ELECTRON MICROSCOPIC RADIOAUTOGRAPHIC STUDY ON MITOCHONDRIAL PROTEIN SYNTHESIS IN COLONIC EPITHELIAL CELLS OF AGING MICE

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Abstract: For the purpose of studying the aging changes of macromolecular synthesis in the colonic cells of experimental animals, we studied 10 groups of aging mice during aging from fetal day 19 to postnatal month 24. They were injected with $^3$H-leucine, a precursor for protein synthesis, sacrificed and the colonic tissues were processed for light and electron microscopic radioautography. On many radioautograms the localization of silver grains demonstrating protein synthesis in colonic epithelial cells in respective aging groups were analyzed qualitatively. The results revealed that the number of mitochondria increased from embryonic day 19 to postnatal adult month 1 and 2, reaching the maximum, then decreased to senile year 1 to 2. On the other hand, the number of labeled mitochondria showing protein synthesis increased from embryonic day 19 to postnatal newborn days, adult month 1 and 2, reaching the maximum and decreased to month 24. To the contrary, the labeling index increased from embryonic day 19 to postnatal day 3, reaching the maximum, then decreased to day 14, increased to month 1, decreased to month 6, increased to month 12 and decreased to month 24, indicating the aging changes. These results demonstrated that intramitochondrial protein synthesis in the colonic epithelial cells increased and decreased again and again for 3 times repeatedly due to aging of individual animals depending upon the cellular activities at respective aging stages

Key words: Mitochondria, Colonic epithelia, EM radioautography, Protein synthesis

INTRODUCTION

The colon is a part of the large intestines in animals and men, among the digestive tubes between the small intestines and rectum. We have studied the macromolecular synthesis of the colonic epithelial cells in the aging mice in 10 groups of litter mates of both sexes, each 3, from embryonic day 19 to postnatal day 1, 3, 7, 14, month 1, 2, 6, 12 (year 1) and 24 (year 2), by means of light and electron microscopic radioautography. We first studied the DNA synthesis in the colon and caecum of aging mice from embryonic day 19 to postnatal month 12 by using $^3$H-thymidine [1-3]. Light and electron microscopic radioautograms (LM and EM RAG) of the colonic and caecal epithelia revealed that some of the nuclei of columnar epithelial cells were labeled with $^3$H-thymidine showing DNA synthesis. The labeled cells were located at the lower half of the crypts, and the labeling index (LI) changed with the aging. A peak of the labeling index of the absorptive cells was found at embryonic day 19, but decreased at the postnatal day 1 and then kept an almost constant value until postnatal month 12. On the other hand, the LI of the goblet cells showed the peak at embryonic day 19, then decreased gradually with aging from postnatal day 1 and completely disappeared from postnatal month 1 onwards.
However, the localizations of silver grains over the mitochondria of these cells were not examined in these studies [1-3]. In the previous studies, we observed the DNA synthesis in the mitochondria of columnar epithelial cells, mainly the columnar absorptive cells, in 10 groups of littermate mice, from embryonic day 19 to postnatal month 24 (2 years). On the other hand, in contrast to the DNA synthesis in nuclei in various cells of colonic and cecal epithelial cells in aging mice, we also found the silver grains due to DNA and RNA syntheses in mitochondria of various cells such as the pancreatic acinar cells, hepatic cells, adrenal cells or renal cells showing intramitochondrial DNA and RNA syntheses [4-7]. We later found that the activities of DNA and RNA syntheses in mitochondria of various cells changed due to aging of individual animals [8-11]. Thus, we have formerly concentrated to study the intramitochondrial protein synthesis in colonic epithelial cells of aging ddY mice at various ages in 10 groups during development and aging from prenatal embryo day 19 to postnatal 2 years at senescence.

MATERIALS AND METHODS

1. The experimental animals: The colonic tissues were obtained from 10 groups of aging normal ddY strain mice, each consisting of 3 litter mates of both sexes, total 30, from prenatal embryo day 19 to newborn postnatal day 1, 3, 7, 14, adult at month 1, 2, 6, 12 (year 1) to month 24 (year 2). They were administered with \(^3\)H-leucine, a protein precursor, and the colonic tissues were taken out, fixed and processed for electron microscopic radioautography. All the procedures used in this study concerning the animal experiments were in accordance with the guidelines of the animal research committee of Shinshu University School of Medicine as well as the principles of laboratory animal care in NIH publication No. 86-23 (revised 1985).

2. Procedures of microscopic radioautography: All the animals were injected intraperitoneally with \(^3\)H-leucine (Amersham, England, specific activity 877 GBq/mM) in saline, at 9 a.m., one hour before sacrifices. The dosage of injections was 370 KBq/gm body weight. The animals were perfused at 10 a.m., one hour after the injection, via the left ventricles of the hearts with 0.1 M cacodylate-buffered 2.5% glutaraldehyde. The distal colon was taken out from each animal, excised into small tissue pieces which were immersed in the same fixative at 4°C for 1 hr., followed by postfixation in 1% osmium tetroxide in the same buffer at 4°C for 1 hr., dehydrated in graded series of ethanol and acetone, and embedded in epoxy resin Epok 812 (Oken, Tokyo, Japan).

For light and electron microscopic radioautography, semithin sections at 0.2 or 0.5 µm thickness were collected and coated with either Konica NR-M2 radioautographic emulsion or Konica NR-H2 radioautographic emulsion (Konica, Tokyo, Japan) by either dipping or wire-loop method, processed for exposure and development similarly to the previous studies [5-7]. The electron microscopic (EM) radioautograms were examined in a JEOL JEM-4000EX electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 400 kV for observing thick specimens.

3. Quantitative analysis of radioautograms: Twenty LM radioautograms based on the light microscopic photographs taken after observation on at least 100 colonic epithelial cells from respective animals were analyzed to calculate the total number of labeled nuclei covered with silver grains by visual grain counting. For EM, 20 EM radioautograms from each group, based on the electron microscopic photographs taken after observation on at least 100 colonic epithelial cells from respective animals were analyzed to calculate the total number of mitochondria in each cell, and the number of labeled mitochondria covered with silver grains by visual grain counting.

RESULTS

1. Morphological observations: We observed the protein synthesis in the mitochondria of columnar epithelial cells, mainly the columnar absorptive cells, in 10 groups of litter mate mice, from embryonic day 19 to postnatal 2 years and the number of labeled mitochondria and the labeling indices of these cells were analyzed.

2. Radioautographic Observations: Observing EM radioautograms of the columnar epithelial cells, the silver grains were found over the nuclei as well as over the cytoplasm including mitochondria of some columnar epithelial cells (Figs. 1), labeled with \(^3\)H-
leucine, demonstrating protein synthesis at respective aging stages from perinatal stages at embryonic day 19 to senescent stage at month 12 and 24. The localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices similarly to other cells such as in the livers [13] or the adrenal glands [14] as reported previously.

3. Quantitative Analysis

3.1. Number of mitochondria per cell: The number of mitochondria and the labeling indices were calculated regardless whether their nuclei were labeled or not. The results obtained from the number of mitochondria in columnar epithelial cells of respective animals in 10 aging groups seemed to show a gradual increase from the prenatal day 19 to postnatal month 24. Counting the number of mitochondria per cell at respective aging stages, it increased from prenatal embryo to month 2, then decreased from month 6 to month 24. The increase of mitochondrial numbers in the colonic columnar epithelial cells from embryonic day 19 to adult stage at postnatal month 2 were considered to be significant at P value <0.01.

3.2. Mitochondrial protein synthesis: The results of visual counting on the number of mitochondria labeled with silver grains obtained from 10 columnar epithelial cells of each animal labeled with $^3$H-leucine demonstrating protein synthesis in 10 aging groups at perinatal stages, from prenatal embryo day 19 (4.7/cell) to adult stages at month 1 (9.8), increased gradually to month 2(10.9), reaching the maximum, then decreased gradually to month 24(9.1/cell). The increases of the numbers of labeled mitochondria from embryo day 19 to postnatal month 2 were stochastically significant (P <0.01).

3.3. The labeling index: Finally, the labeling indices of mitochondrial protein synthesis were calculated. The results showed that the labeling indices increased from prenatal day 19 (90.4%) to postnatal newborn day 1 and 3 (95.3%), then gradually decreased to postnatal day 14(91.7 %), increased again to adult stages at month 1(93.3%), then again decreased to
month 2 and month 6 (90.3%), reaching a minimum, and increased again to month 12 (93.7%) and decreased to month 24 (91.7%). From the results, the increase of the mitochondrial labeling indices in colonic columnar epithelial cells from embryo day 19 to newborn postnatal day 3 was stochastically significant (P <0.01).

**DISCUSSION**

From the results obtained in the present study it was shown that intramitochondrial protein synthesis was observed in the colonic columnar epithelial cells of all the aging stages from prenatal embryos to postnatal senescent adult stages and the number of mitochondria per cell showed increases due to aging, while the number of labeled mitochondria per cell and the labeling index showed increases and decreases due to aging. However, there was a discrepancy between the increases and decreases of the number of total mitochondria and labeled mitochondria that showed single increase and decrease and the two peaks of increase and decrease. The inconsistency may be due to the difference of the timing when the mitochondria synthesized protein at juvenile and young stages from postnatal day 1 to month 2 when the number of mitochondria increased rapidly. These results demonstrated that intramitochondrial protein synthesis in the colonic epithelial cells revealed variations due to aging of individual animals depending upon the cellular activities at respective aging stages.

With regards the macromolecular synthesis in various cells in various organs of experimental animals observed by light and electron microscopic radioautography, it is well known that the silver grains due to radiolabeled ³H-thymidine demonstrate DNA synthesis [1,4,6,10,12-18]. Our previous results obtained from the studies on the hepatocytes of aging mice revealed that silver grains indicating DNA synthesis were observed over the nuclei of some hepatocytes at perinatal stages from postnatal day 1 to day 14 and decreased due to aging [15-18]. Then, we lately observed the intramitochondrial DNA synthesis in the various organs such as the livers [12, 13,19-22] adrenocortical [14,23-26], adrenomedullary cells [14,27,28] and other cells including the pancreatic acinar cells [29-31], at various ages from fetal day 19 to adult month 24. In the present study, further data on protein synthesis obtained from the colonic columnar epithelial cells were added. Anyway, the results obtained from the colonic epithelial cells of aging mice at present should form a part of special cytochemistry [17] in cell biology, as well as a part of special radioautographology [12], i.e., the application of radioautography to the pancreas, as was recently reviewed by the present author including recent results dealing with various organs. We expect that such special radioautographology and special cytochemistry should be further developed in all the organs in the future.

**CONCLUSIONS**

From the results obtained at present, it was concluded that almost all the columnar epithelial cells in the colons of mice at various ages, were labeled with silver grains showing protein synthesis in their mitochondria. Quantitative analysis on the number of mitochondria in colonic columnar epithelial cells showed that they increased from prenatal embryo day 19 to postnatal month 1 and 2, reaching a maximum, then slightly decreased to month 6, 12 and 24. Likewise, the number of labeled mitochondria with ³H-leucine increased from prenatal day 19 to postnatal month 2, reaching a maximum, then slightly decreased to month 24. To the contrary, the labeling index increased from prenatal day 19 to postnatal day 1 and day 3, reaching a maximum, then decreased to day 14, reaching a minimum, increased again to month 1, and decreased again to month 6, increased to month 12 and 24. These results demonstrated that the number of mitochondria increased from prenatal day 19 to postnatal month 2, reaching a maximum, then slightly decreased to month 24, while the number of labeled mitochondria with ³H-leucine showing protein synthesis increased from prenatal day 19 to postnatal month 2, reaching a maximum, then slightly decreased to month 24. To the contrary, the labeling index increased from prenatal day 19 to postnatal day 3, reaching a maximum, then decreased to day 14, reaching a minimum, increased again to month 1, decreased again to month 6, and increased again to month 12 and decreased to month 24 due to aging of animals.

**REFERENCES**

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