Reviewer's report

Title: High-saturate-fat diet delays development of diethylnitrosamine-induced hepatocellular carcinoma

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Reviewer: Hirofumi Uto

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This study indicated that high-saturate-fat (HFD) diet inhibits diethylnitrosamine (DEN)-induced hepatocarcinogenesis in Sprague-Dawley rats. In addition, the inhibition of hepatocarcinogenesis in this study may be due to anti-proliferative and pro-apoptotic effect of HFD. This study is interesting and may provide novel mechanism regarding the inhibition of hepatocarcinogenesis by HFD. However, there were many already-reported facts regarding the effect of high fat diet on hepatocarcinogenesis, and the analysis of mechanism in this study is not fully investigated.

Specific comments.

1. The effect of HFD in this study may depend on total calorie intake rather than fat intake. In fact, HFD restored malnutrition in the DEN-treated rats as stated by authors. Authors should justify the total calorie intake in the HFD+DEN group and NCD+DEN group.

2. Rahman et al concluded that the effect of dietary fat during the initiation phase of AOM-induced hepatocarcinogenesis depends on the type of fat and its fatty acid composition (Ref 18). Sugie et al also showed that the density and the unit area of AOM-induced enzyme altered foci in the liver were significantly lower in the high fish oil group than in the 5% corn oil group and the low fish oil group (Nutr Cancer. 1995;24:187-95.). In addition, Rahman et al reported that fish oil rich in polyunsaturated omega-3 fatty acids could inhibit DEN-induced hepatocarcinogenesis in rats (Jpn J Cancer Res. 1999;90:31-9.). If the effect of HFD in this study depends on fat rather than total calorie intake, authors should clarify whether high-saturate-fat or high-unsaturated-fat is better to inhibit DEN-induced hepatocarcinogenesis.

3. Authors showed the anti-proliferative and pro-apoptotic effect of HFD in rat liver. Are these effects observed in both tumor cells and non-tumor cells (normal hepatocytes)?

4. There was an increase in the hepatic level of caspase-3 in the HFD+DEN group compared to the NCD+DEN group at weeks 10 and 12, and authors concluded that anti-apoptotic effect may be associated with the attenuation of hepatocarcinogenesis. However, the hepatic level of caspase-3 in the HFD+DEN group was significantly lower than that of the NCD+DEN group at weeks 14.

5. Authors stated that there was a more significant reduction in the PCNA expression in HFD+DEN group at weeks 12 and 14 compared to rats in the
NCD+DEN group (page 7, line 23-). However, the PCNA expression (hepatic content of PCNA) in HFD+DEN group at weeks 14 was relatively higher than those in the NCD+DEN group (Fig 2 C).

6. Authors stated that HFD appeared to attenuate the occurrence of HCC and malignant differentiation in rat HCC model induced by DEN (Page 9, 12-). Three steps of progression of hepatocarcinogenesis have been proposed: initiation, promotion, and progression. Authors should discuss this point more in detail.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.