Immunohistochemical analysis employing a monoclonal antibody nm23-H1 (the antibody against nm-23 protein) was performed on archival material, consisting of 12 cases of follicular thyroid carcinoma (FTC), 57 cases of follicular thyroid adenoma (FTA) and 17 cases of nodular goiter (NG). Both cytoplasmic and nuclear immunoreactions for nm-23H1 were observed in cells of FTCs, FTAs and NGs. In oxyphilic adenomas cytoplasmic staining was observed. Eleven (91.7%) cases of FTC, 55 (98.2%) cases of FTA and 14 (82.4%) cases of NG were found to be positive for nm23-H1 protein. There were no statistically significant differences in the mean percentage values of immunopositive cells between carcinomas and adenomas. A significant increase in the number of cases with high percentage (more than 50) of positive cells was found in both carcinomas (FTCs) and adenomas (FTAs) – mainly microfollicular ones, in comparison with nodular goiter. It can be concluded that highly positive immunoreaction for the nm23-H1 protein in the cells of carcinomas (FTCs) and microfollicular adenomas indicates for a high proliferation rate of these tumors.

Introduction

The human nm23 gene is located on chromosome 17q22 and comprises two closely related genes, nm23-H1 and nm23-H2 [1, 2]. These genes encode two polypeptide A and B subunits of nucleoside diphosphate kinase (NDPK) (EC 2.7.4.6), respectively. The NDPK enzyme catalyses the transphosphorylation of nucleoside diphosphates (NDP) to nucleoside triphosphates (NTP) and plays a role in the process of the cell division and also in the signal transduction through G proteins [3, 4]. The nm23 mRNA expression in late S-phase of the cell cycle suggests that nm23 may play a role in mitotic microtubule spindle polymerization [4]. It is hypothesized that proteins of the NDP kinase family may act as positive or negative regulators of metastatic process [3, 5].

The nm23-H1 gene encodes a 17-kilodalton cytoplasmic and nuclear protein that has been recently shown to be reduced in a number of human carcinomas [6]. A relationship between nm23 protein expression and tumor stage was found e.g. in pituitary tumors [7], malignant melanomas [8, 9], laryngeal carcinoma [6, 10], renal cell carcinoma [11, 12] and colorectal carcinomas [5]. The nm23
gene expression in thyroid tissues has not been fully understood as yet [13].

The aim of this study was to examine the expression of nm23-H1 protein in benign and malignant follicular thyroid tumors.

**Material and Methods**

**Material**

Paraffin embedded, archival tissues of 12 cases of follicular thyroid carcinoma, 57 of follicular thyroid adenomas and 17 nodular goiters were studied. All the sections were independently examined by two experienced pathologists (J.S. and S.S.) using a computer microscope and then histopathologically classified, according to the WHO Committee [14].

**Immunohistochemical staining**

Representative paraffin blocks containing tumor from each case were sectioned at 4µm, affixed to silanised slides and dried overnight at 56.7°C. The sections for immunohistochemistry were stained using the avidin-biotin (ABC) method [15]. Deparaffinized sections were treated with 3% hydrogen peroxide (H2O2) in methanol for 5min to block endogenous peroxidase activity. Incubating the sections for 20min with normal goat serum reduced non-specific antibody binding. The slides were incubated with a 1:250 dilution of the primary mouse monoclonal antibody (nm23-H1, clone 37.6 from Novocastra Laboratories Ltd, Newcastle upon Tyne, UK). In the negative control reaction the primary antibody was omitted. The reaction product was demonstrated using the Novostain Super ABC kit (NCL/ABCm) from Novocastra. 3,3’-diaminobenzidine (DAB) was used as chromogen, and the sections were counterstained with Mayer’s haematoxylin, dehydrated and mounted. The paraffin-embedded sections from ductal breast carcinoma were used as positive control. The immunohistochemical staining of cells was estimated by means of the semiquantitative method with the use of Hogg’s net. We considered as positive only the cases displaying cells with nuclear staining or both nuclear and cytoplasmic staining. The results were expressed as percentage of positive cells per 1000 follicular thyroid cells, counted in 10 HPF (objective magnification x40). The relative number of immunoreactive cells was graded as follows: negative reaction (−) – less than 10% of positive tumor cells, positive reaction (+) – 10–50% of tumor cells were stained positively, highly positive reaction (++) – more than 50% of tumor cells were stained positively [16].

**Statistical procedure**

All the parameters represented as the mean percentages of positively staining cells were compared using Mann-Whitney test, where p<0.05 was considered significant. Associations of the categorical variables with nm23-H1 expression were assessed using Fisher’s exact test.

**Results**

In the thyroid follicular carcinomas (FTCs), follicular adenomas (FTAs) and nodular goiters (NGs) the positive immunoreaction for the nm23-H1 protein was found both in the nucleus and the cytoplasm of cells (Fig. 1). The mean percentage values of the positive cells were 54.4 (SD=31.2) in the group of FTCs, 47.7 (SD=22.4) for FTAs and 21.6 (SD=14.2) for NGs. No statistically significant difference in the mean percentage values of the nm23-H1 positive cells was found between FTCs and FTAs. However, the differences between FTCs and FTAs, as compared to NGs, were statistically significant (p<0.001).

In the FTA group, the mean percentage values of the positive cells were 53.8 (SD=21.0) for microfollicular adenomas and 44.7 (SD=23.1) for normo- and macrofollicular adenomas (Table 1).

In oxyphilic adenomas, 30.9% of the cells exhibited positive immunoreaction with prevailing cytoplasmic staining pattern.

A highly positive reaction, i.e., more than 50% of stained tumor cells (++), was observed in 6 (50.0%) cases of follicular carcinoma (FTC), 23 (40.3%) cases of follicular adenoma (FTA) and 1 (5.9%) case of nodular goiter (NG) (Table 2). The differences were statistically significant for comparison FTC/NG (p<0.05) and for comparison FTA/NG (p<0.01). In the group of follicular adenomas (FTAs) a highly positive reaction for nm23H-1 protein was revealed in 16 (51.6%) microfollicular adenomas, 6 (33.3%) normo- and macrofollicular adenomas and 1 (12.5%) oxyphilic adenoma. There were a significantly higher number of cases with highly positive immunoreaction in the group of microfollicular adenomas in comparison with the group of oxyphilic adenomas (p<0.05).

In total, nm23H-1 protein was observed in 11 (91.7%) FTCs, 56 (98.2%) FTAs and 14 (82.4%) nodular goiters.
The differences between values were not statistically significant.

**Discussion**

The results presented in this paper demonstrate that nm23H-1 protein was detected immunohistochemically in cells of both benign and malignant follicular tumors, derived from thyroid follicular cells. The majority of the study cases revealed both nuclear and cytoplasmic staining. The number of cases showing only nuclear or only cytoplasmic immunostaining for nm23H-1 protein was low. Differences were observed in the intensity of nuclear and cytoplasmic staining in all the study groups.

**TABLE 1**

The percentage of nm23H-1 positive cells in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular carcinoma (FTC)</td>
<td>12</td>
<td>54.4*</td>
<td>31.1</td>
<td>2.4</td>
<td>98.6</td>
</tr>
<tr>
<td>Follicular adenoma including:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- microfollicular</td>
<td>57</td>
<td>47.7*</td>
<td>22.4</td>
<td>5.7</td>
<td>93.7</td>
</tr>
<tr>
<td>- normo-and macrofollicular</td>
<td>31</td>
<td>53.8</td>
<td>21.0</td>
<td>14.5</td>
<td>92.5</td>
</tr>
<tr>
<td>- oxyphilic (Hürthle)</td>
<td>18</td>
<td>44.7</td>
<td>23.1</td>
<td>13.5</td>
<td>93.7</td>
</tr>
<tr>
<td>Nodular goiter</td>
<td>17</td>
<td>21.6</td>
<td>14.2</td>
<td>3.2</td>
<td>63.7</td>
</tr>
</tbody>
</table>

N – number of cases, M – mean arithmetic, SD – standard deviation, Min – minimal value, Max – maximal value; * – significantly higher in follicular carcinoma and follicular adenoma than in nodular goiter (p<0.0001).

**TABLE 2**

The number of nm23H-1 positive and negative cases in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>++</th>
<th>N (%)</th>
<th>+</th>
<th>–</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular carcinoma (FTC)</td>
<td>12</td>
<td>6</td>
<td>(50.0)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Follicular adenoma including:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- microfollicular</td>
<td>57</td>
<td>23</td>
<td>(40.3)</td>
<td>33</td>
<td>1</td>
</tr>
<tr>
<td>- normo-and macrofollicular</td>
<td>31</td>
<td>16</td>
<td>(51.6)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>- oxyphilic (Hürthle)</td>
<td>18</td>
<td>6</td>
<td>(33.3)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Nodular goiter</td>
<td>17</td>
<td>1</td>
<td>(5.9)</td>
<td>13</td>
<td>3</td>
</tr>
</tbody>
</table>

(++) – highly positive cells, N – number of cases studied, n (%) – number (percentage) of positive or negative cases
Bertheau et al. [13] found nuclear immunoreaction for nm23H-1 in 93% of cases of normal thyroid tissue, whereas a decrease in its occurrence was observed in benign thyroid tumors (46%), in primary thyroid carcinomas (28%) and in a recurrent and metastatic cancer (31%). In follicular carcinomas, they observed 45% of cases with nuclear staining and 2 cases with immunostained cell membranes. They emphasized this localization as cancer specific. The authors interpreted the nuclear nm23H-1 immunostaining as a good prognostic marker, as far as a disease-free survival was concerned. We are not able to confirm these assumptions, as the period of observation was short in the majority of our cases. However, we should stress that in one patient, in who a FTC dissemination was stated (vertebral metastases), the percentage of immunopositive cells was rather low (25.6). In our material obtained from 12 cases of FTC, nuclear and cytoplasmic immunostaining was observed in 9 cases (75%), while other 3 revealed exclusively cytoplasmatic immunoreaction for the nm23-H1 protein. In 7 out of 9 immunopositive FTCs nuclear staining predominated, whereas in 2 cases the cytoplasmic one.

Various patterns of the nm23H-1 immunostaining in follicular benign thyroid tumors were described by Bertheau et al. [13]. In the group of 10 FTAs they found nuclear staining in 6 (60%) cases and membrane staining in 2 (20%).

The role of the nm23H-1 gene product in the cell and especially its distribution within the cell nucleus, cytoplasm and cell membrane is still being discussed in relation to its suppressor role exerted on the process of metastasis and relapse of various cancers, including thyroid tumors. Zou et al. [17] studied the expression of the nm23H-1 gene at the mRNA transcription level in tissues of nodular goiter, as well as benign and malignant thyroid neoplasms. The authors found the expression of the nm23H-1 gene both in benign and malignant thyroid tumors. However, they did not show any correlation between the level of the nm23H-1 gene in undifferentiated thyroid carcinomas and its anti-metastatic potential. The authors suggested that the high level of nm23H-1 gene product, which they observed in advanced thyroid cancers, might reflect its role in cell proliferation. On the other hand, Arai et al. [18] observed a decrease in the expression of the nm23H-1 gene, but not the nm23H-2 gene in cells of papillary thyroid carcinomas in patients with metastases to lymph nodes. We observed even a higher proportion, with 98.2% of positive results, in FTA. The only negative case was an oxyphilic adenoma. In the group of oxyphilic adenomas cytoplasmic staining was stronger than nuclear reaction. This fact may result from the biological differences demonstrated by these neoplasms in comparison to the typical form of follicular thyroid cancers.

Oberved by us highly positive immunoreaction for nm23H-1 protein in cells of FTCs and FTAs – mainly the microfollicular ones – indicates a high proliferative potential of these tumors.

References


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