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ORIGINAL ARTICLE

Cyclin D1 expression in primary thyroid carcinomas

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Abstract

OBJECTIVES: The aim of the study was to demonstrate and evaluate the expression of cyclin D1, a protein connected with a cell cycle, by means of the immunohistochemical method in malignant thyroid neoplasms. The purpose of the analysis of the results was to explain the relation between cyclin D1 in thyroid cells and neoplasm transformation.

MATERIAL AND METHODS: The study was conducted on thyroid neoplasms from 35 patients who were diagnosed with the thyroid carcinoma (30 women and 5 men). Detection DAKO LSAB + system was applied with use of monoclonal antibodies against cyclin D1. The results of immunohistochemical reaction was described as an index (percentage of cells showing a characteristic brown color in 1000 counted cells). As a positive result of reaction an intensive brown color of carcinomas cellular nuclei was acknowledged.

RESULTS: The mean value of cyclin D1 expression index in papillary carcinoma was 14,44% ± 9,37, in medullary carcinoma 27,35% ± 5,40, in nonpapillary carcinomas originating from A cells 18,0%±10,20.The results were statistically analyzed. In medullary carcinoma the highest values of positive cells cyclin D1 index were revealed.

CONCLUSIONS: The results obtained encourage continued studies on cyclin D1 expression in thyroid neoplasms and a more accurate analysis with a larger number of cases. Perhaps the index of this protein will become a recognized prognostic marker in thyroid neoplasms or an objective risk factor of the thyroid epithelial cells neoplastic transformation.

Introduction

Cyclin D1 is a protein product of a gene CCND1 (PRAD-1,bcl-1) located on chromosome 11 (locus 11q13). It is a protein having mass of 36 kDa built of 259 amino acids and playing a critical role in controlling of a cell cycle. It is a part of a molecular complex regulating transfer of a cell from phase G1 to S of a cell cycle. The cyclin reaches its highest accumulation in a late phase G1 and vanishes when a cell is in a phase S of a cycle. Synthesis of cyclin D1 is stimulated by growth factors inducing non active cells to join a cell cycle [1,2,3,4,5,6].

The disproportionate accumulation of cyclin D1 accompanies excessive proliferation of cells that have a shortened phase G1 and do not react

on the physiological growth factors [1]. Over-expression may be a result of the multiplying of a gene, viral insertions in the cyclin's gene area, translocation of fragments of chromosomes, or caused by an increased sensitivity of neoplasms' cells on an external signals stimulation, e.g. hormones [2,7,8,9]. Impairment of genetic apparatus resulting in changing the level of appropriate factors regulating the cell cycle as well as pathological factors precluding proper function of the internal control points causes homeostasis disorders of the cell. Impairment of the homeostasis is the reason for pathology leading to among other things neoplasmatic transformation. In most of the examined neoplasms of a digestive system and parathyroids, the gene coding cyclin D1 undergoes amplification that causes a considerable rise of its synthesis [9,10]. The amplification of the area 11q13 was revealed in tumors such as: breast, liver, larynx, esophagus, head and neck, ovary, lungs and bladder carcinomas [1,3,5,10,11,12]. The excessive accumulation of cyclin D1 was noted in parathyroid adenomas and carcinomas. It is the result of the translocation of the gene cyclin D1 to locus 11q15 where parathormon gene is located [1].

The gene CCND1 is identical with protooncogene bcl-1 and its increased transcription is observed in malignant mantle-cell lyphomas [1]. A characteristic translocation t(11;14) (q13;32) in these lymphomas is responsible for an increased synthesis of cyclin D1, which leads to joining of the gene CCND1 with the gene of the heavy chains immunoglobulins. As a consequence an increased transcription and translation of cyclin D1 gene occurs which leads to an increased cyclin D1 expression in lymphoma's cells [1,2,3,9,13].

The aim of the study was to assess by means of the immunohistochemical method, cyclin D1 expression in thyroid carcinoma cells as well as investigation of the correlation between cyclin D1 expression and a microscopic type of thyroid gland carcinoma.

Material and methods

The study was conducted on thyroid neoplasms from 35 patients who were diagnosed with the thyroid carcinoma (30 women and 5 men). The analysis was performed on the post-surgical specimens assigned to routine histopathological diagnosis in the year 2002 in the Department of Patomorphology of the Medical University in Lodz. The tumors were classified according to the World Health Organization's Histological Typing of Thyroid Tumors. The material consisted of:18 cases of papillary carcinoma, 1 case of classic type of follicular carcinoma, 8 cases of oxyphilic variant of the follicular carcinoma, 2 cases of insular carcinoma. 2 cases of anaplastic carcinoma, 4 cases of medullary carcinoma.

The average age of the patients was 55,5 ± 17,8 and ranged from 16 to 87 years.

The postoperative material was fixed in 10% buffered formalin and embedded in paraffin. After routine histopathological evaluation and diagnosis, single section was selected for immunohistochemical study. Sections (3μm thick) were deparaffinized and rehydrated through a series of xylene and alcohol. After antigen retrieval with microwave treatment in 10mM citrate buffer (pH 6.0), endogenous peroxidase activity was blocked in 3% hydrogen peroxide. Immunohistochemical staining was performed with mouse monoclonal antibody to cyclin D1 (Clone: DCS-6, DAKO/Denmark) at a 1:30 dilution. For detection the DAKO LSAB+ System, HRP was used. A diaminobenzidine (Liquid DAB+, DAKO/Denmark) was applied as chromogen. The sections were counterstained with modified Mayer's hematoxylin. Either brown and homogenous or granular staining of nucleus was regarded as positive results. Sections of the mantle-cell lymphoma, known to express high level of cyclin D1, were used as positive controls. Negative controls were obtained by omitting primary antibody.

The preparations were evaluated by means of a computer system for microscopic image analysis. The system configuration include an IBM-compatible computer (with Pentium processor) with an ADDA picture digitalization card and Panasonic color video camera, linked with a Olympus study microscope (Tokyo, Japan). The sections were examined in a light microscope at 400 X magnification. Antigen expression was studied in 1000 cells of tumor, calculating the percentage of immunopositive cells, pointed by mouse cursor. The statistical analysis of obtained results was performed using the "Statistica" program, version 5.0 PL.

In order to check the distribution pattern of the results Lillefors (Kolmogorov-Smirnov) test was applied (for all the investigated groups). For conducting a further statistical analysis the following tests were performed: Levenes test for homogeneity of variance for particular types of neoplasms studied with the significance level $p<0,05$ and analysis of the variance of the indexes for particular types of neoplasms studied with the significance level $p<0,05$. Because variances differ slightly, the test of comparing post-hoc mean values was conducted (the smallest significant differences) with the significance level $p<0,05$. For the calculation of the correlation between age (or sex) and the expression of cyclin D1 (regardless the thyroid neoplasm type) the t-Student test was performed.

Results

Positive staining of neoplasmatic cells nuclei was revealed in all thyroid neoplasms studied and analyzed by immunohistochemical methods. Cytoplasmatic pattern of the staining has never been revealed.

For statistical purpose, anaplastic, oxyphilic, insular and follicular carcinomas were grouped into nonpapillary carcinomas originating from A cells. Cyclin D1 expression was presented as an index (% of the cells demonstrating the presence of this protein among 1000 counted cells). The average value and standard deviation was counted for the particular thyroid neoplasms.

The list of mean values of the positive cell percentage for cyclin D1 is presented in Table I.

While researching whether the relationship exists between the age of the patients and cyclin D1 expression in thyroid neoplasms cells regardless the type, the results presented in Table II were obtained. All the patients were divided into two groups younger and equal 50 years and older than 50 years. There were no correlations found between the age of the patients and cyclin D1 expression.

Any correlation between the patients sex and the expression of cyclin D1 in thyroid neoplasms, regardless of its type was not revealed. The results are listed in Table III.

The distribution pattern of the results was as follows (Lillefor's test):

- medullary carcinoma – normal distribution,
- papillary carcinoma – normal distribution,
- nonpapillary carcinomas originating from A cells – normal distribution,
- the age of the patients (≤50 and >50 years) – normal distribution,
- the sex of the patients (women and men) – normal distribution.

From the results of analysis of the variance of the indexes for particular types of neoplasms (Table IV) it may be concluded that:

- medullary carcinoma differs substantially in higher index of cyclin D1 from papillary carcinoma but does not differ from carcinomas originating from A cells,
- papillary carcinoma does not differ significantly in expression of cyclin D1 from nonpapillary carcinomas originating from A cells.

Table I. Percentage of immunopositive cells for cyclin D1. Data are expressed as mean±SEM.

Type of carcinoma expression	Cyclin D1
medullary	27.35 ± 2.7
papillary	14.44 ± 2.2
nonpapillary	18.0 ± 2.8

Table II. Analysis of expression of Cyclin D1 in thyroid neoplasm cells in younger and older groups of patients. Data are expressed as mean±SEM.

Age	No of cases	**Cyclin D1 expression**
≤50	15	18.54 ± 2.43
>50	20	16.27 ± 2.35

Table III. Analysis of the expression of cyclin D1 in thyroid neoplasm cells according to sex. Data are expressed as mean±SEM.

Sex	No of cases	**Cyclin D1 expression**
F	30	16.11 ± 1.86
M	5	24.02 ± 2.36

Table IV. Analysis of variance of the index of cyclin D1 expression for particular types of neoplasm (Levenes test).

Type of carcinoma	papillary index = 14.44	nonpapillary index = 18.0
medullary index = 27.35	0.018380*	0.091781
papillary index = 14.44	–	0.304711

* statistically significant

Discussion

The analysis of cyclin D1 expression by means of immunohistochemical method has been the subject of many studies in human neoplasms. In the current study, the positive immunohistochemical nuclear reaction for cyclin D1 was noted in 100% of the cases of thyroid neoplasms types studied. Wang et al. [2] studying 59 cases of follicular type of papillary carcinoma and 57 cases of follicular adenoma stated a positive reaction in only 63% (37/59) of the cases of follicular type of papillary carcinoma and only 60% (34/57) of the cases of follicular adenoma. Moreover, their studies have shown that normal thyroid tissue does not demonstrate cyclin D1 expression but there occurs a strong expression p27 that correlates with a state of repose of normal thyroid cells.

Similar results concerning cyclin D1 expression in normal thyroid cells were obtained by Saiz et al. [13]. However, when analyzing 8 thyroid follicular carcinomas and 19 papillary carcinomas they demonstrated a positive nuclear reaction in all cases of both tumors types and these data are close to ours. Kim et al. [7] noted cyclin D1 expression in 79%(19/24) cases of papillary carcinoma. Close results were obtained in studies conducted by Khoo et al. [4], where 63,2% cases of papillary carcinoma demonstrated cyclin D1 expression. Lazzereschi et al. [11] stated cytoplasmatic localisation of cyclin D1 expression in 40% of normal thyroid tissue and 72% cases of follicular adenoma. The same authors, in 12 out of 19 cases of papillary carcinoma, noted a positive cytoplasmatic reaction for cyclin D1. The nuclear reaction was demonstrated only in 6/19 cases of this type of neoplasm. According to Lazzereschi et al. [11], cyclin D1 expression is most often a result of amplification or translocation of a gene into the other chromosome location. However, it cannot be acknowledged as the only mechanism leading to the expression of this protein. The amplification of CCND1 gene was also noted in neoplasms originating from other tissues e.g. oesophageal carcinoma, head and neck carcinoma, small cel lung carcinoma.

In our own immunohistochemical studies, no cytoplasmatic location of cyclin D1 was noted, in contrast

to some literature reports. Studies of Wang et al. [5] showed a nuclear reaction in 82% of classical papillary thyroid cancer cases and 70% of cases of a minimally invasive follicular carcinoma. In the case of tall cell and columnar cell variant of papillary carcinoma, percentage of positive cells reached as high as 92%. Moreover, they obtained a positive reaction in 100% cases of insular carcinoma and 77% cases of anaplastic carcinoma. It should be noticed that in the cases of thyroid neoplasms characterizied by more aggressive behavior the percentage of cells showing a positive nucleus reaction was higher in Wang and al. [5] studies. These results which present an increased cyclin D1 expression in a more biologically aggressive thyroid tumors in comparison with a minimally invasive follicular carcinoma suggest that expression of this protein plays a substantial role in a neoplasm progression and may have prognostic significance.

In analyzing the results of our studies it is noted that the percentage of a cyclin D1 positive cells is also higher for a more aggressive types of thyroid neoplasms. For medullary carcinoma this percentage amounts a value of 27,4% while for papillary carcinoma it is 14,4%.

Goto et al. [6] analyzed 146 cases of thyroid neoplasms, where 123 constituted papillary carcinomas, 18 follicular carcinomas, 5 undifferentiated carcinomas and 33 follicular adenomas. Those researchers showed a higher cyclin D1 expression both in well and poorly differentiated thyroid carcinomas than in follicular adenomas. However, they did not find significant differences between the histological carcinoma type and cyclin D1 expression.

Literature differences concerning cyclin D1 expression in thyroid neoplasms became an impulse to conduct our own studies on this subject. Results of our study suggest that the percentage of cyclin D1 positive cells in medullary carcinoma is much higher than in malignant neoplasms originating from follicular epithelium what has been not described so far. Cyclin D1 expression in papillary carcinoma and other carcinomas originating from A cells is close but still significantly different than in medullary carcinoma. Another important aspect is that we found a very low index of cyclin D1 in two cases of insular carcinoma, a rare but very invasive neoplasm.

An important issue arises then, whether intense study of cyclin D1 may give practically useful results. After all, the method is rather simple and possible to apply in routine post-surgical specimens. It is accessible to complete every microscopic study result by the expression index of cyclin D1 that could suggest an invasive tendency of neoplasmatic cells.

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