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Role of Transcription Factor Oct4 in Postnatal Development and Function of the Adrenal Cortex

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We analyzed the expression of transcriptional factor Oct4 in rat adrenal cortical cells during postnatal development. It was found that Oct4 is expressed by typical cortical cells of the zona glomerulosa, zona fasciculata, and zona reticularis in pubertal and postpubertal periods. The maximum number of $Oct4^+$ cells was found in the zona glomerulosa. An inverse correlation between the number of $Oct4^+$ glomerulosa cells and serum level of aldosterone both in pubertal and postpubertal periods was revealed. After puberty, the number of $Oct4^+$ glomerulosa cells directly correlated with the number of $Ki\text{-}67^+$ cells. A hypothesis was put forward that Oct4 is involved in postnatal morphogenesis, regeneration, and functioning of the adrenal cortex.

Key Words: *Oct4; pluripotency; adrenal gland; proliferation; postnatal development*

Self-renewal of cell population during postnatal development is essential for physiological and reparative regeneration of organs. A pool of pluripotent cells in the endocrine glands is required for homeostasis maintenance in various physiological states, in particular, for structural changes. The adrenal cortex is highly sensitive to different physical and chemical factors and hypoxia and is characterized by higher regenerative potential than the adrenal medulla [1,8,9,20]. Cytoarchitectonics of the adrenal cortex changes significantly during prenatal, neonatal, and pubertal period. After birth, the fetal cortex undergoes involution via cell apoptosis [4]. Angiotensin II and adrenocorticotropic hormone promote further morphogenesis of the zona glomerulosa and zona fasciculata. The formation of zona reticularis between the adrenal medulla and zona fasciculata begins later, before puberty and morphogenesis of the adrenals is completed only after puberty. Octamer-binding transcription factor Oct4 is known to be the key player in cell pluripotency [13]. Recent investigations demonstrated that expression of Oct4 is related to the maintenance of pluripotency in not only embryonic stem cells, but also adult cells [13,19]. This function made Oct4 a useful marker in identification of tumor germ cells and an instrument in stem cells studies [2,5,7]. However, Oct4 is considered as tumor marker despite the fact that its role in postnatal morphogenesis and regeneration remains unclear. Oct4 expression in adrenal cortical cells during postnatal development is little studied, because the maintenance of cell pluripotency, their differentiation and proliferation were traditionally associated with the presence of a pool of pluripotent and differentiating cells in the subcapsular zone in human and in undifferentiated zone in several species mainly [10,11].

Here we studied the expression of transcriptional factor Oct4 in cortical cells of different zones of the rat adrenal cortex during postnatal ontogeny.

MATERIALS AND METHODS

The experiments were performed on Wistar rats (n=20). The animals were sacrificed by Zoletil overdose at the age of 42 days (a period between adrenarhe and gonadarhe) and 70 days (at the peak of the adrenal development) [14].

The adrenal glands were fixed in formalin, histological slices were stained with hematoxylin and

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eosin. Computer morphometry of equatorial sections were performed by assessing the areas of surface and cell number per 1 mm^2 in the zona glomerulosa, zona fasciculata, and zona reticularis.

The expression of transcription factor Oct4 was detected by immunohistochemistry with rabbit polyclonal anti-PRH antibodies (Abcam). Sections of rat embryonic tissues was used as the positive control. Proliferation of adrenal cortical cells was evaluated histochemically using anti-Ki-67 antibodies (Cell Marque). The reactions were visualized with the UltraVision LP Detection System (Thermo Scientific). The expression of Oct4 and Ki-67 was assessed as the number of immunopositive cells per 1 mm^2 both on days 42 and 70, as no difference in the density of adrenal cortical cell in pubertal and postpubertal rats was found.

Serum aldosterone concentration were measured by ELISA (Cusabio).

The results were processed statistically using Statistica 7.0 (StatSoft, Inc.). The central tendency and dispersion of quantitative traits with approximately normal distribution were presented as the *M*±*SEM*. Association of the number of Oct4$^+$ and Ki-67$^+$ cells in each cortical zone separately and association of number of Oct4$^+$ glomerulosa cells and serum aldosterone concentration were analyzed by using Pearson correlation coefficients. Quantitative comparisons of independent groups were performed using Student's *t* test with Levene test for the equality of variances. The differences were considered statistically significant at $p<0.05$.

RESULTS

Adrenals of the pubertal rats had well-developed cortex with distinguishable zona glomerulosa, zona fasciculata, and zona reticularis. The undifferentiated zone was presented by concentrically arranged groups of small cells with dark oval or irregular nuclei. Oct4$^+$ cells were seen in zona glomerulosa (Fig. 1, *a*). Their distribution in the zone was focal. Immunohistochemical staining for Oct4 was found only in the nuclei (Fig. 2, *a*). The cells of undifferentiated zone did not express Oct4 (Fig. 2, *a*). In the zona fasciculata, Oct4$^+$ cells were less abundant than in the zona glomerulosa (Fig. 1, *b*, Fig. 2, *b*) and were found mostly in its outer part. Solitary Oct4$^+$ cells were found in the zona reticularis (Fig. 1, *c*, Fig. 2, *c*). The rats showed a concordance between the rates of Oct 4 expression in different cortical layers: the animals with maximum number of Oct4$^+$ glomerulosa cells demonstrated the highest rates of Oct4$^+$ cells in the zona fasciculata and zona reticularis, and *vice versa*.

Evaluation of proliferation capacity of adrenal cortical cells revealed maximum rates in the zona glomerulosa (Fig. 3). However, the number of Ki-67$^+$ cells did not correlate with the content of Oct4$^+$ cells either in the zona glomerulosa, or in zonae fasciculata and reticularis.

A strong inverse correlation was revealed between the number of Oct4$^+$ glomerulosa cells and serum aldosterone levels (r=-0.98, p=0.000) (Fig. 4, *a*).

After puberty when the adrenals reach their maximum of development we revealed changes in the ratio of the structural and functional layers: a decrease in the portion of zona glomerulosa (Fig. 5, *a*) and enlargement of the zona fasciculata (Fig. 5, *b*); the proportion of the zona reticularis remained practically unchanged. The number of Oct4$^+$ cells per 1 mm^2 of the zona glomerulosa was 2-fold higher than during puberty (Fig. 1, *a*) and their distribution became more diffuse. The number of Oct4$^+$ cells in the zonae fasciculata and reticularis

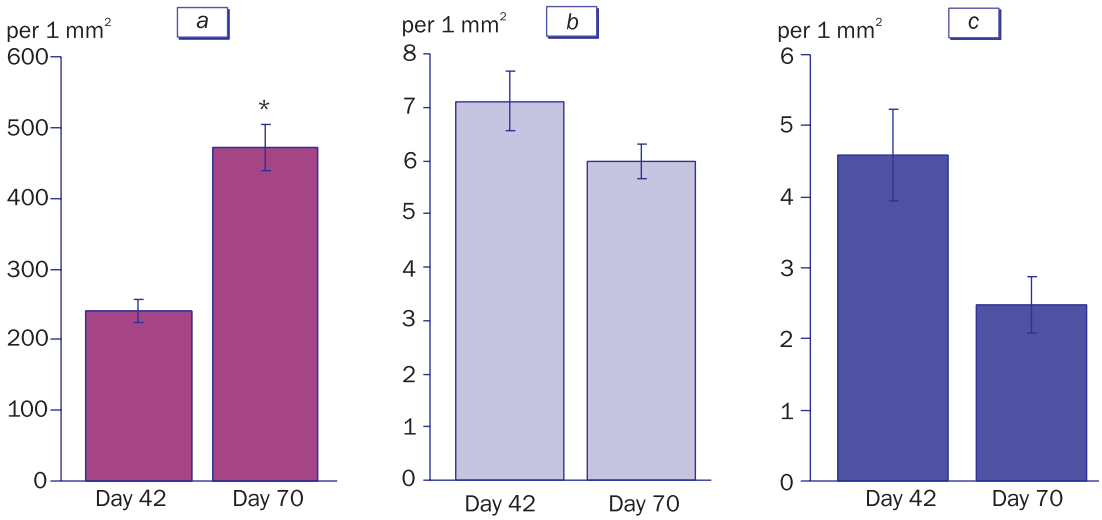

Fig. 1. Content of Oct4$^+$ cells in different structural and functional layers of the adrenal cortex in intact rats during pubertal (day 42) and postpubertal (day 70) periods (*M*±*SEM*). *a*) Zona glomerulosa; *b*) zona fasciculata; *c*) zona reticularis. *$p<0.05$ in comparison with day 42.

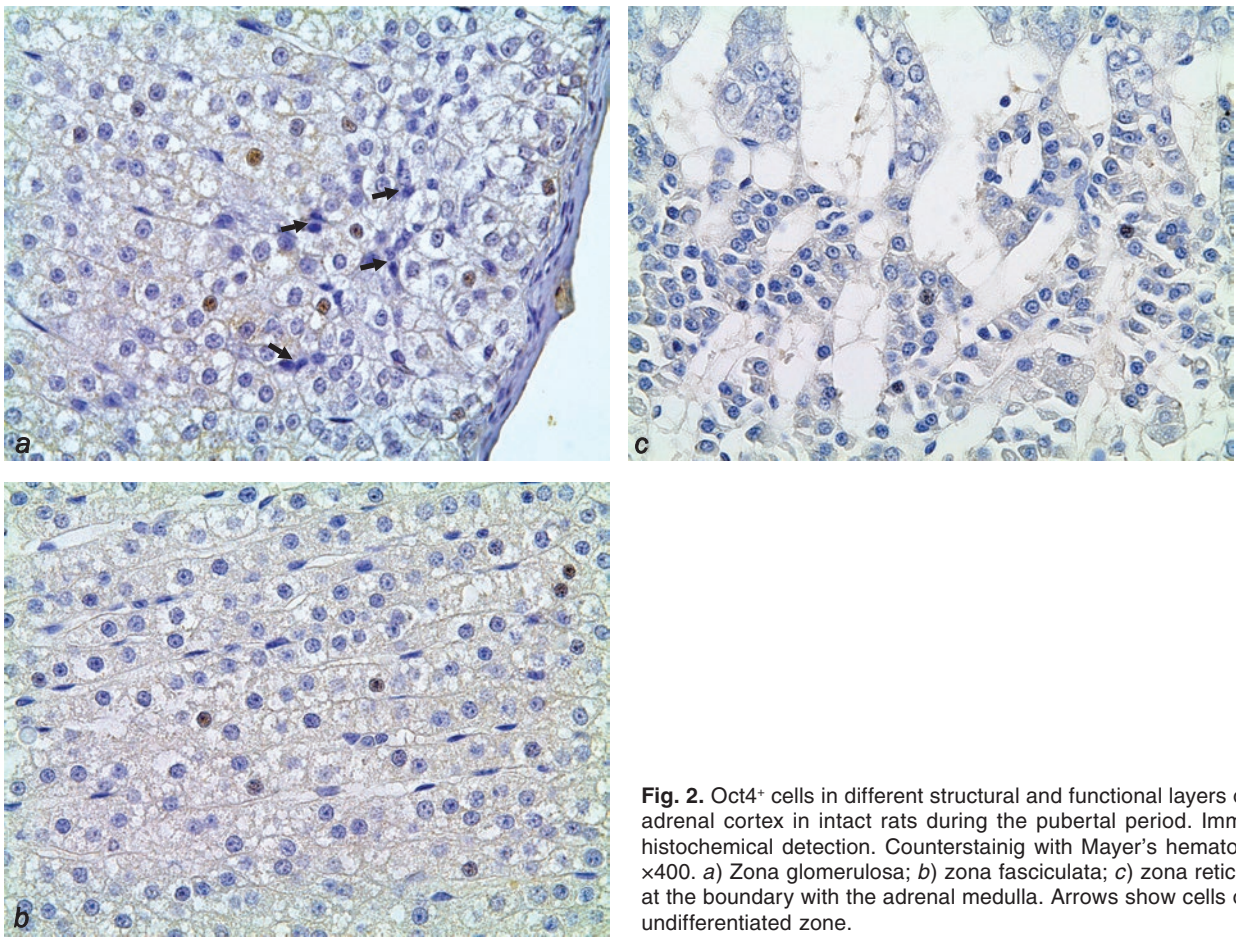

Fig. 2. Oct4$^+$ cells in different structural and functional layers of the adrenal cortex in intact rats during the pubertal period. Immunohistochemical detection. Counterstainig with Mayer's hematoxylin; ×400. *a*) Zona glomerulosa; *b*) zona fasciculata; *c*) zona reticularis at the boundary with the adrenal medulla. Arrows show cells of the undifferentiated zone.

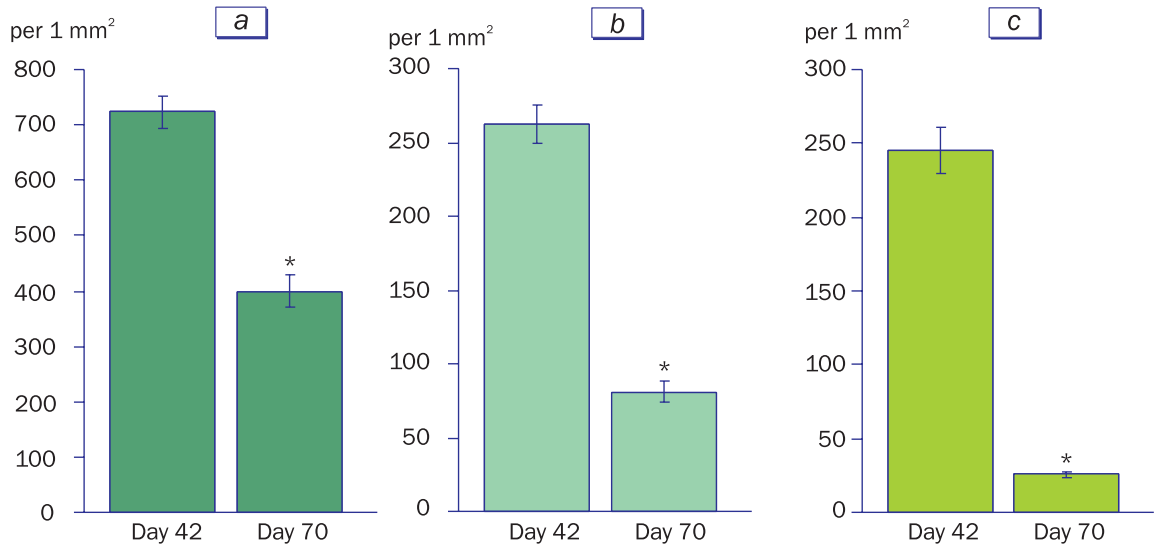

Fig. 3. Content of Ki-67$^+$ cells in different structural and functional layers of the adrenal cortex in intact rats during the pubertal (day 42) and postpubertal (day 70) periods. *a*) Zona glomerulosa; *b*) zona fasciculata; *c*) zona reticularis. *$p<0.05$ in comparison with day 42.

decreased in comparison with that in pubertal rats, but these changes did not reach statistical significance (Fig. 1, *b*, *c*). It should be noted that we observed no differences in the morphology of Oct4$^+$ and surrounding Oct4$^-$ cortical cells in pubertal and adult rats.

Proliferation of adrenal cortical cells after puberty was reduced in all layers compared to pubertal period (Fig. 3). Expression of Oct4 in the zona glomerulosa was strongly associated with the number of Ki-67$^+$ cells (r=0.94; p=0.00013) and demonstrates inverse

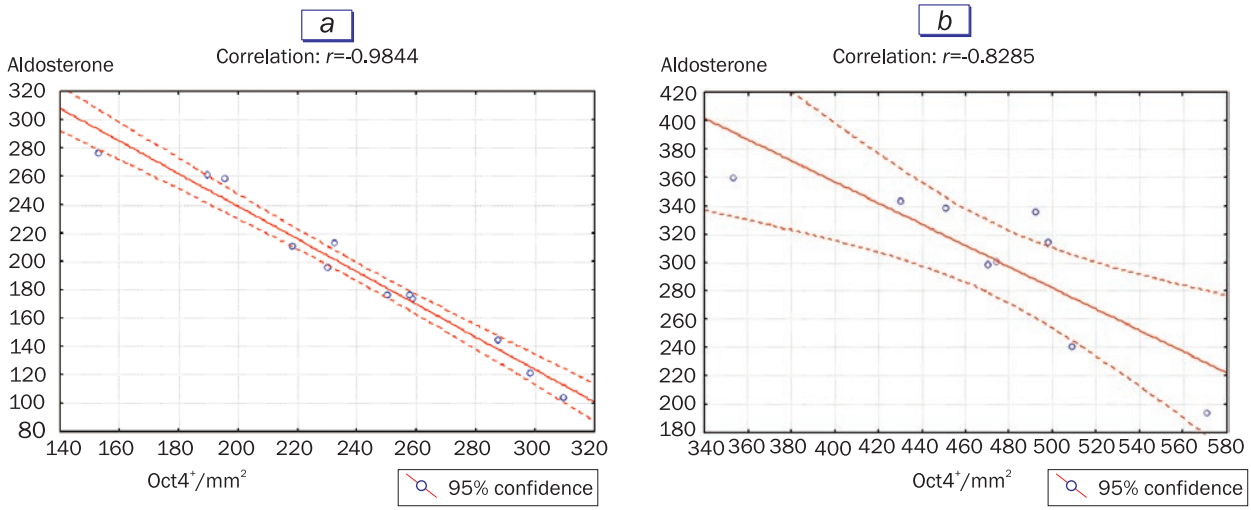

Fig. 4. Analysis of association between the number of Oct4⁺ cells in the zona glomerulosa and serum level of aldosterone in pubertal (*a*) and postpubertal (*b*) rats.

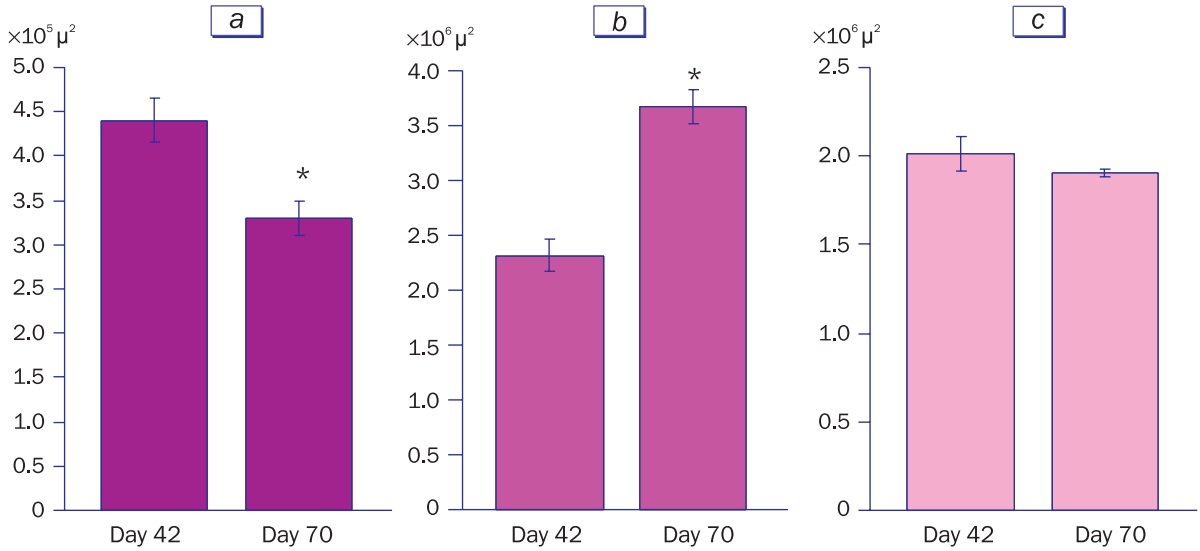

Fig. 5. Areas of structural and functional layers of the adrenal cortex in intact rats during the pubertal (day 42) and postpubertal (day 70) periods. *a*) Zona glomerulosa; *b*) zona fasciculata; *c*) zona reticularis. *$p<0.05$ in comparison with day 42.

correlation with serum aldosterone content (r=-0.83 p=0.0058) (Fig. 4, *b*).

Oct4 is one of the four Yamanaka factors (Oct3/4, Sox2, Klf4, and c-Myc) actively expressed in embryonic cells [13]. They regulate a network of 16 signaling pathways that control cell proliferation, differentiation, and apoptosis [6]. Oct4 expression in pluripotent cells are optimized to maintain their self-renewal and differentiation capacity [15]. It was previously reported that cells of undifferentiated zone between zona glomerulosa and zona fasciculata produce neither aldosterone nor corticosterone, *i.e.* they do not belong to any of these zones [11]. However, expression of steroidogenic factor SF-1/Ad4BP and some other marker proteins typical for cortical steroid producing cells indicated that these cells belonged to cortical parenchyma [10]. The authors supposed that these cells are stem/progenitor cells implicated in the maintenance of functional zonation of the adrenal cortex in rats (in humans, this role is played by subcapsular cells). However, we found no Oct4-expressing cells in the undifferentiated zone or in the subcapsular space. Moreover, nuclear staining for Oct4 was observed only in typical cortical steroid-producing cells, whose cytomorphology did not differ from the surrounding Oct4⁻ steroid-producing cells. These facts are consistent with the data on the ability of differentiated glomerulosa cells to transform into fasciculata cells during regeneration [3]. Comparison of individual levels of Oct4 expression in the zona glomerulosa and serum aldosterone level revealed a strong negative relationship between these parameters indicating readiness of these cells to

self-renewal. The fact that the number of Ki-67$^+$ cells did not correlate with the number of Oct4-expressing cells in pubertal period suggest that the development of the adrenal cortex is not completed by this age.

By day 70, when the adrenal cortex reached its maximum development, the proportion between its morphofunctional zones changed. Despite reduced size of the zona glomerulosa, the number of Oct4$^+$ cells per unit area increased in comparison with that during the pubertal period and their distribution became diffuse. The absence of differences in the structure of Oct4$^+$ and Oct4$^-$ cells attest to pluripotent potential of differentiated cells. Some scientific reports proved the possibility of inducing pluripotency in adult somatic cells through activation of Yamanaka factors [17-19,21]. Detection of Oct4$^+$ parenchymal cells in adult adrenal cortex allows revising the existing concept of postnatal morphogenesis and physiological regeneration of the organ. It is now established that endogenous Oct4 expression is enough for induction of pluripotency in somatic cells [18], whereas upregulation of Oct4 expression in embryonic cells triggered their differentiation [12,16]. This necessitates more thorough studies of the expression of Yamanaka factors in differentiated somatic cells.

Thus, we revealed expression of transcription factor Oct4 in adrenal cortical cells during postnatal development, different rates of Oct4 expression in the functional zones of the adrenal cortex, and the relationship between the morphogenesis and functional activity of the zona glomerulosa.

It can be hypothesized that Oct4 plays a role in postnatal development and function of the adrenal cortex. Taking into account reduction of the zona glomerulosa in the adrenal cortex after puberty and unique metabolic effects of mineralocorticoid hormones, higher content of Oct4$^+$ cells in the zona glomerulosa creates a potential for its physiological regeneration and upregulation of hormone secretion by maintaining the pool of self-renewing cells, in contrast to the zona fasciculata that increases with age and zona reticularis, whose physiological function decreases after activation of steroidogenesis in the gonads. It can be hypothesized that Oct4$^+$ cells in the zona glomerulosa can also participate in the regeneration of the zona fasciculata. These data open new trends in the studies of the regeneration processes in the adrenal gland.

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