

Recombinant Analogs of a Novel Milk Pro-Apoptotic Peptide, Lactaptin, and Their Effect on Cultured Human Cells

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Abstract We recently isolated and characterized a human milk peptide, lactaptin, which induced apoptosis of cultured human MCF-7 cells. Lactaptin was identified as a proteolytic fragment of human kappa-casein. Here, we generated two recombinant analogs of the peptide, RL1 and RL2, containing truncated and complete amino acid sequences of lactaptin, respectively. Analogs were produced in *E.coli*, purified and assayed for biological activity on cultured human MCF-7 cells. RL1 was shown to induce only a small decrease in cell viability, whereas RL2 lowered the viability of MCF-7 cells by 60%. This reduction in MCF-7 cell viability was associated with apoptosis, which was indicated by phosphatidylserine externalization and caspase-7 activation. The viability of A549 and Hep-2 cells was also reduced by RL2, albeit to a lesser degree than seen with MCF-7 cells; this reduced viability was not accompanied by apoptosis. Non-malignant human mesenchymal stem cells (MSC) were completely resistant to RL2 action.

Keywords Apoptosis · Human kappa-casein · Lactaptin · MCF-7

Abbreviations

DFF45	DNA fragmentation factor 45 kDa
FITC	Fluorescein isothiocyanate
MSC	Mesenchymal stem cells
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

PI	Propidium iodide
PS	Phosphatidylserine
TNF- α	Tumor necrosis factor alpha

1 Introduction

Human milk represents a source of proteins and peptides with a broad spectrum of biological activities. In addition to their nutritive functions, milk proteins often possess antiviral and bacteriostatic properties, immune modulating functions and regulatory action on mammalian cell proliferation, differentiation or apoptosis [6, 19]. Many bioactivities in milk are encrypted within the structure of milk proteins, requiring proteolysis for their release from precursors. Furthermore, phosphopeptides derived from caseins inhibit growth of cancer cells and stimulate immunocompetent cell activity [14]. Lactoferrin and its proteolytic fragments induce both apoptotic and necrotic death of mammalian cancer cells in vitro [10, 16]. Complexes containing a human α -lactalbumin folding variant and oleic acid induce the apoptosis of tumor and immature cells [7].

Recently, we isolated and characterized a human milk peptide, lactaptin, that is capable of reducing cell viability and inducing apoptosis of the mammary adenocarcinoma cell line, MCF-7. The milk pro-apoptotic peptide was identified as 8.6 kDa proteolytic fragment of human κ -casein [15]. The mature human κ -casein contains 160 amino acid residues with a hydrophobic N-terminal segment, known as para- κ -casein, and a C-terminal hydrophilic macropeptide. Para- κ -casein is found at the surface of casein micelles and contains cysteine residues that are involved in the formation of disulfide bonds with α -caseins. Posttranslational modifications, including one phosphate

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group and several carbohydrate chains, were detected in the soluble macropeptide [3, 4, 20]. Lactaptin is composed of 74 amino acid residues, 61 of which are associated with para- κ -casein and 13 with the macropeptide moiety of κ -casein. The primary structure of lactaptin includes homologues of the bovine biologically active peptides casoplatelin, casoxin B and, partially, casoxin A. The sequence of lactaptin completely overlaps with the structure of a novel antimicrobial κ -casein peptic fragment discovered by Liepke et al. [12].

Although numerous studies have characterized the antimicrobial, opioid receptor modulating, antihypertensive and antithrombotic activities of peptides derived from κ -casein, little is known about their influence on the viability and apoptosis of mammalian cancer cells. Apoptosis, or programmed cell death, is a natural process responsible for tissue development and integrity, which is commonly associated with a sequence of molecular events leading to elimination of redundant, defective or damaged cells. Dysregulation of apoptosis is implicated in many human pathological processes including oncotransformation and tumour development [5, 22]. Apoptotic cell death is characterized by a series of distinct morphological and biochemical alterations: cell shrinkage, phosphatidylserine (PS) externalization, mitochondrial collapse, nuclear condensation and chromatin fragmentation. The morphological changes seen with apoptosis are orchestrated by caspases, a family of structurally related cysteine proteases [2]. In particular, the activation of caspase-3/7 enzymes is thought to be both the hallmark of the apoptosis execution stage and an essential criterion for distinguish apoptosis from other types of cell death [2, 5, 17, 22].

Our previous findings demonstrated that lactaptin decreased viability and induced apoptotic morphological changes in MCF-7 breast cancer cells raised questions concerning the cell-specificity of cytotoxic and pro-apoptotic actions of this peptide, and concerning the involvement of executor caspases in lactaptin-induced cell death. In the present report we generated recombinant analogs of lactaptin and analyzed the influence of these analogs on viability and apoptosis of cultured human cells from different organs, tissues and malignancy stages. We also examined the involvement of the executioner caspase-7 in apoptosis of caspase-3^{-/-} MCF-7 cells.

2 Materials and Methods

2.1 Materials

Mouse anti-human caspase-7 and mouse anti-human DFF45 antibodies were purchased from BD Pharmingen. Horseradish peroxidase-conjugated antibodies against mouse IgG

were purchased from BioSan, Novosibirsk. Ni-NTA agarose, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fluorescein isothiocyanate (FITC)-conjugated annexin V, propidium iodide (PI) and trypan blue were purchased from Sigma. The GSDI plasmid (pGSDI) was kindly provided by Drs. N.A. Netesova and P.A. Belavin (Vector, Novosibirsk).

2.2 Cells and Cell Culture

MCF-7, A549 and Hep-2 cell lines were obtained from the Russian Collection of Cell Cultures (St. Petersburg, Russia). Cells were maintained in IMDM containing 10% fetal bovine serum (Biolot, St. Petersburg, Russia), 10 mM L-glutamine and 40 mg/L gentamicin (Sigma). Human mesenchymal stem cells (MSC) derived from primary culture of adult adipose tissue were obtained from raw human lipoaspirates, cultured and characterized as described by Zuk et al. [23, 24].

2.3 Expression Constructs

For the construction of RL1, human leukocyte DNA (Medigen, Novosibirsk, Russia) was amplified using the following primers: *P1*, GCCCAGATCTATGAGACCAGCTATAGCAATTAATAATCCAT and *P2*, GCCCGTCGACTTAGTGATGGTGATGGTGATGTGATCCGCCGATGGTAGGATGATTATTTTATCCTG. Also, for the construction of RL2, DNA was amplified using the following primers: *P2* and *P3*, GCCCAGATCTATGAACCAGAAACAACCAGCATGCCATGAGAATGATGAAAGACCA. The PCR products were digested with *SalI* and *BglII*, subjected to agarose gel electrophoresis and purified by gel extraction (Qiagen). The gel-purified products were ligated with *SalI* and *BglII* linearized pGSDI vector and cloned into *E.coli* strain XL Blue (Stratagene). The primary structure of pGSDI insertions were verified by DNA sequencing using the ABI 310 Genetic Analyzer (Applied Biosystems) of CCU SB RAS. pGSDI/RL1 and pGSDI/RL2, encoding RL1 and RL2 lactaptin analogs, respectively, were expressed in the *E.coli* strain BL21(DE3) (Stratagen).

2.4 Purification of Recombinant Proteins

The RL1 or RL2 expressing *E.coli* host cells were lysed in buffer A (50 mM NaH₂PO₄ (pH 8.0), 300 mM NaCl, 10 mM imidazol, 10 mM β -mercaptoethanol, 0.1 mg/mL lysozyme, 1 mM PMSF. Lysates were sonicated at 20 kHz for 5 min and centrifuged for 30 min at 10,000 \times g (5 °C). Supernatants were applied to 1 \times 5 cm Ni-NTA agarose column (Sigma, USA). Unbound proteins were eluted using buffer A. Ni-NTA-adsorbed proteins were recovered using a stepwise elution with increasing concentrations of

imidazole (50 and 250 mM) in buffer A, followed by 6 M guanidine-HCl in the same buffer. Protein fractions were dialyzed against triple changes of buffer B (10 mM NaCl, 10 mM Tris-HCl, pH 7.5) and analyzed using SDS-electrophoresis in 13% PAAG. Fractions containing recombinant analogs were subjected to ion-exchange chromatography using 0.5×1.5 cm DEAE-cellulose and step-wise elution with 50 and 100 mM NaCl in 10 mM Tris-HCl. The RL1 or RL2 lactaptin analogs were collected in fractions eluted by 50 mM NaCl and then stored at -70 °C until use. Protein concentrations were determined using the Bradford assay (BioRad Laboratories). The 98% purity of the isolated proteins was confirmed using RP-HPLC chromatography on a C5 column, Discovery BIO Wide Pore C5 (Sigma), in a water plus 0.5% TFA/acetonitrile solvent system using an HPLC Station (Bio-RAD Laboratories) as well as with a RP-HPLC on a C18 column (ProntosIL) and a Milichrom A-02 station (EcoNova, Novosibirsk).

2.5 MTT Cell Viability Assay

Cells were seeded at 5×10^3 cells/well in a 96-well plates and allowed to adhere overnight in a humidified incubator at 37 °C, 5% CO₂. Lactaptin analogs were added to the medium in the concentration indicated in the figure legends. After incubating the cells with the analogs, 0.5 mg/mL MTT was added and plates were incubated for 1 h at 37 °C. The medium was removed followed by the addition of 0.1 mL isopropanol to each well, the plates were read at 570 and 620 nm using a Apollo LB912 plate reader (Berthold Technologies). Cell viability was determined as absorbance at A570 with reference to A620 and expressed as percent of control \pm SD of triplicate independent experiments.

2.6 Flow Cytometry Analysis of Cell Death

MCF-7 cells (1×10^6) were treated with 60 μ g/mL RL2 for the times indicated. Detached cells were collected with the culture medium. Adherent cells were detached using 0.025% trypsin, washed twice with IMDM containing 10% fetal bovine serum. Adherent and detached cells were pooled, washed and then resuspended in buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂, 1 μ g/mL annexin-V-FITC, 1 μ g/mL PI. Cells were incubated for 10 min at room temperature, and analyzed using the FACSCalibur (BD Biosciences) at CCU FC of SB RAS. Bivariant analysis of FITC fluorescence (FL1) and PI-fluorescence (FL3) gave different cell populations where FITC-negative and PI-negative were designated as viable cells; FITC-positive and PI-negative as apoptotic bodies, and FITC-positive and PI-positive as secondary necrotic cells.

2.7 Western Blot

Cells were lysed in sample buffer (100 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 10% beta-mercaptoethanol). Lysates were heated at 95 °C for 5 min and loaded onto a 12% SDS-PAGE. Proteins were transferred to a Hybond ECL membrane (Amersham Pharmacia Biotech) and the membrane was incubated for 2 h in the blocking buffer (PBS supplemented with 0.05% Tween 20 and 1% gelatin). Primary antibody incubations were done in the blocking buffer for 2 h at 25 °C or overnight at 4 °C. Membranes were washed five times for 10 min with PBS plus 0.05% Tween 20 before being incubated with secondary antibody for 2 h at 25 °C and washed again. After washing, immune complexes were visualized with 0.05% 4-chloronaphtol and 0.01% hydrogen peroxide.

2.8 DNA Fragmentation

For the analysis of DNA fragmentation, 1×10^6 MCF-7 cells were treated with 60 μ g/mL RL2 for times indicated, lysed in 100 mM Tris-HCl buffer (pH 8.5) containing 5 mM EDTA, 0.2% SDS, 200 mM NaCl, and 200 μ g/mL proteinase K. Nucleic acids were recovered by precipitation in one volume of isopropanol and recovered in TE buffer. RNA was digested with 0.1 mg/ml of RNase A, 30 min at 37 °C. DNA equivalent to 2×10^5 cells was fractionated by electrophoresis on a 1.5% agarose gel and stained by ethidium bromide.

2.9 Amino Acid Sequence Accession Number

Numeration of human κ -casein amino acid residues was provided according to GenBank accession NP_005203, including the signal peptide as a subsequence.

3 Results and Discussion

3.1 Construction of *E. coli* Expression Systems and Preliminary Characterization of Lactaptin Analogs

To construct recombinant analogs of lactaptin, we analyzed the amino acid sequence of human κ -casein and selected two fragments that could be efficiently produced by the *E. coli* expression system. The selected fragment of κ -casein was designated RL2 and included lactaptin as a subsequence; fragment RL1 represented the nine-N-terminal amino acid truncated form of lactaptin (Fig. 1a). We constructed the plasmids, pGSDI-BL1 and pGSDI-BL2, that encoded RL1 and RL2, respectively. Both analogs were fused to a C-terminal six His-tag (Fig. 1a).

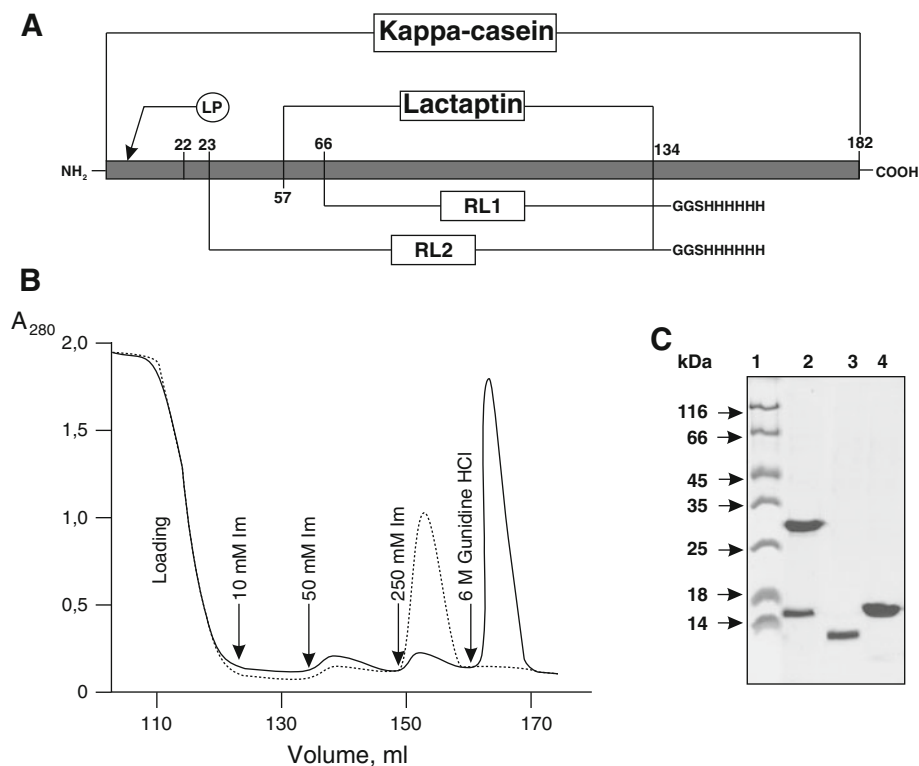


Fig. 1 **a** The relationship of the primary structures of human κ -casein (GenBank NP_005203), its pro-apoptotic proteolytic fragment lactaptin [15] and the two recombinant lactaptin analogs, RL1 and RL2. LP, leader peptide. **b** Elution profiles of RL1 (dotted line) and RL2 (continued line) from Ni-NTA agarose. (See “materials and methods” for experimental conditions.) **c** Analysis of peak fractions from **b** by SDS-PAGE (12%). RL2 preparation involved fractions eluted

from Ni-NTA by 6 M guanidine-HCl; RL1 preparation involved fractions eluted by 250 mM imidazole subjected to electrophoresis in non-reducing conditions (lanes 2 and 3, respectively). RL2-fractions were reduced before loading by 1% 2-mercaptoethanol for 5 min at 98°C (lane 4). Lane 1 shows the protein molecular mass markers as indicated on left. Proteins were stained with colloidal Coomassie blue G250

The plasmids were expressed in *E. coli* and the lactaptin analogs were isolated from cell lysate using affinity chromatography on Ni-NTA agarose. In order to achieve a high efficiency of isolation and to properly remove host cell proteins capable of non-specifically binding to Ni-NTA agarose, we adjusted the chromatography conditions. We determined that, in the course of the chromatography, analog RL1 was completely desorbed by 250 mM imidazole, whereas only 10–15% of RL2 could be eluted at the same conditions. Exhaustive elution of RL2 was achieved by utilizing chaotropic agents such as 6 M guanidine-HCl (Fig. 1b). Apparently the extremely high affinity of RL2 to Ni-NTA agarose can be explained by the low solubility of the protein under conditions of physiological ionic strength and by its tendency to form insoluble high-order oligomers typical for the N-terminal segment of κ -casein [4, 20].

Electrophoretic mobility of RL1 in SDS-PAGE was in agreement with its predicted molecular mass (8.9 kDa). The RL2 analog, in non-reducing conditions, was represented by two isoforms with apparent molecular masses of 14 and 28 kDa in a ratio of 30:70, respectively (lane 1

in Fig. 1c). In the presence of beta-mercaptoethanol, the 28 kDa isoform completely reduced to the 14 kDa (lane 3 in Fig. 1c). Thus, the RL2 analog, produced in *E. coli*, was partially represented by -S-S- covalently linked homodimers. The amino acid sequence of RL2 contains only one cysteine residue, which corresponded to Cys30 of human κ -casein. Consequently, this unique cysteine residue is responsible for the formation of covalent RL2 homodimers.

It is known that α -, β - and κ -caseins form tight multi-subunit complexes (micelles). The hydrophobic core of casein micelle fashioned by the N-terminal segment of κ -casein [4, 20] and amino acid residues of this segment are completely represented in the structure of the RL2 analog but not RL1. Taking into account that RL1 does not form low soluble complexes or covalent homodimers (lane 1 Fig. 1c), it can be concluded that amino acid residues of RL2, corresponding to 23–66 of human κ -casein, are strongly involved in and responsible for the homodimerization and multimerization both of κ -casein and of the RL2 lactaptin analog.

3.2 Influence of RL1 and RL2 on Viability of Cultured Human Cells

Previously, we determined that the human milk peptide lactaptin reduced viability and induced apoptosis of human adenocarcinoma cells MCF-7 [15]. Recombinant analogs of lactaptin, RL1 and RL2, were not equally effective as pro-apoptotic factors for MCF-7 cells (Fig. 2). The truncated analog RL1 induced only a small reduction of viability of the adenocarcinoma cells, whereas incubation of cells with 30–60 $\mu\text{g}/\text{mL}$ of RL2 resulted in up to a 60% reduction in viability. The RL2-mediated reduction in viability, but not the RL1-associated reduction, was accompanied by morphological changes typical for apoptosis: detachment of cells from the substrate; condensation of cells and nuclei; and, the appearance of secondary necrotic cells (see later). Therefore, these data lead us to conclude that the AA-residues of RL2, which corresponded to fragment 23–66 of human κ -casein, are essential not only for multimerization of the peptide but also for promoting the peptide-associated apoptotic action on MCF-7 cells.

In order to determine the cell specificity of the pro-apoptotic activity of RL2, we compared the RL2-induced reduction in viability of the MCF-7 cells to A549 lung carcinoma cells, Hep-2 larynx epidermal carcinoma cells and non-malignant human mesenchymal stem cells exposed to RL2. Although proliferation and viability of A549 and

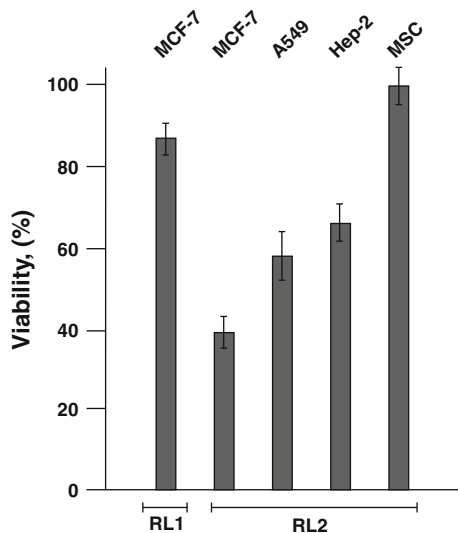


Fig. 2 The influence of RL1 and RL2 on the viability of cultured human cells. MCF-7 human mammary gland adenocarcinoma, A549 lung carcinoma, Hep-2 larynx epidermal carcinoma and non-transformed human mesenchymal stem cells MSC were treated with 60 $\mu\text{g}/\text{mL}$ RL2 or RL1 (as indicated) for 72 h. Cells viability was determined using the MTT assay. The mean and SD from experiments performed in triplicate are shown

Hep-2 cells were noticeably reduced by up to 45 and 30%, respectively (Fig. 2), the reduction was not accompanied by apoptotic changes in the cells (data not shown). The viability of mesenchymal stem cells incubated with RL2 did not differ from the viability of control-treated cells (Fig. 2). Thus, the MCF-7 mammary gland adenocarcinoma cells were the most predisposed to RL2-induced apoptosis and the sensitivity of human cells to the cytotoxic action of RL2 in our experiments was $\text{MCF7} > \text{A549} > \text{Hep-2} \gg \text{MSC}$. Moreover, only oncotransformed cells were sensitive to the cytotoxic action of RL2 whereas non-transformed MSC demonstrated resistance to the protein.

3.3 PS Exposure, Plasma Membrane Alterations and Caspase Activation in RL2 Treated MCF-7 Cells

To confirm that the RL2-induced decrease in viability resulted in apoptosis of the MCF-7 cells, we analyzed subpopulations of PI/annexin V stained cells by flow cytometry. Both RL2-treated and control samples of MCF-7 contained subpopulations of annexin V⁺ and PI⁻ apoptotic bodies as well as secondary necrotic cells. Subpopulations of apoptotic bodies and secondary necrotic cells increased up to 39% after incubation with RL2 for 72 h; untreated samples contained about 6% dead cells. Populations of apoptotic bodies in the RL2-treated cultures represented about 20% of total cell counts after 24 h (Table 1). Therefore, death of MCF-7 cells induced by the lactaptin analog RL2 was accompanied by early PS exposure on the outer leaflet, and later, by cell membrane permeabilization.

It is known that, due to a deletion in the *CASP3* gene, MCF-7 cells are deprived of the pro-caspase 3. The lack of pro-caspase-3 in MCF-7 cells leads to a reduced level of oligonucleosomal DNA fragmentation under conditions of TNF- α -induced apoptosis [8, 9, 21]. Nevertheless, paclitaxel, PBOX-6, vitamin K3 or fatty acids induce the death of MCF-7, which is accompanied by apoptotic nuclear changes and/or oligonucleosomal DNA fragmentation, possibly due to the pronounced activation of the caspase-7 [1, 11, 13, 18].

Table 1 Analysis of RL2-treated MCF-7 apoptosis by flow cytometry of FITC-annexin V/PI stained cells

Populations of MCF-7 (% \pm SD)	Time of incubation (h)			
	24	48	72	72 (untreated)
Valuable	75 \pm 7.2	67 \pm 6.5	57 \pm 6.7	93 \pm 7.3
Apoptotic	19 \pm 3.5	20 \pm 5.1	19 \pm 4.2	3.0 \pm 3.2
Secondary necrotic	5.0 \pm 3.2	11 \pm 3.1	20 \pm 4.5	3.0 \pm 3.5
Unascribed	1.0 \pm 1.1	2.0 \pm 1.1	4.0 \pm 3.2	1.0 \pm 1.1

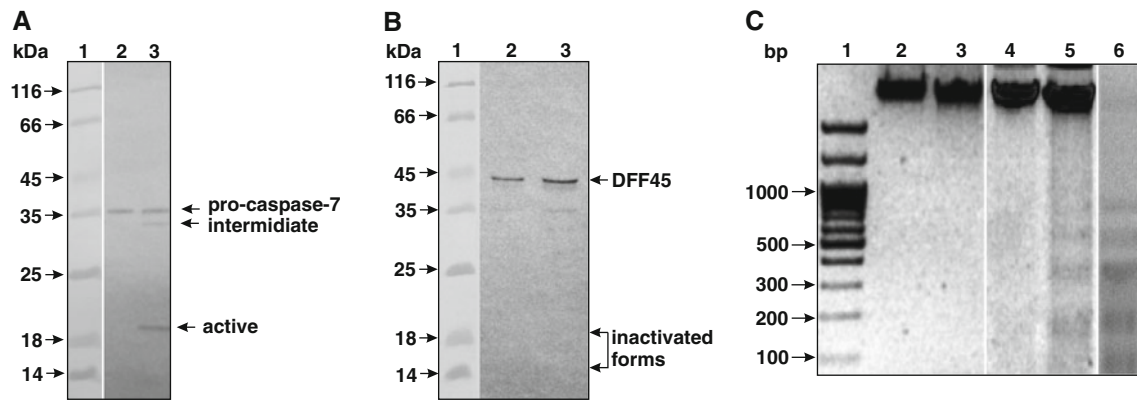


Fig. 3 Western blot analysis of caspase-7 activation (**a**) and DFF45 proteolysis (**b**) of MCF-7 cells treated with 60 µg/mL RL2 for 72 h (lanes 3) and untreated control cells (lanes 2). Whole cell lysates containing equal amounts of total protein were transferred to nitrocellulose and probed with antibodies to caspase-7 (**a**) and DFF45 (**b**). Lanes 1 **a** and **b** show the protein molecular mass markers stained with Amido black 10B. **c**, DNA fragmentation in RL2-treated

(lanes 3, 5) and untreated (lanes 2, 4) MCF-7 cells incubated with 60 µg/mL of the analog for 72 h (lane 3) and 180 µg/mL for 120 h (lane 5). Lane 6 represents the control oligonucleosomal cleavage of MCF-7 nuclear chromatin by Micrococcal nuclease. DNA was extracted from 5×10^6 cells and was fractionated on a 2% agarose gel with ethidium bromide. Lane 1 shows the molecular mass markers of DNA

To determine whether the activation of caspase-7 in the MCF-7 cells occurs during RL2 treatment, we analyzed cell proteins by Western blot using an anti-caspase-7 and anti-DFF45 antibodies. It was demonstrated that RL2-induced apoptosis of the MCF-7 cells was accompanied by the appearance of the active form of caspase-7 (lane 3, Fig. 3a). However, the level of caspase-7 activation was insufficient for the subsequent activation of the DFF40/DFF45 nuclease, because proteolytic fragments of DFF45 were not detected, which indicates inactivation of the inhibitor (lane 3, Fig. 3b).

Inactivity of the apoptotic nuclease DFF40/DFF45 was further confirmed by the absence of oligonucleosomal DNA fragmentation in MCF-7 cells that were treated with RL2 for 72 h (lane 3, Fig. 3c). The low level of DNA fragmentation was observed only after prolonged incubation (120 h) of the cells with the protein (compare lanes 5 and 6, Fig. 3c), when most of the cells were represented by secondary necrotic (41%) and apoptotic bodies (34%). This retarded and moderate DNA fragmentation can be explained by increased permeability of late secondary necrotic cells for the components of culture medium rather than by activation of DFF40/DFF45. Consequently, in agreement with published data regarding caspase-7 activation and DNA fragmentation in TNF- α - or staurosporin-treated MCF-7 cells [21], confined caspase-7 activation in RL2-treated cells was insufficient to promote subsequent activation of DFF40 and fragmentation of nuclear DNA.

Thus, RL2-induced apoptotic death of MCF-7 cells occurs through the activation of caspase-7, which is accompanied by PS exposure on the outer leaflet of the plasma membrane and proceeds without marked internucleosomal DNA cleavage. Similar features of the death

MCF-7 cells were previously described for TNF- α -induced apoptosis, which is known to proceed through the death-receptor mediated caspase 8 activation [8, 9, 21]. In contrast to death-receptor mediated death, the apoptosis of MCF-7 cells that is triggered or accompanied by mitochondrion-dependent caspase 9 activation (paclitaxell, VK3 or free fatty acids) is commonly characterized by dramatic chromatin changes and/or oligonucleosomal DNA fragmentation [1, 11, 13, 18]. This observation indicates that the mechanism of pro-apoptotic action of the recombinant analog of the human milk peptide lactaptin is generally closer to the activity of natural cytokines than to the changes produced by exogenous or synthetic drugs.

4 Conclusion

In this study, we generated two recombinant analogs of the pro-apoptotic human milk peptide lactaptin. These analogs were designated RL1 and RL2 and contained truncated and complete amino acid sequences of lactaptin, respectively. Analogs were produced in *E. coli*, purified to homogeneity and assayed for biological activity using cultured human cells. It has been determined that RL1, which partly lacks the hydrophobic clusters of the para- κ -casein, produced only a small decrease in the viability of MCF-7 cells. Whereas RL2, which contained the complete sequence of the hydrophobic segment of κ -casein, decreased MCF-7 viability by 60%, induced phosphatidylserine externalization and caspase-7 activation typical for apoptosis. The viability of the A549 lung carcinoma and Hep-2 larynx carcinoma cells were reduced by RL2 to a lesser extent than for MCF-7 cells, and the reduction was not due to

apoptosis. In contrast to cancer cells, non-malignant human mesenchymal stem cells were completely resistant to the cytotoxic action of RL2. The mechanism of apoptotic action mediated by lactaptin, and its recombinant analog RL2, on mammary carcinoma cells is unclear and needs to be elucidated in future studies.

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