AN ULTRASTRUCTURAL STUDY OF PRECANCEROUS AND CANCEROUS LESIONS OF THE PANCREAS IN SYRIAN GOLDEN HAMSTERS INDUCED BY N-NITROSOBIS(2-OXOPROPYL)AMINE

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Transmission electron microscopic studies of precancerous and cancerous lesions in the pancreas of hamsters induced by N-nitrosobis(2-oxopropyl)amine (BOP) are presented. BOP was injected subcutaneously once weekly for 10 weeks and hamsters were sacrificed every 5 weeks after initiation of the experiment. The ultrastructural findings indicated that serial changes occurred in the epithelium of the pancreatic duct. The epithelial cells became cuboidal and showed increased secretions at 5 weeks. Probable precancerous cells with prominent nucleoli and irregular rough endoplasmic reticulum were found in the main duct at 10 weeks. At 15 weeks, pancreatic tumors forming a duct arrangement were seen, in good accord with the histological appearance. Well differentiated adenocarcinoma cells showing a tubular pattern had oval nuclei with granular chromatin. Poorly developed rough endoplasmic reticulum was irregularly distributed throughout the cytoplasm and the cell surface was covered with microvilli. Poorly differentiated adenocarcinoma showed poor gland formation and had distorted nuclei with prominent nucleoli. These cells were loosely joined. Mitochondria and rough endoplasmic reticulum were poorly developed, and the tumor cells were devoid of secretory granules.

The most characteristic and common change of the precancerous and cancerous lesions in this experiment was the appearance of numerous microvilli on the luminal surface and loss of cytodifferentiation. These findings were obviously different from those of normal epithelial cells or those seen in inflammation. The findings in this study confirm that the pancreatic carcinoma induced by N-nitrosobis(2-oxopropyl)amine in Syrian hamsters is of duct cell origin. No evidence of acinar cells was obtained.

Key words: Pancreatic carcinogenesis — Transmission electron microscopy — N-Nitrosobis(2-oxopropyl)amine (BOP) — Hamster
found to induce pancreatic cancer in Syrian golden hamsters, thus providing suitable animal model of the human disease.

N-Nitrosobis (2-oxopropyl)amine (BOP) is an extremely potent specific carcinogen for the pancreas in Syrian golden hamsters, and the pancreatic neoplasms induced by BOP are similar in many morphological and biological respects to those of man.11,12,14) It should therefore be possible to observe the early changes of the pancreatic duct in the BOP model by serially sacrificing hamsters after exposure to BOP. In the present investigation we studied the appearance of the induced pancreatic lesions in the hamsters by transmission electron microscopy to clarify the early changes and the site of tumor origin.

MATERIALS AND METHODS

Eight-week-old Syrian golden hamsters (Shizuoka Agr. Co. for Lab. Animals, Hamamatsu) with an initial average body weight of 90g were separated by sex, kept under standard conditions in wire cages, and given basal diet (Oriental MF, Oriental Yeast Co., Tokyo) and water ad libitum. BOP was kindly provided by Dr. P. Pour (Eppley Institute for Research in Cancer, Omaha, N. E., U.S.A.). It was stored at 4°C and dissolved in physiologic saline at a concentration of 2 mg/ml just before the injection. Each group of 40 males and 40 females received a subcutaneous injection of BOP at a dose of 10 mg/kg body weight once weekly for 10 weeks. Controls (10 males and 10 females) were injected with physiologic saline only. About 10 animals from each experimental group were sacrificed at 5-week intervals after the beginning of treatment.

Perfusion was performed via the abdominal aorta and the portal vein using physiologic saline followed by 2.5% glutaraldehyde solution (caco-
dylate buffer, pH 7.3) at a pressure of 40 cm H2O (21G intravenous silicon catheter). The pancreas was removed immediately, and tissue samples from the large duct and the lesions which were seen as white spot nodules in the pancreas were taken and diced into 1 to 2 mm cubes. Tissues were further fixed in 2.5% buffered glutaraldehyde and post-fixed in buffered osmium tetroxide at 4°C, then dehydrated in increasing concentrations of ethanol and embedded in Epon 812. Ultrathin sections were made on an LKB microtome with glass knives, stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-12 transmission electron microscope. The remaining tissues and the pancreas of animals which died during the observation period were placed in 10% phosphate-buffered formalin and processed for routine histopathologic sections.

RESULTS

In the normal pancreatic main duct (large duct) the lumen was lined by a single layer of columnar epithelial cells. The nucleus was spindle-shaped and fine granular chromatin was condensed at the nuclear periphery. The epithelial cells were bordered by a distinct basement membrane and had short microvilli on their luminal surface. The cells contained scanty cytoplasmic organelles with a few secretory granules (Fig. 1). The ductules (interlobular and intralobular ducts) were lined by a single layer of cuboidal epithelial cells with globular nuclei. Endoplasmic reticulum was sparse, but the Golgi complex and pynocytotic vesicles were well

Fig. 1. Normal epithelial cells of hamster pancreatic duct. ×9000.
developed, and a few small mitochondria were seen.

At 5 weeks both regressive and progressive changes were found focally with epithelial cells from the main duct to the small duct. Ultrastructurally these cells showed enhanced secretion with increased cytoplasmic contents and pseudopodial projection of cytoplasm occasionally extended into the lumen. These intraductal lesions were composed of cuboidal cells with increased mitochondria and markedly dilated cisterna of the Golgi complex. In addition, despite the prominent development of cytoplasmic organelles, some cells were small but the nuclear/cytoplasmic ratio was increased (Fig. 2). At this stage, multilayer proliferation of the epithelium was usually found histologically throughout the ductal system and in some peripheral ducts. The centroacinar cell and the ductule near it showed hypertrophic changes with a marked increase in mitochondria. Rough endoplasmic reticulum showed parallel arrays of elongated cisternae around the nucleus in the basal cytoplasm. The Golgi complexes showed frequent distension of cisternae and distended vacuoles.

**Precancerous Lesions** At 10 weeks, the ductular epithelium became histologically pleomorphic with variation in size. The dysplastic cells which grew between adjacent cells were large and the nuclear/cytoplasmic ratio was increased (Fig. 3). Usually a couple of prominent nucleoli were present with pleomorphic and granular chromatin. The cytoplasm contained swollen mitochondria and rough endoplasmic reticulum with irregularly adherent ribosomes as well as free ribosomes scattered throughout the cytoplasm. The surface projected irregularly into the duct lumen and was densely covered by microvilli of various lengths.

**Carcinoma in situ** Histologically, these lesions showed severe dysplasia of the epithelial cells without invasion beyond the basement membrane at 15 weeks. Ultrastructurally, the luminal surface of the cells showed numerous microvilli, and the neoplastic cells had well-developed cytoplasmic...

Fig. 2. The interlobular duct with a single layer of cuboidal epithelial cells at 5 weeks. ×4900.
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Fig. 3. The dysplastic cells at 10 weeks exhibited a high nuclear/cytoplasmic ratio, prominent nucleoli, and a marked increase in the number of microvilli. × 3800.

Fig. 4. In carcinoma in situ, the lumen was retained amongst the proliferating tumor cells with small microvilli on the surface. × 3900.

organelles, as in the cells of well differentiated adenocarcinoma (Fig. 4). At this stage, marked proliferation of ductules and degeneration of acinar cells were seen. Among proliferating ductules, some ductules showed various sizes of lumen consisting of degenerated cells and cancerous cells (Fig. 5) with large nuclei and cytoplasmic organelles, as also seen in poorly differentiated adenocarcinoma.

Cancerous Lesions In the period from 15 to 20 weeks, unequivocal carcinoma was usually found, although a few appeared as early as 13 weeks. Invasion into adjacent tissues and regional lymph node metastases were often present. Ultrastructurally, these carcinomas showed characteristic findings corresponding to their histological appearances.

Well Differentiated Tubular Adenocarcinoma: Tumor cells were columnar and varied in size. The nuclei, which were oval and enlarged, had granularly dispersed chromatin and frequently showed an irregular contour due to infolding and indentation of the nuclear membrane. Nucleoli were enlarged and showed distinct nucleolonema containing both granular and fibrillar components. Mitochondria and rough endoplasmic reticulum, which were often dilated, were usually seen in the apical portion of the nucleus. Poorly developed rough endoplasmic reticulum was irregularly scattered throughout the cytoplasm, but predominantly in the basal side. The luminal surface of carcinoma cells showed numerous microvilli facing the lumen. Basement membrane was not destroyed and tumor cells
were joined tightly by junctional complexes (Fig. 6).

Poorly Differentiated Adenocarcinoma: The tumor consisted of various sized tumor cells, with clear nucleus and cytoplasm. Large tumor cells had distorted nuclei with multiple prominent nucleoli and chromatin dispersed at the nuclear margin. Free ribosomes were present in the cytoplasm, but the number of ribosomes was distinctly less than in well differentiated carcinoma. Mitochondria and endoplasmic reticulum were also poorly developed. The nuclei of the small cells appeared to be irregular with dark nucleoli. The development of cytoplasmic organelles in small cells was less than in large tumor cells. The Golgi complex was not seen clearly, but a few mitochondria and irregular rough endoplasmic reticulum were present. Small tumor cells were loosely joined. Both large and small cells were devoid of secretory granules (Fig. 7).

**DISCUSSION**

Previous ultrastructural descriptions of pancreatic neoplasms induced by 4-hydroxyaminoquinoline 1-oxide\(^1\) or 7,12-dimethylbenz[a]anthracene\(^2\) in rats corresponded to acinar cell tumors rather than ductal tumors. However, under our experimental conditions, BOP was shown to induce ductal carcinomas in Syrian golden hamsters. We were able to observe serially the tumor development from early duct hyperplasia to ductular proliferation, adenoma, intraductal carcinoma or carcinoma *in situ*, microscopic

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Fig. 5. Probable cancerous cell at 15 weeks, resembling a poorly differentiated carcinoma cell. \(\times 3100\).

Fig. 6. Clusters of well differentiated adenocarcinoma at 15 weeks. \(\times 3000\).
adenocarcinoma, and eventually to unequivocal carcinoma. Certain histologic subtypes of carcinomas described in the present study were remarkably similar to those of humans.3) The fine structure of the tumor cells in the present study revealed characteristics of duct cells, distinct from those of acinar cells. The present study also demonstrated the alteration of duct cells in transitional stages during chemical carcinogenesis of the pancreas, involving the nucleus, nucleoli, cytoplasmic organelles, and cytoplasmic membranes of epithelial cells. The fine structure of the tumor cells in the present study was characterized by large nuclei with prominent nucleoli.7) In some types of cancer cells, it is well known that the nuclear and nucleolar size and shape are extremely abnormal, and the abnormal nuclei have been found to coincide with the appearance of aneuploidy in the population.2) Even though the presence of abnormal nuclei is not necessarily considered diagnostic of neoplastic cells, they can usually be used as one of the criteria of malignancy. After 10 weeks, the nuclei, with distinct nucleolonema containing both granular and fibrillar components, were enlarged and showed irregular contours. Both perichromatin and interchromatin granules increased and were irregularly scattered. However, ultrastructural differences between cancerous cells and histologically benign cells, especially in papillary adenoma, were not always distinct, though epithelial cells in untreated hamsters and cancer cells could be distinguished easily.

The second difference between the neoplastic cells and control epithelial cells was the appearance of cytoplasmic organelles. In tumor cells the organelles were poorly developed, resembling those of fetal pancreatic duct cells.16) Although free ribosomes were scattered within the cytoplasm, zymogen granules were rare and the electron density of granule content decreased. Mitochondria and rough endoplasmic reticulum were also decreased, especially the rough endoplasmic reticulum, which was abnormally elongated and string-like in appearance. Various types of structural abnormalities of the cell surface and plasma membrane have been described and were

Fig. 7. Poorly differentiated adenocarcinoma at 15 weeks. ×6600.
Ultrastucture of BOP-Induced Pancreas Neoplasms

Generally characteristic of each type of neoplasm. In this study, the well differentiated cancer showed structures similar to those of normal cells, including junctions between tumor cells and with the connective tissue. In poorly differentiated cancer, although glandular structures were formed with desmosomes present, the lateral side of the cells appeared loose. The most characteristic and common change of the precancerous and cancerous lesions in our experiment was the appearance of numerous microvilli on the luminal surface and loss of cytoidifferentiation. These findings were obviously different from those of normal epithelial cells or those seen in inflammation.

Most human pancreatic carcinomas are classified as being of ductal origin. Some are characterized by excessive mucus production, a condition that might be expected to result from abnormal proliferation of the mucus-secreting cells of the larger pancreatic duct. Others are classified as being ductal origin only on the basis of duct-like structures in the tumor. To clarify the histogenetic origin of these tumors, an animal model is necessary in which sequential observations are possible. The proliferating cells, found by 10 weeks, developed into adenomas and adenocarcinomas, and they appeared to originate from the epithelium of ductules. Takahashi et al. previously showed that these proliferative ductules communicated with the preexisting ductal system, by using India ink, and we also confirmed the connection of the ductal system and ductules to adenomas by preparing resin casts (unpublished data). Ultrastructurally, except for some well differentiated adenocarcinomas, most neoplasms did not contain mucus droplets, but secreted materials often filled proliferating ductules, glands, and cystic spaces. These findings provide additional evidence for a connection between the common ductal system, proliferative lesions, cystic alterations and tumor. The most interesting and significant finding in this study was the generation of neoplastic cells in an ubiquitous ductular proliferation. At 15 weeks, we were able to find carcinoma cells arising from proliferating ductules in some animals. This work directly demonstrated the morphogenesis of pancreatic carcinoma of ductule origin, except for occasional intraductal carcinoma which sometimes appeared in the large duct.

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References