Antitumor Potential of Lactaptin

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Abstract

A number of naturally occurring proteins that selectively induce apoptosis of cancer cells were recently used to develop novel anticancer drug substances. Human milk is a source of multiple bioactive peptides, some of which are activated by proteolysis. Lactaptin is an 8.6 kDa proteolytic fragment of kappa-casein isolated from human milk. This mini review describes apoptotic properties, mechanism of action and antitumor activity of lactaptin.

Keywords: Lactaptin; Antitumor; Proteins

Introduction

Human milk is known to contain proteins and peptides that kill tumor cells (the HAMLET (Human α-lactalbumin made lethal to tumor cells) [1], lactoferrin, lactoferricin [2] and lactaptin. The potential applications of HAMLET, lactoferrin and lactoferricin as pharmaceutical drug candidates and clinical nutrition in the overall management of cancer have been examined in the review Chen et al. [3]. This mini review describes a little-known human milk protein lactaptin.

Origin of Lactaptin

We isolated and characterized the pro-apoptotic peptide from human milk, that was capable of reducing cell viability and inducing apoptosis in cultured tumor cells. We named this peptide lactaptin. Lactaptin was identified as 8.6 kDa proteolytic fragment of human kappa-casein, composed of 74 amino acid residues (residues 57–134 of kappa-casein) [4]. A series of recombinant analogues of lactaptin was constructed but only one of them, RL2, containing the complete amino acid sequence of lactaptin, effectively induced cell death in various human and mouse tumour cells in vitro while having no effect on the viability of nonmalignant MSC cells [5].

Mechanism of Lactaptin Apoptotic Action

We have analyzed biochemical markers of RL2 induced apoptosis of cancer cells. The activation of initiator and effector caspases as well as mitochondrial membrane potential and cytoplasm membrane changes were analyzed using flow cytometry and Western-blot methods. We have found that RL2 induced apoptotic death of tumor cells was accompanied by phosphatidyserine exposure on the plasma membrane surface. It was also shown that RL2 has induced dissipation of mitochondrial membrane potential and resulted in activation of initiator caspases 8, 9 and effector caspase 3 [6]. We demonstrated that RL2 downregulates Bcl-2 expression and induces p53-independent cell death. RL2 penetrates both into cancer and non-transformed cells. Identification of the cellular targets of the lactaptin analogue revealed that α/β-tubulin and α-actinin-1 were RL2-bound proteins [7].

Antitumor Activity of Lactaptin

Mice hepatocarcinoma HA-1 and human breast adenocarcinoma MDA-MB-231 were selected for testing RL2 antitumor activity in vivo. Female A/Sn mice between 10 and 12 weeks of age (colony of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia) weighing 20-25g were used for the in vivo experiments. Ascites HA-1 tumor cells were washed and suspended in saline and 0.1 ml of the suspension containing 2×10^6 cells was inoculated subcutaneously on the dorsal side or intramuscularly in the right thigh of the A/Sn mice. To obtain artificial metastases, 1.5×10^6 tumor cells in 0.2 ml saline were injected into the mice intravenously in the lateral tail vein. Retpetive injections of RL2 (5-50 mg/kg) for 3-5 days effectively inhibited ascites and solid tumor transplant growth when administered intravenously or intraperitoneally, without obvious side effects. The solid tumor inhibitory effect of RL2 (5 i.v. injections, cumulative dose 125 mg/kg) was comparable with that of cyclophosphamide at a therapeutic dose (5 i.v. injections, cumulative dose 150 mg/kg). In combination therapy with cyclophosphamide, RL2 had an additive antitumor effect for ascites-producing tumors. Histomorphometric analysis indicated a three-fold reduction of spontaneous metastases in the liver of RL2-treated mice with solid tumor transplants in comparison with control animals. Repeated RL2 treatment substantially prolonged the lifespan of mice with intravenously injected tumor cells [8,9].

To test in vivo RL2 antitumor activity to human cancer cells a mouse xenograft model of breast cancer was used. A suspension of MDA-MB-231 cells in Matrigel was subcutaneously injected into 6–8-week-old female SCID mice to form solid tumors. Tumors volumes were monitored every 5 days. When tumors reached roughly 100 mm³, RL2 (40 mg/kg) was injected intravenously every 2 days, with a total of three injections. The selected dose of RL2 and the treatment regime were based on a scheme of therapy described previously [8]. Seven days after the last injection the tumours were excised and weighed. RL2 injections significantly delayed tumors growth compared to the control group. The rate of inhibition of tumors growth after RL2 therapy was calculated as 43% [7].

The antitumor effect of RL2 was also examined against primary human endometrial cancer cells. Primary cell cultures of malignant and normal human endometrium were performed by enzymatic digestion of endometrial tissue from biopsy material. Our results indicate that the recombinant analog of lactaptin, RL2, exerts cytotoxic effects against...
primary hormone-dependent endometrial tumor cells in vitro with features of apoptosis [8].

Now the pre-clinical trials of the drug Lactaptin, created on the basis of RL2, have been successfully completed. Like other therapeutic proteins Lactaptin is evenly distributed in organs and tissues, which results in reducing its concentration in the tumor and the antitumor efficiency decrease. Selective delivery of Lactaptin to the tumor site could alleviate this problem and reduce the therapeutic dose by increasing the local concentration of the drug within the tumor to achieve a desired antitumor response.

To increase the antitumor lactaptin efficacy we have chosen tumor specific peptides as the molecular targeting agents and constructed different plasmid vectors producing fusion proteins consisting of tumor specific peptides and RL2. To obtain tumor specific peptides we applied a selection method using phage peptide libraries (Ph.D.™ - Phage Display Peptide Library Kit, New England Biolabs). Two displaying peptides GLHTSATNLYLH and SGVYKV AYDWQH were selected to mice hepatocarcinoma A-1 (HA-1) and Lung adenocarcinoma (LA). The RL2 and fusion proteins were expressed in E.coli and purified. We investigated relative cytotoxic effects in vitro and tumor retention of obtained proteins in vivo.

Breast cancer MDA-MB-231 and MCF-7 cells showed a similar decrease in viability after RL2 and fusion proteins treatment. It was shown, that fusion proteins with targeted peptides are accumulated in HA-1 tumor comparing to RL2.

Conclusion
Thereby we demonstrated that recombinant analog of human milk peptide lactaptin RL2 effectively induced apoptosis of tumor cells in vitro, suppressed the growth of sensitive tumors and metastases in vivo. Lactaptin is not toxic for nonmalignant normal cells and animals and is considered to be the basis for the development of antitumor drugs.

Source of Support

References