

# New Pot-pourri of Markers related to invasive Breast Cancer

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Breast cancer is one of the most frequent malignancies amongst Indian women<sup>1</sup>. About 60-80% of these cases present at a locally advanced stage<sup>2</sup>. This aspect is of constant anxiety to the clinicians, engaged in its treatment. One-way has been, identifying premalignant lesions, followed by conservative treatment and follow-ups. Premalignant lesions are well documented in breast and prostate<sup>3,4</sup>. The other is identification of biological markers helpful in predicting clinical outcome, once the cancer has set in. Several molecular components related to development of breast carcinomas and associated with therapeutic and prognostic value viz. p53, RB, ER, Cerb B-2; have been studied in great detail<sup>5-7</sup>. Identification of newer markers has always been of great interest to the pathologists and clinical onco surgeons, for developing newer therapeutic modalities to combat this disease. While the 1950's saw identification and role of steroid hormones in breast cancer, the researchers in 1980 developed a focus on studying implications of growth factors, including oncogenes and tumor suppressor genes. The 1990's were met with discovery of genes causing familial forms of breast cancer. Final decade ushered advances in cell cycle, DNA repair, cell death (apoptosis) and regulation, with the recent emphasis on identifying markers related to tumor metastasis, which is the major cause of morbidity and mortality in these patients<sup>8</sup>.

A cell, with an intrinsic malignant potential, passes through various stages before assuming a tumor collection. This ranges from early hyperplastic to premalignant to malignant stage, under the influence of wide range of genetic factors and events. The malignant cells further become invasive. Invasive growth of malignant cells is eased by the production of proteolytic activity at the advancing edge of the tumor. Factors relating to these processes, along with those related to uncontrolled cell proliferation and cell dissemination have a major impact on the invasive and metastatic propensity of the tumor. A number of proteins form the underlying basis for tumor invasion.

Cathepsin D is a lysosomal protein that is over expressed and secreted by tumor cells. Its role in invasiveness of breast cancer has been substantiated by a study showing over expression of this protein in node negative, aneuploid tumors, with 60% chance of recurrence in 5 years, thereby poorer survival rates<sup>9</sup>.

Invasion and metastasis of solid tumors like breast requires tumour-biologic factors that promote the dissolution of the surrounding matrix and basement membranes. In this context, a set of serine protease plasminogen activators and inhibitors is of major interest. Increased levels of urokinase type plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) form independent predictors of overall survival rates in breast cancers. uPA, produced by tumor cells and macrophages, has a significant role in angiogenesis. Its levels in cancer cells correlate with microvessel density, vascular invasion, macrophages number and proliferation rate<sup>10</sup>. It has been shown that uPA negative tumors have a better response to tamoxifen treatment than those with uPA positive tumors. Patients with higher levels of plasminogen-

activator inhibitor rates also respond better than those with a similar negative status<sup>11</sup>.

Cell cycle plays a very significant role in development of tumors with the aid of various checkpoints. A set of proteins known as cyclins, regulated by cyclin dependent kinases (CDK's) is responsible for transitions of cells entering into various phases of cell cycle<sup>12</sup>. One such protein molecule is cyclin D, which transfers cells from G1 to S phase of cell cycle. Over-expression of this gene, as seen in many breast carcinomas, even though, its amplification being seen only in a few cases; leads to uncontrolled entry of cells in the S phase, leading to further proliferation<sup>13</sup>.

Certain specific cell-to-cell adhesion molecules have been found to be responsible for embryonic development. One such molecule is a family of "Cadherins". It is a group of genetically related transmembrane glycoproteins, involved in calcium-dependent cell-to-cell adhesion mechanism and are sub classified, based on their binding characteristics and tissue distribution like E-, P- and N-cadherins<sup>14,15</sup>. Significant among these is E-cadherin, also known as uvomorulin, an invasion-suppressor gene. Damsky and colleagues<sup>16</sup> first identified human E-cadherin as cell-CAM 120/80 using polyclonal antibody. In epithelial cells, this transmembrane molecule is considered as one of the key molecules for the formation of the intercellular junctional complex and for establishment of cell polarization<sup>17</sup>. At the structural level, E-cadherin (E-CD) has extracellular portion responsible for homophilic cellular interaction and an intracellular part that provides link to actin cytoskeleton through an association with various catenins, of which  $\alpha$ -catenin has significant role in cell adhesion and signal transduction<sup>18,19</sup>. A positive association between abnormal E-CD expression and occurrence of invasion and metastasis has been reported in cancers of stomach, breast, and other organs<sup>20-22</sup>. Berx et al<sup>23</sup> have reported that protein-truncating mutations in extracellular part of E-CD are responsible for lack of E-CD expression, thereby resulting in characteristic scattered tumor cell growth in infiltrative lobular breast cancer, which further has a propensity for dissemination. Lack of E-CD expression has been known to be linked with invasive tumor types, higher grades and a similar lack of ER expression<sup>24</sup>. Forced expression of E-CD in tumor cell lines has been shown to result in reversion from an invasive to benign tumor cell phenotype<sup>25</sup>. Besides, the reversibility of E-CD down regulation has been demonstrated by the observation that treating the tumor cells with antiestrogen tamoxifen can restore the invasion suppressor activity of E-CD<sup>26</sup>. KA1-1 is another gene responsible for suppressing metastasis and its down regulation is an added factor to loss of E-CD expression, thereby increasing the invasive potential and poor survival in carcinomas, as of breast<sup>27</sup>.

nm-23-H1 and nm-23 H2 (non metastatic proteins) are another set of genes with possible implications in progressive breast carcinomas. They are located on 17q22 and separated from each other by no more than 18 kb. The two have arisen by tandem duplication<sup>28</sup>. Differential colony hybridization between low and

highly metastatic murine K-1735 melanoma cell lines led to identifying function of nm 23, with a ten fold lower expression in the more aggressive cell lines<sup>29</sup>. Its lower expression is associated with higher metastatic potential of breast carcinomas. According to Royds et al<sup>30</sup>, reduced nm 23-H1 expression correlated with increasing grade of invasive duct carcinoma. However, its role in predicting lymph node metastasis is unclear. It has been shown that restoration of nm23 in breast carcinoma cell lines leads to a 50-90% reduction of nm 23 in breast carcinoma cell lines leads to a 50-90% reduction in the invasive potential of the same in human breast carcinoma cell lines<sup>29</sup>. In a study by Hartsough et al<sup>31</sup>, it was concluded that DNA methylation inhibitors like 5-Aza-2'-deoxycyclidine (5-Aza-CdR) can directly or indirectly cause both elevation of nm-23-H1 expression, thereby leading to decreased function in one aspect of metastasis, motility.

Thus, a wide range of newer markers are on their way to help oncologists predict outcome of breast cancer in individual cases. At times, a clinician is faced with the management of node-negative breast cancers due to lack of reliable prognostic parameters that distinguish patients who benefit from adjuvant chemotherapy. Since surgery alone forms a curative mode of management in approximately 70% of such cases, there is an impasse of possible over treatment or under treatment. Traditional CMF regime is added as a compromise, rather than newer effective regimes containing anthracyclins or taxanes. There are relatively recent reports on usage of uPA and PAI-1 for identifying high-risk patients in node negative cancer groups, for intensive effective treatment, in order to reduce groups, for intensive effective treatment, in order to reduce mortality<sup>32</sup>. Reduction of tumour cell mobility could be achieved by restoration of certain adhesion molecules like E-cadherin and proteins like nm-23-H1<sup>25,28</sup>. In this way, a research into development of newer markers, relating to predicting the propensity of tumor cells to metastasise, could form a useful basis to contrive specific drug targets for reducing mortality and morbidity, as a result of breast cancer.

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