Symposium: Innate Immunity and Human Milk

HAMLET Kills Tumor Cells by Apoptosis: Structure, Cellular Mechanisms, and Therapy[1,2]

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ABSTRACT New cancer treatments should aim to destroy tumor cells without disturbing normal tissue. HAMLET (human α-lactalbumin made lethal to tumor cells) offers a new molecular approach to solving this problem, because it induces apoptosis in tumor cells but leaves normal differentiated cells unaffected. After partial unfolding and binding to oleic acid, α-lactalbumin forms the HAMLET complex, which enters tumor cells and freezes their metabolic machinery. The cells proceed to fragment their DNA, and they disintegrate with apoptosis-like characteristics. HAMLET kills a wide range of malignant cells in vitro and maintains this activity in vivo in patients with skin papillomas. In addition, HAMLET has striking effects on human glioblastomas in a rat xenograft model. After convection-enhanced delivery, HAMLET diffuses throughout the brain, selectively killing tumor cells and controlling tumor progression without apparent tissue toxicity. HAMLET thus shows great promise as a new therapeutic with the advantage of selectivity for tumor cells and lack of toxicity. J. Nutr. 135: 1299–1303, 2005.

KEY WORDS: • *tumor* • *apoptosis* • *human milk* • *lactalbumin* • *protein folding*

HAMLET[4] (human α-lactalbumin made lethal to tumor cells) is a molecular complex of α-lactalbumin and oleic acid (1,2) (**Fig. 1**). It induces apoptosis in tumor cells, but normal differentiated cells are resistant to its effect (3). The activity of HAMLET was discovered by serendipity, while using human milk fractions to investigate bacteria adherence to lung carcinoma cell lines. In addition to blocking adherence, one milk fraction actually killed the cells by inducing apoptosis (1). Cell death was accompanied by changes in morphology, nuclear condensation, cytoplasmic blebbing, and formation of apoptotic bodies, similar to cells that undergo classical apoptosis according to Kerr et al. (4).

HAMLET has several unique features. HAMLET kills tumor cells and immature cells but not normal differentiated cells. HAMLET is active against a broad range of tumor cell lines. Because HAMLET kills by an apoptosis-like mechanism, it is unlikely to provoke harmful side effects. HAMLET may contribute to the natural defense against cancer. Three main aspects of the HAMLET project are being investigated:

I. The HAMLET structure and the molecular basis of the novel function.
II. The cellular targets of HAMLET in tumor cells.
III. The in vivo effects of HAMLET on experimental and established tumors.

I. HAMLET: structural aspects

The tumoricidal activity complex was discovered in a casein fraction of milk precipitated at low pH, and was shown to contain α-lactalbumin (5). Native α-lactalbumin is the most abundant protein in human milk and is well known as a coenzyme in lactose synthesis, but the native protein had no effect on tumor-cell viability. Apoptosis induction was linked to a folding change by deliberate conversion of α-lactalbumin to the apoptosis-inducing form. Conversion required unfolding of the protein and a lipid cofactor identified as oleic acid (C18:1, 9*cis*). The chemically defined active complex was named HAMLET (Fig. 1) (2).

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[4] Abbreviations used: HAMLET, human α-lactalbumin made lethal to tumor cells; HPV, human papilloma virus; NAP-1, nucleosome assembly protein 1; TUNEL assay, terminal deoxy nick end labeling.

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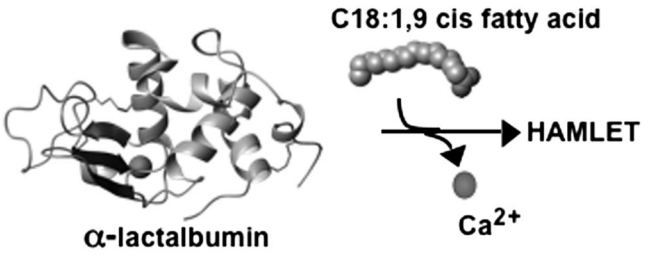

FIGURE 1 The HAMLET complex. HAMLET (human α-lactalbumin made lethal to tumor cells) is a molecular complex of α-lactalbumin and oleic acid (C18:1, 9 *cis*) from human milk. Native α-lactalbumin can be converted to HAMLET by treatment with EDTA, which removes Ca^{2+} and by the addition of the fatty acid C18:1, 9 *cis* (2). The figure is based on the α-lactalbumin crystal structure (31).

α-Lactalbumin can be converted to an apoptosis-inducing complex only in the presence of a lipid cofactor. The complex is formed from pure components (α-lactalbumin and oleic acid), each of which is inactive in the apoptosis assays. The folding change and the lipid cofactor were both necessary to attain this new function. The specificity of the lipid cofactor was investigated using fatty acids differing in carbon-chain length, saturation, or *cis*/*trans* conformation. We identified unsaturated C18 fatty acids in the *cis* conformation as the cofactors that interact with partially unfolded α-lactalbumin and form HAMLET. The interaction between protein and fatty acid was specific, because saturated C18 fatty acids or unsaturated C18:1 *trans* conformers were unable to form complexes with partially unfolded α-lactalbumin, as were fatty acids with shorter or longer carbon chains. Unsaturated *cis* fatty acids other than C18:1, 9 *cis* were able to form stable complexes, but these were not active in the apoptosis assay (6).

Partially unfolded α-lactalbumin does not induce apoptosis in the absence of the lipid cofactor. Mutations in the Ca^{2+}-binding site of bovine α-lactalbumin were used to create an α-lactalbumin conformer that maintained the unfolded conformation at physiologic pH and in the presence of Ca^{2+}. A point mutation at position D87A inactivated the Ca^{2+}-binding site and caused a change in tertiary structure, locking the protein in the partially unfolded conformation (7). This mutant did not induce apoptosis, however, demonstrating that a conformational change in α-lactalbumin is not sufficient to trigger apoptosis. The mutant bovine protein could still be converted to a HAMLET-like complex in the presence of oleic acid, demonstrating that the biological properties of HAMLET are defined both by the protein and the lipid cofactor. Furthermore, the activity of the converted mutant protein demonstrated that a functional calcium-binding site is not required for the apoptotic function of HAMLET (8).

In conclusion, the α-lactalbumin structure can be adjusted by shifting environments and functional diversity can be created by changes in tertiary structure. Also, lipid cofactors enable proteins to adopt stable novel conformations and thus to act as partners in protein folding. In this way, a single polypeptide chain can vary its structure and function, thereby participating in different biological processes in distinct environments.

II. Cellular targets of HAMLET in tumor cells

HAMLET has unique biological properties, because it selectively purges malignant cells by an apoptosis-like mechanism but leaves normal cells unharmed (1,3). This suggests that HAMLET bypasses the different blocks of apoptosis in many tumor cells and that HAMLET activates other cell death pathways that remain active in tumor cells.

Cellular trafficking of HAMLET. The subcellular localization of HAMLET is a potential key to distinguish the cellular responses of sensitive tumor cells from responses by the resistant normal cells (**Fig. 2**). The trafficking of HAMLET in tumor cells and normal differentiated cells was compared by confocal microscopy. The availability of surface receptors is not the limiting step, nor the critical factor determining sensitivity, because both cell types showed rapid surface binding of HAMLET. Translocation of HAMLET to the cytoplasm was detected in both cell types but with different efficiency. Large amounts of HAMLET reached the cytoplasm of the tumor cells and formed cytoplasmic aggregates. Uptake was not blocked by cycloheximide, showing that this step does not require protein synthesis. There was some cytoplasmic accumulation of HAMLET in normal cells. These observations suggested that the translocation into the cytoplasm per se does not distinguish the more sensitive from the less sensitive cells but that massive cytoplasmic accumulation of HAMLET characterizes the tumor cells.

The subsequent redistribution of HAMLET from the cyto-

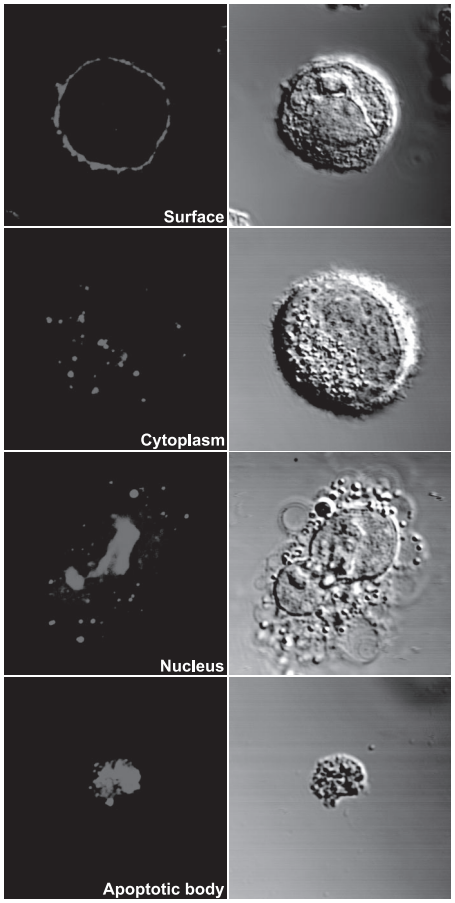

FIGURE 2 Cellular uptake of HAMLET in living tumor cells. HAMLET binds to the cell surface, enters the cytoplasm and accumulates in nuclei of living tumor cells (*left panel*). The cells die and show morphological alterations such as irregular membranes, cytoplasmic vesicles, and reduction in cell volume (*right panel*). Cells were incubated with Alexa Fluor-labeled HAMLET (0.03 mmol/L) and analyzed by confocal microscopy.

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plasm to the perinuclear region occurred only in the tumor cells. Despite the entry of HAMLET into the cytoplasm of normal cells, no trafficking to the perinuclear region was observed. In tumor cells, this effect was abrogated by cycloheximide, demonstrating that the perinuclear translocation of HAMLET required cellular metabolism. The translocation to the perinuclear region was accompanied by the movement of mitochondria, as shown by co-staining with mitochondria-specific markers. Finally, HAMLET was shown to accumulate in tumor-cell nuclei and the apoptotic bodies stained positive for HAMLET (Gustafsson, L., et al., unpublished results).

HAMLET interacts with histones and chromatin in tumor-cell nuclei. The accumulation of HAMLET in tumor-cell nuclei encouraged us to identify molecular targets for HAMLET. The initial studies, using crude cellular fractions, showed that HAMLET binds to histone H3 in nuclear fractions from tumor cells. Using purified histones, HAMLET was shown to interact with histones H2B, H3, and H4. To fold properly, histones need to be present as dimers of H2A–H2B and tetramers of H3–H4. Such natively folded and biologically functional histones were purified from cells and were used to further study the interactions with HAMLET. In affinity chromatography, HAMLET bound all 4 histones, and Biacore assays showed a high affinity binding with very slow dissociation. Mixing histones with HAMLET in solution resulted in precipitation of the proteins, further illustrating the high affinity of the interaction. Both denatured and native histones were precipitated by HAMLET, with a preference for H3 and H4. The relevance of these interactions in vivo was demonstrated in HeLa tumor cells expressing GFP-tagged histones. HAMLET colocalized with histones in cell nuclei and induced changes in the global chromatin structure. The chromatin was condensed to the nuclear periphery or to large, spherical structures. HAMLET was present in both of these chromatin patterns (9).

The histone-binding properties of HAMLET were compared with the known histone chaperone, nucleosome assembly protein 1 (NAP-1). NAP-1 mediates assembly of histones onto DNA to form nucleosomes. NAP-1 or HAMLET were mixed with histones, and short DNA fragments were added. NAP-1 induced a concentration-dependent assembly of nucleosomes, whereas HAMLET completely prevented nucleosome formation. Thus, HAMLET differs from the physiologic histone chaperones that bind histones with affinities low enough to then deliver the histones to DNA. Because of the high affinity for histones, HAMLET disrupted the association between histones and DNA, and thus perturbed the structure and the function of chromatin (9).

HAMLET interacts with all structural and functional conformations of histones, from denatured proteins to natively folded, soluble histones and histones in nucleosomes. This suggests a number of potential functional consequences for the cell. When HAMLET enters the tumor cell, it may interact with newly synthesized histones, compete with chaperones for the histones, and prevent their transport to the nucleus. This would inhibit the chromatin assembly machinery of histones and induce chromatin damage. In the nucleus, HAMLET could bind histones in chromatin and either remove them from DNA or directly bind nucleosomes and impair their function. Alternatively, the binding of HAMLET to chromatin could induce DNA damage. It has been observed that defects in chromatin assembly can lead to double-strand DNA breaks and activation of the S-phase checkpoint (10). However, it is not clear how HAMLET damages the DNA.

In conclusion, HAMLET binds to histones in the nuclei of tumor cells dying after HAMLET treatment. This interaction may disturb the structure and function of the chromatin and could be an important feature of HAMLET-induced cell death.

HAMLET-induced cell death is independent of p53. The strong interaction between HAMLET and histones in tumor-cell nuclei suggest that the nuclear effects of HAMLET may be the trigger of cell death. The *p53* tumor suppressor serves as a guardian of DNA integrity, and *p53*-dependent cell death mechanisms are activated after irreparable DNA damage (11,12). We therefore investigated HAMLET sensitivity of tumor cells as a function of their *p53* status. Surprisingly, there was no difference between cells with mutated or wild type *p53*, suggesting that HAMLET-induced cell death does not require p53 activity.

HAMLET interacts mitochondria and the caspase cascade. HAMLET interacts with mitochondria, as shown by colocalization in living cells and by studies of isolated mitochondria. Furthermore, HAMLET triggers membrane depolarization, release of cytochrome C and activates pro-apoptotic caspases (13,14). However, HAMLET-induced cell death does not rely on caspases, as the pan-caspase inhibitor ZVAD did not prevent cell death (3). HAMLET-induced cell death differs from most classical apoptotic systems in that caspase inhibitors do not rescue cells.

The mechanisms that control the cellular response to HAMLET were further investigated in relation to the Bcl-2 family of proteins. The Bcl-2 protein family regulates cell survival, and Bcl-2 and Bcl-X_L are localized at the mitochondrial outer membrane, where they may block the release of apoptogenic factors from the intermembrane space (15,16). Overexpression of Bcl-2 and Bcl-X_L has also been observed in a variety of cancers (17). Surprisingly, however, overexpression of Bcl-2 or Bcl-X_L did not change the HAMLET sensitivity of tumor-cell lines (3).

We conclude from these and other studies that HAMLET induces apoptosis-like death by a novel mechanism involving trafficking to the perinuclear region and translocation to the cell nuclei. The nuclear accumulation of HAMLET disrupts the chromatin and marks the irreversible stage of tumor cell apoptosis. In parallel, HAMLET activates known effectors of apoptosis, including the caspase cascade.

III. In vivo effects of HAMLET in tumor models

HAMLET induces in vitro apoptosis in cells from carcinomas of lung, throat, kidney, colon, bladder, prostate and ovaries; in melanomas; in glioblastomas of the brain; and in leukemias. The broad activity against such different tumors is quite remarkable, but the in vivo effects may be quite different and must be rigorously tested.

Effects of HAMLET on human skin papillomas. Papillomas are premalignant lesions of the skin and mucosal surfaces (18–21). The human papilloma virus (HPV) can cause condyloma acuminatum, and laryngeal and genital papillomas, and skin lesions, which are extremely common. Therapeutic options are limited and often are ineffective or destructive (23,24). They include cryotherapy, curettage, cautery, salicylic acid, CO_2 laser, photodynamic therapy, antimitotic agents, or immune modulators. Currently, HPV vaccines are being developed to prevent HPV infection, but they are not available for use.

Skin papillomas were selected as a first model to examine the effect of HAMLET in humans (0.9% NaCl) (25). Treatment was performed according to a double-blind, placebo-controlled protocol. HAMLET (0.7 mM in 0.9% NaCl) or placebo was applied topically, once a day for 3 wk. The lesions

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were measured and photographed once a week during the treatment period and at follow-up visits, 1 and 2 mo after completed treatment. The treatment was deemed successful if the patient showed a reduction in lesion volume by ≥75% within 1 mo after completed treatment.

HAMLET treatment reduced the papilloma volume in 100% (20/20) of the patients (88/92 papillomas) compared with 15% in the placebo group (3/20 patients, 15/74 papillomas) ($P < 0.001$). This effect was reproduced in the open study (10/12 patients, 36/41 lesions). No adverse reactions were reported, and there was no difference in efficacy between immunocompetent and immunosuppressed patients (**Fig. 3**).

Topical HAMLET application significantly reduced the volume of skin papillomas. We propose that HAMLET should be further explored as a novel therapeutic agent in patients with HPV-induced diseases.

In vivo effects of HAMLET on glioblastoma xenografts. The majority of intracranial neoplasms originate from neuroglial cells and form a heterogeneous group known as gliomas (26). They account for >60% of all primary brain tumors and have the most unfavorable prognosis. Patients with glioblastomas of WHO grade IV show a mean survival time of less than 1 y (27), and glioblastomas constitute approximately one-fourth of all intracranial tumors in neurosurgical and neuropathological series. In recent years, surgical treatment of glioblastomas has made significant technical advances. Microsurgery and neuronavigation, as well as new diagnostic high-resolution imaging techniques have reduced surgical mortality and morbidity, but there has been no significant improvement in survival. The tumors are inaccessible to complete surgical removal due to their invasive nature and diffuse infiltrating growth, and the current treatment of patients with malignant gliomas is palliative, involving surgery, radiotherapy, and chemotherapy (22).

During our survey of tumor-cell lines, we observed that glioblastoma cells undergo apoptosis in response to HAMLET. Native α-lactalbumin, which was used as a control throughout these studies, did not influence cell viability or cause DNA fragmentation. HAMLET did not induce apoptosis in differentiated brain cells. The normal cells maintained their viability and retained intact DNA after 24 h of exposure to HAMLET.

To investigate the effect of HAMLET on tumor tissue instead of cell lines, human glioblastoma biopsy spheroids were exposed to HAMLET or α-lactalbumin in vitro, and apoptotic cells were detected by the TUNEL assay (29). HAMLET was

C.

	Effect	No effect	Total
HAMLET	20	0	20
Placebo	3	17	20
Total	23	17	40

Effect = a mean volume decrease of >75%, p< 0.001

FIGURE 3 Effect of HAMLET on human skin papillomas. *A.* Study design. A double-blind, placebo-controlled study was performed with HAMLET on human skin papillomas. The substance was applied once a day for 3 wk, and the lesions were measured and photographed once a week during the treatment period and at follow-up visits 1 and 2 mo after completed treatment. *B.* Morphology of papilloma before and after HAMLET treatment. *C.* Treatment effect of HAMLET contra placebo. A ≥75% reduction in lesion volume was observed in 20/20 patients receiving HAMLET and in 3/20 patients receiving placebo ($P < 0.001$).

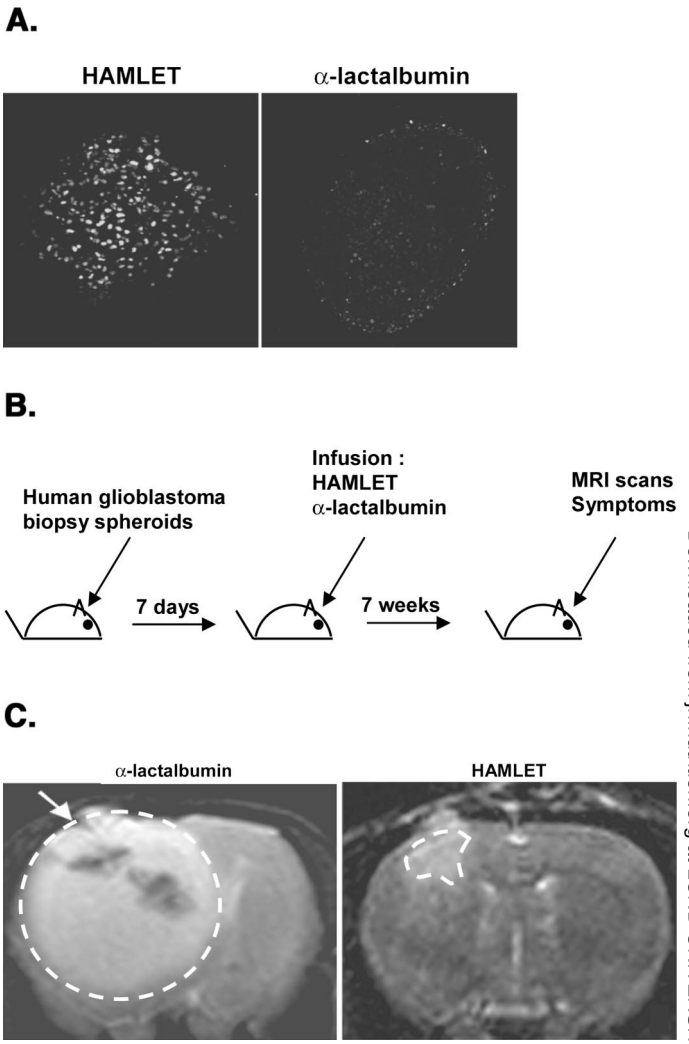

FIGURE 4 Effect of HAMLET on human glioblastomas. *A.* Apoptosis induction in vitro (TUNEL staining). *B.* Xenotransplantation model of human glioblastomas. Biopsy spheroids were injected into the brains of immunodeficient nu/nu rats. Convection enhanced delivery (CED) was used to infuse HAMLET or α-lactalbumin (0.7 mmol/L). Seven weeks later, the tumor volume was determined by MRI. *C.* Tumor volume by MRI. HAMLET showed effect on tumor growth compared with the α-lactalbumin–treated rats ($P < 0.01$).

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shown to induce apoptosis throughout the tumor spheroids, but α-lactalbumin had no effect (**Fig. 4***A*).

The effect of HAMLET was investigated in a rat model of human glioblastoma (28,29). Xenotransplantation of human glioblastoma biopsies into the nude rat brain offers a unique model to study the human disease under experimental conditions, because the xenografts show the infiltrative growth characteristic of human tumors. In this model, human tumor biopsies are allowed to form spheroids in vitro as an intermediate step to obtain standardized inocula of tumor cells. After xenotransplantation, the rats develop pressure symptoms after 8 wk with little variation, and large tumor masses can be detected by MRI scans.

The therapeutic potential of HAMLET was investigated in this model. The tumor cells were allowed 1 wk to integrate in the brain (28,29). HAMLET was administered by infusion into the brain for 24 h with α-lactalbumin as a control, and the rats were observed for another 7 wk (Fig. 4*B*). HAMLET inhibited tumor development (Fig. 4C), as demonstrated by a delayed onset of pressure symptoms. Rats receiving α-lactalbumin developed symptoms significantly earlier than the HAMLET-treated animals ($P < 0.01$).

Apoptosis induction in vivo was examined by subjecting sections from the treated rats to the TUNEL assay. There was extensive apoptosis in the tumor, but the tissue surrounding the tumor did not show TUNEL staining. Furthermore, the infusion of HAMLET did not harm the normal brain and did not produce any neurological symptoms. HAMLET has the potential to act as a selective inducer of apoptosis in patients with malignant glioblastomas.

CONCLUSIONS

HAMLET illustrates the value of human milk as a rich source of molecules with a beneficial effect on a variety of human-disease conditions. The conditions required to form HAMLET are present in the stomach of the breast-fed child, where the low pH may unfold the protein by the release of calcium, and where acid-sensitive lipases hydrolyze milk triglycerides to release oleic acid. We speculate that HAMLET may kill virus-transformed or premalignant cells from the gastrointestinal tract of the breast-fed child. This effect might include lymphoid cells in the gut-associated lymphoid tissue, because breast-fed children have a much reduced frequency of lymphomas compared with age-matched but bottle-fed children (30).

LITERATURE CITED

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

2. Svensson, M., Hakansson, A., Mossberg, A. K., Linse, S. & Svanborg, C. (2000) Conversion of alpha-lactalbumin to a protein inducing apoptosis. Proc. Natl. Acad. Sci. U.S.A. 97: 4221–4226.

3. Svanborg, C., Agerstam, H., Aronson, A., Bjerkvig, R., Duringer, C., Fischer, W., Gustafsson, L., Hallgren, O., Leijonhuvud, I., et al. (2003) HAMLET kills tumor cells by an apoptosis-like mechanism–cellular, molecular, and therapeutic aspects. Adv. Cancer Res. 88: 1–29.

4. Kerr, J. F., Wyllie, A. H. & Currie, A. R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26: 239–257.

5. Svensson, M., Sabharwal, H., Hakansson, A., Mossberg, A. K., Lipniunas, P., Leffler, H., Svanborg, C. & Linse, S. (1999) Molecular characterization of alpha-lactalbumin folding variants that induce apoptosis in tumor cells. J. Biol. Chem. 274: 6388–6396.

6. Svensson, M., Mossberg, A. K., 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: 2805–2814.

7. Anderson, P. J., Brooks, C. L. & Berliner, L. J. (1977) Functional identification of calcium binding residues in bovine alpha-lactalbumin. Biochemistry 36: 11648–11654.

8. Svensson, M., Fast, J., Mossberg, A. K., Duringer, C., Gustafsson, L., Hallgren, O., Brooks, C. L., Berliner, L., Linse, S. & Svanborg, C. (2003) Alpha-lactalbumin unfolding is not sufficient to cause apoptosis, but is required for the conversion to HAMLET (human alpha-lactalbumin made lethal to tumor cells). Protein Sci. 12: 2794–2804.

9. 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: 42131–42135.

10. Ye, X., Franco, A. A., Santos, H., Nelson, D. M., Kaufman, P. D. & Adams, P. D. (2003) Defective S phase chromatin assembly causes DNA damage, activation of the S phase checkpoint, and S phase arrest. Mol. Cell 11: 341–351.

11. Bates, S. & Vousden, K. H. (1999) Mechanisms of p53-mediated apoptosis. Cell Mol. Life Sci. 55: 28–37.

12. Lane, D. P. (1993) Cancer. A death in the life of p53. Nature 362: 786–787.

13. 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: 186–191.

14. Kohler, C., Hakansson, A., Svanborg, C., Orrenius, S. & Zhivotovsky, B. (1999) Protease activation in apoptosis induced by MAL. Exp. Cell Res. 249: 260–268.

15. Vander Heiden, M. G., Chandel, N. S., Williamson, E. K., Schumacker, P. T. & Thompson, C. B. (1997) Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria. Cell 91: 627–637.

16. Green, D. R. & Kroemer, G. (2004) The pathophysiology of mitochondrial cell death. Science 50: 626–629.

17. Reed, J. C. (1999) Dysregulation of apoptosis in cancer. J. Clin. Oncol. 17: 2941–2953.

18. zur Hausen, H. (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat. Rev. Cancer 2: 342–350.

19. zur Hausen, H. (1996) Papillomavirus infections–a major cause of human cancers. Biochim. Biophys. Acta 1288: F55–F78.

20. Tyring, S. K. (2000) Human papillomavirus infections: epidemiology, pathogenesis, and host immune response. J. Am. Acad. Dermatol. 43: S18–S26.

21. Majewski, S. & Jablonska, S. (2002) Do epidermodysplasia verruciformis human papillomaviruses contribute to malignant and benign epidermal proliferations? Arch. Dermatol. 138: 649–654.

22. Grossman, S. A. & Bastor, J. F. (2004) Current management of glioblastoma multiforms. Semin. Oncol. 31: 635–644.

23. Leman, J. A. & Benton, E. C. (2000) Verrucas. Guidelines for management. Am. J. Clin. Dermatol. 1: 143–149.

24. Gibbs, S., Harvey, I., Sterling, J. C. & Stark, R. (2003) Local treatments for cutaneous warts. Cochrane Database Syst. Rev. 3.

25. Gustafsson, L., Leijonhufvud, I., Aronsson, A., Mossberg, A. K. & Svanborg, C. (2004) Treatment of skin papillomas with topical alpha-lactalbumin-oleic acid. N. Engl. J. Med. 350: 2663–2672.

26. Russel, D. S. & Rubinstein, L. J. (1989) Pathology of Tumors of the Nervous System. 5th ed. Williams and Wilkins, Baltimore, MD.

27. Gundersen, S., Lote, K. & Hannisdal, E. (1996) Prognostic factors for glioblastoma multiforme–development of a prognostic index. Acta Oncol. 35 (Suppl. 8): 123–127.

28. Engebraaten, O., Hjortland, G. O., Hirschberg, H. & Fodstad, O. (1999) Growth of precultured human glioma specimens in nude rat brain. J. Neurosurg. 90: 125–132.

29. 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: 2105–2112.

30. Davis, M. K. (1998) Review of the evidence for an association between infant feeding and childhood cancer. Int. J. Cancer (Suppl. 11): 29–33.

31. Acharya, K. R., Ren, J. S., Stuart, D. I., Phillips, D. C. & Fenna, R. E. (1991) Crystal structure of human alpha-lactalbumin at 1.7 A resolution. J. Mol. Biol. 221: 571–581.

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