rate, the models are still limited by relatively poor mimicry of the pathogenesis of human breast cancer.

**Metastasis-controlling genes in breast carcinoma**

Since a working model for tumorigenesis involves mutations of key genes that control cell growth and/or death, it appears plausible that metastasis will also be
controlled by a select set of genes controlling key steps in the cascade. On the basis of this presumption, we will focus on genes that appear likely to be important in either the suppression or promotion of breast cancer metastasis. In this regard, the genetic basis of metastasis would parallel the genetics of tumor formation. Evidence shows that metastasis involves numerous genes (Fidler & Radinsky 1990, Chambers & Matrisian 1997, Price et al. 1997, Welch & Goldberg 1997) that fall into two categories — (1) genes that drive metastasis formation, and (2) genes that inhibit metastasis (Dear & Kefford 1990, Welch et al. 1994, De La Rosa et al. 1995, Dong et al. 1995, Lee et al. 1996, Phillips et al. 1996, Lee & Welch 1997b). The number of identified metastasis-associated genes is growing rapidly. However, their mechanisms of action, their regulation in normal and/or cancer cells, and the universality of function in cancers of different origin remain largely unknown.

The best characterized dominantly acting metastasis gene (i.e. met-oncogene, which drives conversion from benign to malignant) is the activated ras oncogene (Collard et al. 1987, Chambers et al. 1990, Phillips et al. 1990). Transfection and constitutive expression of non-senescent rodent fibroblasts with activated Ha-ras leads to development of tumorigenic and metastatic properties (Muschel et al. 1985, Egan et al. 1987). However, complete induction of metastasis does not occur in all cell lines or cell types (Chambers et al. 1990, Tuck et al. 1990, Jessell & Melton 1992), nor is retention of ras oncogene expression necessary to maintain the metastatic phenotype (Schlatter & Waghorne 1992). In human breast cancer, overexpression of normal or mutant ras in human breast cancer has been associated with increased malignant properties (e.g. reduced responsiveness to estrogens, increased invasiveness, morphological abnormalities (Lundy et al. 1986, Theillet et al. 1986, Fromowitz et al. 1987)), but association with metastatic potential has not been unequivocally demonstrated. Mutations of ras, perse, are relatively uncommon in human breast cancer; so, the importance of ras in controlling breast cancer metastasis is not completely understood.

The prototypical metastasis-suppressor gene, Nm23, was first identified in the murine K1735 melanoma using subtractive hybridization, and its expression is inversely correlated with lung colonization (Steeg et al. 1988, Bevilacqua et al. 1989); however, there are exceptions (Radinsky et al. 1992). The human homolog, Nm23-H1 (also known as NME1), exhibits decreased expression in late-stage, metastatic human breast, endometrial, ovarian, melanoma and colon cancers (reviewed in Freije et al. 1996). However, long-term prognostic value has been questioned in some studies (Kapranos et al. 1996, Russell et al. 1997). Nonetheless, NME1 is a bona fide metastasis-suppressor gene in human breast carcinoma, since transfection of metastatic MDA-MB-435 cells resulted in a significant suppression of metastasis from the mammary gland in experimental mouse models (Leon et al. 1993). The mechanism of action for NME1 remains unknown (De La Rosa et al. 1995), but motility of the transfectants was significantly suppressed (Kantor et al. 1993). NME1 is homologous to Drosophila awd and encodes a 17 kDa protein. NME1’s nucleoside diphosphate kinase homology (Biggs et al. 1990) and function (Steeg et al. 1991) have recently been dissociated from its metastasis-suppressor function (MacDonald et al. 1993, Roys et al. 1994, De La Rosa et al. 1995). Some recent reports suggest that NME1 may be involved in controlling cell cycle progression (Cipollini et al. 1997) and histidine-dependent protein phosphorylation reactions (Freije et al. 1997).

The story for Nm23 becomes more complicated because three additional family members (Nm23-H2/NME2, Nm23-DR, Nm23-H4) have recently been identified and cloned. NME2 has been shown to regulate transcription of the (proto)oncogene, c-myc (Postel et al. 1993, Berberich & Postel 1995, Ji et al. 1995, Seifert et al. 1995). Some studies have shown that NME2 can suppress metastasis (Engel et al. 1993, Mandai et al. 1994, Marone et al. 1996), whereas others have not (Arai et al. 1993, Tokunaga et al. 1993, Yamaguchi et al. 1994, Baba et al. 1995). Nm23-DR is differentially expressed during myeloid differentiation (Venturelli et al. 1995) but association with metastatic potential has not yet been tested in either clinical samples or experimental systems. Nm23-H4 differs structurally from the other homologs in that it appears to have additional N-terminal basic amino acid residues (Milon et al. 1997). However, its mechanism of action and relevance to breast cancer biology have not yet been reported.

A recent study even suggests that expression levels of Nm23-H1 in human breast cancer cell lines (HT115 and MDA-MB-231) can be influenced by diet. Increased consumption of linoleic and arachidonic acids reduced expression, whereas linolenic acid increased expression (Jiang et al. 1997). These conditions lowered invasiveness as measured by in vitro invasion assays. While a significant amount of work needs to be done to determine whether dietary regulation of metastasis is mediated through modulation of Nm23, dietary fat intake has been shown to control breast and mammary tumor metastasis (Hubbard & Erickson 1987, Rose et al. 1994, 1995).

KAI1 (also known as CD82 or C33, members of the TM4SF superfamily of adhesion molecules) was recently discovered as a prostate cancer metastasis-suppressor gene on the p-arm of chromosome 11 (Dong et al. 1995). Other members of the TM4SF family, namely MRP-1/CD9 and CD63/ME491, have been associated with metastatic potential of non small-cell human lung
carcinomas (Ikeyama et al. 1993) and early stage melanomas (Hotta et al. 1988) respectively. Thus, a role for KAI1 in breast cancer metastasis was possible. To test this hypothesis, we measured KAI1 mRNA expression in a panel of human cell lines representing a continuum from nearly normal breast cells (MCF10A) to highly metastatic cells (MDA-MB-435). KAI1 mRNA expression decreased with increasing invasive and metastatic potentials (Yang et al. 1997).

Lower KAI1 expression in metastatic breast cancers correlated well with previous findings that chromosome 11 deletions are common in late-stage breast carcinoma (Deville & Cornelisse 1990, 1994, Mars & Saunders 1990, Negri et al. 1995, Trent et al. 1995). To test directly whether changes on chromosome 11 were responsible for suppressing metastatic potential, we introduced a normal chromosome 11 into metastatic MDA-MB-435 breast carcinoma by microcell-mediated chromosomal transfer. Chromosome 11 significantly reduced the metastatic properties without affecting tumorigenicity (Phillips et al. 1996). Since KAI1 expression was higher in the chromosome 11 hybrids, we hypothesized that KAI1 is the gene responsible for suppressing metastasis. Expression of another TM4SF family member, TAPA-1 which is also encoded on chromosome 11, did not correlate with metastatic potential. Transfection and stable constitutive expression of KAI1 in MDA-MB-435 cells suppressed metastasis from tumors following injection into the orthotopic site—a mammary fat pad (Phillips et al. 1998). However, the cell lines did not maintain transgene expression levels following in vivo growth. This complicated interpretation. Preliminary studies using a panel of human breast specimens of varying grades indicate that KAI1 protein staining was inversely related to grade of disease (X.H. Yang, LL Wei, C Tang & ME Lippman, unpublished observations). Nonetheless, KAI1 appears to meet the criteria described above for a metastasis-suppressor gene in human breast cancer.

Chromosome 1q deletions occur with variable frequency in late-stage human breast carcinomas. Since the recently discovered melanoma metastasis-suppressor gene, KiSS-1, maps to chromosome 1q32 (Lee et al. 1996), we tested whether KiSS-1 could suppress metastasis of the human breast ductal carcinoma cell line, MDA-MB-435. Parental MDA-MB-435 cells did not express KiSS-1, but non-metastatic MDA-MB-231 breast carcinoma cells did. Transfection of a full-length, constitutive mammalian expression construct suppressed metastasis of MDA-MB-435 from the mammary fat pad of athymic mice, whereas vector-only transfectants were unaffected (Lee & Welch 1997b).

The mechanism of action for KiSS-1 has not yet been determined, although its ability to suppress metastasis has been demonstrated in six independently-derived human cancer cell lines of melanoma and breast origin (Lee et al. 1997, Lee & Welch 1997a, b). Based upon the cDNA sequence, the predicted KiSS-1 protein would be a hydrophilic, 164 amino acid protein with molecular mass of 15.4 kDa. The sequence is novel, having no strong homology to any known human cDNA sequences. Four regions within the predicted KiSS-1 protein match consensus as phosphorylation sites for protein kinase C, protein kinase A and a tyrosine kinase (Lee et al. 1997). These sequences suggest that KiSS-1 is a phosphoprotein and our working hypothesis is that it functions within a signal transduction pathway. Thus far, KiSS-1 expression has never been detected in any cells that have metastatic potential. However, all studies have measured mRNA expression since antibodies are not yet available. This deficiency limits our ability to measure clinical correlations, although this is certainly a high priority goal.

Other metastasis-promoting or invasion-promoting genes have been identified in a variety of human and rodent tumor models. The genes include TIAM-1 (Habets et al. 1994), mts1 (Grigorian et al. 1994), mta1 (Toh et al. 1994), TI-241 (Ishiguro et al. 1996), fibroblast growth factor-4 (Dickson & Lippman 1992, McLeskey et al. 1996), and cathepsin D (Rochefort et al. 1990a, b). Transfection of these genes into experimental cell systems (usually fibroblasts) is reported to increase invasiveness and metastasis. Again, the definitive roles of these genes in mammary or breast cancers are not well-defined.

Protein kinase C (PKC) activities are important for several physiological processes relevant to mammary tumor promotion and progression (e.g. proliferation, motility, anchorage-independent growth, responses to growth factors, etc.). In collaboration with Drs Susan Jaken, Sue Kiley and Daniel Medina, we recently compared PKC isoenzyme levels in mouse and rat mammary tumor cell lines (SC Kiley, K Clark, SK Duddy, DR Welch & S Jaken, unpublished observations; Kiley et al. 1996, Jaken et al. 1997). Of particular relevance to this review, 13762NF mammary adenocarcinoma cell clones that have low, moderate and high metastatic potentials were evaluated for expression of PKCs α, δ, ε and ζ. All isoforms were expressed in each of the cell lines; however, PKCζ was significantly greater in highly metastatic compared with poorly metastatic cells. To determine whether this correlation was physiologically relevant, transfections were carried out to increase (full-length PKCζ cDNA in constitutive and inducible expression constructs) or decrease (dominant negative PKCζ regulatory domain (RDζ) in inducible expression constructs) PKCζ expression. Increased expression of PKCζ enhanced clonogenicity in soft agar and metastatic potential, but did not affect anchorage-dependent growth. Expression of the RDζ inhibited metastasis when cells...
were injected into syngeneic rats. Moreover, induction of the RDΔ with doxycycline (which induces the tetracycline-inducible promoter) caused a significant reduction in metastatic potential. Taken together, our results strongly imply that PKCδ is an important regulator of mammary tumor metastasis. Experiments are under way to determine the relevance of RDΔ in controlling human breast cancer metastasis.

Chromosomal changes in breast cancer may predict the location of metastasis-controlling genes

As alluded to above, consistent, non-random rearrangements, deletions and/or amplifications have been instrumental in identifying oncogenes and tumor-suppressor genes involved in the development of human cancer. Over 56 distinct regions of loss of heterozygosity (LOH) have been identified in breast cancer (Kerangueven et al. 1997). The frequency of involvement of each ranges from 20% to >50% depending upon the study, tumor type and markers used. Unfortunately, as tumors progress, they accumulate changes, leading to complex karyotypes. Structural or numerical aberrations for virtually every chromosome have been described in human breast cancer (see Table 2 for an example). Experience has told us that some of the chromosomal changes occur at a frequency higher than could be explained on a random mutational basis. These findings increase the probability that genes associated with tumor progression will be encoded at those sites. LOH has been found in chromosomal regions correlating with parameters associated with breast cancer progression/metastasis (see Table 3). To emphasize the point made above — i.e. that different types of breast cancer exhibit different chromosomal changes — Nishizaki and colleagues (1997) used the comparative genomic hybridization technique to compare lobular and ductal carcinomas. Lobular carcinomas had increased copies of DNA from chromosome 1q in 79% of patient samples and losses of chromosome 16q in 63%. The lobular carcinomas showed higher frequency of 16q loss than ductal carcinomas and lower frequency of 8q and 20q gains (Nishizaki et al. 1997).

In metastases vs primary tumors, karyotypic abnormalities of chromosomes 1, 6, 7, and 11 are particularly prevalent. Among the more common cytogenetic changes in metastases from breast is amplification in the region surrounding band q13 on chromosome 11. The amplicon includes the following genes: int-2 gene (which is syntenic to a site of frequent mouse mammary tumor virus (MMTV) insertional mutagenesis in mice (Lee et al. 1995), but the protein is not usually expressed in human breast tumors); list (which is a member of fibroblast growth factor (FGF) family but this is not expressed at the mRNA level (Nguyen et al. 1988, Theillet et al. 1989)); bcl-1 (which was discovered by involvement in chromosomal translocations in some lymphomas (Tsujimoto et al. 1984, Theillet et al. 1990)); and PRAD-1 (which was initially discovered in parathyroid adenomas (Motokura et al. 1991, Motokura & Arnold 1993), but subsequently found to be cyclin D1 (Motokura et al. 1991, Motokura & Arnold 1993)). Amplification in this region is associated with poor prognosis (Lidereau et al. 1988, Tsuda et al. 1989), presence of lymph node metastases (Zhou et al. 1988, Theillet et al. 1989, Adnane et al. 1991), and ER and progesterone receptor (PR) status (Theillet et al. 1989, Fantl et al. 1990, Borg et al. 1991). While these correlations are compelling, definitive association of 1q13 amplification with metastatic potential has not been demonstrated.

As mentioned above, microcell-mediated chromosomal transfer of chromosome 11 reveals that there exists a metastasis suppressor activity on chromosome 11. However, these types of experiments are complicated because results vary according to the experimental models used. Microcell transfer into MCF7 breast cancer cells revealed that BrCa-1- and p53-independent growth inhibitors (i.e. inhibitors of tumorigenicity) are encoded on chromosome 17 (Casey et al. 1993, Theile et al. 1995, Plummer et al. 1997). Additional growth inhibitors have been described on chromosomes 6 and 11 (Negrini et al. 1994, Theile et al. 1996, Shows et al. 1997). Interestingly, transfer of chromosome 11 suppresses growth in culture and tumor formation in the MDA-MB-231 and MCF7 models, but neither phenotype was significantly, nor consistently affected in MDA-MB-435. These data clearly show that extrapolation based upon data from a single model is ill- advised. However, this problem is not easily solved because of a lack of relevant metastatic models of human breast cancer.

Inadequate models exist to study breast cancer metastasis

Despite the fact that the majority of human breast cancer cell lines have been derived from metastatic lesions, only MDA-MB-435 reproducibly forms macrometastases when evaluated in athymic or SCID mice (Price et al. 1990, Price 1996). This is a serious limitation for investigators wishing to study metastasis of human breast cancer. Several investigators have found that MDA-MB-231 will form lung metastases following injection into the mammary fat pad (Price et al. 1990, Rose et al. 1994) or bone metastases following intracardiac injection (Mbalaviele et al. 1996, Guise 1997). Interestingly, none
Table 2 Percentage of breast carcinomas showing chromosomal aberrations

| Chromosome | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X |
|------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Primary tumor |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Structural (p-arm) | 7 | - | 4 | 1 | 1 | 4 | 1 | 2 | 1 | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Structural (q-arm) | 2 | 5 | 1 | 2 | 1 | 2 | 4 | 2 | 2 | 1  | 2  | 2  | 1  | -  | 2  | 1  | -  | 1  | -  | -  | -  | -  | -  | -  |
| Numerical (gain)   | - | - | - | 1 | - | 2 | - | 1 | - | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | 1  | 1  | -  | -  | -  |
| Numerical (loss)   | 2 | - | 4 | 1 | 2 | 2 | 5 | 5 | 5 | 2  | 2  | 2  | 2  | 5  | 6  | 2  | 5  | -  | 2  | 2  | 2  | 2  | -  | -  |
| Metastases         |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Structural (p-arm) | 22| - | 18 | 6 | 10 | 8 | 8 | 8 | 10 | -  | 8  | 10 | 4  | -  | 2  | -  | 2  | -  | -  | -  | -  | -  | -  | -  | -  |
| Structural (q-arm) | 20| 12| 10 | 4 | 12 | 16 | 4 | 6 | 2 | 14 | 10 | 4  | 2  | 2  | 6  | 2  | -  | -  | -  | 4  | 2  | -  | -  | -  |
| Numerical (gain)   | - | 4 | 6 | 8 | 10 | 2 | 15 | 6 | 4 | 4  | 4  | 6  | 4  | 8  | 4  | 2  | 4  | 10 | 4  | 8  | 10 | 6  | 8  | -  |
| Numerical (loss)   | 10| 14| 6  | 8 | 6  | 4  | 4  | 6 | 8 | 10 | 10 | 8  | 6  | 8  | 6  | 6  | 4  | 6  | 4  | 6  | 10 | 4  | -  | -  |

Data presented here are adapted from Emerson et al. (1993); Hill et al. (1987); Trent et al. (1993) using karyotypic analyses of short term cultures from recently removed breast tissue (primary tumor or metastases). While the overall values vary by study, the relative involvement is consistent with other studies using comparative genomic hybridization (Devilee & Cornelisse 1994; Devilee et al. 1994; Gray et al. 1994; Kallioniemi et al. 1994).
Breast cancer metastasis is not solely due to genetic changes

A heritable component of the metastatic phenotype has been demonstrated numerous times by experimental isolation of metastatic and non-metastatic clones as well as selection of increasingly metastatic variants from heterogeneous tumor populations. For cells to metastasize successfully, they must also interact with a variety of host cells and their secreted molecules and respond appropriately. Thus, any discussion of factors controlling metastasis must include an evaluation of exogenous regulators of the process (or its component steps). Normal breast tissue growth, differentiation and regression after lactation are all exquisitely controlled by hormones. Indeed initiation, promotion and progression of breast carcinomas are strongly regulated by endocrine mechanisms (Dickson et al. 1993, Kaufmann 1997).

Hormones contribute to breast cancer development and metastasis

Hormones have long been implicated in the initiation, development, and progression of breast cancer. Numerous epidemiological studies spanning almost two decades have established that, excluding a genetic predisposition, the reproductive history of a woman is an important risk factor associated with the development of breast cancer. Early menarche and late menopause have been shown to be associated with an increased risk of breast cancer. Epidemiological studies also show that early pregnancy provides a protective effect against breast cancer, but that the protection declines as the age of first pregnancy increases. Taken together, these studies suggest that the length of time between menarche and menopause or menarche and first pregnancy are contributing factors towards the risk or likelihood of breast cancer oncogenesis (Staszewski 1971, Key & Pike 1988, Henderson et al. 1991).

Table 3 Chromosomal location of LOH and the correlation with parameters associated with breast cancer progression/metastasis

<table>
<thead>
<tr>
<th>Chromosome region</th>
<th>Parameter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p</td>
<td>Nodal status</td>
<td>(Borg et al. 1992b)</td>
</tr>
<tr>
<td>1p36.1p34-p35</td>
<td>Nodal status</td>
<td>(Tsukamoto et al. 1998)</td>
</tr>
<tr>
<td>1q21-q24</td>
<td>Stage</td>
<td>(Devilee et al. 1991)</td>
</tr>
<tr>
<td>3p21.3</td>
<td>Metastasis</td>
<td>(Driouch et al. 1998)</td>
</tr>
<tr>
<td>3p21-p25</td>
<td>LOH on 11p, 17p, 17q and aneuploidy</td>
<td>(Devilee et al. 1994)</td>
</tr>
<tr>
<td>7q23</td>
<td>Metastasis-free overall survival</td>
<td>(Bieche et al. 1992)</td>
</tr>
<tr>
<td>8p21.3-p23</td>
<td>Low grade DCIS</td>
<td>(Anbazhagan et al. 1998)</td>
</tr>
<tr>
<td>9q</td>
<td>LOH on 1q, 17p, 18q</td>
<td>(Devilee et al. 1994)</td>
</tr>
<tr>
<td>11p15</td>
<td>Lymph node status</td>
<td>(Takita et al. 1992)</td>
</tr>
<tr>
<td>11p15.5-15.4</td>
<td>Histologic grade</td>
<td>(Karnik et al. 1998)</td>
</tr>
<tr>
<td>13q12-q14</td>
<td>ER content</td>
<td>(Devilee et al. 1994)</td>
</tr>
<tr>
<td>13q12-q14</td>
<td>Ductal carcinoma tumor size</td>
<td>(Andersen et al. 1992)</td>
</tr>
<tr>
<td>13q12-q14</td>
<td>Aneuploidy and S-phase fraction &gt;12%</td>
<td>(Borg et al. 1992b)</td>
</tr>
<tr>
<td>16q22.2-q23.2</td>
<td>Metastasis</td>
<td>(Driouch et al. 1998)</td>
</tr>
<tr>
<td>16q23.2-q24.2</td>
<td>Good prognosis</td>
<td>(Hansen et al. 1998)</td>
</tr>
<tr>
<td>16q24</td>
<td>ER content</td>
<td>(Devilee et al. 1994)</td>
</tr>
<tr>
<td>17q12-q24</td>
<td>c-erb-B2 amplification</td>
<td>(Sato et al. 1991)</td>
</tr>
<tr>
<td>17q12-q24</td>
<td>Age of onset</td>
<td>(Devilee et al. 1994)</td>
</tr>
<tr>
<td>17q12-q24</td>
<td>c-erb-B2 amplification/post-menopausal status</td>
<td>(Andersen et al. 1992)</td>
</tr>
</tbody>
</table>

ER , estrogen receptor negative tumors; DCIS, ductal carcinoma in situ.
The two principal hormones involved both in the onset of menarche and in menopause are the female sex steroids, estrogen (specifically 17β-estradiol) and progesterone. It is well established that estrogen promotes breast cancer by stimulating cell division. Although the main source of estrogen is the ovary in premenopausal women, estrogen can also be synthesized directly in adipose tissue and breast cancer cells via the enzyme aromatase (Yue et al. 1998). Aromatization is typically thought to be the predominant source of estrogens in post-menopausal women (Brodie & Santen 1994, Harvey 1997, Kaufmann 1997). More controversial is the role that estrogens or estrogen metabolites can have in causing or initiating breast cancer. Recent findings suggest that metabolites of 17β-estradiol may be among the culprits leading to DNA damage and subsequently for initiation of breast cancer (Fishman et al. 1995, Cavalieri et al. 1997, Lavigne et al. 1997, Zhu & Conney 1998). However, this interpretation is debatable and additional research will be required to establish this definitively. Nonetheless, there is little doubt that estrogens play a key role in promoting initiated human breast cancer to grow and to progress.

A role for progesterone in breast cancer development is less clear than for estrogen. At one time, it was generally accepted that progesterone was a natural antagonist of estrogen action — suggesting that it would inhibit or block growth-promoting effects of estradiol on breast cells (normal and tumor). This paradigm was based upon findings in the uterus in which progestins reduced or eliminated the risk of estrogen-induced endometrial cancer. Recently, the effect of progesterone (analogs) on normal breast epithelial cells has been re-examined. The mitotic index of normal breast epithelial cells parallels changes in hormone levels during the menstrual cycle. In cyclic women, serum estrogen levels are highest during the follicular phase, with a secondary resurgence in the secretory phase. The mitotic index of endometrial cells parallels serum estrogen levels. In contrast, breast epithelial mitoses are greatest during the secretory phase when serum progesterone levels are maximal (Masters et al. 1977, Meyer 1977, Going et al. 1988). The latter raises the possibility that progesterone may have growth-promoting effects on breast epithelial cells. This supposition is further supported by the following lines of evidence: (1) progestins are mitogenic for established breast cancer cell lines in vitro (Hissom & Moore 1987, Hissom et al. 1989, Manni et al. 1991); (2) progestins promote growth of established mammary tumors (Huggins & Yang 1962, Huggins 1965, Robinson & Jordan 1987); (3) progestins stimulate expression of mitogenic growth factors and/or their receptors (Dickson & Lippman 1988, Murphy et al. 1988, Lanari et al. 1989, Murphy & Dotzlaw 1989, Papa et al. 1991); and (4) anti-progestins induce apoptosis in experimental mammary tumor models (Michna et al. 1989, Schneider et al. 1989). Thus, progesterone exposure may be a contributing factor towards the development of breast cancer (Groshong et al. 1997).

Estrogen and progesterone exert their cellular effects through interactions with nuclear receptor proteins called the estrogen receptor (ER) and the progesterone receptor (PR) respectively. The recognition that these receptors are the primary mediators of estrogen and progesterone action and that their presence within a tumor specimen can help predict the responsiveness of human breast cancer to hormonal therapy is particularly useful. Today, the measurement of ER levels is standard practice and is a useful prognostic marker in determining which patients are most likely to respond to estrogen antagonist therapies such as the anti-estrogen, tamoxifen (also known as Nolvadex). Since PR is an estrogen-induced product, simultaneous detection of PR in the presence of ER from a single tumor is indicative of a functional estrogen receptor pathway and further improves the ability to predict the response to anti-estrogen therapy. Alternatively, the absence of ER and PR is associated with early recurrence and poor survival of the breast cancer patient.

The ER mentioned above refers to the alpha ER (ER-α). Recently, a second ER form has been cloned (ER-β) (Kuiper et al. 1996). ER-α and ER-β both bind 17β-estradiol in traditional binding assays. However, current data suggest that the amount of ER-β relative to ER-α in breast cancer cells is minor (Kuiper et al. 1996, Petersen et al. 1998). In the normal mammary glands of mice, ER-β is undetectable (Couse et al. 1997). Whether ER-β will play an important role in breast cancer biology or etiology remains to be determined, although there have been reports of ER-β mutants in breast cancer cells (Dotzlaw et al. 1997, Vladusic et al. 1998). It is important to remember that many of the studies with ER-β are based upon mRNA, rather than protein, expression. Once more robust protein detection methods/reagents have been developed, the relative importance of ER-β in breast cancers, if any, will be more easily evaluated.

Since almost all breast cancers progress from a hormone-responsive state to a hormone-resistant or hormone non-responsive state, the possibility was raised that mutations in the ER-α (the predominant form of ER in breast cancers) could be a factor leading to anti-estrogen resistance in breast cancer. Several investigators pursued this line of thought and have shown that mutant ERs exist in some breast cancer cell lines and tumor specimens (Graham et al. 1990, Fuqua et al. 1991a, 1992, Scott et al. 1991, Wang & Miksicek 1991). Moreover, mutations of ER can lead to variant estrogen receptor activity which, in turn, may explain estrogen resistance (Fuqua et al. 1991a, 1992). Furthermore, from these and other studies that have
focused on ligand-receptor interactions, it is apparent that variations in ER structure and ligand-specific (estrogen versus anti-estrogen) interactions with ER may lead to altered and unexpected biological responses (Katzenellenbogen 1996, Mcinerney & Katzenellenbogen 1996, Montano et al. 1996, Levenson et al. 1997). This is further complicated by promoter and cell-specific factors (Katzenellenbogen 1996, Yang et al. 1996). Although the existence of mutant ERs is very appealing, their actual contribution to disease progression, particularly anti-estrogen resistance, appears to be small. Furthermore, most of the variant ER data to date have been found at the mRNA level. It is still not known whether they are translated into proteins (Dowsett et al. 1997, Murphy et al. 1997a,b, Tonetti & Jordan 1997).

Although less research has been dedicated towards the identification of variant PR, there are several papers reporting the existence of variant PR mRNA and protein (Wei et al. 1990, Wei & Miner 1994, Leygue et al. 1996a, Richer et al. 1998, Yeates et al. 1998). One variant PR protein form is N-terminally truncated compared with the previously reported A and B PR isoforms. This third form, the C-receptor, has unique transcriptional enhancing properties when in the presence of the two larger PR isoforms and ligand (Wei et al. 1996). From this work and the abundance of other studies, it is becoming apparent that steroid-regulated growth and gene expression involves multiple regulatory factors, of which the steroid receptor is but one component, and that the eventual biological outcome is dependent upon the interaction of steroid receptors with non-receptor proteins (i.e. adaptors) (Katzenellenbogen et al. 1996, Glass et al. 1997, Shibata et al. 1997). Several proteins to date have been associated with gene transcriptional enhancing properties such as SRC-1 (Onate et al. 1995, Spencer et al. 1997), AIB-1 (a member of the SRC-1 family) (Anzick et al. 1997) and RIP140 (Cavailles et al. 1995). Likewise, transcriptional repressor proteins have been identified (Chen & Evans 1995). Steroid-regulated gene expression is further complicated because some neurotransmitters and growth factors (e.g. epidermal growth factor) can activate steroid hormone receptors independently of ligand (Ignar-Trowbridge et al. 1992, Gangolli et al. 1997). Also, some steroid hormones can mimic growth factor action in the absence of steroid hormone receptors e.g. progesterone binds to oxytocin receptor (Grauzini et al. 1998) and estrogen receptor binds to cerbB-2 receptor (Matsuda et al. 1993). Collectively, these studies indicate that steroid-driven gene activation is modulated by multiple factors, of which only one component is the receptor. So, although estrogen and progesterone are key hormones in the regulation of breast cancer growth, there are many additional contributory factors (i.e. growth factors and co-factors) that also regulate breast cancer proliferation.

Although steroid hormone receptor levels can be used as a marker to assess the extent of tumor progression towards malignancy, few studies directly demonstrate a functional role in this regard, especially with regard to metastasis. The most direct test was by Garcia et al. (1992) who transfected the ER-negative MDA-MB-231 breast carcinoma cell line with estrogen receptor (ER-α) and then treated the transfectant cells with estrogens and anti-estrogens. Experimental metastatic potential following intravenous inoculation of cells was inhibited threefold by estradiol whereas the anti-estrogen tamoxifen had little effect (Garcia et al. 1992). Estradiol also increased the invasive capabilities of these transfectants in an in vitro invasion assay using Matrigel; anti-estrogens inhibited these effects. Interestingly, in contrast to the typical stimulatory effect of estradiol on ER-positive breast cancer cell growth, estradiol inhibited the cell proliferation of ER-transfectants. These results must be viewed cautiously until further experiments are done to explain this phenomenon or the experiments are replicated in another cell line.

Endocrine regulation does not act independently to regulate breast tumor cell behavior. The biochemical changes resulting from modified ligand and receptor expression and activation, combined with inter-relationships with other growth factors and intracellular signaling pathways, reveal a byzantine regulatory machinery. Abnormal tissue growth is due to a disruption of the balance between stimulated proliferation and inhibition of cell death. Transformation and progression can be due to: (1) increased production of growth-promoting factors; (2) decreased synthesis of growth-inhibitory factors; (3) decreased responsiveness to growth-promoting factors; or (4) decreased sensitivity to growth inhibitory signals. The latter two mechanisms can be direct, because of alterations in receptors, or via modifications in the downstream signaling pathways. For purposes of this review, only selected growth factors will be presented to provide examples of the complexities of growth regulation of breast cancer growth and progression.

Transforming growth factors

Transforming growth factors (TGFs) were identified initially and named based on their ability to transform selected cell types. This family of growth factors has expanded extensively and is now known to consist of several families of polypeptides (Hartsough & Mulder 1997). These are produced and secreted by normal and cancerous cells. TGF expression can be regulated by steroids as well as by other growth promoting factors, thereby leading to an intricate complex of negative and positive pathways modulating cell cycle progression or
homeostasis. TGF-α and TGF-β represent two distinct families of growth factors that are structurally and functionally distinct.

**TGF-α and epidermal growth factor families**

Many members of the TGF-α family compete with epidermal growth factor (EGF) for binding to the EGF receptor. Like EGF, TGF-α binding results in receptor dimerization, activation of tyrosine kinase activity and eventually leads to stimulation of cell proliferation or differentiation (Massague 1983, Derynck 1988, Todaro 1985, Arteaga et al. 1996). Other members of this family include amphiregulin, heparin-binding EGF, cripto-1, and a subfamily of heparin binding proteins called heregulins (the human homolog) (Bates et al. 1988, Todaro et al. 1990, Higashiyama et al. 1991). Heregulin does not appear to bind the classic EGF receptor, but initially was thought to bind instead to a related EGF receptor protein called erbB-2 (HER-2/neu) (Schechter et al. 1984, 1985, Coussens et al. 1985, Bargmann et al. 1986, Stern et al. 1986, Yamamoto et al. 1986). Studies now indicate that heregulin does not bind directly to erbB-2, but rather to two related receptor forms, erbB-3 (Kraus et al. 1989, Plowman et al. 1990) and erbB-4 (Plowman et al. 1993, Carraway et al. 1994). All four receptor forms (EGF receptor, erbB-2, -3 and -4) have been reported to be present in human breast cancers. In about 30% of human breast cancers, erbB-2 is amplified or overexpressed; this is associated with poor patient prognosis and maintenance of the malignant phenotype (Slamon et al. 1987, Allred et al. 1992).

Overexpression of erbB-2/HER-2/neu and its relationship with other prognostic factors change during the progression of in situ to invasive breast cancer (Van de Vijver et al. 1988, De Potter et al. 1990, Paik et al. 1990, Allred et al. 1992, Gusterson et al. 1992, Toikkanen et al. 1992). Because of this, erbB-2 overexpression was thought to be a key factor that increased the invasive potential of breast cancer cells; however, studies examining comedo-type intraductal carcinomas showed that a higher proportion overexpressed erbB-2 protein compared with invasive cancer, thereby indicating that, although erbB-2 overexpression may play a role in invasion, it does not singly lead to increased invasiveness (Van de Vijver et al. 1988). The roles of erbB-3 and -4 in breast cancer invasion and metastasis are not known.

**TGF-β family**

The TGF-β family of polypeptide growth factors is comprised of several related gene products that form either homodimers or heterodimers. TGF-β isoforms are found in both normal mammary epithelium and in breast tumors. The interactions of these various isoforms is further complicated by the presence of specific binding proteins (Chefietz et al. 1988, Murphy-Ullrich et al. 1992, Wakefield et al. 1992, Butzow et al. 1993). In addition, two TGF-β receptors (type I and type II) have been identified. Four type I receptors have been cloned (Wang et al. 1994b). Type I and type II receptors can heterodimerize. Because there are a wide variety of receptor combinations as well as the existence of multiple TGF-β forms, a diverse number of pathways appear available to regulate breast cancer growth and differentiation.

Most normal epithelial cells are growth inhibited when exposed to TGF-β (Arteaga et al. 1996). Restoration of TGF-β receptors in non-responsive MCF7 cells renders the cells less tumorigenic and less proliferative when grown in the presence of TGF-β (Sun et al. 1994). Because of this, studies on the role of TGF-β in cancer biology have mostly focused on this factor’s effect on growth regulation and tumor formation. However, there is accumulating evidence that TGF-β plays a critical role in tumor invasion and metastasis.

TGF-β overexpression in breast tumors has been associated with a more malignant phenotype (Dickson & Lippman 1996). A specific role in invasion and metastasis was demonstrated when Welch and colleagues (1990) first showed that exposure of mammary adenocarcinoma cell lines to picomolar concentrations of TGF-β1 or TGF-β2 induced production of metalloproteinases, with a corresponding increase in invasiveness and experimental metastatic potential. At these concentrations, growth inhibition was not observed. Similar findings have been reported for the metalloproteinases as well as the urokinases (Walker & Dearing 1992, Agarwal et al. 1994, Walker et al. 1994, Sehgal et al. 1996, Reiss & Barcellos-Hoff 1997, Dong-Le et al. 1998). It is important to note that the source of the TGF-β can be the tumor cells themselves or nearby host cells. Indeed TGF-β can increase stromal cell secretion of urokinase (Hildenbrand et al. 1998). Thus, tumor cells which produce TGF-β could manipulate stromal cells to assist in their malignancy. This concept is substantiated by the known roles of TGF-β in angiogenesis and immunosuppression (Enensteins et al. 1992, Relf et al. 1997, De Jong et al. 1998a,b).

Interestingly, TGF-β expression was originally correlated with increased bone colonization by Walker 256 carcinosarcoma cells (Ort et al. 1993). Since bone is the most common site for breast cancer metastasis, organotropism may be partly explained by differential expression of TGF-β. This hypothesis is, at least partially, supported by Guise and colleagues who showed that TGF-β can alter expression of parathyroid hormone-
related protein (PTHrP) which is, in turn, involved in bone resorption. Expression of PTHrP, with or without exposure to TGF-β, regulates bone colonization by MDA-MB-231 cells (Guise 1997). Still, it must be emphasized that a role for TGF-β in bone colonization by breast cancer has still not been definitively established.

**Other growth factors**

In addition to the EGF and TGF-β families, numerous other growth factor families have been identified and found in breast cancer cells. These include the insulin-like growth factors (IGF-I and IGF-II), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), and vascular endothelial growth factor (VEGF) (Heldin & Westmark 1984, Goustin et al. 1986, Sporn & Roberts 1986, Ferrara et al. 1992). The expression of many of these growth factors can be regulated by estrogen and progesterone (Dickson & Lippman 1996).

Thrombospondin is a 450 kDa adhesive glycoprotein present in high concentrations in the platelet alpha-granule. It is also synthesized by other cells and is incorporated into extracellular matrices. The role of thrombospondin in breast cancer biology is checkered (Walz 1992, Volpert et al. 1995, Qian & Tuszynski 1996, Roberts 1996). Transfection experiments suggest that it can promote cell adhesion, invasion and/or metastasis in some tumor models (Tuszynski et al. 1987a, Pratt et al. 1989, Walz 1992, Arnotetti et al. 1995, Incardona et al. 1995, Wang et al. 1996), whereas it is suppressive in others (Weinstat-Saslow et al. 1994, Zabrenetzky et al. 1994, Qian & Tuszynski 1996). Metastasis-promoting effects are often attributed to changes in adhesion whereas the suppressive effects can be, at least partially, explained by the anti-angiogenic effect of thrombospondin (Dameron et al. 1994a,b, Weinstat-Saslow et al. 1994, Volpert et al. 1995). Interestingly, thrombospondin expression is regulated by progesterone in the endometrium (Iruela-Arispe et al. 1996), opening the possibility that analogous regulation could occur in the breast. Also, thrombospondin-1 (TSP-1) expression appears to be regulated by p53 (Dameron et al. 1994b), which itself has been implicated in breast tumorigenesis (see TP53 in Table 1).

Thus, there are a multitude of interrelated growth factors, receptor types, and steroid hormones in the normal mammary epithelium that tightly regulate and coordinate cell proliferation and differentiation. In breast cancer cells, the intricate balance is perturbed. Invasive and metastatic cells further circumvent the regulation by overexpression or downregulation of growth factors and/or their receptors. Aberrations of downstream signaling cascades further contribute to cellular delinquency. Delineation of these pathways and their impact on angiogenesis, immune response, growth, invasion, and metastasis will require new models.

**Immune regulation of breast cancer metastasis**

There is clear evidence that breast cancer metastasis is based upon the inherent genetic makeup of the tumor cells. However, tumor cells do not exist in isolation and their biological properties are not fully self-determined. Examples are described above but there is one more that merits mentioning. The role of the immune system in cancer is usually considered to be the elimination of tumor cells; however, because metastatic cells and activated leukocytes share many properties, including the ability to attach to endothelium (Hoover & Ketcham 1975, Yong & Linch 1993) as well as degradation of and penetration of basement membranes (Wright & Gallin 1979, Klotz & Jesaitis 1994), it was suggested that, under certain conditions, tumor cells might exploit normal leukocyte function to increase metastatic efficiency (Gorelik et al. 1982, Aeed et al. 1988).

Rats injected with syngeneic 13762NF mammary adenocarcinoma cell clones developed neutrophilia (i.e. tumor-elicted neutrophilia) proportional to the metastatic potential of the primary tumor (Aeed et al. 1988). We showed that the metastatic tumor variants did so by secreting granulocyte-macrophage colony-stimulating factor (GM-CSF) and/or interleukin-3 (IL-3) in proportion to their metastatic propensity (McGary et al. 1995). More importantly, tumor-elicted neutrophils increased metastatic potential and invasiveness of breast cells 2- to 25-fold when co-injected intravenously (Welch et al. 1989), whereas normal circulating neutrophils, protease peptone-elicted and activated neutrophils and phorbol ester-activated neutrophils did not. Alone, these findings may have been merely an experimental curiosity. However, anecdotal clinical data suggest that these types of observations are not altogether uncommon. Leukocytosis (Sawyers et al. 1992), granulocytosis (Hughes & Higley 1952, Suda et al. 1980), eosinophilia (Sawyers et al. 1992) and neutrophilia (Lee et al. 1987) have been described in patients with advanced neoplasms of multiple histological types. This could not be explained solely by infection or tumor necrosis (Aeed et al. 1988). In experimental models, the evidence predominantly supports secretion of factors that stimulate bone marrow precursor cells. Lee and colleagues have shown that GM-CSF levels may be correlated with more advanced mammary tumors (Lee & Baylink 1983, Lee & Lottsfield 1984, Lee et al. 1987). Factor(s) produced by other tumor cell types that elicit bone marrow proliferation vary by tumor type, stage and size (Asano et al. 1977, Wu et al. 1979, Mano et al. 1987, Fu et al. 1991, Nitta et al. 1992,
Sawyers et al. 1992). Takeda et al. (1991) found that 7/14 metastatic transplantable tumors produced GM-CSF mRNA and/or detectable GM-CSF activity, whereas the non-metastatic tumors did not. Taken together, these results demonstrate that breast cancers may modulate their metastatic potential, in part, by manipulation of the immune system.

A molecular genetic model for breast tumor progression

The collection of neoplastic breast diseases are sufficiently distinct that it is unlikely that a single model could describe the genetic changes leading to metastasis. At the root of any model must be a clear understanding of the cell type from which a particular neoplasm developed. Nonetheless, the majority of evidence suggests that cells from the terminal ductal structures are the cells of origin. Insufficient biochemical and molecular markers defining each breast cell type allow for more refinement than that with regard to cellular origin of breast neoplasms. It is believed that the conversion to neoplasia has an intermediary atypical hyperplasia in which the cells have lost some aspects of growth control, but still retain vestigial response to growth controlling signals. During the proliferative phase, cells are responding to the usual milieu of positive and negative endocrine, paracrine and juxtacrine signals. During this hyperproliferative phase, breast epithelial cells accumulate mutations in oncogenes and tumor suppressor genes so that they appear even less “normal” or differentiated and are classified as carcinomas in situ. Further proliferation results in accumulation of mutations, increasing malignant characteristics (i.e. invasion, aneuploidy, angiogenesis, etc.), so that eventually a subset of cells is no longer confined to the breast.

Over 150 genes and genetic loci have been associated with breast cancer development. Of those changes, this review summarizes evidence implicating a role in progression to malignancy for over forty different genes. The magnitude of these numbers highlight the tremendous complexity of breast cancer as a family of diseases. The good news is that all of these markers have been identified, in spite of the extraordinary heterogeneity that exists within breast neoplasms at diagnosis. The bad news is that these changes are only the tip of the iceberg. How, then, can one determine which changes are essential and which are ancillary?

For oncogenes and tumor suppressor genes, the data in breast cancer oncogenesis are relatively mature. While there is still plenty of room for further study, correlative data are often corroborated by functional studies (i.e. transfection with wild-type cDNA followed by bioassay). The mechanism of action is not always known; however, the biological endpoints are unambiguous. The situation is less clear with regard to genes/loci involved in breast tumor progression, invasion and/or metastasis. Only four genes (Nm23-H1, KiSS-1, KAI1 and TSP-1) have been demonstrated to suppress metastasis of human breast carcinoma cells following orthotopic implantation of tumor cells into immunocompromised mice. Of these, only one, NME1 has been studied adequately in the clinical arena to warrant serious consideration as having prognostic value. KAI1 suppressed metastasis at a level comparable to Nm23, but KiSS-1 was more potent than any of the other genes tested with regard to reduction in metastasis incidence. To claim TSP-1 as a metastasis-suppressor gene may be a misnomer since tumor growth was also inhibited. Nonetheless, the tumor cells still expressed the transgene, allowing TSP-1 still to qualify by the criteria listed above.

Considering the number of papers claiming to study metastasis of breast cancer, the number of bona fide functionally tested metastasis-suppressor genes is surprisingly small. In part, this is due to the paucity of models which allow testing in vivo. Indeed most of the functional studies were done using the MDA-MB-435 model. Validation in other models has not been done. Certainly, testing in other breast tumor types has not been attempted. Thus, for the breast cancer metastasis field to advance further, more and better models will be required.

Despite the discovery and identification of four (and probably more) metastasis-suppressor genes, several questions remain regarding control of the metastatic phenotype in human breast cancer. Do the identified genes represent rate-limiting steps? Are these genes functioning in a single pathway or convergent pathways of metastasis control? What are the signals that control these genes? Are the key controlling signals among the correlations already established for breast cancer progression (i.e. hormonal or growth factor control)? While much has been learned, more still remains to be found.

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