Letter

Comparative Aspects of Rat and Human Hepatocellular Preneoplasia and Neoplasia

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In a "comparative histomorphological review of rat and human hepatocellular proliferative lesions", Thoolen and colleagues[1] stated that "there are major similarities in the diagnostic features, growth patterns and behavior of both rat and human proliferative hepatocellular lesions and in the process of hepatocarcinogenesis". I fully agree with this statement, but in view of its far-reaching consequences for the evaluation of rodent carcinogenesis bioassays and the early detection of precursors of human hepatocellular adenomas (HCA) and carcinomas (HCC) I draw your attention to several critical comparisons and conclusions concerning the significance of preneoplastic foci of altered hepatocytes (FAH), called "foci of cellular alteration" in the review.

An appropriate comparison of FAH in rats and humans is hampered by barely considered methodological problems: whereas the nowadays generally accepted definition of FAH in rats[2] was mainly based on studies in tissues containing well preserved cytoplasmic constituents (e.g. glycogen, endoplasmic reticulum, ribosomes, mitochondria)[3], the majority of studies in humans were conducted in formalin-fixed (frequently postmortally taken) tissue. Under these conditions, the hepatocellular cytoplasm shows either a glycogen loss and diffuse, slightly eosinophilic tincture due to autolytic processes or appears transparent following glycogen elution during fixation and/or staining, but becomes "clear" when excessive amounts of glycogen are stored (glycogenosis). Misinterpretation of hepatocellular glycogenosis as "vacuolation", "hydrops", "cell swelling", "ballooning", etc. has been well known from animal experiments with inappropriate tissue preservation or cytochemical evaluation[3]. However, the perception of such incorrect diagnoses is particularly important for the comparison of experimental findings with human data. In human liver pathology, "liver cell dysplasia" including "large cell change" (LCC)[4] and "small cell change" (SCC)[5] has mainly been defined by alterations in cellular, nuclear and nucleolar size, while preneoplastic FAH in rodents were predominantly characterized by changes in cytoplamsic components, notwithstanding that these changes are frequently accompanied by pronounced nuclear and nucleolar alterations[6].

It is, hence, questionable whether basophilic, eosinophilic, and clear cell foci in rats are actually "counterparts of human liver cell dysplasia classified as large cell change and small cell change" as postulated by Thoolen *et al.*[1] For instance, the "classic example of large cell change" demonstrated in Fig. 9 of the review is hardly compatible with LCC defined by Anthony *et al.*[4], but shows typical ground-glass hepatocytes which correspond to acidophilic hepatocytes in rodents, usually storing abundant glycogen as detailed recently[7]. On the other hand, the "eosinophilic cell focus of cellular alteration" depicted in Fig. 10 is most probably poor in, or free of, glycogen, being consistent with "amphophilic" FAH potentially progressing to HCA and HCC in rats exposed to certain chemicals, especially "peroxisome proliferators"[8,9]. Changes resembling these amphophilic lesions have also been observed in cirrhotic human livers, but in this case their significance for neoplastic development has remained obscure[10]. The experimental experience suggests a classification of the various human preneoplastic hepatocellular alterations according to both cytoplasmic and nuclear changes[10] rather than collectively calling them "dysplastic".

An appropriate analysis of hepatic preneoplasia in humans became only possible when well preserved liver tissue was provided by biopsies[11,12] or surgical specimens[13], immediately frozen or fixed by fixatives conserving major cytoplasmic constituents, especially the glycogen. It may have escaped the authors´ attention that detailed investigations on hepatic preneoplasia in appropriately fixed specimens from more than 150 explanted human livers are available[10], in which the cytoplasmic hepatocellular changes were carefully related to "liver cell dysplasia" defined as "large cell change" (LCC)[4] and "small cell change" (SCC)[5], substantiating the argument that LCC should not be considered a preneoplastic change. In contrast, SCC may indicate a preneoplastic condition, but only when appearing inside of certain types of FAH, namely the mixed and variably basophilic types [10].

The statement that "...the role of the clear cell foci in hepatocarcinogenesis is elusive and poorly described..."[1] is unreasonable. It is true that concerns over the significance of the glycogenotic clear cell foci for hepatocarcinogenesis were repeatedly raised[14,15] since their discovery and postulated preneoplastic nature in animals and man[3,11], but this cannot be attributed to their "poor description". The role of clear cell foci and related types of FAH, HCA, and HCC has been studied in numerous animal experiments modelling chemical, viral and hormonal hepatocarcinogenesis[16-19]. Particularly the clear cell foci and their fate were sequentially studied in great detail until neoplasms appeared by light and electron microscopy[3,16], several morphometric approaches[20-24], various cytochemical methods (enzyme histochemistry, immunohistochemistry, radioautography)[25,26], quantitative microbiochemistry using laser-dissected specimens[17,25], and *in situ* hybridization for the expression of genes at the RNA level[17]. A listing of all relevant publications in this letter is impossible, but some reviews[3,7,16,19,25,30] summarizing most of the original articles complement those mentioned by Thoolen *et al.*[1] Deviating opinions appear to result from two main misunderstandings: 1) differences in the classification of FAH, e.g. when early emerging gly-

cogenotic, combined clear/acidophilic FAH are classified as mixed FAH[27], a phenotype which is characteristic of more advanced stages of hepatocarcinogenesis, and should always contain glycogen-poor, basophilic along with clear and/or acidophilic cells[16,17]; 2) the overestimation of FAH in untreated control animals by determination of incidences (sometimes only one focus/animal) at the end of two year carcinogenesis bioassays [27]. This should be avoided by sequential stereological comparisons of the number and size of the various types of FAH and the calculation of their volume fraction in untreated and treated animals[21-24].

The most convincing morphological link between glycogenotic clear/acidophilic cell foci and more advanced types of preneoplastic and neoplastic lesions, namely the intermediate and mixed cell populations composed of clear, acidophilic, basophilic and different forms of intermediate cell types[3, 16, 17, 20–27] were largely ignored by Thoolen *et al.*[1], though they were indirectly mentioned in one sentence: "... eosinophilic or basophilic cells were occasionally present within clear cell foci". Compelling evidence for the most frequently occurring glycogenotic -basophilic preneoplastic hepatocellular lineage has been provided for rodents exposed to various chemicals, hepadnaviridae and hyperinsulinemia[3, 7, 16–26]. Remarkably, however, the basophilic cells appearing in this predominant preneoplastic lineage usually show a more or less strong diffuse basophilia, which may be combined with small cell size, resembling SCC in the human liver[10].

Within the category of basophilic cell foci Thoolen *et al.*[1] noted cells exhibiting a "tigroid" pattern (TCF) which results from an increase in highly organized rough endoplamsic reticulum[28, 29]. This type of focus should be clearly separated from that involved in the glycogenotic-basophilic preneoplastic lineage. TCF have mainly been observed in rats exposed to low (total) doses of chemicals such as aflatoxin, and N-nitrosomorpholine[28, 29]. The occasionally challenged preneoplastic nature of TCF[14] has been substantiated by several studies showing that TCF may progress to HCA[22-24,28,29] and eventually also HCC[29]. Hypertrophied ("xenomorphic") hepatocytes, predominantly localized in perivenular lobular parts, have been identified as precursors of tigroid basophilic preneoplastic and neoplastic lesions[29]. TCF indicate a carcinogenic potential of chemicals tested in bioassays[30], although they have not been explicitly described in human livers.

Another point which should be addressed is the "reversibility" of FAH emphasized by Thoolen *et al.*[1] Several morphometric studies in rats exposed to N-nitrosomorpholine for limited time periods revealed that the total number of FAH not only persisted but even further increased after withdrawal of the carcinogen, while early glycogenotic, clear/acidophilic FAH progressed to more advanced mixed and glycogen-poor, basophilic types[20–24]. However, when high toxic doses of the same chemical were applied, many of the thousands of FAH emerging under these conditions turned out to be phenotypically instable and regressed after withdrawal[23, 31]. Similar observations on FAH, histochemically detected by the expression of gamma-glutamyl transpeptidase or the placental glutathione S-transferase, were made in medium-term carcinogenesis bioassays, in which the test compound is given after partial hepatectomy stimulating cell proliferation, combined with high doses of 2-acetylaminofluorene[32, 33]. But to the best of my knowledge there is not a single report on any of the bioassays proposed showing a complete reversibility of FAH after withdrawal of the test compound. Hence, in any case the development of FAH in carcinogenesis bioassays appears to indicate a carcinogenic potential of the compound tested[30].

As to chronic human liver diseases prone to develop HCC it should be considered that highly toxic conditions comparable to those in the medium-term carcinogenesis bioassays in rodents are usually absent. It is, therefore, unlikely that FAH detected in human liver biopsies belong to the "reversible" category. A more difficult and hitherto unsolved problem is to predict the time course of progression from clear/acidophilic FAH to HCA and HCC. In rodents, the development of hepatocellular neoplasms from low numbers of clear/acidophilic FAH may take months or even years, corresponding to decades in humans. In addition to the definition of the various phenotypes of FAH, and the evaluation of their number and size[20–24], their proliferation kinetics showing a gradual increase from the early emerging clear/acidophilic to the more advanced mixed and basophilic phenotypes is an important prognostic parameter[26]. This also holds for the evaluation of similar findings in the human liver[10].

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Comment from Editor-in-Chief

Editor-in-Chief forwarded the content of the ”letter to editor” written by Dr. Bannasch to the authors of the original paper, and asked them whether and how they would like to respond. In their reply, the authors said that they read the content but would express no comments.

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