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*Review*

# From HAMLET to XAMLET: The molecular complex selectively induces cancer cell death

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**HAMLET (human α-lactalbumin made lethal to tumor cells) is a complex consisting of decalcinated α-lactalbumin and oleic acid. It has been shown to induce massive cell death in various cancers without serious damage to normal tissues in both *in vitro* and *in vivo* experiments. The complexity of the mechanism in the cellular functions of HAMLET results from its heterogeneous compositions as revealed from the structural analyses, as well as its induction of the non-canonical cell responses different from the specific classical programmed cell death. As the remarkable progress was made in the studies of HAMLET in recent years, a variety of HAMLET-like substances have been demonstrated to exert similar biological activities to HAMLET. Here, we termed these species as the XAMLET, and overviewed the current findings in XAMLET studies for a better understanding of their functions and working mechanisms. This review might contribute to the improvement in the application of XAMLET towards clinical cancer therapies.**

**Key words:** XAMLET, α-lactalbumin, cancer cell death.

## INTRODUCTION

HAMLET (human α-lactalbumin made lethal to tumor cells) was first named after the coincidental discovery of the killing of tumor cells when human breast milk fractions were tested to have effects on bacterial attachment to alveolar type II lung carcinoma cells. A casein fraction obtained after low pH precipitation of human milk was determined to be responsible for the lethal impact on tumor cells. The active component of casein fractions was further analyzed by the ion exchange chromatography and the multimeric α-lactalbumin (MAL) was identified. The MAL-containing substance was found to be able to induce apoptosis in a variety of transformed and immature mammalian cells, but not in normal differentiated cells (Hakansson et al., 1995). Strikingly, the native α-lactalbumin (α-LA) proteins had no detectable effect on tumor cells, but the oleic acid (OA, C18:1, 9 cis) was identified as the required co-factor to complex with α-lactalbumin for the killing of cells. Thus, the ethylenediaminetetraacetic acid (EDTA) treatment to remove the chelated calcium ion from the α-LA protein allows the binding of OA that preloaded on an ion exchange matrix for the preparation of HAMLET (Svensson et al., 2003). So far, HAMLET is known as a tumoricidal complex which is comprised of apo α-LA and OA (Pettersson et al., 2006).

HAMLET was demonstrated to effectively destroy a great variety of tumor cells ranging from carcinomas of lung, throat, kidney, colon, bladder, prostate, ovaries, melanomas, to glioblastomas of the brain and leukemia (Gustafsson et al., 2005). Remarkably, the normal and well-differentiated cells were tolerant to HAMLET treatments and were much less affected. As the studies focusing on the structure and mechanisms of HAMLET or

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**Abbreviations: HAMLET,** Human α-lactalbumin made lethal to tumor cells; **MAL,** multimeric α-lactalbumin; **α-LA,** α-lactalbumin; **OA,** oleic acid; **HLA,** human α-lactalbumin; **PI3K,** phosphoinositide 3-kinase; **LC3,** light chain 3; **Atg8,** autophagy-related gene 8.

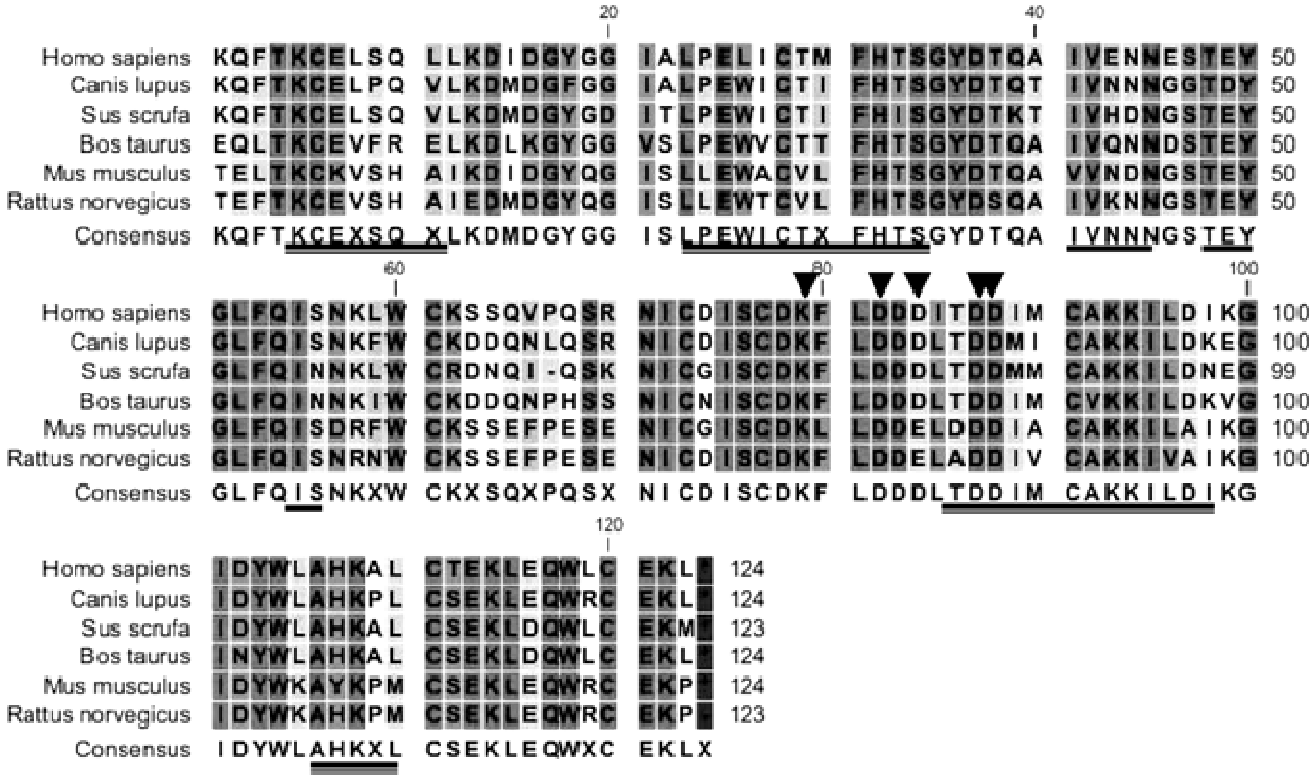


**Figure 1.** The alignment of α-LA amino acid sequences from various species using CLC Sequence Viewer 6. The α-helical domains: amino acids 5–11, 23–34, 86–98 and 106-110 was marked with double underlines; the β-strand domains: amino acids 41–43, 48–50 and 55-56 was single underlined. The calcium-binding loop containing K79, D82, D84, D87 and D88 was indicated by solid triangles.

bovine α-lactalbumin made lethal to tumor cells (BAMLET) go on recently, other molecule complex comprising of protein and oleic acid (OA), including human α-lactalbumin (HLA) orthologues (bovine, equine, porcine, ovine and caprine) (Spolaore et al., 2010) and structural homologue (equine lysozyme) (Wilhelm et al., 2009) were found to have similar structures and functions of HAMLET. We categorize these complexes as the XAMLET, where "X" means the expansion from human to other species, and "A" may refer to other alternative proteins besides the α-LA, like the amyloid beta. In this review, we discussed the preparation, structural analyses, biological activities and the mechanism of XAMLET from recent reports and prospect the application in the related studies.

## STRUCTURE OF HAMLET

Previous studies have indicated that the HAMLET is a complex comprised of apo α-LA and OA, but the structural basis and the function of HAMLET remained unclear. The structure of the α-LA, major protein component of HAMLET, is of great interest in mediating the cytotoxicity. The α-LA is a small (MW 14.2kDa), acidic (pI 4-5) calcium-binding protein in human milk and functions as a co-enzyme of galactosyltransferase in lactose synthesis (Permyakov et al., 2005). The native α-LA consists of two domains in the entire 122–123 amino acid sequence, a large α-helical domain contains four α-helices (amino acids 5–11, 23–34, 86–98 and 106-110), and a small β-strand domain consists of three β–strand segments (amino acids 41–43, 48–50 and 55-56). The two domains are connected by a calcium-binding loop (involving amino acids K79, D82, D84, D87 and D88). Four disulfide bonds (amino acids 6–120, 61–77, 73–91 and 28–111) (Iyer and Qasba, 1999; Pettersson et al., 2006) were determined to stabilize the natural conformation of α-LA. These structures are well-conserved among species (Figure 1). When $Ca^{2+}$ is released following low pH condition, EDTA or heat treatment and α-LA was transformed into the apo state retaining a native-like secondary structure without well-defined tertiary structures (Fast et al., 2005). The apo α-LA is required and important for HAMLET formation in complex with OA to form HAMLET (Figure 2).

Nonetheless, other changes of the α-LA structures have been reported to be able to form HAMLET or BAMLET analogs, for examples, some mutants at the $Ca^{2+}$-binding site mutants of α-LA (Hallgren et al., 2008),

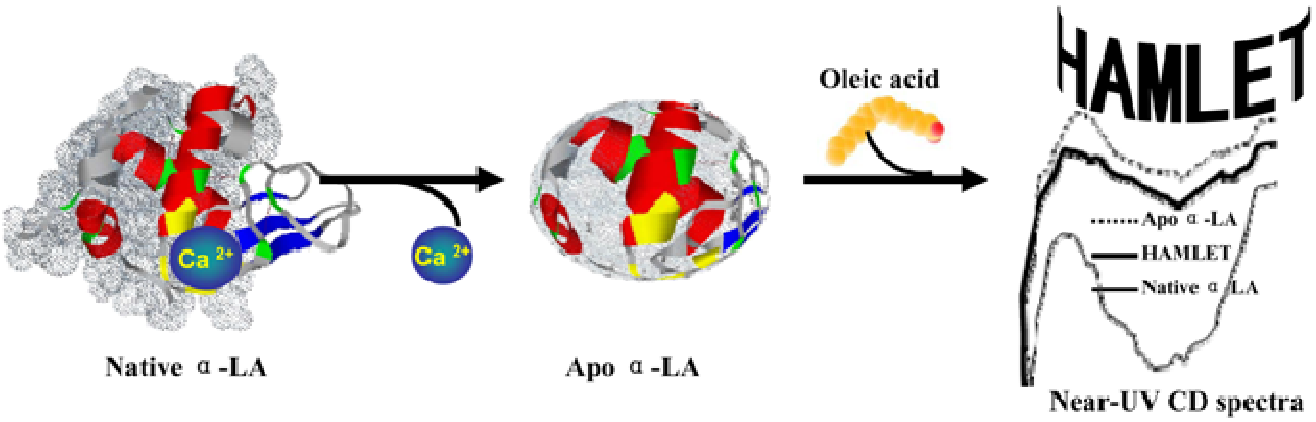


**Figure 2.** The process of HAMLET formation. The calcium ion was released from native α-LA to form apo α-LA. Red: α-helical region; Blue: β-strand region; and Yellow: calcium-binding loop. OA bound to apo α-LA and was incorporated into HAMLET, which showed a similar near-UV CD spectra to apo α-LA and different from that of the native α-LA.

as well as a recombinant variant ($rHLA^{all\text{-}Ala}$) of human α-LA with all eight cysteine residues were substituted by alanines (Pettersson-Kastberg et al., 2009). The HAMLET production using amino acids 1–40 / 104–123, 1–40 / 53–123 and 53–103 fragments of bovine α-LA obtained by limited proteolysis (Tolin et al., 2009), or heat-treated α-LA in the denatured and aggregated forms (Liskova et al., 2010) was shown to be successful. OA-induced aggregation of α-LA processing a BAMLET-like activity was also observed at pH 4.3 ammonium acetate buffers (Zhang et al., 2009). Three common approaches of the HAMLET production involved different methodologies (Table 1): OA-conditioned anion exchange chromatography (Svensson et al., 2000), direct mix under heat denaturation (Kamijima et al., 2008) and phase exchange between water-soluable α-LA and ethanol OA solutions (Zherelova et al., 2009).

Based on these studies, it was believed that the OA-binding inner domains of the α-LA were shaded in the native structure, the removal of $Ca^{2+}$, break of disulphide bonds, or fragmentation following proteolysis allows the hydrophobic domains to be exposed and to interact with OA and form the HAMLET complex. Hypothetically, a pocket-like structure containing α-helices and β-strands could be the peculiar structural base of HAMLET formation and the selectivity to tumor cells.

## MECHANISMS OF TUMOR CELL DEATH

The exact mechanisms of HAMLET-induced cell death in tumor cells appeared to be rather complicated. At least three major responses were observed from different reports: Apoptotic response, autophagic response and chromatin structure disorder, which correlate with the intracellular aggregates and localization of HAMLET. The studies suggested that HAMLET was able to form large cytoplasmic aggregates, traffic to the perinuclear region and accumulate in the nuclei. In malignant glioma cells, however, the native α-LA was shown to bind the cell surface and enter the cytoplasm in small amounts without being translocated into the nuclei. In non-transformed human astrocytes, both HAMLET and α-LA enter the cell much less efficiently. Even when HAMLET entered the cytoplasm, it only formed small aggregates, and failed to be transported into the nuclei (Fischer et al., 2004).

The cytoplasmic aggregates of HAMLET might induce apoptotic and autophagic responses, whereas the nucleic accumulation of HAMLET might lead to the disruption of chromatin structures. Early studies showed that HAMLET induced apoptotic-like responses, including the abnormality of mitochondrial permeability (Kohler et al., 2001) and moderate activation of caspases-9, -3 and -2 (Hallgren et al., 2006). Recent reports have shown that HAMLET aggregates were able to inhibit the proteasome activity by binding to the 20S proteasome and altering the structure of proteasomes (Gustafsson et al., 2009). The activation of the proapoptotic protein Bax was also indicated in recent investigations (Rammer et al., 2010). However, owing to the failure to rescue the cells treated with HAMLET to survive by caspase inhibitors, Bcl-2 overexpression and p53 mutation (Hallgren et al., 2006) showed that the classical apoptotic pathway was not sufficient to explain the underling mechanism of HAMLET-induced cell death. On the other hand, as autophagy is an alternative potent process of programmed cell death, it became an increasing interest to investigate whether HAMLET aggregates lead to the activation of autophagic responses in cancer cells. As is known, autophagic pathway is initiated by class III phosphoinositide 3-kinase (PI3K) and Beclin-1and is involved by two systems composed of microtuble-associated protein light chain 3 (LC3), which is a mammalian homolog of yeast, the autophagy-related gene 8 (Atg8), and Atg4 protease on one hand and the Atg12-Atg5-Atg16 complex on the other (Schmid and Munz, 2007). Finally, the outer membrane of

**Table 1.** Methods for producing HAMLET or its analogs.

| Approach | Steps | Time | Equipment | Advantages | References |
|---|---|---|---|---|---|
| Column chromatography | 6 | 2 days | Specialized chromatography | Classical and reliable | Svensson et al. (2000) |
| Mixing and dialysis | 3 | 1 days | Common laboratory tools | Simple and became popular | Knyazava et al. (2008) and Tolin et al. (2009) |
| Heating and centrifugation | 1 | 20 min | Common laboratory tools | Rapid, maybe for large-scale production | Kamijima et al. (2008) |

the autophagosome fuses in the cytoplasm with a lysosome to form an autophagolysosome where their contents are degraded via acidic lysosomal hydrolases (Rubinsztein et al., 2005). In LC3-GFP-transfected cells, the shift from uniform (LC3-I) to granular (LC3-II) of translocated LC3 and a reduced level of mTOR signals demonstrated the activation of macroautophagy, and this could be blocked by 3-methyladenine, an inhibitor of macroautophagy. An increased autophagic flux was also reflected by the accumulation of LC3-II as determined by Western blot in HAMLET treated cells when lysosomal degradation was inhibited. The suppression of proteins involved in autophagy, Beclin-1, Atg5 and Atg7, inhibited the increase in granular LC3-GFP staining and improved the survival of HAMLET-treated tumor cells (Aits et al., 2009). Therefore, BAMLET accumulated rapidly and specifically in the endolysosomal compartment of tumor cells and induced an early leakage of lysosomal cathepsins into the cytosol followed by the activation of the proapoptotic protein Bax (Rammer et al., 2010). This also suggested that a crosstalk of apoptosis and autophagy might exist and work in coordination to cause HAMLET-induced cell death (Figure 3).

HAMLET was found to bind histones strongly in *in vitro* experiments and hampered their deposition on DNAs (Duringer et al., 2003). In nuclei, histones were also shown to interact with HAMLET as the identified nuclear constituent, where the binding to H3 was the strongest and to much lesser extent of H4 and H2B. The binding specificity of histones and HAMLET was demonstrated by biomolecular interaction and chromatin assembly assays. The co-localization of HAMLET with histones correlated with the alterations of the chromatin structures in tumor cells, and the chromatin-associated HAMLET was resistant to salt extraction as insoluble nuclear fractions.

The studies showed that multiple causes potentially contributed to the formation of HAMLET aggregates, either from the resistance of the degradation by the ubiquitin-proteasome system as misfolded proteins, or from the escape from the endolysosomal compartment by damaged lysosome integrity to avoid the acidic environment of clearance. Additionally, the native α-LA and HAMLET were known to interact with histone and chromatin *in vitro* (Permyakov et al., 2004), thus when OA stabilizes the molten globular like conformation of α-LA, the morphology and integrity of the nuclear membrane may be altered and allow the internalization and aggregation of HAMLET in the nuclei, especially in carcinoma cells (Mossberg et al., 2010).

## APPLICATION OF HAMLET

Although the complete understanding of the mechanisms of HAMLET-induced cell death remained unclear due to its complexity, the preclinical attempts for the application for cancer therapies can be made, especially for the tumors that could not removed completely in surgeries. For examples, HAMLET reduced the intracranial glio-blastoma tumor volume and delayed the onset of pressure symptoms in the tumor-transplanted nude rats. HAMLET was observed to cause apoptosis in the tumor *in vivo* without significant side effects on adjacent intact brain tissue or in non-transformed human astrocytes (Fischer et al., 2004). Local administration of HAMLET was also demonstrated to be of value for potential application in cancer treatments, as it reduced the papilloma volume in 100% (20/20) of the patients (88/92 papillomas) when compared with 15% in the placebo group (3/20 patients, 15/74 papillomas). To date, no adverse reactions were observed, and it was shown to be equally effective in reducing the tumor volumes in both immunocompetent and immunosuppressed patients based on the trials of topical HAMLET application in skin papillomas (Gustafsson et al., 2005). A direct and selective therapeutic effect of HAMLET on bladder cancer tissue *in vivo* was also reported (Mossberg et al., 2007).

## SUMMARY AND PERSPECTIVES

HAMLET, a complex comprised of unfolded α-LA and OA, and has provided the first evidence for a potential novel reagent or an approach for cancer treatments. The re-search progresses following the classical or modified model or principles which do not only helped to understand the mechanism

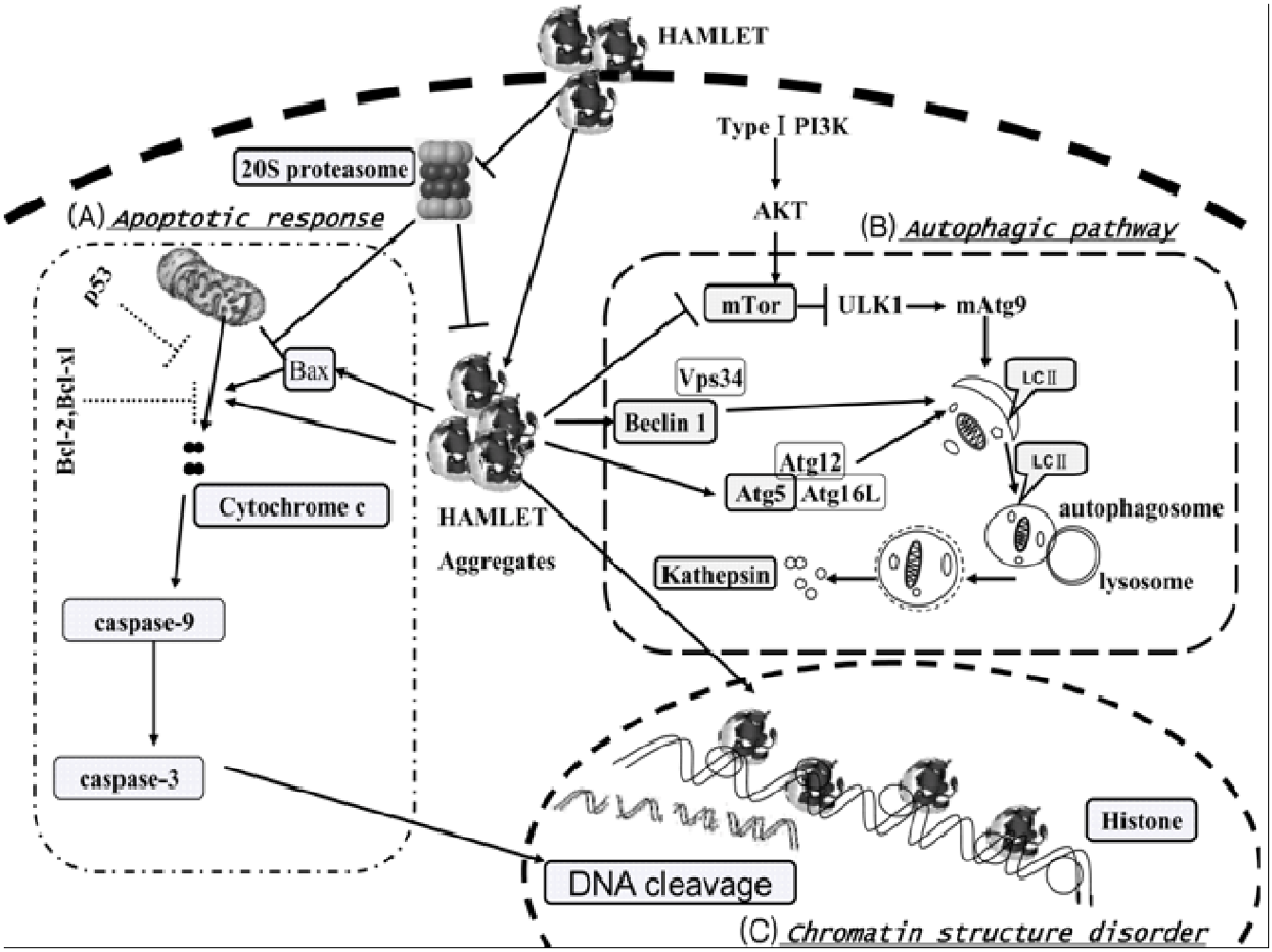


**Figure 3.** The mechanism of HAMLET-induced tumor cell death. HAMLET resists to, degradation by inhibiting the proteasome activity. HAMLET and its aggregates may lead to multiple cellular responses: Apoptosis, autophagy and chromatin alteration. A: The reduction of Bax and the proteasome activity resulted in the leaking of cytochrome c from the mitochondra, and activate caspase pathway, but failed in the rescue by Bcl-2 overexpression and p53 mutations. B: HAMLET aggregates activate autophagy by inhibiting the mTor pathway, or activation of Beclin 1 or Atg5. The leakage of cathepsins can be observed when HAMLET is distributed into the lysosomal compartment. C: HAMLET aggregates in the nuclei will lead to chromatin structure disorder by interacting with histone.

that HAMLET works, but also greatly expanded the knowledge of the HAMLET-like products termed XAMLET in this review.

Future studies in HAMLET-related fields will be expected to bring answers or solutions to the following questions. 1) What is the most simple and cost-effective method for the industrialized production of HAMLET-like reagents? The structure analyses apparently will facilitate the improvement of HAMLET preparation. The engineering of recombinant proteins, such as the human α-LA, has already shown their advantages. In addition, the common approaches, including heating, EDTA extraction and column chromatography, or their combinations for HAMLET production is being optimized in various reports. 2) Is there a standard or dedicated measurement for the biological activities of HAMLET? Or, is there a unique subspecies within the HAMLET complex that is much more potent for the induction of cancer cell deaths? These questions have to be answered, at least in part, prior to HAMLET species for their applications in clinical trials. Currently, the CD or OD measurements appeared to be practical for the quality controls for the biochemical prosperities. However, the biological activity of HAMLET, especially the specific index, remained to be established. Noticeably, although simple assays to characterize HAMLET is desired, the combinational tests may still be valuable for the application and laboratory researches of HAMLET or HAMLET-like substances. 3) For the mechanism of HAMLET-induced cell death, the predominant underlining pathways, especially the selectivity to eliminate tumor cells, will be the top interest for the researchers. As some evidences already indicated that

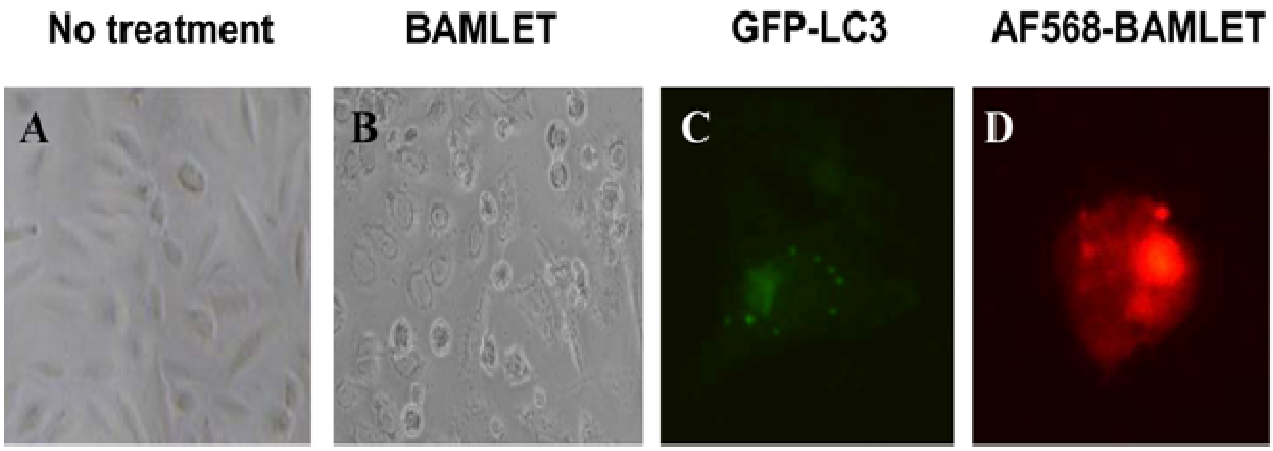


**Figure 4.** The cell death was induced by BAMLET in cells. HeLa cells without (A) or with (B) the treatment of BAMLET (0.2 mg/ml) for 6 h. C: The translocation of LC3 to autophagosomes shown as granular LC3-GFP (green) after the treatment of BAMLET for 6 h. D: The nuclear localization of BAMLET (red) prepared with AlexaFluor-568 pre-labeled bovine α-lactalbumin.

apoptosis might not likely to be a typical response following HAMLET treatments in tumor cells, autophagy and epigenetic modifications like the alteration of chromatin structure have been taken into consideration in many ongoing studies. In our own laboratory, when HeLa cells were treated with BAMLET, the activation of the autophagic death pathway was observed (Figure 4). Nonetheless, it is important to know that the natural HAMLET responses can be complicated, involving multiple pathways of programmed cell death, and it can be different among different cell types or models. 4) As we pointed out, XAMLET can originate from a broader source of native cellular components, such as amyloid beta (Qahwash, 2007). Can the HAMLET-like responses and the cellular tolerance of HAMLET exposure be a critical biological process in the survival of native cells? This can be an intriguing question that may expand the HAMLET-related studies and may pose more interesting concerns for the mechanistic studies and therapeutical applications of XAMLET.

**REFERENCES**

Aits S, Gustafsson L, Hallgren O, Brest P, Gustafsson M, Trulsson M, Mossberg AK, Simon HU, Mograbi B, Svanborg C (2009). HAMLET (human alpha-lactalbumin made lethal to tumor cells) triggers autophagic tumor cell death. Int. J. Cancer, 124(5): 1008-1019.

Duringer C, Hamiche A, Gustafsson L, Kimura H, Svanborg C (2003). HAMLET interacts with histones and chromatin in tumor cell nuclei. J. Biol. Chem. 278(43): 42131-42135.

Fast J, Mossberg AK, Nilsson H, Svanborg C, Akke M, Linse S (2005). Compact oleic acid in HAMLET. FEBS. Lett. 579(27): 6095-6100.

Fischer W, Gustafsson L, Mossberg A K, Gronli J, Mork S, Bjerkvig R, Svanborg C (2004). Human alpha-lactalbumin made lethal to tumor cells (HAMLET) kills human glioblastoma cells in brain xenografts by an apoptosis-like mechanism and prolongs survival. Cancer Res. 64(6): 2105-2112.

Gustafsson L, Aits S, Onnerfjord P, Trulsson M, Storm P, Svanborg C (2009). Changes in proteasome structure and function caused by HAMLET in tumor cells. PLoS One, 4(4): e5229.

Gustafsson L, Hallgren O, Mossberg AK, Pettersson J, Fischer W, Aronsson A, Svanborg C (2005). HAMLET kills tumor cells by apoptosis: structure, cellular mechanisms, and therapy. J. Nutr. 135(5): 1299-1303.

Hakansson A, Zhivotovsky B, Orrenius S, Sabharwal H, Svanborg C (1995). Apoptosis induced by a human milk protein. Proc. Natl. Acad. Sci. USA, 92(17): 8064-8068.

Hallgren O, Aits S, Brest P, Gustafsson L, Mossberg AK, Wullt B, Svanborg C (2008). Apoptosis and tumor cell death in response to HAMLET (human alpha-lactalbumin made lethal to tumor cells). Adv. Exp. Med. Biol. 606: 217-240.

Hallgren O, Gustafsson L, Irjala H, Selivanova G, Orrenius S, Svanborg C (2006). HAMLET triggers apoptosis but tumor cell death is independent of caspases, Bcl-2 and p53. Apoptosis, 11(2): 221-233.

Iyer LK, Qasba PK (1999). Molecular dynamics simulation of alpha-lactalbumin and calcium binding c-type lysozyme. Protein Eng. 12(2): 129-139.

Kamijima T, Ohmura A, Sato T, Akimoto K, Itabashi M, Mizuguchi M, Kamiya M, Kikukawa T, Aizawa T, Takahashi M, Kawano K, Demura M (2008). Heat-treatment method for producing fatty acid-bound alpha-lactalbumin that induces tumor cell death. Biochem. Biophys. Res. Commun. 376(1): 211-214.

Kohler C, Gogvadze V, Hakansson A, Svanborg C, Orrenius S, Zhivotovsky B (2001). A folding variant of human alpha-lactalbumin induces mitochondrial permeability transition in isolated mitochondria. Eur. J. Biochem. 268(1): 186-191.

Liskova K, Kelly AL, O'Brien N, Brodkorb A (2010). Effect of denaturation of alpha-lactalbumin on the formation of BAMLET (bovine alpha-lactalbumin made lethal to tumor cells). J. Agric. Food Chem. 58(7): 4421-4427.

Mossberg AK, Puchades M, Halskau O, Baumann A, Lanekoff I, Chao Y, Martinez A, Svanborg C, Karlsson R (2010). HAMLET interacts with lipid membranes and perturbs their structure and integrity. PLoS One, 5(2): e9384.

Mossberg AK, Wullt B, Gustafsson L, Mansson W, Ljunggren E, Svanborg C (2007). Bladder cancers respond to intravesical instillation of HAMLET (human alpha-lactalbumin made lethal to tumor cells). Int. J. Cancer, 121(6): 1352-1359.

Permyakov SE, Pershikova IV, Khokhlova TI, Uversky VN, Permyakov EA (2004). No need to be HAMLET or BAMLET to interact with histones: binding of monomeric alpha-lactalbumin to histones and basic poly-amino acids. Biochemistry, 43(19): 5575-5582.

Permyakov SE, Pershikova IV, Zhadan AP, Goers J, Bakunts AG, Uversky VN, Berliner LJ, Permyakov EA (2005). Conversion of human alpha-lactalbumin to an apo-like state in the complexes with basic poly-amino acids: toward understanding of the molecular mechanism of antitumor action of HAMLET. J. Proteome. Res. 4(2):

564-569.
Pettersson-Kastberg J, Mossberg AK, Trulsson M, Yong YJ, Min S, Lim Y, O'Brien JE, Svanborg C, Mok KH (2009). alpha-Lactalbumin, engineered to be nonnative and inactive, kills tumor cells when in complex with oleic acid: a new biological function resulting from partial unfolding. J. Mol. Biol. 394(5): 994-1010.
Pettersson J, Mossberg AK, Svanborg C (2006). alpha-Lactalbumin species variation, HAMLET formation, and tumor cell death. Biochem. Biophys. Res. Commun. 345(1): 260-270.
Qahwash IM, Boire A, Lanning J, Krausz T, Pytel P, Meredith SC (2007). Site-specific effects of peptide lipidation on beta-amyloid aggregation and cytotoxicity. J. Biol. Chem. 282(51): 36987-36997.
Rammer P, Groth-Pedersen L, Kirkegaard T, Daugaard M, Rytter A, Szyniarowski P, Hoyer-Hansen M, Povlsen LK, Nylandsted J, Larsen JE, Jaattela M (2010). BAMLET activates a lysosomal cell death program in cancer cells. Mol. Cancer Ther. 9(1): 24-32.
Rubinsztein DC, DiFiglia M, Heintz N, Nixon RA, Qin ZH, Ravikumar B, Stefanis L, Tolkovsky A (2005). Autophagy and its possible roles in nervous system diseases, damage and repair. Autophagy, 1(1): 11-22.
Schmid D, Munz C (2007). Innate and adaptive immunity through autophagy. Immunity 27(1): 11-21.
Spolaore B, Pinato O, Canton M, Zambonin M, Polverino de Laureto P, Fontana A (2010). alpha-Lactalbumin Forms with Oleic Acid a High Molecular Weight Complex Displaying Cytotoxic Activity. Biochemistry, 49: 8658-8667.
Svensson M, Hakansson A, Mossberg AK, Linse S, Svanborg C (2000). Conversion of alpha-lactalbumin to a protein inducing apoptosis. Proc. Natl. Acad. Sci. USA, 97(8): 4221-4226.
Svensson M, Mossberg AK, Pettersson J, Linse S, Svanborg C (2003). Lipids as cofactors in protein folding: stereo-specific lipid-protein interactions are required to form HAMLET (human alpha-lactalbumin made lethal to tumor cells). Protein Sci. 12(12): 2805-2814.
Tolin S, De Franceschi G, Spolaore B, Frare E, Canton M, Polverino de Laureto P, Fontana A (2009). The oleic acid complexes of proteolytic fragments of alpha-lactalbumin display apoptotic activity. FEBS J. 277(1): 163-173.
Wilhelm K, Darinskas A, Noppe W, Duchardt E, Mok KH, Vukojevic V, Schleucher J, Morozova-Roche LA (2009). Protein oligomerization induced by oleic acid at the solid-liquid interface-equine lysozyme cytotoxic complexes. FEBS J. 276(15): 3975-3989.
Zhang M, Yang Jr. F, Yang F, Chen J, Zheng CY, Liang Y (2009). Cytotoxic aggregates of alpha-lactalbumin induced by unsaturated fatty acid induce apoptosis in tumor cells. Chem. Biol. Interact. 180(2): 131-142.
Zherelova OM, Kataev AA, Grishchenko VM, Knyazeva EL, Permyakov SE, Permyakov EA (2009). Interaction of antitumor alpha-lactalbumin-oleic acid complexes with artificial and natural membranes. J. Bioenerg. Biomembr. 41(3): 229-237.