Commentary

Epithelial stem cells in the mammary gland: casting light into dark corners

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Abstract

The epithelial structures of the human breast or the mouse mammary gland are derived from a relatively small number of multipotent, tissue-specific stem cells, of which we are surprisingly ignorant. We do not know how many are required to produce a complete mammary gland, how many times they divide during the process, where they are situated in the gland, or even what they look like. We want to know the answers to these questions, not just to satisfy intellectual curiosity, but also because the answers may shed light on the evolution of breast cancer. Now, studies carried out by Kordon and Smith at the National Cancer Institute have pointed the way toward a new understanding of mammary stem cells and their progeny.

Keywords: breast cancer, epithelial stem cell, mammary gland

Kordon and Smith [1] have refined a widely used model of transplanting mouse mammary epithelium into the cleared mammary fat pads of syngeneic or athymic nude mice [2], and have shown that just one cell can give rise to a complete and fully functional gland. To reach this remarkable conclusion, Kordon and Smith took advantage of the CzechII mouse strain. Like other strains, these mice become infected congenitally with the mouse mammary tumour virus (MMTV) through milk [3]. Unlike other strains, however, the CzechII mice have no endogenous MMTV-like sequences in their genome, so viral insertions can be detected by Southern analysis as long as a large enough number of cells contain the same insertion. The only way that this can happen is if the population being analyzed is clonal, and thus use of the CzechII mice represents an elegant advance on previous methods for detecting transplanted populations in cleared mammary fat pads.

One cell, one mammary gland

Accordingly, Kordon and Smith [1] took small fragments of mammary epithelium from CzechII MMTV-infected mice and transplanted them into cleared mammary fat pads of syngeneic hosts. These hosts were mated and 1 day after parturition about 80% of each of the reconstituted glands was removed for analysis, leaving the remainder intact for subsequent serial transplantation. As expected, most of the transplanted epithelial fragments expanded during pregnancy to become complete and functional mammary glands. If these glands had been derived from several different progenitors, no clear pattern of MMTV-insertional events would have been detected. If, on the other hand, the outgrowths were clonal a distinct and easily detected pattern of MMTV insertion sites would have been seen, as was the case in 20 of the 30 different outgrowths examined by Southern analysis. As a

MMTV = mouse mammary tumour virus.

control, the intact contralateral glands of the host mice were analyzed in the same way and, as would be expected for a polyclonal tissue derived from several progenitors, no clear pattern of insertional events could be seen. The inescapable conclusion of this first part of the work of Kordon and Smith was that most of the reconstituted mammary glands were clonal. Fragments of these clonal glands were then transplanted into new hosts where they were shown to retain the same pattern of MMTV insertion sites, providing evidence of self-renewal of the original stem cell. Additional MMTV insertion sites were often detected, however, suggesting the acquisition of more mutations during clonal expansion.

Two lineage-specific progenitors come from one stem cell

The above work confirmed, very elegantly, what had been strongly suspected but not proven – that the outgrowths derived from transplantation of mammary epithelial fragments are clonal in that they are the product of a single, self-renewing stem cell. Because this progenitor gave rise to fully functional outgrowths, we might have assumed safely that it is multipotent. Nevertheless, Kordon and Smith [1] decided to test this experimentally by transplanting limiting numbers of cells derived from third-generation outgrowths into cleared fat pads. These outgrowths were first placed into primary culture to allow accurate quantitation of the number of cells to be implanted. Injection of 14000 cells into cleared fat pads resulted in outgrowths in three out of eight cases, indicating that there was less than one clonogenic component per injection. Only one of the three outgrowths completely filled the fat pad with a lactating ductolobular structure, however. The other two showed limited growth and one was composed of primitive ducts, whereas the other comprised lobules. These results support previous studies by Chepko and Smith [4] by suggesting the presence of a single multipotent stem cell that gives rise to two lineage-specific progenitors of a more limited proliferative capacity. These lineage-specific progenitors might also be considered multipotent or at the very least oligopotent because they appear to be the precursors of both luminal epithelial and myoepithelial cells. An interesting corollary was that lineage-specificity must be an intrinsic property because the limiting dilution experiments were carried out under hormonal conditions (i.e. pregnancy) that are known to support both ductal and lobular development, but only one type or the other was achieved.

The final part of the studies of Kordon and Smith [1] comprised estimation of the number of cells that could be derived from a single progenitor. This was achieved by measuring the total DNA content of fat pads bearing lactating outgrowths and then determining the proportion contributed by the epithelial component. Using 6 pg as the DNA content of a single diploid mouse cell [5], Kordon and Smith arrived at a range of $23–77 \times 10^6$ epithelial cells per lactating outgrowth. Extrapolation of these data indicated that a single cell would have to undergo 25–27 doublings to produce a lactating outgrowth. In practice, the number of doublings required was probably rather less than this. In previous experiments [6], Smith estimated that the number of stem cells capable of repopulating a mammary fat pad was about 1 in 2500 of all mammary epithelial cells. If the population dynamics were the same in the clonal outgrowths as in the normal gland, then the single progenitor would have undergone self-renewal by symmetrical mitosis 11 times to produce an equivalent number of stem cells which, in turn, would contribute to repopulation of the fat pad.

Stem cells have prodigious proliferative capacity

Mammary epithelial outgrowths become senescent after approximately four serial transplants [7] meaning that the original stem cell could undergo a total of 40–50 doublings. Rather satisfyingly, this figure agrees with the 'Hayflick number' (i.e. the maximum number of divisions a eukaryotic cell can undergo before replicative senescence occurs [8]). This also makes it clear that stem cells have prodigious proliferative capacity; just one cell could give rise to a further $10^{12}–10^{13}$ multipotent progenitors during its lifetime. It is also clear, however, that no stem cell in the mammary gland actually realizes this potential within the lifetime of the mouse or woman. It appears that the signals that initiate proliferation are very tightly regulated so that, under normal circumstances, stem cells cannot divide uncontrollably. These control mechanisms are poorly understood, but one possibility is that stem cell proliferative activity is influenced by position in relation to more differentiated progeny, to the basement membrane and to stromal cells. It is unclear what these 'positional cues' are, although we do know that division competent cells in both the mouse mammary gland and the human breast are separate from, but adjacent to cells that express receptors for oestrogen, implying that this steroid controls proliferation indirectly [9,10]. Transforming growth factor-β is a prime candidate for the role of a negative growth regulator secreted by differentiated progeny surrounding the stem cells [11, 12].

Human breast development

Thus, through a combination of inspired experiments and thoughtful interpretation of their data, Kordon and Smith [1] have made considerable progress toward answering some of our questions about stem cells. We now know that just one stem cell can generate a complete mammary gland, during which process it probably undergoes about 11 self-renewing symmetrical divisions to give rise to other stem cells and the more committed, lineage-specific precursors. Along the way, evidence has been found to suggest that lineage-specificity is intrinsic and that lineage-specific

precursors are oligopotent. What are the implications for human breast development and the evolution of cancers? Although there are important differences between the mouse mammary gland and the human breast in terms of the timing of maximal growth and differentiation, there is evidence to suggest that the human breast epithelium is derived also from multipotent stem cells scattered throughout the ductal structure. This has been demonstrated most clearly in studies in which contiguous patches of epithelium were shown to be clonal in terms of their pattern of X-chromosome inactivation [13]. We know that most human hyperplasias and tumours arise from the luminal epithelial population and that they are clonal [14,15], but we do not know much more because of the lack of suitable *in vitro* or *in vivo* models for the study of human breast epithelial stem cells. It is to be hoped that results of studies on the mouse mammary gland can be used to accelerate the development of these, much needed, models.

Cancer prevention and treatment

As far as cancer is concerned, there is indirect evidence that stem cells persist in the mammary gland throughout life, where they must be regarded as prime targets for oncogenic transformation. Perhaps it is time for us to adjust our preconceptions as to exactly what is oncogenic transformation, however. The work of Kordon and Smith makes it clear that immortalization is not necessary as a single progenitor can already produce more than enough cells to kill someone of breast cancer. Perhaps the earliest steps in tumourigenesis are those that allow a stem cell to escape from the constraints imposed by its position. Once escape has been achieved, it seems highly likely that further genetic alterations will accumulate as suggested by Kordon and Smith after they demonstrated that additional MMTV insertions occurred during clonal expansion. We can speculate that these further mutations might enhance sensitivity to growth promoters such as oestrogen, switch on production of angiogenic factors or confer metastatic potential.

Finally, it is clear that successful breast cancer treatment or prevention strategies must eradicate stem cells. Most current chemotherapeutic and endocrine agents induce apoptosis, but whether mammary stem cells are susceptible to programmed cell death is not known. Studies on the mouse small intestine suggest that there may be two populations of stem cells. One of these undergoes spontaneous apoptosis as part of the homeostatic mechanism restricting the number of stem cells present at any one time [16]. The other, smaller, population is resistant to radiation-induced apoptosis, undergoes DNA repair and, presumably, is responsible for repopulation of the damaged intestinal epithelium. The model of transplanted mouse mammary gland refined by Kordon and Smith should allow us to determine whether mammary stem cells exhibit similar properties and to develop the means to overcome resistance to apoptosis.

Conclusion

We believe that the future of breast cancer research lies in the elucidation of the mechanisms governing mammary gland stem cell activity and numbers. Kordon and Smith [1] have provided us with an important new tool for studying clonal populations and the stem cells from which they are derived. We now need to apply ourselves to the exhaustive study of the ontogeny and biology of human breast stem cells that they recommended.

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