**Reprogramming stem cells is a microenvironmental task**

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LBNL/DOE funding & contract number: DE-AC02-05CH11231

That tumor cells for all practical purposes are unstable and plastic could be expected. However, the astonishing ability of the nuclei from cells of normal adult tissues to be reprogrammed—given the right embryonic context—found its final truth even for mammals in the experiments that allowed engineering Dolly (1). The landmark experiments showed that nuclei originating from cells of frozen mammary tissues were capable of being reprogrammed by the embryonic cytoplasm and its microenvironment to produce a normal sheep. The rest is history.

However, whether microenvironments other than those of the embryos can also reprogram adult cells of different tissue origins still containing their cytoplasm is of obvious interest. In this issue of PNAS, the laboratory of Gilbert Smith (2) reports on how the mammary gland microenvironment can reprogram both embryonic and adult stem neuronal cells. The work is a follow-up to their previous report on testis stem cells that were reprogrammed by the mammary microenvironment (3). They demonstrated that cells isolated from the seminiferous tubules of the mature testis, mixed with normal mammary epithelial cells, contributed a sizable number of epithelial progeny to normal mammary outgrowths in transplanted mammary fat pads. However, in those experiments they were unable to distinguish which subpopulation of the testis cells contributed progeny to the mammary epithelial tree.

The current work adds new, compelling, and provocative information to our understanding of stem cell plasticity. Booth *et al.* (2) use neuronal stem cells (NSCs) isolated from WAP-*cre/R26R* mice combined with unlabeled mammary epithelial cells that subsequently are implanted in cleared mammary fat pads. In this new microenvironment, the NSCs that are incorporated into the branching mammary tree make chimeric glands (Fig. 1) that remarkably can also express the milk protein β-casein, progesterone receptor, and estrogen receptor α. Remarkably, the primary transplants are capable of maintaining chimerism through serial transplantation. When the chimeric glands are explanted back into NSC growth media, cells with NSC markers were present only in explants from the first transplant and not from explants in the subsequent serial transplants, suggesting that there is a window of time and an unknown but specific context for becoming NSC again.

There is considerable literature on the stroma's ability to reprogram other tissues (for an example, see ref. 4). Nevertheless, lingering doubts persist about the possibility that host stem cells residing either in the stroma or the circulation could be the source of the reprogramming. Furthermore, the inability to elucidate the molecular mechanisms underpinning these phenomena have dimmed enthusiasm for this body of work and many still strongly believe that ''terminal differentiation'' has to be cell-autonomous and truly terminal.

The mouse mammary gland provides a versatile and robust experimental model for evaluating developmental potential of both mammary and other cell types (5, 6). Nearly 50 years ago (7) DeOme and his colleagues reported that prepubertal mouse mammary tissue could be surgically removed at 21 days postpartum from inguinal glands, leaving the remaining empty mammary fat pad available for transplantation of mammary epithelium and analysis of its developmental capacity. What followed was the discovery that dispersed mammary epithelial cells from primary cell culture (8), and fragments of mammary tissue possess the ability to recapitulate an entire epithelial component of the mammary gland on transplantation to the cleared fat pad. Stellar experiments by Sakakura and colleagues (9) showed that rudimentary mammary epithelia

were able to recapitulate normal mammary morphogenesis in the mouse mammary fat pad of females in the absence of reproductive hormones. Pulmonary, pancreatic, and salivary rudiments showed no morphogenetic response within the adult mammary fat pad, but rudimentary hair follicles underwent extensive follicle development. These studies suggested that the signals from the stroma of the juvenile to adult mammary fat pad are directed more specifically to mammary and epidermal epithelia than to the epithelia from alternate organs. Heterotypic recombinants of embryonic salivary mesenchyme and embryonic mammary epithelium underwent development into structures similar to the salivary gland, but at lactation, the isografts synthesized α-lactalbumin, a milk protein (10). Thus, the ability of the juvenile through adult mouse mammary fat pad to support mouse mammary morphogenesis is well established.

An important aspect of mammary gland regeneration by epithelial mammary fragment transplants is the observation that all portions of the epithelium regardless of age or reproductive history maintains the ability to recapitulate an entire functional gland when transplanted. This fact alone indicates that stem cell activity is either uniformly distributed throughout the mammary epithelium, or that the mammary stroma can reprogram mammary cells from different parts of the mammary gland. In addition, the potency of this activity, at least qualitatively, is una_ected by age or reproduction as demonstrated by serial transplantation for many generations (11, 12).

However, with the advent of genetically engineered mice, it became clear that ductal morphogenesis and secretory alveologenesis may be developmentally distinct activities. Smith used limiting dilution transplantation of dispersed mammary epithelial cells in full-term pregnant hosts to determine whether duct and lobule developments could be independently and selectively separated in fat pads (13). The result indicated that perhaps secretory lobule development and duct morphogenesis were activities in distinctly different mammary epithelial cell compartments [niche/lineage-limited progenitors; see also Bissell and LaBarge (14)]. Kordon and Smith (15) demonstrated that an entire functional mammary gland could comprise the progeny of a single mammary cell. Within this clone, they demonstrated the distinct lobule-limited versus duct-limited developmental activities in limiting dilution transplantation experiments.
Smith and Boulanger (16) conducted serial transplantation studies with retrovirally tagged epithelial clones in pregnant hosts, and demonstrated that duct and secretory lobule development were independently lost as mice senesced, further evidence for lobule-limited and duct-limited progenitor activity in mouse mammary epithelia generated from an individual retrovirally tagged precursor or mammary stem cell. Hierarchical mammary stem/progenitor cell activity has been documented in the mouse, the rat (17), and in the human (18, 19).

A Cre-lox recombinase system has been used to target reporter activation in mammary epithelial cells by using the mouse mammary tumor virus (MMTV) and whey acidic protein (WAP) promoters to express Cre in female mice carrying one or the other of these transgenes in combination with Rosa26/flox/flox-LacZ reporter (20). The WAP promoter most efficiently targeted the mammary epithelial cells and showed a pattern of hormonally regulated expression nearly identical to the endogenous WAP gene. Despite this, a population of activated LacZ+ epithelial cells remained after glandular involution following lactation. These LacZ+ cells were discovered to be self-renewing, multipotent cells that were responsible for regeneration of secretory lobules on ensuing pregnancies (21, 22). Subsequently, these cells were named parity-

identified mammary epithelial cells and found to be present in nulliparous mammary gland. It is this conditional model that Smith and coworkers used here (2) to demonstrate that cells from both the seminiferous tubules of mature male mice (3), and neural stem cells isolated from WAP-Cre/Rosa26LacZ reporter mice were reprogrammed to produce mammary epithelial cell progeny when mixed with mammary epithelial cells before inoculation into juvenile mammary fat pads. These seminal experiments show beautifully the dominance of signals from the mammary microenvironment, including those from somatic mammary epithelial cells, the fat pad stroma, the extracellular matrix, and soluble factors over the apparent commitment of stem/progenitor cells from a ‘‘foreign’’ tissue.

Crucial questions remain: Would NSCs differentiate into mammary epithelial cells in the postpubertal mammary microenvironment? What are the crucial factors and the overall mechanism by which the microenvironment directs neuronal stem cells? What components of the microenvironment described are essential? Can nonmammary epithelial cells mixed with NSCs accomplish the same end? Are there effects of parity or age on reprogramming? Nu/Nu mice do not have mature T cells. Would this humoral defect affect the results? And finally, can we turn MEPs or at least NSC-turned MEPs into NSCs (again)? And could we turn MEPs into neuronal cells, or at least could the converted NCSs go back to being NCSs if placed in a NSC-like microenvironment? Such questions could take a long time and much ingenuity and resources to answer. Smith and colleagues have contributed much to our appreciation of the mammary niche, but clearly additional answers are crucial not only for understanding the basic biology and how we may have evolved from stem cells, but also for stem cell therapy if we are to fulfill our promise to the public.

## Acknowledgments

The work from the authors was supported by grants from the Office of Biological and Environmental Research (OBER), awards from the National Cancer Institute, Innovator awards from the Department of Defense breast cancer program, and a Distinguished Fellowship Award from the OBER, Department of Energy (M.J.B.).

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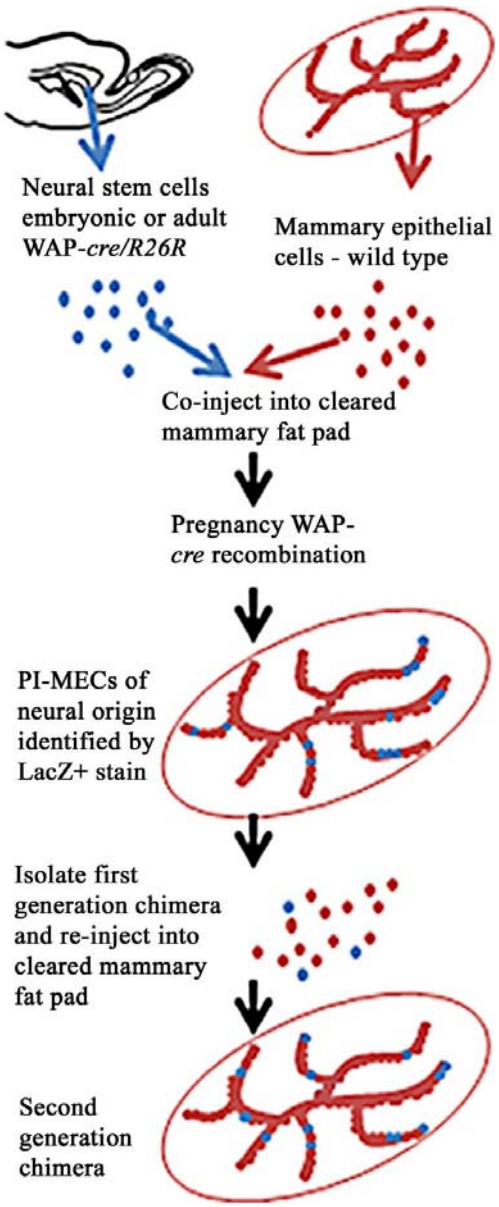
Figures

FIGURE 1




The initial outgrowths in the fat pad contained 5–7% cells of neural origin as shown by LacZ+ stain postinvolution. Cells of neural origin in the chimeric glands displayed mammary differentiation markers ERα and PR, and made the milk protein β-casein. Serial transplantations were chimeric.