Case Report

A Hepatocellular Adenoma in a Diet-induced Obese Mouse

Kouji Kawai¹, Tetsuya Sakairi¹, Masaharu Tanaka², Junko Shinozuka¹, Mika Ide¹, Hiroko Sato¹, Toshihisa Fujiwara¹, Fumiko Sano¹, and Eisuke Kume³

¹Safety Research Laboratory, Mitsubishi Tanabe Pharma Corporation, 1–1–1, Kazusakamatari, Kisarazu, Chiba 292-0818, Japan
²Advanced Medical Research Laboratory, Mitsubishi Tanabe Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 227-0033, Japan
³Safety Research Laboratory, Mitsubishi Tanabe Pharma Corporation, 2–2–50, Kawagishi, Toda, Saitama 335-8505, Japan

Abstract: A hepatic nodule was noted in a C57BL/6J mouse with diet-induced obesity at 53 weeks of age. Macroscopically, a protruding yellowish white nodule was observed on the visceral surface of the left lateral lobe. Light microscopy demonstrated clear demarcation from the compressed adjacent parenchyma, with loss of the distinct lobular pattern. The proliferating cells of the lesion varied in shape and showed cellular atypia and prominent nucleoli along with vacuoles of various sizes. Some of the cells contained various-sized eosinophilic inclusion bodies in their cytoplasm, and electron microscopy revealed the presence of lipid droplets in the rough endoplasmic reticulum. Eosinophilic inclusions were observed as electron dense granular material in the rough endoplasmic reticulum, with one or a few low density central cores. A diagnosis of hepatocellular adenoma was made based on these findings. (J Toxicol Pathol 2010; 23: 59–62)

Key words: Hepatocellular adenoma, diet-induced obese mouse, inclusion body

Introduction

Diet-induced obese (DIO) mice are widely used for studies of human obesity and insulin resistance and for evaluating the efficacy of various kinds of anti-obesity agents¹. Although the pathogenesis of obesity in DIO mice has been intensely studied, there are few reports on histopathological analysis of associated hepatocellular proliferative lesions. We describe here a hepatocellular adenoma in a DIO mouse in a vehicle control group of a pharmacological study in our research laboratory. Three-week-old male C57BL/6J mice were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) and fed a high-fat diet (59% fat by calories, 5.578 kcal/g, Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. At 51 weeks of age, the animal was assigned to the vehicle-treated group and given 0.5% hydroxypropyl methylcellulose/0.1% Tween 80 orally for 2 weeks. At necropsy (53 weeks of age), a hepatic nodule was noted in the mouse. For light microscopy, the nodule and surrounding liver tissue was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (4 μm) were stained with hematoxylin and eosin (HE) and Watanabe's silver stain and examined under a light microscope. For electron microscopy, small pieces of the nodule were fixed with 2.5% glutaraldehyde solution and 2.0% formalin, postfixed in 1% osmium tetroxide and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined under a JEM-1210 electron microscope (JEOL, Tokyo, Japan).

Macroscopically, a protruding nodule approximately 10 mm in diameter was observed on the visceral surface of the left lateral lobe (Fig. 1). The surface of the liver was pale brown, whereas the nodule was yellowish-white. No other findings were noted in the other lobes of the liver or in the other organs grossly.

On light microscopy, the lesion was found to be well demarcated from the surrounding tissue, and compression of the adjacent parenchyma was noted (Fig. 2). Compressed adjacent hepatic cords and loss of the lobular pattern within the lesion were prominent on Watanabe silver staining (Fig. 3). Some bile ducts were partly noted within the lesion. The lesion was characterized by proliferating hepatocytes that had weakly basophilic cytoplasm with vacuoles of various sizes. The proliferating cells did not form distinct hepatic cords and showed architectural and cellular atypia with prominent nucleoli (Fig. 4). Some appeared packed with
Hepatocellular Adenoma in a Diet-induced Obese Mouse

Fine vacuoles throughout the entire cytoplasm, showing a ground glass-like appearance (Fig. 4). Such vacuoles were much less prominent in the hepatocytes of the surrounding tissue. In addition, some proliferating cells contained various-sized eosinophilic inclusion bodies in their cytoplasm (Fig. 4).

On electron microscopy, various sized lipid droplets of medium electron density were observed within the rough endoplasmic reticulum. Areas with a ground glass-like appearance by light microscopy were characterized by numerous small lipid droplets also in the rough endoplasmic reticulum (Fig. 5). Eosinophilic inclusions were observed as electron dense granular material in the rough endoplasmic reticulum, with one or more medium density central cores (Fig. 6).

A diagnosis of hepatocellular adenoma was made based on the clear boundary, the compression of the surrounding tissue, loss of the lobular architecture and the proliferation and cellular atypia. The eosinophilic cytoplasmic inclusions were also thought to support the diagnosis because such inclusions are characteristically found in murine hepatic neoplasms\(^2\)–\(^5\). Also, the ultrastructure of the inclusions was
the same as in previous reports2–5. The fact that they were found in the rough endoplasmic reticulum was presumably the result of abnormal transportation of proteins and lipids6,7.

Interestingly, areas were noted in the lesion where the degree of atypia was relatively low and bile ducts were present. In contrast, they were absent in areas of high atypia compressing the adjacent parenchyma. These findings may indicate that the lesion consisted of areas of atypical proliferating cells, and that intact bile ducts remained between the atypical areas.

The effect of the high-fat diet on the present case was mainly observed as numerous lipid droplets in the proliferating cells. Interestingly, these lipid droplets were noted in the endoplasmic reticulum, which is known to be characteristic of fatty liver7. Also, in a different obese control mouse, another hepatocellular adenoma was noted with similar histologic features (data not shown). While it is known that a high-fat diet increases tumor incidence and promotes growth in various organs of experimental tumor models of mice8–14, the influence on the lesions remains unclear. More such cases and further studies are needed to clarify the promotion effects of high-fat diet on tumorigenesis in DIO mice.

**Acknowledgments:** The authors gratefully acknowledge the assistance of Dr. T. Harada and Dr. R. Maronpot in critically reviewing the slides. We appreciate the generous provision of the DIO mouse by Dr. K. Arakawa and also thank S. Kurabe and E. Ohtsuka for technical assistance in tissue preparation.

**References**