A developmental hypothesis to explain the multicentricity of breast cancer

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Abstract

IN THIS ARTICLE THE AUTHOR PROPOSES that the multicentricity of breast cancer might be explained by a developmental hypothesis. Genetic alterations ("hits") occurring in epithelial stem cells during the development of the breast may be transmitted to populations of daughter cells during growth. As a result, areas of the breast may be predisposed to malignant transformation with the occurrence of further genetic hits. Areas with the same predisposition should be anatomically connected, and earlier hits during breast development should result in larger areas of predisposition. The multicentricity of breast cancer would be explained if multiple lesions — monoclonal for the predisposing genetic hit and polyclonal for subsequent hits — developed within a predisposed area. Multiple lesions arising from the spread of disease by extension would be expected to share many genetic hits. The author discusses the implications that further evidence supporting the developmental hypothesis would have for the prevention and treatment of breast cancer.

Résumé

DANS CET ARTICLE, L'AUTEUR SOUTIENT qu'il est peut-être possible d'expliquer par une hypothèse liée au développement le caractère multicentrique du cancer du sein. Des altérations génétiques survenues dans les cellules souches épithéliales au cours du développement du sein peuvent être transmises à des populations de cellules filles au cours de la croissance. Il s'ensuit que des zones du sein peuvent être précédées à une transformation maligne s'il se produit d'autres altérations génétiques. Des zones qui ont la même prédisposition devraient être reliées anatomiquement et des altérations plus précoces au cours du développement du sein devraient produire des zones de prédispositions plus étendues. La multicentricité du cancer du sein serait expliquée si des lésions multiples — monoclonales dans le cas de l'alteration génétique prédisposante et polyclonales dans les cas des altérations subséquentes — font leur apparition dans une zone de prédisposition. On s'attendrait par conséquent à trouver de nombreuses altérations génétiques communes dans des lésions multiples découlant de la propagation de la maladie. L'auteur discute des répercussions que d'autres données probantes à l'appui de l'hypothèse liée au développement auraient sur la prévention et le traitement du cancer du sein.

Breast cancer tends to be multicentric.1–4 Multicentricity refers to the "presence of separate independent foci of carcinoma within the breast — separate from the lesion which is clinically or mammographically evident."5 These separate foci reflect "de novo development of malignant epithelium" and are distinguished from multifocal carcinomas, which can result from intraductal spread from a single, primary carcinoma.6

Operationally defined, lesions are considered multicentric if situated in different quadrants of the breast or more than 5 cm apart, and multifocal if situated in the same quadrant as the primary lesion or less than 5 cm apart.7 However, the concept of quadrants is surgical and does not reflect the anatomical distribution of ductal systems.8

Education

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Patterns of disease

Carcinoma in situ illustrates the tendency toward multicentricity. Ductal carcinoma in situ (DCIS) can involve large areas of the breast, a pattern that tends to be associated with multicentric foci. Some breasts with invasive carcinoma have large numbers of DCIS lesions. Lobular carcinoma in situ (LCIS) is also characterized by multicentricity. Its distribution is nonrandom, tending to involve radial mammary sectors, often skipping contiguous radii, which suggests that LCIS may be distributed along ductal systems.

Fisher and associates found microscopic foci of multicentric cancer in 121 (13.4%) of 904 breasts surgically removed for the treatment of invasive cancer. Although the frequency of multicentricity has had implications for the treatment of breast cancer, it also requires explanation in terms of the pathogenesis of the disease, as do the patterns of disease described above. Understanding the pathogenesis of breast cancer may help to prevent and treat the disease more effectively.

Etiology

All cancers are caused by altered gene expression. The genetic alterations ("hits") are transmitted from transformed cells to their daughter cells. Genetic hits can cause malignant transformation by activating oncogenes or inactivating tumour suppressor genes. Many such hits have been described for breast cancer. Oncogenes can be activated by chromosomal rearrangements, increases in chromosome number, gene amplification and point mutations. Oncogenes act dominantly: one activated allele is tumourigenic. Tumour suppressor genes can be inactivated by chromosomal deletions or by point mutations. These genes act recessively: one functioning allele (the heterozygous condition) is protective, and only the loss of function of both alleles (loss of heterozygosity [LOH]) is tumourigenic.

Women can inherit mutated tumour suppressor genes, increasing their risk of breast cancer. The genetic hits that initiate breast cancer may occur early in life, while the breast is still developing. The effects of these events are modulated by other factors. A high fat intake during childhood results in an earlier age at menarche, which increases risk, whereas high levels of physical activity delay menarche, which reduces risk. Subsequent events can modulate risk further. An early age at first full-term childbirth reduces risk, whereas a later age increases risk. Weight gain in adulthood increases risk, whereas high levels of physical activity reduce it. Alcohol consumption, oral contraceptive use and hormone replacement therapy increase risk. Tobacco use during adolescence may increase risk among some women, although there is conflicting evidence.

Given the factors that affect the risk of breast cancer, opportunities to reduce risk and prevent breast cancer may exist throughout life. I suggest that the multicentricity of breast cancer may be a consequence of the prolonged period required for the breast to complete its development. If the developmental hypothesis is correct, it would reinforce the notions that the pathogenesis of breast cancer may be a lifelong process and that efforts to prevent the disease should begin in childhood.

Explanatory hypotheses

One hypothesis is that both multicentric and multifocal breast cancer result from intraductal spread, in which case the lesions should be genetically similar. An alternative, but not mutually exclusive, explanation is the developmental hypothesis: genetic hits to epithelial stem cells during breast development may be transmitted to daughter cells during growth, causing areas of the breast to be predisposed to malignant transformation with the occurrence of further genetic hits. The multicentricity of breast cancer would be explained if multiple lesions — monoclonal for the predisposing genetic hit and polyclonal for subsequent hits — developed within a predisposed area.

Both of these hypotheses predict that breast cancer should follow the anatomical distribution of the ducts. If multiple lesions developed from independent events, unrelated to spread or an underlying predisposition, they should be randomly distributed within the breast and genetically dissimilar.

The supporting evidence

Intraductal-spread hypothesis

The comedo type of DCIS is characterized by tumour cells arising in ducts and extending along them. Although multicentric and multifocal cancers have not yet been studied with 3-dimensional reconstruction, this technique was used to demonstrate that unicentric invasive breast cancer can spread by extending through the ductal tree. Such spread could result in the development of tumours at multiple sites within a breast.

Noguchi and colleagues’ studied X-chromosome inactivation to determine whether multiple breast carcinomas arose independently or spread from a single lesion. Very early in embryogenesis, before organogenesis, one of the two X chromosomes in every cell of the female embryo is randomly inactivated — daughter cells always inherit the same inactive X chromosome. Noguchi and colleagues...
studied 3 patients with 3 or 4 separate cancer foci; in each patient the same X chromosome was inactivated in all foci. This was interpreted as evidence that the foci arose from spread from the primary tumours.

Teixeira and colleagues carried out cytogenetic analyses on multifocal breast cancers and found clones with similar abnormalities in different lesions, indicating spread.

Clinical evidence suggests that intraductal spread accounts for lesions arising near resected invasive carcinomas. Among patients treated with breast-conserving surgery, the presence of an extensive intraductal component predicts local recurrence, which usually occurs near the primary tumour. Quadrantectomy, excising the involved ductal system, and breast irradiation to treat residual disease have been recommended.

**Developmental hypothesis**

For simplicity, I have restricted the developmental hypothesis to consideration of events within a single breast, since the breasts develop separately. The effects of inherited genetic hits will not be considered, since they involve all breast cells.

The breasts begin as dimples on the skin of the chest 6 weeks after conception (Fig. 1a). Infolding occurs, forming ducts that extend into subcutaneous tissue (Fig. 1b and 1c). At puberty the ducts grow and divide dichotomously, forming club-shaped terminal end buds. After menarche, alveolar buds appear, their numbers increasing with age.

If a cell in a dimple acquires a genetic hit that is not repaired (Fig. 1d), its daughter cells could carry the genetic hit into multiple developing ductal systems (Fig. 1e and 1f). If a similar event occurs in a cell in the tip of one of the ducts (Fig. 1h), the daughter cells in that duct could carry the genetic hit, but adjacent ducts might not (Fig. 1i) — “genetic mosaicism.” Earlier genetic hits (Fig. 1d) should result in larger altered areas situated closer to the orifice of the duct at the nipple.

A study of X-chromosome inactivation in normal female breast tissue suggests that the breast develops in such a way that the above events could occur. Tsai and associates showed that the epithelium is a mosaic of discrete regions in which all cells have the same inactive X chromosome, suggesting that each region developed from the same stem cell. Entire lobules and large ducts had the same X chromosome inactivated. These findings could provide an alternative explanation to that of intraductal spread postulated by Noguchi and colleagues. The lesions could have arisen within a population of altered cells that arose from one stem cell, as the result of genetic hits that occurred after X-chromosome inactivation.

If one or more genetic hits were acquired early in breast development and were transmitted to daughter cells during growth, the areas that would develop from the cells with the genetic hit(s) could be predisposed to malignant transformation with further hits. Moolgavkar

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*Fig. 1: Sequence of normal breast development in utero and 2 possible sequences illustrating developmental hypothesis to explain multicentricity of breast cancer. Normal development (top row): (a) dimples develop on skin on either side of chest; (b) as dimples deepen, infolding occurs and ducts are formed; (c) ducts extend into the subcutaneous tissue. Development with genetic alteration (hit) at very early stage (middle row): (d) cell in primordial dimple acquires genetic hit (dot); (e and f) daughter cells carry hit into 3 developing ductal systems. Development with genetic hit at later stage (bottom row): (g) primordial dimple develops normally; (h) cell in tip of primordial duct acquires genetic hit; (i) daughter cells carry hit into parts of duct that developed later but not into adjacent ducts.*
and Knudson\textsuperscript{36} developed a mathematical model on the premise that cancer can develop from 2 genetic hits. They explained that the expansion of the population of cells with one hit would increase the probability of the second hit required for malignant transformation. Their model predicted the shape of age-specific incidence curves of breast cancer in 6 countries.\textsuperscript{37}

The clonal expansion of cells with such acquired genetic hits conforms to the definition of tumour promotion.\textsuperscript{38} Although the potential importance of increased cell division in carcinogenesis and epidemiologic studies of the effects of ionizing radiation in women supports the notion that developing breasts are more vulnerable to carcinogens,\textsuperscript{40,42} the possible tumour-promoting role of normal growth is only beginning to be appreciated.\textsuperscript{41,45}

Evidence from experimental studies of chemical carcinogenesis and epidemiologic studies of the effects of ionizing radiation in women supports the notion that developing breasts are more vulnerable to carcinogens.\textsuperscript{40,42} After the first full-term pregnancy, epithelial stem cells differentiate and become less susceptible to carcinogenic agents.\textsuperscript{41,42}

Multicentric lesions occurring within a predisposed region should share the initial predisposing genetic hit(s) and possess additional hits that occurred subsequently at different loci. O’Connell and colleagues\textsuperscript{43} found that 50% of benign proliferative breast lesions and 80% of DCIS lesions shared specific LOH patterns with more advanced lesions from the same breast. That the shared LOH patterns occurred in multiple anatomically and histologically distinct lesions could be explained by an early LOH expanded by normal breast growth.

Deng and associates\textsuperscript{46} compared the DNA from breast cancer tissue with the DNA from adjacent, phenotypically normal terminal ductal-lobular units (TDLUs), separated by microdissection. In 8 of 30 cases LOH was found in both the cancerous tissue and the adjacent TDLUs. In all 8 cases the same allele was missing in both types of tissue. Distant TDLUs showed no LOH. Deng and associates concluded that the LOH may indicate “a localized predisposed region from which the cancer arises [from] genetic aberrations [acquired] prior to mammary gland differentiation.” Apparently, the loss of both functioning alleles of a tumour suppressor gene is compatible with maintaining the normal phenotype: more than 2 events must be required for malignant transformation. Additional evidence for the existence of clones of predisposed cells within mammary glands comes from animal experiments and a study of microsatellite alterations in atypical breast hyperplasia.\textsuperscript{47,48}

**Continuing the search for evidence**

Further research is needed to determine whether the developmental hypothesis is correct. The hypothesis would be falsified if the parts of the localized predisposed regions were not anatomically connected to the same ducts. Dyes injected into cannulated ducts could demonstrate the presence or absence of connections. Using this technique, Love and Barsky\textsuperscript{49} found that ductal systems “do not anastomose. The individual ductal systems . . . intertwine like branches of a tree. . . . [D]ucts which appear adjacent on a two-dimensional tissue section do not necessarily belong to the same ductal system.” Fluorescent in situ hybridization, with the use of specific DNA probes labelled with fluorescent dyes applied to tissue sections, could be used to demonstrate the distribution of LOH or gene amplification among breast ducts.\textsuperscript{50} Polymerase chain reaction with in situ hybridization could also be used to reveal more subtle genetic hits, such as point mutations.\textsuperscript{51}

The emergence of further convincing evidence supporting the developmental hypothesis would have implications for both the prevention and treatment of breast cancer. It would emphasize the need to begin preventive efforts early in life and to protect young women against carcinogenic exposures. The margins of breast tissue resected for tumour could be examined with molecular genetic techniques to identify patients who may be predisposed to additional tumour foci.\textsuperscript{52} Eventually, with the development of suitable imaging techniques, it might be possible to excise the entire ductal systems involved in carcinoma in situ while sparing uninvolved areas of the breast.\textsuperscript{53}

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**References**


