

## Tumor Grading and Staging as Biology of the Transformation Event in Breast Carcinoma

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**Abstract:** Biology of breast carcinoma appears an integral composite of various proliferative and infiltrative events that characteristically are acquired through a malignant transformation event. Grading and staging of a given neoplasm are biologic attributes of the lesion that would incorporate not only proliferation and stromal infiltration but also a full range of inter- reactive changes also contributing to the formation of the lesion. Attempts at recapitulation of the native breast tissues appear to fall short of actual ductal differentiation in many breast carcinomas and this appears essentially reflected in an infiltration of the proliferating stroma that reacts desmoplastically to the neoplastic cells. It is in such terms that breast lesions ranging from adenosis to hyperplasias of variable atypia would relate to a stroma that proliferates concurrently with the epithelial component, as prominently seen with fibroadenomas. Intraductal epithelial lesions would attest to a differentiation of ductal-type structures. The role of hormonal cyclical activity would determine parameters of concurrent proliferation of the stroma and ductal epithelium in further progressing to a potentially infiltrative lesion.

**Key words:** Tumor, biology, breast carcinoma, neoplasm

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### INTRODUCTION

An integral operability of the whole endocrine system appears to be maintained throughout the body both under normal circumstances and also in disease. In breast carcinoma patients, there would operate retinoic acid receptor beta, epidermal growth factor receptors, p53, BRCA1 and BRAC2 as risk factors in disease development<sup>[1]</sup>. Hormones may possibly become abnormally interactive in carcinogenesis. Excessive estrogenic action may be conducive to carcinogenesis only in the full context of such operatively integral endocrine system in the body. Progesterone receptor status also determines breast carcinoma biology in terms particularly of estrogenic function.

Even the receptor status in breast carcinomatous cells appears to develop from a complex interaction involving estrogen and progesterone levels in the blood, a phenomenon that would contribute to genesis and early development of the primary breast neoplasm.

Age of first pregnancy and completion of a full pregnancy would protect against breast carcinoma in terms of a dramatic change in the hormonal milieu ; high progesterone levels in serum would, in particular, reduce the effectively direct influence of any excessively high estrogen levels in blood

mRNA expression data, when combined with proteomic information, may provide more detail regarding

breast carcinomatous cells that are altered in their anti-estrogen-resistant versus anti-estrogen-sensitive state<sup>[2]</sup>.

In the normal menstrual cycle, estrogen and progesterone phases would prove balancing phenomena directed towards tissues such as endometrium affecting its growth, differentiation to a secretory phase and also its shedding and subsequent replacement.

The ovary appears particularly implicated in a complementary evolutionary pathway that is both cyclical and interactive.

Indeed, it is the cyclical nature of phenomena of exposure of a particular organ such as the breast that would contribute either to protection or else to carcinogenetic influence of estrogen and progesterone action.

It is perhaps an abnormal cyclical bioavailability of estrogen that would prove critical in breast carcinogenesis. Estrogen induces proliferation and differentiation of breast tissues and also is related to the promotion of somatic genetic mutation. In this regard, microarray allows a global analysis of gene expression at the level of transcription<sup>[3]</sup>.

Estrogen may very well play a pivotal role in promoting a whole array of abnormalities in ductal epithelial cells. It is the potential for such abnormalities that would account for an increased incidence of breast carcinoma in women rather than in men<sup>[4]</sup>.

On the other hand, infiltrating lobular carcinoma, despite a quite favorable biologic phenotype, carries a prognosis that is no better than for patients with infiltrating ductal carcinoma<sup>[5]</sup>.

**Estrogen receptivity is a biologic marker of the malignant transformation event in breast carcinoma:**

Estrogen receptor status in breast carcinoma appears fully dependent on characteristics of the malignant transformation process, especially with regard to loss of differentiating potential of the neoplastic cells. Loss of hormonal receptor status in tumor cells might participate in carcinogenesis as part of an intrinsic genetic heterogeneity<sup>[6]</sup>.

Susceptibility of the premenopausal woman to the development of breast carcinoma may relate differently to the malignant transformation than in the postmenopausal woman. It is true to note that a drop in serum estrogen levels concomitant with the menopause may in some way affect the biologic attributes of breast carcinomatous cells, including insitu ductal carcinoma. HER-2/neu overexpression in the latter patients appears to contribute to a higher risk of recurrence<sup>[6]</sup>.

A tumor that has developed premenopausally and subsequently traversed the menopausal period may be little affected if the neoplasm was initially estrogen-receptor negative. Distinct pathogenic pathways in the development of either estrogen-receptor-positive or estrogen-receptor-negative tumors may be implicated. In this manner, some distinct histologic subtypes of breast carcinoma, such as medullary carcinoma, are generally estrogen-receptor-negative in contrast to many invasive lobular carcinomas. This would be suggestive of a close association of histologic subtype with estrogen-receptor status in terms of specific pathogenesis involving, for example, a hormonally-related reduction or promotion effect in carcinogenesis.

Particularly in the premenopausal woman, the absence of estrogen receptors on the tumor cells would prove an additional abnormality contributing to carcinogenesis. Estrogen receptivity would appear a marker of fundamental importance in understanding the susceptibility of breast ductal cells to undergo malignant transformation, either in the premenopausal or postmenopausal woman.

**Intrinsic biology of the malignant transformation event:**

It is noteworthy that definite tubular differentiation of a breast carcinoma is particularly significant in determining the grade of the neoplasm. Such a feature appears to be at least as significant as nuclear grading. Possible relationships may exist pathobiologically in the

histopathologic grading as patterns of neoplastic growth or nuclear grading.

The growth pattern of a breast carcinoma may reflect biologic attributes of the lesion, and the nuclear grade would reflect cytologic manifestations of the malignant transformation even as a predominantly nuclear phenomenon.

The specific growth pattern would reflect the nature of the specific transformation process leading to a carcinoma that incorporates not only the ability to differentiate but also the propensity to spread locally and involve vessels. Protease-activated receptors (PAR1), in particular, correlate with invasion by breast carcinoma, partly through the formation of focal contact complexes on activation and also through angiogenesis<sup>[8]</sup>. As such, it is a reflection more of the interactive effects of microenvironment with the cellular malignant phenotype. Cellular phenotype may translate mainly in terms of biologic behavior and of nuclear morphology.

The essential attributes of the malignant transformation process would give rise to growth patterns and nuclear grade that reflect biology of the carcinomatous lesion itself.

One might speak of the individual biologic nature of a breast carcinoma<sup>[9]</sup> as reflected in the initial malignant transformation. An essential spectrum of biologic variability would characterize the process of carcinogenesis giving rise to a particular neoplasm. The specific biology of a given neoplasm would strictly refer to the specific biology of the malignant transformation event giving rise to that neoplasm.

The malignant transformation process is an integral event in its own right with a point of origin, course, outcome and also susceptibility to factors that may increase its progression or reverse it.

It is on the basis of distinct features of the response or susceptibility of the malignant transformation event as a reflection of the intrinsic biology of the tumor cells that eradication of the neoplasm may prove biologically feasible.

**Tumor grade is determined by biology of the infiltrative component:**

The true grade of a primary breast carcinoma refers strictly to its invasive component. Grading is intrinsically applicable only in terms of the most rapidly progressive portion of the lesion.

Tumor grading reflects essentially a progressive criterion that evolves according to dynamic changes in proliferative rate and increasing anaplasia or de-differentiation as phenomena affecting many tumor types ranging from gliomas to carcinomas.

The tumor grade as a reflection of the progressive nature of the neoplasm would evolve and constitute essentially a short-term marker of biology of the neoplasm.

The validity of tumor grading would be fully dependent on infiltrative behavior of the neoplastic cells and would be less applicable as an assessment of the insitu breast carcinoma component.

Distinct parameters governing infiltrative behavior of malignant neoplasms in general would relate to tumor grading as a biologic criterion.

**Tumor differentiation reflects biologic attributes of the malignant transformation process:** It would seem that the essential malignant transformation process giving rise to a specific neoplasm would underlie integrity of innumerable mechanisms associated with the basic biologic cause of the lesion, especially as a progressive lesion with proliferative and metastatic potential.

**Neoplastic cells are prone to undergo necrosis due largely to their high proliferative rates:** Tumor necrosis is a recognized poor prognostic feature in primary breast carcinoma, with reference particularly to the first two years of followup of the patient. Such a phenomenon would appear to closely correlate with some basic pathologic attributes of the carcinoma.

Metastatic potential of a neoplasm would relate to necrosis of clones of tumor cells that are injured genetically or by hypoxia. Breast carcinomatous cells appear overtly susceptible to the action of calcium as a mediator of 1,25 dihydroxyvitamin D<sub>3</sub>-induced apoptosis<sup>[10]</sup>.

A highly proliferative carcinoma would tend to spread and metastasize early and also to show foci of tumor necrosis.

Necrosis of tumor cells may be possibly associated with a dissociation of the cells with a tendency to penetrate vessels. Matrix metalloproteinases are implicated in extracellular matrix degradation<sup>[11]</sup>. Tumor necrosis would thus prove a marker of an enhanced tendency for spread via the blood stream or lymphatics. Abnormal interactions between neoplastic cells, adhesion molecules and extracellular matrix proteins including dystroglycan complex are implicated<sup>[12]</sup>.

It would appear that an essential inability to adapt to a potentially varied range of homeostatic and nutrient requirements is a central attribute of tumor cells.

Tumor cells may undergo necrosis more readily due to their unresponsiveness or lack of adaptability to significant hypoxia or ischemia reflecting secondarily high proliferative rates.

**Proliferating neoplastic cells and reactive changes to these cells integrally make up the neoplasm:** It appears invalid to speak in terms of strictly independent prognostic variables in patients with primary breast carcinoma. The individual criteria of grading, both nuclear and histopathological, would be very intimately related to

each other in determining overall grading of the tumor. Expression profiling of RNAs or proteins would allow differentiation between histopathologically defined classes of breast carcinoma. Intermediate filament proteins, thymosins, and cathepsin C are particularly expressed<sup>[13]</sup>.

Stage and grade of the tumor are probably very closely inter-linked biologically. The great majority of primary breast carcinomas tend to be intermediate in grade.

Also, various parameters such as primary tumor size, status of regional lymph nodes, presence of lymphatic or blood vessel invasion intratumorally and peritumorally, even the presence of any lymphoplasmacytic infiltrates, would characterize any carcinomatous lesion. Multifocal carcinoma carries a higher risk of axillary lymph node metastases with earlier spread. This is not dependent on tumor volume alone<sup>[14]</sup>.

An appreciation of the full interactive pathobiology of these variables would somehow summate as an integral neoplastic lesion that progresses.

Certain parameters are more significant prognostically, and one main variable may counteract the effects of another variable.

Variable interactivity would hence appear a complex integration of quantitative and qualitative attributes with more or less prognostic significance. Increased stability of mRNA in particular, as influenced by ELAV (embryonic lethal abnormal vision)-like protein HuR, may be associated with higher tumor grade and increased cyclooxygenase-2 expression in breast carcinoma<sup>[15]</sup>.

Both the neoplastic cell proliferation and the reactive changes to the lesion all develop from the same individual host tissues.

**Desuppression of stromal regulation of epithelially derived cells rather than active infiltration by carcinomatous cells:** Lesions such as fibroadenomas, sclerosing adenosis, florid adenosis, microglandular adenosis, and other breast lesions such as tubular adenoma, blunt duct adenosis and adenosis in fibroadenomas may represent a primary disturbance in a postulated normal suppressive effect of stroma around lobular or ductal epithelium. These morphologically distinct lesions are characterized essentially by concurrent proliferation of stromal elements in association with a proliferating epithelial component. Angiogenesis, expressed as microvessel density, may constitute a biologic attribute of various proliferative breast lesions<sup>[16]</sup>.

It is this occurrence of concurrent stromal and epithelial proliferation that would be suggestive of interrelated deregulation between these two components. In particular, beta1 integrin receptors are of fundamental importance to transmembrane invasion by breast

carcinoma cells<sup>[17]</sup>. Widespread infiltration by carcinomatous cells would reflect also a stromal proliferation that characterizes the tumor very early in its inception.

**Intraductal carcinoma of the breast as failed stromal infiltration rather than as intraductal cell proliferation:**

The absence of a myoepithelial cell layer in cases of intraductal breast carcinoma, particularly with the comedo subvariant, would call into question the crucial distinction between proliferation of neoplastic cells within a pre-existing duct and the ductal growth pattern of carcinomatous cells. Ductal carcinomatous cells may actually possess the ability to form ductal-type structures as a direct result of the neoplastic proliferation itself. Ductal carcinomatous cells may be capable of forming ductal-type structures that closely resemble normal ducts except for the severe attenuation of myoepithelial cells or even their complete absence. Also, vitronectin derived from leakage of vessels may be implicated in breast-carcinoma interaction with the plasminogen activation system and integrins<sup>[18]</sup>.

Infiltrating tumor cells may very well represent a failure to closely recapitulate normal structural components of the breast, including ducts.

An intraductal lesion that is associated with foci of micro-invasion would represent a more aggressive lesion<sup>[19]</sup>.

The intraductal component might very well constitute a potentially infiltrative lesion but differentiation of the duct-type component would restrict such infiltration of the stroma.

Infiltrative behavior of the neoplastic cells would be better characterized as also a strict phenotypic feature rather than simply as a pathobiologic event. Data indicate that atypical lobular hyperplasia and insitu ductal carcinoma are molecularly similar to adjacent invasive ductal carcinoma<sup>[20]</sup>.

Local recurrence of intraductal carcinoma appears mainly a problem with the comedo type. This may be a reflection of a more aggressive nature of the lesion with a concomitant inability to closely re-capitulate ductal-type structures, and also a reflection of the tumor necrosis. In fact, quantitative and qualitative differences in protein abundance are found between breast intraductal carcinomas, reflecting histologic and pathologic status of the lesions (11). The comedo-type central zonal necrosis would perhaps be better interpreted as a fundamental tendency for whole subpopulations of tumor cells to be more susceptible to serious genetic damage and to subsequent necrosis.

The question of whether positive estrogen-receptor-status of tumor cells actually represents an intrinsically lower grade of neoplasia would be consistent with the high rate of positivity for estrogen receptors in intraductal carcinoma.

Hence, there may arise a situation whereby negative estrogen-receptor- status would be a direct index of the degree of differentiation and grade of the neoplastic cell pool.

The occurrence of combined lobular and ductal types of growth in a given neoplasm might be more a function of a cell of origin in the terminal ductule-lobular complex which prior to malignant transformation has still retained the ability to differentiate along either ductal or lobular pathways. However, specific changes in gene expression distinguish lobular from ductal breast carcinoma<sup>[21]</sup>.

Actual metastases to regional lymph nodes in some cases of intraductal carcinoma, especially of comedo type, would attest to an intrinsic tendency for duct-type structures to infiltrate rather than to a purely intraductal proliferation of cells.

**REFERENCES.**

1. Keen, J.C. and N.E. Davidson, 2003. The biology of breast carcinoma. *Cancer.*, 97: 825-833.
2. Huber, M., I. Bahr, J.R. Kretschmar and A. Becker *et al.*, 2004. Comparison of proteomic and genomic analyses of the human breast cancer cell line T47D and the antiestrogen-resistant derivative T47D-r. *Mol. Cell Proteomics.*, 3: 43-55.
3. Goldsmith, Z.G. and N. Dhanasekaran, 2004. The microevolution: Applications and impacts of microarray technology on molecular biology and medicine (review). *Intl. J. Mol. Med.*, 13: 483-495.
4. Gennari, R., G. Curigliano, B.A. Jereczek-Fossa and S. Zurrida *et al.*, 2004. Male breast cancer: A special therapeutic problem. Anything new?. (Review) *Intl. J. Oncol.*, 24: 663-670.
5. Arpino, G., V.J. Bartou, G.M. Clark and R.M. Elledge, 2004. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. *Breast. Cancer Res.*, 6:149-156.
6. Chang, J., G.M. Clark, D.C.Allred, S. Mohsin, G. Chamness and R.M. Elledge, 2003. Survival of patients with metastatic breast carcinoma: Importance of prognostic markers of the primary tumor. *Cancer.*, 97: 545-553.
7. Rodriguez, N.A., D. Dillon, D. Carter, N. Parisot, B.G. Haffty, 2003. Differences in the pathologic and molecular features of intraductal breast carcinoma between younger and older women. *Cancer.* 97: 1393-1403.

8. Yin, Y.J., Z. Salah, S. Grisaru-Granovsky and I. Cohen, *et al.* 2003. Human protease-activated receptor-1 expression in malignant epithelia: A role in invasiveness. *Arterioscler. Thromb. Vasc. Biol.*, 23:940-944.
9. Page, D.L., 2004. Breast lesions, pathology and cancer risk. *Breast J.*, 10 : 3-4.
10. Sergeev, I.N., 2004. Calcium as a mediator of 1,25-dihydroxyvitamin D3-induced apoptosis. *J. Steroid. Biochem. Mol. Biol.*, 90 :419-425.
11. Tam ,E.M., C.J. Morrison, Y.I. Wu, M.S. Stark, and C.M. Overall, 2004. Membrane protease proteomics: Isotope-coded affinity tag MS identification of undescribed MTI and matrix metalloproteinase substrates. *Proc. Natl. Acad. Sci., USA* 101 (18): 6917-6922.
12. Brennan, P.A., J. Jing, M. Ethunandan and D. Gorecki, 2004. Dystroglycan complex in cancer. *Eur. J. Surg. Oncol.*, 30: 589-592.
13. Tammen, H., H. Kreipe, R. Hess and M. Kallman, *et al.* 2003. Expression profiling of breast cancer cells by differential peptide display. *Breast. Cancer Res. Treat.*, 79: 83-93.
14. Andea, A.A., D. Bouwman, T. Wallis and D.W. Visscher, 2004. Correlations of tumor volume and surface area with lymph node status in patients with multifocal/multicentric breast carcinoma. *Cancer*, 100: 20-27.
15. Denkert, C., W. Weichert, K.J. Winzer and B.M. Muller, *et al.*, 2004. Expression of the ELAV-like Protein HuR is associated with higher tumor grade and increased cyclooxygenase-2 expression in human breast carcinoma. *Clin. Cancer Res.*, 10: 5580-5586.
16. Offersen, B.V., M. Borre and J. Overgaard, 2003. Quantitation of angiogenesis as a prognostic marker in human carcinomas: A critical evaluation of histopathological methods for estimation of vascular density. *Eur. J. Cancer*, 39: 881-890.
17. Berry, M.G., A.W. Gonde, J.R. Puddefoot, G.P. Vinson and R. Carpenter, 2003. Integrin beta1-mediated invasion of human breast cancer cells: an *ex vivo* assay for invasiveness. *Breast. Cancer*, 10: 214-219.
18. Aaboe, M., B.V. Offersen, A. Christensen and P.A. Andreasen, 2003. Vitronectin in human breast carcinomas. *Biochim. Biophys. Acta.*, 1638: 72-82.
19. Friedrich, M., R. Felberbaum, S. Kramer, R. Axt-Flidner and K. Diedrich, 2003. Ductal carcinoma in situ of the breast: Diagnosis and management *Onkologie.*, 26: 588-595.
20. Jeffrey, S.S. and J.R. Pollack, 2003. The diagnosis and management of pre-invasive breast disease: Promise of new technologies in understanding pre-invasive breast lesions. *Breast. Cancer Res.*, 5: 320-328.
21. Somiari, R.I., A. Sullivan, S. Russell and S. Somiari *et al.*, 2003. High-throughput proteomic analysis of human infiltrating ductal carcinoma of the breast. *Proteomics.*, 3: 1863-1873.
22. Korkola, J.E., S. DeVries, J. Fridlyand and E.S. Hwang *et al.*, 2003. Differentiation of lobular versus ductal breast carcinomas by expression microarray analysis. *Cancer Res.*, 63: 7167-7175.