Commentary

A new model for ductal carcinoma *in situ* suggests strategies for treatment

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Received: 5 June 2002
Revisions requested: 25 June 2002
Revisions received: 4 July 2002
Accepted: 8 July 2002
Published: 19 July 2002

Breast Cancer Res 2002, **4**:176-178

(Print ISSN 1465-5411; Online ISSN 1465-542X)

Abstract

Human ductal carcinoma *in situ* (DCIS) of the breast is now diagnosed quite frequently, due largely to the introduction of mammographic screening. It has been shown in a cell culture system that activation of c-erbB-2, but not the epidermal growth factor receptor, results in a DCIS-like phenotype. Since overexpression of c-erbB-2 occurs in 60% of DCIS, this suggests that it could be a target for treatment in this disease.

Keywords: ductal carcinoma *in situ*, growth factor receptor, tyrosine kinase inhibitor

Introduction

Breast pathologists have for many years been seeking to demonstrate a sequence of morphologically distinguishable cellular changes that define a linear sequence of transformation of normal breast ductal epithelium to the fully malignant state. It is still not entirely clear that this has been achieved, but a general model consists of premalignant changes (a variety of hyperplasias) followed by DCIS with subsequent invasive disease [1,2]. There are alternative views that these changes are neither linear nor do they always entail each intermediate phenotype [3]. Clinically, however, there are clear distinctions between these stages in terms of the risk to the patient, which affects the choice of treatment.

It has been hoped that the description of the underlying molecular changes accompanying these morphologically defined stages, and at least a partial understanding of their significance, would enable pathologists to confirm or refute their models and to allow individual cases to be placed in the sequence. This has to some extent been achieved; many molecular changes at the level of the DNA have been found in DCIS but not in premalignant disease. Somewhat disappointingly, however, little if anything is known definitively about any additional molecular changes that are presumed to result in the transition from DCIS to invasive disease. Research continues into this problem using methods that minimise preconceptions such as genomic analysis and gene expression analysis.

The molecular changes seen in DCIS include mutations in the *p53* gene [4], and gene amplification and overexpression at the protein level of the c-erbB-2 receptor tyrosine kinase [5]. There is a higher prevalence of both these events in more undifferentiated DCIS (known as comedo cancer in previous terminologies) [6], suggesting a functional significance, but this had not been definitively established.

Growth factor receptor activation leads to a DCIS-like phenotype

The hypothesis that growth factor receptor activation leads to a DCIS-like phenotype is tested in an article by

 DCIS = ductal carcinoma *in situ*; EGFR = epidermal growth factor receptor.

Muthuswamy *et al*. [7]. Two groups cooperated in that work, each bringing a different technological contribution. Mina Bissell, the doyen of breast cancer cell biologists, developed three-dimensional cell culture models of normal breast epithelial cells over several years [8]. Joan Brugge explored the mechanisms of growth factor receptor signalling and developed a system in which ectopically expressed receptors can be selectively activated at will by the addition of drugs [9].

The c-erbB-2 protein, which is overexpressed in DCIS, is a member of the epidermal growth factor receptor (EGFR) family. This family consists of the EGFR, c-erbB-2 and two more receptors (c-erbB-3 and c-erbB-4) [10,11]. Each protein has an extracellular domain, which in some cases recognises an activating ligand, a transmembrane domain and a cytoplasmic domain with protein tyrosine kinase enzyme activity. Ligand binding induces receptor dimerisation, activation of the kinase and phosphorylation of tyrosine residues in the cytoplasmic domain. The phosphorylated protein then recruits and activates a variety of intracellular second messenger systems that induce changes to the cytoskeleton and that can stimulate the rate of cell division.

Brugge and colleagues [9] have constructed altered receptors in which the extracellular domain is derived from the nerve growth factor receptor and cytoplasmic domains from either the EGFR or the c-erbB-2 receptor. In addition, however, there is a sequence fused to the C-termini of the constructs derived from the FK506-binding protein. The membrane-permeable bivalent compound AP1510 binds to this region and induces dimerisation, phosphorylation and intracellular signal transduction, allowing selective, acute or chronic receptor activation [8].

In the study reported in *Nature Cell Biology* [7], Muthuswamy *et al*. introduced the chimeric EGFR and c-erbB-2 separately into MCF10A cells (immortal but untransformed human mammary epithelial cells) and established stable lines expressing moderate levels of either receptor. In the absence of AP1510, these grew in three-dimensional cultures, indistinguishable from the untransfected cells, as structures highly reminiscent of mammary epithelial acini. Each acinar structure contained approximately 20–40 cells with basally located nuclei and a lumen. Staining for cell adhesion molecules showed that the cells were polarised and surrounded by a collagen layer similar to a basement membrane.

The chimeric EGFR was able to stimulate growth of the MCF10A cells in monolayer culture, demonstrating that it was functional, but activation by the addition of AP1510 had no effect on cell behaviour or morphology when the cells were grown in three-dimensional cultures. Strikingly, however, activation of the c-erbB-2 protein caused marked changes in the polarised acinar structures. These lost their polarised organisation and grew into structures described as 'consisting of multiple acinar-like units with filled lumina'; in some cases, a hundred times the size of untreated structures. Muthuswamy *et al*. [7] conclude that the EGFR and c-erbB-2 'have different abilities to affect polarised and growth arrested acini' and that 'acute activation of c-erbB-2 results in the generation of multi-acinar structures that share properties with structures associated with carcinoma in situ'.

Significance of the results for future treatment

DCIS is often treated surgically by mastectomy rather than breast-conserving surgery since it is commonly multifocal and sometimes extensive [12–14]. Failure to completely remove the disease is associated with a low but significant probability of the subsequent development of invasive cancer. It would be highly desirable to identify effective, low-toxicity adjuvant therapy that might justify the use of more limited surgery.

The formal demonstration by Muthuswamy *et al*. that overexpression of c-erbB-2 in normal breast ductal epithelial cells leads to a DCIS-like phenotype provides strong support for studying such treatments in more detail in model systems. In independent work, Bundred and coworkers [15] have developed a xenograft system in which small pieces of human DCIS are implanted subcutaneously in nude mice where the tissue survives and grows. Treatment of the mice with herceptin (a monoclonal antibody to the human c-erbB-2 protein now used for the treatment of some invasive breast cancers) did not have any effect, but treatment with iressa (a small-molecule tyrosine kinase inhibitor that acts principally on the EGFR but that will also, at higher concentrations, inhibit c-erbB-2) did inhibit cancer cell growth. It is also possible that heterodimers between EGFR and c-erbB-2 are important in signalling in this context, and that these will also be inhibited to a degree by the tyrosine kinase inhibitor.

The presented results suggest that antibodies may not be the first method of choice for treatment of DCIS of the breast because they are large molecules and may have limited access to the interior of the cancer-filled duct. Orally available small-molecule tyrosine kinase inhibitors are more promising, and several pharmaceutical companies have selective c-erbB-2/EGFR inhibitors in development. Should these studies prove encouraging, there would be a rationale for testing these inhibitors in cases of ductal carcinoma of the breast in clinical trials.

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