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The role of HAMLET in cancer treatment

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Abstract

HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells) is a new antitumor protein-lipid complex that largely kills cancer cells while protecting healthy cells. HAMLET is formed by the incorporation of incompletely folded alpha-lactalbumin oleic acid found in large quantities in human milk. HAMLET is thought to activate apoptosis by targeting pathways that maintain cell viability in cancer cells. Clinical studies with HAMLET have identified HAMLET as an anticancer agent that kills tumor cells. In this proceeding we will review HAMLET as anti-tumor agent.

Introduction

HAMLET (Human Alpha-lactalbumin Made Lethal to Tumor cells) is a complex formed by binding oleic acid to alpha-lactalbumin found in human milk to destroy tumor cells [1]. While this complex is capable of promoting apoptosis for cancer cells, it has no lethal effect on healthy cells. The term HAMLET first entered the literature in 1995 [2]. It has been reported that a structure in human milk encourages tumor cells to die, and healthy cells are resistant to this structure. It was later discovered that this structure is the oleic acid-alpha lactalbumin complex [2]. Antitumoral effects have been reported in *in-vitro* and clinical studies with HAMLET [3]. In this review, recent studies investigating the molecular structure and mechanisms of action of HAMLET and its effects on cancer cells are discussed.

Molecular structure of HAMLET

Three-dimensional structure of alpha-lactalbumin, the main protein component of the human milk contains four alpha helices, a triple beta layer, and a calcium binding site [4]. In this state, it is a cofactor for lactose synthesis and does not lead to cell death. For this structure to be antitumoral, the protein structure must be opened and the oleic acid compound must be attached to this structure [5]. There is no information in the literature regarding the *in-vivo* formation of HAMLET, but the acidic condition of the stomach is thought to be a suitable environment for the HAMLET formation. It is known that the low pH of the stomach causes the α-lactalbumin to be partially opened by releasing the strongly bound calcium ion [6]. During the HAMLET *in-vitro* formation process, α-lactalbumin is removed from the calcium ion with ethylenediamine tetraacetic acid (EDTA) or acid and partially opened. Then, the oleic acid binds to the protein and the complex structure are completed [7].

Mechanisms of action of HAMLET on tumor cells

Cancer cell death can occur in various ways such as apoptosis, necrosis, and autophagy. However, cancer cells have death escape mechanisms such as gene amplification, gene, and chromosomal mutations. In tumor treatment, cell death is promoted by targeting apoptosis pathways and mitochondrial signaling pathways that lead to phagocytosis with ionizing radiation and chemotherapeutic agents. Hamlet binds to the cell surface in tumor cells,

invades cells, causes mitochondrial membrane disruption, increases cytochrome c release, initiates caspase activation, and thus performs apoptosis [8]. Tumor cells exposed to HAMLET cause an excessive increase in unfolded protein load, which contributes to the death of cells by activating 20S proteosomes [9]. When tumor cells encounter HAMLET, they reach the nucleus after about one hour. H3 and H4 histones in the nucleosome structure bind to proteins with high affinity and form insoluble chromatin complexes in the nucleus. As a result of this cytotoxic effect, DNA damage and degradation occur [10]. It is thought that the mechanism of macroautophagy is induced by enlargement, damaged mitochondria, and formation of double membranes in the cytoplasmic vacuols of the tumor cells exposed to HAMLET [8].

HAMLET's effect on cancer

There are *in vitro* and clinical studies in the literature investigating the therapeutic efficacy of HAMLET in cancer types such as bladder cancer, colon cancer, and glioblastoma. HAMLET has been reported to reduce tumor growth in mouse bladder cancer [11]. It has been reported that the residence time of HAMLET in the body of mice with tumor development is longer than in the healthy control group. In addition, it has been reported that HAMLET treatment reduces tumor volume and there is a substantial reduction in new tumor formation [12].

In a study focusing on the effect of HAMLET in colon cancer induced mice, it was reported that it accumulates especially in tumor tissue, and it has been reported that the secretion of important oncoproteins, cyclooxygenase-2 inhibitor, and vascular endothelial growth factor (VEGF) are significantly decreasing after HAMLET administration [13].

In a study investigating the effect of HAMLET on glioblastomas, it was stated that HAMLET promoted apoptosis in tumor cells by invading the tumor cell. Studies investigating the effects of HAMLET on bladder cancer and skin papillomas are limited. In a clinical study investigating the effects of HAMLET in bladder cancer, patients with bladder cancer were given HAMLET intravesically five times a day before surgery and it was observed that dead tumor cells were excreted in the urine for five days. In the study data, it has been shown that the majority of cancer cells have the signs of apoptosis. It has been reported that cells undergoing apoptosis are in the majority in cystoscopy biopsy specimens in which the tumor size is reduced during the bladder surgery [14].

In a study investigating the effectiveness of HAMLET on skin papillomas, it was found that a significant reduction in lesion volume was seen when HAMLET was administered to a patient with severe, treatment-resistant papilloma for 3 weeks. It has also been reported that in approximately 83% of patients treated with HAMLET, all lesions healed completely after two years [15].

Conclusion

In line with the studies investigating the effects of HAMLET in cancer treatment, it can be considered an alternative treatment method to chemotherapy and radiotherapy. While it was reported to have a cytotoxic effect in *in-vitro* studies, these effects were not fully studied in *in-vivo* studies. However, due to the small number of studies, it is not yet possible to qualify HAMLET as a stand-alone treatment. It can be concluded whether HAMLET is an alternative treatment by increasing the number of studies trying it in different types of cancer.

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