Overexpression of Prolactin Receptors during Intrahepatic Transplantation of RS1 Rat Cholangiocellular Carcinoma Cells

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Immunohistochemical study showed that expression of prolactin receptors in intrahepatically transplanted cells of RS1 cholangiocellular carcinoma 2-fold exceeds that in cholangiocytes of intact rats. The number of prolactin receptors significantly increased in tumor cell nuclei of male and female animals. The RS1 transplant induced overexpression of prolactin receptors in hepatocytes and increased their number in nuclei of these cells.

Key words: *prolactin receptor; cholangiocellular carcinoma; cholangiocytes; hepatocytes; rat*

Prolactin is a pituitary hormone involved in the regulation of function and proliferative activity of various cells, including hepatocytes. Prolactin receptor (PrlR) plays a role in intracellular signal transduction, which is mainly realized via the JAK-STAT mechanism. PrlR expression in liver cells of animals [2] and humans [1,6] increases during proliferation.

Here we studied expression and compartmentalization of PrlR in intrahepatic transplants of RS1 cholangiocellular carcinoma and liver tissue of male and female recipient animals.

MATERIALS AND METHODS

Experiments were performed on adult outbred rats weighing 180-240 g. The animals were divided into groups (5-12 rats per group). The control group included intact rats. The RS1 tumor strain was obtained from the Bank of Tumor Strains (N. N. Blokhin Russian Cancer Research Center). This strain was maintained by subcutaneous transplantation into the hip (1 time per 25-30 days). The suspension of tumor cells from of a subcutaneous transplant was diluted with modified Hanks solution (Sigma) to obtain 40% suspension. Intrahepatic transplantation to narcotized rats was performed. The suspension of tumor cells (0.1 ml) was punctured into the central hepatic 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. We examined the samples of tumor tissue, surrounding liver tissue, and native tissue from the nontumor hepatic lobe. For histological study the tissues were stained with hematoxylin. PrlR localization in tissue samples was determined by indirect immunoperoxidase method with monoclonal U_5 antibodies against rat PrlR (Sigma). We examined 2 treated (in the presence of anti-PrlR antibodies) and 2 control sections (in the absence of anti-PrlR antibodies) from each tissue sample. The study involved 50 tumor cells in tumor tissue, 50 cells in periportal (PP) and pericentral (PC) zones of the liver,

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and all cells in sections of bile ducts. Expression of PrlR did not differ in hepatocytes of PP and PC zones. The results of studying this parameter were combined. Immunopositive staining of PrlR was studied in whole cell, nucleus, and cytoplasm. The degree of PrlR expression was estimated by a semi-quantitative analysis of images from sections obtained under an Axioplan microscope (Zeiss) equipped with a KAF 400 camera (Photometrix) with a CCD matrix using 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 [9]. The data did not conform to a normal distribution (Fig. 1). The results were expressed as medians and upper and lower quartiles (arb. units) and analyzed using Statistica 6.0 software. The significance of differences was evaluated by nonparametric Kruskal—Wallis test and median test. The differences were significant at $p<0.05$.

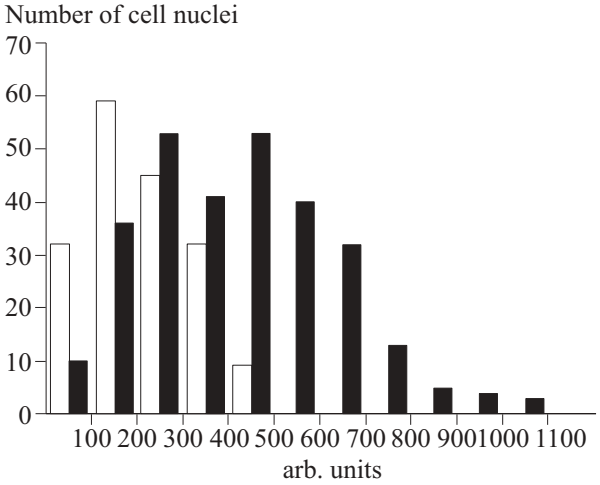

Fig. 1. Immunostaining for prolactin receptors in RS1 tumor cell nuclei of male (light bars) and female rats (dark bars).

RESULTS

PrlR-positive staining was revealed in cell membranes, cytoplasm, and nuclei of cholangiocytes from intact rats (Fig. 2, *a*). Expression of PrlR in the nuclei and cytoplasm of cholangiocytes did not differ in females and males and corresponded to expression in the whole cell (Fig. 3).

PrlR expression in cell compartments of cholangiocytes in female rats was higher than in male rats. Sex differentiation of PrlR expression in cho-

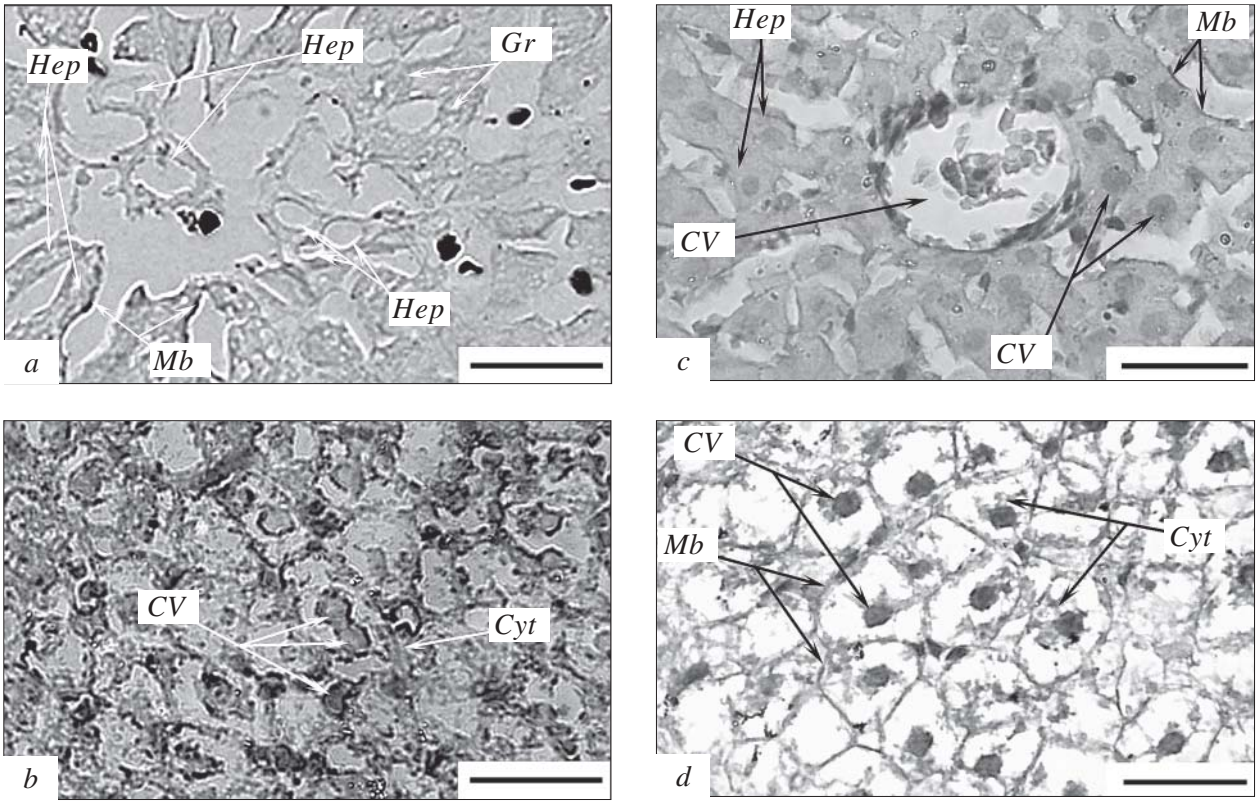

Fig. 2. Immunoperoxidase identification of prolactin receptors in liver cells from the intact female rat (*a*) and RS1 tumor cells from the female rat with intrahepatic RS1 transplant (*b*). Hematoxylin staining of the tumor-surrounding tissue in male (*c*) and female rats with Intrahepatic RS1 transplant (*d*). Arrows: bile duct (*BD*), hepatocyte (*Hep*), central vein (*CV*), cytoplasm (*Cyt*), cytoplasmic granules (*Gr*), nuclei (*N*), and cytoplasmic membrane (*Mb*). Scale 30μ.

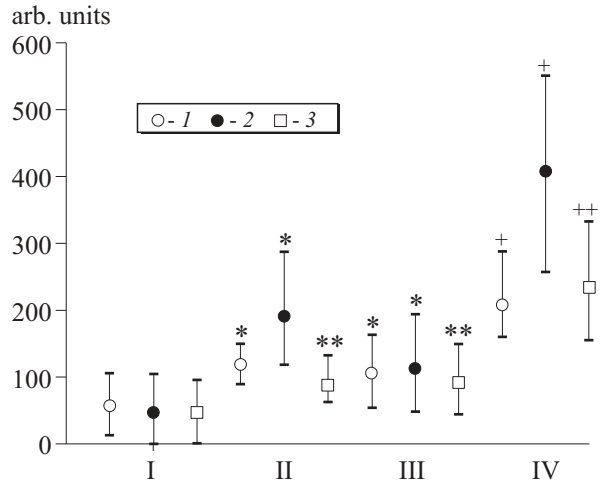

Fig. 3. Expression of prolactin receptors in cell compartments of cholangiocytes from intact male (I) and female rats (III) and intrahepatically transplanted RS1 cells from male (II) and female rats (IV). Expression of prolactin receptors in whole cell (*1*), nuclei (*2*), and cytoplasm (*3*). **p*<0.001 and ***p*<0.01 compared to intact males; ^{+}p<0.001 and ^{++}p<0.01 compared to intact females.

langiocytes of intact rats probably depends on the positive effect of estrogens [5].

Histological study of intrahepatic RS1 tumor on days 25-30 after transplantation confirmed the development of cholangiocellular carcinoma. PrlR-positive staining was found in the nucleus, cytoplasm, and cell membranes of intrahepatically transplanted RS1 tumor cells (Fig. 2, *b*). PrlR-positive staining in the whole cell increased similarly in males and females (by 2.1 and 2 times, respectively; Fig. 3). PrlR expression in RS1 tumor cell nuclei of males and females increased by 4.1 and 3.6 times, respectively, compared to normal cholangiocytes. PrlR expression in the cytoplasm of tumor cells in females increased more significantly than in males (by 2.5 and 1.9 times, respectively, compared to PrlR expression in the cytoplasm of normal cholangiocytes). However, no differences were revealed in sex differentiation of tumor cells.

RS1-surrounding liver tissue and native tissue in males and females retained normal structure of the hepatic lobule (Fig. 2, *c*, *d*). As differentiated from males, hepatocytes in females were vacuolated. PrlR-positive staining was found in the cytoplasm, cell membrane, and nucleus of hepatocytes from intact animals (Fig. 2, *a*). PrlR expression in hepatocyte nuclei was similar to that or lower than in the cytoplasm. The intensity of PrlR-immunopositive staining in hepatocytes of female rats was higher than in males, which agrees with published data [8]. The intrahepatic RS1 transplant induced an increase in PrlR-positive immunoreactivity in hepatocytes of the surrounding and native liver tissue (compared to hepatocytes of intact males and females, Table 1). The intrahepatic RS1 transplant induced accumulation of PrlR in cell nuclei of adjacent and distant hepatocytes. PrlR expression in hepatocyte nuclei from the surrounding tissue of males and females increased by 7.7 and 7 times, respectively, compared to normal hepatocytes. This tumor most significantly affected the liver of female rats. PrlR expression in the whole cell of surrounding tissues in females increased more significantly than in males. The number of PrlR in cell nuclei of native tissue in males and females increased by 5.1 and 7.2 times, respectively, compared to normal hepatocytes. The degree of hepatocyte vacuolation in surrounding and native tissue of female rats was higher than in male rats.

Intrahepatic transplantation of RS1 tumor cells not only increased the sensitivity to prolactin, but also induced accumulation of PrlR in nuclei (compared to normal cholangiocytes of male and female animals). Our previous studies revealed an increase in PrlR expression in nuclei of proliferating hepatocytes and cholangiocytes after ligation of the common bile duct [2,4]. It can be hypothesized that tumor progression is associated with a direct effect of prolactin on the nucleus [3]. The influence of

TABLE 1. Immunocytochemical Staining for Prolactin Receptors (arb. units) in Hepatocytes of Intact Rats and Hepatocytes of Surrounding and Native Liver Tissue in Rats with Intrahepatic RS1 Transplant

Tissue sample	Whole cell	Nuclei	Cytoplasm
Hepatocytes from intact males	62 (33;103)	40 (26;90)	68 (39;100)
Hepatocytes from tumor-surrounding tissue of males	151* (116; 191)	308* (227; 381)	124** (100; 184)
Hepatocytes from native liver tissue of males	170* (138; 215)	202* (145; 293)	141*** (90; 181)
Hepatocytes from intact females	81 (46; 122)	59 (41; 101)	89 (50; 122)
Hepatocytes from tumor-surrounding tissue of females	262^{+} (190; 336)	415^{+} (266; 581)	312^{+} (241; 384)
Hepatocytes from native liver tissue of females	194^{+} (144; 238)	427^{+} (260; 578)	239^{+} (165; 326)

Note. Median value. First number in brackets, lower quartile; second number in brackets, upper quartile. **p*<0.001, ***p*<0.01, and ****p*<0.05 compared to intact males; ^{+}p<0.001 compared to intact females.

prolactin under these conditions is probably realized via the nuclear signaling pathway typical of Nb2 lymphoma cells. This mechanism results in proliferation and growth of cells and is based on the interaction of prolactin with intranuclear cyclophillin B and activation of Stat5a protein [7]. We believe that the nuclear mechanism for prolactin action on tumor cells in males plays a greater role than in females.

Overexpression of PrlR in intrahepatically transplanted RS1 tumor cells and hepatocytes of the surrounding and native liver tissue, as well as accumulation of PrlR in nuclei of these cells, can serve as a specific marker of cholangiocarcinoma.

It remains unclear whether prolactin increases proliferation of RS1 tumor cells or protects the liver under pathological conditions. Our results provide indirect evidence that prolactin has a promoter effect on RS1 tumor growth, which is more pronounced in females. Further studies should be performed to develop new methods for sex-specific therapy of patients with liver carcinoma.

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