Increase in Sex-Dependent Expression of Prolactin Receptors after Intrahepatic Transplantation of H27 Rat Hepatoma Cells

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Immunohistochemical study revealed overexpression of prolactin receptors in central and peripheral cells of intrahepatic grafts from H27 rat hepatoma without changes in compartmentalization compared to hepatocytes of intact animals. Experiments with gonadectomized animals showed that the hormonal regulation of prolactin receptors in both types of tumor cells does not differ from that in normal liver cells (positive estrogen-dependent regulation and negative androgen-dependent regulation).

Key words: *prolactin receptor; H27 hepatoma; hepatocytes; sex hormones; rat*

Adenohypophyseal hormone prolactin is involved in the regulation of various functions in humans and animals (*e.g.*, function of liver cells). The effects of prolactin are mediated via prolactin receptors (PrlR). Signal transduction in these receptors is realized via a variety of pathways. The major pathway is JAK-STAT. Hepatocytes in animals [2,5,10] and humans [6] are characterized by high number of PrlR, especially in females. Prolactin participated in the regulation of proliferative processes in the liver.

Here we studied the expression and compartmentalization of PrlR in intrahepatic grafts of H27 hepatoma and liver tissue of male and female recipient animals and their dependence on the concentration of sex hormones.

MATERIALS AND METHODS

Experiments were performed on intact outbred albino rats and mature gonadectomized animals weighing 180-240 g. Each group consisted of 4-12 rats. The control group included intact rats not subjected to tumor transplantation. H27 hepatoma is a cell line of rat hepatocellular carcinoma. The strain of H27 hepatoma was obtained from the Bank of Tumor Strains (N. N. Blokhin Russian Cancer Research Center) and maintained by subcutaneous transplantations in the thigh region (one time per 12-14 days). The suspension of tumor cells from the subcutaneous transplant was diluted with modified Hanks solution (Sigma) to obtain 40% suspension. Intrahepatic transplantation was performed on narcotized rats. The suspension of tumor cells (0.1 ml) was punctured into the central liver lobe. The site of puncture was glued with a MK-7M tissue glue. The liver with implanted tumor tissue was isolated after 25-30 days; samples of tumor tissue and native tissue from the nontumor liver lobe were examined. Tissue samples were fixed in 4% paraformaldehyde (Sigma), embedded into paraplast (Sigma), and 3-μ slices were stained with hematoxylin.

PrlR localization in samples was determined by the indirect immunoperoxidase method with U_5 monoclonal antibodies against rat PrlR (Sigma). Two treated (in the presence of anti-PrlR antibodies) and

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two control slices (in the absence of anti-PrlR antibodies) from each tissue sample were examined; 100 tumor cells in the tumor tissue, 50 cells in the periportal zone, and 50 cells in the pericentral zones of the liver were analyzed. PrlR expression in hepatocytes of the periportal and pericentral zone was comparable in all cases. Therefore, these data were combined. Immunopositive staining for PrlR was studied for the whole cell and for the nucleus and cytoplasm separately. The intensity of PrlR expression was estimated by a semiquantitative analysis with images of slices obtained under an Axioplan microscope (Zeiss). We used KAF 400 camera (Photometrix) with a cooled CCD matrix and PMIS 2.1 image processing software. The difference between shades of gray in treated and control samples was proportional to the relative concentration of labeled compound [11]. Since experimental data did not fit normal distribution, the results were expressed in relative units (median, upper and lower quartiles) [2].

The data were analyzed by means of Statistica 6.0 software. The significance of differences was evaluated by nonparametric Kruskal—Wallis test and median test. The differences were significant at $p<0.05$.

RESULTS

Histological study of H27 intrahepatic tumor in male and female rats on days 25-30 after transplantation revealed hepatoma with 2 types of tumor cells (central and peripheral cells). Central tumor cells spread diffusely and nonstructurally as focuses of different size and were often surrounded by peripheral cells (Fig. 1, *a*). Central cells had small size and oval shape. A large round or oval nucleus occupied nearly the total volume of the cell (Fig. 1, *b*). The nucleus-cytoplasm ratio in these cells surpassed that in normal hepatocytes. Peripheral tumor cells (Fig. 1, *c*) often formed rosette-like structures and were larger than central tumor cells, but smaller than normal hepatocytes. The nucleus-cytoplasm ratio in these cells slightly exceeded that in hepatocytes from intact animals. Peripheral tumor cells sometimes formed the periportal and pericentral zone of the hepatic lobule.

In tumor cells of both types, the PrlR-positive staining was seen in the nucleus, cytoplasm, and cell membrane (Fig. 1, *d*). No differences were found in PrlR expression in the cytoplasm and nuclei of tumor cells of both types from male and female rats. In further studies, PrlR expression in

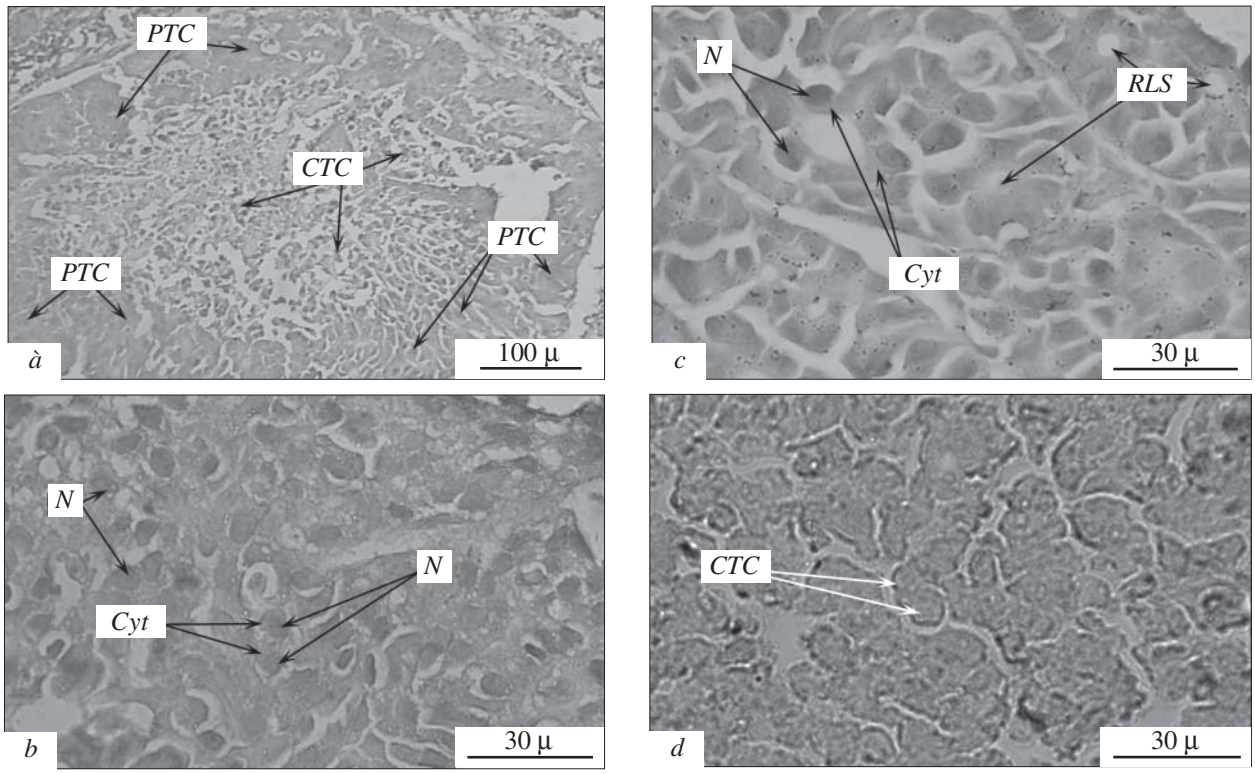

Fig. 1. Histology and expression of PrlR in the intrahepatic graft of H27 rat hepatocellular carcinoma. *a*) intrahepatic H27 graft in male rat; *b*) tumor cells in the central tumor zone of male rat; *c*) tumor cells in the peripheral tumor zone of male rat; *d*) immunoperoxidase identification of PrlR in central tumor cells of female rat. Hematoxylin staining (*a-c*). *CTC*, central tumor cell; *PTC*, peripheral tumor cell; *Cyt*, cytoplasm; *N*, nuclei; *RLS*, rosette-like structure.

tumor cells and hepatocytes from animals of various groups was evaluated for the whole cell.

In male and female animals PrlR expression in central tumor cells surpassed that in normal hepatocytes from (by 1.7 and 3.3 times, respectively, Table 1). In peripheral tumor cells immunopositive staining for PrlR increased less significantly (by 1.3 in males and by 1.4 times in females). These data suggest that central tumor zones are more sensitive to prolactin signals than the peripheral zone (particularly in females).

PrlR expression in the liver of intact animals is regulated by sex steroids (positive estrogen-dependent regulation and negative androgen-dependent regulation) [10]. The hormone regulation of PrlR expression was similar in tumor cells of H27 intrahepatic grafts. Castration of males was followed by a 1.6-fold increase in immunopositive staining for PrlR in central and peripheral tumor cells (Fig. 2). In ovariectomized females, PrlR expression significantly decreased in central and peripheral cells by 2.1 and 1.7 times, respectively.

Native liver tissue (from the lobe not involved into the tumor process) in male and female rats retained normal structure of the liver lobule. PrlR distribution in hepatocytes from native tissue did not differ from that in hepatocytes of intact animals [2].

Tumor growth did not change PrlR expression in native liver lobe of females, but 1.5-fold increased it in males (Table 1), which probably reflects more potent toxic effect of the tumor on hepatocytes in males.

Our results indicate that hormone regulation of PrlR in intrahepatic grafts of H27 hepatoma cells does not differ from that in normal liver cells (positive estrogen-dependent regulation and negative androgen-dependent regulation). Hence, PrlR can serve as a therapeutic target for sex-specific therapy of liver tumors.

Intrahepatically transplanted H27 tumor cells are characterized by overexpression of PrlR compared to hepatocytes from intact animals and cells of other prolactin-sensitive organs [5]. Our findings suggest that these changes depend on the cell origin of the tumor. We previously showed that PrlR are intensively expressed and accumulated in RS1 rat cholangiocellular carcinoma cells after their intrahepatic transplantation [2]. It was hypothesized that prolactin has a direct proliferative effect on nuclei of these cells [2,3]. A similar effect is typical of Nb2 lymphoma cells [9]. This effect is mediated by the formation of the intranuclear prolactin/cyclophilin B complex, which induces the Stat5-dependent gene expression. The direct nuclear pathway for action of prolactin on H27 hepatoma probably does not play a role, since PrlR expression is similar in the nucleus and cytoplasm of tumor cells. It can be hypothesized that PrlR trigger the major JAK-STAT cascade producing a proliferative effect in H27 hepatoma cells (similarly to normal hepatocytes) [5], while overexpression of PrlR in tumor cells amplifies this effect. An alternative pathway was demonstrated for mouse breast cells: prolactin directly maintains a constant level of nuclear transcription factor NF1-C2, which activates tumor suppressor gene p53 [7]. Thus, the oncogenic and antioncogenic effects of prolactin probably depend on the fine balance between proliferative and antiproliferative intracellular signaling pathways that are activated by this hormone. Moreover, it should be emphasized that PrlR has 2 main isoforms: the

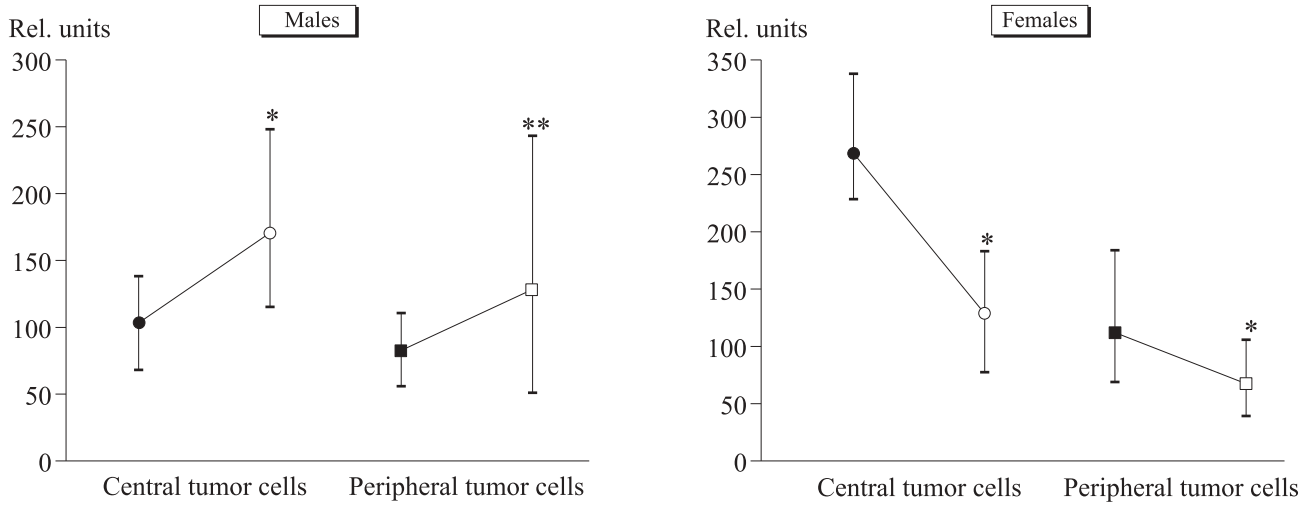

Fig. 2. PrlR expression in H27 hepatocellular carcinoma cells from animals with intact gonads (dark marker) and gonadectomized rats (light marker). The results are presented as the median and lower and upper quartiles. *p<0.001 and **p<0.01 compared to the corresponding type of cells in animals with intact gonads.

TABLE 1. PrlR-Positive Staining in Various Types of Cells from Male and Female Rats after Intrahepatic Transplantation of H27 Hepatoma (rel. units)

Group of animals		Hepatocytes	Tumor cells	
			central	peripheral
Males	intact	61.5 (33; 103)	—	—
	with graft	41* (20; 65)[x]	103.5** (69; 138)	82.5** (55; 111)
Females	intact	81 (46; 122)	—	—
	with graft	80 (56; 122)[x]	268.5** (227; 336)	112** (68; 184.5)

Note. The median values are presented and the lower and upper quartiles are shown in brackets. [x]Hepatocytes from the nontumor liver lobe are considered as hepatocytes of native tissue in animals with graft. *$p<0.05$ and **$p<0.001$ compared to hepatocytes from intact animals.

long isoform triggers the JAK-STAT cascade, while the short isoform serves as a negative regulator of signals from the long isoform [4,8]. For understanding of the role of prolactin in hepatocarcinogenesis it is necessary to take into account not only the number of PrlR, but also the ratio between PrlR isoforms. However, PrlR undoubtedly play an important role in the pathogenesis of some liver tumors.

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