Subcapsular Cell Hyperplasia in the Mouse Adrenal Glands

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ABSTRACT. Subcapsular cell hyperplasia in the adrenal glands of ICR mice, aged from 2 to 25 months, were investigated for incidence and morphological characteristics. The lesions were first observed in the 4-month-old mice of both sexes and increased gradually in incidence until reaching a plateau at 19 months of age when the incidence of males was 59% and of females was 91%. Females showed higher incidences than males at any ages examined. Subcapsular cells in the earliest lesion were of fusiform type (Type A cells) and formed scattered foci of small cellular aggregates just beneath the capsule. There were no morphological changes in the zona glomerulosa cells. The subcapsular cells then proliferated to expand their area vertically as well as horizontally and tended to form wedge-shaped hyperplastic foci. Large hyperplastic foci were composed of fusiform cells and polygonal cells (Type B cells) containing abundant cytoplasm. Fat staining showed lipid droplets in both Type A and B cells. Electron microscopic examination on the subcapsular cells revealed basement membranes, desmosomes and dense bodies which developed into intracytoplasmic vacuoles. Some subcapsular cells gathered together to form nests similar to the glands of zona glomerulosa.—**KEY WORDS:** adrenal gland, aging, mouse, hyperplasia, pathology, subcapsular cell.

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Adrenal glands of older mice occasionally have proliferation or hyperplasia of fusiform and/or polygonal cells beneath the capsule, which have been termed “subcapsular cells” by Westberg *et al.* [14]. The kind of lesion has been observed in many strains of mouse [1, 13, 14, 17] and is one of the most common changes in aged mice, which are commonly employed in testing substances for chronic toxicity or carcinogenicity. Dalton [2] and Dunn [5] reported that adrenal cortical tumors of type A and B adenomas or carcinomas seemed to arise from these subcapsular cells. However, few studies have been conducted on how aging influences the occurrence of the subcapsular cells [13]. Investigators have been unable to clarify their origin, physiological roles or a factor(s) which initiates proliferation of these cells. Also it is still controversial whether these cells can be classified as a group of cortical cells [1, 4, 12, 15].

The present report describes incidences of

hyperplasia of the subcapsular cells in relation to aging and their morphological characteristics under light and electron microscopes.

MATERIALS AND METHODS

Animals: One-hundred ninety three males and 206 virgin females of ICR SPF mouse were examined. They were obtained at 4 weeks of age, housed 4 animals of same sex per an aluminum cage (W215×D330×H180 mm), kept at an ambient temperature of 24°C and a humidity of 55%, and maintained on a pulverized diet M (Oriental Yeast Co., Ltd.) and tap water *ad libitum*.

Light microscopy: The animals were sacrificed by incision of the posterior vena cava and the diaphragm under ether anesthesia at intervals of 2, 4, 13, 19 and 25 months of age. Numbers of mice examined at each stage are shown in Table 1. The bilateral adrenal glands were removed and immersed

in 10% buffered neutral formalin. Paraffin embedded tissues were prepared by cross sectioning near the center of the glands, and stained with hematoxylin and eosin, or with AZAN and Watanabe's method for silver impregnation. Oil red O and Sudan black B stains for lipid were also performed on the frozen specimens.

Electron microscopy: Six males and seven females of 19-month-old were used for electron microscopy. Ether anesthetized animals were perfused through the left ventricle with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), while the right atrium was cut to drain excess blood and fluid. Following the perfusion, the adrenal glands were removed and further fixed for three hours with same fixative. The specimens were then divided into two hemispheres and postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for 2 hours, and embedded in Quetol 812. Sections of 1 μ m in thickness were stained with 0.1% toluidin blue for light microscopy as well as orientation of the lesions. Ultrathin sections were stained with 2% uranyl acetate and lead hydrate, and observed with a transmission electron microscope operated at 70 kv.

RESULTS

Incidences of subcapsular cell hyperplasia for mice of each sex at 5 different intervals of age are shown in Table 1. The lesion was first found in the 4-month-old animals with an incidence of 8 of 19 (42%) in males or 11 of 20 (55%) in females. No foci were detected in either sex of 2-month-olds. The incidence increased with age, until reaching a plateau at 19 months of age, when it was 59% in males and 91% in females. The incidence was always higher in females than in males at all ages.

The hyperplasia of subcapsular cells in the adrenal cortex were observed under light

Table 1. Incidences of subcapsular cell hyperplasia in the adrenal gland of ICR mice of different ages

Age (months)	Males	Females
2	0/ 6 (0) ^{a)}	0/ 8 (0)
4	8/ 19 (42)	11/ 20 (55)
13	21/ 48 (44)	39/ 48 (81)
19	10/ 17 (59)	20/ 22 (91)
25	64/103 (62)	100/108 (93)

a) No. of mice with the lesion/No. of mice examined (%).

microscopy and a schematized morphology is illustrated in Figure 1. In the early stage of subcapsular cell hyperplasia, fusiform cells were seen in a small aggregation just beneath the capsule (Fig. 2). These subcapsular cells arranged parallel to the capsule and, similar to the capsular cells, contained flat to oval, chromatin rich nuclei and scant basophilic cytoplasm. However, they were fuller and had a somewhat irregularly shaped cell contour. The mitotic index of subcapsular cells was very low. The cortical cells lying beneath the hyperplastic foci revealed no degenerative changes except for a slight compression of the zona glomerulosa.

With expansion of the hyperplastic lesion, subcapsular cells extended downward into the cortex along a narrow space of stroma between cell cords of the cortical cells to form wedge-shaped foci that founded their base on the capsule. The peripheral or the apical region of these wedge-shaped foci were generally comprised of fusiform cells (Fig. 3). However, in the center of large foci, polygonal cells having a pale oval nucleus and more abundant cytoplasm were seen among the fusiform cells. The transitional cells between the fusiform cells and polygonal cells were also observed. Small vacuoles were often found in the cytoplasm of these polygonal cells. Sometimes, there were small aggregations of polygonal cells forming nests of spherical shape or glandular structure. Some of them resembled the

Fig. 1. A schematic diagram of subcapsular cell hyperplasia in the mouse adrenal gland. C; capsule, G; zona glomerulosa, F; zona fasciculata, M; medulla, ●; lipid droplet.

Fig. 2. The early stage of subcapsular cell hyperplasia in a 4-month-old female mouse showing fusiform subcapsular cells just beneath the capsule. No degenerative changes were observed in the adjacent cortical cells. HE. $\times 150$.

Fig. 3. A wedge-like focus in a 13-month-old female mouse consisting of subcapsular cells proliferating to extent downward into the space of stroma between the cell cords of zona fasciculata. HE. $\times 200$.

Fig. 4. A focus in a 19-month-old female mouse consisting of fusiform cells (Fu), polygonal cells (P), and nest forming cells (N). C; capsule, G; zona glomerulosa, F; zona fasciculata. Toluidine blue. $\times 550$.

structure of the zona glomerulosa (Fig. 4). In older animals, the foci, formed by fusiform and polygonal cells, arose multicentrically, while in young animals they were generally observed as a single lesions consisting of only fusiform cells.

The foci also grew horizontally to extend their base along the capsule and then fused with the neighboring ones.

In a progressive stage, the apex of the wedges which reached the cortico-medullary boundary ran along with the boundary as it seemed to wrap the medulla (Fig. 3). A large part of the adrenal cortex in some older mice with severe hyperplastic lesions were often replaced by proliferating subcapsular cells.

Oil red O and Sudan black B strains demonstrated many minute lipid droplets in the cytoplasm of both fusiform and poly-

Fig. 5. An adenoma originated from subcapsular cells in a 25-month-old female mouse. HE. $\times 150$.

gonal subcapsular cells. The droplets in the fusiform cells were much smaller than those in the polygonal cells.

Besides these hyperplastic lesions, whitish brown nodules were observed unilaterally in the adrenal glands of two females of 25-month-old. The nodule sizes were 6 and 15 mm in diameter. Neoplastic proliferation of fusiform or polygonal cells, similar in appearance to the subcapsular cells in the hyperplastic foci, was observed in these nodules, but the majority of them was fusiform type. They showed a sheet-like proliferation occasionally interrupted by narrow strands of collagen fibers and capillaries (Fig. 5). The polygonal cells often tended to proliferate in a glandular pattern. These cells destroyed the normal architecture by rupturing the capsule and/or distorting the glands. No metastasis was observed.

The subcapsular cells were divided into

Fig. 6. An electron micrograph of subcapsular cells at the superficial layer. A series of "S1-S5" shows morphological changes from fusiform to polygonal cells. C; capsular cell. $\times 6,000$.

Fig. 7. A high magnification of superficial subcapsular cells showing junctional complexes (A) and basement membrane-like structure (B). $\times 30,000$.

four types ultrastructurally according to their locations and morphological characteristics, (a) fusiform cells just beneath the

capsule or superficial layer of the hyperplastic foci, (c) fusiform cells located except superficial layer, (c) polygonal cells at the

Fig. 8. Fusiform subcapsular cells locating except superficial layer. D; dense body. $\times 14,000$.

inner region of the foci, and (d) nest forming cells.

(a) *Fusiform cells just beneath the capsule or the superficial layer of the hyperplastic foci:* There was no marked demarcation between the capsular cell layer and the foci of the subcapsular cells. Transitional cells between these two cell types were usually observed on the boundary. The capsular cells were very elongated cells with delicate cytoplasmic protrusions and poorly developed cytoplasmic organelle, being separated in parts from neighboring cells by abundant collagen fibers in the intercellular space. They resembled so-called "fibrocytes" distributed in the capsules of other abdominal organs. On the other hand, the subcapsular cells were fusiform or polygonal in shape (Fig. 6). The nuclei locating in the center of these cells were elongated or oval in shape and rich in heterochromatin. Oval mitochondria, rough surfaced endoplasmic reticulums and lysosomes were rather sparsely distributed in the cytoplasm. A considerable number of

pinocytotic vesicles were found in the periphery of the cytoplasmic membranes. These subcapsular cells were closely attached to the adjacent cells by well-developed cytoplasmic protrusions and desmosomes. A basement membrane was readily observed around these subcapsular cells in contrast to the fibrocytes in the capsule layer (Fig. 7). There were a few bundles of collagen fiber and thin fibrillar materials in the intercellular space of these subcapsular cells.

(b) *Fusiform cells located except superficial layer:* In the peripheral and apical region of the wedge-shaped foci and on the cortico-medullary boundary, fusiform subcapsular cells were found less frequently than in the superficial layer (Fig. 8). These cells were characterized by a chromatin-rich nucleus with prominent nucleolus and occasional dense bodies surrounded by limiting membranes, oval mitochondria and a few lysosomes in the cytoplasm. The dense bodies were round, usually from 0.5 to 1 μm in

Fig. 9. A polygonal subcapsular cell containing several dense bodies (D) in the cytoplasm. Basement membrane is observed around the cytoplasmic membrane. $\times 14,000$.

diameter, contained moderately electron dense material, and their distribution corresponded to the lipid droplets under the light microscopy. Focal dense areas of the cytoplasmic membrane were occasionally observed on the attached sites of two fusiform cells. These apparatus were similar to the desmosomes observed in the subcapsular cells at superficial layer, though the interspace between the cell membranes was often wider. A basement membrane was not detected around these fusiform cells.

(c) *Polygonal cells at the inner region of the foci*: Subcapsular cells located at the inner region of the foci revealed more polymorphism and sometimes bigger than the fusiform cells, and contained large nuclei with substantially higher amounts of chromatin. The nuclei were sometimes constricted. There were small amounts of mitochondria, lysosomes, vacuoles with electron lucent matrix and dense bodies of various size in the cytoplasm (Fig. 9). Large dense bodies occasionally had electron lucent areas in the

dark monotonous matrix. Some of the cells lacked the basement membrane.

(d) *Nest forming cells*: In the center of the foci, a few polygonal subcapsular cells occasionally gathered together to form nests which resembled the glands of zona glomerulosa (Fig. 10). They were characterized by large nuclei with moderate chromatin, and oval to short tubular mitochondria, a few Golgi apparatus, and sparsely scattered dense bodies or vacuoles in the cytoplasm. Mitochondrial cristae in these cells were rod or villous in shape similar to those in the adrenocortical cells or other endocrine cells (Fig. 11). Desmosomes and basement membranes were generally present.

DISCUSSION

The subcapsular cell hyperplasia in ICR strain were detected in the animals of both sexes over 4-month-old. The lesions were not observed in younger animals though the presence of macroscopical and/or microscopical

Fig. 10. Nest forming subcapsular cells containing one vacuole of transitional phase (V) from a dense body (D). Basement membrane is also observed. $\times 9,000$.

Fig. 11. A high magnification of a nest forming cell showing several vacuoles (V), oval mitochondria with villous cristae (M) and Golgi apparatus (G). $\times 28,000$.

pical foci were reported in the JAX dba mouse of 2 months of age [16]. Females showed higher incidences than males at any age and the same trend was reported in C3H mouse [14]. The incidences in ICR mice are much higher than in BALB/C female mice which developed the lesion at an incidence of 23.8% in the animals ranging from 864 to 1001 days of age [13].

Female adrenal cortex is generally prone to disorganization compared with that of males [14]. Wooly and Little [17] reported that proliferation of subcapsular cells was accelerated by ovariectomy. This suggests that the outbreak of subcapsular cell hyperplasia is presumed to be phenomena relating to hormonal alterations induced by changes of steroidogenesis in the adrenal glands, reproductive organs, and/or pituitary glands after sexual maturation. The blastemic cells progressing to the subcapsular cells in the female adrenal glands may be more sensitive to the hormonal alteration than those in the male.

In the morphological examinations, the findings indicate that the foci of subcapsular cells grow anywhere in the subcapsular region, and that the original type of subcapsular cells was a type represented by fusiform cells which would differentiate later to polygonal cells or nest forming cells. These results are comparable to the reports by Wooly and Little [17] and other authors [5, 8], in which fusiform cells were described as Type A cells and polygonal cells were as Type B cells. However, the fat staining in the present study revealed small lipid droplets in the cytoplasm in Type A cells, which were reported to be negative for fat staining in a previous paper [5]. Electron microscopic findings further suggest that the subcapsular cells should be included in the cortical cells. The subcapsular cells at the superficial layer of foci in the earliest stage are presumed to be a prototype of succeeding subcapsular cells, because they already

have junctional complexes and basement membranes, denying their mesenchymal origin, and show obvious different features from capsular fibrocytes. The other three types of subcapsular cells, fusiform cells located except in the superficial layer, polygonal cells and nest forming cells, further demonstrated characteristics that are consistent to the adrenal cortical cells; basement membranes, partially vacuolated dense bodies which stored non-saturated and/or saturated fatty acid, indicating possible steroidogenesis [3, 10]. Polygonal cells sometimes forming nest-like arrangement are similar in appearance to those of the zona glomerulosa. Therefore, their morphological variations seem to be consistent to the degrees of differentiation from the subcapsular cells on the boundary of the capsule to the parenchymal cells.

These morphological characteristics of the subcapsular cells indicate that the lesion is an abnormal hyperplasia of blastemic [7] or reserve cells [12] of the cortical cells, presumably those of zona glomerulosa. It is quite unlikely that the lesion was formed as the result of a regenerative reaction followed by damages of the cortical cells, or a fibrous degeneration [4] or scar tissue [15] in the cortex. The hyperplastic lesions were sometimes associated with considerable proliferation of collagen fibers. A previous report [11] indicated that mesodermal cells, the source of adrenal capsule and cortical cells, induced a growth of interstitial components following their proliferation.

Diagnostic criterion differentiating hyperplasia from tumor of subcapsular cells is very confusing, and it is hard to distinguish them clearly in morphology [5, 6], because both fusiform and polygonal cells distribute almost equally in both non-neoplastic and neoplastic foci, and mitotic indices of the cells are usually very low in both foci. Therefore, the two cortical adenomas occurring in the 25-month-old females were di-

agnosed on the basis of their size and the degree of their destructive nature toward normal tissue of the adrenal glands [9]. The morphological similarity between the hyperplastic cells and neoplastic cells could indicate that the latter can be derived from the former. However, as far as the evidences obtained in the present study is concerned, it is difficult to say whether or not the hyperplastic cells were transformed into neoplastic cells.

REFERENCES

1. Berdijis, C. C. 1958. Structure of the zone glomerulosa of the adrenal cortex as an indication of age and sex variations in normal mice. *Arch. Ital. Anat. Embriol.* 63: 223-232.
2. Dalton, A. J. 1943. A spontaneous, transplantable, adrenal cortical tumor arising in a strain C mouse. *J. Natl. Cancer Inst.* 4: 329-338.
3. Dean, H. W. 1951. Physiological regulation of zona glomerulosa of the rat's adrenal cortex, as revealed by cytochemical observations. p. 31. In: Pituitary-adrenal function, (Christman, R. C. ed.), AAAS, Washinton, D. C.
4. Delost, P., Delost, H., and Vincent, A. M. 1958. Etude pondérale, morphologique et histochemique de la surrénale chez la Souris âgée multipare. *C. R. Soc. Biol. (Paris)* 152: 1059-1062.
5. Dunn, T. B. 1970. Normal and pathologic anatomy of adrenal gland of the mouse, including neoplasms. *J. Natl. Cancer Inst.* 44: 1323-1389.
6. Dunn, T. B. 1979. Tumors of the adrenal gland. pp. 475-486. In: Pathology of tumors in laboratory animals vol. II. (Turusov, V. S. ed.), International Agency for Research on Cancer, Lyon.
7. Eguchi, Y. 1963. Experimental studies on the fetal mouse adrenal cortex. *Bull. Univ. Osaka Pref., Ser. B.* 14: 211-247.
8. Frith, C. H. 1983. Adenoma and Carcinoma, Adrenal Cortex, Mouse. pp. 49-56. In: Endocrine System. (Jones, T. C., Mohr, U. and Hunt, R. D. eds.), International Life Sciences Institute, Springer-Verlag.
9. Goodman, D. G. 1983. Subcapsular-Cell Hyperplasia, Adrenal, Mouse. pp. 66-68. In: Endocrine System. (Jones, T. C. Mohr, U., and Hunt, R. D. eds.), International Life Sciences Institute, Springer-Verlag.
10. Jone, I. C. 1949. The relationship of the mouse adrenal cortex to the pituitary. *Endocrinology* 45: 514-536.
11. Raftery, A. T. 1973. Regeneration of parietal and visceral peritoneum: An electron microscopical study. *J. Anat.* 115: 375-392.
12. Sato, T. 1967. Age and sex differences in the fine structure of the mouse adrenal cortex. *Nagoya J. Med. Sci.* 30: 225-251.
13. Sheldon, W. G., and Greeman, D. L. 1979. Spontaneous lesions in control BALB/C female mice. *J. Environ. Pathol. and Toxicol.* 3: 155-167.
14. Westberg, J. A., Bern, H. A., and Barnawell, E. B. 1957. Strain differences in the response of the mouse adrenal to oestrogen. *Acta. Endocrinol.* 25: 70-82.
15. Whitehead, R. 1932. Abnormalities of the mouse suprarenal. *J. Pathol.* 35: 415-418.
16. Wooly, G. W. 1950. Experimental endocrine tumors with special reference to the adrenal cortex. *Recent. Progr. Hormone Res.* 5: 383-405.
17. Wooly, G. W., and Little, C. C. 1945. The incidence of adrenal cortical carcinoma in gonadectomized female mice of the extreme dilution strain. I. Observations on the adrenal cortex. *Cancer Res.* 5: 193-202.

要 約

マウスの副腎被膜下細胞過形成：吉田明由・真板敬三・白須泰彦（残留農薬研究所）——マウスの副腎における被膜下細胞過形成の発生頻度と形態学的特徴を2～25ヵ月齢のICR系マウス雌雄について検索した。病変の発生は雌雄ともに4ヵ月齢より認められ、以後19ヵ月齢時に雄で59%、雌で91%に達するまで加齢とともに増加した。雌はいずれの検査時期においても、雄に比べて高い発生頻度を示した。初期病変は副腎被膜下に好塩基性核を有した細胞質の乏しい紡錘形細胞（A型細胞）の小集簇巣として観察されたが、病変部周囲の球状帯細胞には形態学的異常は認められなかった。病変はその後、水平および垂直方向に拡大し、皮質に浸潤する楔形病巣を形成した。このような発達した病巣では紡錘形細胞とともに、細胞質がより豊富でしばしば細胞質内に空胞を有する多角形細胞（B型細胞）が観察された。脂肪染色では紡錘形細胞および多角形細胞の両細胞質内に脂肪滴を認めた。電顕観察では基底膜、デスモソームおよび液胞への分化を示す暗調体（dense body）が認められ、数個の被膜下細胞が集簇して球状帯細胞に類似した腺様構造を形成した。