Distribution of cathepsin L in human umbilical cord tissues

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The extracellular matrix components show specific distribution patterns within various structures of the umbilical cord, among which Wharton's jelly is especially collagen-rich tissue. Cathepsin L is a potent cysteine protease engaged in degradation of extracellular matrix proteins, including collagens. We evaluated the activity and expression of cathepsin L, and the inhibitory effect of cysteine protease inhibitors in the umbilical cord arteries, vein and Wharton's jelly. Cathepsin L activity and anti-papain inhibitory effect of cysteine protease inhibitors were quantified in extracts of separated umbilical cord tissues using fluorogenic substrates. The results were calculated per DNA content. The enzyme expression was assessed by Western immunoblotting. The active cathepsin L activity (without activation by pepsin digestion), its percentage in the total activity (after pepsin activation), and the expression of the mature single-chain enzyme were the lowest in the umbilical cord arteries and the highest in Wharton's jelly. The effect of cysteine protease inhibitors showed similar distribution as in the case of the active enzyme, being the highest in Wharton's jelly. Distribution of the activity and expression of mature cathepsin L within the umbilical cord probably results from distinctions in the proenzyme activation process. Differences in the action of cysteine protease inhibitors can partly restrict divergences in the enzyme activity that could reflect its expression alone. Differential enzyme action seems to contribute to tissue-specific collagen turnover within the umbilical cord cells, especially those of Wharton's jelly.

Key words: cathepsin L, cysteine protease inhibitors, umbilical cord, umbilical cord artery, umbilical cord vein, Wharton's jelly

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Abbreviations: AMC, 7-amido-4-methylcoumarin; CATB, cathepsin B; CATL, cathepsin L; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulphonate; CPIs, cysteine protease inhibitors; EDTA, ethylenediaminetetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ECM, extracellular matrix; GAG; glycosaminoglycan; hc-CATL, heavy chain of double-chain cathepsin L; IGF-I, insulin-like growth factor-I ; IGF BP-3, insulin-like growth factor binding protein-3; pro-CATB, procathepsin B; pro-CATL, procathepsin L; sc-CATL, single-chain cathepsin L; TGF-β, transforming growth factor-beta; UC, umbilical cord; UCA, umbilical cord artery; UCV, umbilical cord vein; WJ, Wharton's jelly; Z–FR–AMC, benzyloxycarbonyl-phenylalanyl-arginyl-7-amido-4-methyl-coumarin; Z-FY(tBu)-DMK, Z-Phe-Tyr(t-Bu)-diazomethylketone

INTRODUCTION

The vascular system of the placenta plays a key part in the intrauterine foetal growth and development (Bole-hovská *et al.*, 2015). The umbilical cord (UC) is the vital life-sustaining structure connecting the foetus with the placenta (Can & Karahuseyinoglu, 2007; Romanowicz & Galewska, 2011; Corrao *et al.*, 2013; Bosselmann & Mielke, 2015). It contains one vein delivering oxygenated and nutrient-rich blood to the foetus and two arteries carrying deoxygenated blood and metabolic waste products to the placenta (Romanowicz & Galewska, 2011; Bosselmann & Mielke, 2015). The vascular wall is rich in many extracellular matrix (ECM) macromolecules (collagen, elastin, proteoglycans and glycoproteins) necessary for its proper structure and functions (Ferguson & Dodson, 2009; Romanowicz & Galewska, 2011).

The umbilical cord vessels are embedded in Wharton's jelly (WJ), which is a mucous connective tissue consisting of scarce cells immersed in abundant ECM components, mainly collagen, hyaluronate and several proteoglycans with various sulphated glycosaminoglycan (GAG) chains (Sobolewski *et al.*, 1997; Gogiel *et al.*, 2003; Can & Karahuseyinoglu, 2007; Ferguson & Dodson, 2009; Corrao *et al.*, 2013). The abundant ECM components in this tissue provide hydration and supramolecular scaffold that protects its vessels from constriction, overdistension, torsion and bending, caused by foetal grasping or movements and uterine contractions (Can & Karahuseyinoglu, 2007; Ferguson & Dodson, 2009; Corrao *et al.*, 2013; Bosselmann & Mielke, 2015). Additionally, the Wharton's jelly is a rich storage of numerous peptide growth factors that support stromal cells proliferation, differentiation, synthesis and remodelling of the ECM (Sobolewski *et al.*, 2005; Corrao *et al.*, 2013).

ECM homeostasis is conditioned on balanced secretion and degradation of its various components (Corrao *et al.*, 2013) and matrix remodelling is highly affected by activities of proteolytic enzymes, such as cysteine cathepsins (Lalmanach *et al.*, 2015). These include the lysosomal endopeptidase cathepsin L (EC 3.4.22.15; CATL), a member of the papain family of cysteine proteinases that is ubiquitously expressed in many cells and tissues (Kirschke, 2004; Turk *et al.*, 2012; Tan *et al.*, 2013; Pišlar & Kos, 2014; Lalmanach *et al.*, 2015; Sudhan & Siemann, 2015). It is synthesized as a preproenzyme consisting of 333 amino acid residues, processed to an inactive latent precursor and transported to lysosomes (Ishidoh *et al.*, 1998; Tan *et al.*, 2013). The proenzyme is converted to a mature single-chain CATL (devoid of the propeptide), which is further cleaved into a double-chain form (Ishidoh *et al.*, 1998).

Cathepsin L plays a major role in the lysosomal degradation of both intracellular and endocytosed proteins (Sudhan & Siemann, 2015) under acidic conditions (Kirschke, 2004; Turk *et al.*, 2012; Tan *et al.*, 2013; Sud-

han & Siemann, 2015). The enzyme cleaves a wide range of ECM components, including collagens, proteoglycans, fibronectin, laminin and elastin (Ishidoh & Kominami, 1995; Kirschke, 2004; Turk *et al.*, 2012; Tan *et al.*, 2013; Sudhan & Siemann, 2015). Moreover, it can indirectly facilitate matrix breakdown by proteolytic activation of latent proforms of other degrading enzymes, including urokinase plasminogen activator (Kirschke, 2004; Sudhan & Siemann, 2015), pro-heparanase (Sudhan & Siemann, 2015), other cathepsins (Turk *et al.*, 2012; Lalmanach *et al.*, 2015; Sudhan & Siemann, 2015), and certain matrix metalloproteinases (Kirschke, 2004; Turk *et al.*, 2012; Lalmanach *et al.*, 2015; Sudhan & Siemann, 2015) or by cleavage and inactivation of inhibitors, such as alpha 1-proteinase inhibitor and secretory leucoprotease inhibitor (Lalmanach *et al.*, 2015).

The activity of CATL is regulated by endogenous cysteine protease inhibitors (CPIs) of the cystatin superfamily including stefins, cystatins and kininogens (Turk *et al.*, 2012; Novinec & Lenarčič, 2013; Pišlar & Kos, 2014; Lalmanach *et al.*, 2015; Sudhan & Siemann, 2015). These competitive, reversible, tight-binding, proteinaceous inhibitors (Turk *et al.*, 2012; Novinec & Lenarčič, 2013; Pišlar & Kos, 2014; Lalmanach *et al.*, 2015) are widespread in human cells, tissues and body fluids (Turk *et al.*, 2012; Novinec & Lenarčič, 2013; Pišlar & Kos, 2014; Lalmanach *et al.*, 2015), where they trap and neutralize an excessive peptidase activity (Pišlar & Kos, 2014). A balance between active cysteine cathepsins and CPIs is crucial for proper cellular and tissue homeostasis (Novinec & Lenarčič, 2013).

The distribution of cathepsin L and cysteine protease inhibitors in the umbilical cord has not hitherto been investigated. Therefore, we decided to compare the activity and expression of CATL, and the inhibitory effect of CPIs in the umbilical cord arteries, umbilical cord vein and in Wharton's jelly of healthy newborns.

MATERIALS AND METHODS

The investigation protocol was approved by the Committee for Ethics and Supervision on Human and Animal Research of the Medical University of Bialystok, Poland (Approval No R-I-002/169/2011; 28 April, 2011).

Tissue material. Studies were performed on the umbilical cord arteries (UCAs), umbilical cord veins (UCVs) and Wharton's jelly taken from 10 newborns delivered at term by healthy mothers aged 20–30. The tissue samples and extract preparation were the same as we previously described in detail (Gogiel *et al.*, 2012).

Cathepsin L activity assay. Cathepsin L activity was measured fluorometrically as previously described (Kirschke *et al.*, 1982) with modifications including performing the assay in 96-well black microtiter plates. The final incubation mixture (200 µl) contained tissue extract with fluorogenic substrate benzyloxycarbonyl-phenylalanyl-arginyl-7-amido-4-methylcoumarin (Z–FR–AMC; Peptide Institute, Inc.; 10 µM) in 0.1 M citrate/phosphate buffer pH 5.5, supplemented with 3 mM ethylenediaminetetraacetic acid (EDTA), 4 mM dithiothreitol, 0.05% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulphonate (CHAPS), 7.5 µM pepstatin and the specific cathepsin B inhibitor N-(L-3-*trans*-propylcarbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline (CA-074; Bachem; 1 µM), added to avoid interference of cathepsin B (Werle *et al.*, 1995). Fluorescence of the released 7-amido-4-methylcoumarin (AMC) was read continuously at 1-minute intervals with a Tecan Infinite® 200 PRO multimode microplate reader (Tecan Group Ltd., Männedorf, Switzerland) at the excitation and emission wavelengths of 354 and 442 nm, respectively. Measurements were standardised with 10 µM AMC. Initial rates were monitored for less than 5% of total substrate conversion. Duplicate samples additionally contained the cathepsin L-specific inhibitor Z-Phe-Tyr(t-Bu)-diazomethylketone (Z-FY(tBu)-DMK, Enzo Life Sciences; 10 µM). The difference between values for samples without and with Z-FY(tBu)-DMK corresponded to CATL activity. One unit of enzyme activity (U) was defined as the amount of enzyme releasing 1 µmol of product (AMC) per minute at 37°C.

Latent CATL was activated with pepsin as previously described (Gianotti *et al.*, 2008) with modifications. Briefly, 100 µl of UCA, UCV and WJ extracts, 100 µM AMC in the extraction buffer (standard), or the extraction buffer alone (negative control) were incubated with 100 µl of 0.1 M citrate/phosphate buffer, pH 3.2, containing 7 mg/ml pepsin, for 60 min at 37°C (the pH during this step was 3.5). Duplicate samples were kept on ice in the absence of pepsin. The reaction was stopped by adding 300 µl of 0.1 M citrate/phosphate buffer, pH 6.1, containing 4 mM EDTA and 25 µM pepstatin. All the samples were then subjected to CATL activity assay. The activities measured for samples incubated with pepsin were defined as total CATL activities. The values for samples incubated without pepsin represented active CATL activities. The differences between total and active CATL activities corresponded to the latent enzyme activities.

Determination of inhibitory effect of CPIs in the UC tissue extracts. The inhibitory effect of cysteine proteinase inhibitors was assayed against papain after heat treatment of tissue extracts (at 100°C for 10 min), using fluorogenic substrate, Z-FR-AMC, as we previously described (Gogiel *et al.*, 2015). One unit of CPI effect (U) was defined as the amount of inhibitory proteins required to totally inhibit the release of 1 µmol of product (AMC) by papain per minute at 37°C.

Western blot analysis. Samples of UCA, UCV and WJ extracts were mixed with a cocktail of protease inhibitors (catalogue number P8340; Sigma) at a ratio 100:1 (v/v). They were normalized according to their DNA content (0.8 µg) and resolved on 10% SDS-polyacrylamide gel under reducing conditions according to the method of Laemmli (1970). Separated proteins were transferred to nitrocellulose membranes (Sigma) and probed with rabbit polyclonal antibodies against human cathepsin L (catalogue number 219387; Merck; 1:2000), overnight at 4°C. Bound antibodies were detected using horseradish peroxidase-conjugated mouse anti-rabbit secondary antibody (clone RG-16, catalogue number A2074; Sigma; 1:20 000) and developed with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). The blots were striped and reprobed with anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) rabbit polyclonal antibody (catalogue number G9545; Sigma; 1:20 000), overnight at 4°C, followed by an incubation with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody for 1 hour at room temperature and detection with BCIP/NBT reagent (catalogue number B1911; Sigma).

Determination of DNA content in the UC tissues. DNA concentration was determined by the fluorimetric method of Downs and Wilfinger (1983), with the use of *bis*-benzimidazole Hoechst 33258 dye (Sigma) and modifications, as we previously described (Gogiel *et al.*, 2015).
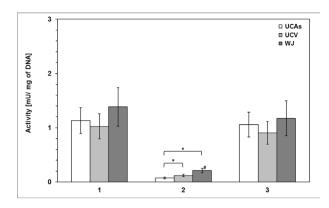
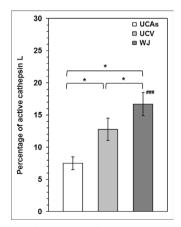**Figure 1. Activity of cathepsin L in umbilical cord tissues.**
The extracts of umbilical cord arteries (UCAs), umbilical cord vein (UCV) and Wharton's jelly (WJ) were assayed using Z–FR–AMC as a fluorogenic substrate and Z-FY(tBu)-DMK as a specific inhibitor. Mean values ± SEM (n=10) of total (1), active (2) and latent (pepsin-activatable; 3) cathepsin L activities per milligram of DNA contained in the examined tissues are shown. Data were analyzed using Friedman repeated-measures ANOVA (#P<0.05) and *post-hoc* Wilcoxon signed-ranks testing corrected for multiple comparisons by the Bonferroni-Holm method (*P<0.05).
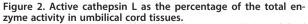
Statistical analysis. Data are shown as the mean values from 10 assays and standard errors of mean (SEM). Friedman ANOVA was performed followed by multiple *post-hoc* Wilcoxon signed-ranks tests corrected by Bonferroni-Holm method. Differences in results with $P<0.05$ were assumed as statistically significant. Statistical analysis was carried out using Statistica 10 software (StatSoft, Inc; Hill & Lewicki, 2007).

RESULTS

Cathepsin L activity

The activities of cathepsin L calculated per milligram of DNA in UC tissues are presented in Fig. 1. Both total and latent CATL activities were almost evenly distributed within the UC tissues (Fig. 1, parts 1 and 3, respectively;

all $P>0.05$). On the other hand, the active CATL activity in UCV was over 1.5 times higher, and that of WJ – nearly 3 times higher than in the UCAs (Fig. 1, part 2; both $P<0.05$). The values for active CATL (Fig. 1, part 2) were much lower than those of the total and latent enzyme (Fig. 1, parts 1 and 3, respectively).

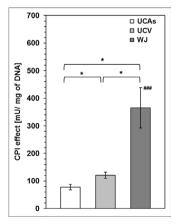### Active cathepsin L activity as the percentage of the total enzyme activity

In order to evaluate the efficiency of CATL activation we decided to compare the activities of the active enzyme expressed as percentages of those of the total enzyme, measured for individual materials. It is apparent from Fig. 2 that the activity of active CATL constituted only 7.5–16.7% of the total activity, depending on the UC tissue (Fig. 2). As can be seen, this percentage for the WJ was over 2 times higher than for the UCAs and almost 1.5 times higher than for the UCV, and that of UCV – over 1.5 times higher when compared with the UCAs (Fig. 2, all $P<0.05$).
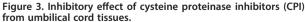
Inhibitory effect of cysteine protease inhibitors

Figure 3 shows CPI effect of the UC tissue extracts. This effect in the WJ was over 4.5 times higher than in the case of UCAs and about 3 times higher than in the UCV, whereas that of UCV – over 1.5 times higher than in the UCAs (Fig. 3, all $P<0.05$).

Expression of cathepsin L protein in the umbilical cord tissues

Western immunoblot analysis of cathepsin L protein expression in the extracts of UC tissues is presented in Fig. 4. Several specific bands were detected. These included: bands of 37–41 kDa, corresponding to procathepsin L (pro-CATL; Kirschke, 2004), 34 kDa band, probably reflecting the presence of partial zymogen activation intermediate (see asterisk; Reilly *et al.*, 1989), 28 kDa band of the single-chain mature CATL (sc-CATL), and 24 kDa band of a heavy chain of the fully mature double-chain enzyme (hc-CATL; Kirschke, 2004). The intensity of pro-CATL bands (37–41 kDa)

Figure 2. Active cathepsin L as the percentage of the total enzyme activity in umbilical cord tissues.
Cathepsin L activities in umbilical cord arteries (UCAs), umbilical cord vein (UCV) and Wharton's jelly (WJ) were determined using Z–FR–AMC as a substrate and Z-FY(tBu)-DMK as a specific inhibitor. Active and total enzyme activities were measured in individual tissue extracts. Mean values ± SEM (n=10) are presented. Data were analyzed with Friedman ANOVA (###, $P<0.001$) followed by *post-hoc* Wilcoxon signed-ranks tests with Bonferroni-Holm corrections (*P<0.05).

Figure 3. Inhibitory effect of cysteine proteinase inhibitors (CPI) from umbilical cord tissues.
The CPI effect of heat-treated extracts of umbilical cord arteries (UCAs), umbilical cord vein (UCV) and Wharton's jelly (WJ) against active site titrated papain was measured using Z–FR–AMC as a substrate. Mean values ± SEM (n=10), expressed per milligram of DNA contained in umbilical cord tissues, are presented. Data were analyzed with Friedman ANOVA (###, $P<0.001$) followed by *post-hoc* Wilcoxon signed-ranks tests with Bonferroni-Holm corrections (*P<0.05).
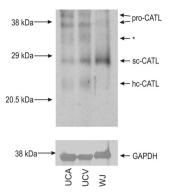
Figure 4. Western immunoblot analysis of cathepsin L in pooled extracts of umbilical cord tissues.

Samples were applied based on DNA content (0.8 µg/ lane). Proteins were resolved by SDS-PAGE (10%) under reducing conditions and immunoblotted with antibodies to human cathepsin L (upper panel) and human GAPDH (lower panel). UCA – umbilical cord artery; UCV – umbilical cord vein; WJ – Wharton's jelly. The positions of pre-stained molecular mass markers (catalogue number 1610318; Bio-Rad Laboratories) which were pre-calibrated with unstained standards (catalogue number 17-0446-01; Pharmacia) by SDS/PAGE are indicated on the left. Immunolabelled cathepsin L bands are marked on the right: pro-CATL – procathepsin L; sc-CATL – single-chain mature cathepsin L; hc-CATL – heavy chain of fully processed double-chain mature cathepsin L; an intermediate form of partially processed pro-CATL is marked with asterisk.

was highest in the UCAs (Fig. 4, lane 1), slightly lower for the UCV (lane 2) and very weak for WJ (lane 3). The intermediate band of 34 kDa was more intense for the UCV (lane 2) than for the UCAs (lane 1), but only trace for WJ (lane 3). In contrast, the band of sc-CATL (28 kDa) was stronger for the UCV (lane 2) than for the UCAs (lane 1) and by far the most prominent for WJ (lane 3). On the other hand, the intensity distribution of hc-CATL band (24 kDa) was similar to that of the intermediate band, being more prominent for the UCV (lane 2) than for the UCAs (lane 1), and only trace for WJ (lane 3). However, the overall intensity of the two mature CATL bands was highest for WJ because of that of the single-chain form, which was the most distinct in the whole blot (Fig. 4, lanes 1–3).

DISCUSSION

Cathepsin L plays major biological role in various physiological processes. Apart from being involved in general protein turnover, it participates in antigen presentation, cardiac function, skin homeostasis, bone resorption, reproduction, cell development and differentiation (Kirschke, 2004; Turk et al., 2012; Novinec & Lenarčič, 2013; Tan et al., 2013; Lalmanach et al., 2015; Sudhan & Siemann, 2015). It was suggested to be necessary for normal embryo implantation and development, uterine decidualization and placentation (Afonso et al., 1997). The umbilical cord is a foetus-derived structure; therefore it may provide unique insight into processes taking place within the developing foetus (Ferguson & Dodson, 2009; Corrao et al., 2013).

We investigated the distribution of cathepsin L in all the umbilical cord tissues. The catalytically active CATL is located within the cells (Tan et al., 2013; Sudhan & Siemann, 2015), therefore we quantified its activity against DNA content, which is widely used to assess the cell number in tissues. The results presented here showed that active (not pepsin-treated) CATL activity, calculated per milligram of DNA, was highest in WJ and lowest in the UCAs. These differences strongly correlated with those of the expression of mature CATL forms, as assessed by Western immunoblotting.

It is known that the activity of lysosomal cysteine proteases is a delicate balance of their synthesis, targeting, zymogen activation, inhibition by endogenous inhibitors and degradation (Turk et al., 2012). It should be noted that the total CATL activity was similar in both UC vessels and in WJ, thus pointing to comparable biosynthetic activity of cells from all UC tissues. Therefore, the observed differences in the activity and expression of the mature enzyme seem to be mainly caused by divergences in the process of pro-cathepsin L activation. It is supported by a positive correlation of active CATL activity and its percentage in the total enzyme activity, as well as by a negative correlation between the expressions of mature enzyme forms and pro-CATL in the UC tissues. These differences may result from specific features of cells residing in the individual tissues of the umbilical cord. The WJ cells, initially described as myofibroblasts (Can & Karahuseyinoglu, 2007; Ferguson & Dodson, 2009; Corrao et al., 2013), have properties of mesenchymal stem cells, because they can differentiate towards various mature cell types derived from all the three germ layers (Can & Karahuseyinoglu, 2007; Corrao et al., 2013). On the other hand, endothelial cells and smooth muscle cells are located in the UC vessels (Can & Karahuseyinoglu, 2007; Ferguson & Dodson, 2009). However, mesenchymal stem cells, able to differentiate into (at least) adipocytes and osteoblasts, were reported to reside also within the UCV endothelial/subendothelial layer (Romanov et al., 2003). Such localisation might thus explain an intermediate activation level of pro-CATL in this tissue.

Differences in glycosaminoglycan content and composition can also contribute to dissimilar pro-CATL activation among the UC tissues. It was reported that GAGs can stimulate autocatalytic conversion of pro-CATL into the mature enzyme, with hyaluronate and keratan sulphate being among the most effective (Ishidoh & Kominami, 1995). It is of interest that both the total GAG content and the amount of hyaluronate and keratan sulphate are much higher in Wharton's jelly in comparison to the UCA wall (Sobolewski et al., 1997). Moreover, the amount of these and the other (heparin, heparan sulphate) strongly activating GAGs tends to be higher in the UCV than in the UCAs, although the total GAG content is similar in all UC vessels (Romanowicz et al., 1998). Therefore, it cannot be excluded that tissue-specific GAG-mediated activation mechanism of pro-CATL mentioned above is responsible for higher expression and activity of mature CATL forms in the UCV, and especially in Wharton's jelly.

The distribution of active CATL activities positively correlated with the amount of collagen in the UC tissues. It was previously reported that Wharton's jelly contains about 4 times more (Sobolewski et al., 1997) and UCV – 3 times more collagen (Romanowicz & Sobolewski, 2000) in comparison to the UCA wall (Sobolewski et al., 1997), when calculated per gram of defatted tissue. One can estimate that differences in collagen content per cell within UC tissues are much higher, as the amount of DNA negatively correlates with that of CATL, being 4.5 times less abundant in WJ in comparison to the UCAs and almost 2.5 times less than in the UCV, as we reported previously (Gogiel et al., 2012). One may suppose that such differences would be even higher without differential action of CATL, which can moderate an excessive deposition of collagen and other ECM proteins by cells

of those tissues. Alternatively, the divergent abundance of mature CATL may contribute to prominent collagen accumulation in WJ and, to a lower extent, in the UCV, by indirectly stimulating the ECM production by cells of those tissues. It was reported that CATL is involved in intracellular degradation of insulin-like growth factor binding protein-3 (IGF BP-3; Zwad et al., 2002), which plays the most important role in insulin-like growth factor-I (IGF-I) binding in the umbilical cord (Bańkowski et al., 2002). One may speculate that differences in actual CATL activity distribution in cells of UC tissues contribute to opposite expression pattern of IGF BP-3 as a result of distinctions in its CATL-mediated breakdown. The degradation of this binding protein is thought to increase bioavailability of IGF-I, thus facilitating its binding to cell receptor and leading to enhanced synthesis of ECM components, including collagens (Bańkowski et al., 2002), which are especially abundant in Wharton's jelly (Sobolewski et al., 1997; Ferguson & Dodson, 2009; Corrao et al., 2013; Bosselmann & Mielke, 2015) and, to some extent, in the UCV (Romanowicz & Sobolewski, 2000).

We also determined the total inhibitory effect of CPIs, an important factor controlling the activity of CATL (Turk et al., 2012; Lalmanach et al., 2015; Sudhan & Siemann, 2015). It is apparent that the combined effect of these inhibitors was lowest in the UCAs and the highest in WJ. It was reported that the expression and secretion of cystatin C, one of the most abundant CPIs (Novinec & Lenarčič, 2013; Qin et al., 2013), by various cell types, including fibroblasts, can be stimulated by transforming growth factor-β (TGF-β; Sokol & Schiemann, 2004; Kotajima et al., 2010; Kasabova et al., 2014; Lalmanach et al., 2015). It is of interest that the amount of this factor (per microgram of DNA) is 42-fold higher in WJ than in the UCA (Sobolewski et al., 2005). Therefore, exceptionally high CPI effect in WJ may be caused by the accumulation of TGF-β in this tissue.

Differences in the inhibitory effect of CPIs on ECM-degrading cysteine cathepsins in the UC tissues can favour a prominent ECM accumulation in WJ and, to a lower extent, in the UCV, when compared with the UCAs. A positive correlation between CPI effect and mature CATL expression in the examined tissues may contribute to the lower differences in the enzyme action that could be estimated from its expression alone.

It is apparent from our study that the CPI-mature CATL complexes, apart from the proenzyme, can be responsible for the latent enzyme activity (measured after pepsin digestion). Such complexes seem to significantly contribute to a prominent latency of CATL, as the zymogen expression did not significantly prevail over that of the mature enzyme (sc-CATL and hc-CATL) regardless the tissue examined. Moreover, the latent enzyme activity did not correlate with pro-CATL expression. It is especially evident in the case of WJ, which contained almost exclusively single-chain form of CATL, despite similar latent activity of the enzyme compared to the other UC tissues. Thus, the major part of mature enzyme activity can be masked by such inhibitors and can only be detected after their degradation by pepsin. Interactions with intracellular inhibitors of CATL, stefins A and B, were suggested to completely prevent the detection of the mature enzyme in liver homogenates unless they were preincubated at acidic pH (Mason et al., 1985). Moreover, substantial amount of CATL in human lung tissue was found to be complexed with kininogen, which resulted in low CATL activity values in the tissue extracts (Werle et al., 1995).

The activity and expression patterns of CATL in the examined tissues differed from those for catepsin B (CATB; EC 3.4.22.1), a ubiquitous lysosomal cysteine proteinase (Aggarwal & Sloane, 2014). We previously reported that the highest CATB activity and expression are found in Wharton's jelly and such distribution pattern is mainly caused by differences in the expression and the activity of the latent (pepsin-activatable) enzyme (Gogiel et al., 2012). It is apparent that the activity and expression of the two enzymes are regulated independently.

The expression of CATB is augmented by the action of collagen (Koblinski et al., 2002; Roshy et al., 2003) and TGF-β (Reisenauer et al., 2007) in various cell types, including fibroblasts. In contrast to that, the expression and activity of CATL are only weakly stimulated by TGF-β (Reisenauer et al., 2007). Taking into account the exceptional abundance of collagen (Sobolewski et al., 1997) and TGF-β (Sobolewski et al., 2005) in Wharton's jelly, these factors may be responsible for selective prevalence of only CATB (but not CATL) in this tissue.

Individual forms of these two enzymes also show separate distribution patterns within the umbilical cord, probably because of the differences in lysosomal sorting, conversion and activation. Proforms of both CATB (Aggarwal & Sloane, 2014) and CATL (Ishidoh et al., 1998) undergo conversion to single-chain forms in lysosomes. However, these two proteinases do not co-localize to the same vesicle, pointing to lysosomal sorting pathways typical for each of them (Gopal et al., 2006). Moreover, the proforms of cysteine cathepsins can be constitutively secreted by some cell types, such as macrophages and fibroblasts (Lalmanach et al., 2015). The secretion of procatepsin B (pro-CATB) can be stimulated by collagen (Koblinski et al., 2002), whereas no such effect on pro-CATL secretion was reported. One may suppose that secretion of pro-CATB within the UC tissues positively correlates with the amount of collagen (Sobolewski et al., 1997; Romanowicz & Sobolewski, 2000). As a result, the cells of WJ and, to a lower extent, those of the UCV, secrete relatively more and retain less proform of CATB for intralysosomal activation than in the case of pro-CATL. One cannot exclude that these processes are responsible for different pattern of the activity and expression of mature CATL, and of pro-CATB, within the UC tissues.

In summary, the results of our present study show that the active CATL activity and the expression of the mature single-chain enzyme are lowest in the UCAs and highest in WJ, which may result from specific features of the proenzyme activation process within the cells of UC tissues. The distribution of CPIs is similar to that of sc-CATL, therefore it can partly restrict differences in the enzyme activity that could be estimated from its expression alone. Proteolytic action of CATL can favour degradation of collagen and other ECM proteins by cells within UC tissues or, alternatively, may indirectly stimulate the ECM production by increasing bioavailability of IGF-I following IGF BP-3 cleavage. Therefore, differential enzyme effect may contribute to cell-specific collagen turnover within the umbilical cord, especially in Wharton's jelly. Different morphological and biochemical alterations in the umbilical cord tissues were observed in a variety of pregnancy-associated pathological conditions, including genetic disorders, pre-eclampsia, gestational diabetes, foetal distress and growth retardation, and even in foetal death (Ferguson & Dodson, 2009; Romanowicz & Galewska, 2011; Corrao et al., 2013). Although our work concerns healthy subjects, it may facilitate future studies explaining several aspects of various prenatal pathological conditions.

Conflicts of interests

The authors declare no conflicts of interest.

Acknowledgements

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