Morphogenesis of Non-neoplastic Nodular Lesions of the Pancreas in Aged Rats: Light Microscopic, Immunocytochemical and Autoradiographic Study

Isao NARAMA, Minoru TSUCHITANI, and Yoshitaka SUZUKI¹⁾

Hamamatsu Seigiken Research Laboratory Co., 95-10 Aoi-cho, Hamamatsu, Shizuoka 433, and ¹⁾Department of Veterinary Anatomy, Gifu University, 1-1 Yanagido, Gifu 501-11, Japan

(Received 14 October 1985/Accepted 22 January 1986)

ABSTRACT. Non-neoplastic nodular lesions of the pancreas were histopathologically and immunocytochemically investigated with forty three 85 to 114 week-old F344 rats. Similar lesions were also studied by autoradiography with 10 Sprague-Dawley rats. Microscopically, the pancreatic lesion essentially was replacement of the exocrine tissue by proliferated duct-like structures. The often enlarged nuclei of epithelial cells lining these duct-like structures showed mitotic figures and active synthesis of DNA by autoradiography. Single acinus or small aggregates of endocrine cells were seen among these duct-like structures. There were also many transitional type cells between immature cubic cells resembling ductal epithelium and mature exocrine cells. These transitional type cells also revealed active DNA synthesis. The results indicate that the nodular lesions under study are involved with the regenerating change of the pancreatic tissue. Immature epithelial cells of duct-like structure appear to have the potential to differentiate into both ductal and endocrine cells. The possible morphogenesis of these regenerating nodules was discussed.—**KEY WORDS:** aged rat, nodular lesion, pancreas, regeneration.

Jpn. J. Vet. Sci. 48(2): 389–398, 1986

Nodular pancreatic lesions of non-neoplastic nature are common in many strains of aged rats [3, 7, 9]. They have been described as lobular or acinar atrophy [9] or multifocal atrophy [7]. The essential histologic change was the proliferation of ductal structures. Similar ductal proliferation in the pancreas has also been observed in rats by partial ligation [19] or treatment with DL-ethionine [11], various carcinogens [13, 16, 18], or in guinea pigs with experimental pancreatitis [17]. The proliferation of the ductal structure is considered in the literature to be a regenerative change [10, 17] or pre-neoplastic change [18].

The true nature and morphogenesis of these pancreatic lesions in aged rats remains unclear, because these incidental findings have not been studied systematically. However, this change is seemingly significant because of its high incidence in some rat strains [7].

The present report describes the detailed histological appearances of the lesion and attempts to discuss the morphogenesis using autoradiography.

MATERIALS AND METHODS

Nineteen male and 24 female F344 inbred rats (Shizuoka Agricultural Cooperative Association for Laboratory Animal, Japan) of 85 to 114 weeks of age were used for histopathological study. They did not received any previous treatment for collecting the background data of this strain. They were housed 3 or 5 per wire mesh cage in a barrier sustained animal room conditioned at $24\pm2^{\circ}\text{C}$ temperature, $55\pm5\%$ relative humidity under 12 hours artificial light cycle. All rats were fed an autoclaved commercial diet (Funabashi MM-2, Funabashi Farm Ltd., Japan) and tap water ad libitum. For autoradio-

Fig. 1. Small focus of non-neoplastic nodular lesion in the pancreas. The pancreatic tissue is replaced by adenomatously proliferated duct-like structure containing several acini and small aggregates of islet cells. HE stain. $\times 130$.

graphic study, 5 male and 5 female Sprague-Dawley rats from a closed colony (110 week-old), kept under the above-mentioned conditions were used. They were injected intraperitoneally with 1 μ Ci of 3 H-thymidine per gram of body weight just 1 hour before autopsy.

All animals were sacrificed by bleeding from the abdominal aorta under ether anesthesia. Pancreatic tissues were sampled with representative organs and fixed immediately in 10 per cent formalin solution, dehydrated in automated processor and embedded in paraffin. Sections were cut at 5 μ m and stained by hematoxylin and eosin, PAS reaction and Heidenhain's azocarmine (AZAN). Immunohistochemical staining was carried out using the Dako PAP kit (Dako corporation, Santa Barbara, Calif., USA) from a commercial source. On some representative sections of the pancreas, immunoperoxidase reaction for insulin, glucagon and somatostatin was applied, and these sections were counterstained with hematoxylin. Sections for autoradio-

graphy were cut also at 5 μ m. The deparaffinized sections were dipped in emulsion Type NR-M2 (Konishiroku Photo Industrial Co., Japan) at 40°C. The coated sections were placed in a light-tight box with a desiccant and exposed for 6 days at 4°C. The coated sections were developed in FD-111 (Fuji Photo Film Co., Japan). The autoradiographs were immediately stained with hematoxylin and eosin.

RESULTS

Morphology: All lesions were focal or multifocal and detectable only by microscopic examination (Fig. 1). Although only several acini were affected in the smallest lesion, most lesions spread over a whole lobule and involved several other lobules. A few lesions involved the whole thickness of the pancreas and were sharply circumscribed from normal tissue by the delicate interlobular connective tissue septa (Fig. 2).

The essential change of the lesion was

Fig. 2. Widespread pancreatic lesion involving the whole thickness. The lesion is sharply circumscribed from normal tissue by delicate interlobular septa. AZAN stain. $\times 75$.

Fig. 3. Adenomatous proliferation of duct-like structure, newly formed acini and aggregates of islet cells in the pancreatic lesion. Lymphocytic infiltration into somewhat conspicuous interstitium. Some ducts have developed cystic dilatation of the lumen. HE stain. $\times 130$.

adenomatous proliferation of duct-like structure in the pancreatic tissue (Fig. 3).

The epithelial cells lining the duct-like structure took a varied appearance in the lesion, and showed various stages of transformation from immature cubic cells to mature acinar cells. The immature cubic cells had round or ovoid clear nuclei, with sparse chromatin and no obvious nucleolus. They had a profile similar to that of the epithelial cells of the intercalated duct or centroacinar cells. Occasional mitotic figures and enlargement of the nuclei were detected among these cells (Fig. 4). Some duct-like structures contained one or more cubic cells incorporating a few zymogen granules in the apical side of their cytoplasm. The epithelial cells became taller and tended to be pyramid-shaped with a spherical nucleus near their base and contained more zymogen granules in their cytoplasm. Immature cubic cells and pyramid-shaped cells rich in zymogen granules were often observed in the same duct-like structure (Fig. 5). The epithelial cells showing advanced maturation had basophilic cytoplasm

Fig. 4. Mitotic figure of ductal epithelium in a small lesion. HE stain. $\times 520$.

in their basal portion and became very similar to the normal acinar cells. Such fully matured duct-like structures were very similar to the normal acini except for the slightly enlarged luminal space. The epithelial cells showing acinar differentiation had dark nuclei with a conspicuous nucleolus and definite nuclear wall. Mitotic figures and enlarged or irregular-shaped nuclei were also detected in these cells.

The epithelium lining the duct-like structure also contained islet cells with many granules in their cytoplasm. They resembled B cells which are most numerous in rat pancreatic islets. Usually, several islet cells formed a small islet surrounded by thin connective tissue (Fig. 6). Some of these newly formed islets had a small central space which seemed to be derived from the lumen of the duct-like structure. Several adjacent aggregates of islet cells often formed a larger mass. They were similar to the normal pancreatic islets except for the connective tissue septa separating them into units of several cells. When a large group of duct-like structures

Fig. 5. Early stage of exocrine differentiation of ductal cells. A duct is lined by immature flat cells and larger cells rich in zymogen granules. HE stain. $\times 780$.

Fig. 6. Newly formed small islets in the pancreatic lesion. Aggregates of islet cells are surrounded by thin connective tissue suggesting duct-like structure origin. HE stain. $\times 780$.

Fig. 7. Complication of duct-like structure, exocrine acini and aggregates of islet cells. The direction of transformation is decided by the unit of a duct. HE stain. $\times 780$.

was transformed into the mass of islet cells, the lesion assumed an appearance of islet-cell adenoma containing several acini within.

Differentiation of the epithelial cells varied in each duct-like structure. Therefore, newly formed acini, small aggregates of islet cells and duct-like structure containing immature exocrine cells were complicated in some lesions (Fig. 7). While the degree of maturation in exocrine cells varied in each duct-like structure, most islet cells were in the advanced stage of maturation. Thus, most duct-like structures showed a biphasic character of transformation, but some developed cystic dilatation of the lumen without exo- or endocrine elements (Fig. 8).

Infiltration of lymphocytes and mastocytes was occasionally detected, but inflammatory changes were rather slight and inconsistent. An increase in the volume of interacinar connective tissue fiber was also prominent in some areas, but this was attributed to the reduction of tissue volume of each duct com-

Fig. 8. Pancreatic nodular lesion developing cystic dilatation of the lumen. HE stain. $\times 75$.

pared with those in normal acinus and not to the fibrosis. Yellow pigment deposition was also observed in the interstitial tissue of many cases, but this change was not always associated with other pancreatic changes.

Immunocytochemistry: All islet cells in the nodular pancreatic lesions showed a positive reaction for insulin, glucagon or somatostatin by immunohistochemical method. There were also a few immature cubic cells with duct-like structure having positive granules for these pancreatic endocrine hormones. Among these islet cells and immature cubic cells of the duct-like structure in pancreatic lesion, insulin-positive cells were most abundant. Fewer glucagon-positive cells and a small number of somatostatin-positive cells were observed in the lesion. The proportion of the number of insulin-, glucagon- and somatostatin-positive cells was similar to that of normal islet tissue. The somatostatin-positive cells were slender and located in the peripheral area of aggregates of islet cells. The location of glucagon-positive cells was also analogous to normal islet tissue.

Table 1. Number of labeled cells in pancreatic lesions

Sample	Cell component of the lesion				Normal acinar cell
	Acinar	Mixed	Duct	Islet	
No. 1	188/2450		5/273	0/186	1/2129
No. 2		39/565			1/523
No. 3		22/293		0/80	1/304
No. 4		39/659	17/385		1/2342
No. 5	35/291	28/368		0/57	1/357

Number of labeled/Number of examined.

Fig. 9. Many labeled nuclei of the newly formed acinar cells in the pancreatic lesion (right) by the treatment of 1 μ Ci of 3 H-thymidine one hour before killing. $\times 260$.

Fig. 10. Several labeled cells (arrows) of ductal type in a nodular pancreatic lesion by the treatment of 1 μ Ci of 3 H-thymidine one hour before killing. $\times 260$.

Autoradiography: Five out of 10 aged Sprague-Dawley rats examined by autoradiography had pancreatic lesions. For the evaluation of the autoradiographic slides, all pancreatic lesions on tissue sections were divided into acinar-, duct-, and islet types by morphological characters of their lining epithelial cells. However, some cells appeared to be transitional types between acinar and duct cells, so these cells were considered a mixed type. The number of all lining cells of all lesions were countered along with the number

of labeled cells. The numbers of labeled cells in pancreatic lesions and normal pancreatic tissues of these 5 rats were summarized in Table 1. In contrast to the fact that the acinar cells in the normal pancreatic tissues of 5 rats were labeled at a rate of one to 304–2342, all acinar, mixed and duct type cells in the pancreatic lesion were labeled as much as about 20–180 times (Figs. 9 and 10), but no cell of islet type showed any apparent evidence of DNA synthesis.

Fig. 11. Diagrammatic representation showing the possible morphogenesis of nodular pancreatic lesion in aged rats. (a) adenomatous proliferation of duct-like structure, (b) early stage of acinar and insular transformation of ductal epithelium, (c) late stage of differentiation of acinar cells predominant area, (d) late stage of differentiation, mixture of acini and islet tissue in the unit of a duct, (e) late stage of differentiation of islet cells predominant area, (f) cystic dilatation of lumen of the duct with flattening the epithelial cells.

DISCUSSION

The essential histological change of the pancreatic lesions in the rats under study was focal replacement of the pancreatic tissue by proliferating duct-like structures. The lesions were analogous to those described as most common non-neoplastic change in the aged rats [3, 7, 9], and reported to be common in male and female rats of various strains such as BN/Bi, WAG/Bi, WAG/Ri, WAG X BNF1 [7], F344 [6, 9] and Sprague-Dawley [3]. Andrew [1] first described the nodular proliferation of the ducts in the pancreas of aged rats and considered that this change

indicated a decreased function of the exocrine tissue. Since then, many authors [7, 9] described this change as an atrophic one, taking the duct-like structure lined with cuboidal cells for a regressive transformation of acini resulting from shrinkage of acinar cells associated with complete loss of cell granules. In contrast to these authors, Goodman *et al.* [12] alone listed duct and islet hyperplasia in addition to atrophic lesions in aging F344 rats. However, the correlation between both lesions was not clear.

In the present cases, the morphological evidence of the nucleus, including abundant mitotic figures and autoradiographic findings,

confirmed that this pancreatic change with duct-like structure formation is not a simple atrophic change of exocrine tissue but that at least some of their lining cells have a proliferative nature.

Some immature cells differentiating the acinar cell looked like intermediate types between ductal epithelium and acinar cells. As these epithelial cells formed the acinus with a somewhat dilated lumen in contrast to the rarely detectable lumen of the normal acinus, they might be regarded as the atrophic acinus which have been observed in the uremic human and primate pancreas [2, 5]. However, the nuclei of these cells showed a high concentration of silver grains in the autoradiogram of the pancreatic lesions in the ^3H -thymidine treated rats. Therefore, these transitional type cells also possess the same proliferating activity as the cubic cells of the duct-like structure.

Fitzgerald [11] described the regenerative changes in the pancreas of the rats treated intraperitoneally with DL-ethionine and identified the transitional cell types between duct cells and pre-acinar cells. A similar regenerating change of exo- and endocrine cells from duct-like structure was also observed in taurochlorate-induced acute pancreatitis in guinea pigs [6]. These authors considered that acinar cells of the pancreas have a great regenerative ability and the possible presence of precursor cells develops into ductal or acinar cells. The regenerating acinar cells observed in the present study seem to have originated from cubic cells of duct-like structure which have been shown to reflect a very active DNA synthesis [19].

Duct-like structures similar to the present cases were observed in the pancreas of rats or of guinea pigs treated with various carcinogens [13, 18]. The histological features of these changes were very similar to the pancreatic lesions in old rats, but they are considered to be a precancerous or cancerous state of the pancreas tissue of duct origin. The proliferating

nature of the pancreatic lesions in the rats under study may be different from that of a precancerous change, because no malignant transformation was observed in the lesions and the cells comprising the lesions were extremely well differentiated.

Cantenys *et al.* [8] described the pancreatic regeneration in newborn rats after the destruction of B cells by streptozotocin and revealed that islet cells also were transformed from ductal cells. The cubic epithelial cells of the duct-like structure in the rats in the present study also seem to transform into islet cells. Structural characteristics of newly formed large islets such as separation by connective tissue septa and the presence of a few acini suggested that the giant islet originated from more differentiated duct-like structures. These giant islets were very similar to the pancreatic islet adenoma or insulinoma in aged rats [3, 7]. Some author has described a tubular or acinar structure in a part of these pancreatic islet tumors of rats [7], although their morphogenesis and significance are still obscure.

The possible morphogenesis of the pancreatic lesions in aged rats was illustrated in Fig. 11. Adenomatous proliferation of the ducts appears to be the earliest lesion, although a primary change of the pancreatic tissue which seems to pre-date this change is still undetectable. However, the location and size of the lesions suggest that primary changes of the pancreatic tissue may be caused by the occlusion of some parts of pancreatic ductular system. However, neither dilatation of the ductular system nor obturator has been observed in our cases. Some epithelial cells of duct-like structure developed cystic dilatation of the lumen indicating the atrophic change of epithelial cells. This change also seems to be caused by occlusion of the ductular system, although the cause of the primary change is unexplainable. Duct-like structures also have an appearance very similar to normal intercalated duct, but the nuclei of the epithe-

lial lining cells show active DNA synthesis. The epithelial cells of the duct-like structure transform into both acinar and islet cells. The direction of the epithelial transformation seems to be determined by the unit of a duct, so an aggregate of several islet cells and immature acini consisting of ductual cells containing a small amount of zymogen granules are often observed side by side. In the area which the ductual epithelial cells transform predominantly into exocrine cells, the lesion has features similar to normal exocrine tissue. The lesion in which the ductual epithelium transformed predominantly into islets cells finally forms large islets separated by thin connective tissue. In the small part of these large masses of islets cells, sometimes are one or two exocrine acini in the periphery which seem to be formed by the exocrine transformation of the ductual cells.

The pancreatic islet cells and other intestinal amine precursor uptake and decarboxylation (APUD) cells were considered to be of neural crest (ectodermal) origin for long time [14, 15], but a recent report suggested that intestinal APUD cells differentiate directly from the intestinal epithelium [4]. The pancreatic lesions in the present cases also strongly suggest that pancreatic islet cells have an endodermal origin. Identical proportions of three cell types of the newly formed islet to normal islet tissue makes one consider possible factors regulating the differentiation of ductual cells. This unknown factor might also regulate the direction of differentiation of ductual cells into both exocrine and endocrine tissue.

REFERENCES

1. Andrew, W. 1945. Senile changes in the pancreas of wistar institute rats and of man with special regard to the similarity of locule and cavity formation. *Am. J. Anat.* 100: 97-127.
2. Baggenstoss, A. H. 1948. The pancreas in uremia: A histopathologic study. *Am. J. Pathol.* 24: 1003-1017.
3. Berg, B. N. 1967. Longevity studies in rats. II. Pathology of aging rats. pp. 749-786. In: *Pathology of Laboratory Rats and Mice* (Cotchin, E., and Roe, F.J.C. eds.), Blackwell Scientific Publications, Oxford and Edinburgh.
4. Bosman, F. T., and Lowerens, J. K. 1981. APUD cells in teratomas. *Am. J. Pathol.* 104: 174-180.
5. Bronson, R. T., Strauss, W., and Wheeler, W. 1983. Pancreatic ectasia in uremic Macaques. *Am. J. Pathol.* 106: 342-347.
6. Bubis, J. J., Orda, R., Wiznitzer, Th., and Bawnik, J. B. 1980. Ductual proliferation in late stage of experimental acute pancreatitis. *Exp. Pathol.* 18: 474-476.
7. Burek, J. D. 1978. Age-associated pathology. pp. 53-58. In: *Pathology of Aging Rats*, CRC Press Inc, Florida.
8. Cantenys, D., Portha, B., Dutrillaux, M. C., Hollande, E., Roze, C., and Picon, L. 1981. Histogenesis of the endocrine pancreas in newborn rats after destruction by streptozotocin. An immunocytochemical study. *Virchows Arch. [Cell Pathol.]* 35: 109-122.
9. Coleman, G. L., Jonas, A. M., Hoffman, H., Barthold, S., Osbaldiston, G., and Foster, S. 1977. Pathological changes during aging in barrier reared Fischer 344 male rats. *J. Gerontol.* 32: 258-278.
10. El-Wahed, H. A., and Norr, A. 1956. Regeneration in Pancreas der Ratte nach Alloxan-Schädigung. *Z. Zellforsch. Microsk. Anat., Abt. Histochem.* 44: 185-188.
11. Fitzgerald, P. J. 1960. The problem of the precursor cell of regenerating pancreatic acinar epithelium. *Lab. Invest.* 9: 67-85.
12. Goodman, D. G., Ward, J. M., Squire, R. A., Chu, K. C., and Linhart, M. S. 1979. Neoplastic and non-neoplastic lesions in aging F344 rats. *Toxicol. Appl. Pharmacol.* 48: 237-248.
13. Konishi, J., Ikeda, T., Kawabata, A., Katada, H., Higashiguchi, R., Maruyama, H., and Yoshimura, H. 1980. Carcinogenic effect of N-bis-(2-hydroxypropyl) nitrosamine in newborn rats. *Exp. Pathol.* 18: 136-140.
14. Pearse, A.G.E. 1973. Cell migration and the alimentary system: Endocrine contributions of the neural crest to the gut and its derivatives. *Digestion* 8: 372-385.
15. Pearse, A.G.E., and Polak, J. M. 1971. Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. *Gut* 12: 783-788.
16. Pour, P., Mohr, U., Cardesa, A., Althoff, J., and Kruger, F. W. 1975. Pancreatic neoplasms in an animal model: morphological, biological and comparative studies. *Cancer* 36: 379-389.
17. Reddy, J. K., Rao, M. S., Sroboda, D. J., and

- Prasad, J. D. 1975. Pancreatic necrosis and regeneration induced by 4-hydroxyaminoquinoline-1-oxide in the guinea pig. *Lab. Invest.* 32: 98-104.
18. Roebuck, B. D., and Longnecker, D. S. 1977. Species and rat strain variation in pancreatic nod-
ule induction by Azaserine. *J. Natl. Cancer Inst.* 59: 1273-1277.
19. Walker, N. I., and Pound, A. W. 1983. An autoradiographic study of the cell proliferation during involution of the rat pancreas. *J. Pathol.* 139: 407-418.

要 約

老齡ラットの非腫瘍性結節性膵病変の形態発生・光顕的・免疫組織化学的ならびにオートラジオグラフィーによる検索：奈良間 功・土谷 稔・鈴木義孝¹⁾ ((株)生物科学技術研究所, ¹⁾岐阜大学農学部家畜解剖学教室) —43例の老齡 F344 ラットの非腫瘍性結節性膵病変について病理組織学的に検索し, 老齡SDラットの同病変をオートラジオグラフィーを用いて検索した。病巣は基本的には膵外分泌組織の増殖した腺管様構造による置換であった。腺管を裏うちする上皮細胞の核はしばしば腫大し, 有糸分裂像も散見された。これらの上皮細胞の核には活発な核酸合成がオートラジオグラフィーにより確認された。これらの腺管上皮は腺管単位で腺房あるいは島細胞集簇に移行し, 介在部導管上皮に類似した未熟な円柱上皮と腺房細胞のさまざまな過程の移行型細胞が認められた。腺管上皮から移行した島細胞は免疫組織化学的にインスリン, グルカゴンおよびソマトスタチン陽性を示し, これら3種の島細胞の比率, 形態, 等も正常島におけるものに類似していた。以上の結果から, 老齡ラットの非腫瘍性結節性膵病変が再生性病変であることが示唆された。また, 未熟腺管上皮が腺房細胞と島細胞の双方に分化し得る可能性も示唆された。