Biology of mammary fat pad in fetal mouse: capacity to support development of various fetal epithelia *in vivo*

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Summary

Epithelia from the lobular part of submandibular salivary gland, glandular stomach, intestine and colon of 14-day C3H/HeN fetuses, and from pituitary gland and pancreas of 12-day fetuses were recombined with 14-day mammary fat pad precursor tissue and syngrafted under the kidney capsule. The normal organogenetic development typical of the epithelium occurred. The same epithelia taken from earlier stage fetuses did not develop normally. Thus, 14-day fetal mouse mammary fat pad precursor tissue has the capacity to support normal organogenesis of various fetal epithelia of developmentally advanced stages. This supportive capacity is decreased in the fat pad precursor tissue of 17- to 18-day fetal mice and is entirely lost postnatally.

Key words: mammary fat pad, mouse, epithelium, graft, organogenesis.

Introduction

In our previous study, two different mesenchymes that affect mouse mammary gland development were identified histologically (Sakakura, Sakagami & Nishizuka, 1982). One is fibroblastic mammary mesenchyme closely surrounding the epithelial rudiments and the other is fat pad precursor tissue. Of these two tissues, the fibroblastic mesenchyme has been verified as the element determining absence of nipples of the mammary glands in male mice (Kratochwil & Schwartz, 1976; Drews & Drews, 1977; Durnberger & Kratochwil, 1980; Heuberger, Fitzka, Wasner & Kratochwil, 1982). In female mice and also in male mammary glands at the stage prior to the phenotypical sex differentiation, this fibroblastic mesenchyme may have an inductive function, determining and fixing the ability of fetal mammary epithelium to grow in the fatty stroma (Propper, 1968; Sakakura, 1983). Very recently a novel extracellular matrix protein, which was previously described as myotendinous antigen (Chiquet & Fambrough, 1984) and now named tenasin by Chiquet-Ehrismann, Mackie, Pearson & Sakakura (1986), was detected by immunohistochemical study specifically in the fibroblastic mesenchyme. Neither fat pad precursor tissue nor dermis was stained by anti-tenasin. The function of tenasin is not clear yet but it is presumably a growth promoter. When the primary mammary tumour cells were incubated on tissue culture wells coated with tenasin, these cells were able to continue growing without fetal calf serum as determined by \[^3H\]thymidine incorporation. However, when the wells were coated by other substrates such as laminin, fibronectin or collagen I or IV, these cells stopped growing in the serum-free condition (Chiquet et al. 1986). The same result was observed in transplantation studies. When fetal mammary epithelium was combined with this fibroblastic mesenchyme and transplanted under the kidney capsule, the epithelium underwent development by ductal hyperplasia. However, when the epithelium was combined with fat pad precursor tissue, the morphogenesis of the resulting gland was that of the typical mammary gland (Sakakura et al. 1982). Thus, fibroblastic mesenchyme may be important for the determination of
mammary gland and the epithelial growth at an early developmental stage.

The mammary fat pad precursor tissue, on the other hand, is first recognized at the 14-day stage, slightly distant from the mammary epithelial rudiments, as a mass of condensed mesenchyme. At this stage, the fat pad precursor tissue is fairly large in size occupying more than half of the subcutaneous space of the trunk. Then, at the late 16-day stage, these cells start to differentiate into adipocytes and complete the conversion to adipocytes 48 to 72 h after birth (Slavin, 1979; Sakakura et al. 1982). With respect to the function of this tissue, numerous publications describe how mammary fatty tissue determines the unique morphogenesis of normal mammary parenchyma in both fetal (Sakakura, Nishizuka & Dawe, 1976, 1979a; Sakakura et al. 1982) and adult (DeOme, Faulkin, Bern & Blair, 1959; Hoshino, 1962; Sakakura, Sakagami & Nishizuka, 1979b; Sakakura et al. 1982; Daniel, Berger, Strickland & Garcia, 1984) mice. Additionally, mammary fat pad has been used as a preferable site for transplantation of the various tissues obtained from syngeneic mice. Tissues that survive better in the fat pad than subcutaneously include normal (DeOme et al. 1959; Hoshino, 1962; Moretti & Blair, 1966; Outzen & Custer, 1975), preneoplastic (DeOme et al. 1959) and neoplastic (Outzen & Custer, 1975; Miller, Medina & Heppner, 1981) mammary tissues, and normal pancreas epithelium (Outzen & Leiter, 1981). Furthermore, several recent papers have dealt with the growth-promoting activity of mammary fibroblasts and adipocytes towards mammary epithelium in culture: mammary fibroblasts of adult (Enami, Enami & Koga, 1983) and fetal (Taga, Sakakura & Oka, 1983) mice, rat mammary preadipocytes (Rudland, Davies & Tsao, 1984), 3T3-Li adipocytes (Levine & Stockdale, 1984) and adult fat pad explants (Carrington & Hosick, 1985). Thus, many publications suggest the growth-promoting activity of mammary fat pad. However, little has been written to define the precise function of this tissue.

In the present study, we have found an important function of fetal mammary fat pad. This paper describes evidence that mammary fat pad precursor tissue isolated from 14-day mouse fetuses has a capacity to induce several foreign epithelial types to proceed along their own predetermined developmental pathways.

Materials and methods

**Animals**

Male and female mice of C3H/HeN strain, originated in 1975 at the Small Animal Section, Veterinary Resources Branch, Bethesda, Maryland and maintained in this laboratory were used throughout the present experiments. The developmental stages of the fetuses were counted from the day a copulation plug was observed (day 0).

**Recombination and transplantation of SE and FP**

Submandibular salivary gland rudiments were dissected from 13- and 14-day fetuses aseptically in 2 % fetal calf serum (FCS) in Hank’s balanced salt solution (HBSS). The salivary epithelium (SE) was separated from mesenchyme by collagenase digestion and subsequent dissection using fine cataract knives as described previously (Kusakabe, Sakakura, Sano & Nishizuka, 1984). The epithelial component taken from each lobule was considered as one SE fragment. Prospective mammary fat pads (FP) were taken from no. 4 mammary glands of 14-day (the stage before fat droplets appear) or 17- to 18-day (after fat droplets appear) female fetuses, and from newborn and 3-week female mice all by the dissection methods described previously (Sakakura et al. 1982). Dermis was also separated from skin of the back of 14-day fetuses by collagenase digestion.

Each FP was cut into three or four pieces. In experiments with dermis, tissue pieces of a similar size were prepared. In the middle of each piece of small slit was made into which one 14-day SE was inserted. After incubation for 2 h in 50 % FCS in HBSS, the tissue recombinants were transplanted under the kidney capsule of syngeneic young male mice. Animals were killed 1–2 weeks after transplantation. All explants were fixed in 10 % formalin and were prepared for morphological examinations as wholemounts as described previously (Sakakura et al. 1979b). Some of the tissues were also observed histologically after thin sectioning using standard techniques.

**Preparation of epithelial rudiments from other organs**

Stomachs and intestines were obtained from 11- and 14-day fetuses, colons from 12- and 14-day fetuses, pituitary glands and pancreas from 10- and 12-day fetuses, of both sexes. Separation of the epithelial components from these organs, reassociation of the epithelia with FPs of various ages and subsequent renal subcapsular transplantation of recombicants were performed by the same method as in the submandibular gland experiments. The various epithelia were also transplanted without mesenchymal tissues. Histological examination of the resulting glands, harvested 2 weeks after transplantation, was performed by serial thin sections. Glandular stomach epithelium, intestinal epithelium, colon epithelium, anterior pituitary epithelium and pancreatic epithelium were abbreviated GstE, IntE, CoIE, PitE and PanE, respectively.

**Criteria for morphogenesis of various organs**

The morphogenesis of various organs was characterized by the following histological features: submandibular gland, formation of ducts and secretory endpieces of seromucous acini; glandular stomach, glandular pit structures consisting of mucus-secreting cells, chief cells and parietal cells; small intestine, crypt villi structure consisting of columnar cells, goblet cells and Paneth cells; colon, single tubular gland structure of columnar cells and many goblet cells; anterior pituitary, formation of Rathke’s pouch and pars distalis.
characterized by epithelial proliferation on one side of the
tubuloalveolar gland structure and development of islets of
Langerhans.

Test for the contamination of epithelia with
mesenchymal tissue

The validity of tissue recombination studies is based on the
assumed purity of the separated epithelium and mesenchyme. In order to rule out the possibility that any
mesenchymal component might have remained in the
separated epithelia, we performed recombination exper-
iments using tissues from two different strains of mice that
have strain-specific markers for the tissues under consider-
ation. FP from BALB/c mice was recombined with various
epithelia taken from C3H/HeN and FP from C3H/HeN
mice was recombined with BALB/c epithelia. These re-
ciprocal recombinant tissues were transplanted under the
kidney capsules of nude mice. The resulting glands were
then stained with an antibody against C3H mice. The
antibody was produced by immunization of (BALB/c ×
SJL)F1 female mice with the fractions containing glucose-
phosphate isomerase (GPI) of livers and muscles of
C3H/HeN mice. The methods for the production of the
antibody and immunohistochemistry were described in a
previous paper (Kusakabe, Sakakura, Sano & Nishizuka,
1985). In this case, the method of immunohistochemistry
was slightly changed by using the FITC-labelled avidin-
biotin technique instead of an indirect immunofluorescent
staining method.

Application of these methods has proved that epithelia
isolated from the various organs were not contaminated
with mesenchymal cells. Sometimes we found a fairly large
number of fibroblasts surrounding the transplanting ep-
ithelium, but these cells have originated from FP, not from
the epithelial component. Immunohistochemical staining of the
resulting glands of SE and FP recombination between
C3H/HeN and BALB/c mice is shown in Fig. 1A,B. The
dense fibroblasts surrounding the epithelia were positively
stained by anti-C3H antibody when the FP was isolated
from C3H/HeN (Fig. 1A), but were not stained when the
FP was from BALB/c mice (Fig. 1B). Therefore, this result
indicates that the origin of the dense fibroblasts is FP. These
FP-derived fibroblasts have been shown to play a significant
role in epithelial morphogenesis; these observations will be
described elsewhere (T. Sakakura et al., unpublished data).

Results

Morphogenetic behaviour of the recombinants of SE
with FP

Table 1 summarizes the morphogenetic patterns in
SEs of 13- to 15-day fetuses recombined with FPs of
various ages. When SEs taken from 13-day fetuses
were recombined with 14-day FPs, about 40 % of the

Fig. 1. Sections of reciprocal combination between C3H/HeN and BALB/c strains. Immunofluorescence study of
recombinant tissues of 14-day fetal SE and FP, a week after transplantation in nude mice, staining for C3H-strain-
specific antigen. (A) BALB/c-SE/C3H-FP, (B) C3H-SE/BALB/c-FP. ×160. Dense fibroblasts (DF) surrounding
epithelium and FP are positively stained in A, but not stained in B tissue. This clearly indicates that these fibroblasts are
derived from FP, but not from contaminated mesenchyme in epithelium.
Table 1. Developmental behaviour of the recombinants of SE with FP observed 2 weeks after transplantation

<table>
<thead>
<tr>
<th>Recombinants</th>
<th>SE (age)</th>
<th>FP (age)</th>
<th>No. of grafts</th>
<th>No. recovered</th>
<th>Cyst</th>
<th>Ductal branching*</th>
<th>Salivary gland (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13-day</td>
<td>14-day</td>
<td>40</td>
<td>22</td>
<td>11</td>
<td>9</td>
<td>2 (9.0%)</td>
</tr>
<tr>
<td></td>
<td>14-day</td>
<td>14-day</td>
<td>57</td>
<td>51</td>
<td>4</td>
<td>8</td>
<td>39 (77.2%)</td>
</tr>
<tr>
<td></td>
<td>15-day</td>
<td>14-day</td>
<td>19</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>17 (94.4%)</td>
</tr>
<tr>
<td></td>
<td>14-day</td>
<td>—</td>
<td>22</td>
<td>7†</td>
<td>0</td>
<td>0</td>
<td>10 (43.4%)</td>
</tr>
<tr>
<td></td>
<td>17-day</td>
<td>14-day</td>
<td>30</td>
<td>23</td>
<td>2</td>
<td>11</td>
<td>6 (30.0%)</td>
</tr>
<tr>
<td></td>
<td>18-day</td>
<td>14-day</td>
<td>34</td>
<td>20</td>
<td>1</td>
<td>13</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Newborn</td>
<td>(0–3 days)</td>
<td>14-day</td>
<td>20</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Dermis</td>
<td>14-day</td>
<td>14-day</td>
<td>14</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

* In this case, ductal branching means an irregular branching pattern.
† All seven recovered tissues survived but without cell proliferation.

Fig. 2. Wholemounts (A–D) and section (E) of the resulting glands of SEs combined with 14-day FPs transplanted under the kidney capsule. Cyst formation of 13-day SE (A, x39), irregular ductal development of 13-day SE (B, x41) and acinar development of 14-day SE (D, x41), with FP 2 weeks after the transplantation. (C) Early stage of the development of 14-day SE with FP, 5 days after the transplantation. The development of the SE resembles the early stage of salivary gland embryogenesis (x41). (E) Histological section of 14-day SE with FP. Two large cysts, numerous small ducts and seromucous acini are seen. Note 14-day FP converted to fatty tissue around the developed salivary gland (x50).
glands degenerated or disappeared. Cyst formation (Fig. 2A) or irregular ductal branching (Fig. 2B) was seen in many of the recovered grafts. In only two cases of 13-day SE grafts, the typical salivary gland pattern of closely grouped adenomeres was observed. When SEs from 14- or 15-day fetuses were recombined with 14-day FPs, the epithelial rudiments branched out, recapitulating submandibular gland embryogenesis (Fig. 2C) and about 80-90% of the recovered grafts showed salivary gland development (Fig. 2D). Histological examination revealed the presence of a large number of small ducts and seromucous acini (Fig. 2E). If 14-day SEs were transplanted without supporting mesenchyme, neither growth nor submandibular gland morphogenesis occurred. All such SEs either survived without signs of cell proliferation or else disappeared. Thus, SEs from 14- or 15-day fetuses can interact with 14-day FP so as to undergo a normal developmental sequence. However, 13-day SE only responds weakly to mammary FP.

To investigate the age specificity of FP capacity for supporting submandibular gland development of SE, FPs taken from donors of different ages were recombined with 14-day SE. As indicated clearly in Table 1, 14-day SE did not develop when recombined with neonatal FP. 17- and 18-day FPs support 14-day SE development in 43-3% and 30% of cases, respectively; the proportion of positive responses decreases with FPs from the older animals. When 14-day SE was recombined with 14-day fetal dermis, which is similar in appearance to FP at this stage, six of fourteen SEs formed branching ducts of a type not characteristic of salivary gland and the remaining eight developed into cysts. Thus, 14-day dermis cannot support the normal development of 14-day SE.

Responsiveness of various other epithelia to FP

Table 2 summarizes the developmental behaviour of GstE, IntE, ColE, PitE and PanE recombined with 14-day FP. When 11-day GstE and IntE or 12-day ColE were transplanted with 14-day FP, a large number of the grafts formed cysts without any other characteristic morphology. Cysts of GstE were lined by a single layer of cuboidal cells without differentiation of parietal and chief cells (Fig. 3A). In contrast, among the cells lining IntE and ColE cysts, the characteristic cells, such as Paneth cells in IntE cyst and goblet cells in ColE cyst, were observed (Fig. 3B,C). All of the 10-day PitE recovered formed cysts lined by two or three layers of cuboidal cells (Fig. 3D). All the grafted 10-day PanEs were resorbed. When epithelia from older fetuses were recombined with 14-day FPs, more than half of the transplants developed more typically, forming the characteristic glandular pit structures with mucous, parietal and chief cells (Fig. 4A), crypt villi structure including both goblet cells and Paneth cells (Fig. 4B) or deep crypts composed of many goblet cells (Fig. 4C). When 12-day PitE was transplanted with 14-day FP, the epithelial cells proliferated forming pars distalis (Fig. 4D). In the case of 12-day PanE transplants, the recovered transplants developed only into ducts and islets without pancreatic acinar formation (Fig. 4E).

Table 2. Developmental behaviour of recombinants of epithelial components of various organs with 14-day FP

<table>
<thead>
<tr>
<th>Epithelium</th>
<th>No. of grafts</th>
<th>No. recovered</th>
<th>Cyst</th>
<th>Characteristic morphogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GstE</td>
<td>11-day</td>
<td>12</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>14-day</td>
<td>15</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>14-day without FP*</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>IntE</td>
<td>11-day</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>14-day</td>
<td>13</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>14-day without FP*</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ColE</td>
<td>12-day</td>
<td>16</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>14-day</td>
<td>18</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>14-day without FP*</td>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PitE</td>
<td>10-day</td>
<td>30</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>12-day</td>
<td>18</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>12-day without FP*</td>
<td>20</td>
<td>18</td>
<td>18†</td>
</tr>
<tr>
<td>PanE</td>
<td>10-day</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12-day</td>
<td>18</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>12-day without FP*</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Epithelia were transplanted alone without support of FP.
† 12-day PitE became cysts of 5-10 layers of cells. No morphogenesis was found, but some of those cells differentiated into ACTH-producing cells. Cited from our previous report (Kusakabe et al. 1984).
Fig. 3. Sections of the resulting glands of fetal GstE (A, x44), IntE (B, x162), ColE (C, x50) and PitE (D, x47) taken from developmentally early stage, with 14-day FPs transplanted under the kidney capsule. These epithelia became cysts without unique morphogenesis. The cells lining GstE cyst are cuboidal. Neither parietal nor chief cells are observed. The cells lining IntE and ColE cyst are goblet and Paneth cells (inset, x420), and goblet cells (inset, x427), respectively.

Table 3. Age-dependent capacity of FP to induce epithelial morphogenesis

<table>
<thead>
<tr>
<th>Epithelium: FP (age)</th>
<th>No. of grafts</th>
<th>No. recovered</th>
<th>Cyst</th>
<th>Characteristic morphogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-day GstE: f14-day</td>
<td>15</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>14-day: f17- to 18-day</td>
<td>16</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>: newborn</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>14-day IntE: f14-day</td>
<td>13</td>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>14-day: f17- to 18-day</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>: newborn</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14-day ColE: f14-day</td>
<td>18</td>
<td>13</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>14-day: f17- to 18-day</td>
<td>16</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>: newborn</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12-day PitE: f14-day</td>
<td>18</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>12-day: f17- to 18-day</td>
<td>16</td>
<td>15*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>: newborn</td>
<td>10</td>
<td>6*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12-day PanE: f14-day</td>
<td>18</td>
<td>5</td>
<td>0</td>
<td>5†</td>
</tr>
<tr>
<td>12-day: f17- to 18-day</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>: newborn</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Epithelial cells survived without forming any characteristic structure.
† All of these recombinants developed ducts and islets, but not acini.
If these epithelia were transplanted alone, many of them underwent almost the same morphogenesis as that observed when the epithelia from younger fetuses were recombined with 14-day FP. GstE, IntE and ColE made cysts lined by single layers of cells. Goblet cell differentiation occurred slightly in the IntE and ColE cysts. Eleven of twelve 12-day PitEs survived under the kidney capsule but without any sign of cell proliferation. None of the 12-day PanE were recovered at autopsy.

In summary, 14-day FP had the capacity to promote normal development of various fetal epithelia of advanced fetal age, but not epithelia of younger ages. Neither 17- to 18-day FP nor newborn FP promoted normal development of GstE, IntE or ColE from 14-day fetuses, or of PitE or PanE from 12-day fetuses. The transplanted epithelia either disappeared or became cysts (Table 3).

**Discussion**

Fetal mammary epithelium undergoes normal morphogenesis when transplanted into epithelium-free mammary fat pads of postnatal mice either 3 or 40 weeks of age (Hoshino, 1962; Daniel & DeOme, 1965; DeOme, Miyamoto, Osborn, Guzman & Lum, 1978; Sakakura et al. 1979a). The resulting glands respond normally to lactogenic hormones. Thus, postnatal mammary stroma is capable of promoting

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**Fig. 4.** Sections of the resulting glands of fetal GstE (A, ×100), IntE (B, ×101), ColE (C, ×163), PitE (D, ×48) and PanE (E, ×160) taken from developmentally advanced stage with 14-day FPs transplanted under the kidney capsule. (A) 14-day GstE developed characteristic glandular pit structure with mucous, parietal and chief cells. (B) 14-day IntE formed crypt villi structures with goblet and Paneth cells (inset, ×400). (C) 14-day ColE developed some crypts with numerous goblet cells. (D) 12-day PitE formed Rathke's pouch (R) and pars distalis (arrow) by epithelial cell proliferation. (E) 12-day PanE formed ducts and islets (arrows).
normal development of both fetal and adult mammary parenchyma. However, epithelia from other organs, including salivary gland, lung and pancreas, fail to undergo morphogenesis when transplanted into mammary fat pad of postnatal mice (Sakakura et al. 1979a). In the present study we have now demonstrated that mammary fat pad precursor tissue from 14-day fetuses does have the ability to support development of fetal epithelia from a variety of organs. This supportive ability, however, is already greatly decreased in the fat pads of 17-day fetuses. One question may arise as to whether there is a regulating effect of the fat pad in older animals which might have inhibited the development of the transplanted foreign epithelia. As shown in Table 2, if these epithelia were transplanted alone, many of them were lost and the rest of the epithelia survived without growth but with slightly differentiated cells. This result clearly shows that fetal epithelia cannot undergo morphogenesis autonomously at the site of the renal subcapsular region, if they are transplanted without the support of mesenchyme. Therefore, the possibility that the fat pad in older animals may regulate autodifferentiation potentials that the various epithelia have, is not conceivable in this case.

As it matures into adipose tissue, therefore, fetal mammary fat pad precursor tissue loses its ability to interact successfully with foreign (but not native) fetal epithelia. Our observations thus suggest that the mechanism(s) of interaction of foreign epithelium with fetal fat pad is different from that of native epithelium with the fat pad. A variety of changes is known to occur in unspecified mesenchyme cells as they mature into adipocytes. Lipid droplets appear in adipose precursor tissue at 16–17 days of gestation in the mouse (Slavin, 1979; Sakakura et al. 1982). Associated with lipid accumulation are changes in the quantities of numerous enzymes that participate in lipid metabolism (e.g. Wise & Green, 1979; Cook & Kozak, 1982). In addition, a distinct basal lamina accumulates around adipose cells during their maturation (Kuri-Harcuch, Arguello & Marsh-Moreno, 1984). We have recently identified two substances characteristic of extracellular basal lamina, laminin and proteoheparan sulphate, in 17-day FP but not 14-day FP (Kimata, Sakakura, Inaguma, Kato & Nishizuka, 1985). The extracellular biomatrix is now known to be of fundamental importance in supporting growth and differentiation of mammary and other epithelia (Rojkind, Gatmaitan, MacKensen, Gambone, Ponce & Reid, 1980; Wicha, Lowrie, Kohn, Bagavandoss & Hahn, 1982) and subtle changes in composition of biomatrix can have a large impact on epithelial cell function (Rojkind et al. 1980). Thus one likely possibility is that changes in composition of extracellular matrix underlie the altered interaction of developing fat pad with epithelia and we plan to test this possibility by comparing bioactivity of various mesenchyme-derived matrices.

There is also some more-specific evidence available to suggest the molecular basis for interaction of mammary epithelium and mesenchyme. Enami and his associates (1983) have described a growth factor produced by adult mammary mesenchyme that specifically stimulates mammary epithelial cell proliferation. They have partially purified this factor from culture medium conditioned by fibroblasts from adult mouse mammary gland. Taga et al. (1983) have also described a substance in medium conditioned by 17-day fetal mesenchymal cells that affects proliferation and cytodifferentiation of mammary epithelium. Note that the mesenchymal cells from which these factors were isolated (i.e. from 17-day fetuses and adults) fail to support morphogenesis of foreign epithelia. It is likely that a different mesenchymal substance(s) plays this role. We now have in progress experiments similar to those described above but using a newly developed culture system (Inaguma, Nishi, Sakakura, Kusakabe & Hoshick, 1987) in which we seek to identify specific mammary mesenchyme-produced molecules that will support function of nonmammary epithelia.

The fetal FP tissues used in the present study are heterogeneous in cellular composition, being made up of presumptive adipocytes, fibroblasts, endothelial cells, nerve cells and, perhaps, other cells types. We do not yet know which cell type actually provides the stimulatory molecules. Furthermore, it is not yet known if adult or fetal mesenchyme from other organs, especially those rich in adipose tissue, can have similar stimulatory effects. Fetal dermal mesenchyme fails to support development of SE, suggesting that the stimulatory ability is not universal among all fetal mesenchymes. We cannot contend that the biological activity of fetal FP is entirely specific without extensive testing of many more tissue combinations using mesenchyme from mice of various ages. This is beyond the scope of the present paper, in which we are interested primarily in the nature of fetal FP.

Early work by Grobstein (1953) suggested that salivary epithelium is responsive only to salivary mesenchyme in vitro. Subsequently, however, Cunha (1972) showed that mesenchyme of urogenital sinus origin can induce morphogenesis of salivary epithelium in vivo and Lawson (1974) has reported that lung mesenchyme will support budding and cytodifferentiation of salivary epithelial rudiments in vitro. We report here a third heterologous tissue, mesenchyme of 14-day-old fetal mouse mammary gland, that can support salivary morphogenesis of 14- to 15-day-old SE. However, SE from 13-day fetus did not
respond to mammary mesenchyme. Apparently the SE became responsive to fetal FP between days 13 and 14, simultaneously with the first evidence of salivary-like branching in situ. Results with other mesenchymal/epithelial combinations of different ages suggest such a critical period of ‘determination’ in these organs as well. The system described in this paper should prove useful for continuing our ongoing exploration of the biological and biochemical bases of the role of mammary fat pad in prenatal and postnatal mammary development.

The results presented suggest that we will be able to learn how interaction with the fat pad precursor tissue promotes epithelial morphogenesis in the mammary gland, rather than just epithelial growth which is the focus of work by several other groups. Our goal is to learn the molecular basis by which mammary fat pad precursor tissue stimulates epithelial morphogenesis; the feasibility of our approach is greatly increased by the present observations and also by a newly developed culture system for fetal tissues (Inaguma et al. 1987).

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