Pathological studies of apoptosis in the normal breast

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Abstract
Apoptosis in the normal breast has been demonstrated to be related to the menstrual cycle. The factors that influence apoptosis in the breast are reviewed, together with related variations in the degree of apoptosis to disease pathogenesis. Evidence for a relationship between mitosis and apoptosis is provided, and the implications of pregnancy and prolonged postlactational involution are discussed. Future progress will depend on active collaboration between molecular biologists and pathologists to dissect the complex signalling pathways that appear to control the apoptotic and mitotic cascades and their inter-relationships.

Introduction
Three issues are encountered immediately on considering the topic ‘pathological studies of apoptosis in the normal breast’, each concerning matters of definition. First, the glands that unite the mammalian genus are generally referred to as ‘mammary’, whereas ‘breast’ defines the human organ, which is considered here. The second issue concerns the term ‘normal’, for here we have to consider the variety of states in which the breast may be so described. Indeed, the breast is apparently unique in that its lobular development is achieved without the stimulus of copulation or pregnancy (Short 1974, Monaghan et al. 1990). The degree and uniformity to which this parenchymal development is achieved varies considerably, both in the number of lobular units and their location within the mammary disc in different individuals. Stereomicroscopic views of the postmenarchal breast parenchymal elements reveal the basic structure of the resting state as being very similar to an inverted bush or tree, with the lobules representing the buds or leaves. It is from this resting state that the differentiated lactating lobules develop during pregnancy, to give rise to full lactation after delivery. At some suitable time thereafter, and with the withdrawal of suckling, the process of involution takes place, returning the organ to its mature resting state. The rate and degree to which this takes place over a period of time remain somewhat uncertain because of the limited microscopic ‘snapshots’ taken of these events. In the absence of pregnancy, there is repeated cycling of the resting state in parallel with menstruation until finally, with the menopause, there follows a gradual process of senile involution by which the parenchymal elements are replaced by fibrosis or fat, or both. From this it should not, however, be inferred that the lobule and ductule structures are rapidly lost, because persistent lobule formation has been commonly observed in post-menopausal breasts.

It can be appreciated that the expression ‘normal’ breast encompasses a considerable range of states. The following comments are restricted to the physiologically stimulated mature organ. The studies from which they are drawn are ‘pathological’ in the sense that that is the discipline in which they took place - thus accounting for the third issue of definition.

When does apoptosis occur?
In the early 1980s, the frequency of apoptosis in the normal breast terminal duct lobular unit was reported to show a notable fluctuation during the menstrual cycle, with the maximum occurring around the period of menstruation (Anderson et al. 1982), following a peak of mitosis that occurred some 3-4 days earlier. The apoptotic bodies are readily perceived on light microscopy and their location within the duct lining is characteristic. Also characteristic is the wide scatter in the frequency of the apoptotic/mitotic events about the mean; this can be likened to the stuttering of a motor-cycle petrol engine as it is repeatedly kick-started before the ignition catches. It is the herald for a more sustained frequency of both mitotic and apoptotic events at a higher level in pregnancy (Battersby & Anderson 1988).

By the late 1980s, a series of more than 300 samples had been assessed for proliferation, with the establishment of a higher rate of proliferation amongst oral contraceptive users (Anderson et al. 1989). A higher rate of apoptosis would also be anticipated. The accumulation of this large series of cases made it possible to undertake a multivariate
analysis of independent factors that might significantly influence normal breast proliferation and, by assumption, apoptosis. Those found to be significant are summarised in Table 1. These findings, supported by others (Potten et al. 1988, Williams et al. 1991), have led to the acceptance of a difference between the breast and the endometrium in their response to menstrual cycles. The major agent(s) affecting this difference in response remains to be defined (Clarke et al. 1997b); this is equally true for the particular events triggering breast apoptosis, as is apparent from other contributions in this issue. The regular occurrence of apoptosis in healthy tissues is, however, not in any doubt.

Similar results have been obtained concerning the frequency of mitosis and apoptosis in the normal breast in relation to the menstrual cycle - whether natural or during oral contraceptive use (Williams et al. 1991). In addition, the same group have reported specifically on the frequency of apoptosis in the normal breast epithelium in relation to the nature of accompanying breast pathology (Allan et al. 1992). They distinguished three groups of pathological conditions, namely fibroadenomas, fibrocystic change and carcinomas, and assessed the frequency of events in the accompanying normal tissue components. In specimens obtained from more than 200 premenopausal women, the investigators observed a reduced frequency of apoptosis in the last two groups. It is worth commenting that the report presents something of a biostatistical challenge, as the conclusions drawn were based on differences in incidence ratios derived from measures of proliferation or cell death. The study was a commendable attempt to associate variations in the ‘balance’ of those events with the genesis of pathology. There were, however, inequalities in parity and in the use of oral contraceptives between the different groups of women, which may be confounding - an issue discussed below. The findings of another study of morphologically normal tissue in cancer breasts (Hassan & Walker 1998) are in agreement with those of Allan et al. (1992) with respect to decreased apoptosis, yet the data on menstrual and parity status of the groups are not known, for either the cancer or the control cases. The demonstration of a clear relationship between the frequency of apoptosis and the pathogenesis of disease has yet to emerge.

<table>
<thead>
<tr>
<th>Phase of menstrual cycle</th>
<th>Chronological age</th>
<th>Breast age</th>
<th>Oral contraceptive use (especially nulliparous)</th>
<th>Recent parity</th>
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**Where does apoptosis occur?**

With respect to the microanatomy of the breast terminal duct lobular unit, there is consistency in the reporting of a greater frequency of events in the ductules of the lobule than within the minor ducts that feed into them. Unfortunately, the random branching of the ductular tree within the intralobular stroma detracts from the assessment of order and progression of apoptotic events within the microanatomical structures; this puts the breast studies at a disadvantage in comparison with those of crypts and villi of the alimentary canal (Potten 1996). The regional variation in reactivity of the terminal duct lobular unit within a given breast is another feature that requires better definition, but has obvious practical impediments. Nevertheless, an important comment has been made in relation to the polarisation of mitoses in the luminal epithelium of the ductules (Ferguson 1988): in the great majority of instances, the polarity is perpendicular to the lumen, and only very occasionally does it occur in parallel; furthermore, the location of apoptosis is more usually in the lower portions of the ductule wall, and is seldom observed in the ‘restin’ ductule lumen. The inference can therefore be made that apoptotic cells may be derived from recently mitotic cells. Evidence establishing this as a matter of fact from in vitro studies has proved elusive (Mukherji 1983).

**The influences of pregnancy and lactation**

The fully differentiated state of the breast is achieved with pregnancy and lactation yet may undergo this differentiated state in the virgin breast, usually in association with abnormal prolactin stimulation, for example by drugs. There is a higher level of proliferation in the first half of pregnancy than in the second, and equivalent levels of apoptosis are observed (Battersby & Anderson 1988). In contrast, the morphological series of changes taking place during postlactational involution in the breast are incompletely documented because of an inadequacy of appro-priate samples. In addition, the time-span over which involution is completely achieved has yet to be established. An under-recognised phenomenon is that of prolonged postlactational involution (Battersby & Anderson 1989). This appearance is recognisable, although not always uniformly, in more than 50% of women within 18 months after parturition, and is still present in almost one-third up to 5 years. The significance of this observation lies in the fact that lobules with such prolonged involutionary characteristics also show very low levels of proliferation and apoptosis, and they are immunohistologically unreactive for steroid receptors (Battersby et al. 1992). Together, these observations may have a bearing on the findings of Allan et al. (1992)
mentioned above, suggesting that there may have been some significance in the dissimilarity of parity status between their study groups. The fibroadenoma group, with the greatest frequency of apoptosis, were 70% nulliparous and also had the greatest use of oral contraceptives; in the other two groups, nulliparity was 30% or less and use of oral contraceptives was less frequent. With the information we now have on the breast effects of these two factors, the equal comparability of the study groups weakens, as does the evidence for a relationship of apoptotic frequency with disease states. It can only be concluded that more attention needs to be given to this neglected aspect of the ‘normal’ range.

Another way of looking at the relative inactivity of breast tissues characterised as having prolonged involutionary change is to compare the level of second messenger systems with those present in other biological states. Among these differing states that occur during a woman’s reproductive years, the level of cyclic AMP binding proteins is at its lowest in prolonged postlactational involution (Battersby et al. 1994a) and the phosphorylation of membrane proteins is similarly affected (Battersby et al. 1994b). The mechanisms by which the lobule is maintained in this ‘anergic state’ have not been characterised, but may well be the consequence of paracrine factors and cytokines produced by the low-level chronic inflammatory cell element that is present. We thus have a further example of the biological diversity within the breast. Understanding the mechanisms by which to maintain - or even exogenously initiate - this particular state has obvious implications for the field of cancer prevention. It also focuses attention on the importance of factors that ‘communicate’ between different cell components of the terminal duct lobular unit in the regulation of events.

Pathways of apoptotic regulation

Two years ago, the status of the normal mammary epithelium was summarised as a balance between factors that were pro-apoptotic, represented by Bax, or anti-apoptotic, represented by Bcl-2, Mcl-1 and Bcl-X (Reed 1996). Since then, there has been an expansion of this family of proteins and the definition of their involvement in regulating the apoptotic event; there is also a growing awareness of their inter-relationship with other intracellular factors mediating cell cycle regulation. If it is necessary, not only to discriminate between different varieties of lobule, but also to localise and interpret the immunohistochemical reactivity to several biological factors in the different tissue compartments, then the histopathological definition of tissues increases both in complexity and in importance. Such observations may apply as much to the stromal elements as to the epithelium, if we are to draw analogies from animal model systems (Walden et al. 1998) and to pursue leads from in vitro human tissue studies (Clarke et al. 1997a). The potential and limitations for immunohistochemical localisation of relevant growth factors have still to be fully explored in human systems. As a model, the terminal duct lobular unit does indeed present a considerable challenge. Deductions must be made from a restricted series of random snapshots rather than a sequence, akin to the ‘moving’ picture and, as explained in the preceding sections, the need to acknowledge a number of confounding factors. To meet this challenge, molecular biologists and histopathologists must collaborate; only then can we sort out the ‘wood’ from the ‘trees’. Despite comments to the contrary (Russo et al. 1990), the breast terminal duct lobular units are outstanding by virtue of their diversity and sometimes full microanatomical development in the virgin state. They are, in fact, the best model system from which to glean information about the practical relevance to humans of breast apoptosis and its regulation.

References


Anderson: Normal breast apoptosis


