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A New Nuclear Protease With Cathepsin L Properties Is Present in HeLa and Caco-2 Cells

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ABSTRACT

Recently many authors have reported that cathepsin L can be found in the nucleus of mammalian cells with important functions in cell-cycle progression. In previous research, we have demonstrated that a cysteine protease (SpH-protease) participates in male chromatin remodeling and in cell-cycle progression in sea urchins embryos. The gene that encodes this protease was cloned. It presents a high identity sequence with cathepsin L family. The active form associated to chromatin has a molecular weight of 60 kDa, which is higher than the active form of cathepsin L described until now, which range between 25 and 35 kDa. Another difference is that the zymogen present in sea urchin has a molecular weight of 75 and 90 kDa whereas for human procathepsin L has a molecular weight of 38–42 kDa. Based on these results and using a polyclonal antibody available in our laboratory that recognizes the active form of the 60 kDa nuclear cysteine protease of sea urchin, ortholog to human cathepsin L, we investigated the presence of this enzyme in HeLa and Caco-2 cells. We have identified a new nuclear protease, type cathepsin L, with a molecular size of 60 kDa, whose cathepsin activity increases after a partial purification by FPLC and degrade in vitro histone H1. This protease associates to the mitotic spindle during mitosis, remains in the nuclei in binuclear cells and also translocates to the cytoplasm in non-proliferative cells. J. Cell. Biochem. 111: 1099–1106, 2010.

KEY WORDS: NUCLEAR CATHEPSIN L; CYSTEIN PROTEASE; CHROMATIN; CELL CYCLE

For a long time, it was thought that mammalian cysteine proteases of the papain family were important only in terminal degradation of proteins in the lysosomes [Chapman et al., 1997]. Recently, a number of observations support the evidence that cystein proteases may also be involved in processing distinct protein substrates in the nucleus [Irving et al., 2002; Goulet et al., 2004, 2007; Ceru et al., 2010] and also during development and differentiation of embryonic mouse stem cells [Duncan et al., 2008] and in early development of sea urchins [Concha et al., 2005a,b; Puchi et al., 2006; Morin et al., 2008].

After fertilization in sea urchins, male chromatin remodeling precedes initiation of cleavage cell cycles. We have reported that a nuclear cysteine protease, ortholog to cathepsin L, degrade sperm histones while CS histone variants remain unaffected [Imschenetzky et al., 1997; Morin et al., 1999a,b; Iribarren et al., 2008]. This protease was present in the nucleus of unfertilized eggs as an inactive precursor and was activated and mobilized into male pronucleus after fertilization. It persists in the nucleus of the zygote during S phase of the initial cell cycle and co-localizes with α-tubulin in the mitotic spindle at the first cleavage division. The inhibition of this protease after insemination blocks the sperm histones degradation that normally follows fertilization and severely disturbs DNA replication. It also alters the organization of the mitotic spindle and aborts the early development at the initial cleavage division [Concha et al., 2005a,b]. When the activity in vivo was blocked by injecting anti-protease antibodies into one of the two initial blastomeres, the cytokinesis was arrested, the chromatin failed to decondense and BrdU incorporation into DNA was inhibited [Puchi et al., 2006]. Since the N-terminal sequence of this protease [Concha et al., 2005a,b; Puchi et al., 2006] and the gene encoding the SpH-protease in the sea urchin *Sphaerechinus granularis* (unpublished results) are homologous to cathepsin L family of proteases, we investigated if the deleterious effect on the cell cycle is related to its cathepsin L activity. We observed an arrest of cell-cycle progression when cathepsin L inhibitor I (Z-Phe-Phe-CH2F) was added to embryos at the first mitosis (80 min post-insemination), similar as when we inhibited the protease by antibodies microinjection [Puchi et al., 2006; Morin et al., 2008].

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In humans, the presence of orthologs to this enzyme are yet unknown. However, the over-expression of cathepsin L variants has been implicated in the metastatic potential of cells derived from prostatic cancer [Colella and Casey 2003], gastric cancer [Saleh et al., 2003], and melanomes [Rousselet et al., 2004; Rousselet and Frade, 2006, 2008]. The implications of nuclear cathepsin L activity in cell-cycle control have only recently been documented in NIH3T3 cells, Ha-ras-V12 transformed 3T3 cells [Goulet et al., 2004, 2007], in colorectal cancer [Sullivan et al., 2009] and in mouse embryonic fibroblasts [Ceru et al., 2010].

We investigated the existence of this cathepsin L variant in HeLa and Caco-2 cell lines using a polyclonal antibody produced in our laboratory that recognizes the active form of the 60 kDa cystein protease in sea urchin, ortholog to cathepsin L. The results indicate that a cathepsin L variant with a molecular weight of 60 kDa, homologous to sea urchin SpH-protease, exists in human cells. The localization varies in function of the cell-cycle stage. In non-proliferative cells its localization is mainly cytoplasmatic, is mobilized to the nucleus when the cells are in a proliferative state and is located in the spindle during mitosis. The localization of the protease in HeLa and Caco-2 cells and the capacity of degrade histones in vitro are concordant with the results published for sea urchin embryos.

METHODS

CELL CULTURE AND SYNCHRONIZATION

HeLa cells (epithelial cells of uterine cervic adenocarcinoma) were cultured in high glucose-DMEM (13.4 g/L of DMEM, 2 mM L-glutamine, and 4.5 g/L glucose) supplemented with 5% fetal bovine serum (FBS) and penicillin–streptomycin (100 U/ml and 100 μg/ml, respectively) at 37°C in 5% CO_2.

Go arrested Hela cells were obtained by incubating 50% confluent culture for 24 h in DMEM without FBS. In order to arrest HeLa cells at the G1/S boundary, thymidine was added to a final concentration of 2 mM for 18 h. Then the cells were washed with PBS 1× and were incubated in a standard HeLa cells growth medium for 9 h. After the release period, a second thymidine block was performed in presence of 2 mM thymidine for 17 h. In order to synchronize the cells in the G2-M transition, cells were incubated with 2 mM thymidine for 18 h. Then they were washed and incubated for 3 h in normal medium. Finally, they were treated with 100 ng/ml of nocodazole for 12 h [Whitfield et al., 2000].

Caco-2 cells (human colorectal adenocarcinoma) were cultured in high glucose-DMEM (13.4 g/L of DMEM, 2 mM L-glutamine, and 4.5 g/L glucose) supplemented with 10% FBS, non-essential amino acids 1× (HyClone), penicillin–streptomycin (100 U/ml and 100 μg/ml, respectively) at 37°C in 5% CO_2. Go arrested Caco-2 cells were obtained by incubating 60% confluent culture for 24 h in DMEM without FBS. Thymidine was added to a final concentration of 2 mM for 16 h to obtain Caco-2 cells synchronized in G1/S. Then the cells were washed with PBS 1× and incubated in normal medium for 9 h. After the release period, a second thymidine block was performed in presence of 2 mM thymidine for 16 h. In order to synchronize the cells in the transition G2-M, cells were incubated with 100 ng/ml of nocodazole for 24 h.

NUCLEAR EXTRACTS PREPARATION

Cells were lysed in buffer A containing 10 mM HEPES pH 7.5, 10 mM KCl, 1 mM EGTA pH 8.0, 0.2 mM EDTA pH 8.0, 1 mM DTT, 0.5 mM PMSF, 100 μg/ml TPCK, and 1 μg/ml leupeptin and centrifuged at 1,000*g* for 15 min at 4°C. The pellet that contains the nuclei was incubated for 1 h in buffer B containing 20 mM HEPES pH 7.5, 420 mM KCl, 25% glycerol, 0.2 mM EDTA pH 8.0, 1 mM EGTA pH 8.0, 1 mM DTT, 0.5 mM PMSF, and 100 μg/ml TPCK at 4°C. Later, it was centrifuged at 16,000*g* for 15 min. Soluble nuclear protein extracts were separated by electrophoresis in 12% polyacrylamide gels and Western blot analysis were performed as described by Towbin et al. [1979]. In order to identify the presence of cathepsin L, Western blots were incubated with antibodies against SpH-protease of sea urchin (1/4,000) and against different human cathepsin L antibodies such as anti-human-cathepsin L H-80 (against the N-terminal, 1/5,000, Santa Cruz Biotechnology), anti-human-cathepsin L CPLH-2D4 (against procathepsin L, 1/200, Santa Cruz Biotechnology) and anti-human cathepsin L C-18 (against the C-terminal, 1/4,000, Santa Cruz Biotechnology). The secondary antibodies used were anti-rabbit-HRP (1/10,000, GE Healthcare), anti-mouse-HRP (1/10,000, Santa Cruz Biotechnology), and anti-goat-HRP (1/10,000, Santa Cruz Biotechnology).

CATHEPSIN L ACTIVITY

Cathepsin activity was measured using Z-Phe-Arg-MCA (carbobenzoxy-phenyl-arginyl-4-methyl coumaryl-7-amide) (Peptides International) as substrate as described by Barrett and Kirschke [1981]. The amount of 7-amino-4-methyl coumarin liberated from the substrate was monitored fluorometrically in a Shimadzu Spectrofluorophotometer RF-5301PC with excitation at 370 nm and emission at 440 nm. Cathepsin L inhibitor I (Calbiochem) and E-64 d (Sigma) were used as inhibitors of the cathepsin L activity.

PARTIAL PURIFICATION BY FPLC

The protease was partially purified by FPLC (Superdex-75 HR 10/30 column; 13–15 μM agarose-dextran beads) from chromosomal proteins released in 420 mM KCl from chromatin of HeLa cells. The elution of the proteins was performed in 10 mM buffer phosphate pH 6.0. Each 1 ml of the fractions collected was eluted with a flow of 0.5 ml/min and the presence of the protease was followed by ELISA immuno-assays. Cathepsin L activity of fraction 12 was measured as previously mentioned.

IN VITRO H1 CLEAVAGE ASSAY

Sperm histone H1 (SpH1) were labeled with [14C]-formaldehyde essentially according to the method of Rice and Means [1971]; 0.3 mg of the proteins to be labeled were dissolved in 86 μl of 100 mM HEPES buffer pH 7.5; 4.4 μl of [14C]-formaldehyde (53 mCi/mmol) and 100 μl of 200 mM sodium cyanoborohydride were added. The mixture was incubated for 2 h at room temperature and dialyzed overnight to remove low-molecular-weight components. The specific activities for [methyl-14C]SpH1 were 85,000 cpm/μg.

The nuclear extracts were incubated with [14C]SpH1 for 2 h at 37°C in 0.05 M phosphate buffer pH 7.5 containing 1 mM DTT, 1 mM EDTA, and 0.1% Triton X-100 [Imschenetzky et al., 1997]. The products resistant to proteolysis were analyzed by electrophoresis in

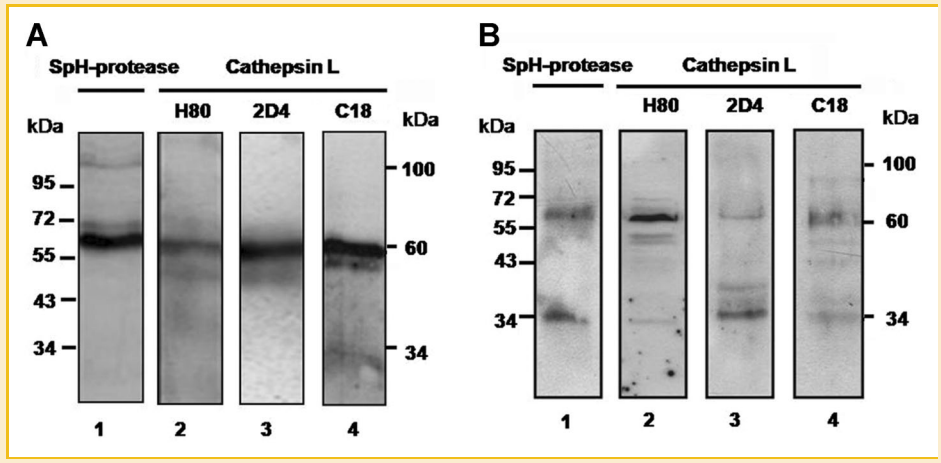

Fig. 1. Detection of cathepsin L in HeLa and Caco-2 cells. Ten micrograms of proteins from asynchronic HeLa cells (A) and Caco-2 cells (B) cultures were analyzed by Western blot in 12% SDS–PAGE and revealed with antibodies against SpH-protease of sea urchin (1), and commercial antibodies against human cathepsin L H-80 (2), human cathepsin L CPLH-2D4 (3), and human-cathepsin L C18 (4).

SDS–PAGE. The radioactivity associated to each electrophoretic band was detected by fluorography as described by Chamberlain [1979].

IMMUNOFLUORESCENSE STAINING

HeLa and Caco-2 cells were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 in buffer 10 mM Tris-phosphate pH 7.8 and blocked with 1% BSA for 1 h. Cells were incubated with rabbit polyclonal anti-sea urchin SpH-protease antibody produced in our laboratory against the N-terminal of the protein (1/250, title 1/9.600) and with a monoclonal anti α-tubulin antibody (1/500, Sigma) overnight at 4°C. The secondary antibodies used were anti-rabbit-TRITC (1/250, Sigma) and anti-mouse-FITC (1/250, Sigma). Fluorescent signals were observed by a Nikon Eclipse TE2000-U confocal microscope.

RESULTS

DETECTION OF A NEW NUCLEAR PROTEASE IN HeLa AND Caco-2 CELLS

We have previously described a 60 kDa nuclear cysteine protease (SpH-protease) that degrades sperm histones (SpH) during male chromatin remodeling after fertilization in sea urchins [Imschenetzky et al., 1997]. It was shown that this enzyme was localized in

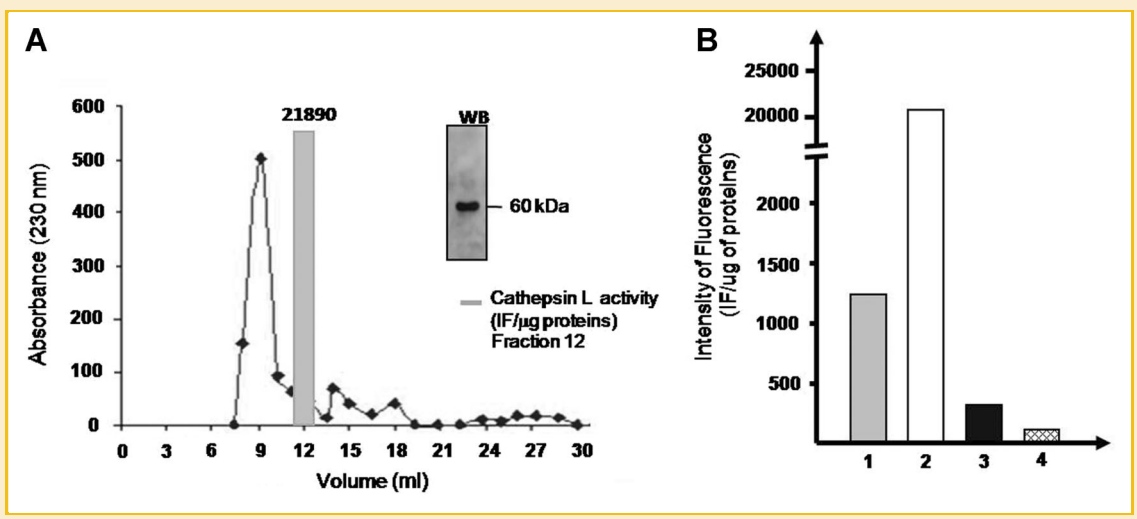

Fig. 2. Partial purification of cathepsin L by FPLC from HeLa cells. A: The protease was purified by FPLC (Superdex-75: agarose-dextran) from 800 µg of chromosomal proteins released in 420 mM KCl from chromatin. The elution of the proteins was performed in 10 mM buffer phosphate pH 6.0 and the presence of the protease was followed by ELISA immuno-assays. Cathepsin L activity of 10 µg of fraction 12 was measured with Z-Phe-Arg-MCA as substrate using the method of Barrett and Kirschke [1981]. The amount of 7-amino-4-methyl coumarin (MCA) release from the substrate was monitored fluorometrically with excitation at 370 nm and emission at 440 nm in a fluorescence spectrometer. B: The activity associated to 10 µg of a nuclear extract obtained from asynchronic HeLa cells culture was measured before (lane 1) and after their purification by FPLC (lane 2). The activity for the purified fraction was also made in presence of cathepsin L inhibitor I (20 µM) (lane 3) and E-64 d (50 µM) (lane 4).

the mitotic furrow and was involved in cell-cycle control in sea urchin embryos [Concha et al., 2005a,b]. Since the N-terminal sequence and the gene encoding this protease was consistent with human cathepsin L family, we investigate, using antibodies against the SpH-protease of sea urchin, if similar proteases are present in HeLa (Fig. 1A) and Caco-2 cells (Fig. 1B). Three proteins with molecular masses around 60, 55, and 34 kDa were detected by inmunoblots of nuclear extracts by anti-sea urchin SpH-protease, anti-human-cathepsin L H-80, anti-human-cathepsin L CPLH-2D4 and anti-human cathepsin L C18 antibodies. These results suggest that exist at least three nuclear forms of protease with homology to cathepsin L in nuclei of human cells.

THE NUCLEAR PROTEASE HAS CATHEPSIN L PROPERTIES

The protease was partially purified by FPLC (Superdex-75: agarose-dextran) from chromosomal proteins released in 420 mM KCl from chromatin of HeLa cells (Fig. 2A). Proteins were eluted with 10 mM buffer phosphate pH 6.0 and the presence of the protease was followed by ELISA immuno-assays. Fraction 12 of FPLC chromatogrphy was analyzed by Western blot and the presence of a protease of 60 kDa was confirmed in HeLa cells. The same results were obtained with the protease isolated from sea urchins (data not shown). This fraction has cathepsin L activity with Z-Phe-Arg-MCA and is sensitive to inhibitors as cathepsin L inhibitor I and E-64 d as shown in Figure 2B. Cathepsin L activity in nuclear extracts is low (lane 1) and increase after its purification by FPLC (lane 2). This result suggests the presence of a potential inhibitor in the nuclear extracts.

To investigate if the protease present in nuclear extracts of HeLa and Caco-2 cells can degrade histone H1 in vitro, in a similar way as the protease from sea urchin, we have analyzed the activity of the nuclear protease against [14C]-SpH1 as substrate. The products of digestion were characterized by an analysis on SDS–PAGE followed by fluorography. Figure 3 shows that the protease present in nuclear extract from HeLa cells (lane 1) and in fraction 12 of FPLC (lane 2) degrade SpH1 just as the protease present in sea urchin nuclear extract (lane 3). In lane 4 the undigested H1 is observed. Nuclear cathepsin L activity was measured in the fraction corresponding to

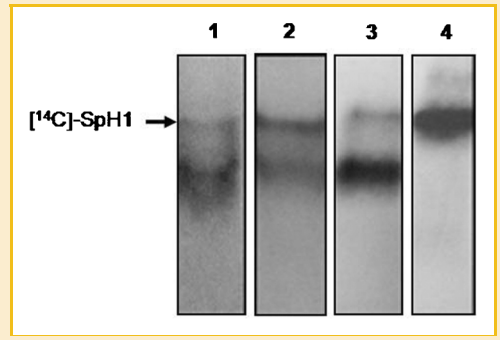

Fig. 3. Nuclear cathepsin L degrade sperm histone H1. Sperm histone H1(14C-SpH1) were incubated with nuclear extract from HeLa cells (1), with fraction 12 obtained by FPLC (2), and with chromatin extract from sea urchin (3). One microgram of undigested sperm histone H1(14C-SpH1) was used as control (4). The products obtained after each incubation were analyzed by SDS–PAGE followed by fluorography.

the chromosomal proteins that are soluble in 420 mM KCl of Caco-2 cells with [14C]SpH1 (Fig. 4A) and Z-Phe-Arg-MCA (Fig. 4B) as substrates and in presence of cathepsin L inhibitor I and E-64 d. These results confirm that the nuclear protease can degrade H1 and has cathepsin L activity.

SUBCELLULAR LOCALIZATION OF CATHEPSIN L VARIES DURING THE CELL CYCLE

Antibodies against the synthetic KLH-peptide that represent the N-terminal sequence of the SpH-protease were obtained in rabbit. The serum IgG fraction was purified as previously described [Concha et al., 2005a]. Since the N-terminal sequence of this protease [Concha et al., 2005a; Puchi et al., 2006] and the gene encoding the SpH-protease in sea urchin *S. granularis* (unpublished results) are homologous to human cathepsin L family, we subsequently investigated its presence at different stages of the cell cycle in mammalian cells.

We found that in HeLa (Fig. 5) and Caco-2 (Fig. 6) cells the intracellular localization of this protease varies according to

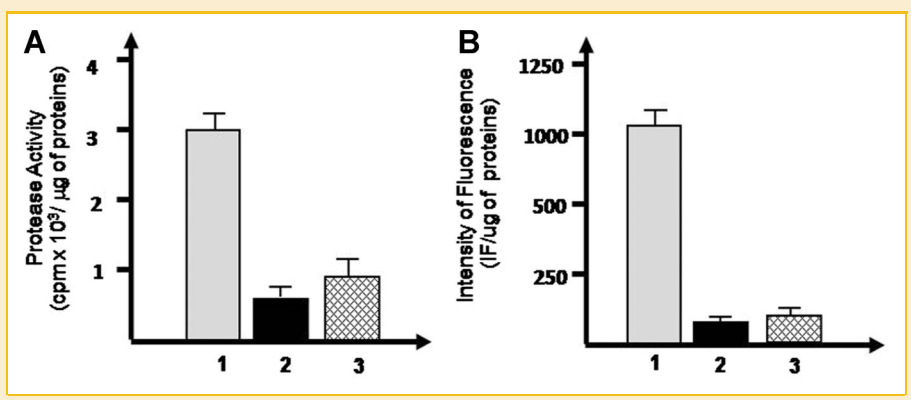

Fig. 4. Substrate selectivity of cathepsin L variant in nuclear extracts from Caco-2 cells. The activity was measured with sperm histone H1 (14C-SpH1) from sea urchin (A) and Z-Phe-Arg-MCA (B) as substrate. The activity associated to 10 µg of proteins obtained from Caco-2 cells synchronized in G1 was measured in absence (1) and presence of cathepsin L inhibitor I (20 µM) (2) and E-64 d (50 µM) (3).

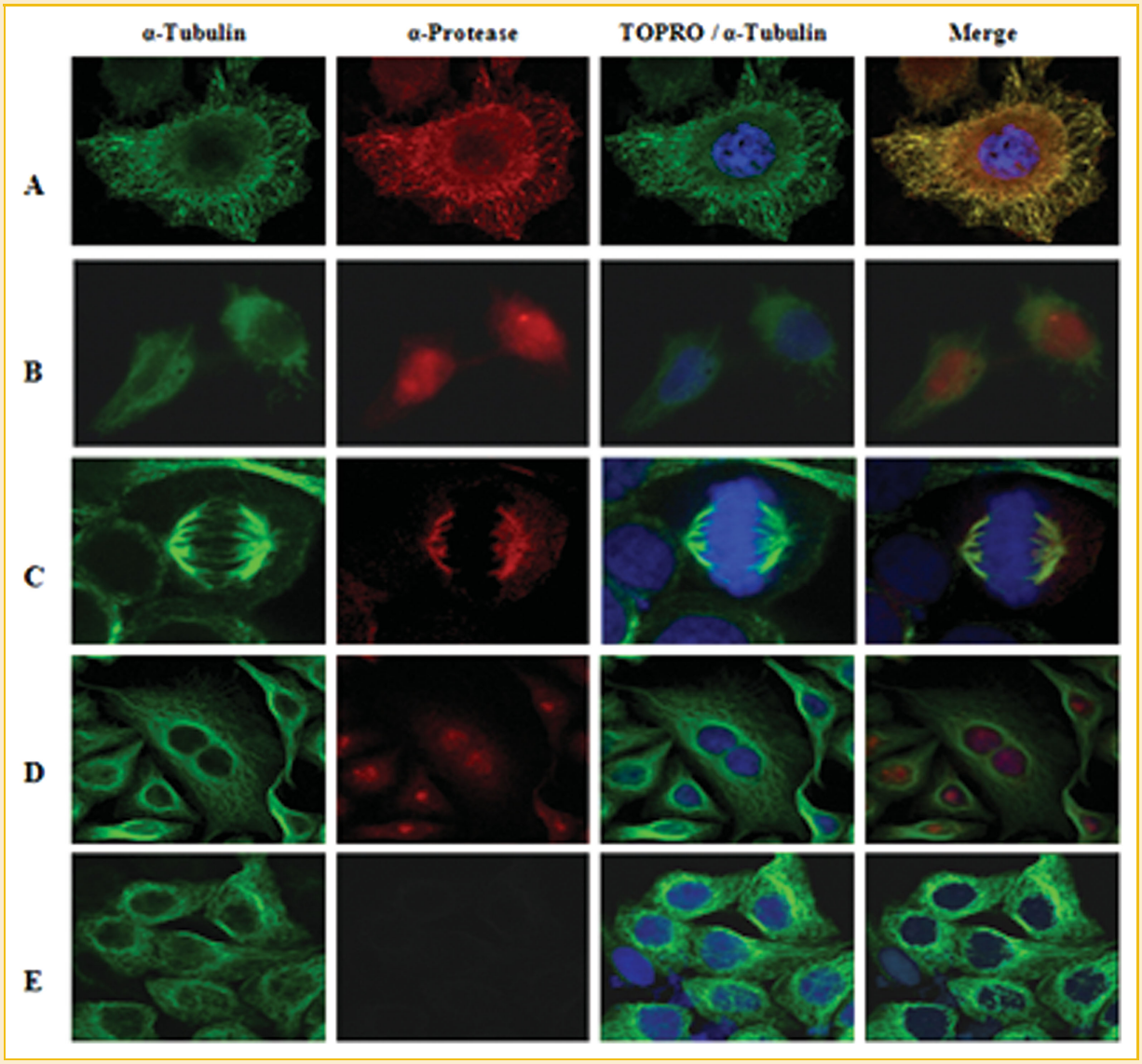

Fig. 5. Immunodetection of the protease during the cell cycle in HeLa cells. Hela cells were fixed from synchronized cultured obtained at Go (A), S phase (B), mitosis (C), and after cell division (D). The cells were incubated with rabbit polyclonal anti-SpH-protease produce in our laboratory (1/250, title 1/9.600) and with monoclonal anti α-tubulin (1/500, Sigma). The secondary antibodies used were anti-rabbit-TRITC (1/250, Sigma) and anti-mouse-FITC (1/250, Sigma). To demonstrate the specificity of the antibodies, the serum was previously immunodepleted by incubation with the commercial peptide representing the N-terminal of the protease used as antigen (E). To visualize the DNA, the cells were stained with TOPRO. The cells were visualized in a confocal microscopy Nikon (inverted Eclipse TE 2000-U).

proliferative status of the cells that were analyzed. In serum-deprived cells (Go), the protease is localized in the cytoplasm and exhibits a perinuclear location (Figs. 5A and 6A). The protease is found in the nucleus of the cells that were arrested at the beginning of S phase (Figs. 5B and 6B). Subsequently it localizes on the mitotic spindle during mitosis (Figs. 5C and 6C) to finally segregate into the two nucleus of the binuclear cells generated after mitosis (Figs. 5D and 6D). Asynchronic cells were stained with rabbit immunodepleted IgG serum as shown in Figures 5E and 6E to demonstrate the specificity of the antibodies against cathepsin L. We conclude that the localization is dependant on the stage of proliferation.

DISCUSSION

On this research, we demonstrate the presence of a new nuclear cathepsin L isoform in HeLa and Caco-2 cells. This protease was detected using an antibody directed against the first 15 amino acids of the amino terminal end of a cystein protease homologous to cathepsin L family present in sea urchin [Morin et al., 2008] and using commercial antibodies against human cathepsin L. This protein has a molecular mass of 60 kDa and its location depends on the proliferative state of the cell. This protease is located in the cytoplasm in a non-proliferative phase, in the spindle during mitosis and in the nucleus after the first cell division. The difference in the

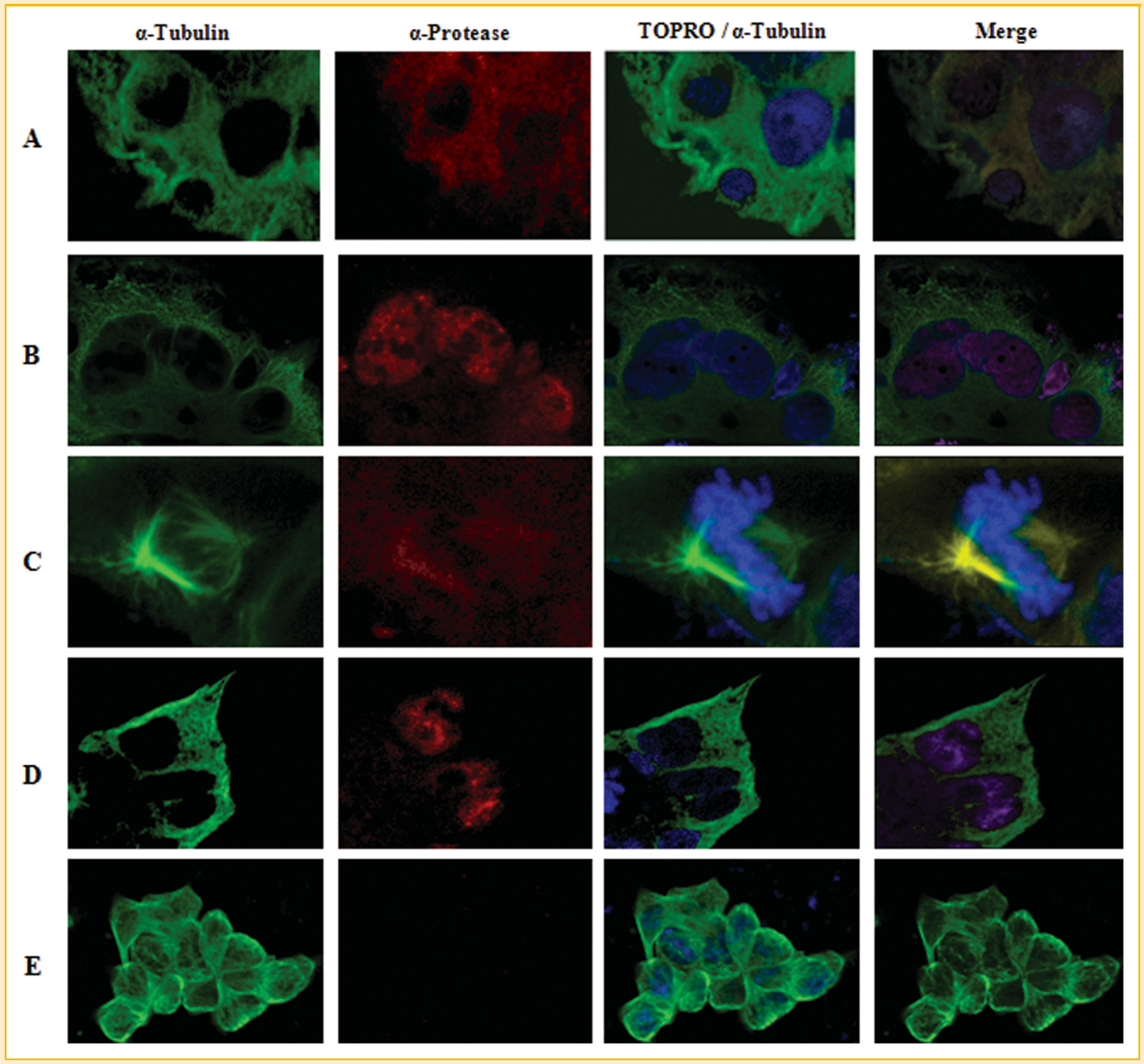

Fig. 6. Immunodetection of the protease during the cell cycle in Caco-2 cells. Caco-2 cells were fixed from synchronized cultured obtained at Go (A), S phase (B), mitosis (C), and after cell division (D). To demonstrate the specificity of the antibodies, the serum was previously immunodepleted by incubation with the commercial peptide representing the N-terminal of the protease used as antigen (E). The cells were visualized in a confocal microscopy Nikon (inverted Eclipse TE 2000-U). The antibodies used were the same that were described in the previous figure.

location between proliferative and non-proliferative states has not been reported for cathepsin L nor the existence of larger variants in eukaryotes.

The new isoform of nuclear cathepsin L found in HeLa and Caco-2 cells is very similar to the enzyme present in sea urchin. The electrophoretic migration on SDS–PAGE and its localization on the mitotic spindle of the protease are consistent with sea urchin protease, strongly suggesting that orthologs of this protease are present in human cells. The 60–57 kDa isoforms partially purified by FPLC in HeLa cells, exhibit a cathepsin L activity that was measured using Z-Phe-Arg-MCA as substrate. The nuclear enzyme present in HeLa and Caco-2 cells degrades histone H1, similar to the activities found in sea urchins, and the activity is inhibited by inhibitors of cathepsin L [Imschenetzky et al., 1997; Morin et al., 2008].

There are several evidences that demonstrate the presence of a nuclear variant of cathepsin L in the nucleus. Goulet et al. [2004, 2007] describe the presence of a nuclear cathepsin L present in NIH3T3 cells with a molecular mass of 35 kDa. The function of this protease is to degrade the transcription factor CDP/Cux allowing the transition G1/S. The cleavage induced by cathepsin L generates one isoform of the transcription factor of 110 kDa that interacts with greater affinity with DNA, stimulating the expression of several genes in the transition G1/S, among them is the gene of DNA polymerase α [Truscott et al., 2003]. Goulet et al. [2004] demonstrated that this isoform of nuclear cathepsin L is the result of the translation of a protein from an internal initiation codon giving rise to a protein with a shorter pro-dominion which lacks the sequence of signaling to the route endosome/lisosome. Sansanwal

et al. [2009] demonstrate that a truncated human cathepsin L is encoded by a new splice variant whose localization is nuclear, perinuclear, or cytosolic. This isoform is cytotoxic to mammalian cells.

Irving et al. [2002] have demonstrated in NIH/3T3 and CV-1 cell lines that a chromosomal serpin (MENT) participates in the remodeling and condensation of chromatin through its direct interaction with the inhibitor of the nuclear cystein protease type papain. In addition, Riccio et al. [2001] detected the presence of cystatin B in the nucleus of somatic cells in mammals and Ceru et al. [2010] found that stefin B (cystatin B) localized in the nucleus and interacts with histones and cathepsin L. Since cystatins are endogenous inhibitors of cathepsin activity, their presence in cellular nucleus suggests that they could have a role on its regulation. One of our results is in agreement with the presence of inhibitors of cathepsin L in the nucleus. The activity in nuclear extracts is very low but strongly increase after different steps of purification.

Duncan et al. [2008] identified a nuclear cathepsin L that processes histone H3 tail during mouse embryonic stem cell differentiation and their results suggest that their cleavage may be regulated by covalent modifications present on the histone tail. One of the functions of SpH-protease in male pronucleus formation during sea urchin development is to degrade sperm specific histones leaving the maternal cleavage stage histone variants intact. This substrate specificity is regulated by pos-translational modification of the substrates either by poly(ADP-ribosylation) or by phosphorylation [Morin et al., 1999a,b]. Since the protease present in HeLa and Caco-2 cells can degrade in vitro histone H1 and other histones (not shown), we postulate that this could be another function of this protease and other cathepsin L during cell-cycle progression.

Additionally, Sullivan et al. [2009] reported the existence of a nuclear cathepsin L in colorectal cancer whose proenzyme has a molecular size of 50 kDa and the active form has a size of 25 kDa. Recent work from Zheng et al. [2009] has demonstrated that cathepsin L may also regulate cancer cell resistance to chemotherapy. The combination of the cathepsin L inhibitor with doxorubicin strongly suppressed the proliferation of drug-resistant tumors in nude mice. An investigation of the underlying mechanism(s) led to the finding that the active form of this enzyme shuttles between the cytoplasm and nucleus. Fei et al. [2009] recently discovered that olomoucine, a cyclin-dependent kinase inhibitor, may exert neuroprotective effects through inhibiting cathepsin L nuclear translocation and activating autophagy in primary dopaminergic neurons from rat embryos.

In the case of cathepsin L, until now, three substrate have been identified in nucleus as target of the protease, the transcription factor CDP/Cux [Goulet et al., 2007], topoisomerase II [Fei et al., 2009], and histone H3 [Duncan et al., 2008]. There is no doubt that other proteins must be targeted in normal and particularly in cancer cells. The activity of the protease must be regulated, and a possibility is with the presence of some inhibitors in the nucleus like the family of cystatin cathepsin inhibitors, cystatin B [Riccio et al., 2001].

A role of cathepsin L in mitosis has been only reported in equinoderms but never in human cells until now [Concha et al., 2005b; Puchi et al., 2006; Morin et al., 2008]. The immunocolocalization of cathepsin L with tubulin and the coimmunoprecipitation of both proteins in cells harvested at mitosis (data not shown) suggest new functions for this protease that must be investigated in the future. Our results confirm that a nuclear protease with cathepsin L properties and with a higher molecular weight exists in mammalian cells.

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