Establishment of health monitoring with oxidative stress markers
-Oxidative DNA damage in mouse liver with chronic inflammation-

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Introduction
Oxidative damages including DNA, lipid and protein damages occur in physiological or/and pathological process. Of those, the role of oxidative DNA damage in carcinogenesis and ageing has been widely investigated. The generation of reactive oxygen species has been documented in various inflammatory status, but it is poorly understood whether the inflammations lead to increased oxidative DNA damage. Recently, it has been reported that mice with NF-kB-inducing kinase (NIK) mutation develop multiple organ chronic inflammation. In this study, we used NIK mutant mice as an inflammation model and studied 8-hydroxydeoxyguanaine (8OHdG), typical of oxidative DNA damage in inflamed liver.

Materials and Methods
Mice: Female NF-kB-inducing kinase (NIK) mutant (aly/aly) and its wild type (C57BL/6) mice were used. Young and aged mice used were 10-11 wks and 56-60 wks, respectively. Tissue collection: For DNA isolation use, liver was immediately frozen and stored at -80°C. For histological use, liver was embedded in OCT and sectioned. Sections were stained by H&E. Detection of 8OHdG: DNA was extracted from liver tissue and digested into nucleosides under anaerobic condition. 8OHdG and dG were detected by HPLC with an electrochemical detector and UV monitor. Oxidative DNA damage are calculated from 8OHdG and dG contents and expressed as 8OHdG/10²dG.

Results and Discussion
In this study, we used 8OHdG as an indicator and investigated oxidative DNA damage in the liver of NIK mutant and wild type mice. Figure 1 shows a representative chromatograph data on 8OHdG detected by HPLC. We did not observe significant difference in 8OHdG between young mutant and wild type mice (Figure 1 & 2). In contrast, 8OHdG levels in aged mutant and wild type mice were higher than those in young mice, but the significant difference was only found between aged and young mutant mice. Interestingly, we found that 8OHdG levels in aged mutant mice were significantly higher than that in age-matched wild type mice. These findings suggest that pathological conditions may have more contribution to elevated 8OHdG than ageing. Since chronic inflammation develops in multiple organs in NIK mutant mice at certain age, we compared liver histology between age-matched mutant and wild type mice (Figure 3). In surport of our 8OHdG data, we observed the remarkable inflammatory cell infiltrates in the portal tract and perivascular areas of liver in aged NIK mutant mice whereas no obvious inflammation was found in the liver of aged wild type, young mutant and wild type mice.

In conclusion, our study implies that chronic inflammation may be high risk for carcinogenesis through enhancing oxidative DNA damage.