Differentiation of Mammosomatotrophs in Swine Adenohypophysis

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The existence and distribution of mammosomatotrophs (MS cells) containing growth hormone (GH) and prolactin (PRL) were detailed in swine adenohypophysis. MS cells were positively identified and were first recognized in the adenohypophysis of fetuses with 33.0 cm in body length, when GH cells existed earlier and PRL cells occurred. The proportion of MS cells transiently increased in the glands of three month-old females and were densely populated in the fore ventral (FV) and the hind ventral (HV) region except for the zona tuberalis (ZT). The distribution was similar to GH cells and PRL cells. MS cells were divided into three subtypes by the intracellular intensity of both GH and PRL stainings. Type I MS cells which were strongly stained with anti-GH antiserum, constituted 100%

of MS cells in fetuses and the proportion decreased with age in females. Type II MS cells which were evenly stained with both anti-GH and PRL antisera were detected in all animals except for fetuses, and were proportioned moderately. Type III MS cells which contained more PRL than GH existed predominately in three and six month-old females, castrated males and aged males. The findings indicated that an amount of GH and PRL within MS cells was changeable in response to physiological conditions. The results suggest that GH cells may transform into PRL cells, with the MS cell acting as an intermediate cell in swine adenohypophysis, and that the GH and PRL in MS cells are synthesized synchronously or asynchronously according to the secretory activities.

Key words: Swine adenohypophysis, Mammosomatotrophs, Distribution, Differentiation, Functional heterogeneity

I. Introduction

Growth hormone (GH) and prolactin (PRL) are protein hormones that are produced in corresponding specific pituitary secretory cells, somatotrophs (GH cells) and mammotrophs (PRL cells). Recently, existence of a new cell type that secretes both GH and PRL was first described in the adenohypophysis of adult male rats by using a reverse haemolytic plaque assay [7]. The bihormonal cells were ascertained immunocytochemically in intact adult rats and designated as mammosomatotrophs or somatomammotrophs (MS cells) [24]. The existence of MS cells has been reported in rats [17, 22, 24, 25], the musk shrew [17], bats [18], mice [29, 30], the rhesus monkeys [1],

humans [21, 23], bovine [9, 12], sheep [37, 38] and goats [31]. It is now proposed that possible functions of MS cells are as transitional cells for the conversion of GH cells to PRL cells [2, 3, 14, 26, 27], or as progenitor cells for GH cells and PRL cells [23]. However, the developmental and functional significance of MS cells is not fully understood, although their occurrence is closely related to the differentiation of GH cells and PRL cells [5, 16].

In swine adenohypophysis, Dachux reported that no one cell can synthesize and store both GH and PRL under normal physiological conditions [6]. Thereafter, surprisingly, no information is available about the existence and distribution of MS cells in swine adenohypophysis. Since MS cells are occasionally present throughout adenohypophysis in sheep [37, 38], mice [29] and goats [31], further extensive and precise observations are needed to conclude if MS cells exist or not in swine adenohypophysis. The present study is focussed on the existence and distribution of MS cells in swine adenohypophysis, and on

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the mutual relationship of MS cells, and GH cells and PRL cells for differentiation.

II. Materials and Methods

Animals

Swine of three-way crosses of Duroc and Landrace $\times$ Large white F1 sows were used in the present study. The swine consisted of three female fetuses of 17.5 cm and 33 cm body lengths, three females of three months-old, three females and three castrated males of six months-old, and three boars of two or three years-old. The castration of male swine was carried out on day 35–45 postpartum.

Tissue preparation

The pituitary glands were freshly removed and bisected at the midsagittal plane. The tissues were removed from three regions of the midsagittal plane, the fore ventral (FV) and hind ventral (HV) region, and the zona tuberalis (ZT), and diced into pieces about 1 mm³. The pieces were fixed in Zamboni's solution [34] for 6 hr at 4°C, and post-fixed in a 1% osmium tetroxide solution for 1 hr at 4°C. After the fixation, all tissue blocks were rinsed thoroughly with a cacodylate buffer (0.1 M, pH 7.4), dehydrated in a graded series of ethanol and embedded in Spurr's resin.

Immunohistochemistry

A couple of serial sections, consisting of 0.5 μ m sections, were cut from each block by an ultramicrotome (Ultracut S, Reichert-Nissei, Wien, Austria). The sections were mounted on glass slides previously coated with poly-L-lysine and treated with sodium methoxide for 4 min to remove the resin. The sections were incubated in a 0.05% saponin solution for 20 min, and then washed with PBS. After the treatment of normal goat serum for 20 min, one of the serial sections was incubated with anti-porcine GH rabbit antiserum (1 : 3,200; Biogenesis, Poole, England), and the other with anti-porcine PRL rabbit antiserum (1 : 3,200; Biogenesis) for 14 hr at 4°C. After washing three times in PBS, the immunoreactive cells were visualized by immunogold silver staining (IGSS) [15]. Briefly, the sections were incubated with gold-labeled anti-rabbit IgG antibody (5 nm, 1 : 60; BioCell International, Cardiff, England) for 1 hr, and immersed in physical developer for 45 min according to the method of Fujimori and Nakamura [8]. After washing in distilled water, the counterstain was performed with Carazzi's hematoxylin. To investigate the specificity of GH and PRL immunoreactions, the primary antibody was omitted or replaced by irrelevant rabbit IgG at about the same protein concentration in which the specificity was ascertained by a total loss of the staining.

Percentages of MS cells

The photomicrographs were obtained from the FV, HV and ZT of several sections at $\times 400$. The number of

cells that contained only GH or PRL, and both GH and PRL, was counted and the percentage (immunoreactive cells/total cells $\times 100$) was calculated. A total of 2,000 to 20,000 cells in each region was observed on several photomicrographs.

Statistical analysis

The statistical significances of difference were evaluated by Student's *t* test for the difference of cell frequency, and were determined by an Analysis of Variance followed by Tukey's Multiple Range Test for the difference among each region.

III. Results

Existence and distribution of MS cells

In swine adenohypophysis, numerous cells were immunostained with each anti-GH and anti-PRL antiserum. GH cells and PRL cells were first detected in fetuses of 17.5 cm and 33.0 cm body lengths, respectively. GH cells increased in number until just before delivery. The occurrence of MS cells was precisely evaluated on two serial sections. MS cells were first identified in fetuses of 33.0 cm in body length on about the 105th day of gestation in swine (Fig. 1), and occasionally appeared as a group of two or three cells. In the fetus adenohypophysis, GH cells existed predominantly over PRL cells. In three month-old females, the existence of MS cells was significantly greater in FV and HV where GH cells and PRL cells were densely populated, but the proportion was much lower than that of GH cells and PRL cells (Table 1). In ZT where GH cells and PRL cells were less frequent, the proportion of MS cells was very low (Table 1). MS cells were mainly solitarily distributed, and occasionally grouped in the cell clusters of GH cells and PRL cells.

Changes of MS cell proportion

The proportion of MS cells was compared in the FV region where the numeral density was the highest in female adenohypophysis of three-months old (Table 2). MS cells were detected at the rate of 0.2% in female fetuses with a 33 cm body length, increased to 5.5% in three month-old females and decreased to 1.5% in six month-old females. The proportion was still lower in aged boars. At six months-old, the proportion of MS cells in castrated males was significantly greater than that of females (Table 2).

Functional heterogeneity of MS cells

In swine adenohypophysis, some MS cells were evenly stained with both anti-GH and anti-PRL antisera, but other cells were heterogeneously immunostained by only one of them. To investigate the mutual relationship between an amount of intracellular GH and PRL, and the function of MS cells, the cells were divided into three subtypes by the relative intensity of GH and PRL immunostainings. Type I MS cells were immunoreactive for anti-GH antiserum stronger than anti-PRL antiserum

Fig. 1. A couple of serial sections of fetal adenohypophysis with 33cm body lengths immunostained with GH (a) and PRL (b) antiserum. MS cells immunoreactive for both antisera are distinguishable as Type I cells by the predominance of GH staining (arrows). S and M show GH cells and PRL cells, respectively. Bar = 10 μ m.

(Fig. 1). All MS cells in fetuses were Type I MS cells, which proportionally decreased in females with age (Table 2). Type II MS cells were evenly immunostained with both anti-GH and anti-PRL antisera and were detected in three and six month-old females, castrated males and aged males except for fetuses (Table 2). A large number of Type II MS cells were strongly immunostained with both

Table 1. Distribution of MS cells, GH cells and PRL cells in three different regions of swine adenohypophysis.

Region	MS cells	GH cells	PRL cells
FV	5.5 $\pm$ 1.5 ^b	25.7 $\pm$ 4.7 ^b	44.9 $\pm$ 5.5 ^b
HV	2.7 $\pm$ 0.8 ^b	28.2 $\pm$ 3.8 ^b	34.5 $\pm$ 6.4 ^b
ZT	0.8 $\pm$ 0.4 ^a	8.7 $\pm$ 1.7 ^a	13.8 $\pm$ 3.4 ^a

Values are mean percentages $\pm$ S.D. of adenohypophysial cells from three animals of three months-old. Significantly different means ($p < 0.05$) are identified by different letters in same column.

Table 2. Changes of MS cells and their subtypes in swine adenohypophysis.

Animals	% of MS cells	% of MS cell subtypes		
		I	II	III
Fetuses				
(17.5 cm body length)	0.0	0.0	0.0	0.0
Fetuses				
(33.0 cm body length)	0.2 ^a	100.0	0.0	0.0
Females				
(3 months-old)	5.5 ^c	13.1	33.8	53.2
Females				
(6 months-old)	1.5 ^b	1.5	34.3	64.2
Castrated males				
(6 months-old)	5.8 ^c	14.4	29.2	56.4
Boar				
(2–3 years-old)	0.7 ^a	16.7	20.8	62.5

Significantly different means ($p < 0.05$) are identified by different letters in % of MS cells.

antisera (Fig. 2) and these cells frequently existed in six month-old females and aged males. Type II MS cells which were weakly immunostained with both antisera were relatively more common in 3 month-old females and castrated males (Fig. 3). Type III MS cells which were more heavily immunostained by anti-PRL antiserum than anti-GH antiserum (Fig. 4), predominated in three and six month-old females, castrated males and aged males (Table 2). The fluctuation of GH and PRL in MS cells indicated that the cells are functionally heterogeneous depending upon physiological conditions. However, in some cases, especially in six month-old females, it was hard to clearly distinguish Type III MS cells because of the scant staining of GH.

IV. Discussion

The present study initially demonstrated the substantial existence of MS cells in swine adenohypophysis by two serial sections combined with IGSS methods. Dacheux, however, did not succeed in immunological detection of MS cells under normal physiological conditions for swine adenohypophysis [6]. The experiments were carried out with electron immunocytochemical methods, which is insufficient to observe the entire adenohypophysial constituents. Since MS cells were low in number and their existence changed regionally and age-dependently in swine adenohypophysis, a difference between our findings and the above mentioned work may be partly due to the difference in methods used to identify MS cells.

The reliable identification of MS cells in the present study revealed the differential distribution in the three regions of swine adenohypophysis. The proportion of MS cells was higher in the region where GH cells and PRL

Fig. 2. Type II MS cells which are strongly immunostained by both GH (a) and PRL (b) antisera in female adenohypophysis of six months-old (arrows). The cells exist adjacently to GH cells (S) and PRL cells (M). Bar = 10 μ m.

Fig. 3. Type II MS cells which are weakly immunostained by both GH (a) and PRL (b) antisera in female adenohypophysis of three months-old (arrowhead). The cells occasionally appear together with other types of MS cells (arrows) within a cluster of GH cells (S) and PRL cells (M). Bar = 10 μ m.

Fig. 4. Type III MS cells which are immunostained by PRL antiserum (b) stronger than GH antiserum (a) in castrated-male adenohypophysis of six months-old (arrows). In some PRL cells, GH is scantily immunostained (arrowheads). S and M show GH cells and PRL cells, respectively. Bar = 10 μ m.

cells were densely populated, and was lower in ZT which consists of almost only basophils and chromophobes, but not acidophils [40]. In addition, the occurrence of MS cells was later than for GH cells, and was almost simultaneous with PRL cells during the fetal period. MS cell distribution similar to GH and PRL cells suggests that

their occurrence may be closely associated with the cyto-genesis of GH and PRL cells.

Although several researchers have reported the proportional differences of MS cells for ages and sexes [7, 18, 20], the data of the relative proportion cannot be directly compared, because the mode of data description is

different. Kineman *et al.* reported that pituitary cell cultures contained fewer MS cells in gonad-intact males when compared with castrated males in bovine adenohypophysis [19]. In swine adenohypophysis, the proportion of MS cells in castrated males was more than that for gonad-intact females of the same age. From these findings, it is supposed that the changes of hormonal milieu after castration correlate with the induction of MS cells, although the proportional changes of MS cells between the different sexes remains unclear. On the other hand, MS cells showed an age-related decline in swine adenohypophysis. The proportion of PRL cells, however, increased with age (data not shown). Shinkai *et al.* [31] reported that the number of MS cells at 21 months old increased to more than five times the average of 12–13 months old in female rat adenohypophysis, and the number of PRL cells also steadily increased with age by a flow cytometric study. Since GH and PRL within the MS cells are of a variety owing to the difference in physiological states of the animals, it is likely that some MS cells are classified in the category of PRL cells by an immunocytochemical method when the GH within MS cells is scanty.

The IGSS method has been developed to detect low levels of fixed antigens in tissue sections [11, 32]. This method enabled us to accurately observe the intracellular GH and PRL within MS cells on the sections. The subtypes of MS cells uniquely changed with age in female swine. Unexpectedly, MS cells in fetuses were all Type I cells. Type III MS cells age-dependently increased as Type I MS cells decreased. In regard to the reciprocal changes of GH and PRL within MS cells, there is little available information. Ishibashi and Shiino [18], however, have reported that MS cells show remarkable seasonal fluctuations, and the granules with GH and/or PRL proportionally change throughout the reproductive cycle in the female Japanese house bat. They suggested that the MS cell itself gradually enhanced its ability to produce PRL rather than GH, and ultimately changed into a pure PRL-producing cell. Considering these findings, it is speculated that the subtypes of MS cells in swine adenohypophysis result in functional heterogeneity under different secretory activities although the localization of GH and PRL within the same granules remains to be investigated.

The mechanism responsible for cytogenesis of MS cells in swine adenohypophysis is not fully understood. Our investigations showed that 1) MS cells occurred almost simultaneously with PRL cells while GH cells preexisted, 2) MS cells are regionally distributed similar to GH cells and PRL cells and 3) the hormone contents of MS cells changed from GH to PRL with age. From these findings, it is conceivable that MS cells originate in GH cells that preexisted in swine adenohypophysis. The possibility that MS cells are a transitional stage of conversion from GH cells to PRL cells has been reported during the embryonic period [19, 36, 39]. The increase in MS cells in mice transgenic for GHRH suggests that the generation of

MS cells is connected to the differentiation of GH cells [33]. Using the thymidine kinase obliteration system, Borrelli *et al.* [3] reported that both GH cells and PRL cells derive from a common GH-expressing stem GH cell, which terminally differentiates into GH and PRL cell populations in transgenic mice. These findings support a possible transformation of GH cells into PRL cells, with MS cells acting as an intermediate cell in swine adenohypophysis as previously described in the adenohypophysis of rats [5, 13, 26, 35], humans [10] and bats [18].

In conclusion, we identified MS cells producing both GH and PRL in swine adenohypophysis, and revealed that the frequency of occurrence of MS cells changes relatively under normal physiological conditions. The proportional divergence in MS cells suggests that the hormonal milieu of adenohypophysis plays an important role in cell differentiation. A full understanding of the mechanism of how MS cells functionally correlate to GH cells and PRL cells will require additional investigation by an accurate localization of GH and PRL within the secretory granules of MS cells.

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VI. References

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