Short Communication

Gamma-glutamyl transpeptidase in putative precancerous thyroid lesions of rats treated with diisopropanolnitrosamine

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Gamma-glutamyl transpeptidase (γ GT) has been widely used by many investigators as a useful histoenzymic marker for preneoplastic hepatocellular lesions during experimental hepatocarcinogenesis (Kalengayi *et al.*, 1975, Hirota & Williams, 1979, Farber, 1980), since the appearance of this enzyme in rodent livers exposed to hepatocarcinogens was reported as an oncofoetal property by Fiala *et al.* (1972). We recently demonstrated a rather widespread histochemical distribution of γ GT activity in various kinds of human thyroid lesions, either neoplastic or non-neoplastic, with little or no activity present in the normal thyroid tissues and with the strongest reaction for γ GT occurring in papillary carcinoma among the thyroid neoplasms (Moriyama *et al.*, 1982). In the present study, a significant γ GT activity was found in putative precancerous thyroid lesions in rats treated with thyroid carcinogen.

Injection (s.c.) of diisopropanolnitrosamine (DIPN, Nakarai Chem., Ltd., Kyoto) 750 mg kg⁻¹ was given, once weekly for 10 weeks, to 7 male and 4 female Wistar rats, weighing 400 g and 300 g, respectively. Animals were maintained on a commercial diet (CE-2, Oriental Yeast, Co., Ltd., Tokyo) throughout the experiment. One male animal was sacrificed at 5 weeks after the commencement of the experiment. Two males were killed at 10 weeks and the remainder of the males and all the females at 18 weeks. At autopsy, the thyroid tissues were removed immediately. Frozen sections (6 μ m) were prepared in a cryostat for histochemical staining of γ GT as described by Rutenburg *et al.* (1968).

No γ GT activity was present in the thyroid tissues from untreated control rats, except for a positive γ GT stain in the squamous epithelial nests. As early as 5 weeks after the first injection of DIPN, the thyroid glands showed, microscopically,

diffuse hyperplasia of small thyroid follicles. The thyroid glands in 2 animals killed at 10 weeks were similarly hyperplastic. γ GT staining remained negative in these hyperplastic follicles. At 18 weeks focal proliferative lesions of atypical cells with prominent nuclei (designated “altered foci”), characterized by the appearance of ectatic follicular structures, were found within the hyperplastic thyroid tissues. Some of these “altered foci” were strongly positive for γ GT (Figure 1), while the other foci were weakly positive or even negative. Nodular proliferative lesions (designated “neoplastic nodules”), which exhibited strong activity of γ GT (Figure 2), also occurred at 18 weeks. Some foci were present around the nodules. Three out of 4 male rats exhibited either foci or nodules at 18 weeks. Among these, 6 out of an aggregate of 17 foci were γ GT positive, as were 2 nodules one of which was present in each of 2 rats. By contrast, only diffuse hyperplasia of the thyroid with no γ GT-positive lesions was observed in the female rats at 18 weeks, indicating that in the latter the thyroid as a target organ is either less sensitive to DIPN or the enzymes metabolizing the agent are present to a much lesser extent since the ultimate carcinogen is a metabolite of DIPN.

This is the first report to our knowledge on the occurrence of γ GT activity in the atypical proliferative focal or nodular lesions in thyroids during experimental thyroid carcinogenesis. These discrete γ GT-positive lesions induced by a long-term treatment with DIPN, which is known to be a thyroid carcinogen (Mohr *et al.*, 1977), are reminiscent of altered (hyperplastic) foci or neoplastic (hyperplastic) nodules during hepatocarcinogenesis. In view of the morphological features and γ GT staining, we consider that “altered foci” or “neoplastic nodules” of the thyroid in rats treated with DIPN are possibly precancerous in nature. Mohr *et al.* (1977) reported that DIPN at the same dose level as employed in the present experiment induced thyroid cancer 26 weeks following the initial injection. However, so far, no histoenzymatically altered foci or nodules have been described. Because increased γ GT activity has not

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Figure 1 A γ GT-positive “altered focus” (in the lower left) in the thyroid of a male rat at 18 weeks after the initial DIPN treatment. A squamous epithelial nest also showing positive staining (in the upper right). γ GT reaction without counterstain. $\times 240$.

Figure 2 Strong γ GT activity in a “neoplastic nodule” surrounded by hyperplastic thyroid tissues in a male rat at 18 weeks after the initial DIPN treatment. γ GT reaction, counterstained with hematoxylin. $\times 160$.

been documented in rat foetal thyroid tissue, discussion of the oncofoetal property of this enzyme in the thyroid is not relevant. Nevertheless, the situation seems close to that of experimental hepatocarcinogenesis in rats, in which γ GT is a significant histoenzymic marker of preneoplastic or neoplastic changes, with an implication of induction of foetal enzymic activity. These preliminary data

suggest that γ GT might be a useful, functional marker for preneoplastic changes in experimental thyroid carcinogenesis.

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