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Significance of the appearance of α-fetoprotein in the early stage of azo-dye hepatocarcinogenesis*

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It is well known that α-fetoprotein (AFP) appears in the sera of rats with azo-dye hepatomas. This appearance is explained by the fact that hepatoma cells have function similar to that of fetal liver cells. However, little is known about the transient appearance of AFP which occurs in rat sera at the 4th to the 6th week of azo-dye feeding[10].

We have extensively studied the pathological changes of rat liver during the course of 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) carcinogenesis from histological, histochemical, ultrastructural, radioautographic and biochemical viewpoints[2~5,7,8], and obtained results to support the transient appearance of AFP in the early stage.

The first noticeable histological changes found in the liver of rats fed with a diet containing 0.06% of 3′-Me-DAB were degeneration and disappearance of hepatocytes in the periportal area with a decrease in glucose-6-phosphatase activity in the liver homogenate. At the 4th week of azo-dye feeding, a marked proliferation of cholangiolar cells, the so-called "oval cells", was seen in the periportal area, and at the 6th week, small hepatocytes with somewhat basophilic cytoplasm were seen occupying almost the entire space of the liver lobule. Studies on the fluctuation of cell populations in the liver lobule revealed a reverse proportion of oval cells and small hepatocytes, suggesting transformation of oval cells into small hepatocytes[3]. A follow up study of radioisotope labeled cells at this stage indicated transformation of oval cells into small hepatocytes during two successive cell divisions[2]. Therefore, the small hepatocytes occupying almost the entire space of the liver lobule at the 6th week of 3′-Me-DAB feeding are considered to be renewed hepatocytes which matured from proliferated oval cells. 3′-Me-DAB is metabolized in hepatocytes and turns into carcinogenic and cytotoxic metabolites. Hepatocytes actively metabolize azo-dye and hence are degenerated, whereas oval cells can not metabolize azo-dye and are not affected.

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A transient appearance of AFP occurred almost at the same time as the maturation of oval cells into small hepatocytes. Fluorescent antibody technique applied on frozen sections revealed that many transitional cells from oval cells to small hepatocytes and small hepatocytes were AFP positive at the 4th week of 3′-Me-DAB feeding (Fig. 1), but at the 6th week, only

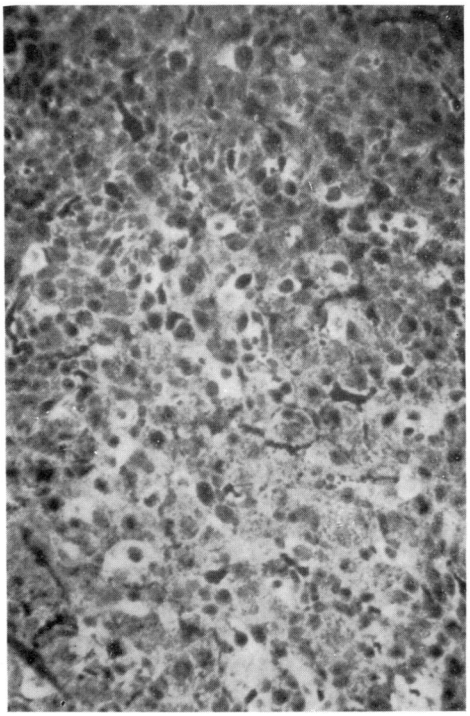

Fig. 1. AFP positive cells at the 4th week of 3′-Me-DAB feeding.

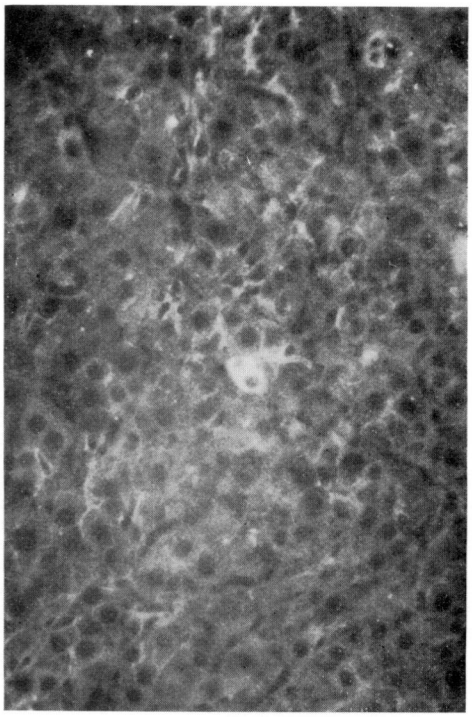

Fig. 2. AFP positive cells at the 6th week of 3′-Me-DAB feeding.

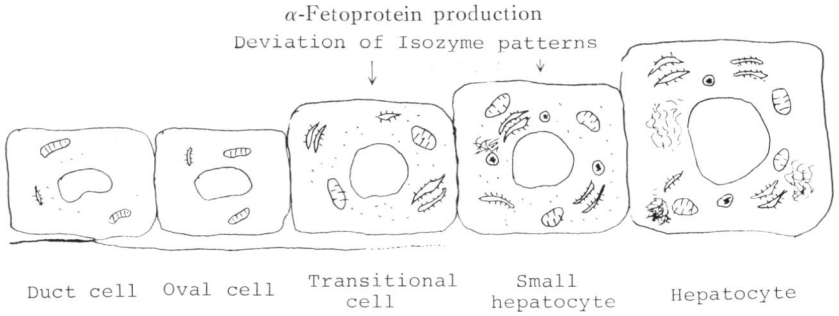

Fig. 3. Schematic representation of interrelation between cell maturation and expression of ontogenetically immature cell function during the early stage of 3′-Me-DAB hepatocarcinogenesis.

a few small hepatocytes were AFP positive (Fig. 2). Maturation of small hepatocytes seems to reflect the decrease in the number of AFP positive cells as well as the decrease in the amount of AFP in the serum.

On the other hand, Rutter and Schapira have pointed out the deviation in isozyme pattern of aldolase from liver type to muscle type in hepatomas. Endo *et al.*[1] found the occurrence of such deviation in the early stage of azo-dye carcinogenesis. Histochemical demonstration of aldolase isozyme in the liver of rat at the 6th week of 3′-Me-DAB feeding was attempted with using fructose diphosphate (FDP) and fructose monophosphate (FMP) as substrates. A small hepatocyte population showed only a slight liver type aldolase activity as compared to original hepatocyte population. On the other hand, small hepatocytes were as high as original hepatocytes in muscle type aldolase activity. Such deviation of isozyme patterns from adult type into fetal type was also seen biochemically in acid phosphatase and non-specific esterase in the early stage of 3′-Me-DAB feeding[4,5].

In conclusion, drastic changes of cell population in the liver occur in the early stage of 3′-Me-DAB carcinogenesis, at which stage the transient appearance of AFP and the deviation in isozyme patterns of various enzymes are observed. Such changes are explained as an expression of ontogenetically immature function of the cells in the process of transformation of oval cells into small hepatocytes. However, a question remains as to whether such deviations characterize the precancerous liver cells or not. Further studies on this point are necessary, but results of our recent experiments[6] seem to provide some clues to this problem. In the rat fed with 0.06% of 3′-Me-DAB for 4 weeks, the frequency of abnormal mitotic figures in the liver 31 hours after partial hepatectomy increased up to 19%, even after the cessation of azo-dye feeding for 24 weeks. The frequency of abnormal mitotic figures in normal rat liver after partial hepatectomy is 3% in younger individuals, and 8% in aged ones. Changes which occurred in the liver around the 4th week of 3′-Me-DAB feeding may play a very important role in azo-dye hepatocarcinogenesis.

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