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In vivo and in vitro evidence of basic fibroblast growth factor action in mouse mammary gland development

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Abstract Basic fibroblast growth factor (bFGF) stimulated [^{3}H]thymidine incorporation at all stages of development, although the magnitude of this effect was the greatest in cells derived from pregnant mice. Cells primed with insulin and bFGF synthesized more casein than cells not exposed to either hormone. bFGF inhibited casein synthesis and decreased the amounts of β-casein and α-lactalbumin transcripts in cells from pregnant animals simultaneously incubated with insulin, hydrocortisone and prolactin. bFGF content in mammary gland increased with puberty and pregnancy, but decreased markedly in lactation; the number of bFGF receptors in epithelial cells changed in parallel. These data suggest that bFGF may have a physiological role both in stimulating growth and in inhibiting functional differentiation of normal mouse mammary epithelial cells.

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Key words: Basic fibroblast growth factor; Growth factor; Milk protein; Mammary gland; Gene expression

1. Introduction

The process whereby embryonic mouse mammary epithelial cells (MMEC) develop into secretory cells capable of producing milk involves a series of growth and developmental changes that occur over a relatively long period of time. The mammary tissue of virgin animals is developmentally dormant and it contains a relatively small number of epithelial cells. During pregnancy, MMEC multiply extensively to form the lobulo-alveolar structure, although their ability to produce milk still is suppressed. Following parturition, the mammary tissue expresses its differentiated function by synthesizing and secreting milk and then regresses to the undifferentiated states upon weaning [1]. Mouse mammary growth and differentiation that occur during pregnancy and lactation are controlled by systemic steroid and polypeptide hormones as well as local regulatory growth factors [2]. Although the importance of endocrine hormones such as estrogen, progesterone, prolactin and growth hormone in the mammary gland has been well documented, these hormones are virtually incapable of stimulating mammary epithelial cell growth in vitro [3]. Thus, the growth stimuli for mammary cells need clarification. In recent years, epidermal growth factor (EGF), insulin-like growth factor-I, transforming growth factor α and β have been implicated in mammary gland development [2,4]. Basic fibroblast growth factor (bFGF or FGF-2) is a member of a large multigene family of heparin-binding growth factors [5–7], which can act locally in a paracrine, autocrine or intracrine fashion [8]. Although originally described as a mitogen for fibroblasts, bFGF is also a potent mitogen agent for diverse cell types [9]. Some studies have indicated the presence of bFGF in the mammary tissue [10–14]. Northern blot analysis has revealed three bFGF mRNA transcripts of 7.5, 4.4, and 2.2 kb in human mammary epithelial cells (HMEC) and in the immortal mammary cell line HBL-100 [10]. However, of the four mammary-tumor-derived cell lines, only the Hs578T cells produced detectable levels of bFGF mRNA. Western blot analysis of cell lysates using an anti-bFGF monoclonal antibody detected three distinct molecular weight species of bFGF protein (18, 24 and 27 kDa) in normal HMEC strains, in HBL-100 and HS578T cells, but not in the other tumor cell lines [10]. FGF-1 (acidic fibroblast growth factor or aFGF), FGF-2, FGF-4 and FGF-7 are expressed during the ductal stage of mammary development, the majority of FGF-1 gene expression was in the luminal epithelial cells, whereas FGF-2 expression was in the mammary stroma and possibly the myoepithelial cells [13]. Several lines of evidence suggest a role for bFGF in the growth and differentiation of the mammary gland. bFGF stimulates the growth of rat mammary myoepithelial and stromal cell lines in culture [15], mammary epithelial cell line COMMDA-1D [16] and also T-47 and MCF7 breast cancer cells [17,18]. bFGF also stimulates growth and inhibits casein production in virgin mouse mammary cells [19]. These findings prompted us to examine the effect of bFGF on proliferation and milk protein gene expression in normal MMEC differentiating in culture and to study the changes in the content of bFGF and the number of bFGF receptors in these cells during the development of the mouse mammary gland.

2. Materials and methods

2.1. Chemicals

Sources were as follows: protosol and [^{3}H]amino-acid mixture (1 mCi/ml), NEN-Dupont (Boston, MA, USA; [methyl-^{3}H]thymidine (68 Ci/mmol) and [α-^{32}P]UTP (800 Ci/mmol), Amersham (Arlington Heights, IL, USA); Na^{125}I, Comisión Chilena de Energía Nuclear (Santiago, Chile); Riboprobe system, Promega (Madison, WI, USA); T_7 and T_3 RNA polymerases, Stratagene (La Jolla, CA, USA); human recombinant bFGF, Chiron (Emeryville, CA, USA); mouse EGF (culture grade), Collaborative Research (Bedford, MA, USA); goat-anti-rabbit IgG, Miles Lab. (Elkhart, IN, USA); Affi-Gel heparin gel (90–180 μm), Bio-Rad (CA, USA); Vitrogen 100 (purified collagen), Celtrix Pharmaceuticals (Santa Barbara, CA, USA); DME and fetal bovine serum medium, Gibco (Grand Island, NY, USA);

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Abbreviations: bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; F, hydrocortisone; I, insulin; MMEC, mouse mammary epithelial cells; P, prolactin

Table 1
Effect of bFGF and EGF on thymidine incorporation of cultured MMEC at different physiological states

Physiological state	[^{3}H]Thymidine incorporation (cpm/dish/24 h)$\times 10^{-5}$		
	Control	bFGF	EGF
Virginity	1.09 ± 0.06	1.68 ± 0.15**	1.63 ± 0.09*
Pregnancy	0.61 ± 0.01	2.07 ± 0.17**	1.48 ± 0.06**
Lactation	0.48 ± 0.04	0.79 ± 0.12	1.15 ± 0.12*

MMEC from virgin (3 months old), pregnant (18 days) or lactating (8 days) mice were plated at the same starting cell density and cultured for 2 days in medium alone or either 5 ng/ml bFGF or 10 ng/ml EGF. Medium was changed daily and on the last day of incubation cells were pulsed with 1 μCi/ml [^{3}H]thymidine. Each point represents the mean ± S.E.M. (n=3). *$P<0.05$ and **$P<0.01$ are significantly different from cells incubated with medium alone.

collagenase (CLS III, 118–120 U/mg), Worthington (Freehold, NJ, USA); hydrocortisone, Calbiochem (San Diego, CA, USA). Crystalline porcine zinc insulin was a gift from Eli Lilly (Indianapolis, IN, USA). Ovine prolactin (NIDD-oPRL 19) was obtained from the Hormone Distribution Program, NIDDK, NIH. HEPES, bicinchoninic acid, heparin, Nonidet P-40, leupeptin, CHAPS, gelatin, SDS, PMSF, anti-rabbit IgG (made in goats, affinity purified), reagents for SDS-PAGE and Western blot, polyclonal anti-bFGF antibody against human recombinant bFGF, Sigma (St. Louis, MO, USA).

2.2. Animals

C3H/HeN mice in different physiological states (virgin, pregnant and lactating), were obtained from the Animal Breeding Facility of the University of Chile. Day 0 of pregnancy was the day in which a vaginal plug was observed, and the day of parturition was counted as day 0 of lactation.

2.3. Primary mammary cell culture

MMEC from female mice at different stages of the lactogenic cycle (virginity, pregnancy and lactation) were isolated and cultured as previously described [20]. Microscopic analysis revealed that MMEC comprised over 95% of the total population. The remaining cells (fibroblasts, endothelial and myoepithelial cells) were estimated to be less than 5% of the total cell population in agreement with our previous study [20]. Since myoepithelial cells were a minority component in our cell system, our findings described here on proliferation, milk protein gene expression, bFGF receptors and bFGF content correspond to the mammary epithelial cell component. The concentrations of hormones and growth factors were: insulin (I), 5 μg/ml; prolactin (P), 5 μg/ml; hydrocortisone (F), 1 μg/ml; EGF, 10 ng/ml; and bFGF, 5 ng/ml, unless stated otherwise. For studies of cell proliferation, cells were cultured for 3 days. For cell differentiation, cells were primed with bFGF for 2 days to stimulate cell proliferation, washed 4 times in medium without hormones and were then induced to differentiate for 3 days in the presence of IFP.

2.4. DNA synthesis

DNA synthesis was determined by the incorporation of [^{3}H]thymidine (0.1 μCi/ml) into trichloroacetic acid (TCA)-insoluble material [10].

2.5. Casein and total protein synthesis

Casein synthesis was determined by incubation of MMEC in the presence of ^{3}H-amino acid mixture (10 μCi/ml), unless noted otherwise. After 24 h, culture medium was collected and used for the determination of labeled casein which had been secreted. Cells were homogenized with 1.5 ml PBS solution containing 1% (w/v) Triton X-100. The homogenate was centrifuged at $105\,000\times g$ for 60 min and the resultant supernatant was used for the determination of intracellular casein synthesis. Radiolabeled casein was separated by indirect immunoprecipitation using rabbit IgG anti-mouse casein antiserum and goat anti-rabbit IgG antiserum [21]. Total casein synthesis was obtained by combining the values for the secreted and intracellular caseins. Total secreted protein was assessed from the incorporation of ^{3}H-amino acid into TCA-insoluble material [21]. Both casein and total protein synthesis increases were almost completely inhibited by cycloheximide in MMEC obtained from pregnant mammary tissue, discarding that this increase was due to an enhanced intracellular specific activity of the precursor pools.

2.6. Milk protein mRNA expression

Total cellular RNA was extracted from mammary cells as previously described [22]. The RNA concentration was determined by its absorbance at 260 nm. Dot blot analysis were performed as previously described [22]. Radiolabeled riboprobes complementary to mouse β-casein and mouse α-lactalbumin mRNA, respectively, were used as hybridization probes. [^{32}P]Casein riboprobe was synthesized from the cDNA cloned into pBS(−) plasmid vector using T_7 RNA polymerase and [α-^{32}P]UTP according to the manufacturer's protocol. The labeled riboprobe corresponds to the portion of mouse β-casein cDNA between bases 278 and 930 [23]. Mouse α-lactalbumin cDNA, originally cloned in plasmid pBR 322, was recloned into the pBS(−) vector; an [α-^{32}P]lactalbumin riboprobe was synthesized as above. Although normalization to a constitutively expressed mRNA such as actin or GAPDH is often done, this may not be too informative because the levels of these mRNA also change, depending on the developmental stage of mammary gland. Data were expressed on the basis of total RNA.

2.7. bFGF radioimmunoassay and Western blot analysis

For quantitation of bFGF we used human recombinant [^{125}I]bFGF as a ligand and a polyclonal anti-bFGF antibody against human recombinant bFGF as the binder. bFGF was labeled with ^{125}I according to the chloramine-T method [24]. After that, the procedure follows the one published for the bFGF RIA [25]. Each mammary sample was analyzed separately, weighed and mixed with 4 vol. of cold 0.4 M NaCl in 50 mM Tris-HCl, pH 7.4, with 10 mM $MgCl_2$, 1% Nonidet P-40, 1 mM PMSF, 1 μg/ml leupeptin. The tissue was then briefly homogenized by Ultraturrax and centrifuged at $12\,000\times g$ for 10 min at 4°C. The supernatant was absorbed at 4°C overnight (with gentle rocking) to swollen heparin-Sepharose (1 ml per 10 g of wet tissue) equilibrated with 0.6 M NaCl, 0.2% gelatin, 0.02% CHAPS and 20 mM NaH_2PO_4 (pH 7.4). The non-adsorbed supernatant was decanted by centrifugation for 8 min at $6000\times g$ and the beads were poured into a column and washed with 20 column vol. of 0.6 M NaCl, 0.2% gelatin, 0.02% CHAPS and 20 mM NaH_2PO_4 (pH 7.4). The column was then eluted with 0.6 ml fractions of 2 M NaCl, 0.2%

Table 2
Effect of bFGF on casein secretion to the culture medium, intracellular casein and total amount of casein in a two-step culture mammary system

Condition	β-Casein (cpm per cell culture system/24 h$\times 10^{-3}$)		
	Intracellular	Extracellular	Total
Control	2.7 ± 0.3	5.4 ± 1.0	8.1 ± 1.4
I	21.1 ± 1.9**,#	43.2 ± 1.5**,#	64.2 ± 3.4**,#
bFGF	7.6 ± 0.8*,#	9.1 ± 0.4#	16.8 ± 1.2*,#
I+bFGF	31.0 ± 0.9**	56.9 ± 1.1**	87.9 ± 2.0**

MMEC from mid-pregnant mice were cultured with the medium alone or containing I (5 μg/ml), bFGF (5 ng/ml) or both in the first incubation period (2 days) and further cultured with IFP and ^{3}H-amino acid mixture (10 μCi/ml) in the second incubation period (2 days). Casein secreted to culture medium and intracellular casein were determined by immunoprecipitation. Total casein was obtained by adding intracellular and secreted casein. Values are the mean ± S.E.M. (n=4). *$P<0.05$ and **$P<0.01$ vs. control; $^{\#}P<0.001$ vs. I+bFGF.

Table 3
Effect of bFGF in β-casein and α-lactalbumin transcript induction on two step culture

1st incubation	2nd incubation	Relative amounts of transcripts	
		β-Casein	α-Lactalbumin
None	IFP	100±8	100±7
I	IFP	300±8**,#	147±7**,#
bFGF	IFP	182±6**,#	137±3**,#
I+bFGF	IFP	338±10**	170±8**

MMEC were cultured with the indicated agents in the first incubation period (2 days) and further cultured with IFP in the second incubation period (2 days). After the last incubation period, total cellular RNA was extracted and the amount of β-casein and α-lactalbumin transcripts was examined by dot blot hybridization. The amount of β-casein and α-lactalbumin transcripts in cultured tissue was assigned the value 100. Values are the mean±S.E.M. ($n=6$). ** $P<0.01$ significantly different from control; # $P<0.01$ significantly different from I+bFGF.

gelatin, 0.02% CHAPS and 20 mM NaH_2PO_4 (pH 7.4). The fractions were collected in polypropylene tubes and frozen at −80°C.

For bFGF Western blot analysis the proteins binding to the heparin-Sepharose beads were eluted by boiling the beads for 5 min in 2× SDS-PAGE sample buffer (0.125 M Tris, pH 6.8; 4% SDS, 20% glycerol and 200 mM dithiothreitol). The samples were then analyzed by 15% SDS-PAGE and the separated proteins were blotted to a nitrocellulose membrane. The Western blots were blocked with 5% (w/v) non-fat powdered milk in PBS; washed three times with PBS-0.05% Tween 20 and incubated sequentially with a polyclonal anti-bFGF antibody and anti-rabbit IgG (made in goats, affinity purified and labeled with ^{125}I). Then nitrocellulose sheets were washed three times, dried and exposed for radioautography at −70°C with intensifier screens.

2.8. FGF binding assays

The binding of [^{125}I]bFGF to MMEC was performed as described previously [26]. MMEC (1–10×10^6 cells) were incubated with [^{125}I]bFGF (1.5–2.5×10^4 cpm) and increasing concentrations of bFGF in 250 μl of HEPES binding buffer (100 mM HEPES, pH 7.4; 150 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 8.8 mM dextrose, 0.05% gelatin and heparin 0.3 μg/ml) at 4°C for 18 h. At the end of incubation, the cells were washed (3×1 ml) with cold PBS followed by an extraction with 20 mM HEPES containing 2 M NaCl (1×1 ml). Subsequently, the cells were lysed with 0.5% SDS (2×250 μl), and radioactivity in the NaCl and SDS extracts was measured in a Packard γ-counter. Bound cpm were normalized according to protein content of SDS extracts as measured by bicinchoninic acid protein assay [27].

2.9. Statistics

Data were analyzed by a statistic computer program (GB-STD version 3.0, USA). An ANOVA test followed of the Tukey's protected *t*-test were used to analyze the heterogeneity of the experimental groups and to set significance of paired values with a value of $P<0.05$.

Table 4
Effect of bFGF on the synthesis of casein in cultured MMEC

bFGF (ng/ml)	IFP	Casein (cpm per cell culture system/24 h×10^{-3})
0	+	1.40±0.11
0.1	+	1.47±0.10
0.5	+	1.26±0.09
1	+	0.54±0.09*
5	+	0.54±0.07*
10	+	0.62±0.05*
50	+	0.55±0.07*

Mammary cells from late pregnant mice were cultured for 3 days in medium alone (NH) or containing IFP and the indicated concentration of bFGF. Labeled amino acid mixture (10 μCi/ml) was added to the medium after a 24-h plating period and was present throughout the culture period. At the end of incubation the amount of [^{3}H]casein presented in the medium was determined by immunoprecipitation. Each point represents the average±S.E.M. ($n=3$). *Significantly different from those cells cultured without bFGF ($P<0.01$).

3. Results

3.1. The effect of bFGF concentration on the growth of primary MMEC in culture

As measured by incorporation of [^{3}H]thymidine into DNA, both human and bovine recombinant bFGF stimulated proliferation of primary MMEC from pregnant mice in culture with similar kinetics (data not shown). The maximal stimulation was observed at 5 ng/ml bFGF with ED_{50} values of approximately 0.5 ng/ml in mouse pregnant mammary epithelial cells. The addition of cytarabine, a well known DNA synthesis inhibitor, to the medium of the cultured MMEC showed that [^{3}H]thymidine incorporation was significantly inhibited (>85%) in the presence or absence of the growth factor. This control rules out that an increased [^{3}H]thymidine incorporation is due to an increased uptake by MMEC. In addition to the increase in DNA synthesis as measured by [^{3}H]thymidine incorporation there was an increase in cell number as observed microscopically or by counting. As shown in Table 1, bFGF (5 ng/ml) stimulated growth of MMEC from virgin, pregnant and lactating mammary glands ($P<0.05$); the magnitude of the stimulatory effect was largest on cells derived from pregnant mice. As shown previously [20], mammary cell proliferation was also stimulated by EGF but to a lesser extent than with bFGF in cells from pregnant animals.

3.2. bFGF action on mammary cell differentiation in vitro

MMEC were primed with insulin and growth factors to stimulate proliferation and were then induced to differentiate by further incubation with insulin, hydrocortisone and prolactin [22]. This two-step culture system simulates the stages of proliferation and differentiation in the mammary gland during pregnancy and lactation, respectively. As shown in Table 2, cells primed with insulin and bFGF together produced a greater amount (about 11 times) of casein than those not primed. This was true for both secreted and intracellular casein. The effects of insulin and bFGF were additive, paralleling the relative stimulation of cell proliferation by the two factors in single cultures (results not shown). Cells primed with insulin and bFGF also showed a 4-fold increase in the synthesis of total proteins relative to controls (results not shown). Table 3 shows that the amounts of β-casein and α-lactalbumin mRNAs (relative to the appropriate controls) were also increased substantially by insulin and bFGF, indicating that at least part of the increase in milk protein synthesis is regulated at the level of transcription. MMEC produce milk proteins (albeit to a limited extent, as shown in Table 2) in response to IFP in a one-step culture system.

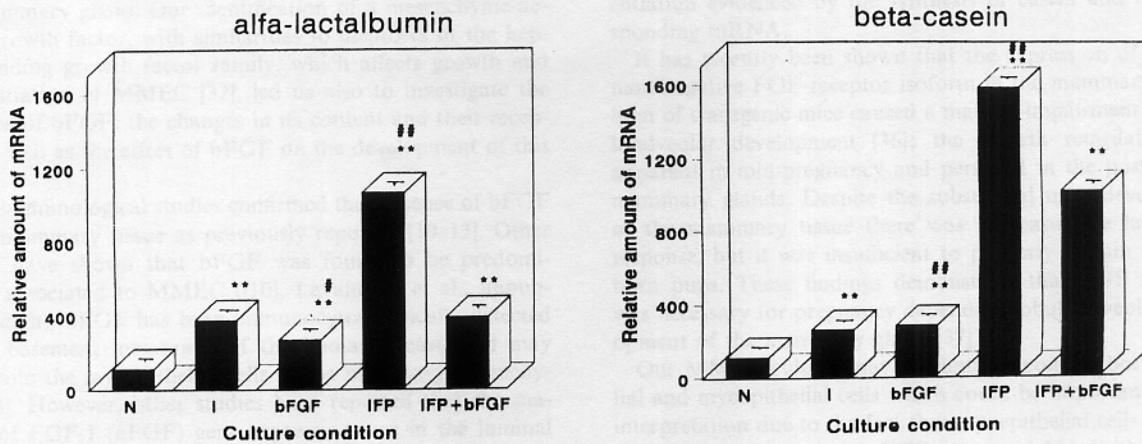

Fig. 1. Effect of bFGF on milk protein gene expression. MMEC from pregnant mice were cultured for 2 days with incubation medium alone (N), insulin (I; 5 μg/ml), bFGF (5 ng/ml), IFP or containing IFP and bFGF (5 ng/ml). Total cellular RNA was extracted, dotted on a nitrocellulose membrane, hybridized with β-casein or α-lactalbumin riboprobes, respectively, and the extent of hybridization was determined. The amount of transcripts in uncultured tissue was assigned the value 100. Values are the average ± S.E.M. ($n = 3$). ** $P < 0.01$, significantly different from control (N); # $P < 0.05$ and ## $P < 0.01$ vs. IFP+bFGF.

When bFGF is added to such cultures, casein production is decreased in a dose-dependent manner (Table 4) and maximally by 63% ($P < 0.001$). In the same single incubation culture system, casein and α-lactalbumin gene expression was also substantially decreased by bFGF (Fig. 1).

3.3. Content and receptor density of bFGF in the mammary tissue

The content of bFGF in mammary tissue changes markedly during development and in lactation, rising at puberty and again in early pregnancy. From mid-pregnancy onwards and during lactation, the content falls to the pre-pubertal level. These variations are reflected in parallel changes in the number of bFGF receptors in MMEC from animals at the same stages of development (Table 5).

The results of Western blot analysis using a polyclonal anti-bFGF antibody showed that bFGF proteins were identified in the extracts of the mammary tissue. Bands were seen at 18, 26 and 45 kDa in the mid-pregnant tissue, the 18-kDa bFGF species being predominant (> 90%). A lower amount of bFGF proteins was observed in the lactating mammary tissue and the 18-kDa band was not detected. The 26- and 45-kDa forms were present in similar abundance in the lactating mammary tissue.

4. Discussion

An increasing body of evidence has indicated that growth factors may play an important role in the control of mammary cell growth and differentiation [2–4]. Such growth factors may be synthesized locally or originate in other tissues. Studies both in vitro and in vivo have demonstrated the importance of EGF [20,21], insulin-like growth factor-1 [28,29] and transforming growth factors [30,31] in the development of

Table 5
Content of bFGF and receptor density of bFGF during the development of the mammary tissue

Stage	n	Mammary weight (g)	bFGF mammary content (ng/g)	FGF receptor density (fmol/mg protein)
Virginity				
Prepuberty	6	0.20 ± 0.04	62 ± 11	ND
Puberty	6	0.47 ± 0.09**	192 ± 45**	6 ± 3 ($n = 3$)
Pregnancy				
Early	4	0.74 ± 0.10**	185 ± 22**	ND
Middle	5	0.90 ± 0.09**	260 ± 10**	27 ± 10* ($n = 3$)
Late	5	0.88 ± 0.12**	58 ± 12	ND
Lactation				
Middle	6	1.45 ± 0.28**	41 ± 5	12 ± 4 ($n = 3$)
Post-lactation	6	0.76 ± 0.16**	105 ± 20	ND

Female mice at different stages of the lactogenic cycle (prepuberty, 3–4 weeks; puberty, 8 weeks; early pregnancy, 7 days gestation; middle pregnancy, 8–15 days gestation; late pregnancy, 16–20 days gestation; middle lactation, 10–12 days post-parturition; post-lactation, 15–20 days weaning) were sacrificed and abdominal and thoracic mammary gland were excised and weighed. The bFGF mammary content was determined by RIA as described in Section 2. In other experimental groups, the number of high affinity FGF binding sites was determined in plasma membranes obtained from MMEC as also described in Section 2. Values are the mean ± S.E.M; n = number of animals; ND: not done. * $P < 0.05$ and ** $P > 0.01$ are significantly different from prepuberal animals.

the mammary gland. Our identification of a mesenchyme-derived growth factor, with similarities to members of the heparin-binding growth factor family, which affects growth and differentiation of MMEC [32], led us also to investigate the presence of bFGF, the changes in its content and their receptors as well as the effect of bFGF on the development of this tissue.

The immunological studies confirmed the presence of bFGF in the mammary tissue as previously reported [10–13]. Other studies have shown that bFGF was found to be predominantly associated to MMEC ([10], Lavandero et al., unpublished data). bFGF has been immunocytochemically detected in the basement membrane of the human breast, and may arise from the myoepithelial cells of the mammary parenchyma [33]. However, other studies have reported that the majority of FGF-1 (aFGF) gene expression was in the luminal epithelial cells, whereas bFGF expression was in the mammary stroma and possibly the myoepithelial cells [13,34]. We have detected the presence of aFGF in the myoepithelial cells (Lavandero et al., unpublished data). The presence of bFGF protein in the mouse mammary tissue extracts was confirmed by Western blot analysis using an anti-bFGF polyclonal antibody, which showed three distinct molecular weight species of bFGF protein (18, 26 and 45 kDa). The 18-kDa band migrates in the same position as human recombinant bFGF. Three species of bFGF have also been detected in human mammary cell lines (18, 24 and 27 kDa) [10], the expression of the 18-kDa species in cell lines having an epithelial morphology and the 24- and 26-kDa bands in myoepithelial-like cells [35]. Although preabsorption of the bFGF antibody with bFGF was shown to be specific, our data do not discard that the 26- and 45-kDa proteins are not related to bFGF. The highest and lowest levels of the 18-kDa bFGF species were detected by immuno-Western blot in pregnant and lactating mammary tissue, respectively. These data correlated well with bFGF content assessed by RIA (Table 5) and our immunocytochemistry studies (data not shown).

It has previously been shown that bFGF increases the number of MMEC from virgin animals [19]. We have now confirmed these observations, extended them to cells from animals at different stages of mammary development and examined the effects on milk protein synthesis in cells cultured in a two-step culture system that simulates the physiological process. Both EGF and bFGF increase proliferation, but the effect of bFGF was greater in cells from pregnant animals. This corresponded both with the increased level of bFGF and its receptors in the mammary tissue in vivo. The bFGF receptors were detectable by the binding of [^{125}I]bFGF to MMEC in agreement with one study reported previously [34] in which only high affinity [^{125}I]bFGF binding sites on rat fibroblastic and myoepithelial-like cell lines were detected but not on mammary epithelial cell lines [36]. Priming of MMEC with bFGF and/or insulin, followed by induction with the lactogenic hormones, greatly increased the synthesis of milk proteins. Expression of the milk protein genes was also increased. The difference between the increase of milk protein (3-fold) and mRNA (10-fold) is noteworthy. It is possible that posttranscriptional control may play a role in addition to the transcriptional regulation. The additive nature of the responses to a mixture of the two factors indicates differences in the mode of action of bFGF and insulin. Basic FGF resembles EGF in inhibiting the precocious functional differentiation evidenced by the synthesis of casein and its corresponding mRNA.

It has recently been shown that the expression of a dominant-negative FGF receptor isoform in the mammary epithelium of transgenic mice caused a marked impairment of lobulo-alveolar development [36]; the growth retardation was apparent in mid-pregnancy and persisted in the post-partum mammary glands. Despite the substantial underdevelopment of the mammary tissue there was a measurable lactational response, but it was insufficient to properly sustain the newborn pups. These findings demonstrate that FGF signaling was necessary for pregnancy dependent lobulo-alveolar development of the mammary gland [37].

Our MMEC culture may presumably contain both epithelial and myoepithelial cells which could be important to data interpretation due to the fact that myoepithelial cells can produce FGF and/or present FGF receptors. Myoepithelial cells are most abundant in the lactating gland in which they may comprise roughly 10–20% at best. These cells are much less in pregnant and virgin glands. With regard to this point our results showed: first, MMEC obtained from pregnant mammary tissue comprise 95% of the total population; and second, the effect of bFGF is most prominent in mammary cells from pregnant mammary gland. This makes it very unlikely that the effect of FGF takes place on myoepithelial cells – if this is the case, we should see the biggest effect of FGF on the cells from lactating mammary tissue.

Our data provide evidence of the existence of an autocrine and or paracrine regulatory loop for FGF in the mouse mammary epithelial cells. This is supported by the fact that bFGF appears to be produced by the MMEC. Although the quantitation of bFGF content was done in the mammary tissue, our immunocytochemical studies showed that bFGF and aFGF were mainly found in the MMEC and myoepithelial cells, respectively. High affinity FGF receptors were detected in MMEC which could be activated by bFGF or aFGF. bFGF is able to activate FGF receptor. Third, the biological effects of bFGF ([^{3}H]thymidine and ^{3}H-amino acid incorporations, milk gene expression) also take place in the MMEC.

In summary, our data showed that bFGF is present in the mouse mammary tissue and that the content of bFGF and its receptors in mammary tissue changes markedly during the different physiological stages of development. bFGF not only stimulates growth but has also an inhibitory effect on functional differentiation of MMEC. As such, bFGF may be added to the growing list of putative regulatory growth factors known to modulate development of mammary epithelial cells.

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