

# Lobar Anatomy of Human Breast and Its Importance for Breast Cancer

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I have heard a good anatomist say, “the breast is so complicated I can make nothing clear of it.”

Astley Paston Cooper, *On the Anatomy of the Breast* (Cooper 1840).

When a powerful new method emerges the study of those problems which can be dealt with by the new method advances rapidly and attracts the limelight, while the rest tends to be ignored or even forgotten, its study despised.

Imre Lakatos, *Proofs and Refutations: The Logic of Mathematical Discovery* (Lakatos 1976).

## 2.1 Introduction: Anatomy of Human Breast as a Subject of Scientific Study

Some believe that anatomy has experienced the fate described by Lakatos (Marusič 2008). But, while molecular explanations are more highly favored in biology, no single “thought-style” (Fleck 1979) can explain a process as complex as breast cancer, influenced by events at every scale from molecules to society and the environment, occurring over time scales from less than a second to a human lifetime. This chapter addresses a neglected order of breast organization which deserves closer attention: its partitioning into *lobes*.

Reasons for this neglect include a human tendency not to notice gaps in our knowledge. Anatomist and

pioneer senologist Sir Astley Cooper may also be partly responsible: a serious commentator as recently as the mid-twentieth century could suggest that Cooper had said all that needed to be said about the large-scale anatomy of the breast (Brock 1952), including its lobar structure.

Cooper’s own comment (“it was absolutely necessary to give an account of the natural structure of the breast, before its morbid changes could be properly explained or understood”; Cooper 1840) was true then, and is true today. But while *On the Anatomy of the Breast* still contains more original data about lobar organization of human breast tissue than almost any twentieth or twenty-first century primary source, Astley Cooper did not say the last word on the subject, and would, I think, have been surprised by such an idea; so it is satisfying that new work on morphology of human breast is being undertaken (Ramsay et al. 2005; Going 2006; Geddes 2007; Rusby et al. 2007).

Cooper was one of the last first-rate anatomists not also to be a microscopist, though he did see it in use (Cooper 1843). Joseph Jackson Lister had invented the achromatic microscope in the 1820s, but the rise of histology as a discipline (von Gerlach 1848) waited on theoretical and practical developments including cell theory (Schleiden and Schwann), Virchow’s insight that all cells arise from preexisting cells (“*omnis cellula e cellula*”), development of the microtome by Wilhelm His, senior, and improved staining, all of which emphasized *the cell* as the fundamental unit of tissue organization.

The molecular revolution of the twentieth century may, then, seem to place large-scale aspects of anatomy at two removes from contemporary biological science, but the breast parenchyma and its stroma remain the theater of the cellular and molecular dramas of normal mammary development

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and breast disease during infancy, adulthood, and old age, and as these processes occur on multiple spatial scales, including that with which we are concerned (the lobe), its present relevance is greater than ever.

## 2.2 Limitations of Classical Microscopy

While microscopy is well adapted to the study of microscopic entities, including small portions of human breast tissue, and an entire murine mammary gland can be easily embedded in a single block of paraffin wax for histology, only a tiny portion of a complete human breast is easily handled like this. Giant histological sections do address this problem, with advantages recognized by breast pathologists from Cheatle (1920) to Eusebi (Foschini et al. 2006) and Tot (Tot et al. 2000), but their use is routine only in a few dedicated laboratories.

Also, histology is naturally two dimensional: 3D information can be extracted only with considerable effort, especially for larger objects. Virtuoso serial section studies of 3D anatomy and embryology have been performed from the time of His onwards, but the knotty problem of describing the lobar anatomy of the breast has received relatively little attention, being correctly perceived as difficult (Osteen 1995); incorrectly as having been done already (a perception suggested by its neglect), and perhaps also as lacking particular significance for breast cancer, which in recent years has been widely seen as a disease of the mammary lobules.

### 2.2.1 The “Lobular Origins” Hypothesis of Breast Cancer

It has never been obvious where breast cancers come from. Early investigators saw cells looking like cancer cells lining ducts and glandular acini of cancer-associated breast tissue (Cheatle 1906, 1920). It seemed plausible that these cells were progenitors of invasive breast carcinomas, and indeed, they could sometimes be observed apparently in the act of exiting ductal or glandular structures to invade the adjacent stroma.

Even before Foote and Stewart (1941) published their definitive description of lobular carcinoma in situ (LCIS), lesions recognizable as LCIS had also been illustrated (e.g., plate II in Cheatle and Cutler 1931:162). Ewing (1940:563) distinguished between “duct carcinoma arising from the lining cells of ducts” and “acinar carcinoma arising from the epithelium of the acini.”

Given morphologically distinct ducts and lobules of the mammary parenchyma, and site-of-origin as a classifier for neoplasia, the formula of ductal and lobular carcinoma in situ, arising from epithelium of ducts and lobules respectively, as precursors of ductal and lobular invasive carcinoma is neat, tidy, and plausible, but seemed to have been dealt a severe blow by studies of subgross breast anatomy in the 1970s onward, which emphasized that many duct-like structures colonized by neoplastic cells were actually enlarged and distorted (“unfolded”) lobules (Wellings et al. 1975). This has given rise to a frequently stated belief that breast cancer is of lobular origin, which appears to contradict the notion of a “sick lobe.”

This “lobular origins” viewpoint was strongly endorsed by John Azzopardi in his discussion of work by Wellings and colleagues in his influential book *Problems in Breast Pathology* (Azzopardi 1979). He observed “the first fundamental conclusion that stems from this superb work is that the vast bulk of breast disease, much of which has been traditionally regarded as of ductal origin, is in fact of lobular and/or terminal duct origin.” Other workers have published data interpreted to support the lobular hypothesis (Ohuchi et al. 1985; Faverly et al. 1992), and the degree to which it has been accepted by the senological community as a “scientific fact” can be confirmed by any student of the literature from how often it is asserted without citation of primary data to support it.

A key argument advanced by proponents of the lobular origins hypothesis is that structures considered to be ductal by workers advocating a ductal cancer origin were really expanded lobules which the pioneers had failed to recognize. It is difficult to assess the degree to which this is true, but insightful observers like Lenthal Cheatle were perfectly able to recognize even highly deformed lobules, and illustrate them in their works (e.g., Cheatle 1920:288, Fig. 204). Cheatle also recognizes that discriminating between abnormal ducts and lobules may be difficult, and notes the utility of serial sections in making this distinction. The same

publication also illustrates a case (page 290, Fig. 208) in which, based on his study of giant histological sections, Sir Lenthal explicitly proposes a lobar distribution of epithelial proliferation leading to cancer.

### 2.2.2 A Critique of the “Lobular Origins” Theory

Subgross studies *do not* reveal the origins of breast cancer.

If we accept the proposition that neoplasia are monoclonal (Fialkow 1976), we accept that all neoplastic cells in a tumor (not counting stromal, inflammatory, and other nonneoplastic cells) are descendents of a somatic cell, which have acquired (epi)genetic changes sufficient to confer a neoplastic phenotype. (We may note that this point of view is not universally accepted (Parsons 2008), but shall not explore the arguments here.)

If tumor monoclonality is correct, to identify where breast cancers begin could be easily taken to mean this: to identify where in the mammary parenchyma that cell was located from which all the neoplastic cells of a particular cancer are descended, maybe years or decades even before that cancer became detectable clinically or by screening. It is safe to say that this has never been achieved for even one case of breast cancer, let alone often enough to allow anything to be said about the origins of breast cancer in general, and I intend to argue there is in reality *no such thing* as a “founder” cell to which all the cells in a cancer can be traced back in anything other than a trivial sense.

### 2.2.3 What Does It Mean to Speak of a Cancer’s “Origin”?

Further reflection shows how elusive this idea is.

In the standard model, invasive breast cancer and most other cancers are usually thought to be clonally descended from proximate precursor lesions variously called severe dysplasia, high-grade intraepithelial neoplasia, or carcinoma in situ (essentially synonymous terms for lesions with which most invasive carcinomas are intimately associated) (Sinn 2009). If so, the origin of the in situ carcinoma is as legitimate a “beginning”

of the whole process as the moment a tumor cell first broke through a basement membrane.

But the same argument applies to in situ carcinomas: many of these arise on a background of atypical hyperplasia, itself likely to be clonally descended from still earlier ancestors, perhaps hyperplasias of usual type; columnar cell change; otherwise altered lobules; normal-looking but abnormal parenchyma; truly normal parenchyma; or even a remote ancestor cell in existence before the breast even began to form, early in fetal life; or the zygote itself, in which a germ line mutation in *TP53*, *BRCA1*, *BRCA2*, *CDH1*, *STK11*, or *PTEN* would already represent a major step on the long road to breast cancer (Campeau et al. 2008).

The common Icelandic 999del5 *BRCA2* mutation has been causing breast cancer since the mid-sixteenth century (Thorlacius et al. 1996). Many women who have experienced breast cancer would never have done so had they not inherited this defective gene. In what sense, exactly, would it be incorrect to say these cancers began in the sixteenth century, or even earlier?

### 2.2.4 Cancers Have No “Beginning” in Time or Space

To sum up, cancers do not begin at a definable point in time or space. Even if a one-celled cancer was conceivable, which it isn’t, it would only be apparent after many cellular generations that is what it was, when there is no prospect that unique individual cell can be identified. Even if the existence of such a cell could be inferred from indirect evidence, the chain of molecular causation extends over many cellular generations, into a remote past, and there is no reason to confer privileged status on the last to occur of the set of mutations driving the transformed phenotype, over all others: the first of them to occur may be as necessary as the last for the phenotype, and on that basis have a better claim to have begun the process of cancer formation.

To accept these arguments is to accept that “breast cancer *begins* in the terminal duct lobular units” is a *meaningless statement* which posits nothing with which one might agree or disagree. It will be obvious that the statement “breast cancer begins with a sick lobe” is equally meaningless: which is not to say “sick lobes” (and lobules) are not deeply and intimately connected with the evolution of breast cancer in an individual.

### 2.2.5 Another Reason for Doubting the “Lobular Hypothesis”

There are usually many abnormal lobules in both cancer-bearing and noncancerous breasts. Jensen, Rice, and Wellings (Jensen et al. 1976) found a median of 15 atypical lobules in cancer-containing breasts (3rd quartile 51, maximum 225) and a median of 5 in breasts without cancer (3rd quartile 11, maximum 91).

Each of these atypical lobules *must* have arisen independently, *if* their existence is to support a “lobular origin” theory of breast cancer, because, if they do share some common causation, their mere existence implies the involvement of a greater portion of breast parenchyma (necessarily including ducts as well as lobules) in that process.

At most, we may perhaps say that changes associated with breast cancer visibly affect lobules earlier than other parts of the mammary parenchyma.

A “lobular origins” theory *would* be supported by the existence of monolobular neoplasia, analogous to aberrant crypt foci or monocryptal adenomas in colon, which implicate the colonic crypt as a niche in which a founder cell was resident (Preston et al. 2003). Establishing the existence of isolated lobules colonized by recognizably neoplastic cells, but not involving any other lobules in the neighborhood of the abnormal one would require careful examination of all adjacent lobules not merely in the plane of a single histological section, but in other planes as well. This, subgross studies such as those of Wellings and others, including even the careful 3D studies of Ohuchi (Ohuchi et al. 1984a, b, 1985; Ohuchi, 1999) and Holland (Faverly et al. 1992) which found in almost every case multiple abnormal lobules, have not done. And even if they had, it would still not exclude the existence of a field in which a phenotypically silent mutation was present.

## 2.3 Evolution of Breast Cancer Precursors: Clonal Expansion

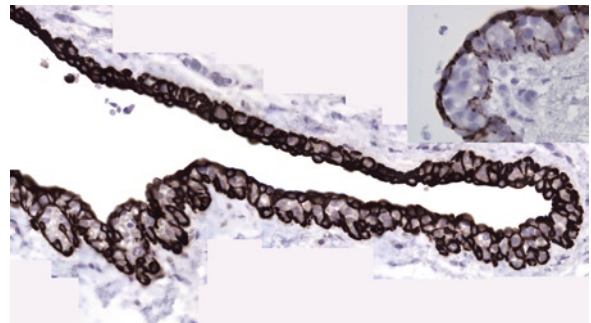
So, we should forget about where breast cancer or its putative precursors “begin” and ask instead, how do they evolve? The distribution in space of morphologically and genetically abnormal parenchymal cells in a cancer-containing breast is clearly informative about events in the evolution of a breast cancer.

The evolution of neoplasia in Barrett’s esophagus, which is more accessible to direct observation over time than breast parenchyma, affords an instructive comparison. In Barrett’s esophagus, a clone of cells with a mutation giving a selective advantage is capable of colonizing >10 cm of a long-segment Barrett esophagus, and of out-competing other clones (Maley 2007).

If this process is completed, the mutation is said to have “gone to fixation” by a “selective sweep” (Maley et al. 2004). If such events also occur in the breast, they would be expected to create a “sick lobe” if confined to one lobe, analogous to a “sick segment” of Barrett’s esophagus. Cells with a markedly abnormal morphological phenotype, such as those of DCIS and LCIS, can be observed in the act of colonizing preexisting parenchymal structures including ducts and lobules, more or less extensively (Fig. 2.1), and DCIS does sometimes appear to colonize whole lobes.

This can occur in a continuous and possibly also a discontinuous fashion (Faverly et al. 1992), but in both cases, expansion is likely to be confined to parenchyma of the lobe in which the abnormal clone of cells is expanding, at least prior to the emergence of any invasive elements capable of transgressing basement membranes.

In the absence of an obviously abnormal morphological phenotype, such clonal expansion would be harder to observe. However, the expansion of a clone of cells at increased risk of completed neoplastic



**Fig. 2.1** Neoplastic epithelial cells can infiltrate widely within the epithelial bilayer of mammary ducts and lobules. In this immunostained duct, both basal and luminal cells express cyokeratin 5 strongly. Pale, moderately atypical, sometimes vacuolated cells individually and in clumps characteristically colonize the virtual space between basal and luminal cells. This example of so-called “Pagetoid spread” by atypical lobular hyperplasia was an incidental finding following surgical reduction of a breast contralateral to a previously treated cancer. Inset: Loss of E-cadherin expression is also characteristic of these cells

transformation could manifest itself indirectly by the emergence of *in situ* or invasive neoplasia throughout a field of genetically altered but morphologically normal or minimally abnormal cells.

Another possibility is that a mutation in a cell of the prepubertal breast or even the fetal breast anlage could give rise to a clone of cells from which a large part of the mature breast might be derived, and if that mutation was associated with increased risk of neoplastic transformation, it could manifest itself as a field of increased risk in which multifocal mammary neoplasia might develop.

A prescient, but little-noticed paper (Sharpe 1998) looking at breast cancer origins had received only five citations by October of 2009 (ideas which do not resonate with fashionable thought-styles are not attacked, but ignored). In it, Sharpe suggests that breast cancer multifocality could arise by intraductal spread of abnormal precursor cells or by a developmental mechanism in which anatomically connected branches of developing mammary duct trees might be populated by cells derived from a mutant precursor arising early in development. These first of these ideas would correspond to an initially healthy lobe becoming sick, and the second to a lobe “born sick” *ab initio*.

Both could be true. The maximal sensitivity of the human breast to radiation-induced carcinogenesis – before the age of five – is at least compatible with the latter concept. Females exposed to radiation in the atomic bombings at Hiroshima and Nagasaki experienced an increased incidence of breast cancer. This excess relative risk (ERR) was greatest (4.6) for women exposed as very young girls (0–4 years) (Tokunaga et al. 1994). Land (1995) found this surprising, given the much smaller mass of breast epithelium in this age-group, but women who were irradiated as infant girls for “thymic enlargement” have a comparable ERR (3.6) for breast cancer, so the susceptibility of the infant female breast to breast cancer initiation by ionizing radiation is well established, and the mutagen N-nitroso N-methylurea is likewise more carcinogenic to the mammary gland of sexually immature than of mature rats (Ariazi et al. 2005).

A radiation-induced mutation in a mammary precursor cell could be inherited by many descendents following thelarche, even perhaps to the extent of being disseminated throughout a complete lobe. The well-known ability of a single precursor cell to reconstitute an entire rodent mammary gland (Kordon and Smith

1998) highlights the potential for one cell to create an extensive glandular domain for itself.

While multifocal neoplasia might also occur following exposure of all the parenchyma of the breast to a common environment promoting neoplasia, e.g., an external carcinogen or an endocrine influence, one would expect such a process to be nonlobar.

## 2.4 The Need for Whole-Breast Parenchymal Visualization

To investigate fully the evolution of breast neoplasia up to cancer formation in its parenchymal context requires the ability to visualize morphology of parenchymal systems (lobes) in complete breasts, a scale much larger than is commonly attempted in 3D histological studies.

The need for this capability is imposed by the prediction that clonal expansion setting the scene for multifocal mammary carcinogenesis is likely to act over and within a lobe, as in the case of an abnormal clone spreading along ducts after the adult breast structure has been established following thelarche, or of a mutation disseminated in the descendents of a cell belonging to the prepubertal breast, in which early branching by the mammary anlage is established well before birth.

Growth (elongation and branching) of individual duct systems at thelarche could offer an opportunity for the expansion of mutation-bearing clones of cells possessing a growth advantage, which, while not necessarily having a morphologically abnormal phenotype, might be able to colonize more than their fair share of the developing breast and set the scene for future neoplastic development. It is known that breast lobe development is highly unequal (Going and Moffat 2004).

Because the emphasis over the last 30 years has been so strongly on the lobule as the relevant unit of organization of human mammary parenchyma, this larger scale, long-range structure of the breast has been neglected and techniques for its study are not mature. Nevertheless, possibilities for development in this area are attractive.

The rest of this chapter describes central and peripheral ductal/lobar anatomy of breast, as far as it is known; examines evidence for anastomoses within and between lobes, by which intraepithelial neoplasia might be able to spread from lobe to lobe; looks at

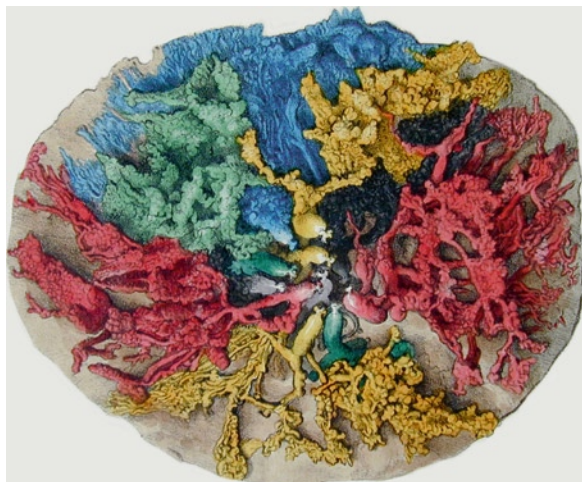


whether precursors of breast cancer and cancer itself are distributed in a lobe-like manner, in keeping with the “sick lobe” hypothesis; and considers how gaps in our knowledge of lobar breast anatomy might be filled, and the scope for developing techniques allowing morphological and molecular data to be optimized in research and diagnostic settings.

## 2.5 Lobar Anatomy of the Breast

Many published illustrations of lobe anatomy in human breast are at best artist’s impressions, attractive but without primary evidential value.

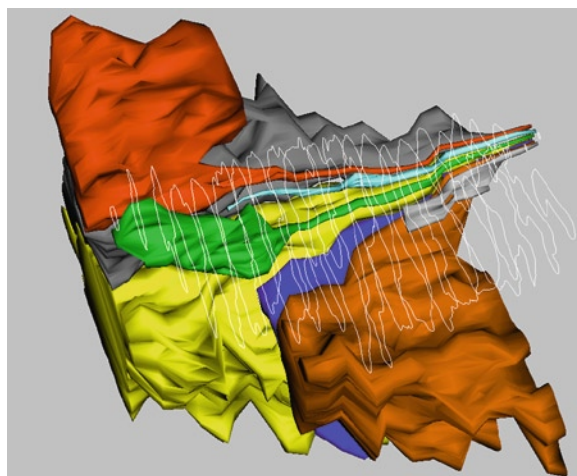
Cooper’s original illustrations, in contrast, are primary research data. In these illustrations, the most noticeable features are the ducts, variable in caliber, radiating from the center, branching and rebranching, with the last branches terminating in glandular parenchyma. Note that glandular tissue is present in all parts of the breast, not just the periphery, although in the nipple itself lobules are said to be sparse (Stolier and Wang 2008). There is noticeable variation in the extent of different lobes (Fig. 2.2), and to some degree their branches intertwine, but not to the extent that their distributions overlap greatly.



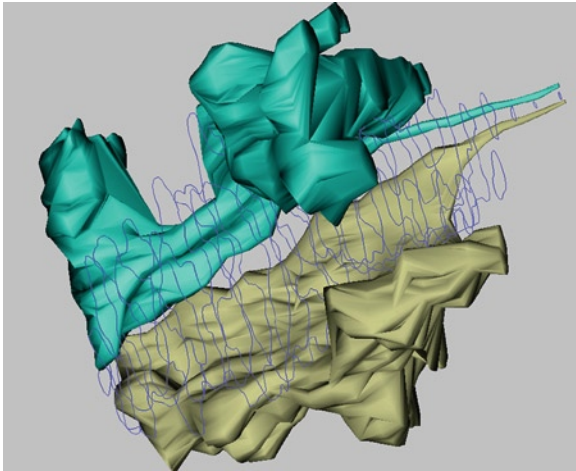
**Fig. 2.2** On the Anatomy of the Breast, Plate VI, Fig. 3 (Cooper 1840). Individual lobes have been injected separately with different colored waxes. This figure is less often reproduced than Fig. 2 of the same plate, in which breast segmentation into lobes is more uniform. This may reflect an esthetic bias in favor of uniformity, which may account for the many published artists’ impressions of lobes in human breast which emphasize a regularity of development and arrangement not sanctioned by any primary source

The tracing of all ducts and their branches in an autopsy breast of a young woman by Moffat and Going (Moffat and Going 1996; Going and Moffat 2004) was a rare attempt to capture duct branching lobe-by-lobe in a complete human breast. Such studies are dauntingly laborious by manual methods (Osteen 1995), but the scope for developing more streamlined procedures has yet to be fully exploited (Going 2006).

Features of different ducts systems (lobes) revealed in these studies include great variability in total extent (Figs. 2.3 and 2.4): one lobe can account for as much as 25% of a whole breast, as little as 1%, or even less; variability in envelope profile (including predominantly convex, concavo-convex, and cuneiform or wedge-shaped); variability in the length of the central duct before first branching (short or long); and the existence of vestigial or abortive lobes with relatively long ducts penetrating deeply into the central breast, but little or no peripheral branching or associated glandular parenchyma. Minimal lobes with longish but unbranching ducts imply that duct elongation is allowed even when side branching is inhibited (Going and Moffat 2004),



**Fig. 2.3** Variation in mammary lobe morphology, I. Seven representative lobes of a single breast vary greatly in size and distribution. Each duct system was traced through serial subgross sections. In each slice, the area occupied by branches of any one duct system may have complex borders, but can be drawn around. Lobes are visualized in “Reconstruct” (Fiala 2005). Boissonnat surfaces are shown for six lobes; the seventh by wire-frame outlines, so as not to obscure the central part of the model behind this lobe. Obvious lobe-to-lobe differences include size; early branching close to the nipple and the breast surface (tan) versus late branching in the depths of the breast (orange, green), or none at all (sky blue). This last is a vestigial lobe in the form of a duct with no peripheral branching at all. This was the longest of several “failed lobes” in this breast, which was studied by Moffat and Going (1996, 2004)



**Fig. 2.4** Variation in mammary lobe morphology, II. Three other lobes belonging to the breast shown in Fig. 2.3. The turquoise lobe has two largely separate domains, one close to the surface, and a deep extension. Both lobes (represented by their Boissonnat surfaces) are wrapped around the blue system represented in wire-frame section profiles. One can speculate that the blue system developed in advance of the other two

perhaps implying a mechanism in humans akin to asymmetric (monopodial) branching characteristic of rodent mammary morphogenesis (Davies 2002).

The existence of a largely convex lobe with a concave lobe wrapped around it (Fig. 2.4) seems to suggest that growth of the convex lobe was dominant over growth of the concave lobe. Possibly the “convex” lobe began its growth earlier or grew more rapidly than the concave lobe, and hence growth of duct branches belonging to the concave lobe into virgin territory was inhibited by the fact that elongating branches of the convex lobe had got there first. This apparent competition between lobes in breast growth is of interest in the context of a possible role for the female human breast as a signifier of reproductive fitness (Møller et al. 1995), and the relationship between breast symmetry and cancer risk (Scutt et al. 2006).

### 2.5.1 Are There Anastomoses Between Lobes?

An abnormal clone of cells expanding within an epithelial domain bounded by a basement membrane must remain limited to that domain as long as the clone is confined by the basement membrane. In the case of a breast lobe, the expanding clone would remain monolobar,

provided the lobe was isolated from neighboring lobes. We ignore for the time being the theoretical possibility of cells of the clone escaping from the lobe into the epidermis of the nipple, and entering another lobe via its duct opening on the nipple surface.

If lobes are not isolated from each other, but are linked by epithelium-lined anastomotic ducts, then a clone might escape from its lobe of origin into an adjacent lobe to which it was connected; thence it might spread to any lobe that second lobe was also connected to; and so on, potentially putting any part of the entire breast parenchyma within reach of such an expanding clone. Such a process would be analogous to the dissemination of pneumococcal lobar pneumonia throughout a lung via the interalveolar pores of Kohn.

Anastomoses between lobes could also influence sampling of the mammary environment by techniques such as duct lavage and duct endoscopy (Tondre et al. 2008; Dooley 2009), and might have a physiological role in lactation, by providing alternate pathways for drainage of milk from parenchyma to nipple, by which a duct blockage might be bypassed. This could help to maximize effective lactating tissue mass, as impaired milk drainage inhibits milk secretion via feedback inhibitors of lactation (Wilde et al. 1995), one of which is thought to be serotonin acting on the 5HT7 receptor in both human breast and murine mammary glands (Stull et al. 2007). Whether anastomoses exist is therefore important, but an entirely satisfactory answer has not yet been given.

### 2.5.2 The Challenge of Lobar Anatomy

Lobes remain intractable objects of study. To define a lobe completely, all its “branches” (ducts) and “leaves” (lobules) must be visualized. Ducts are thin-walled, embedded in tough fibrous tissue, and can ramify extensively, branching again and again. One breast contains many lobes, and neither macroscopic nor microscopic examination of breast tissue gives any clues to lobe boundaries.

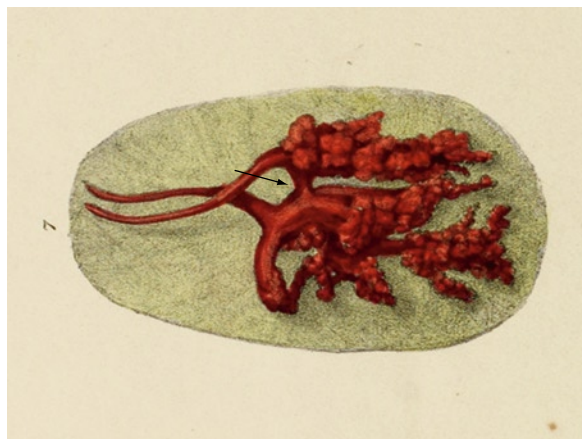
Practically, lobes can be defined by injection with a marker fluid (colored wax, resin, latex, urethane, mercury), or by tracing through serial thick (“subgross”) sections, after they have been stained and cleared. Giant histological sections of conventional thickness may hint at the lobe architecture, but a sampling gap of 3–5 mm between sections does not allow confident duct tracing from slice to slice.

### 2.5.3 Duct Injection Studies

Cooper was a pioneer in this area (it is salutary to remember that he began to research normal breast when he was already 67 years old). Sir Astley's opinion is clear: physiological anastomoses do not connect separate duct systems (lobes): "The mammary ducts do not communicate with each other, as is easily shown by throwing injections of different colours into the ducts, or by injecting one duct only."

"If various colours are thrown into each duct, they proceed to the gland without any admixture of colour. If one duct be most minutely injected with quicksilver, it does not escape into any other. And this remark is also applicable to the mammary glands of other animals, where there are many, as in the hare, the bitch and the pig, the ducts are separate and distinct from those of the other gland."

"I have only seen one instance to the contrary of this position, in injecting a milk tube from the interior of the gland towards the nipple, two large branches of ducts crossing each other, where they laid in contact, the injection found its way by rupture, or by a deviation from the natural structure, from the one into the other duct, of which I have given a figure [Plate VIII, Fig. 7] (Fig. 2.5); and as this has only occurred once in more than two hundred times, it shows that it is not the result of a common structure." (Cooper 1840).



**Fig. 2.5** On the Anatomy of the Breast, Plate VIII, Fig. 7. Rare anastomosis (arrow) between ducts of separate lobes. Cooper remarks this figure is "taken from a preparation which shows a rare deviation from a general law, viz., of two ducts communicating, of which this is the only instance I have seen. One of the ducts was injected from a branch near the circumference of the gland, and the injection was thrown towards the nipple, when either by laceration or unusual communication, two ducts became filled"

Cooper used a technique well adapted to the detection of anastomoses, in "more than two hundred" injection experiments, a breadth of experience unparalleled before or since.

Moffat and Going could find no anastomoses when tracing all identifiable branches of all ducts in subgross sections of an autopsy breast (Going and Mohun 2006).

Further evidence that anastomoses between lobes are rare is the absence of any reference in the galactography literature to retrograde filling of another central duct following injection of contrast medium down one central duct (Fig. 2.6). Love and Barsky detected no anastomoses in their studies (Love and Barsky 2004) which included a review of many galactograms performed by Otto Sartorius in Santa Barbara, California. Likewise, I am not aware of any published evidence of retrograde flow of fluid during nipple duct lavage, although such flow might not always be detected.

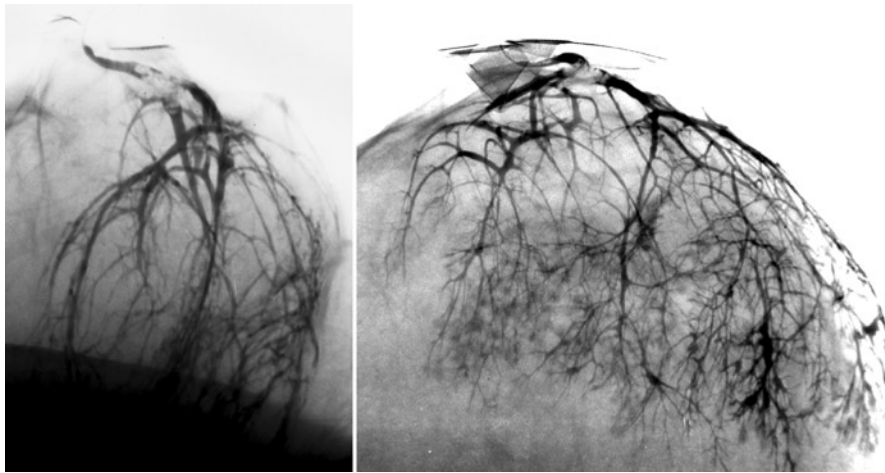
A theoretical consideration is that during mammary gland development, elongating mammary ducts mutually inhibit each others' continuing growth, and both rodent (Faulkin and DeOme 1960) and human (Going and Moffat 2004) mammary gland duct distributions show clear evidence of repulsion (Fig. 2.7), which would be calculated to interfere with the formation of anastomoses (Faulkin and DeOme 1960). TGF $\beta$  is likely to be a critical negative regulator of this mammary duct spacing (Lee and Davies 2007).

Ohtake et al., on the other hand, do describe interlobar and intralobar duct anastomoses in their subgross studies (Ohtake et al. 1995, 2001). This interesting and important question will be resolved only by further careful morphological studies. Experience of recording *x*, *y*, and *z* coordinates of all branch points and duct terminations of a complete mammary lobe (Going 2006) makes one aware of how fatally easy it is in such studies to confuse branches, and some of the apparent anastomoses identified by Ohtake et al. could have been a consequence of duct mis-tracing, however carefully they tried to avoid this.

### 2.5.4 Are Breast Cancer Precursors Lobar in Their Distribution?

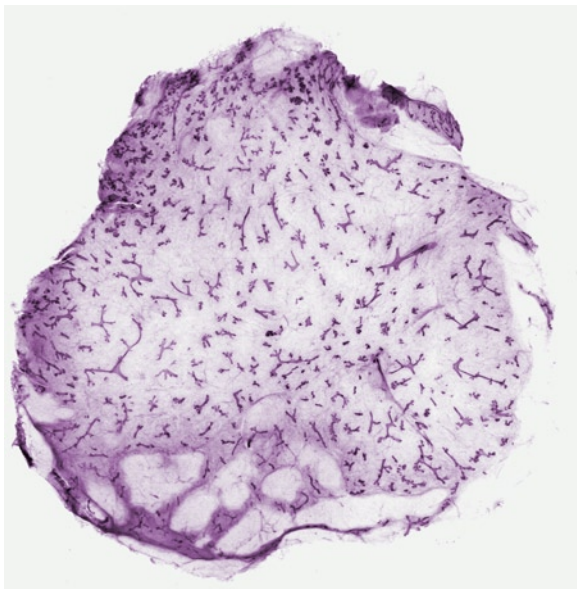
There is only a distant relationship between breast quadrants and lobes, so studies of breast cancer and its precursors which look only at the distribution of disease between quadrants tell us little or nothing about the distribution of disease between lobes.





**Fig. 2.6** Two views of the same lobe in a galactogram (oblique on left, cranial–caudal on right). This extensive system accounts for a significant fraction of breast volume. In the craniocaudal view glandular tissue is clearly visible. A filling defect (\*) visible in both views is due to an intraductal papilloma. Note the

single central duct and absence of retrograde filling of any other duct system. Galactograms, courtesy of Dr Jean Murray, South East Scotland Breast Screening Centre. Images have been contrast-reversed to maximize duct visibility



**Fig. 2.7** Hematoxylin-stained subgross section cleared in methyl salicylate (oil of wintergreen). Section is in the coronal plane through a complete (autopsy) breast. Spacing of parenchymal elements maximizes the distance between adjacent units (implying repulsion during development)

Extensive intraductal carcinoma is a risk factor for local recurrence (Holland et al., 1990a, b), and finding a small or large, often wedge-shaped area of DCIS is common experience for the practicing breast

pathologist. Other published studies support this segmental distribution of disease in breast cancer, in keeping with a lobar process (Johnson et al. 1995). The proposal that segmental treatment should be employed seems plausible, but the lobar hypothesis is not thereby proved, and the difficulty of doing this rigorously has been pointed out (Osteen 1995): “to prove the segmental anatomy of breast cancer would require serial sectioning of the breast in such a way as to establish the continuity of each duct and lobule. Such a monumental task is probably beyond the resources of any department and the patience of any individual.” In the same editorial, Osteen reviewed findings by Holland et al. (1990b) of lobe-like regional DCIS in 81/82 mastectomies they subjected to subgross examination, but points out that while consistent with a segmental (lobar) distribution, such a distribution was not thereby established, because the lobe anatomy was unknown even in this thorough study. Indeed, few have attempted to extract such anatomy, and the small numbers of cases examined reflect the difficulty of the task.

In this same editorial, we also find another adumbration of the “sick lobe,” in the remark that “some patients with breast cancer may have a segment that is, in some biologically definable terms, ‘bad’... These cases raise the question of whether other markers, such as atypical lobular hyperplasia,

microcalcifications in benign epithelium, or some genetic or molecular biologic markers, might identify ‘bad segments’ that require wide excision or mastectomy for treatment.”

A recent review (Jain et al. 2009) usefully surveys the literature concerning multicentric and multifocal ipsilateral breast cancer.

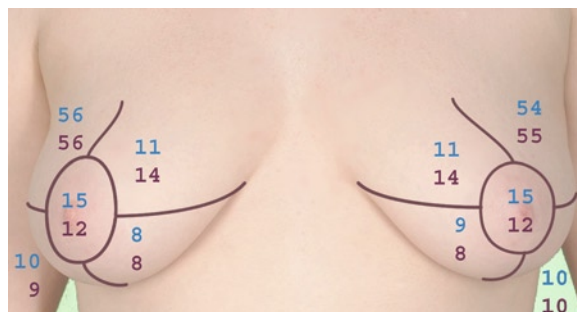
In the case of lobular neoplasia (ALH/LCIS), the segmental distribution of the process is less obvious. Lobular neoplasia is often presented as a marker of risk rather than a lineal precursor of breast cancer. The relationship is not entirely clear, but a 2003 paper by David Page and colleagues indicating an approximately 3:1 ipsilateral:contralateral ratio for invasive cancers diagnosed subsequently to a diagnosis of ALH strongly implies more than a marker function for ALH (Page et al. 2003).

### 2.5.5 Abnormalities of “Normal” Breast Tissue in the Vicinity of Cancers

There is now a considerable body of evidence that breast tissue which looks normal histologically may not be normal on genetic, epigenetic, or other molecular analysis (Ellsworth et al. 2004a, b; Meeker et al. 2004; Yan et al. 2006; Tripathi et al. 2008; Chen et al. 2009). These data are certainly in keeping with the idea of a sick lobe, but again, in the absence of anatomical data to anchor it in a lobar context, other possibilities are not excluded.

Chen et al. (2009) undertook global gene-expression microarray analysis of 143 histologically normal or non-atypical benign breast tissue samples from 90 patients with breast cancer. Eleven samples showed expression profile features in common with invasive carcinoma. Genes involved in cell proliferation and the cell cycle featured strongly in a “malignancy risk” expression signature derived by the authors from their data.

The finding of an increased frequency of molecular abnormalities in morphologically unremarkable tissue in the outer quadrants of the breast is of interest given the greater incidence of breast cancer in the outer and, especially, the upper outer quadrant of the breast (Ellsworth et al. 2004a). See also Fig. 2.8.



**Fig. 2.8** Location of in situ and invasive carcinoma in human breast by side and quadrant. Data from Perkins et al. (2004). The percentages are based on the recorded locations of 223,053 invasive carcinomas (numbers in brown) and 36,280 in situ carcinomas (numbers in blue) in US cancer registries. The distributions of in situ and invasive carcinoma are closely matched. The upper outer quadrant is at greatest risk of cancer

### 2.5.6 Inhomogeneity of Breast Cancer Risk by Quadrant

A majority of cancers occur in the outer breast, especially the upper outer quadrant. This applies equally to in situ and invasive cancers. While this may reflect a greater bulk of parenchymal tissue at risk, there is no definite evidence for this. Ellsworth et al. (2004a) found a greater prevalence of loss of heterozygosity in normal-looking breast tissue in outer than inner quadrants of cancer-bearing breasts, and thought that this might imply “field cancerization.”

A unique feature of the parenchyma of the upper outer quadrant of the breast which may be relevant is its superolateral extension around the inferomedial border of pectoralis major to form the axillary tail (of Spence). If the growth of individual lobes is a competitive process, any competitive advantage possessed by ducts of a developing duct system might favor their arrival first in areas of the developing breast furthest from the nipple, which might therefore be most likely to harbor growth-promoting changes. Very marked variation in the depth of branching exists not only between lobes (Going and Moffat 2004) but also between divisions of individual lobes (Going 2006). This is a testable idea, in that it would be possible to look at molecular changes in normal-looking parenchyma in the axillary tail and other locations in the breast, and in relation to depth of duct branching associated with these different areas.



Apropos any relationship between depth of duct branching and breast cancer risk, many studies of breast size and cancer risk have yielded inconsistent results, but a large study (Kusano et al. 2006) of 89,268 participants in the Nurses' Health Study II did find a moderate excess risk in women with larger breasts, but only for those with body mass index  $<25 \text{ kg/m}^2$ , in whom obesity is not a confounding factor.

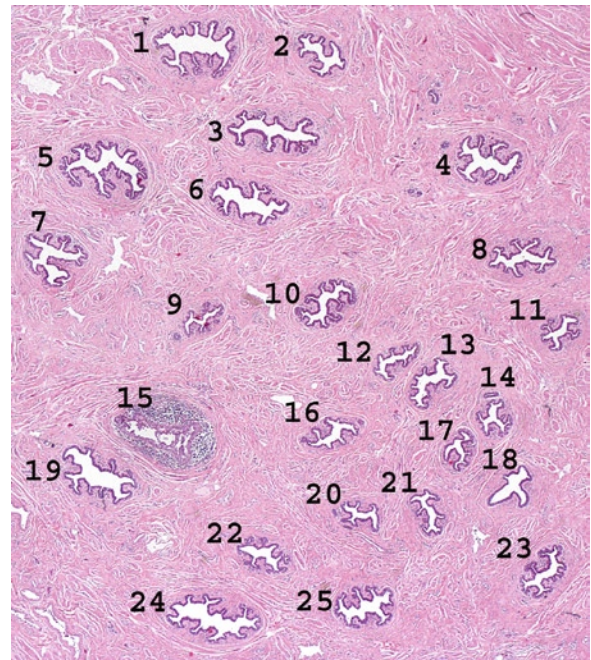
## 2.6 The Nipple and Its Anatomy

The large number of ducts in the central duct bundle in the nipple has been mentioned already. These vary in size and open on the apex of the papilla. Similar ducts opening on the lateral aspects of the papilla and in the areola constitute the glands of Montgomery. Several ducts may apparently share a single ostium (Rusby et al. 2007); this could go some way toward explaining the apparent discrepancy between the large number of ducts in the nipple duct bundle and the substantially smaller duct numbers from which milk may be observed to issue during lactation, or which may be cannulated at the apex of the papilla. With hindsight, this feature of the human breast ducts in the nipple is hinted at in older publications; Cooper's atlas includes an illustration which hints strongly at ostium sharing, and Cheatele and Cutler (1931) include a photomicrograph of an ostium into which two separate ducts clearly discharge their secretions.

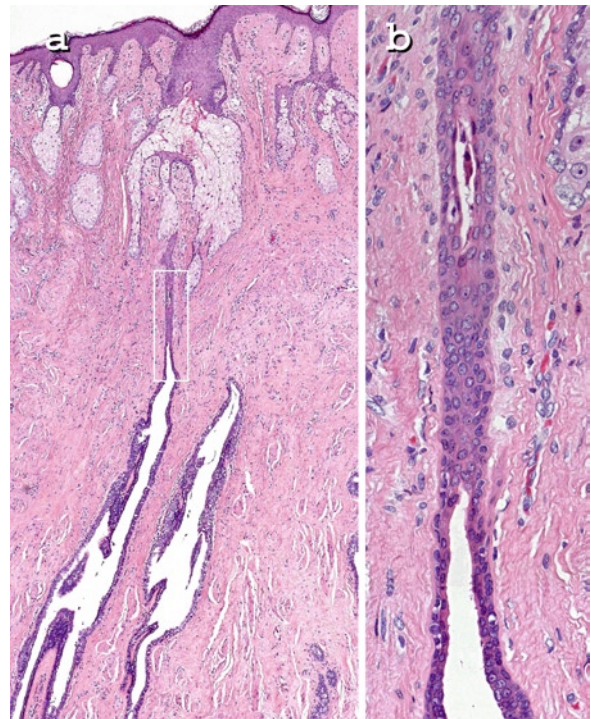
Figure 2.9 shows a cross section of the nipple duct bundle, illustrating the large number of ducts and their characteristically convoluted profile.

Figure 2.10 shows the squamocolumnar junction between the characteristic epithelial/luminal–myoepithelial/basal bilayer of the duct systems of the breast and the keratinizing squamous epithelium of the nipple epidermis. It is not uncommon to see a single nipple duct colonized by DCIS, but no evidence of Paget's disease; it appears that nipple epidermis usually resists colonization by DCIS, but in Paget's disease of the nipple, colonization of nipple epidermis does occur.

*HER2* amplification and Her2 overexpression by about 85% of Paget's disease suggest an important role in its pathogenesis. Heregulin- $\alpha$  is a motility factor made and released by epidermal keratinocytes, and Paget cells express heregulin receptors Her3 and Her4 as well as their coreceptor Her2 (Schelfhout et al. 2000). Heregulin binding to the receptor complex on



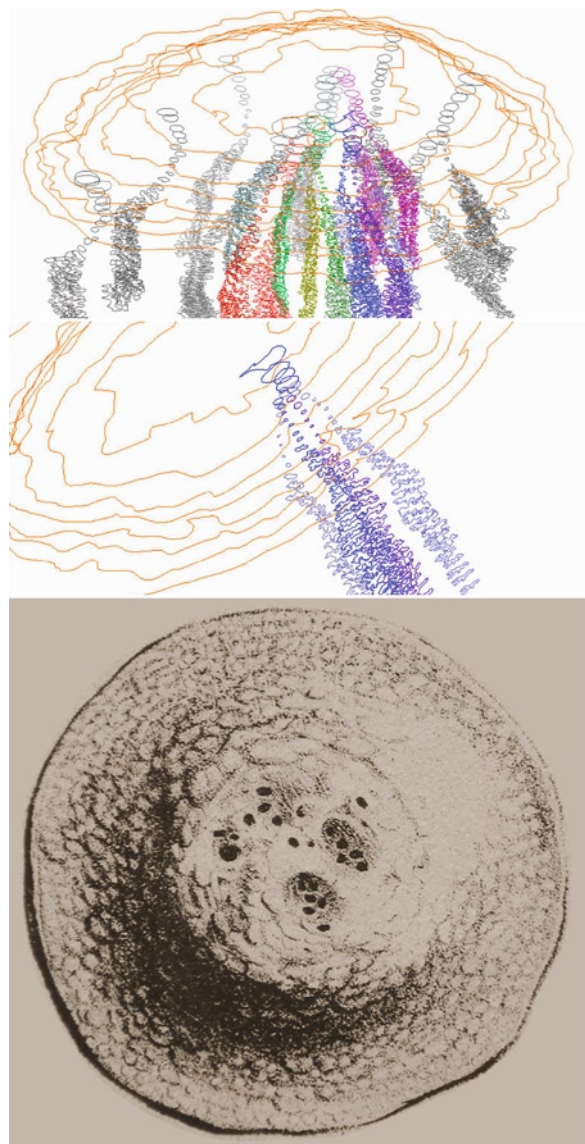
**Fig. 2.9** Duct bundle in the papilla, H and E, low power. Twenty-five individual ducts are present, implying twenty-five individual lobes, not all of which would have developed to a significant degree. Only one system (15) is involved by DCIS



**Fig. 2.10** Squamocolumnar junction in a duct approximately 0.5 mm deep to the epidermal surface of the papilla; Low (a) and high power views (b). Notice how small the duct is at this point

Paget cells is probably responsible for their migration into nipple epidermis. As normal mammary duct epithelium also expresses heregulins (de Fazio et al. 2000), this mechanism could equally promote expansion of Her2-positive DCIS in the breast itself.

Figure 2.11 shows a (previously unpublished) 3D reconstruction by the author of all the ducts in a mastectomy nipple as they approach the apex of the papilla,



**Fig. 2.11** Three dimensional reconstruction of ducts approaching the apex of the papilla in a mastectomy breast. Wire-frame views. Top view: all ducts shown. Middle: ostium sharing by four ducts. Below: ostium sharing depicted by Sir Astley Cooper (Cooper 1840)

and a closer view of ducts sharing a single ostium. Clearly, it would be difficult to cannulate these ducts separately. This figure also reproduces a figure illustrating ostium sharing from Cooper's atlas (1840).

### 2.6.1 Clear Cells of Nipple Epidermis: Toker Cells

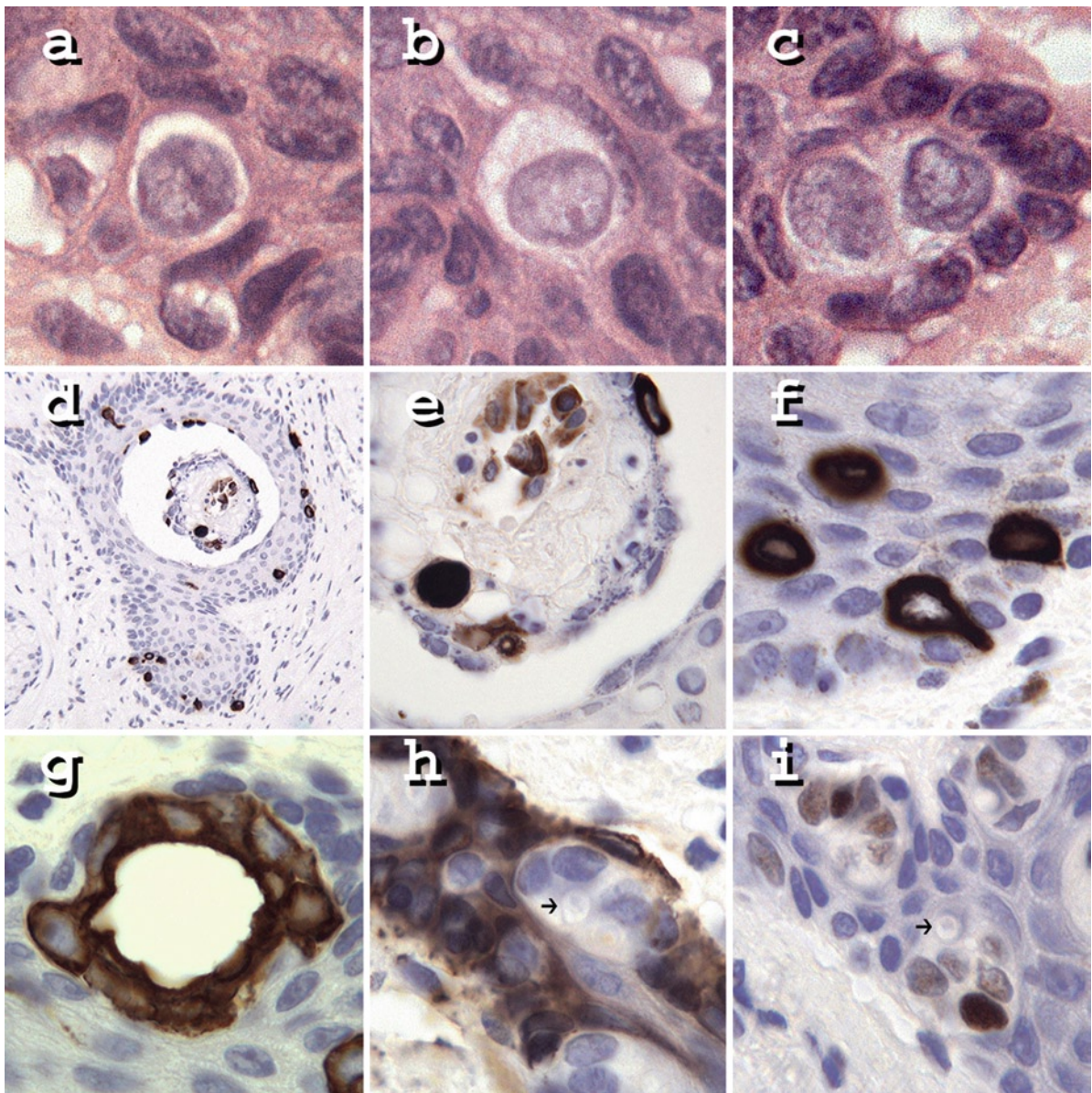
Finally, we take note of a population of cells to be found in many breasts, which have features which raise the possibility that they could act as vectors of risk in the creation of a "sick lobe" at increased risk of neoplastic transformation. These are the "clear cells of nipple epidermis" described by Cyril Toker (Toker 1970), and now known as Toker cells (Figs. 2.12 and 2.13).

Obviously, abnormal cells like those of high-grade DCIS can spread widely, to the extent of colonizing the ductal and glandular tissue of whole lobes. Less highly atypical cells of lobular neoplasia do the same. There is no a priori reason why other cells predisposed to neoplastic development should not do likewise, but if they did not have an obvious morphological phenotype, they would blend into the parenchymal background. Could Toker cells be representatives of such populations?

Toker cells are characteristically found in nipple epidermis in the vicinity of duct ostia. They express low molecular weight cytokeratins (cytokeratin 7, 19) in common with breast luminal epithelium and it has been plausibly suggested that they are of mammary origin (Marucci et al. 2002). Although inconspicuous in H and E sections (being observable in about 10% of cases), immunostaining with a marker such as cytokeratin 7 will reveal them in a much greater proportion of breasts (70–80%). They vary in numbers from scanty individual cells to so many, singly and in clumps there may be a possibility of mistaking them for Paget cells (which usually show much greater cytological atypia).

Their distribution implies an ability to migrate within nipple epidermis, and morphological features including the formation of lamellipodium- and filopodium-like cellular projections support this idea (unpublished observations by the author; Fig. 2.13). Despite apparently expressing steroid hormone receptors (although the literature is not entirely concordant on this point: Garijo et al. 2009), they can be just as numerous in breasts long postmenopausal as in breasts prior to the menopause. Also, their occasional presence in dead keratin suggests





**Fig. 2.12** Clear cells of nipple epidermis (Toker cells). (a–c) Hematoxylin and eosin. (a, b) Individual Toker cells resembling mammary small and large light cells. c Paired Toker cells; (d–g) CK7 immunostaining. (d, e) Numerous clear cells in the epidermis surrounding a duct ostium. CK7+ cells are also present in the keratin plug filling the lumen. (f) A suprabasal

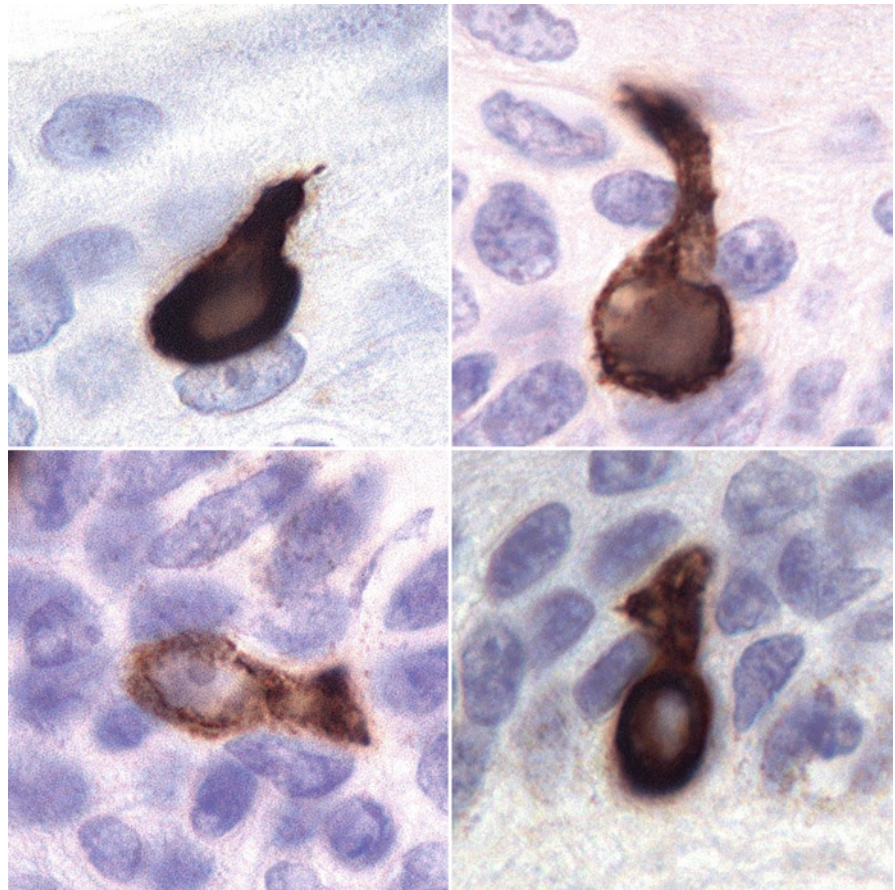
location is usual but a projection onto the basal lamina may give a gourd-like shape. (g) An acinus formed of CK7+ clear cells. (h) Clear cells negative for CK14 in contrast to surrounding keratinocytes. The arrow in this figure and in (i) indicates lumen formation. (i) Variable expression of estrogen receptor by Toker cells

an ability to survive in a situation in which they might have been expected to undergo anoikis (Fig. 2.12), suggesting apoptosis resistance. These hints at Toker cell autonomy and motility suggest a possible role not merely in relation to Paget's disease, with which a connection has been proposed, but more generally in breast cancer,

especially as possible vectors of risk in the genesis of a "sick lobe."

Unfortunately, there are at present no specific markers allowing Toker cells to be recognized in mammary epithelium. Their expression profile for molecules related to cell motility, cell adhesion

**Fig. 2.13** Clear cells of nipple epidermis (Toker cells) immunostained for cytokeratin 7, illustrating features compatible with motility. *Top left:* this cell has a broad lamellipodium-like extension from which a filopodium (“microspike”) extends. *Top right:* a long clavate projection. *Bottom left and right:* two cells with wedge-shaped projections with a flattened “contact surface” on a neighboring keratinocyte



molecules, and receptors (e.g., Her3, Her4) for possible motogens (including heregulins) would be worth investigating.

## 2.7 Cellular Supercompetition in the Making of a Sick Lobe

Clinically “early” neoplasia is nothing of the kind. Waves of clonal expansion (at the expense of neighboring cells) over many years establish and consolidate mutations in tissues and, by increasing after each new event the number of cells in which the new mutation and earlier mutations are present, pave the way to eventual malignancy.

Scope for competitive clonal expansion would be increased by any reduction in the degree to which stem cells remain tightly bound to a specific tissue niche. (Any reduction in the ability of a cell and its

descendents to repair DNA damage would also favor accumulation of further mutations, and several such mechanisms are well known.) Recent research interests have focused on cell competition and supercompetition as a mechanism in carcinogenesis.

Cell competition is well attested in *Drosophila* (Morata and Ripoll 1975). Cells heterozygous for Minute ribosomal gene mutations grow into phenotypically normal flies, but in chimeric flies, M/wt cells lose ground to wt/wt cells. The same occurs with *dmyc* mutations (Johnston et al. 1999), and even more strikingly, overexpression of *dmyc* creates “supercompetitor” cells (Moreno and Basler 2004) which outcompete wild-type cells. Supercompetitor cells may also be created by aberrant Salvador/Warts pathway signaling (Tyler et al. 2007). Particularly important is that a population of “winner” cells can expand at the expense of “loser” cells in a tissue without any visible histological alteration. Perhaps Toker cells are supercompetitors.



## 2.8 Prospects for Improved Understanding of Breast Lobe Anatomy

### 2.8.1 Injection Studies

Injecting individual duct systems (lobes) with colored or radioopaque tracer fluids, gels, resins, polymers, liquid metals, and waxes (in vitro and in vivo) has a long history. These techniques have advantages but many disadvantages. Suitable fluids can define even fine duct branches, which certainly is an advantage, but human milk ducts are delicate and extraductal rupture and leakage are frequent; furthermore, few studies appear to record successful injection of anything approaching the number of ducts really present in a human breast. Primary sources for accurate ducts counts are hard to find in the literature but Going, who counted duct profiles in complete cross sections through the nipple duct bundle at the base of the papilla in cancer mastectomy breasts, found a median of 27 ducts (range 11–41; Q1 21, Q3 30) (Going and Moffat 2004), a number greater than the usual 10–20 or so quoted in secondary sources.

While many of these systems may be rudimentary or vestigial, in the absence of good data, this is speculative. At all events, many injection studies investigate far fewer systems. Khan et al. (2004) studied ducts yielding nipple aspirate fluid and were able to lavage and inject 39 systems in 28 breasts (1.4 per breast). On the other hand, Love and Barsky (2004) observed milk flow from a median of 5 nipple openings in lactating women and Ramsay et al. (2005) observed a mean of 9 ducts in right and left breasts of fully lactating women. Ultimately, these data are still difficult to explain fully. Some systems may be rudimentary, with little functional parenchyma; alternately, duct nonpatency could also be a factor, as a system disconnected from the nipple would not establish lactation, in that nondrainage inhibits lactation by the negative feedback mechanism mentioned earlier. Such nonpatency of main or branch ducts would also interfere with injection studies.

### 2.8.2 Duct Tracing

The other main technique for lobe studies has been tracing ducts as they ramify through serial thick stained and cleared (so-called subgross) sections.

Subgross techniques have a long history, going back at least to the studies of Werner Spalteholz (Spalteholz 1914). They were extensively applied by Adolf Dabelow (Dabelow 1957) and later workers including Wellings and colleagues (Wellings et al. 1975; Jensen et al. 1976), and remain widely used in developmental biology and experimental pathology most often in the form of wholemount preparations. Even in this venerable technique there are new developments: many fluorescent DNA-intercalating stains are incompatible with the classical hydrophobic clearing agents like benzyl alcohol/benzyl benzoate or methyl salicylate. Recently, thiodiethanol (refractive index = 1.52) was introduced into confocal microscopy as a water-miscible, low-toxicity (Reddy et al. 2005) high refractive index mounting medium compatible with many intercalating DNA dyes (Staudt et al. 2007; Appleton et al. 2009) and facilitating microscopy of substantially thicker specimens. The prospect of an improved, fluorescent subgross technique applicable to breast tissue is exciting.

Subgross techniques have the great advantage of allowing all parenchyma in a breast to be stained and visualized, but as a method of studying lobar breast anatomy, although all the data is present, the challenges remain great. Tissue distortions during sectioning and processing create a difficult registration problem, that is, points of correspondence between adjacent sections may be hard to identify, and duct tracing correspondingly difficult. (These difficulties were referred to above in the discussion of the work of Ohtake et al.)

Large sections certainly allow a greater appreciation of relationships over longer distances than conventional small histological sections in the size range 15–25 mm, but although 3D data can be inferred, great caution is required in the evaluation of duct connections. If a tissue block is 3 mm thick, a duct traversing that block at an angle of 10° to its surface will sustain more than 15 mm of lateral displacement. This makes inferring duct connections from histological sections of tissue blocks as little as 3 mm thick highly unreliable.

Conventional x-ray galactography is now little used with ready availability of other imaging modalities including ultrasound, but MRI galactography has potential in the area of defining lobe anatomy. However, it faces all the challenges of other duct injection techniques including contrast extravasation, the difficulty of injecting more than a few ducts, and (even if multiple ducts could be injected) it might be difficult to discriminate between systems.

Now, it would be a good time to bring together complementary techniques to advance the study of breast biology and pathology. Molecular and morphological analyses are powerful separately, but even more powerful together. Breast cancer is a disease of astonishing complexity. Neither approach on its own is optimal. The “sick lobe” hypothesis asks questions about the development of breast cancer in time and space, and observes that concentrating on events in a few cubic millimeters of tissue is not enough. To be able to analyze molecular events in different parts of duct trees, with a knowledge of how those ducts are physically connected, would allow for the testing of otherwise untestable hypotheses.

Almost all the necessary tools are available: fixatives less deleterious than formaldehyde to nucleic acids, proteins, and other important biological molecules; sensitive and specific fluorescent dyes to reveal structure; a new tissue clearing agent, thiodiethanol (Staudt et al. 2007), compatible with these dyes; data processing techniques for storage, extraction, processing, and visualization of that structure; and the whole gamut of molecular techniques.

Strangely, one of the challenges looks as if it ought to be easy, but isn't: making stacks of serial thick sections without distortion, essential for accurate duct tracing from section to section, and lobe reconstruction. Classically, investigators have used prolonged formaldehyde fixation, and deep-frozen the fixed tissue in agar for slicing. Egan introduced the slicing of deeply chilled tissue (Egan et al. 1969; Egan 1982). Neither is optimal, or free from artifacts.

The real challenge is to take unfixed breast tissue straight from the operating theater – be it a diagnostic biopsy, wide local excision, or mastectomy – and slice it within minutes into a stack of 2–3 mm thick slices, each collected on a dimensionally stable substrate for optimal fixation, staining, tissue clearing, visualization, and data collection for subsequent 3D analysis; followed by tissue processing for classical histology, immunohistochemistry, and any other including molecular analyses as indicated by clinical necessity. All to be done on a time scale no longer than we now accept for conventional histology. There are no grounds for thinking that this is not possible. Such a technique could allow us to be more accurate in our evaluation of diagnostic issues such as completeness of excision of in situ and invasive cancer, and achieving it is a highly desirable goal.

## 2.9 Conclusion

Astley Cooper's researches have been a theme in this chapter, and it is fitting to take final look at Sir Astley's work. His plate V, Fig. 1 (Fig. 2.14) illustrates different degrees of glandular development between areas of a lactating breast, to which Sir Astley draws particular attention. This may be the first published suggestion of significant variation in differentiation potential between human mammary gland lobes, and a very early hint at the possibility of a “sick lobe,” given the possibility that failed attempts to establish lactation (in keeping with impaired glandular differentiation) may be associated with increased breast cancer risk (Yang et al. 1993).

Continuity in thought is interesting, and it is gratifying that such an “old” subject as the lobar organization of human breast tissue is, if anything, even more important in the postgenomic era than in 1840 when Cooper first laid the foundations for scientific senology.



**Fig. 2.14** On the Anatomy of the Breast, Plate V, Fig. 1. Cooper's caption reads “Lactiferous tubes, injected with red wax, in a woman who died during the period of lactation. Twelve ducts have been filled and ligatures are placed on their orifices. The ducts are seen forming large reservoirs at the roots of the mamillary tubes; these reservoirs are seen to be produced by the union of numerous branches from the ducts. The ducts are perceived to terminate at the margin of the gland in branches, but in some parts, in glandules.” Glandular tissue is most obvious at 3–5 o'clock and 10–11 o'clock. This may be the first published suggestion of significant biological variation between human mammary gland lobes



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