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Morphological alterations of basal cells of vaginal epithelium in neonatally oestrogenized mice[1]

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Summary. Neonatal oestrogen treatment results in the development of vaginal cancers in the mouse. A morphological sign which probably indicated early invasion of altered vaginal cells into the stroma through gaps in the basal lamina was first seen at 10 months of age in neonatally oestrogenized C57Black mice. Prior to this, a decrease in the numbers of cellular attachment organelles such as half desmosomes and desmosomes was observed by 3 months.

Neonatal treatment of mice with sex steroids causes a hormone-independent, irreversible proliferation of cornified vaginal epithelial cells, which is finally converted to neoplastic growth at an advanced age[2-4]. It is also known in the human that vaginal cancers appear in the daughters of mothers given diethylstilbestrol at a critical stage during pregnancy[5]. Since it has been reported that absence and/or disruption of the basal lamina may occur at an earlier stage of local invasion of cancer cells[6], we have attempted to investigate the morphological evidence suggesting early signs of the invasive growth of the mouse vaginal cells possibly converted to neoplastic cells by neonatal exposure to oestrogen.

Materials and methods. Mice used in this experiment were C57Black/Tw strain. This strain originated from C57Black/6 transferred to the Zoological Institute, Faculty of Science, University of Tokyo in 1963 from the National Institute of Genetics, Mishima, Japan, and was maintained by the strict brother × sister mating. 70 female mice were injected s.c. with 20 µg of 17β-oestradiol in 0.02 ml of sesame oil for the first 5 days after birth. 81 controls were given the vehicle only. Mice of both groups were sacrificed at intervals from 3–24 months of age. Histological and electron microscopic studies were performed on the vaginae of oestrogenized and control mice. Paraffin sections of vaginae were stained with haematoxylin and eosin or the alcian blue-PAS technique[7]. For electron microscopy, thin sections of vaginae, fixed in glutaraldehyde-paraformaldehyde, post-fixed in osmium tetroxide, and embedded in epoxy resin, were stained with uranyl acetate and lead citrate. Numbers of half desmosomes, desmosomes and mitochondria per vaginal basal cell cross section were counted on electron microscope photographs.

Results and discussion. In control mice, the basement membrane of the vaginal epithelium stained red with the alcian blue-PAS technique, indicating the presence of glycoproteins (fig. 1a). At 3–10 months of age, vaginal basal cells rested on a basal lamina with a distinct and continuous contour. At 12 months and thereafter, duplication and folding of the basal lamina were observed in some areas; and at 18 months, it was not infrequently found to be discontinuous. In oestrogenized mice, the basement membrane stained red with the alcian blue-PAS technique; however, the intensity of staining was usually weak and its contour was obscure (fig. 1b). Further disruption of the basal lamina was often observed at 3 months, and frequent-

Numbers of half desmosomes, desmosomes and mitochondria per basal cell cross section*

Groups	Age at autopsy (months)	Half desmosomes	Desmosomes	Mitochondria
Oestrogenized	3	$14.2 \pm 0.5^{abc**}$	7.9 ± 0.4^{g}	11.0 ± 0.5^{k}
	12	11.0 ± 0.7^{ad}	7.2 ± 0.5^{h}	11.4 ± 0.7^{l}
	18	11.2 ± 0.5^{b}	7.2 ± 0.3	11.7 ± 0.8
Control	3	21.9 ± 0.9^{ce}	10.4 ± 0.4^{gi}	16.8 ± 0.6^{km}
	12	19.7 ± 0.7^{df}	10.8 ± 0.4^{hj}	14.7 ± 0.6^{ln}
	18	12.1 ± 0.6^{ef}	5.8 ± 0.5^{ij}	10.6 ± 0.7^{mn}

* Numbers of half desmosomes, desmosomes and mitochondria per vaginal basal cell cross section were calculated for 30 cells from each mouse. All groups consisted of 5 mice and data obtained were assessed by Student's t-test. ** Mean values which have the same superscripts are significantly different each other at 0.01 level.

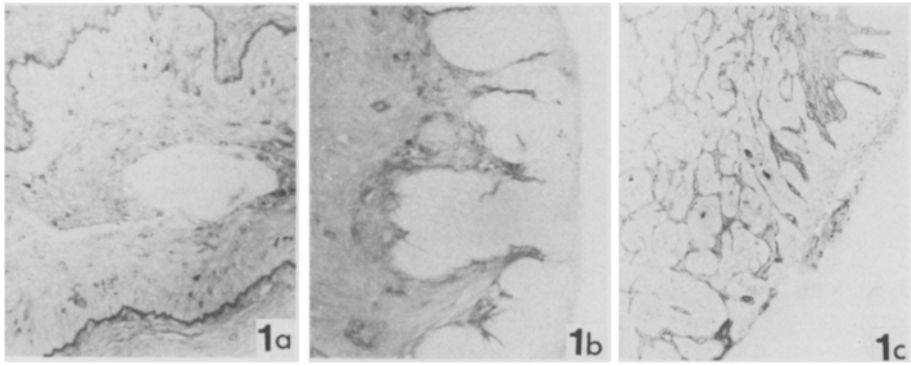

Figure 1. Alcian blue-PAS staining of the vaginae of a 12-month-old control mouse (a, × 113) and of an oestrogenized mouse (b, × 113) and of an epidermoid cancer of the vagina of a 15-month-old oestrogenized mouse (c, × 56). Note distinct basement membrane (a) and weakly stained membrane (b). The stain has disappeared in basement membranes of epidermoid cancer (c).

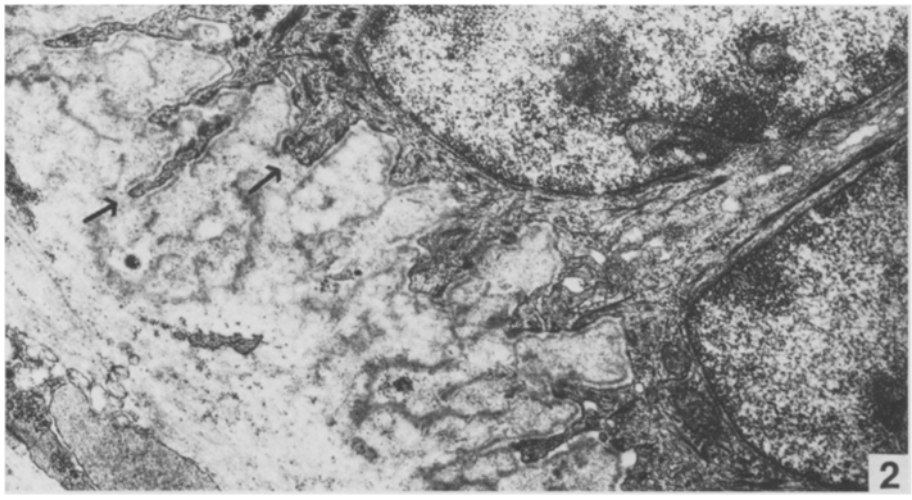

Figure 2. Vaginal basal cells of a 10-month-old oestrogenized mouse. Note folding and disruption of basal lamina and cytoplasmic projections of basal cells (arrows). × 11,800.

ly at 10 months (fig. 2). Protrusion and penetration of cytoplasmic processes of basal cells into the connective tissue through gaps and destructive sites of the basal lamina were observed at 10 months. Afterwards, such destruction of the basal lamina, often accompanied by multiple layering, extensive folding and irregular thickening became more prominent and invasive downgrowth proliferation of basal cells were found in some cases. As described previously, epidermoid cancers were found at 9–12 months of age in this system using C57Black mice[8]. Complete disappearance of staining of glycoproteins was observed in the basement membranes of squamous cell carcinoma (fig. 1c). As for cellular organelles such as half desmosomes and desmosomes, their numbers decreased much prominently in the oestrogenized vaginae with increasing age, compared with those in controls (table). Together with morphological alterations of the basal lamina, such decreases in cellular organelles participating in cellular adhesion and attachment[9], would be connected with invasive downgrowth proliferation of vaginal cells among which transformed cells possibly existed. It is also reported in human cervical squamous cell carcinoma that a decrease in the number of desmosomes is closely correlated with a decreased capacity for cell attachment and with anaplastic morphology of cancer cells[10].

It is known that thickening, duplication and folding of the basal lamina, and a marked decrease in the number of mitochondria, are found in the epithelial tissue as a consequence of senescent conditions at advanced ages[11,12]. It seemed of significance that in oestrogenized mice, the number of mitochondria in the basal cells did not show such a gradual decrease with age. Namely, by 3 months, basal cells contained smaller numbers of mitochondria compared with those in age-matched controls. Thereafter, no numerical change was observed (table). Mitotic activity is higher in the altered vaginal cells than in the normal cells[13]. Therefore, it is possible that the rate of cell division is too rapid to duplicate a larger number of mitochondria. The absence of numerical change probably reflects that the vaginal basal cells, when exposed to oestrogen at a critical neonatal age, acquired abnormal biological characters which were somehow correlated with lack of hormone dependency and higher risk of cancer development.

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