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The Immunohistochemical Study of Apoptosis in Prostatic Tissue

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The prostate has been shown to be androgen dependent. It has been predicted that apoptosis may be related to shrinkage or atrophy of the prostate caused by androgen deprivation.

Present immunohistochemical study was aimed to clarify the relationship between shrinkage of prostate and apoptosis caused by androgen deprivation during the time of course of various hormonal conditions.

Male Sprague-Dawley rats were used to prepare several endocrinological enviroments as followed .1) control, 2) castration,3) castration plus exogenous testosterone, 4)castration plus exogenous testosterone and anti-androgen.The prostate was removed at 3,7,10,14,28,and 56days after castration. Immunohistochemical studies were carried out to observe the appearance of apoptosis.Similar study was also performed for human prostate cancer.

In the control group,apoptosis was rarely seen in the nucleus of glandular epithelial cells.In the castration group, apoptosis increased with a peak at the 3rd day after castration and then decreased until the 56th day. The castration plus exogenous testosterone group demonstrated almost the same conditions as the control group.The castration plus exogenous testosteron and anti-androgen group showed the same conditions as the castration group.The apoptosis was also observed in human prostate cancer as well as rat prostate tissue under same conditions.

The present data suggest that apoptosis in the prostate including prostate cancer was induced by androgen deprivation and recoverd by androgen administration.

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Electron microscopic observation on TUNEL positive nuclei of rat jejunal crypt cells to which apoptosis was induced by adriamycin

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The localization of DNA strand breaks in control and adriamycin (a topoisomerase II-targeting antitumor drug)-treated rat jejunal crypt cells was electron microscopically investigated by the method of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL). In control rats, there was almost none of "spontaneously" apoptotic cells in jejunal crypt. By adriamycin treatment, in contrast, the apoptotic cells markedly increased in number and clustered in the lower neck regions of crypt. On observing those apoptotic cells electron microscopically, the reaction products of TUNEL were randomly localized in the entire nucleoplasms and showed crescent shape condensation frequently. Those features are clearly different from those of terminally differentiating jejunal absorptive cells undergoing apoptosis "spontaneously" . The majority of the labeled nuclei shrank and fragmented into membrane-bounded apoptotic bodies which contained extremely condensed and crescent shaped chromatin. As the fragmentation proceeded, cells follen into apoptosis are phagocytosed by neighboring epithelial cells and showed diffuse TUNEL staining in their remnant cytoplasms which may be due to the leakage of DNA fragments out of the nuclei during the digestion process. These findings suggest that the degradation of DNA in adriamycin-induced apoptosis is far rapid and abrupt clearly differing from that occurred in "spontaneous" apoptosis of the absorptive cells.

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THE SIMULTANCE OBSERVATION OF APOPTOTIC NUCLEI BY TUNEL METHOD AND METHYL GREEN NUCLEI COUNTERSTING IN THE ABSORPTIVE EPITHELIAL CELLS OF RAT SMALL INTESTINE WITH CLSM.

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The usefulness and valuability of the methyl green (MG) nuclear counterstaining in histochemical staining have widely been appreciated. While we were observing MG counterstained sections by CLSM, it was incidentally found that a fluorescence ray was emitted through MG and was clearly detected by the CLSM. It was generally accepted fact that MG reacts specifically to the double strand (dbs)-DNA and this was experimentally proved in our laboratory (Umemura, S. ibid.). In the present report, MG stained nuclei of ① the absorptive cells (terminal differentiating cells) of rat intestinal villi and ② cells in rat intestinal crypts undergoing apoptosis, which was induced by Adriamycin (ADM) administration, were observed by a CLSM, and the changes of the intensity and distribution pattern of MG staining were precisely examined. The apoptotic cells of ① also appreciated as slowly apoptotic cells. The apoptosis is thought to occur during the process of the terminal differentiation, which takes about 48 hours. To examine those apoptotic changes, TUNEL method, in which 3'ends of fragmented dbs-DNAs are end-labeled with histochemically detectable dUTP, was employed.

【Results and Discussion】 In the observation of ① cells, the most intense and diffuse MG staining was observed in the crypt regions(base of the villi). White those cells are moving up to the mid portions and tips of the villi in the process of differentiation, MG reacted substances are translocated to the peripheries of nuclei (heterochromatin regions) and the MG fluorescency in the heterochromatin regions was gradually weakened. On the contrary, TUNEL reaction products, which are solely localized in the heterochromatin regions, increased their amounts during the process. The heterochromatin regions to the site of the dbs-DNA degradation. In experiment ②, ADM damaged cell in the intestinal crypts showed typical "apoptotic bodies" which exhibited the intense TUNEL staining. In those cells, MG reacting substances are irregularly fragmented and diminished, and localized along the margins of TUNEL positive lesions. MG staining appears to reflect the amount of intact dbs-DNA rather accurately in those damaging cells.

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Effect of rifampicin on carbon tetrachloride-induced DNA fragmentation in hepatocytes of mice

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To examine whether rifampicin prevents carbon tetrachloride (CCl_4)-induced DNA injury in hepatocytes, DNA fragmentation in livers of adult male ICR mice treated with rifampicin (200mg/kg, once a day for 4 days) followed by a single injection of CCl_4 (400 μl/kg) was detected by in situ nick translation (ISNT) or in situ end labelling (TUNEL). In addition, denatured DNA in livers of the animals was detected by in situ hybridization (ISH) with poly (A) probe. Animals treated with rifampicin (rifampicin-treated) or CCl_4 alone (CCl_4-treated), or those treated with saline and corn oil instead of the reagents (control) were also examined. Livers of the animals were perfusion-fixed with buffered 4% paraformaldehyde and embedded in paraffin. Paraffin sections were cut and subjected to ISNT, TUNEL or ISH. In livers of control or rifampicin-treated animals, very few ISNT- or TUNEL-positive hepatocytes were occasionally seen around the central venule. Although many ISNT- or TUNEL-positive hepatocytes were found in the perivenular region in CCl_4-treated animals, a few positive hepatocytes were found in narrow area around the central venule in animals treated with rifampicin followed by CCl_4. Negligible hybridization signal was seen in the sections from control or rifampicin-treated animals. A strong hybridization signal was observed in almost all perivenular hepatocytes in CCl_4-treated animals, whereas the signal was seen in a few hepatocytes adjacent to the central venule in animals treated with rifampicin followed by CCl_4. Thus rifampicin prevented CCl_4-induced DNA injury in hepatocytes of mice.