IMMUNOHISTOCHEMICAL STUDY

Immunohistochemical detection of MRP1 protein in normal and hyperplastic human thyroid gland

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Abstract

In this study we describe the localization of MRP1 in normal and hyperplastic thyroid tissues. To demostrate this protein we have used the enzyme imunohistochemical method with monoclonal antibody MRPm6. We have found MRP1 to be present in follicular epithelial cells of normal thyroid tissue and higher expression in the same cells of hyperplastic thyroid tissue. The brown colour of the diaminobenzidine reaction product allowed us to define the distribution of stain clearly. ***(Fig. 4, Ref. 16.)***
Key words: immunohistochemistry, thyroid gland, MRP1.

The resistance to anticancer drugs is the major problem in the treatment of tumors. A variety of mechanisms causing resistance have been observed. One of these mechanisms is called multidrug resistance (MDR), which is associated with the elevated expression of multidrug transporter proteins.

Several transporter proteins can be involved in MDR. The first one to be discovered was P-glycoprotein, which is a cell membrane glycoprotein. It was identified by Juliano and Ling in 1976 (Juliano and Ling, 1976). The second one was MRP1 (multidrug resistance-associated protein) found in 1992 (Cole et al, 1992) and LRP (lung tesistance-related protein) found one year later (Scheper et al, 1993). The last multidrug transporter protein is BCRP (breast cancer resistance protein), which has been identified recently (Doyle et al, 1998).

For the first time, MRP1 (multidrug resistance-associated protein) was recognized in 1992 from a multidrug-resistant small cell lung cancer cell line (H69AR) by Cole et al. It is a membrane glycoprotein and belongs to the ATP-binding cassette superfamily of membrane proteins (Cole et al, 1992).

MRP1 was found in all human tissues and in all cell types from peripheral blood (Zaman et al, 1993; Burger et al, 1994). MRP1 expression is relatively high in lung, adrenal gland, heart muscle, and skeletal muscle, and low in liver and brain (Flens et al, 1996; Zaman et al, 1996). In lung, MRP1 is expressed in bronchial epithelium and in alveolar macrophages (Flens et al, 1996; Thomas et al, 1995). MRP1 is also present in epithelial cells in the esophagus, the gastrointestinal tract and the bladder, and in cells with endocrine functions as the adrenal cortex, the islets of Langerhans in the pancreas and the Leydig cells in the testis (Flens et al, 1996). Based on its prominent presence in many epithelia lining external surfaces and in cells with endocrine functions, MRP1 may play a role in the protection of human tissues against xenobiotics and in hormone transport.

The subcellular distribution of MRP1 in normal cells is remarkably different from P-gp. MRP1 is predominantly present in the membranes of intracellular vesicles (Hipfner et al, 1994).

MRP1 gene, encode MRP1 protein, is located on chromosome 16p13.1 (Cole et al, 1992). MRP1 protein consists of 1531 amino acids, involving two halves, each containing an ATP-binding domain and a number of transmembrane segments (Cole et al, 1993). MRP1 is post-translationally modified by glycosylation and by phosphorylation.

This protein is a GSH S-conjugate carrier (GS-X pump). GS-X pumps have a relatively broad substrate specificity. They transport substrates containing a hydrophobic part and at least two negative charges (Jansen et al, 1993; Ishikawa, 1992). MRP1 transporter exports toxic GSH S-conjugates from cells and releases the inflammatory mediator leukotriene C4 from mast cells. This protein may also mediate the release of oxidized glutathione from cells under conditions of oxidative stress (Ishikawa, 1993). MRP1 may transport anionic metabolites of substances rather

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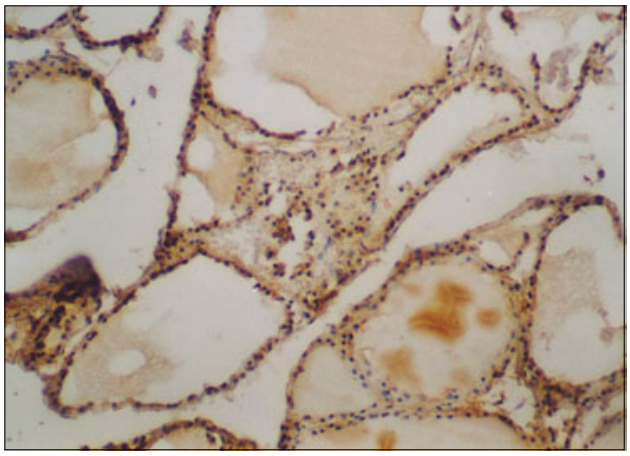
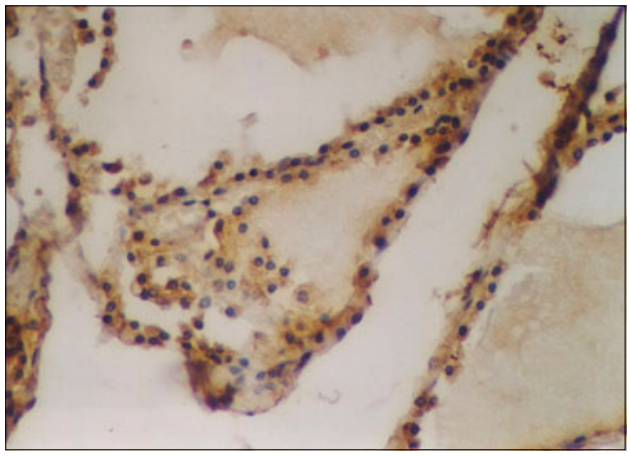
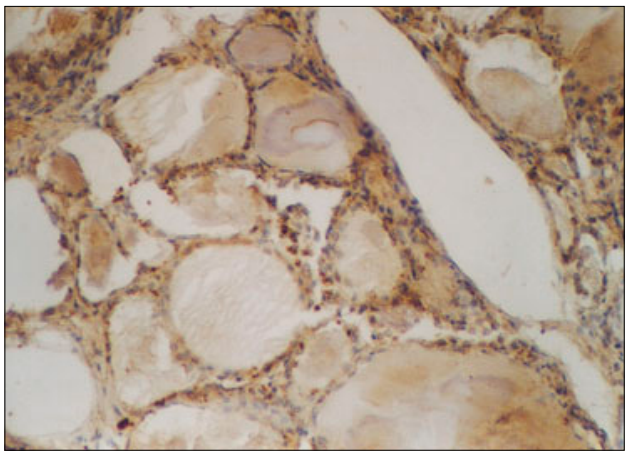
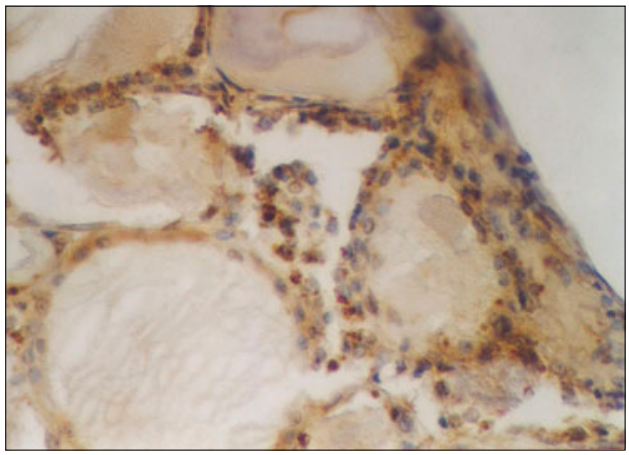




Figs 1, 2. Demonstration of MRP1 expression in normal human thyroid gland as detected by immunostaining of parafine tissue sections with MRPm6. We can see MRP1 positivity as a dark brown staining of follicular cells (Fig. 1 – 10x10, Fig. 2 – 20x10).





Figs 3, 4. MRP1 reactivity of hyperplastic thyroid gland with the MRPm6 monoclonal antibody shows higher expresion in cytoplasma of follicular cells (Fig. 3 – 10x10, Fig. 4 – 20x10).

than substances themselves (Jedlitschky et al, 1994). There are several ways by which substances can be modified within the cell and that add negative charge, i.e. conjugation to GSH, glucuronidation, sulfonation.

Material and methods

Positive control

Indirect enzymatic immunohistochemical method was used to detect MRP1 in paraffine sections of normal (n=5) and hyperplastic (n=10) thyroid tissue. Paraffine sections of tissue samples were cut 4 μm, they were then deparaffinized and washed with phosphate-buffered saline containing 0.05 % Tween-20 (PBS-Tw), pH 7.6. Endogenous peroxidase activity was blocked using 3 % H_2O_2 in methanol for 30 minutes at room temperature. Sections were pretreated in citrate buffer solution in a microwave owen for 20 minutes. MRP1 staining continued by exposing the primary antibody MRPm6, in humidified chamber at 4 °C overnight. All sections were properly washed in PBS-Tw 3x5 minutes and subsequently incubated with secondary antibody (prediluted biotinylated horse antibody – Vector Laboratories, USA) for 30 minutes. After rinsing the streptavidine-peroxidase (prediluted R.T.U. Vectastain – Vector Laboratories, USA) was applied for 30 minutes. The slides were then incubated with 3,3'-diaminobenzidine tetrachloride (DAB) at a concentration of 0.5 mg/ml in Tris buffer, pH 7.6 and 0.015 % H_2O_2. Sections were stream-rinsed with tap water, washed in tap water, mounted and coverslipped.

Negative control

Negative control included PBS-Tw alone in place of primary antibody.

Results

We have detected the localization of MRP1 in normal and hyperplastic thyroid tissue. We have found MRP1 to be present in follicular epithelial cells of normal thyroid tissue. Higher expression was found in the same cells of hyperplastic thyroid gland.

The brown colour of the diaminobenzidine product allowed us to define the distribution of stain clearly.

The negative control served as a proof of correct immunostaining methodology, sensitivity of antibodies and other chemicals we have used. In this case no reactivity was observed and no MRP1 positive structure was found. The whole tissue section stayed light grey showing no MRP1 positivity.

Discussion

Using immunohistochemistry we have found MRP1 to be present in normal and hyperplastic parenchyma of thyroid gland.

The thyroid gland, brownish-red and highly vascular, is placed anteriorly in the lower neck. Its undersurface localization allows easy surgical removing for other histochemical procedures. The thyroid parenchyma is separated into follicles, each with a central coloid core surrounded by simple epithelium and basal lamina. Proper follicular cells vary from squamous to columnar, depending on their activity, mainly controlled by circulating hypophyseal thyrotropin (TSH). Prolonged high levels of circulating TSH induce follicular hypertrophy and even hyperplasia with progressive resorption of coloid. In the absence of TSH, follicular cells are squamous and resting with luminal coloid being abundant. Follicular epithelial cells produce tri-iodothyronine (T3) and tetra-iodotyronine (T4). Thyroid parenchyma contains another, parafollicular cell type (C cells) producing the peptide hormone thyrocalcitonin.

The thyroid gland is not directly exposed to xenobiotics (i.e. lung or kidney) and the expression of MRP1 is not so high as in the above organs. We have detected higher concentration of MRP1 in follicular cells of hyperplastic in comparison with normal parenchyma of thyroid gland. We would like to find an explanation for the differing levels of MRP1 in normal as opposed to hyperplastic thyroid gland.

There are several mechanisms, which lead to hyperplasia (goiter). The major cause is a deficiency of iodine in diet (iodized salt is an effective dietary prevention). The other conditions include familial dyshormonogenesis (a genetic deficiency of any one of the six enzymes that are required to synthesize and secrete T3 and T4), goitrogenic food or medications (e.g. cyanates, isocyanates, resorcinols, cobalt, phenothiazines, lithium ets.) and periods of excessive hormonal activity (adolescence, pregnancy, menopause).

We have found some relation between one of these mechanisms and overexpression of MRP1. It is difficult to demostrate whether the goitrogenic food or medications are responsible for higher expression of MRP1 in follicular cells of hyperplastic thyroid gland. Our hypotesis is supported by the fact, that MRP1 may transport anionic metabolites of substances having the same chemical properties as substances of goitrogenic food or medications. Both of these substrates have a negative charge, so they should be substrates of MRP1.

Our suggestion has not been confirmed by any similar experimental studies, i.e. we have not found any information on this problem so far.

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