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Analysis of p27^{KIP1} Protein and Ki-67 Expression in Aggressive Fibromatosis (desmoid tumor)

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Aggressive fibromatosis (desmoid tumor) is an uncommon locally invasive non-metastasizing neoplasm lesion. Desmoid tumor consists of fibroblasts, miofibroblasts and a significant amount of extracellular matrix. p27^{KIP1} (p27) protein is a member of the universal cyclin-dependent kinase inhibitor (CDKI) family that regulates progression through the cell cycle. In various human neoplasms the decreased level of p27 was observed.

There were analysed 42 specimens of aggressive fibromatosis, in which there were 24 abdominal and 18 extra-abdominal cases. There was performed immunohistochemical analysis employing a monoclonal antibody against p27 protein and Ki-67 (Novocastra, UK). The sections for immunohistochemical study were stained using the streptavidin – biotin method. The average percentage of cells stained positively for all cases for p27 and Ki-67 was 22.1% (SD=29.2) and 6.0% (SD=8.8) respectively. There was no statistically significant difference between Ki-67 or p27 expression in abdominal and extra-abdominal location.

Analysis of p27 and Ki-67 expression levels might indicate that low proliferating activity of desmoid fibroblasts is connected with another mechanism than the one, in which p27 takes part.

Introduction

Aggressive fibromatosis, also referred to as desmoid tumor or deep fibromatosis, is a mesenchymal neoplasm, which develops from muscle connective tissue, fasciae and aponeuroses [29]. Although it presents a tendency to infiltrate the surrounding tissues and structures, it rarely metastasizes. Desmoid tumor consists of fibroblasts, miofibroblats and a significant amount of extracellular matrix (ECM), especially collagen bundles [1]. Taking under consideration its location, aggressive fibromatosis may be classified as: extra-abdominal, abdominal and intra-abdominal [29].

The aetiology of desmoid tumor is not clear. Nevertheless, few causes of this neoplasm are indicated such as: genetic factor, trauma or endocrine agent (review Ferenc et al. [8]) [10, 24]. Although aggressive fibromatosis is a benign tumor, it tends to recur after complete resection [16].

A multifunctional p27^{KIP1} (p27) protein, together with p21^{WAF1} and p57^{KIP2} is a member of CIP/KIP family which takes part in cell cycle regulation. The proteins gathered in this family act as inhibitors of cyclins-CDK kinases such as cyclin E-CDK2, cyclin A-CDK2, cyclin D-CDK4 and cyclin B-CDK1 [4, 11, 21, 27, 28]. The lack or depleted level of p27 is often a hallmark of many tumors and might be a predictor of poor diagnosis [6, 17, 23, 25].

The aim of this study was to estimate the level of p27 and Ki-67 expression in the examined cases of aggressive fibromatosis and to consider its importance in cell cycle regulation in this neoplasm.

Material and Methods

Material

42 formalin-fixed, paraffin-embedded tissue blocks of aggressive fibromatosis (desmoid) were studied, among

which there were 24 abdominal and 18 extra-abdominal cases. All the sections were independently examined by two experienced pothologists (A.K. and J.S.), using a conference microscope and were histopathologically classified, as recommended by Weiss and Goldblum [29].

Immunohistochemical screening

Each tumor section, 4 μm thick, was deparaffinized and subjected to antigen retrieval by microwaving in 10 mM of citrate buffer (sodium citrate, pH 6.0) for 15 min or by incubation in water bath in Target Retrival Solution Citrate pH 6.0 (code: S2369, DAKO Cytomation, Denmark) for 40 min at 95–99°C, respectively for Ki-67 and p27. Endogenous peroxidase activity was blocked by incubating the deparaffinized sections in 3% hydrogen peroxide (H_2O_2) for 5 min. Nonspecific antibody binding was reduced by incubation of the sections for 10 min with normal horse serum only for Ki-67 staining. The sections were incubated with anti-Ki-67 (clone: MM1, Novocastra, UK) and anti-p27 (clone: 1B4, Novocastra, UK) monoclonal antibodies at dilution of 1:120 and 1:50 respectively, for 1 hour at room temperature. In the negative control reaction the primary antibody was omitted. This was followed by incubation with the Novocastra Universal Detection Kit (NCL-RTU-D) for Ki-67 and with LSAB®+SYSTEM-HRP reagent (code: 0690, DAKO Cytomation, Denmark) for p27. 3,3'-diaminobenzidine (DAB) was used as a chromogen to yield brown reaction products. The sections were counterstained with Mayers hematoxylin, dehydrated and mounted.

The paraffin embedded sections from tonsil were used as positive control for Ki-67 and p27. For each case of desmoid the immunohistochemical reaction for CD34 (clone: QBEnd/10, Novocastra, UK) was performed.

The immunohistochemical staining of cells was estimated by means of the qualitative method using computer program IMAGEJ v. 1.34. The percentage of positive cells per 250–900 aggressive fibromatosis cells, counted with 40× magnification of objective lens, was used to express the results, which were graded into four groups: (-) – less than 10% of tumor cells stained positively; (+) – 10–50% of tumor cells stained positively; (++) > 50% of tumor cells stained positively. Lesions scored as (++) were considered as showing high expression (overexpression) of the protein.

Statistical procedure

The basic statistical analysis (arithmetic mean and standard deviation) was performed. All the parameters represented as the mean percentage of positively stained cells were compared using Pearson test, where $p<0.05$ was considered significant.

Results

Two groups of desmoid tumors were examined dependently on their location: abdominal and extra-abdominal. Group one included 24 (100%) specimens collected from women, while group two 12 (66.7%) specimens collected from women and 6 (33.3%) from men. There was a high correlation between female sex and presence of aggressive fibromatosis in both groups.

The average percentage of cells stained positively for all cases for p27 and Ki-67 was 22.1% (SD=29.2) and 6.0% (SD=8.8), respectively. Mean values of both proteins expression for abdominal and extra-abdominal group are presented in Table 1. There was no statistically significant difference between Ki-67 expression in abdominal and extra-abdominal location (Table 2). Similar result was observed for p27 expression as well (Table 3).

TABLE 1
The average percent of p27 and Ki-67 positive cells in the studied groups

Protein	p27			Ki-67		
Group (tumor location)	N	M	SD	N	M	SD
Abdominal	24	27.0	4.9	23	6.4	2.0
Extra-abdominal	18	15.7	8.8	11	5.2	4.9

N – number of cases; M – arithmetic mean; SD – standard deviation

TABLE 2
The number and the percentage of Ki-67-positive and negative cases in the studied groups

Group (tumor location)	- n (%)	+ n (%)	++ n (%)
Abdominal	17 (73.9)	6 (26.1)	0 (0.0)
Extra-abdominal	10 (90.9)	1 (9.1)	0 (0.0)

(-) – < 10% of positive cells; (+) – 10–50%; (++) – > 50%; n (%) – number (percent) of positive/negative cases

TABLE 3
The number and the percentage of p27-positive and negative cases in the studied groups

Group (tumor location)	- n (%)	+ n (%)	++ n (%)
Abdominal	13 (54.2)	3 (12.5)	8 (33.3)
Extra-abdominal	12 (66.7)	3 (16.7)	3 (16.7)

(-) – < 10% of positive cells; (+) – 10–50%; (++) – > 50%; n (%) – number (percent) of positive/negative cases

TABLE 4
The percentage of abdominal and extra-abdominal cases in various age groups

Group (tumor location)	A %	B %	C %	D %
Abdominal	4.2	62.4	16.7	16.7
Extra-abdominal	38.9	33.3	5.6	22.2

A – 20 years old and less; B – over 20 and no more than 30 years old; C – over 30 and no more than 40 years old; D – over 40 years old

The abdominal location of desmoid tumor was predominant in the group of patients aged between 20 and 30 years and this frequency was statistically significant in all the analysed age groups ($p<0.05$). Also the differences among age groups for extra-abdominal location of the aggressive fibromatosis were noticed but there was no statistical significance (Table 4).

Discussion

From among 34 cases, analyzed as regards the level of Ki-67 expression, only in 7 (20,6%) of them, the expression was observed, whereas overexpression of this protein was not found at all. These results coincide with the data reported by other authors. Kouho et al. [14] did not observe Ki-67 expression in the examined cases of desmoid tumor or the expression was visible only in single cells, which was regarded as negative result. Hoos et al. [12] obtained similar results in 24 analyzed desmoid cases.

Leithner et al. [15] noted Ki-67 expression in 20 out of 80 cases of aggressive fibromatosis. They assumed the value of expression at the level of 5% to be a positive result. The expression over 10% was observed only in two cases. In turn, Hoos et al. [13] demonstrated high level of Ki-67 expression (overexpression) in 18 and its lack in 35 out of 53 cases of desmoid tumor, assuming the value of expression at the level of 20% proving overexpression.

Aggressive fibromatosis, despite the tendency to local infiltration of the surrounding organs, is a benign neoplasm and it does not metastasize [16, 24]. In our studies Ki-67 expression was not observed in 90.9% of desmoid cases with abdominal location and in 73.9% of cases with extra-abdominal location. These results concerning Ki-67 expression point to low proliferative activity of desmoid cells, which was reported by other authors, too [12, 13, 14, 15]. This may indicate that increased proliferative activity of fibroblasts is not the base of proliferative mechanisms of this neoplasm. However, the fact is that fibroblasts found in this tumor synthesize significant amount of extracellular matrix, particularly type 1 collagen [1]. Collagen fibers are organized in bundles which markedly limit cell-to-cell contact [29]. Transforming Growth Factor β (TGF-β) has additionally stimulating effect on collagen synthesis and on depositing extracellular matrix components by fibroblasts [7, 9]. TGF-β expression in aggressive fibromatosis cells is significantly elevated in relation to normal fibroblasts [18, 19].

Reception of external signals stimulating and inhibiting cell division is one of the functions of p27 protein [2, 20]. The level of p27 protein expression is high in cells in resting phase or in those exposed to antimitogenic factors. Then, when a mitogen acts on a cell and there comes to the decrease of p27 level, the cell passes G_1 phase of the cell cycle and gets ready for further phases [2, 17]. p27 protein is also an inhibitor of the activity of cyclin E-CDK2, cyclin A-CDK2 and cyclin D-CDK4 complexes, responsible for pRb protein phosphorylation, which enables realization of subsequent cell cycle phases but its activity is higher with regard to cyclin E-CDK2 complex than to cyclin D-CDK4 or cyclin A-CDK2 complexes [2, 4, 22, 27]. In turn, overexpression of p27 protein in a cell causes arrest of cell cycle in G_1 phase [22, 27].

The change of the level of p27 protein expression occurs in post-transcriptional stage because the level of p27 mRNA does not change in the course of cell cycle [2, 22]. However, the level of active protein in a cell changes significantly during subsequent phases of the cell cycle. It is possible because p27 is found in a cell associated with cyclin-CDK complexes. Its affinity to these complexes is significantly higher than to subunits forming them [4]. Although p27 is an inhibitor of activity of various cyclin-CDK complexes, its ability to their functions inhibition is not the same [4, 22, 27]. In a cell, p27 protein is found in the form of stable bindings with cyclin D-CDK4 and cyclin D-CDK6 complexes not affecting significantly their activity. At the same time, the protein itself does not fulfil its biological function that is, it does not arrest the cell cycle [2, 4]. However, when antimitogenic factor affects the cell, p27 is released from cyclin D-CDK4/6 complexes and binding with cyclin E-CDK2 or cyclin A-CDK2 complexes it blocks their activity [2, 27].

Majority of normal cells, which have already reached the final stage of differentiation have a high level of nuclear p27 protein [2]. p27 acts as an inhibitor of cell cycle when it is located in the cell nucleus, whereas when it is found in cytoplasm it does not demonstrate inhibitory effect on the cycle course [26]. Cytoplasmic location of p27 protein favours the progression of cell cycle and contributes to the transformation of a noeplastic cell. Accumulation of p27 protein in cytoplasm has been observed in many neoplasms [2].

Decrease or loss of p27 protein expression in tumor cells has been observed in numerous cases of neoplasms, which is often associated with poor diagnostication. Authors suggest that the depleted level of p27 protein in a cell, more frequently than its loss may contribute to the development and progression of numerous neoplasms (review in Belletti et al. [2]) [17]. Among the examined cases of hepatocellular carcinoma, in over 90% of them, the expression of p27 protein was not observed [6]. Decreased expression of p27 was also noted in prostatic gland adenocarcinoma cells as compared to normal cells. Furthermore, the more malignant histologically the neoplasm was the lower was the level of p27 protein [5]. Similar results were obtained in cervical intraepithelial neoplasia [25]. In basaloid squamous cell carcinoma of the larynx high expression of the level of p27 protein was observed in 40% of patients in whom no symptoms of this disease were found [23]. Also in this study the authors demonstrated an association of low level of p27 protein expression and the increase of larynx carcinoma aggressiveness. The level of p27 protein expression may also be a prognostic factor in lung carcinoma. Catzavelos et al. [3] demonstrated in the analyzed cases with lung carcinoma, that in patients with low level of p27 protein expression there came to recurrence or death, whereas high level of p27 was a good prognostic sign for a patient with this carcinoma.

So far, no data have been published on the level of p27 protein expression in desmoid cells. In this study, 42 cases of desmoid tumors were examined as regard the presence of p27 protein. In both groups – abdominal and extra-abdominal in over a half of cases there was lack of p27 protein expression but overexpression was found in 33.3% and 16.7% of the cases respectively. In majority of cases with the observed p27 protein expression, the protein was located in the cell nucleus. However, in 20% of the cases p27 was located in cytoplasm. It should be assumed that in these cases p27 protein was not an inhibitor of cell cycle. Taking into account the obtained results of p27 protein and Ki-67 expression, a hypothesis may be considered that some other mechanism than that dependent on p27 protein forms the basis for low proliferative potential of desmoid cells. However, to verify this hypothesis for desmoid tumors further analysis is needed of the expression of p27 protein and other proteins engaged in the cell cycle regulation.

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