CLONAL POPULATIONS OF THE MOUSE MAMMARY CELL LINE,
COMMA-D, WHICH RETAIN CAPABILITY OF
MORPHOGENESIS IN VIVO

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SUMMARY

Clonal populations were isolated from the mouse mammary cell line, COMMA-D, by transfection with
a dominant-selectable gene, pSV2Neo, which confers resistance to the antibiotic, G418. Seven of
twenty-four clones isolated retained the ability of the parental line to repopulate cleared mammary fat
pads in vivo as ductal-alveolar hyperplasias. Two sublines designated CDNR2 and CDNR4 retained
hyperplastic growth potential after multiple passages in vitro with low incidence of tumor formation. A
third subpopulation, CDNR1, contained a single integration site for the pSV2Neo plasmid indicating a
bonafide clonal origin for this subline. CDNR1 cells displayed heterogeneous growth phenotypes in
vivo including hyperplasia, adenocarcinoma, and bone formation. Functional differentiation of CDNR1
cells organized as alveolar-like structures in vivo or on floating collagen gels in vitro was observed as
determined by immunoperoxidase staining for the milk-specific protein, casein. Overall, the results
indicate that a subset of cells from the COMMA-D cell line may be functionally analogous to stem cells
existing in the mammary gland.

Key words: mammary; epithelial; clones; morphogenesis; casein synthesis.

INTRODUCTION

The mammary glands originate from epidermal
derivatives and consist of a branching network of ducts
and alveoli within an adipose stroma. During maturation
of the female mouse, the duct system enlarges to fill a pad
of predominantly fatty tissue (30). Upon pregnancy,
further ductal branching occurs and clusters of alveoli
grow out from the sides and ends of the ducts. Each
alveolar cell originates from a ductal cell and is a
complete milk-producing unit, capable of synthesizing
milk fat, protein, and carbohydrate in response to
lactogenic hormones (4). In addition to these two
epithelial cell types, myoepithelial cells are present
surrounding the alveolar buds. During lactation, the
myoepithelial cells contract in response to the pituitary
hormone, oxytocin, causing ejection of milk products
from the alveoli.

The possibility that a mammary stem cell population
exists at the terminal end buds of the growing ductal tree
has been postulated by several investigators. Williams
and Daniel (41) have described a layer of proliferating
cuboidal epithelial cells at the end bud tip which seems
undifferentiated morphologically and seem to give rise to
myoepithelial cells surrounding the ducts. These cap
cells may be pluripotent stem cells in the mammary
gland, although evidence for a direct cell lineage to the
lumenal cell population was not presented by the authors.
Similarly, Dulbecco and his co-workers (8) have shown by
immunofluorescent staining that an epithelial cell
population at the tip of the growing end bud shares
several marker proteins common to both myoepithelial
and ductal lumenal cells. However, a separate lineage
was proposed for lumenal cells destined to differentiate into
alveolar cells.

To date, little work has been done to isolate and
identify a defined mammary cell population capable of complete regeneration of a mammary gland tree.
Transplantation studies have demonstrated that an
epithelial stem cell component must exist in the mouse
mammary gland throughout life. Samples taken from any
portion of the mammary gland at any age and at any
developmental state, including full functional differenti-
ation, gave rise to mammary epithelial outgrowths with
complete developmental capacity (15,35). Ormerod and
Rudland (28), demonstrated that rat mammary ductal
tissue free of lateral and alveolar buds can give rise to branching outgrowths within interscapular adipose tissue of syngeneic hosts. Isolated terminal end buds gave rise to similar structures which contained ducts, lateral buds, and terminal end buds. Alveolar buds containing casein-secreting cells also appeared if the outgrowths were allowed to develop for 15 to 16 wk. The authors hypothesized that uncommitted stem cells arose from severed ductal ends, forming end buds which could then regenerate the entire mammary gland tree. Smith and Medina (35) described the presence of morphologically distinct cells distributed sporadically among the mammary epithelium whose behavior in vivo and in vitro suggests that they might represent a latent stem cell population. However, proof of the existence of such a stem cell rests on the ability to isolate in vitro a cloned population which is capable of producing an entire mammary gland tree. To directly address this possibility, we have derived clonal cell populations from the COMMA-D cell line, a mammary cell line known to repopulate the mammary fat pad, and tested them for outgrowth potential. We describe here the growth properties in vivo of four of these clonal subpopulations.

MATERIALS AND METHODS

Cell culture and transfection procedure. The mouse mammary cell line, COMMA-D, has been described previously (5). It was routinely propagated in growth medium containing Dulbecco’s modified Eagle’s (DME) and Ham’s F12 (1:1 mixture) supplemented with 2% fetal bovine serum (FBS), bovine pituitary extract (10 μg/ml), Mito+ serum extender used at a 1:1000 dilution, and gentamicin sulfate (5 μg/ml). Cultures were passaged by treatment of cell monolayers (50% confluent) with dispase (type II), used at a concentration of 2.4 mg/ml in HEPES-buffered saline, designated solution A (34). After 15 to 20 min incubation in the dispase solution at 37 °C, the cells were dislodged by gentle pipetting. The COMMA-D cell line was previously confirmed to be free of mycoplasma by examination of cell monolayers stained with the DNA-specific fluorescent dye, Hoechst 33258 (5).

COMMA-D cells were transfected with the plasmid pSV2Neo (37) by the calcium phosphate precipitation technique (13). Cell monolayers (50% confluent) were washed with Hank’s balanced saline solution (HBSS) and treated with polyethylene glycol 6000 in growth medium at a concentration of 0.5 g/ml for 10 min at 37 °C (38). The cultures were washed twice with HBSS, and then plasmid DNA (5 μg for approximately 1 × 10⁶ cells) was introduced into cells as a calcium phosphate coprecipitate. After 4.5 h at 37 °C, the cultures were washed once with growth medium, and fresh growth medium was added. Within 48 h, the neomycin analog G418 was added to the growth medium at a concentration of 500 μg/ml several days after plating.

Culture of cells on collagen gels. CDNR cells were seeded on a collagen gel to determine inducibility of casein synthesis in vitro. A collagen gel mixture containing 5% Matrigel was prepared as previously described (9,42) and allowed to gel at room temperature. Cells were seeded on the gel in the presence of growth medium, which was changed after 24 h and thereafter every 2 to 3 d. After 12 d incubation, the gel was gently released from the bottom of the dish using a sterile Pasteur pipette and induction medium consisting of DME:F12 supplemented with 5% FBS, prolactin (5 μg/ml), aldosterone (5 μg/ml), and hydrocortisone 15 was discovered that G418-resistant colonies of COMMA-D cells could also be generated by introducing DNA into cells in the presence of polybrene, a polycation reagent which facilitates adsorption of DNA to cell membranes (21,27). COMMA-D clones resistant to G418 were designated CDNR1, CDNR2, CDNR3, etc.

To prepare primary cultures of CDNR cells derived from outgrowths in vivo, mammary fat pads containing 3- to 4-mo.-old outgrowths were removed aseptically and minced finely. Epithelial cell clumps were released by enzymatic dissociation in a solution of DME:F12 containing 0.1% collagenase (type III) for 4 to 5 h at 37 °C (10). The cell clumps were collected by centrifugation, washed in growth medium and seeded in 100-mm-diameter culture dishes. To ensure that recultured cells were derived from the cloned populations, the neomycin analog G418 was added to the growth medium at a concentration of 500 μg/ml several days after plating.

FIG. 1. Southern blot of CDNR clones 1, 2, 3, and 4 transfected with pSV2Neo. Total genomic DNAs isolated from G418-resistant CDNR clones were digested separately with EcoRI and PstI. A ten microgram quantity of each digested DNA was separated on a 0.8% agarose gel and transferred to a nitrocellulose filter. Filter was hybridized to the pSV2Neo 4.8 kb Ham HI-EcoRI fragment which had been nick translated with [³²P]dCTP to a specific activity of 2 × 10⁶ cpm/μg. A, EcoRI enzyme digest of total CDNR DNA, Lanes 1, 2, 3, 4; CDNR clones 1, 2, 3, and 4, respectively. B, PstI enzyme digest of total CDNR DNA, Lanes 1, 2, 3, 4; CDNR clones 1, 2, 3, and 4, respectively. DNA molecular size markers are indicated on the left in kilobase pairs.