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Downregulation of Genes Encoding for Subunits of Adaptor Complex-3 in Cervical Carcinomas

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Abstract—We explored the expression of four genes encoding for subunits of AP-3 in cervical tumors and cancer cell lines. Using RT-PCR we demonstrated more than twofold decrease in the levels of mRNA of *AP3D1*, *AP3B1*, *AP3M1*, and *AP3S1* in 32, 28, 23, and 26% tumors in comparison with normal tissues of uterine cervix, respectively. The level of mRNA of at least one subunit was decreased in 28 out of 47 (60%) of tumors and in four out of five cancer cell lines in comparison to tissues adjacent to tumors. The suppression of expression of any of the subunits was revealed in 15 out of 28 cases (54%). The expression of two and more subunits was decreased simultaneously in different combinations in 13 cases (46%). This fact testifies to the lack of a common mechanism of downregulation of four subunits in tumors. There is a tendency to more frequent suppression of AP-3A expression in tumors associated with lymphatic node metastases as compared with tumors without metastases ($P = 0.034$). Thus, here we demonstrate for the first time the decrease in expression of genes encoding for AP-3A subunits in tumors.

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Key words: gene *AP3D1*, gene *AP3B1*, gene *AP3M1*, gene *AP3S1*, intracellular transport of proteins, uterine cervix cancer, metastases

The arising and development of tumors is a multistage process associated with disturbances of functions and expression of various gene groups. When analyzing genes expressed differentially in tumors and normal tissues of the uterine cervix, we have observed a decrease in the level of mRNA for the *β3A-adaptin* gene encoding a subunit of the adaptor complex AP-3A [1]. Four adaptor complexes, AP-1 through AP-4, are known in mammals. They are homologous in structure (consist of four subunits) and function similarly (are involved in the intracellular transport of proteins between the *trans*-Golgi network, plasma membrane, endosomes, and lysosomes). The AP-3A complex includes two large subunits, δ- and β3A-adaptins, the medium subunit μ3A-adaptin, and the small subunit σ3A-adaptin, which are encoded by genes *AP3D1*, *AP3B1*, *AP3M1*, and *AP3S1*, respectively. These genes are expressed in all studied mammalian tissues. The AP-3A complex plays a crucial role in the intracellular transport of proteins from the *trans*-Golgi network and early endosomes to lysosomes and the related organelles (melanosomes in melanocytes, dense granules in platelets, etc.) [2].

In mice, natural inactivating mutations were found in the genes of the two large AP-3 subunits, and this revealed some functions of the complex [2]. In humans, mutations in the *β3A-adaptin* gene were detected in patients with Hermansky–Pudlak syndrome type 2 (HPS2). In all cases, carriers of the mutant alleles had a similar phenotype. They had prolonged bleeding caused by disorders in the protein transport in platelets and hypopigmentation of the coat and eyes because of defects in the biogenesis of melanosomes. In these patients, functions of the immune system were disturbed, and they suffered from recurrent microbial infections, which seemed to be associated with a decrease in the protein transport to lysosomes of the protein CD1b necessary for presentation of bacterial antigens [3]. In these patients, the complex AP-3 deficiency was also associated with disorders in functioning of lytic granules in cytotoxic T-lymphocytes, which resulted in the loss of their functions [4].

Studies on cell lines obtained from mice with natural mutations and patients with the Hermansky–Pudlak syndrome type 2 established some specific features of disorders in the AP-3A-mediated transport of proteins. The lack of any large subunit of the complex (δ- or β3A-adaptins in mice, β3A-adaptin in human) is associated

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not only with disorders in the assembly of the fully functional complex capable of binding to membranes of lysosomes and endosomes, but also with destabilization of other subunits [5-8]. Cells with a decreased level of AP-3A are characterized by disorders in the direct transport of the lysosome-associated proteins from the *trans*-Golgi network and endosomes to lysosomes manifested by increased circulation of these proteins across the plasma membrane of the cell [5, 9, 10]. The AP-3A functions can be affected not only as result of mutations in the genes of the subunits, but also because of estrogen-induced changes in gene *Ap3d* expression in mouse brain [11].

So far there are no data on changes in functions or expression of the adaptor complexes, including the AP-3A, in tumors, although disorders in functions and expression of other proteins involved in vesicular transport have been described [12]. Translocations of the genes *AF1-p*, *EEN*, and *CALM*, products of which are involved in endocytosis, have been described in various leukemias. Amphiphysins I and II are involved in formation of clathrin-associated vesicles. Amphiphysin I is hyperexpressed in breast cancer; amphiphysin II binds to the proto-oncogene *c-Abl* and strengthens its transforming capacity. Disorders in functions of caveolin-1 located on the cell plasma membrane and involved in transport of proteins and also the regulation of signaling pathways have been found in various tumors, including cervical cancer [13].

In the present work the expression of genes *AP3D1*, *AP3B1*, *AP3M1*, and *AP3S1* encoding four subunits of the heterotetrameric adaptor complex AP-3A was studied in cervical tumors and cell lines of cervical carcinoma. It is shown for the first time that the level of mRNA of at least one of four subunits was more than twofold decreased in 60% of the tumors and in four of five cell lines, compared to the normal tissues adjacent to the tumor. A tendency for more frequent and pronounced decrease in the level of mRNA of at least one subunit of the AP-3A complex was also found in tumors with metastases into the regional lymph nodes compared to tumors without metastases.

MATERIALS AND METHODS

Clinical material. Forty-seven specimens of squamous cell cervical carcinoma and 40 specimens of the adjacent morphologically normal tissues were obtained from patients of the N. N. Blokhin Russian Cancer Research Center (RCRC), Russian Academy of Medical Sciences (RAMS). The tissue specimens were frozen immediately after the ablation and stored in liquid nitrogen. All tumor specimens were classified by the TNM-classification in accordance with requirements of the International Union Against Cancer (UICC). The tissues were identified histologically in the Department of Tumor Pathomorphology, Institute of Clinical Oncology, N. N. Blokhin RCRC, RAMS. Histological studies have shown the absence of tumor cells in the morphologically normal tissues.

Microdissection was performed on serial cryostat sections of tumor specimens of 5 μm in thickness. Sections stained with hematoxylin and eosin were used for verification of the clinical diagnosis and choosing the tumor region for analysis. The cells of the chosen region of the tumor were taken from the serial section with a drop of the lysing buffer from the kit for isolation of RNA on RNeasy® Micro Kit microcolumns (Qiagen, USA). RNA was isolated from the resulting lysates on the microcolumns according to the producer's protocol.

Cells. Five cell lines of cervical carcinoma were used: C-33A, C-41, SiHa, HeLa, and CaSki. The cells were cultured on DMEM medium supplemented with 5% fetal calf serum. The cells were treated with demethylating agent 5-azadeoxycytidine (Sigma, USA) for three days. 5-Azadeoxycytidine was added into the culture medium to the final concentration of 5 μM.

RNA was isolated from the cells and tissues frozen in liquid nitrogen using the routine guanidine isothiocyanate method with DNA and RNA separation by centrifugation in CsCl density gradient medium [14].

RT-PCR. cDNA was prepared from 1 μg total RNA with Superscript II RNase H^- reverse transcriptase (Invitrogen, USA) and a hexaprimer, following the producer's protocol. In the case of microdissectional RNAs, the entire material was used in the reaction. Then cDNA was amplified in PCR with primers specific for each gene (Table 1). The housekeeping genes *HPRT1* and *GAPDH* were used as the control of cDNA quantity. The PCR conditions were matched for each gene to perform the reaction in the zone of linear dependence of amplification on the number of cycles. The reaction was performed in 25 μl of incubation medium which contained 60 mM Tris-HCl (pH 8.3), 16 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 μM each of four deoxynucleoside triphosphates, 50 pM of each primer, and 1 unit *Taq*-polymerase (Institute of Bioorganic Chemistry, Russia). The amplification conditions were as follows: 94°C, 30 sec; 60°C, 30 sec; 72°C, 45 sec; the number of cycles for each gene is shown (Table 1). To monitor the absence of genomic DNA admixtures, in the case of genes *GAPDH* and *AP3S1* the same conditions as those of the reverse transcription reaction were used but without the addition of reverse transcriptase. In the case of other genes, the sequences of genome DNA complementary to the primers are located at the distance from one another of no less than 7 kb. In no case did we observe amplification of DNA admixtures in the RT-PCR (data not presented).

Gel electrophoresis in agarose. The amplification products were separated in 2% agarose gel at the ethidium bromide concentration of 0.5 μg/ml, and electric field strength of 10-15 V/cm for 30-45 min. The results were recorded by fluorescence in ultraviolet light and pho-

Table 1. Primers and PCR conditions

Gene	Primers	Number of cycles	Product size
GAPDH	fpr: 5'-accacagtccatgccatcac-3' rpr: 5'-tccaccaccctgttgctgta-3'	23	455 bp
HPRT1	fpr: 5'-ctggattacatcaaagcactg-3' rpr: 5'-ggattatactgcctgaccaag-3'	30	230 bp
AP3D1	fpr: 5'-cgcatgttcgacaagaatctg-3' rpr: 5'-gtgccttcgtgaaagctctg-3'	28	284 bp
AP3B1	fpr: 5'-tgccctctggactggaacct-3' rpr: 5'-aatcatcccaacaatccgctt-3'	28	281 bp
AP3M1	fpr: 5'-cctgtcatttcaacacctcac-3' rpr: 5'-tgttagattcggtagccagtg-3'	28	247 bp
AP3S1	fpr: 5'-acagcaaatcatcagggagac-3' rpr: 5'-gcatcaatttgtgtaacaatctc-3'	28	345 bp

Note: fpr, forward primer; rpr, reverse primer.

tographed with a video system DNA Analyzer (Litekh, Russia).

Processing of results. Photographs of electrophoretic separation of PCR products were analyzed using computer programs Molecular Dynamics ImageQuant version 3.3 and Microsoft® Excel 2002. For each specimen of tumor or normal tissue the normalization factor was obtained as the geometric mean of mRNA levels of the control genes *HPRT1* and *GAPDH*. The geometric mean was used to more accurately average variations of the different gene expression [15]. Then the mRNA amount of each subunit of the complex was divided by the normalization factor corresponding to this specimen. Based on the relative content of mRNA of the subunits in 40 specimens of normal tissues of uterine cervix, the mean RNA content for the corresponding subunit in normal tissue and the confidence interval at 95% reliability were calculated. The significance of correlations between changes in the gene expression and clinical characteristics of tumors was calculated using the non-parametric Mann–Whitney *U*-test with the StatSoft Statistica computer program version 6.0.

RESULTS

Expression of AP-3A subunits in primary cervical tumors. The level of mRNA of AP-3A subunits in the specimens of primary squamous cell cervical carcinoma and adjacent morphologically normal tissues was determined using semiquantitative RT-PCR. Figure 1 shows results of a typical experiment. The first two specimens (patients 20 and 22) exemplified cervical carcinomas with unchanged level of mRNA of either subunit of the adaptor complex AP-3A. In the following three specimens (patients 6-8), the amount of mRNA of the different AP-3A subunits was twofold and more decreased. It should be noted that in some cases the mRNA level was decreased even in the adjacent normal tissue (genes *AP3D1*, *AP3M1*, and *AP3S1* in specimen 7, gene *AP3D1* in specimen 8). The mRNA level for three of four genes under study was decreased in some conventionally normal tissues (Table 2). For 40 paired specimens the decrease in the mRNA level in the tumors was evaluated with respect to the level of mRNA of the appropriate subunit in both the adjacent morphologically normal tissue and the mean level of mRNA in these specimens of normal tissues. For all tumors (40 specimens), there were no discrepancies in

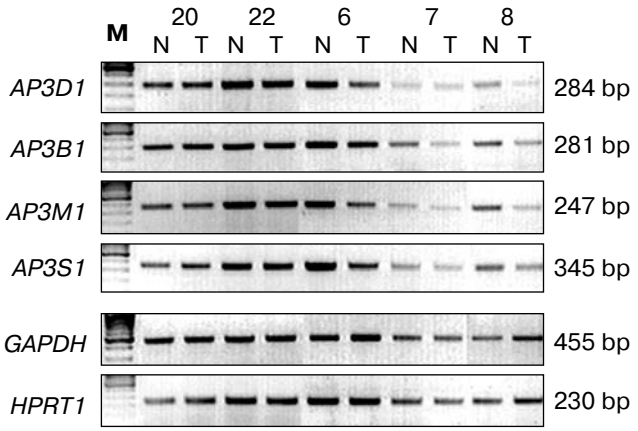

Fig. 1. Level of mRNA of the adaptor complex AP-3A subunits in squamous cell cervical carcinomas determined by semiquantitative RT-PCR. The amplification products were revealed in 2% agarose gel in the presence of ethidium bromide. The figures show the numbers of specimens of normal (N) and tumor (T) tissue of uterine cervix. M is the 100-bp molecular marker. To the right the size of the amplification products is shown in base pairs.

results obtained by the two manners of evaluation; therefore, the data on the mRNA level with respect to the mean content of mRNA of the AP-3A subunit genes are presented. The mean content of mRNA in the normal tissues relative to the control genes (*HPRT1* and *GAPDH*) was 0.97 ± 0.19 for *AP3D1*, 1.06 ± 0.14 for *AP3B1*, 1.00 ± 0.11 for *AP3M1*, and 0.96 ± 0.13 for *AP3S1*. The decrease in the mRNA level was considered significant if its relative content in the specimen was decreased twofold and more compared to the mean normal content. Data on the expression of the genes studied in squamous cell cervical carcinomas are presented in Fig. 2 (a and b) and Table 2. The mRNA levels for all four genes were decreased in the tumors, but the incidence of the decreases varied from 23 to 32% (Table 2). The decreased expression of the level of mRNA of at least one subunit was recorded in 28 of 47 tumor specimens (60%) and in 7 of 40 specimens of normal tissue (17.5%). Statistical analysis revealed that the expression of the AP-3A complex subunits was suppressed in tumors significantly more frequently than in normal tissues (P from 10^{-3} to 10^{-6}). Because the mRNA level of the AP-3A complex genes was decreased in the conventionally normal tissue, it was suggested that the adaptor complex should be involved in carcinogenesis even during its early stages.

The level of mRNA of only one subunit was suppressed in 15 of 28 squamous cell cervical carcinomas (54%) and of two or more subunits (in varied combinations) in 13 cases (46%); thus, there was no coordination in the deregulation of transcription of the four genes of the subunits in tumors.

The adaptor complex AP-3A is expressed in all mammalian tissues studied [16]. Therefore, the loss of expression can be masked when RNA specimens are studied in the total clinical material of the tumor, which includes not only tumor cells but also normal tissue cells (those of blood, vessels, connective tissue). To decrease the amount of nontumor elements in the specimen, we microdissected tumor cells from the section of tumor. In three specimens of carcinomas which were earlier found to lack a markedly inhibited expression of the complex subunits (Fig. 2, a and b: 18, 45, and 47), the population of enriched tumor cells prepared by microdissection was revealed to have more than twofold decreased level of mRNA of gene *AP3D1* (Fig. 2c).

Thus, microdissection confirmed the decrease in mRNA level in tumor cells and increase in frequency of detecting changes in expression in the tumor cells compared to the morphologically normal ones.

Expression of AP-3A subunit genes in cervical carcinoma cell lines. Cell lines present populations consisting only of tumor cells, without admixtures of normal cells. Therefore, five cervical carcinoma cell lines were studied (C-33A, C-41, SiHa, HeLa, CaSki). The level of mRNA of the AP-3A subunits in the cell lines was compared with the mean levels of these mRNA in adjacent normal tissue of uterine cervix. The results are shown in Fig. 3a. The level of mRNA of the *AP3D1* and *AP3M1* subunits was suppressed in three of five cell lines and of the *AP3B1* subunit in two cell lines. In four of five cell lines, the expression of at least one subunit was suppressed. Thus, the decrease in the level of mRNA of the AP-3A complex subunits is a specific feature of tumor cells.

Gene expression is likely to be suppressed, in particular, by the mechanism of DNA methylation. To elucidate the possible role of DNA methylation in the regulation of complex AP-3A expression, we compared the level of mRNA of the complex subunits in three cell lines of cervical carcinoma (SiHa, HeLa, CaSki) before and after treatment of the cells with the demethylating agent 5-azadeoxycytidine. After the treatment with 5-azadeoxycytidine, the level of mRNA of two subunits of the complex was increased only in HeLa cells (Fig. 3b). The amount of subunit *AP3D1* mRNA was increased 1.8-fold and of *AP3B1* subunit mRNA was increased 1.5-fold. Thus, the expression of the AP-3A complex subunits could be partially recovered by DNA demethylation. Possibly, DNA methylation contributes to the regulation

Table 2. Incidence of inhibition of expression of AP-3A complex subunit genes in specimens of normal tissue and cervical tumors

	AP3D1	*AP3B1*	*AP3M1*	*AP3S1*	At least one subunit
Normal tissue of uterine cervix	5/40 (12.5%)	0/40 (0%)	2/40 (5%)	4/40 (10%)	7/40 (17.5%)
Primary squamous cell cervical carcinomas	15/47 (32%)	13/47 (28%)	11/47 (23%)	12/47 (26%)	28/47 (60%)
Statistical validity* (P)	10^{-3}	$1.6 \cdot 10^{-5}$	10^{-4}	$1.6 \cdot 10^{-3}$	10^{-6}

* Significance of differences between incidence of inhibition of expression of complex AP-3A subunits in specimens of squamous carcinomas and normal tissue of uterine cervix (Mann–Whitney *U*-test).

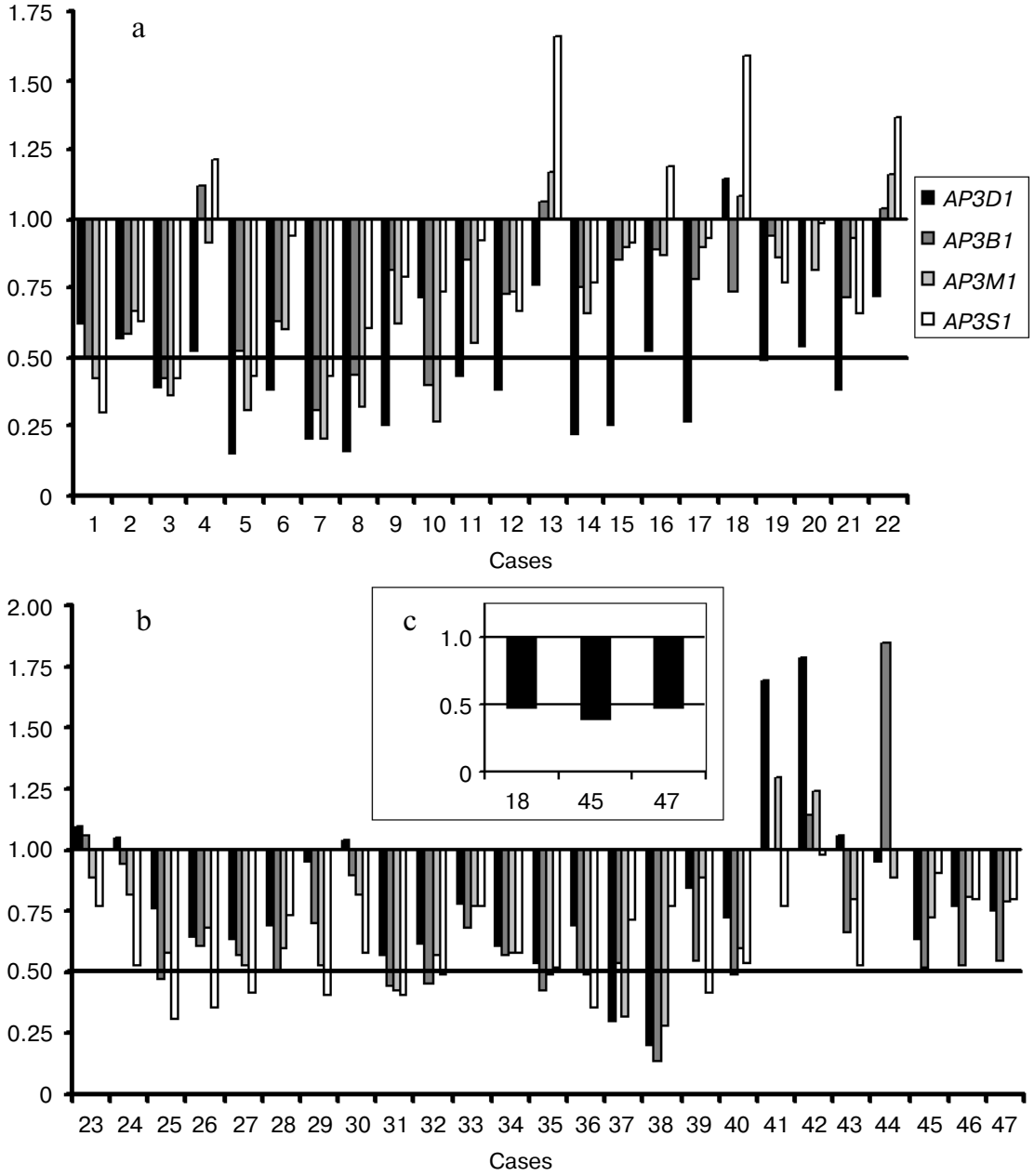

Fig. 2. Level of mRNA of AP-3A complex subunits in specimens of squamous cell cervical carcinomas normalized relatively to average content in normal tissue. The mean content of the subunits in the normal tissue is taken as the unit. a) Carcinoma specimens from patients with metastases into regional lymph nodes. b) Carcinoma specimens from patients without metastases. c) Analysis of carcinomas by microdissection.

of expression of adaptor complex AP-3A in cervical carcinoma, in addition to other mechanisms.

Correlation of changes in AP-3A subunit expression with clinical and pathological characteristics of patients. We attempted to find a possible correlation between the loss of level of mRNA of complex AP-3A subunits and traditional clinical and pathological parameters of patients. No correlations were found between the loss of level of mRNA of the subunits and age, differentiation degree, and tumor size. However, in the tumors with metastases into the regional lymph nodes the level of mRNA of at least one AP-3A subunit was decreased in 15 specimens of 22 (68%, Fig. 2a), and in the tumors without metastases such a decrease was observed in 13 of 25 specimens (52%, Fig. 2b). Moreover, the degree of the mRNA level decrease was different in these two groups of tumors: decrease to 0.25 and lower was observed in eight tumors with metastases (specimens 5, 7, 8, 9, 10, 14, 15,

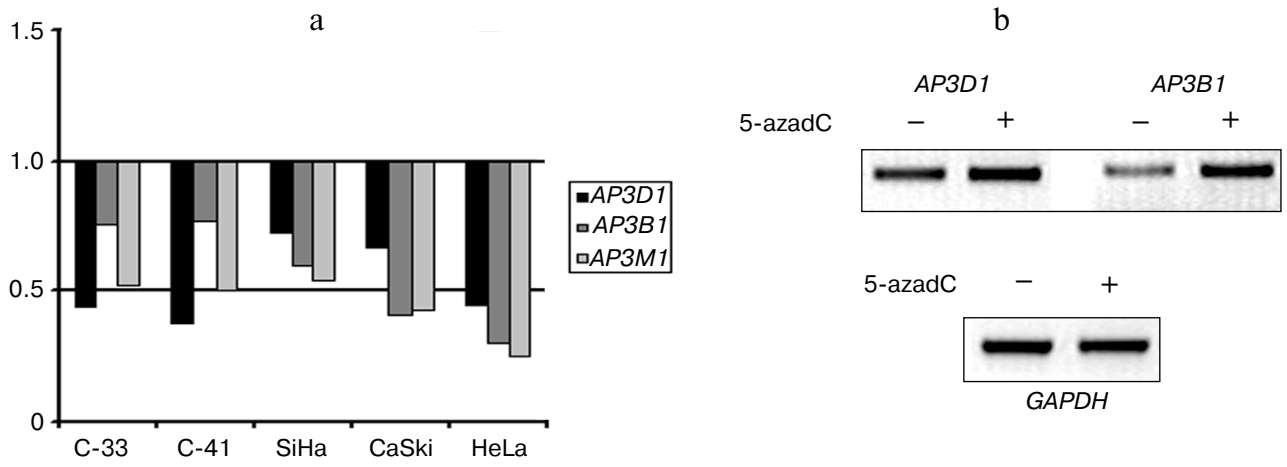

Fig. 3. Expression of AP-3A complex in cervical carcinoma cell lines. a) Level of mRNA of AP-3A complex subunits in cervical carcinoma cell lines compared to the average level in normal tissue. b) Increase in mRNA level of AP3D1 and AP3B1 subunits in HeLa cells under the influence of 5-azadeoxycytidine (5-azadC). The amplification products of the semiquantitative RT-PCR were detected in 2% agarose gel in the presence of ethidium bromide.

17; Fig. 2a), whereas a similar degree of decrease was recorded in only one tumor without metastases (specimen 38, Fig. 2b). Based on the degree of the decrease in every case when analyzing the significance of differences between the two groups of tumors, we obtained the value of $P = 0.034$ (Mann–Whitney *U*-test).

Thus, there is a tendency for more frequent and pronounced decrease in AP-3A expression in tumors with affected regional lymph nodes compared to tumors without ones.

DISCUSSION

The present study of mRNA level of adaptor complex AP-3A subunits has revealed a significantly higher frequency of their decreased expression in carcinomas compared to normal uterine cervix tissue (60%). The significant difference between the incidence of decreased expression of subunits in carcinomas and normal tissues confirms the specificity of this phenomenon for the tumor (Table 2). The decrease in the level of mRNA of the complex subunits in some specimens of normal tissues seems to reflect the involvement of adaptor complex AP-3A in cervical carcinogenesis even during its early stages. It is unclear whether papilloma virus, which is an etiological agent of cervical carcinoma, plays a role in disorders of adaptor complex AP-3A functioning. This question needs further investigation.

The expression of the four subunit genes in tumors is suppressed with no coordination. Such a pattern can reflect the absence of a common mechanism of suppressing the expression of the complex subunits. The expression of one subunit can be lowered due to different causes, in particular, because of the loss of an allele. In the case of the suppressed expression of several subunits, synchronous deletions of their genes are unlikely because the genes of the AP-3A complex subunits are located on different chromosomes. In humans, the *AP3D1* gene is located on chromosome 19, and genes *AP3B1* and *AP3S1* are located on chromosome 5 in different loci (5q14.1 and 5q22), and gene *AP3M1* is located on chromosome 10. Along with the allelic deletions, the expression can also be suppressed by direct methylation of the promoter region of the subunit genes or through indirect methylation of the transcriptional factor (factors) promoter regulating the expression of AP-3A complex subunits. Although we have observed increased level of mRNA of genes δ- and β3A-adaptins in the cell culture under the influence of demethylating agent, no methylation of the CpG-island of gene *AP3B1* was found either in the cervical carcinoma cell lines or in tumors [1]. To elucidate whether the gene promoters of other subunits are methylated in tumor cells or the expression of complex AP-3A subunits is suppressed through methylation indirectly, further studies are necessary.

What consequences for the assembly and functioning of AP-3A complex can cause a decrease in the content of even one of four subunits in a tumor cell? Studies on complex AP-3A composition and transport of its protein targets in human and mouse cells with natural inactivating mutations in the genes encoding δ- or β3A-adaptins have shown that the presence of all subunits is essential not only for the assembly of the functional complex but also for the stability of individual subunits. In particular, in mutant mice β3A subunit deficiency was shown to be associated with a virtually complete degradation of the μ3A subunit. The absence of functional complex AP-3A in cells obtained from the mutation carriers in all cases was associated with missorting of AP-3A target proteins

[5-8]. Data on the individual subunit properties also confirm the importance of the presence of all subunits for the adequate functioning of the complex. Thus, in particular, a certain group of transported proteins possessing a unique tyrosine signal interacts with the AP-3 complex via the medium subunit (μ3-adaptin). Inhibition of this interaction (in particular, by suppressing the subunit expression) leads to disorders in the transport of the corresponding proteins [10, 17]. Thus, in cell cultures the deficiency of each of three subunits (δ, β3A, μ3A) leads to disorders in functions of the adaptor complex AP-3A and finally to damage of the AP-3A-mediated vesicular transport.

It is interesting that notwithstanding the approximately equal incidence of suppression of all four subunits of the complex, we observed more frequently a decrease in the level of mRNA of two large subunits, and in the great majority (86%) of the tumors with repressed subunit(s) expression of one or both large subunits was suppressed. In mammals natural mutations are found only in the δ- and β3A-adaptins, and they are very frequently suppressed in cervical tumors, hence the large subunits are suggested to play a key role in the regulation of complex AP-3A functioning, which needs further investigations.

It should be noted here that we have observed in some tumors increase in the level of mRNA of individual subunits (less than twofold) compared to the conventionally normal tissues (Fig. 2). In these cases, the level of mRNA of other subunits was unchanged or decreased. Considering all that was said above about the limiting role of the amount of each of the subunits for the complex assembly, it was suggested that the increased expression of individual subunits could not compensate the shortage of other subunits in the cells.

In addition to lysosomes, the AP-3A complex is mainly located in early endosomes and *trans*-cisterns of the Golgi network of the cell [18]. Such location allows the complex to be involved not only in the transport of tissue-specific proteins into specialized structures (melanosomes, lysosomes, etc.) but also in the regulation of the functioning duration of the proteins located on the plasma membrane. Because disorders in AP-3A functions are associated with increased transport of lysosomal proteins to the plasma membrane, the lowering of the functional AP-3A complex is suggested to prolong the term of activity of such proteins on the membrane [2].

Proteins CD63 and CD164 are complex AP-3A target proteins, and their increased expression on the plasma membrane is observed with the shortage of AP-3A in cells [5-8, 17, 18]. CD63 discovered as an antigen of early stages of melanomas belongs to the family of tetraspanines and is involved in the regulation of cell mobility and adhesion [19]. Various tetraspanines, including CD63, produce on the cell plasma membrane numerous complexes to one another and also with other plasma proteins. In particular, CD63 interacts with some receptors of adhesion from the family of integrins, and this provides for its involvement in the regulation of cell mobility [20]. It is suggested to be realized not only via regulation of the integrin interaction with the extracellular matrix but also through the regulation of their intracellular transport. It seems that CD63 can act as an adaptor between integrins and the intracellular transport system due to its interaction with adaptor complexes, including the AP-3A. Another protein transported by the AP-3A complex, sialomucin CD164 (endolin) is involved in proliferation, adhesion, and differentiation of some types of cells, but up to now there are no data on its relation with carcinogenesis [18].

In the light of the aforesaid, it seems that the observed tendency for more frequent and pronounced decrease in expression of AP-3A complex subunits in tumors with metastases can be associated with disorders in the transport of these, and possibly, also other targets of AP-3A.

Thus, we are the first to show the suppressed expression of adaptor complex AP-3A subunits in tumors.

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REFERENCES

1. Petrenko, A. A. (2000) *Study on DNA Methylation in Cervical Carcinoma*: Author's abstract of Candidate's dissertation [in Russian], Russian Cancer Research Center, Moscow.
2. Boehm, M., and Bonifacino, J. S. (2002) *Gene*, **286**, 175-186.
3. Sugita, M., Cao, X., Watts, G., Rogers, R. A., Bonifacino, J. S., and Brenner, M. B. (2002) *Immunity*, **16**, 697-706.
4. Clark, R. H., Stinchcombe, J. C., Day, A., Blott, E., Booth, S., Bossi, G., Hamblin, T., Davies, E. G., and Griffiths, G. M. (2003) *Nat. Immunol.*, **4**, 1111-1120.
5. Dell'Angelica, E. C., Shotelersuk, V., Aguilar, R. C., Gahl, W. A., and Bonifacino, J. S. (1999) *Mol. Cell*, **3**, 11-21.
6. Peden, A. A., Rudge, R. E., Lui, W. W., and Robinson, M. S. (2002) *J. Cell. Biol.*, **156**, 327-336.
7. Huizing, M., Scher, C. D., Strovel, E., Fitzpatrick, D. L., Hartnell, L. M., Anikster, Y., and Gahl, W. A. (2002) *Pediatr. Res.*, **51**, 150-158.
8. Jung, J., Bohn, G., Allroth, A., Boztug, K., Brandes, G., Sandrock, I., Schaffer, A. A., Rathinam, C., Kollner, I., Beger, C., Schilke, R., Welte, K., Grimbacher, B., and Klein, C. (2006) *Blood*, **108**, 362-369.
9. Yang, W., Li, C., Ward, D. M., Kaplan, J., and Mansour, S. L. (2000) *J. Cell. Sci.*, **113**, 4077-4086.
10. Le Borgne, R., Planque, N., Martin, P., Dewitte, F., Saule, S., and Hoflack, B. (2001) *J. Cell. Sci.*, **114**, 2831-2841.
11. Lee, J. Y., Kim, J. H., Hong, S. H., Lee, J. Y., Cherny, R. A., Bush, A. I., Palmiter, R. D., and Koh, J. Y. (2004) *J. Biol. Chem.*, **279**, 8602-8607.

12. Floyd, S., and de Camilli, P. (1998) *Trends Cell. Biol.*, **8**, 299-301.
13. Chan, T. F., Su, T. H., Yeh, K. T., Chang, J. Y., Lin, T. H., Chen, J. C., Yuang, S. S., and Chang, J. G. (2003) *Int. J. Oncol.*, **23**, 599-604.
14. Sambrook, J., Fritsch, E. E., and Maniatis, T. (1989) *Molecular Cloning*, Cold Spring Harbor Laboratory Press, N. Y.
15. Vandesompele, J., de Preter, K., Pattyn, F., Poppe, B., van Roy, N., de Paepe, A., and Speleman, F. (2002) *Genome Biol.*, **3**, 0034.1-0034.12.
16. Boehm, M., and Bonifacino, J. S. (2001) *Mol. Biol. Cell*, **12**, 2907-2920.
17. Rous, B. A., Reaves, B. J., Ihrke, G., Briggs, J. A., Gray, S. R., Stephens, D. J., Banting, G., and Luzio, J. P. (2002) *Mol. Biol. Cell*, **13**, 1071-1082.
18. Ihrke, G., Kytta, A., Russell, M. R., Rous, B. A., and Luzio, J. P. (2004) *Traffic*, **5**, 946-962.
19. Boucheix, C., Duc, G. H., Jasmin, C., and Rubinstein, E. (2001) *Expert Rev. Mol. Med.*, **31**, 1-17.
20. Berditchevski, F. (2001) *J. Cell. Sci.*, **114**, 4143-4151.