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Appearance of a Carcinoid-like Pattern in Rat Hepatic Tumors Induced by 3′-Methyl-4-dimethyl-aminoazobenzene

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This study investigated the histological distribution of argyrophilic cells in experimental hepatic neoplasms, the number of these cells, and the proportion of neoplasms with such cells. Seventy 6-week-old male Donryu rats were given a 0.06% 3′-methyl-4-dimethyl-aminoazobenzene (3′-MeDAB) diet for 10 weeks, followed by an ordinary diet for an additional 10 weeks. Of the 70 rats, 50 were used for this investigation; 29 had hepatic tumors, 18 had cholangiofibrosis, and the other three had oval cell proliferation only. Hepatic tissues were stained with Grimelius and Fontana-Masson stains as well as routine hematoxylin-eosin stain. Argyrophilic cells were found in the hepatic neoplasms of 8 rats without argentaffin cells, while cholangiofibrosis was associated with argentaffin cells in almost all cases. Of the 8 rats with argyrophilic cells, three had an abundant population of these cells. The argyrophilic cells were found in areas of the neoplasms with a glandular, trabecular, tubular, or poorly differentiated pattern. Electron microscopy revealed that the neoplastic cells with a positive argyrophil reaction contained small round electron-dense endocrine granules. In addition, in the areas of cholangiofibrosis, two different types of gut endocrine cells were present (G and EC cells). These results suggest that 3′-MeDAB might induce hepatic carcinoid under certain conditions, though we have yet to confirm the development of a pure hepatic carcinoid due to this substance.

Key words: Carcinoid-like pattern — 3′-MeDAB-induced rat hepatic tumor

There are few reports available on human hepatic carcinoids.[1,2] Concerning the histogenesis of hepatic carcinoids, it has been suggested that these tumors arise from the argentaffin cells of the intrahepatic bile ducts,[3] but this is no more than an unconfirmed speculation. The presence of gut endocrine cells has been demonstrated in the extrahepatic biliary system,[4,5] but not in the intrahepatic cholangioepithelium or in the parenchymal cells of the liver, except in some reports which indicated by electron microscopy and histochemical methods that gut endocrine cells were possibly present in the portal areas, sinusoidal walls, or subcapsular region.[6,7] We have been investigating whether these endocrine cells are normally present or only appear under certain conditions in the livers of rats given 3′-methyl-4-dimethyl-aminoazobenzene (3′-MeDAB). Histochemical and electron microscopic studies have shown that they appear in the areas of cholangiofibrosis and hepatic tumor tissue sporadically but quite commonly.[8]

In this study, 3′-MeDAB was administered to rats to produce hepatic tumors, and we studied the histological pattern at the site of gut endocrine cell proliferation, the frequency of such changes, the ultrastructure of granules in the endocrine cells, and the possibility of producing carcinoid experimentally in the liver, as Tahara *et al.*[9] did in the rat stomach.

MATERIALS AND METHODS

Seventy 6-week-old male Donryu rats were used. The rats were given 0.06% 3′-MeDAB in a solid diet (Oriental Yeast Industries) that was administered for 10 weeks, followed by a standard solid diet (MF) for an additional 10 weeks. At 20 weeks after the commencement of administration the rats were killed (some had already died of tumors before the 20th week). Liver tissue was fixed in 10% formalin and stained with hematoxylin-eosin (H-E), Grimelius, or Fontana-Masson stains for light microscopy. For electron microscopy, several pieces of tumor tissue were obtained from fresh specimens. These were immediately immersed in 2.5% glutaraldehyde solution for 2 h in phosphate-buffered 1% osmium tetroxide. After dehydration in a graded series of ethanol baths, the specimens were embedded in epoxy resin. Small pieces of tissue were also obtained from the paraffin blocks which contained a so-called carcinoid pattern. After deparaffinization, these were prepared for embedding as were the fresh specimens. The blocks were sectioned on a Porter-Blum ultramicrotome. Semithin sections were stained with toluidine blue and observed for orientation by light microscopy. Ultrathin sections were placed on copper grids and doubly stained with uranyl acetate and lead citrate. All sections were examined using a Hitachi electron microscope.

RESULTS

Of the 70 rats, 50 were available for this investigation and 29 developed hepatic tumors. In addition, 18 had cholangiofibrosis and the other 3 had oval cell proliferation only. The neoplastic tissues of these 29 rats were stained with Grimelius and Fontana-Masson stains as well as routine H-E stain. Argyrophilic cells were found in the tumors of 8 rats, but no argentaffin cells were found.

On the other hand, in the tissues showing cholangiofibrosis (Fig. 1a), both argyrophilic cells (Fig. 1b) and argentaffin cells were seen sporadically. Table I shows the histological findings in the 8 hepatic neoplasms having silver-reactive cells, the histological architecture at the site of argyrophilic cell proliferation, and the frequency of argyrophilic cells. All 8 tumors were of the mixed type,[10] and argyrophilic cells were found mainly in regions with a poorly differentiated (Fig. 2), glandular or tubular (Fig. 3), and trabecular pattern (Fig. 4). The mixed type showed a mixture of various histologic patterns, such as trabecular, poorly differentiated, papillary or glandular with a predominantly medullary architecture consisting of small basophilic neoplastic cells. These tissues often showed an apparently carcinoid-like histological pattern, i.e., trabecular, ribbon-like, cord-like, or solid form structures composed of relatively uniform neoplastic cells. The number of endocrine cells in tumor tissue was investigated, and when it was more than 50 cells/mm^2, this was defined as active argyrophilic cell proliferation (AACP). The tumors of 3 rats (K-14, 15 and 69 in Table I), showed the presence of AACP. No positive cells were found in the tumor regions, indicating that they were well or moderately differentiated, and no argentaffin cells were found in any of the tumors.

Table I. Hepatic Tumors with Argyrophilic Cells

No.	Histological types	Tumor structures associated with argyrophilic cells	Number of argyrophilic cells (cells/mm^2)	Argentaffin cells
K14	mixed type	glandular trabecular	50–60	negative
K15	mixed type	nodular glandular tubular	80–90	negative
K37	mixed type	glandular tubular	20–30	negative
K67	mixed type	poorly diff.[a)]	30–50	negative
K68	mixed type	poorly diff.[a)]	30–50	negative
K69	mixed type	poorly diff.[a)] glandular trabecular	80–160	negative
K73	mixed type	tubular	10–20	negative
K47	mixed type	tubular	20–30	negative

a) Poorly differentiated tubular adenocarcinoma.

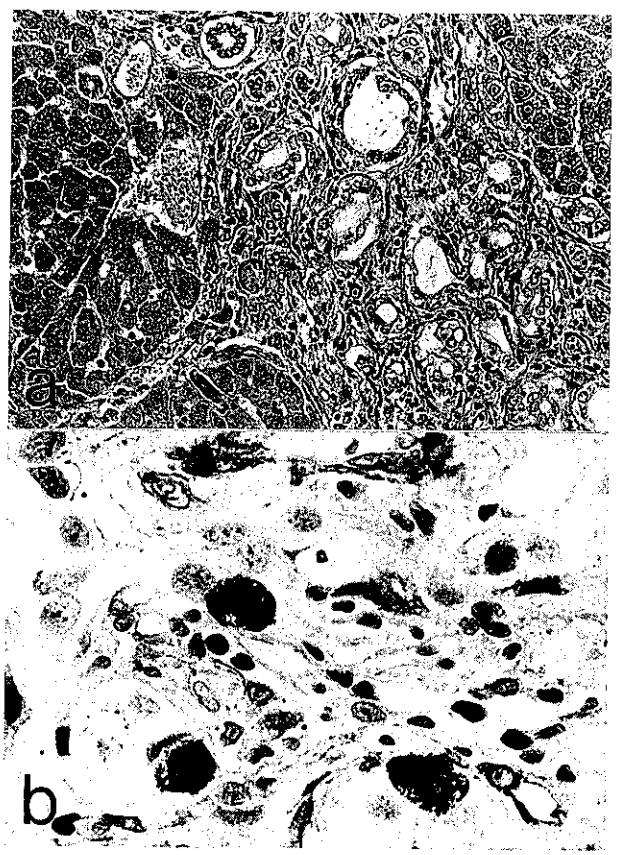

Fig. 1. a. Cholangiofibrosis: Convoluted bile ductules and surrounding areas of fibrous proliferation are seen (H-E, ×170). b. Argyrophilic cells are seen among the bile duct cells in the regions of cholangiofibrosis (Grimelius stain, ×680).

Electron microscopy demonstrated electron-dense, round granules of 150–200 nm in diameter in the cytoplasm of tumor cells in two tumors (Fig. 5). Endocrine cells in the areas of cholangiofibrosis also possessed two different kinds of endocrine granules, the G (Fig. 6) and EC types.

DISCUSSION

It is well known that some gut endocrine cells exist in ordinary carcinoma tissues.[11–17] The incidence ranges from 1.7% to 26% in gastric carcinoma[12–14, 17, 18] and is 2% in carcinoma of the large bowel.[11] The appearance of endocrine granules in some of the neoplastic cells in

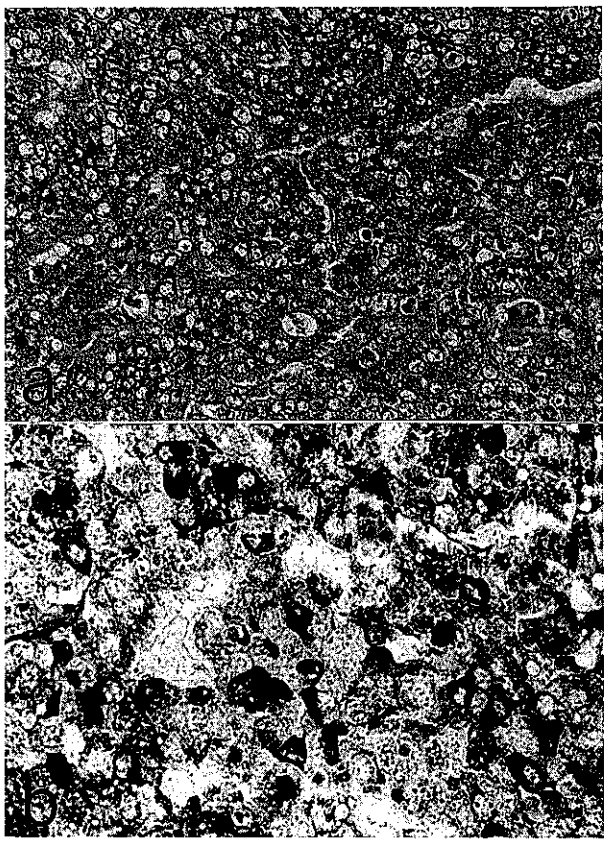

Fig. 2. a. Tumor tissue of K69. A poorly differentiated tubular adenocarcinoma with slight nuclear atypism showing partly cord-like structures (H-E, ×50). b. Grimelius staining of the same area as in Fig. 2a. Many argyrophilic cells are seen throughout the lesion (×520).

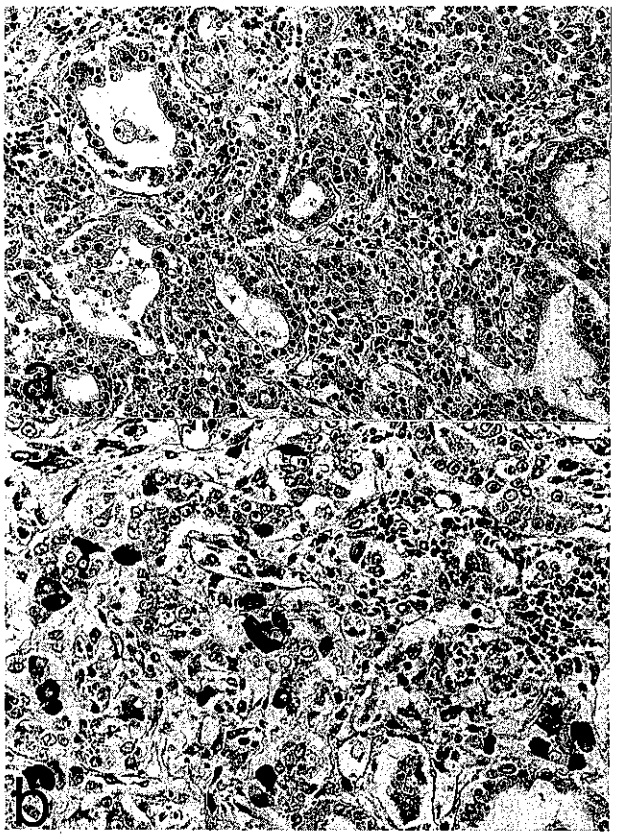

Fig. 3. a. Tumor tissue of K14. Glandular or tubular structures are composed of relatively uniform neoplastic cells with a round or oval nucleus (H-E, ×170). b. Grimelius staining of the same area as shown in Fig. 3a. Argyrophilic cells are mostly scattered but show some aggregates (×260).

cancer tissues has also been recognized by electron microscopy.[14, 16] Regarding the peptide hormones supposed to be produced by these endocrine cells, several kinds of hormones (gastrin, somatostatin, glucagon, etc.) have been detected by Tahara *et al.*[16] and Bonar and Sweeney[18] using immunohistochemical procedures.

Concerning the histogenesis of these tumors, Soga[19] has hypothesized that some primordial cells with the capacity for multidirectional differentiation might differentiate into both endocrine cells and non-endocrine cells during carcinogenesis. Tahara *et al.*[16] also hypothesized that these endocrine cells differentiate from totipotent immature cells of endodermal origin, as is the case with other epithelial cells. Some reports have been published on the appearance of endocrine cells in rat neoplasms produced by chemical carcinogens.[20–26] Kobori *et al.*[22, 23] confirmed by electron microscopy that endocrine cells with uniform round granules were present in the neoplastic tissue of tumors of the rat glandular stomach produced by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). Jonas *et al.*[21] reported that some endocrine cells were noted in 40% of gastric cancer tissue and 37.5% of carcinoma of the small bowel. Three gastric carcinomas showed a very high frequency of argyrophilic cells. Shimamoto *et al.*[25] reported that among 122 rat gut carcinomas induced by 1,2-dimethylhydrazine, 42 (34.3%) possessed argyrophilic cells. Furthermore, Tahara *et al.*[9] successfully produced gastric carcinoid in 20% (6/30) of inbred Wistar rats using MNNG, although no identification of gut hormones by immunohistochemical methods was performed. In these tumors, 2 types of endocrine cells (argentaffin and argyrophilic cells) were recognized by electron microscopy and/or silver impregnation staining.

Argyrophilic cells were not found in the normal hepatic tissue of the rat. But, in some pathologic conditions,

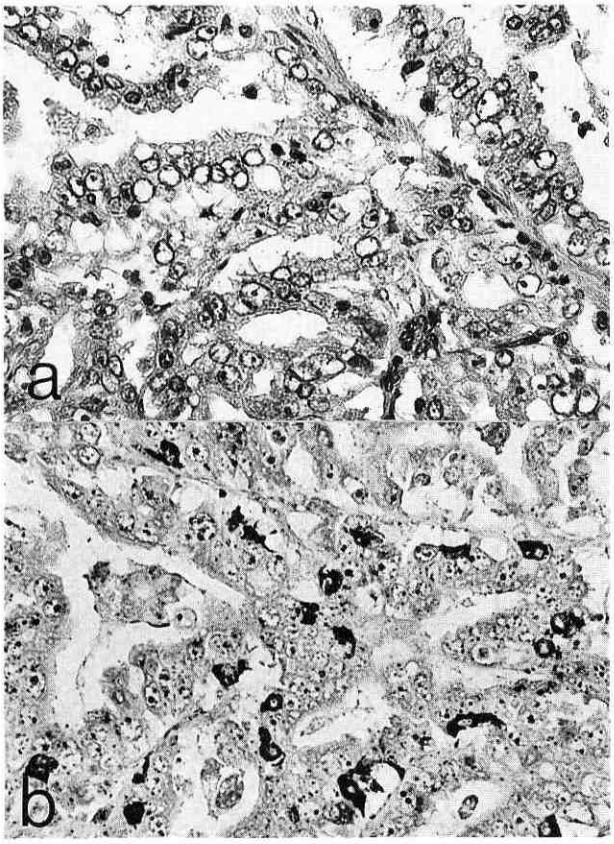

Fig. 4. a. Tumor tissue of K69. Neoplastic cells form a trabecular pattern that is similar to that seen in a carcinoid (H-E, ×340). b. Grimelius staining of the same area as in Fig. 4a. Many argyrophilic cells are scattered among the neoplastic cells (×340).

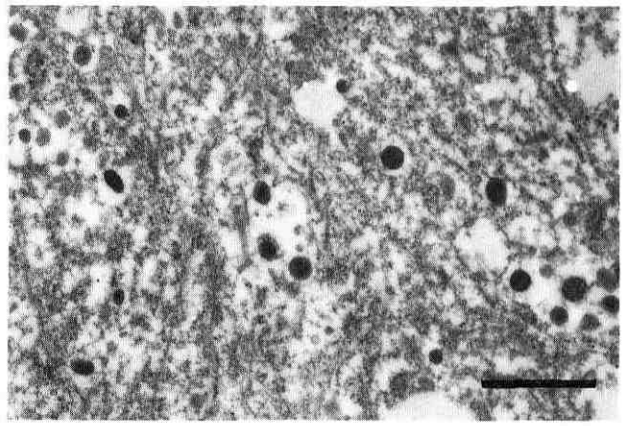

Fig. 5. Electron microscopic image of tumor tissue of K67 taken from a paraffin block. Electron-dense granules of 150–200 nm are seen (×15,000; scale: 1 μm).

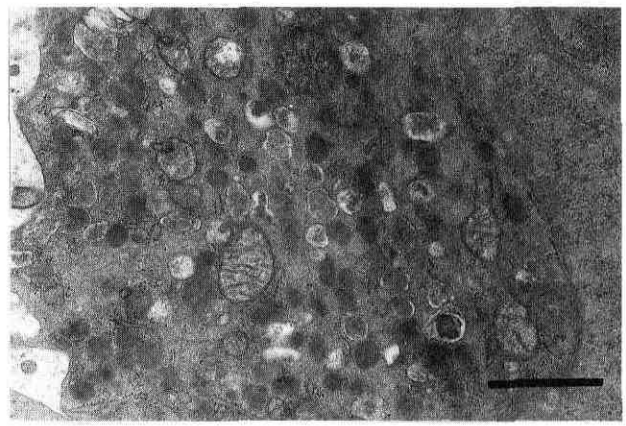

Fig. 6. A G cell seen in cholangiofibrosis (×15,000; scale: 1 μm).

cells of this type are present in the hepatic tissue.[27,28] In the early stage of azo-dye hepatocarcinogenesis, oval cell (ductular cell) proliferation occurs after severe injury of normal hepatocytes.[29-33] Based on electron microscopical, histochemical and autoradiographic studies, Inaoka[30] concluded that the oval cells are able to transform into hepatocytes during severe and prolonged injury and the oval cells play a leading role in the course of azo-dye hepatocarcinogenesis. This hypothesis has been supported by many investigators.[31-33] Terao *et al.*[27] reported a typical intestinal cell metaplasia including goblet cells, enterochromaffin cells and Paneth cells in the region of cholangiofibrosis produced by administration of 3′-MeDAB. Epithelial elements of cholangiofibrosis were speculated to be derived from oval cells.[33,34] Therefore, the oval cells (ductular cells) may be blast cells capable of multidirectional differentiation, as are the undifferentiated cells of crypt epithelium of the small intestine and the neck mucous cells of the gastric epithelium. In this context, we considered as did some other researchers[35] that the oval cells as blast cells transform in one direction into hepatocellular carcinoma through hyperplastic nodules and in another direction into adenocarcinoma (cholangiocarcinoma) or undifferentiated or poorly differentiated carcinoma. Therefore, in the course of the DAB-induced hepatocarcinogenesis, the appearance of gut endocrine cells is likely. We speculate that the properties of DAB-induced tumors with or without gut endocrine cells or intestinal-type cells are due to neoplastic conversion in the course of differentiation of the oval cells.

Onoe *et al.*[28] pointed out that endocrine cells were often detected by electron microscopy in DAB-induced

hepatic tumors. In addition, Yoshida *et al.*[36] have noted cells with electron-dense granules of about 200 nm diameter in tumor regions showing an undifferentiated pattern, a finding similar to ours in the present study. These were all studies at the electron microscopic level and there appears to be no report on histochemical investigations, except for ours. Our study also revealed electron microscopically detectable endocrine cells, and the presence of sporadic or aggregated argyrophilic cells in some tumors was confirmed by silver staining. In addition, the tumor model used in this study was a standard one, so such cells may have been present at a considerable frequency among the DAB-induced hepatic tumors produced by many researchers in the past. We found active argyrophilic cell proliferation in 10.3% of all tumors, i.e., these cells appeared to be quite common. Thus it seems possible that carcinoids may be experimentally produced in the liver as they have been in the stomach of Wistar rats.[9]

Such argyrophilic cells did not appear throughout the neoplasms, but only appeared densely in the regions showing a carcinoid-like pattern. These neoplasms could thus hardly be said to be typical carcinoids, although endocrine granules were demonstrated by electron microscopy.

Even if typical carcinoids were not demonstrated, the appearance of endocrine cells at a high density seems to be a very interesting phenomenon in relation to the histogenesis of carcinoma or carcinoid in the liver and in general.

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