A Review of Nomenclature and Diagnostic Criteria for Proliferative Lesions in the Liver of Rats by a Working Group of the Japanese Society of Toxicologic Pathology

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Abstract: The final version of the international harmonized nomenclature for proliferative lesions in rats was issued on June 21, 2000. The recommended nomenclature for proliferative lesions in the liver includes focus of cellular alteration, regenerative hepatocellular hyperplasia, cholangiofibrosis, cholangiofibroma, oval cell hyperplasia, hepatocellular adenoma, hepatocellular carcinoma, bile duct hyperplasia, cholangioma, cholangiocarcinoma, hepatocellularadenoma, and hepatocellular carcinoma. Foci of cellular alteration are further classified into the following phenotypes: amphophilic, diffusely basophilic, tigroid basophilic, clear cell, eosinophilic, and mixed (basophilic/eosinophilic). Hepatocellular carcinomas are divided into 3 types based on their growth patterns: acinar, solid, and trabecular. In consideration of this international harmonized nomenclature, the current classification, terminology, and diagnostic criteria for proliferative lesions in the liver of rats recommended by the Japanese Society of Toxicologic Pathology (JSTP) were reviewed by a Working Group of the JSTP. The hepatic proliferative lesions reviewed by the present Working Group included lesions of hepatocellular, cholangiocellular, mixed hepatocellular/cholangiocellular, sinusoidal, and hemangioendothelial origins. Any comments and questions on these lesions were discussed among pathologists in the Working Group and the results of discussions were presented at the 1st seminar on the continuing education program of the JSTP in November 2000. (J Toxicol Pathol 2003; 16: 1–17)

Key words: harmonization, nomenclature, proliferative lesions, liver, rat

Introduction

Classification, terminology, and diagnostic criteria for proliferative lesions in the liver of rats were first proposed by Squire and Levitt¹ at a workshop 1975 and their proposal was discussed worldwide among pathologists especially in the United States, Europe, and Japan. After that, several versions of revised or modified nomenclature and diagnostic criteria have been published²–⁶ as shown in Table 1. However, there are still some controversies over these issues among pathologists in different countries. Since many chemicals including pharmaceuticals and pesticides are now used worldwide, toxicological data are evaluated by many regulatory agencies in the world. In addition, histopathology still takes an important part for risk assessment. Therefore, an international standardized nomenclature and diagnostic criteria for the lesions observed in toxicology studies are essential to improve the reliability of the pathology data for interpretation.

Harmonization of nomenclature and diagnostic criteria for lesions observed in laboratory animals is one of the important goals for pathologists working in the field of toxicologic pathology. On this point of view, initiatives had been started in the late 1980s in the United States by the Society of Toxicologic Pathologists (STP) and in Europe by the Registry of Industrial Toxicology Animal-data (RITA...
Table 1. History of Nomenclature and Diagnostic Criteria for Proliferative Lesions in the Rat Liver

<table>
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<th>Year</th>
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Table 2. International Harmonized Nomenclature for Proliferative Lesions in the Rat Liver

1. Hepatocellular lesions
   1) Focus of cellular alteration
      (1) Basophilic focus (tigroid basophilic and diffusely basophilic)
      (2) Eosinophilic focus
      (3) Clear cell focus
      (4) Amorphophilic cell focus
      (5) Mixed basophilic/eosinophilic focus
   2) Regenerative hepatocellular hyperplasia
   3) Hepatocellular adenoma
   4) Hepatocellular carcinoma (acinar, solid, and trabecular)

2. Cholangiobiliary lesions
   1) Bile duct hyperplasia
   2) Oval cell hyperplasia
   3) Cholangiobiliary hyperplasia
   4) Cholangiofibroma
   5) Cholangioma
   6) Cholangiocarcinoma

3. Mixed hepatobiliary lesions
   1) Hepatobiliary adenoma
   2) Hepatobiliary carcinoma

database group), which both lead to internationally recognized publications (“SSNDC: Guides for Toxicologic Pathology”, “WHO/IARC: International Classification of Rodent Tumours”). In 1994 the “Joint STPs/ILSI Committee on International Harmonization of Nomenclature and Diagnostic Criteria in Toxicologic Pathology” was established with the objective to coordinate the international activities for the standardization of diagnostic criteria of proliferative lesions in mice and to reconcile the already published nomenclature of proliferative lesions in rats. Consequently, the “Rat Nomenclature Reconciliation Subcommittee” was formed during the 1998 Annual STP meeting in Vancouver with the goal to eliminate discrepancies existing between the published nomenclature systems and to reach a common consensus. As a result of this work, the final version of international harmonization of rat nomenclature was issued in Spring 2000. The harmonized nomenclature for rat hepatoproliferative lesions is shown in Table 2. Currently the diagnostic criteria published by the STP and WHO/IARC are being checked for consistency with the goal to identify major discrepancies.

In these circumstances, the Japanese Society of Toxicologic Pathology (JSTP) established the “Continuing Education Committee” in Spring 2000 and subsequently a Working Group for rat liver proliferative lesions. The Working Group reviewed the current classification, terminology, and diagnostic criteria for proliferative lesions in the liver of rats recommended by the JSTP in comparison with the final version of international harmonization of rat nomenclature. Any comments and questions were discussed among pathologists in the Working Group and the results of discussions were presented at the 1st seminar on the continuing education program of the JSTP which was held in the University of Tokyo on November 29, 2000. This manuscript describes a review of the current nomenclature and diagnostic criteria for proliferative lesions in the rat liver recommended by the JSTP in consideration of the international harmonization of rat nomenclature and also includes the results of discussions among pathologists in the Working Group and participants at the seminar.

Hepatocellular Lesions

Focus of cellular alteration

Synonyms: Phenotypically altered hepatocellular foci; Hyperplastic foci; Enzyme-altered foci; Prenecoplastic foci

Origin: Hepatocyte

Histological features: Foci of cellular alteration can be identified even in routine paraffin sections stained with hematoxylin and eosin (H&E). Hepatic foci vary in size from small focus comprising several altered hepatocytes to large one occupying an area equivalent several adjacent lobules. Large foci may have slight compression of adjacent parenchyma around a portion of its circumference, but merge imperceptibly with the surrounding hepatic cords. The foci identified in H&E stained sections are generally classified as basophilic, eosinophilic (acidophilic), amphophilic, clear cell, vacuolated, or mixed cell foci based on their cytological features and tinctorial properties, although there are still some discrepancies among pathologists6,8–12.
Basophilic foci are characterized by increased cytoplasmic basophilia and have 2 variations of tigroid basophilic type and diffusely basophilic type especially in rats. Tigroid basophilic foci generally consist of small hepatocytes with increased cytoplasmic basophilia in clumps or dense linear arrangements which may represent cisternae of rough endoplasmic reticulum (Fig. 1). The hepatic cords may be slightly tortuous and some hepatocytes have a pyknotic nucleus. In contrast, the cells in diffusely basophilic foci are of normal size or larger than unaffected hepatocytes and the cytoplasm is homogeneously basophilic (Fig. 2). Nuclei also are usually enlarged and vesiculate with prominent nucleoli, and sometimes there is a single prominent central nucleous with a clear space surrounding it (so-called “bull’s eye” appearance). The hepatic cord may be slightly disrupted with cells in a disassociated or jumbled pattern. Ultrastructurally, the cells have been shown to contain increased free ribosomes.

Eosinophilic foci usually consist of hepatocytes with an increased amount of eosinophilic cytoplasm. The cytoplasm is often pale (Fig. 3) and has a “ground-glass” (Fig. 4) or fibrillar appearance. These cells may contain increased smooth endoplasmic reticulum and/or excess glycogen. The cells in eosinophilic foci tend to be larger than the surrounding hepatocytes and may cause compression of the adjacent parenchyma.

Clear cell foci are usually irregularly shaped, occasionally poorly circumscribed, and have different extents of cytoplasmic clear spaces (Fig. 5). The cells are of normal size or slightly enlarged and often have...
hyperchromatic nuclei. The clear cytoplasmic spaces have been shown to contain excess glycogen which is dissolved out by aqueous fixative. Since eosinophilic foci also contain clear cells to some extent, both eosinophilic and clear cell foci have been referred to as glycogen storage foci.

Amphophilic foci consist of enlarged hepatocytes with diffusely eosinophilic cytoplasm which also contains a definite basophilic tint resulting in an amphophilic appearance (pale purple in H&E stain) (Fig. 6). The cytoplasm is dense and homogeneous and has a decreased glycogen content. The cytoplasmic eosinophilia has been suggested to be mainly due to a multiplication of mitochondria and peroxisomes.

Vacuolated foci consist of hepatocytes with various-sized round or oval clear cytoplasmic vacuoles which represent fat droplets (Fig. 7). The morphological features of this lesion are similar to those of focal lipidosis and sometimes it is difficult to distinguish one from the other. Therefore, vacuolated foci tend to be excluded from the classification of hepatic foci and diagnosed as focal fatty change (lipidosis) by some pathologists.

Mixed cell foci are comprised of two or more of any of the cell types described above in varying proportions (Fig. 8). The international harmonized nomenclature recommend that only a combination of two phenotypes of eosinophilic and basophilic cells (half-and-half in proportion) can be referred to as mixed cell foci, but the others be classified by the dominant cell type.

**Differential diagnosis:** Hepatocellular adenoma and regenerative hepatocellular hyperplasia are frequent...
differential diagnoses for hepatic foci. Hepatocellular adenomas are well-circumscribed lesions usually occupying an area greater than one liver lobule and causing distinct compression of adjacent parenchyma. Compressive atrophy of the borderline hepatocytes is an evidence of the physical pressure of growing tumor cells. In addition, adenomas usually have an absence of normal lobular architecture and portal triads. They show an altered growth pattern and the hepatic plates at the margins impinge at sharp angles to the surrounding normal liver plates. These morphological characteristics are helpful for the distinction between adenomas and foci of cellular alteration. On the other hand, regenerative hepatocellular hyperplasia can be distinguished from hepatic foci or adenomas by the following characteristics: presence of discrete nodules (often multiple) of hepatocytes and evidence of prior or ongoing hepatic parenchymal damage including necrosis, fibrosis, and inflammatory changes in the surrounding tissue. In addition, the regenerative hepatocytes are generally uniform and slightly basophilic but have a relatively normal appearance and lack cytologic evidence of neoplasia.

Comments: Foci of cellular alteration occur spontaneously in various animal species including rats and mice and increase in frequency with age, although the incidence and phenotype of foci differ considerably among species, strain, and sex. The incidence of spontaneous hepatic foci is highest in rats and reaches nearly 100% in F344 rats by the age of 2 years. Since the incidence of spontaneous hepatocellular tumors is still low even in rats maintained for their entire lifetime, it is considered that most spontaneous foci do not progress to neoplasia in rats. Classification of hepatic foci by phenotype indicates that the spontaneous incidence of tigroid basophilic foci is high in rats, whereas diffusely basophilic and amphophilic foci are very rare. However, these diffusely basophilic and amphophilic foci have been shown to increase significantly in rats treated with various genotoxic hepatocarcinogens. Thus, such specific foci may have a potential to progress to neoplasia.

In addition to conventional H&E-staining, foci of cellular alteration also can be identified by various histochemical and immunohistochemical markers such as γ-glutamyl transpeptidase (GGT), glutathione S-transferase placental form (GST-P), glucose-6-phosphatase (G6P), and iron exclusion (Table 3). The enzyme markers of GGT and GST-P have been extensively used to identify hepatic foci in short-term or medium-term initiation/promotion rat liver carcinogenesis models and GST-P is recommended as one of the most sensitive and reliable markers. However, GST-P fails to identify foci induced by peroxisome proliferators. In that case, other markers should be applied. In general, GST-P is available for identification of eosinophilic or clear cell foci but not for other phenotypes.

The biological or toxicological significance of hepatic foci is still controversial. In rat initiation/promotion liver carcinogenesis models, hepatic foci occur prior to development of hepatocellular neoplasms. Consequently, they are generally regarded as “preneoplastic” lesions. These chemically-induced foci differ in phenotype depending on the class of chemicals, but they are morphologically similar to naturally occurring foci. Spontaneous foci increase in frequency and variation with age, but the occurrence of hepatocellular tumors is low. Thus, it is considered that most spontaneous foci do not progress to neoplasia as described above. However, it has been shown that the occurrence of spontaneous foci is accelerated by nongenotoxic mitogenic chemicals such as phenobarbital (enzyme inducer) and clofibrate (peroxisome proliferator) and hepatocellular tumors are developed after long-term exposure. Genotoxic chemicals such as aflatoxin B1, diethylnitrosamine, and azo dye are also shown to induce specific types of foci which progress to neoplasia.

Spontaneous or chemically-induced initiated cells may have a potential to progress to neoplasia, but they do not
always develop into tumors. Certain conditions including enhanced cell proliferation, inhibited apoptosis, and disturbed intercellular communication could be required for promotion and progression of initiated cells. In addition, genetic changes such as activation of protooncogenes and inactivation of tumor suppressor genes may be essential for malignant transformation. Therefore, initiated cells do not progress to neoplasia when placed on insufficient conditions. In fact, we often encounter the results from experiments of nongenotoxic chemicals in that hepatic foci increased with dose levels, but tumor induction was observed only in the high dose group. In this case, it is considered that lower dose levels did not provide sufficient growth advantage to initiated cells. This suggests that nongenotoxic chemicals may have a threshold to induce tumors. It was reported that hepatic foci induced by certain nongenotoxic chemicals decreased in number after cessation of treatment\(^\text{20}\). This result indicates that hepatic foci induced by nongenotoxic chemicals may have no autonomous growth activity as well as spontaneous foci. These foci may progress to neoplasia only when placed on a sufficient condition at carcinogenic dose level. In contrast, genotoxic hepatocarcinogens have both initiation and promotion effects and may induce specific types of foci which have a high potential to develop into neoplasia\(^\text{10,18}\). Some of these foci might have an autonomous growth activity.

**Histological features:** Regenerative hepatocellular hyperplasia is characterized by presence of focal or multifocal discrete nodules of hepatocytes and evidence of prior or ongoing hepatic parenchymal damage such as necrosis (Fig. 10). The regenerative hepatocytes are generally uniform and slightly basophilic but have a relatively normal appearance and lack cytologic evidence of neoplasia. In prolonged cases, fibrosis, bile duct hyperplasia, oval cell proliferation, evidence of chronic inflammation, and distortion of the lobular architecture are seen in the surrounding tissue\(^6\).

**Differential diagnosis:** Focus of cellular alteration and hepatocellular neoplasia are major differential diagnoses for regenerative hyperplasia. Evidence of prior or ongoing hepatic parenchymal damage and lack of cytological evidence (cellular atypia) of neoplasia are major distinctions between regenerative hyperplasia and hepatic foci or neoplasia.

**Comments:** Regenerative hepatocellular hyperplasia may occur in the liver involved in prolonged parenchymal damage caused by repeated chemical exposure, infection, and nutritional disorders. For instance, the regenerative lesion is often noted in choline-deficient rats, LEC rats, and rats involved in advanced leukemia. In prolonged cases, repeated parenchymal damage may result in distortion of the lobular architecture leading to cirrhosis. Occasionally, hepatocellular foci or neoplasia might occur within the lesion.

**Hepatocellular adenoma**

**Synonyms:** Neoplastic nodule; Hyperplastic nodule; Hepatic adenoma; Benign liver cell tumor; Benign hepatoma

**Origin:** Hepatocyte

**Histological features:** Hepatocellular adenomas are well-demarcated nodular lesions usually occupying an area greater than one lobule (sometimes several lobules) and causing distinct compression of adjacent parenchyma (Fig. 11). They are composed of neoplastic hepatocytes that are variable in size and tinctorial characteristics. The tinctorial appearance of the cytoplasm may be eosinophilic, basophilic, clear, vacuolated, or an admixture of them in various proportions\(^1\). Vacuolated cytoplasm may be due to fatty change. Adenomas usually have an absence of normal lobular architecture and show an altered growth pattern. They may appear solid but more often trabecular and the cells occur in irregular plates one to three cell layers thick. Pseudoglandular pattern is rarely seen. Cellular atypia may be present and mitotic figures are observed with varying frequency. The sinusoids are usually compressed but occasionally dilated. The hepatic plates at the margins impinge at sharp angles to the surrounding normal liver plates. Central veins and portal triads are not easily apparent although some may be trapped in the expanding mass near the periphery. Occasionally, encapsulated adenomas which are surrounded by fibrous connective tissues may occur in some cases.

**Differential diagnosis:** Foci of cellular alteration, regenerative hepatocellular hyperplasia, and hepatocellular carcinomas are major differential diagnoses for hepatocellular adenomas. Foci merge imperceptibly with the surrounding hepatic cord, while adenomas are sharply

- **Fig. 10.** Regenerative hepatocellular hyperplasia in a control F344 rat. Note nodular lesion consisting of uniform and slightly basophilic hepatocytes. In the vicinity of the lesion, necrosis and fibrosis can be seen (at left lower part of the photograph). H&E stain.
circumscribed. This is a good distinction between foci and adenomas. Cytomorphological features, growth pattern, and compression of adjacent parenchyma are also helpful for distinction. As described in the above section, regenerative hyperplasia can be distinguished from adenomas by the evidence of prior or ongoing parenchymal damage. Thickness of hepatic cords (one to three cell layer in adenoma vs three or more cell layer in carcinoma) and degree of cellular atypia are a major distinction between adenomas and carcinomas.

**Comments:** Adenomas are grossly round or oval nodules and usually solitary but may be multiple when induced by chemicals. They may have enzyme alterations as well as foci of cellular alteration, but the variation is greater than that in foci and considerably differs among animal species. Occasionally, adenomas may have a focus of malignant cells within the lesions (carcinoma in adenoma), reflecting a malignant transformation of tumor cells. This case would be better to be diagnosed as carcinoma. As described above, encapsulated adenomas are rarely seen but its pathogenesis is unclear. If an evidence of prolonged hepatic parenchymal damage is present in the surrounding tissue, it is conceivable that the adenoma might arise within the lesion of regenerative hyperplasia. Otherwise, it still remains obscure. The incidence of spontaneous adenomas is usually 2–5% in rats and higher in males, although it differs among strains. Chemically-induced adenomas may occur in many animal species including rats treated with hepatocarcinogens such as aflatoxin B1 and diethylnitrosamine. The term of “neoplastic nodule” proposed by Squire & Levitt in 1975 had been used for a long time, but at the present time it tends to be referred to as adenoma in accordance with that in other epithelial tissues.

**Hepatocellular carcinoma**

**Synonyms:** Hepatic/hepatocellular adenocarcinoma; Hepatic/liver cell carcinoma; Malignant hepatoma; Hepatoma

**Origin:** Hepatocyte

**Histological features:** Hepatocellular carcinomas are not always well demarcated from the surrounding tissue, but are characterized by an abnormal growth pattern or cytological atypia consistent with malignancy. They may show variable growth patterns such as trabecular, acinar/glandular, and/or solid within the lesions. These lesions are further divided into the following three subtypes: well differentiated, moderately differentiated, and poorly differentiated tumors based on the degree of differentiation in consideration of structural and cellular atypia. Undifferentiated type of carcinomas may have a higher production level of α-fetoprotein. Hepatocellular carcinomas sometimes may show local invasion and metastasis to other tissues and organs such as neighboring lymph nodes, spleen, and lungs. The lung is the most frequent site of metastasis.

The trabecular pattern is the most common type and is characterized by hepatic cords three or more cell layers in thickness (Fig. 12). This type of carcinoma often has the appearance of packets or sheets of neoplastic cells and sometimes the trabeculae are separated by dilated vascular spaces. Where the trabeculae are thick, the tumor cells in the central area may be necrotic, mimicking a glandular pattern. Well differentiated trabecular carcinomas consist of relatively larger hepatocytes with inconspicuous nuclear atypia and have sinusoidal structures resembling those in normal tissue. The tumor cells usually have an eosinophilic cytoplasm and nuclei with condensed nuclear membrane and enlarged nucleoli. Cellular atypia is more evident than in adenomas, but nuclear atypia and polymorphism are relatively mild and mitotic figures are less frequent than other types. In moderately differentiated type, the trabeculae are more thickened with multiple cell layers, irregular in

![Fig. 11. A hepatocellular adenoma in a F344 rat treated with a carcinogen (methylnitrosourea). The lesion is sharply demarcated from the surrounding tissue. A distinct compression of the adjacent parenchyma is seen. H&E stain.](image1)

![Fig. 12. A hepatocellular carcinoma in a F344 rat treated with a carcinogen. Note trabecular growth pattern with multilayered hepatic cords and enlarged sinusoids. Cellular and nuclear atypia is evident. H&E stain.](image2)
shape, and the sinusoids may be inconspicuous. The tumor cells may be smaller in size than those in well differentiated carcinomas and have an increase in nucleus/cytoplasm ratio. They also have increased cytoplasmic basophilia and anisokaryosis. Poorly differentiated trabecular carcinomas are more solid and have poorly organized structures. The tumor cells are generally basophilic and sometimes vacuolated with lipidosis. They have prominent anisocytosis/anisokaryosis with increases in cellular/nuclear atypia, mitotic figures, and nucleus/cytoplasm ratio. Multinucleated giant cells and atypical small neoplastic cells may be seen.

A less common pattern for hepatocellular carcinomas is the acinar or glandular (pseudoglandular) form (Fig. 13). However, the acinar or glandular patterns rarely occupy more than 50% of the carcinoma. This type of carcinoma is characterized by a central clear space surrounded by neoplastic hepatocytes that are generally a single layer thick. The thickness of cell layers surrounding the space depends on the degree of differentiation. The acini are sometimes enlarged (cystic) and filled with finely granular material. Ultrastructurally, the central space has been shown to be a dilated bile canalculus. Genuine acinus formation should be distinguished from the pseudoglandular pattern that results from necrosis of individual or small groups of cells in the center of a thick trabecula. True acini have a basement membrane and an empty lumen, while pseudoacini do not have any basement membrane and contain necrotic debris within their lumens. The distinction between this type and other types of carcinomas may depend on the presence of acinar or glandular form.

The third pattern is a solid type where the neoplastic cells do not form any recognized pattern of organization. Tumor cells in this solid type are generally anaplastic, have basophilic cytoplasm, and often show considerable nuclear pleomorphism and atypia. Mitotic figures are also frequently observed. Since this type of carcinoma is morphologically similar to poorly differentiated trabecular carcinoma, the distinction between these tumors would be difficult.

**Differential diagnosis:** Hepatocellular adenomas and mixed hepatocellular carcinomas are major differential diagnoses. Adenomas also show both solid and trabecular growth patterns, but cellular atypia is mild and the trabecular thickness is within two or three cell layers. In addition, mitotic figures are less frequent. Based on these findings, adenomas can be distinguished from carcinomas. As to hepatocellular carcinomas, they are composed of neoplastic hepatocytes and neoplastic bile duct epithelial cells. The neoplastic growth of both cell components (hepatocytes and bile duct cells) is an essential distinction for differential diagnosis of this lesion. This tumor should not be confused with other hepatic neoplasms that simply contain a number of normal or hyperplastic bile ducts.

**Comments:** Hepatocellular carcinomas may arise from any lobe of the liver. Grossly, they tend to be roughly spherical although the border is frequently irregular. At necropsy, the tumor masses are generally soft and the color of cut surface tends to show reddish brown in well differentiated carcinomas and whitish in undifferentiated carcinomas. The color may be influenced by the presence of hemorrhage, necrosis, or fibrosis. Nitrosamine-induced trabecular carcinomas sometimes contain a blood lake within the tumor mass which may result in abdominal hemorrhage leading to animal death. In the hemorrhagic area of the liver, the tumor cells may be degenerative and show an irregular growth pattern which resembles the morphological features of hemangiosarcomas.

The incidence of spontaneous hepatocellular carcinomas is low (less than 1%) in control rats, but higher in LEC rats or choline-deficient rats. Chemically-induced hepatocellular carcinomas may occur in many animal species treated with hepatocarcinogens such as diethylnitrosamine, aflatoxin B1, and azo dye. Occasionally, encapsulated carcinomas may occur. It is known that the encapsulated carcinomas are also found in humans, especially in Japanese.

**Cholangiocellular Lesions**

**Bile duct hyperplasia**

**Synonyms:** Bile duct proliferation

**Origin:** Intrahepatic bile duct epithelium

**Histological features:** Bile duct hyperplasia consists of several small bile ducts and is confined to the area of portal triads. The small bile duct proliferation is also referred to as simple hyperplasia. These bile ducts may be of normal size, sometimes dilated, and frequently accompanied by periductal fibrosis and/or mononuclear cell infiltration (Fig. 14). The epithelial cells resemble those of normal bile ducts in the periportal areas, but may exhibit degenerative and/or

![Fig. 13. A hepatocellular carcinoma with a glandular pattern in a F344 rat treated with a hepatocarcinogen. The glandular spaces are filled with proteinaceous material and are surrounded by a single layer of neoplastic hepatocytes. Cellular and nuclear atypia is also evident. H&E stain.](image-url)
atrophic changes in aged rats. In some cases, the proliferative bile ducts show cystic dilatation.

**Differential diagnosis:** Cholangiocellular adenoma (simple type) is a differential diagnosis. The simple type of cholangiocellular adenoma is well-circumscribed and often compressing adjacent parenchyma. These findings would be helpful for distinction between simple type of cholangiocellular adenoma and bile duct hyperplasia.

**Comments:** Bile duct hyperplasia is a common aging lesion in rats. The spontaneous incidence of the lesion is approximately 96.8% for males and 34.7% for females in F344 rats. Bile duct hyperplasia is also induced by treatment with some chemicals or by ligation of the common bile duct. When induced by 2-acetylaminofluorene in rats, it may appear within several weeks and the lesion is accompanied by periductal inflammation. It is also known that bile duct epithelial degeneration and necrosis occur prior to development of bile duct hyperplasia in rats treated with α-naphthylisothiocyanate (ANIT).

**Oval cell proliferation/hyperplasia**

**Synonyms:** Bile ductule cell hyperplasia

**Origin:** It is suggested that oval cells may be derived from intrahepatic terminal bile ductule epithelium (Hering canal cells) that is potent to differentiate into both hepatocyte and bile duct epithelium.

**Histological features:** Oval cell hyperplasia is a multifocal lesion and usually widespread throughout the liver. The oval cells are small oval to round cells with scanty basophilic cytoplasm and pale blue oval nuclei (Fig. 15). They usually appear first in the periportal region, later infiltrate into the hepatic acinus, and tend to form incomplete duct-like structures. The oval cells are morphologically similar to bile ductular cells and poor in cytoplasmic organelles. They have desmosome and tight junction for cell adhesion and short microvilli extending into an intercellular space at the junctional area.

**Differential diagnosis:** Bile duct hyperplasia may be a differential diagnosis. By the infiltrative proliferation and incomplete duct-like structure, oval cell hyperplasia can be distinguished from bile duct hyperplasia.

**Comments:** Oval cell hyperplasia is an uncommon hepatic lesion in control rats, but frequently noted in LEC rats and choline-deficient rats. This lesion is also induced in rats and mice treated with some chemicals such as carbon tetrachloride, aflatoxin B1, 2-acetylaminofluorene, and azo dye, but not reported in other animal species. It has been reported that oval cells may have a potential to differentiate into both hepatocytes and biliary epithelia cells. This fact may support the concept that hepatocellular and cholangiocellular carcinomas can sometimes arise from oval cells. However, it is still controversial.

**Cholangiofibrosis**

**Synonyms:** Adenofibrosis; Bile duct adenomatosis

**Origin:** Intrahepatic bile duct epithelium

**Histological features:** Cholangiofibrosis is characterized by atypical bile ducts surrounded by abundant collagenous connective tissue (Fig. 16). The ducts are often irregular, dilated, sometimes glandular, and contain mucinous material, cellular debris, and white blood cells in the luminal space (Fig. 17). The epithelium varies from low cuboidal to tall columnar with variable numbers of goblet cells. The epithelium often exhibits degeneration/necrosis and is sometimes discontinuous, allowing the mucinous material to come in contact with the connective tissue and stimulating an inflammatory reaction. Connective tissue surrounding the ducts is dense and sclerosis often occurs in the central area of the lesion. In the peripheral area, the connective tissue often is arranged concentrically around the ducts or glandular structures. The surrounding hepatic tissue is retracted from all directions towards the lesion. Multifocal areas of cholangiofibrosis often are continuous.
with one another.

**Differential diagnosis:** Cholangiofibroma and cholangiocellular carcinoma (cholangiocarcinoma) are major differential diagnoses. Since cholangiofibrosis and cholangiofibroma are morphologically quite similar to each other, it seems difficult to distinguish one from the other. However, the following points may be used for distinction: the hepatic tissue surrounding cholangiofibrosis tends to be retracted from all directions towards the lesion (contraction), while cholangiofibroma undergoes an expansive growth with compression of the adjacent parenchyma (expansion). On the other hand, cholangiocellular carcinoma can be distinguished from other cholangiocellular tumors based on the following histological characteristics of the lesion: higher degree of cellular atypia, multiple layered lining cells, strongly basophilic epithelial cytoplasm, high mitotic index, and/or metastasis to other tissues.

**Comments:** Cholangiofibrosis varies in size and large lesions are visible grossly as firm and pearly white areas (Fig. 18). The lesions are usually multifocal. Spontaneous occurrence of cholangiofibrosis is rare, but the lesion may be induced in rats treated with chemical carcinogens such as trihalomethane, azo dye, and 2-acetylaminofluorene. There have been no reports on this lesion in other animal species. The biological nature of cholangiofibrosis is still unclear and may vary depending on the inducing agent, length of treatment, and strain of rat. Thus, the interpretation of cholangiofibrosis is still controversial in that some pathologists regard it as a preneoplastic lesion, but some others do not.

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**Cholangiofibroma**

**Synonyms:** Nodules of cholangiofibrosis

**Origin:** Intrahepatic bile duct epithelium

**Histological features:** Cholangiofibroma is composed of atypical bile ducts and large amounts of collagenous connective tissue. The epithelium of the ducts usually consists of one cell layer and contains intensely basophilic cells and goblet cells. Necrosis and mitotic figures are often present in the lining cells. The lumens of the ducts are frequently filled with mucous substances containing necrotic cellular debris and leukocytes. In the central part of cholangiofibroma, the epithelium of the ducts may be partly or completely denuded so that the mucus-filled luminal space directly is in contact with connective tissue. Rarely, cysts lined by a flat epithelium and free of mucus may be found within the lesion. The connective tissue of cholangiofibroma consists predominantly of fibroblasts and more or less abundant collagenous fibers. The fibroblasts contain intermediate filaments of vimentin type which are characteristic of both normal and neoplastic mesenchymal cells. The adjacent liver parenchyma is often compressed...
due to the expansive growth of the lesion.

**Differential diagnosis:** Cholangiofibrosis and cholangiocellular carcinoma (cholangiocarcinoma) are differential diagnoses. As described in the above section, the hepatic tissue surrounding cholangiofibrosis is retracted from all directions towards the lesion. This is one of the most important criteria for differential diagnosis between cholangiofibroma showing an expansive growth and cholangiofibrosis. The differential diagnosis of cholangiofibroma and cholangiocellular carcinoma may be difficult, but the following histological characteristics of cholangiocarcinoma may be helpful for distinction: multilayered epithelial cells with intensely basophilic cytoplasm; glandular, solid, or papillary in structure; and invasive growth into the surrounding tissue and blood/lymph vessels. In addition, the absence of metastasis in cholangiofibroma is a reliable distinction between both lesions.

**Comments:** According to the international harmonized nomenclature for rat liver proliferative lesions, cholangiofibroma is classified as a benign tumor. Although the biological behavior of cholangiofibroma is still unclear, it may have the potential to develop into cholangiocarcinoma because the rat study of furan conducted by the NTP demonstrated the lesions persisted following cessation of treatment at 13 weeks and ultimately developed into cholangiocarcinomas in nearly 100% of the rats at 2 years.

**Cholangiocellular adenoma**

**Synonyms:** Cholangioma; Bile duct adenoma; Biliary adenoma; Bile duct cystadenoma; Biliary cystadenoma

**Origin:** Intrahepatic bile duct epithelium

**Histological features:** Cholangiocellular adenomas (cholangiomas) are divided into two types: simple and cystic. Simple cholangiocellular adenomas are well circumscribed, expansively growing lesions consisting of proliferating bile ducts and varying amounts of stroma (usually small amount). The ducts are lined by cuboidal cells which are similar to normal bile duct epithelial cells and rarely show mitotic figures. On the other hand, cystic cholangiocellular adenomas form unilocular or multilocular cysts which are lined by a single-layered flat or cuboidal epithelium and sometimes may occupy large portions of liver lobes (Fig. 19). The cysts are usually surrounded by a basement membrane and a small rim of connective tissue. In large lesions, the lining epithelium occasionally may be multilayered and sometimes shows a papillary growth or small ductular formation (Fig. 20).

**Differential diagnosis:** Bile duct hyperplasia and multiloculated biliary cysts are main differential diagnoses. The simple type of cholangiocellular adenomas can be distinguished from bile duct hyperplasia by the well circumscribed nature and expansive growth, while the cystic type of adenomas may be distinguished from biliary cysts by the greater cellularity in the lining epithelium which sometimes shows a papillary growth or small ductular formation.

**Comments:** Gross appearance of cholangiocellular adenomas varies from firm, gray-white nodules to lesions with a spongy texture when cystic structures are present. Both simple and cystic types of adenomas are clearly benign tumors, showing no evidence of either metastasis or local invasion. The occurrence of the tumors is rare in control rats, but it may be induced by chemical carcinogens.

**Cholangiocellular carcinoma**

**Synonyms:** Cholangiocarcinoma; Cholangiolar adenocarcinoma

**Origin:** Intrahepatic bile duct epithelium

**Histological features:** Cholangiocellular carcinoma is characterized by proliferation of atypical bile ducts with irregular forms along with a collagenous tissue stroma. The atypical duct epithelia are usually composed of cuboidal to
columnar cells with intensely basophilic cytoplasm and prominent hyperchromatic nuclei (Fig. 21). The atypical epithelial cells show papillary, glandular, or solid growth patterns and a high mitotic index. In dilated glands, the epithelial linings are often piled up (multilayered), but sometimes they are partly or completely missing. Mucin production is highly variable. The connective tissue stroma is generally abundant and scirrhous. Cholangiocellular carcinomas usually exhibit an invasive growth into the surrounding tissues including blood vessels and may metastasize to other organs.

**Differential diagnosis:** Cholangiofibrosis, cholangiofibroma, and hepatocholangiocellular carcinoma are major differential diagnoses for cholangiocellular carcinoma. The distinction from cholangiofibrosis/cholangiofibroma may be difficult, but the following histological characteristics of cholangiocellular carcinoma would be useful as criteria: presence of areas of florid epithelial proliferation (solid nests, papillary growth, and/or multilayered epithelial linings), relatively lower amounts of mucus and collagenous stroma, and invasive growth. On the other hand, hepatocholangiocellular carcinoma can be distinguished from cholangiocellular carcinoma by the presence of neoplastic hepatocytes and absence of abundant collagenous stroma and mucous production.

**Comments:** Cholangiocellular carcinomas usually are noted as firm, white to gray masses with irregular borders at necropsy (Fig. 22). They may protrude from the surface of the liver. In cystic areas, they may have a spongy texture and exclude clear or yellow fluid from the cut surface. The spontaneous occurrence of this tumor is very rare in rats, but it may be induced by some carcinogens such as N-nitrosomorpholine and furan.

**Mixed Hepatocholangiocellular Lesions**

**Hepatocholangiocellular adenoma**

**Synonyms:** Mixed hepatobiliary adenoma

**Origin:** Both hepatocyte and intrahepatic bile duct epithelium

**Histological features:** Hepatocholangiocellular adenomas are morphologically similar to hepatocellular adenomas, but they also contain areas of neoplastic bile duct proliferation. The neoplastic bile duct epithelium usually forms slightly dilated acini lined by cuboidal or flattened cells (Fig. 23). The neoplastic biliary component appears to infiltrate areas of the hepatocellular adenoma.

**Differential diagnosis:** Hepatocellular adenoma with bile duct hyperplasia is a main differential diagnosis for hepatobiliary adenoma. In the former, hyperplastic bile ducts are usually lined by cuboidal epithelial cells and are often surrounded by a small amount of connective tissue with inflammatory cells. In addition, the hyperplastic bile ducts occur in small groups that are often lined up in a row. In the latter, both hepatocellular and biliary elements must be neoplastic.

**Comments:** Hepatocholangiocellular adenomas are grossly indistinguishable from hepatocellular adenomas, but histologically distinguishable as described above. Spontaneous occurrence of hepatobiliary adenomas is very rare (less than 1% in rats and mice) and no reports in other animal species. However, it may be induced by certain chemicals. It was reported that hepatobiliary adenomas and carcinomas occurred with relatively high frequency in the neonatal rat hepatocarcinogenesis model that was initiated by diethylnitrosamine.
Hepatocholangiocellular carcinoma

**Synonyms:** Mixed hepatocholangiocellular carcinoma

**Origin:** Both hepatocyte and intrahepatic bile duct epithelium

**Histological features:** Hepatocholangiocellular carcinoma is characterized by admixture of neoplastic hepatocytes and neoplastic bile duct epithelial cells. The neoplastic hepatocytes show a trabecular, solid, or glandular growth pattern, while the neoplastic biliary elements form acini usually lined by cuboidal/columnar cells or small nests without distinct lumens (Fig. 24). On occasion, both neoplastic elements may line the same glandular structure. Ultrastructural features of the biliary components of these mixed neoplasms include the presence of a distinct basement membrane, absence of cytoplasmic glycogen, and microvilli on the free surface of the cell membrane.

**Differential diagnosis:** Hepatocellular carcinoma and cholangiocellular carcinoma are main differential diagnoses. Hepatocholangiocellular carcinoma can be distinguished from these tumors by the presence of both neoplastic hepatocytes and neoplastic bile duct epithelial cells. In addition, both elements must have evidence of malignancy.

**Comments:** Hepatocholangiocellular carcinomas are grossly similar to hepatocellular carcinomas. Spontaneous occurrence of hepatocholangiocellular carcinomas is very rare (less than 1% in rats and mice and no reports in other animal species). However, it may be induced by certain chemical carcinogens. The tumor induction was reported in rats treated with diethylnitosamine or azo dye and also in mice receiving 2-acetylaminofluorene or benzidine dihydrochloride. On occasion, hepatocellular carcinoma and cholangiocellular carcinoma may occur separately, but after growing they merge into a single mass within the liver. Such a case must be distinguished from hepatocholangiocellular carcinoma.

Sinusoidal Cell Lesions

**Spongiosis hepatis**

**Synonyms:** Cystic degeneration

**Origin:** It is suggested that the lesion may arise from sinusoidal stellate cells (Ito cells).

**Histological features:** Spongiosis hepatis is a multilocular cystic lesion containing a finely granular or flocculent eosinophilic material (Fig. 25) and occasionally erythrocytes; the presence of erythrocytes may be due to ruptured sinusoidal blood vessels. The cystic structures are not lined by epithelial or endothelial cells; India ink studies have shown no communication between blood sinuses and...
cystic spaces of spongiosis hepatis. Ultrastructurally, the walls of cystic cavities consist of cells resembling fibroblasts with long cytoplasmic extensions in contact with neighboring cells. They are covered by a basement membrane-like lining, containing bundles of collagen fibers with a regular cross-striation pattern. Osmophic droplets characteristic for Ito cells are retained within the cytoplasm of lining cells.

Spongiosis hepatis may occur in normal appearing liver tissue but also more often within hepatocellular proliferative lesions including foci of cellular alteration, adenomas, and carcinomas. Areas of spongiosis hepatis are generally less than one lobule in size, but tend to be larger in the hepatocellular proliferative lesions. This lesion (even large lesion) does not compress adjacent normal or neoplastic liver tissue. When present within foci or hepatocellular tumors, it should not be diagnosed separately.

**Differential diagnosis:** Biliary cysts and sinusoidal dilatation (pelirosis) are main differential diagnoses. These lesions can be distinguished from spongiosis hepatis by the presence of epithelial or endothelial cells.

**Comments:** Spongiosis hepatis is infrequently found in control rats, but more frequently in rats treated with hepatocarcinogens such as N-nitrosomorpholine, dimethylnitrosamine, nitrosopyrrolidine, and 2-acetylaminofluorene. The incidence of spontaneous spongiosis hepatis in rats may be as high as 30% in males, but is much lower in females, although it differs among strains, animal colonies, and laboratory conditions including diet. Since the biological nature or behavior including carcinogenic potential of spongiosis hepatis has not been clearly demonstrated, the interpretation of this lesion is still controversial among pathologists. One group has proposed that spongiosis hepatis may be a preneoplastic and/or neoplastic lesion because of its proliferative properties and persistent increased cell turnover rate in stop experiments with hepatocarcinogens, and the assumption that it can develop into a sarcoma. In contrast, the other group has suggested that the lesion may be a secondary/ reparative change but not a preneoplastic or neoplastic lesion, because spongiosis hepatis was more associated with hepatocellular hypertrophy or hepatotoxicity, rather than hepatocarcinogenicity in 12 oncogenicity studies in rats with induced spongiosis hepatis. In addition, the group has indicated that persistent proliferation is also seen with other nonneoplastic lesions and spongiosis hepatis does not have neoplastic histomorphologic characteristics.

**Kupffer cell hyperplasia/proliferation**

**Synonyms:** Histiocytic cell hyperplasia/proliferation

**Origin:** Sinusoidal Kupffer cell

**Histological features:** Kupffer cell hyperplasia is characterized by diffuse or multifocal proliferation of oval to spindle cells along sinusoids. The cells resemble histiocytes in other tissues and may contain phagocytized cellular debris or erythrocytes. They grow along with sinusoids and form sheets, nests, or nodules, but the edge of the lesions is generally indistinct. Small foci of Kupffer cells surrounding necrotic hepatocyte debris or foreign bodies are often observed in aged rats. They are generally referred to as microgranuloma or granuloma rather than Kupffer cell hyperplasia.

**Differential diagnosis:** Oval cell hyperplasia may be a differential diagnosis. Evidence of phagocytosis and immunohistochemical detection of Fc receptor characteristic for Kupffer cells would be helpful for distinction.

**Comments:** Spontaneous incidence of Kupffer cell hyperplasia is very low (less than 1%) in rats, but it may be induced in rats after ingestion of exogenous foreign particles such as iron. In addition, it is known that proliferation and phagocytic activity of Kupffer cells are accelerated in rats treated with estrogen. It has not been established whether Kupffer cell hyperplasia is a neoplastic lesion.

**Kupffer cell sarcoma**

**Synonyms:** Histiocytic sarcoma

**Origin:** The cell of origin may be sinusoidal Kupffer cell or circulating macrophage from the bone marrow.

**Histological features:** Kupffer cell sarcomas are composed of oval to spindle cells resembling macrophages. The cells have foamy cytoplasm, indistinct cell boundaries, and may contain phagocytized cellular debris or erythrocytes. They grow along sinusoids and often involve other organs such as the spleen and lung, suggesting early metastasis or a multicentric origin. Characteristically, Kupffer cell sarcomas form nodules within the liver which may contain necrosis in the central area of the nodule (Fig. 26). Giant cells may be present in some cases.

**Differential diagnosis:** Malignant fibrous histiocytoma (MFH) and histiocytic sarcoma are potential differential diagnoses. MFH may be distinguished from Kupffer cell sarcoma by the presence of collagenous component and storiform pattern. Immunohistochemical detection of Fc...
receptor for Kupffer cells also might be a helpful distinction. On the other hand, the distinction between histiocytic sarcoma and Kupffer cell sarcoma is extremely difficult because of their morphological similarity. In addition, it is also difficult to determine whether tumor cells involving various tissues are due to metastasis from the liver or multicentric origin. Therefore, both lesions tend to be referred to as histiocytic sarcoma. When involving only the liver, it is often referred to as Kupffer cell sarcoma.

**Comments:** Kupffer cell sarcomas are grossly found as multiple gray nodules that occur randomly through the liver. Spontaneous incidence of this sarcoma is very rare in rats (less than 1%) and almost no reports in other animal species. Experimentally, it was reported that the lesion was induced in rats treated with trypan blue, dimethylnitrosamine, or 2-acetylaminofluorene.

**Hemangioendothelial Lesions**

**Hemangioma**

*Synonyms:* Benign hemangioendothelioma  
*Origin:* Endothelial cell of blood vessels  
*Histological features:* Hemangiomas are characterized by a single layer of densely packed endothelial cells forming various-sized vascular cavities (Fig. 27). The lesions are divided into capillary hemangioma or cavernous hemangioma based on the size of vascular spaces (smaller in the former and larger/dilated in the latter). The endothelial cells are uniform and lack pleomorphism and have indistinct cytoplasm containing dense, round to oval nuclei. Variable amounts of blood may be present in the dilated channels. Hemangiomas start as proliferating endothelium along existing hepatic sinusoids. The proliferating endothelial cells are relatively larger than normal endothelium and may be flattened, oval, or round with mild cellular atypia. As growth continues, the endothelial cells develop independently of hepatic plates and may have a definite connective tissue stroma. Their morphological features are less diverse than those in hemangiosarcomas.  
*Differential diagnosis:* Angiectasis (telangiectasis/peliosis hepatis) with dilated vascular spaces is a main differential diagnosis. This vascular lesion can be distinguished from hemangiomia by the absence of proliferating endothelial cells lining the dilated vascular spaces.  
*Comments:* Hemangiomas may be recognized as irregular dark spots on the liver surface and tend to bleed. Spontaneous occurrence of this lesion is low (less than 1%) in rats, but it may be induced by certain chemicals. Experimental induction of hemangiomas was reported in mice treated with diethylnitrosamine and dogs receiving N-ethyl-N’-nitro-N-nitrosoguanidine.

**Hemangiosarcoma**

*Synonyms:* Malignant hemangioendothelioma  
*Origin:* Endothelial cell of blood vessels  
*Histological features:* Hemangiosarcomas are characterized by a proliferation of rather atypical endothelial cells which line the sinusoids in single layers or form multilayered masses (Fig. 28). In the early stage of the tumor development, the neoplastic endothelial cells may be arranged in solid sheets or nodules, but the increased cell proliferation rapidly leads to an irregular dilatation of the sinusoids. The endothelial cells are spindle-shaped or polyhedral, and they are much larger than normal cells. Nuclei are often large, diverse in structure, and are undergoing mitosis. Necrosis and thrombosis are frequently present. The neoplastic cells irregularly merge with adjacent parenchyma, actively invade adjacent sinusoids, and occasionally may metastasize to other organs.  
*Differential diagnosis:* Hemangioma is a main differential diagnosis. Hemangiosarcoma can be distinguished from hemangiomia by that the former often shows solid or nodular growth of endothelial cells with evident cellular atypia and polymorphism, while the endothelial cells of the latter exhibit relatively normal cellular appearance and uniform single-cell pavementing of hepatocyte surfaces.

Fig. 27. Hemangioma in a control F344 rat. Various-sized enlarged vascular spaces filled with blood are seen. H&E stain.

Fig. 28. Hemangiosarcoma in a control F344 rat. Note atypical endothelial cells invading adjacent tissue. H&E stain.
Hemangiosarcomas are also distinguished from other sarcomas by their tendency to form blood-filled vascular channels. In addition, primary hepatic hemangiosarcomas should be distinguished from metastatic hemangiosarcomas from other sites, particularly the spleen. The lack of the lesions at other sites is an available evidence for a primary hepatic origin.

**Comments:** Hemangiosarcomas can be seen grossly when located closed to the liver surface. They are not sharply demarcated and usually show more or less extended dark red areas within the tissue. Rupture of the lesions leads to hematoperitoneum. Hemangiosarcomas tend to metastasize to the lung. Spontaneous occurrence of this tumor is rare in rats, but it may be induced by certain chemicals. Chemically-induced hemangiomas tend to be multicentric. In other animal species, induction of this lesion was reported in mice treated with diethylnitrosamine and in dogs receiving N-ethyl-N'-nitro-N-nitrosoguanidine.

**Others**

**Mesenchymal tumors**

Mesenchymal tumors such as lipoma, liposarcoma, and fibrosarcoma may occur in the liver of rats, but they are extremely rare.

**Conclusion**

The present review by the Working Group indicates that the current classification, terminology, and diagnostic criteria for the rat liver proliferative lesions recommended by the JSTP are generally consistent with the international harmonized nomenclature. However, it should be recognized that there are still discrepancies especially in the interpretation of certain lesions among pathologists in the world even after the international harmonized nomenclature was issued. For instance, the biological behavior and toxicological significance of spongiosis hepatis/cystic degeneration and cholangiofibrosis/cholangiofibroma are still controversial issues and are open to discussion. In addition, the term of “cholangiofibroma” listed in the group (category) of epithelial neoplasms seems to be misleading because it may be a term to be used for mesenchymal tumors rather than neoplasms of epithelial origin. These subjects should be discussed among the JSTP pathologists in the near future. The international harmonization of nomenclature and diagnostic criteria for lesions observed in laboratory animals is one of the goals for toxicologic pathologists. The next important goal would be harmonization of interpretation of lesions observed in toxicology studies, since it is very important for human risk assessment of chemicals used worldwide. Thus, we need further investigations to clarify the biological nature and behavior of both spontaneously-occurring and chemically-induced lesions in more details.

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