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Expression, Intracellular Localization, and Maturation of Cysteine Cathepsins in Renal Embryonic and Cancer Cell Lines

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Abstract—Cysteine cathepsins play an important role in tumor development and metastasis. The expression of these enzymes is often increased in many types of tumor cells. Cysteine cathepsins contribute to carcinogenesis through a number of mechanisms, including proteolysis of extracellular matrix and signaling molecules on the cell surface, as well as degradation of transcription factors and disruption of signaling cascades in the cell nucleus. Distinct oncogenic functions have been reported for several members of the cysteine cathepsin family in various types of cancer, but a comparative study of all eleven cysteine cathepsins in one experimental model is still missing. In this work, we assessed and compared the expression, localization, and maturation of all eleven cysteine cathepsins in embryonic kidney cells HEK293 and kidney cancer cell lines 769-P and A-498. We found that the expression of cathepsins V, B, Z, L, and S was 3- to 9-fold higher in kidney tumor cells than in embryonic cells. We also showed that all cysteine cathepsins were present in varying amounts in the nucleus of both embryonic and tumor cells. Notably, more than half of the cathepsin Z or K and over 88% of cathepsin F were localized in tumor cell nuclei. Moreover, mature forms of cysteine cathepsins were more prevalent in tumor cells than in embryonic cells. These results can be further used to develop novel diagnostic tools and may assist in the investigation of cysteine cathepsins as potential therapeutic targets.

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INTRODUCTION

Cysteine cathepsins are lysosomal proteases belonging to papain-like enzyme family. The group of cysteine cathepsins includes 11 enzymes: cathepsins B, C, F, H, K, L, O, S, V, W, and Z (X). They have a common domain structure but differ in molecular mass and functions. The main role of cysteine proteases is protein degradation and the regulation of key cellular processes [1]. Based on the amino acid sequences of their prodomains, cathepsins are classified as L-, B-, and F-like cathepsins [2].

Cathepsins consist of an N-terminal signalling peptide, a prodomain, and a catalytic domain [3]. Cathepsins are synthesized as inactive proforms. The multistep maturation process includes the cleavage of the signaling peptide and prodomain from the proform, which results in the generation of a mature enzyme. In some cases, one or several intermediate forms of cathepsins are produced in the course of maturation [4].

Almost all cells in an organism express cysteine cathepsins. Some of them have pronounced expression in specific organs of the human body. Among immune cells,

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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cathepsin S is abundant in antigen-presenting cells [5], and cathepsin W is expressed primarily by the NK cells and T lymphocytes [6]. Overexpression of cathepsin has been observed in brain tumors [7-9], breast cancer [10-13], colorectal cancer [14, 15], melanoma [16], non-Hodgkin's lymphoma [17], squamous cell carcinoma [18], lung cancer [19], stomach cancer [20], thymoma [21], kidney cancer [22, 23], thyroid cancer [24, 25], and large granular lymphocytic leukemia [26]. It is known from the literature data that patients with renal cell cancer exhibit overexpression of cathepsin B in comparison with the adjacent non-tumor cells [27]. Cathepsin K is used as a diagnostic marker in the TFE3/TFEB-rearranged renal cell carcinoma and renal epithelioid angiomyolipoma [23]. Enhanced expression of cysteine cathepsins in tumor cells is often associated with poor prognosis [7, 28], low overall survival rate [29], and metastasis [30].

Cysteine cathepsins are considered potential markers and targets for diagnostics and treatment of certain types of cancer. Cathepsin B [27], cathepsin H [14], as well as cathepsin K [23] could be considered as potential markers of cancers. Inhibition of cathepsin B activity in the cells of hepatocellular carcinoma results in the reduction of tumor cell growth [31, 32]. Peptides produced from the substrates of the papain-like cysteine proteases, triticain-alpha, decrease the migration of kidney tumor cells and the formation of spheroids and colonies [22]. Knockouts of the cathepsins Z and B in breast cancer result in a decrease in metastasis and tumor size [33].

Despite the fact that initially all cysteine cathepsins were described as lysosomal proteases, they have been found in the cell nuclei as well as some other intracellular compartments [25, 34, 35]. In general, the functional role of the "nuclear" cysteine cathepsins is poorly understood so far. However, it was shown that the presence of nuclear cathepsin L is associated with a poor survival prognosis for oncology patients [36].

Numerous studies focus on the investigation of one particular cysteine cathepsin. Despite the fact that many cathepsins are expressed in particular cancer types, there are almost no studies aiming to investigate the expression of all cysteine cathepsins simultaneously. With the goal of filling this gap, in this work we investigated levels of expression of all 11 cysteine cathepsins in human embryonic kidney cells HEK293 and in the kidney tumor cell lines 769-P and A-498 and determined the localization and degree of maturation of the cysteine cathepsins present in these cells.

MATERIALS AND METHODS

Cell line cultivation. Cell lines of human kidney carcinoma 769-P, A-498, and human embryonic kidney cells HEK293 were obtained from the American Type Culture Collection. 769-P and A-498 cells were propagated in flasks in a RPMI-1640 medium supplemented with 10% fetal bovine serum (*v/v*) and 1% of antibiotic mixture penicillin-streptomycin (*v/v*) (Gibco, USA) at 37°C under 5% CO_2. HEK293 cells were propagated in a DMEM medium with the same supplements and under the same conditions.

Production of antibodies against cathepsin L. Polyclonal antibodies against cathepsin L were produced and purified using common procedure [37]. Briefly, purified recombinant cathepsin L (1 mg/ml) was mixed at a ratio 1 : 1 with 1 ml of Freund's adjuvant solution and subcutaneously injected to a rabbit. One month after immunization was enhanced by administration of recombinant cathepsin L (1 mg/ml) mixed with incomplete Freund's adjuvant at the same ratio. Two weeks after second immunization rabbit blood was taken and antibodies were purified using affinity chromatography. For this purpose, 1.2 mg of recombinant cathepsin L was mixed with 0.6 ml of CNBr-activated sepharose (GE Lifesciences, USA) according to manufacturer's instructions. Blood serum was diluted 2-fold with PBS and loaded onto an immunoaffinity column. Antibodies were eluted from the column with a 0.2 M glycine solution (pH 2.5) followed by neutralization of the eluate with 1 M Tris-HCl (pH 9.0). Next antibodies were precipitated by adding ammonium sulfate powder to 75% of saturation. Precipitated antibodies were separated by centrifugation, resuspended in an ammonium sulfate solution at 50% saturation, and stored at 4°C.

Fractionation of cellular structures. Fractionation of the cell contents into cytoplasmic and nuclear fractions was carried out according to the protocol described in [38] with some modifications. Cells were washed from the medium with cooled PBS. A sucrose buffer [0.32 M sucrose, 3 mM $CaCl_2$, 2 mM magnesium acetate, 0.1 M EDTA, 10 mM Tris-HCl, 1 mM DTT, 0.5% NP-40 (*v/v)*, cocktail of proteinase inhibitors (Sigma-Aldrich, USA)] was added to the cells followed by scraping the cells from the plastic surface and transferring them to an ice-cold tube. After 30-min incubation on ice with periodic mixing by pipetting the samples were centrifuged for 15 min at 3000*g* and 4°C. Supernatant containing cytoplasmic fraction was collected after centrifugation, and precipitate containing nuclei was resuspended in a sucrose buffer without addition of NP-40. After centrifugation at 3000*g*, 4°C for 5 min nuclei were resuspended first in a low-salt buffer [20 mM HEPES, pH 7.9, 20%-glycerol (*v/v*), 1.5 mM $MgCl_2$, 0.02 M KCl, 0.2 mM EDTA, 0.5 mM DTT, cocktail of proteinase inhibitors (Sigma-Aldrich)], and next in a high-salt buffer [20 mM HEPES, pH 7.9, 20% glycerol (*v/v*), 1.5 mM $MgCl_2$, 0.8 M KCl, 0.2 mM EDTA, 1% NP-40 (*v/v*), 0.5 mM DTT, cocktail of proteinase inhibitors (Sigma-Aldrich)]. Nuclei were incubated for 30 min on ice and centrifuged at 16,000*g* and 4°C for 15 min. The collected supernatant comprised

a nuclear fraction. Cytoplasmic and nuclear fractions were stored at −80°C. Fractions were mixed with a 2× loading buffer (125 mM Tris-HCl, pH 6.8, 20% glycerol (*v/v*), 4% SDS (*w/v*), 0,004% bromophenol blue (*w/v*), 10% 2-mercaptoethanol (*v/v*) and heated for 5 min at 96°C. PAAG was loaded with 6 μg of cytoplasmic fraction and 60 μg of nuclear fraction.

Preparation of cell lysate samples. Cells were carefully washed with a cooled PBS and lysed in a RIPA-buffer [150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 1% NP-40 (*v/v*), 0.5% sodium deoxycholate (*w/v*), 0.1% SDS (*w/v*)] with addition of a cocktail of proteinase inhibitors (Sigma-Aldrich) for 30 min on ice. Cell lysate was centrifuged at 16,000*g* and 4°C for 15 min. The obtained cell lysate was mixed with a 2× sample loading buffer [125 mM Tris-HCl, pH 6.8, 20% glycerol (*v/v*), 4% SDS (*w/v*), 0.004% bromophenol blue (*w/v*), 10% 2-mercaptoethanol (*v/v*)] and heated for 5 min at 96°C. An aliquot (30 μg) of the cell lysate was applied onto a PAAG.

Western blot. Prepared samples were separated in a 14% PAAG and transferred onto a PVDF-membrane (Merck Millipore, USA). Antibodies against cathepsins V, C, B, K, W, Z, O, H, S (Abcam, United Kingdom), cathepsin L (produced in this study), cathepsin F, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), lamins A/C (Thermo Fisher Scientific, USA), as well as secondary antibodies conjugated with horseradish peroxidase (Thermo Fisher Scientific) were used in the study. The membranes were processed using a Clarity Western ECL Substrate kit (Bio-Rad, USA). Densitometry analysis was carried out with the help of the GelAnalyzer 19.1 software. The obtained data were normalized to GAPDH and presented in arbitrary units. Purity of cytoplasmic and nuclear fractions was tested based on the content of GAPDH protein and lamin nuclear proteins.

Fluorescence microscopy. Cells were washed in a cooled PBS, fixed in a 4% PFA (*w/v*)/PBS for 10 min and permeabilized in a 0.25% Triton X-100 (*v/v*) for 10 min. To block non-specific binding sites cells were blocked in a 1% solution of BSA (*w/v*)/1× PBS-T/100 mM in glycine for 30 min. Next cells were incubated with the primary antibodies described in the Western blot section for 1 h at room temperature. After that secondary antibodies labeled with fluorophore (Thermo Fisher Scientific) were added to the cells and incubated for 1 h. Cell nuclei were stained with a nuclear stain DAPI (Thermo Fisher Scientific), next cells were embedded into a mounting medium (Sigma-Aldrich). Cells were visualized using a C2 confocal microscope with an Eclipse Ti-E platform (Nikon, Japan). Analysis of fluorescence images was carried out with the help of ImageJ 1.53c software.

Analysis of results. Statistical analysis of the result was performed using the GraphPad Prism 8.4.3 program. Results of comparative analysis of expression are presented as a mean ± standard deviation.

RESULTS

The expression of cysteine cathepsins differs in embryonic and tumor cells. To determine the level of expression of cysteine cathepsins in kidney embryonic and tumor cells, Western blotting of cell lysates was performed (Fig. 1). The results of the analysis demonstrated that all 11 cysteine cathepsins are present both in the human embryonic kidney cells HEK293 and in the two kidney tumor cell lines 769-P and A-498. However, significant differences were observed between the embryonic and tumor cells in the level of expression, as well as in the presence of various forms of cysteine cathepsins emerging due to different stages of the enzyme maturation. It was found that the kidney tumor cells produce on average more cathepsin V (9.34-fold), cathepsin Z (5.5-fold), cathepsin L (3.34-fold), cathepsin S (9.2-fold), and cathepsin B (3.34-fold) in comparison with the embryonic kidney cells. It is worth mentioning that in addition to the enhanced expression of cathepsins L and B, higher amounts of mature forms of these enzymes were detected in the tumor cells.

The content of the proform and intermediate form of cathepsin C was shown to be higher in the tumor cells than in the embryonic ones, while the content of the mature cathepsin C was lower. The amount of the proform and intermediate form of the cathepsin O, on the contrary, was lower in the tumor cells in comparison with the content in the embryonic cells, and mature cathepsin O with a molecular mass around 20 kDa was detected exclusively in the tumor cells. Cathepsin K was present in the kidney tumor cells predominantly in its mature form, and embryonic cells conversely contained more of the cathepsin K proform. Quantity of the proform and intermediate form of the cathepsin H was different in all three cell lines. However, a lower amount of the mature form of cathepsin H was found in the tumor cells than in the embryonic cells.

Expression of all F-like cathepsins (cathepsins F and W) was found to be higher in the embryonic kidney cells in comparison with the expression in the tumor cells. In addition to the observed changes in the levels of expression of cathepsins in embryonic and tumor cells, the presence of the intermediate forms of the cathepsins V, K, C, and H in the tumor cells should be mentioned.

Hence, based on the obtained data it could be concluded that all 11 cysteine cathepsins are produced by both embryonic and tumor cells, however, the levels of their expression, as well as the content of particular forms typical for each maturation stages could be significantly different. Furthermore, the kidney tumor cells contain significantly higher amounts of cathepsins V, B, Z, L, and S in comparison with the embryonic kidney cells.

Cysteine cathepsins are present in the nuclei and cytoplasm of kidney embryonic and kidney tumor cells, but in different ratios. The next step of this work involved

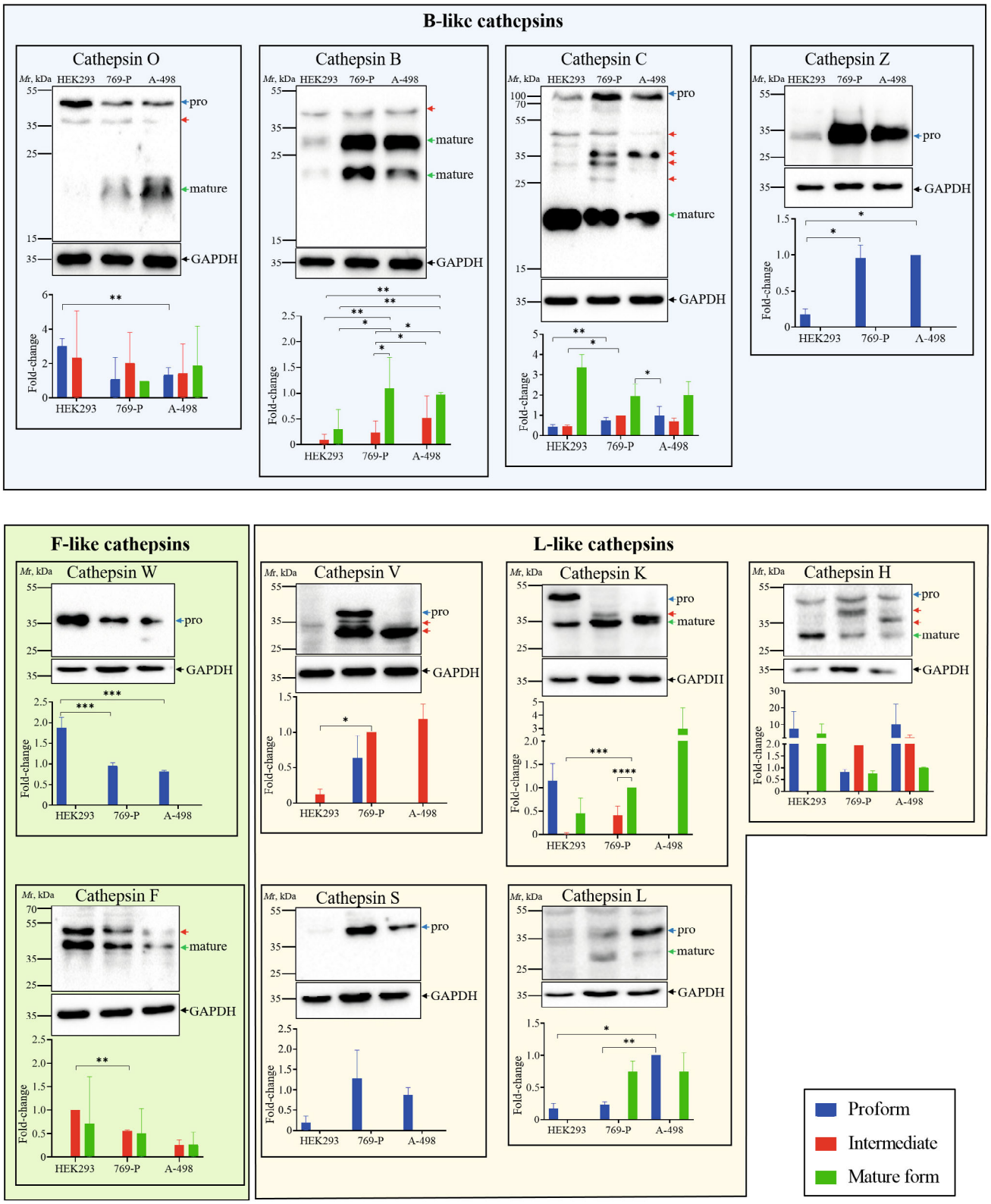

Fig. 1. Expression of B-, F-, and L-like cysteine cathepsins in the embryonic kidney cells HEK293 and in the kidney tumor cells 769-P and A-498. Analysis of cell lysates was carried out using Western blotting. Membranes were stained with the help of antibodies against 11 cysteine cathepsins, as well as against GAPDH for the purpose of controlling sample loading. Pro – proform of cathepsin, Mature – mature form of cathepsin, arrows without designation – intermediate forms of cathepsins. Results of Western blotting are presented as histograms (mean value and standard deviation). Fold-change of expression was calculated as a ratio of the protein band intensity signal to the median value of the protein band intensity signal. Median was selected among the values of the signal of protein band intensity for the proteins, which are presented in the figure for the particular cathepsin. All protein band intensity values were normalized to GAPDH. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

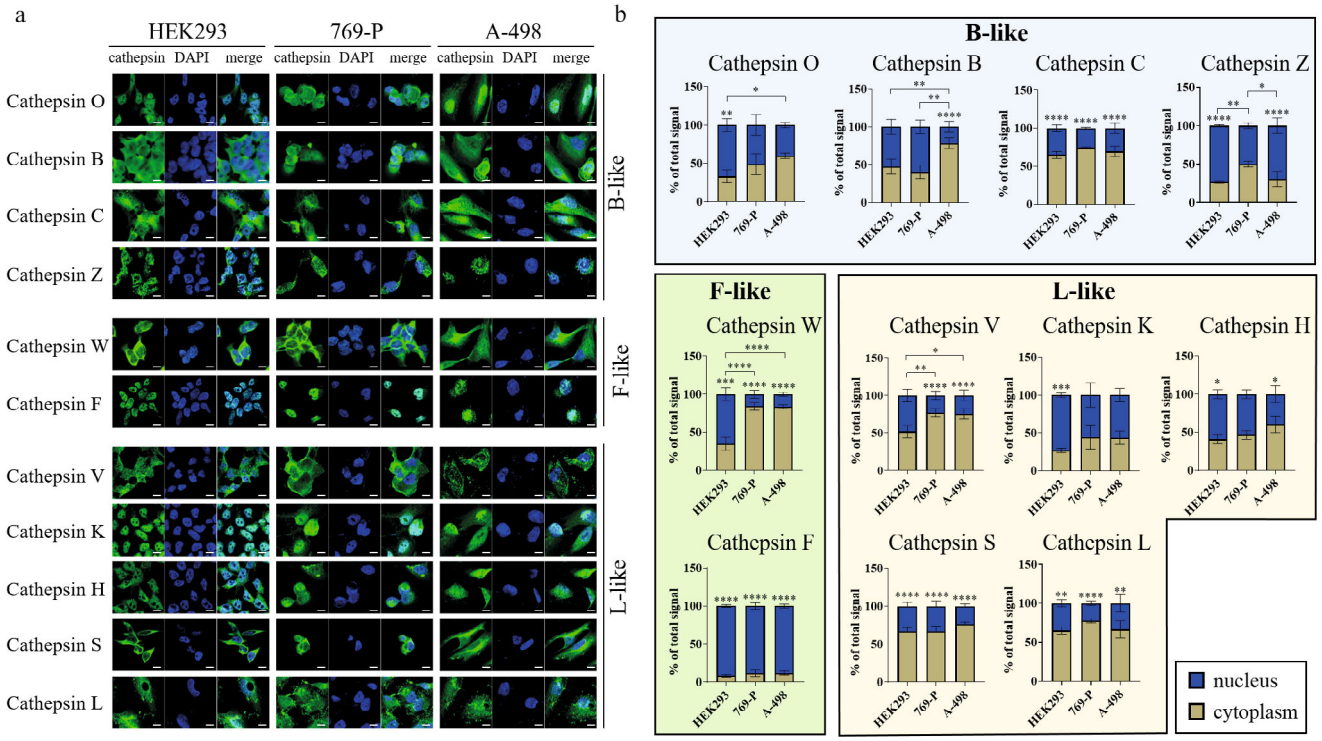

Fig. 2. Intracellular localization of B-, F-, and L-like cysteine cathepsins in embryonic kidney cells HEK293 and kidney tumor cells 769-P and A-498. a) Microphotographs obtained with the help of confocal microscopy. Cysteine cathepsins were stained with the help of specific antibodies and simultaneously cells' nuclei were stained with the help of nuclear dye DAPI. Scale bar: 10 μm. Merge – merging images. b) Results of calculating of the ratio of fluorescence signals for conjugated antibodies detected in the cytoplasm or nucleus in the cell lines HEK293, 769-P, and A-498 (mean value and standard deviation). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

the investigation of intracellular localization of the cysteine cathepsins in kidney embryonic and tumor cells. To this end, they were stained using antibodies labeled with fluorescent dyes and visualized by confocal microscopy (Fig. 2). Nuclei were stained with a nuclear stain DAPI.

The results of the analysis of microscopic images demonstrated that all cysteine cathepsins were present both in the cytoplasm and nuclei of the kidney embryonic and kidney tumor cells. Nevertheless, the distribution of particular proteins between the cytoplasm and nucleus was significantly different. In particular, almost 90% of the cathepsin F signal was observed from the nuclei of embryonic and tumor cells. Based on the distribution of the fluorescence signals it was concluded that in the case of 769-P cells, the major part of cathepsins Z, B, O, K, and H (more than 50% of the total signal) is associated with the nuclei, while in case of A-498 cells the nuclei contain more of cathepsins Z and K.

Signals of some cysteine cathepsins in the cytoplasm and nucleus differed between the kidney embryonic and tumor cells. In the embryonic cells 49% of the cathepsin V signal and 65% of the cathepsin W signal were detected in the cell nucleus, while in769-P and A-498 cells, signal of nuclear cathepsin V decreased to 22.94% and 24.76% and of nuclear cathepsin W to 16% and 16.46%, respectively.

Analysis of the distribution of cathepsins B, O, and Z in the embryonic and tumor cells revealed statistically significant differences only in 769-P or A-498 cell lines in comparison with the embryonic cells. In particular, the level of cathepsin Z decreased from 73.35% to 50.1% in the nuclei of the 769-P tumor cells, and nuclear localization of the cathepsin B decreased significantly in the tumor cells A-487 from 55.68% to 21.65% and of cathepsin O from 66.93% to 39.88%. At the same time fluorescence signals of the cathepsins S, H, K, L, and C detected in the cytoplasm and in the nuclei of embryonic and tumor cells did not differ significantly.

The obtained results allow concluding that the cysteine cathepsins are present in the nuclei and cytoplasm of both kidney embryonic cells and kidney tumor cells. However, the ratio of the contents of individual enzymes in the nucleus and in the cytoplasm could be significantly different depending on the cell type.

Cysteine cathepsins mature more effectively in kidney tumor cells than in embryonic kidney cells. The final step of our study involved determination of various forms of the cathepsins generated as a result of enzyme maturation and contained in the cytoplasm and nuclei of embryonic kidney cells and kidney tumor cells. Cytoplasmic and nuclear fractions of the cells were obtained using

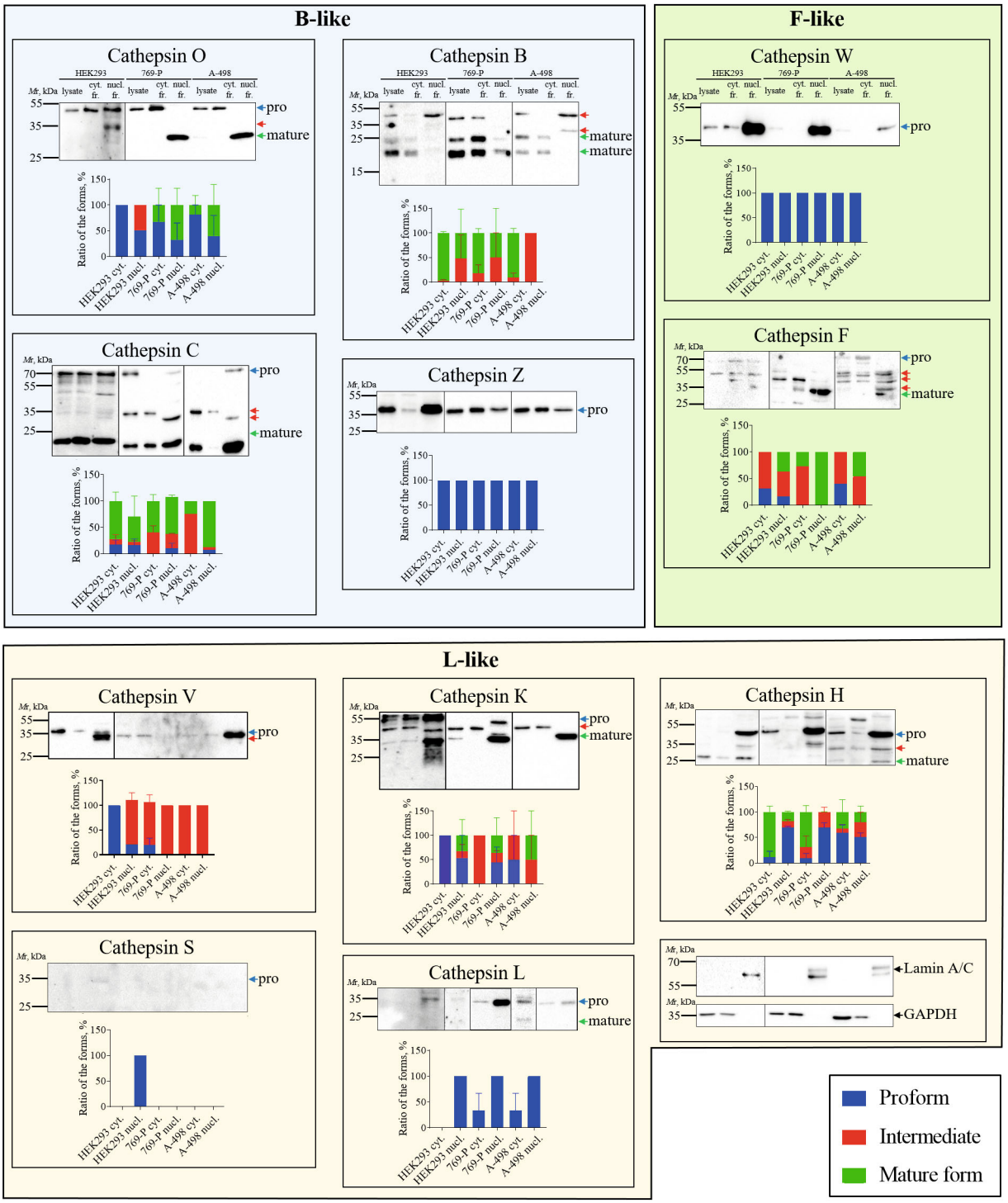

Fig. 3. Determination of different forms of B-, F-, and L-like cysteine cathepsins in HEK293 embryonic kidney cells and kidney tumor cells 769-P and A-498. Analysis of cell lysates and fraction was carried out with the help of Western blotting. Membranes were stained with the help of antibodies against 11 cysteine cathepsins. Antibodies against GAPDH and lamins A/C were used to test purity of cytoplasmic and nuclear fractions. Pro – proform of cathepsin, mature – mature form of cathepsin, arrows without designations – intermediate forms of cathepsin, cyt. fr. – cytoplasmic fraction, nucl. fr. – nuclear fraction. Results of processing of Western blots are presented as histograms (mean value and standard deviation).

fractionation and analyzed by means of Western blotting using antibodies against all 11 cathepsins (Fig. 3).

Analysis of the Western blotting data showed that the cathepsins Z, W, V, and L are present in cells as proforms, which could be detected both in the cytoplasm and nucleus (Fig. 3). Proforms as well as mature forms of the cathepsins K, C, F, and H were found in the nuclear fractions isolated from all three cell lines.

Analysis of the fractions for cathepsin S revealed the presence of its proform only in the nuclear fraction of kidney embryonic cells. Active mature forms of cathepsin O were detected in the nuclear fraction obtained from the tumor cells, while the nuclear fraction of embryonic cells contained exclusively profrom and intermediate forms. Cathepsin B was predominantly detected in the cytoplasmic fraction as an intermediate and mature forms, while the nuclear fraction contained only a minor amount of the intermediate form of the enzyme.

Based on the obtained results presented above it can be concluded that the cysteine cathepsins are present in the cell nuclei in all possible cases. Different forms of the cathepsins O and B are present in the nuclear fractions of embryonic kidney cells and kidney tumor cells. Moreover, the processes of cathepsin maturation occur more effectively in the nuclei and cytoplasm of tumor cells than in the nuclei and cytoplasm of embryonic cells.

DISCUSSION

The results obtained in this study support and complement the data available in the literature indicating that the expression of almost all cysteine cathepsins is enhanced in tumor cells [7-26]. At the molecular level, overexpression and secretion of cysteine cathepsins leads to proteolysis of the extracellular matrix proteins and surface membrane proteins, which, in turn, result in disruption of intercellular contacts, increasing the migration of tumor cells and tumor metastasis [39].

In this study, we observed overexpression of cathepsins B and L in kidney cancer cells. An increased level of expression of the cathepsins B and L has also been reported for other tumor cells, such as pancreatic cancer cells [40, 41]. Using the mouse model of pancreas carcinogenesis RIP1-Tag2 employing islets of Langerhans, it was demonstrated that cathepsins B and L participate in proteolysis of the tumor suppressor E-cadherin [42], which resulted in the activation of epithelial-mesenchymal transition. This, in turn, facilitated metastasis and chemoresistance in tumor cells [43]. We suggest that a similar cleavage of E-cadherin could occur in kidney tumor cells with the help of cathepsins B and L. Overexpression of the cathepsins H, K, and Z was also documented, and molecular mechanisms of the participation of these enzymes in the development of prostate [44, 45] and liver [46] cancer have been established. Moreover, the results obtained in this work are in agreement with the literature data on overexpression of the cathepsins in brain [7-9, 47], breast [10-13], lung [19], colon [14, 15], skin [16], and prostate [44, 45, 48, 49] cancers.

Interestingly, it was observed that the ratio of cathepsin K and cathepsin O forms in the tumor cells was shifted towards the mature enzymes. The mature form of cathepsin K is catalytically active and commonly generated inside liposomes [1]. Correspondingly, the predominance of the mature forms of cathepsins K and O in tumor cells could indicate more complete processing of these enzymes in lysosomes, leading to their full activation. It is logical to suggest that the oncogenic role of the cathepsins K and O in kidney cancer development could be due to their full activation. The hypothesis stating that the effective maturation of the cathepsins K and O mediates their active involvement in carcinogenesis is in agreement with the recently published data on the role of these cathepsins in the development of prostate [45] and breast [11] cancer.

The availability of multiple intermediate forms of cathepsins in kidney tumor cells could be explained by the catalytic activity of cysteine proteases. It is known that the cathepsins K and V can cleave cathepsin C with the formation of several intermediate forms [50]. The molecular weights of these intermediate forms correspond to the sizes of cathepsin fragments found in this study in kidney tumor cells.

Analysis of the expression of the cysteine cathepsins revealed that the content and composition of cathepsins O, C, V, H, and L differ in the tumor cells 769-P and A-498. The tumor cell lines used in this study, 769-P and A-498, belong to the group of kidney carcinomas. Some researchers consider the A-498 cell line a papillary renal cell carcinoma, while the 769-P cell line belongs to the group of clear-cell renal cell carcinomas. These groups exhibit different molecular and histological traits [51, 52]. In particular, different levels of cell growth were observed for 769-P and A-498 cell lines upon the addition of plant extracts [53], and the cell responses to the TRAIL-induced apoptosis were also different [54]. Due to the differences in the abovementioned molecular, morphological, and biochemical properties of 769-P and A-498 cells, one could expect different levels of expression of the cysteine cathepsins and different patterns of their processed forms.

We have shown in this work that all 11 cysteine cathepsins are present in the nuclei of both embryonic and tumor kidney cells. These results are confirmed by the literature data demonstrating the nuclear localization of a number of cathepsins in cancer cells [15, 23, 25, 36, 49-58]. At the same time, we were the first to demonstrate that cathepsins O, W, Z, and K are present not only in the cytoplasm but also in the cell nucleus.

The nuclear localization of the cysteine cathepsins in the kidney cancer cells observed in this study agrees with the literature on the carcinogenic role of nuclear cathepsins. In particular, an active, mature form of cathepsin L has been found in the nucleus of the colorectal carcinoma cells HCT116, where it participates in the disruption of the cell cycle [57]. Moreover, these cells lack stefin B, physiological inhibitor of cathepsin L. The molecular mechanism underlying the carcinogenic effect of cathepsin L in the cell nucleus involves

proteolysis of the transcription factor CDP/Cux, which participates in the regulation of the cell cycle [59] and could play a role as an oncogene [60]. Another example involves nuclear membrane-associated cathepsins B, K, L, and S regulating the nuclear transport of the SMAD proteins, which are important components of the TGF-β signaling pathway. This pathway plays an important role in cancer development [61] and in anticancer immunity [62]. Another representative of the cysteine cathepsins family, cathepsin V, has also been found in the nuclei of the cells of thyroid carcinoma. Its truncated N-terminal nuclear form likely participates in the disruption of the cell cycle and causes hyperproliferation [24].

The results of the current study show that mature cysteine cathepsins are partly localized in the nuclei of kidney tumor cells. It is known from the literature that cathepsins are activated at an acidic pH [63] or with the help of other proteases [50]. On the contrary, an increase in pH commonly results in the loss of activity by cathepsins [64]. We suggest that under neutral pH in the cell nucleus, cathepsins could either partly lose their activity or change their substrate specificity, which would prevent the non-specific degradation of nuclear proteins. In addition, the catalytic activity of cathepsins in the cell nuclei could be regulated by their selective inhibitors, with their nuclear localization being confirmed by the literature [65].

CONCLUSIONS

In this study, we compared the levels of expression of cysteine cathepsins in kidney embryonic and kidney tumor cells. We established that the expression of cysteine cathepsins V, B, Z, F, and H is increased in tumor cells. Based on the analysis of the content of mature cathepsin forms, we concluded that cathepsin maturation occurs more effectively in tumor cells than in embryonic cells. Furthermore, we compared the localization of all 11 cysteine cathepsins in one cellular model and showed that all these enzymes are present in the cell nucleus to varying degrees. Importantly, several cathepsins were found in their active form in the nucleus, suggesting that cysteine cathepsins are functionally active not only in the cytoplasm, but also in the cell nucleus. Although the functions of some cathepsins with nuclear localization are being actively studied, the molecular mechanisms of their action in the nucleus are not yet fully understood. We hope that the results of this study on the expression and localization of cysteine cathepsins will aid in the identification of novel tumor-specific biomarkers in the tissues of patients with kidney cancer.

Contributions. A.S.F., A.A.Z. – concept of the study and work supervision; A.S.F., N.K.T., I.I.K. – conducting experiments; A.S.F., E.Yu.Z., A.P., K.I.I., A.A.Z. – discussion of the results; A.S.F., A.A.Z. – writing the paper; A.S.F., K.I.I., A.A.Z. – editing of the paper.

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