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Mastoparan is a membranolytic anti-cancer peptide that works synergistically with gemcitabine in a mouse model of mammary carcinoma

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ABSTRACT

Anti-cancer peptides (ACPs) are small cationic and hydrophobic peptides that are more toxic to cancer cells than normal cells. ACPs kill cancer cells by causing irreparable membrane damage and cell lysis, or by inducing apoptosis. Direct-acting ACPs do not bind to a unique receptor, but are rather attracted to several different molecules on the surface of cancer cells. Here we report that an amidated wasp venom peptide, Mastoparan, exhibited potent anti-cancer activities toward leukemia (IC_{50} ~ 8–9.2 μM), myeloma (IC_{50} ~ 11 μM), and breast cancer cells (IC_{50} ~ 20–24 μM), including multidrug resistant and slow growing cancer cells. Importantly, the potency and mechanism of cancer cell killing was related to the amidation of the C-terminal carboxyl group. Mastoparan was less toxic to normal cells than it was to cancer cells (e.g., IC_{50} to PBMC = 48 μM). Mastoparan killed cancer cells by a lytic mechanism. Moreover, Mastoparan enhanced etoposide-induced cell death in vitro. Our data also suggest that Mastoparan and gemcitabine work synergistically in a mouse model of mammary carcinoma. Collectively, these data demonstrate that Mastoparan is a broad-spectrum, direct-acting ACP that warrants additional study as a new therapeutic agent for the treatment of various cancers.

1. Introduction

Over the past 5 years for which there are data, delay-adjusted cancer death rates declined by a modest 1.8 and 1.4% in men and women, respectively [1]. Hence, cancer remains a major health concern, as it represents the second leading cause of death in the United States, and is expected to surpass heart disease as the leading cause of death over the next few years. Therefore, there is a continued need for improvements in cancer screening, diagnosis, and treatment. To this end, anti-cancer peptides (ACPs) represent a potential untapped reservoir of effective adjunctive therapies for the treatment of cancer.

ACPs are small polypeptides (<50 residues) that exhibit preferential toxicity towards cancer cells. ACPs are predominantly composed of cationic and hydrophobic amino acids, giving them an overall positive charge and amphipathic structure that promotes binding to negatively charged cell membranes [2]. In contrast to normal cell membranes, which are largely charge neutral, cancer cell membranes carry a net negative charge due to an abundance of anionic phospholipids (e.g., phosphatidylserine) [3], proteoglycans (e.g., heparan sulfate proteoglycans) [4], O-glycosylated mucins [5], and sialoglycoproteins [6]. Following membrane binding, ACPs kill the cancer cell by causing irreparable membrane damage followed by cell lysis (i.e., direct-acting ACPs), or by initiating a cell death pathway that results in death by apoptosis (i.e., indirect-acting ACPs) [7]. Unlike conventional chemotherapeutic agents, ACPs do not only target rapidly dividing cells; therefore, certain ACPs are also toxic to slow growing cancers [8]. Moreover,

Abbreviations: ACP, anti-cancer peptide; AML, acute myeloid leukemia; DMSO, dimethyl sulfoxide; HMEC, human mammary epithelial cell; IP, intraperitoneal; LDH, lactate dehydrogenase; LUV, large unilamellar vesicle; MDR, multidrug-resistance; PBMC, peripheral blood mononuclear cell; PI, propidium iodide; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; Q2D, every 2 days; Q7D, every 7 days; T-ALL, T cell acute lymphoblastic leukemia.

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because most ACPs act at the level of the cell membrane, ACPs are able to target multidrug-resistant (MDR) cancer cells, including those that overexpress MDR proteins [8,9]. Certain ACPs potentiate killing by conventional chemotherapeutic agents in vitro, suggesting that they hold potential for use as novel adjunctive therapies [8]. Importantly, many ACPs, including direct-acting ACPs, exhibit potent anti-tumor activities toward primary tumors and their metastases without causing harm to normal tissues [10]. Selected direct-acting ACPs can completely eliminate primary tumors, but perhaps more importantly treatment with direct-acting ACPs can initiate an anti-tumor immune response that not only protects the tumor-bearing animal from re-challenge, but can also be adoptively transferred to recipient mice [11,12]. To date there has not been any report suggesting that cancer cells can develop resistance to direct-acting ACPs. This is likely because ACPs are attracted to several different surface molecules rather than a unique targeted receptor. Collectively, these findings suggest that ACPs, particularly those that are direct-acting, have considerable potential for use as novel adjunctive therapies for the treatment of various cancers.

One of the challenges of identifying ACPs is that many peptides with anticancer activity are also toxic towards normal cells. Accordingly, screening approaches to identify novel ACP sequences must be complemented by screening against normal cells to establish whether a given ACP will be selective for tumor cells. Therefore, the purpose of the present study was to screen a small library of short (<20 residues) cationic peptides with various biological activities (e.g., anti-microbial, anti-biofilm, and immune-modulatory properties) to identify novel ACPs that selectively kill cancer cells. The Piscidins NRC-03 and NRC-07 were included as positive controls [8,13]. This screening approach identified amidated Mastoparan as the most potent, selective ACP. Mastoparan is a 14-residue peptide isolated from wasp venom [14]. Recently, non-amidated Mastoparan (i.e., Mastoparan-COOH) was shown to induce apoptosis in melanoma cells [15]. Here, we show that Mastoparan capped with a C-terminal amide (i.e., Mastoparan-NH_2), as is observed with the natural peptide, is 8–11-fold more potent than Mastoparan-COOH. Interestingly, it is also shown that Mastoparan amide (herein referred to as Mastoparan) clearly kills cancer cells by a lytic mechanism, which is in contrast to the non-amidated derivative [15]. Mastoparan was toxic to leukemia, myeloma, and breast cancer cells, and showed toxicity towards both slow-growing and MDR cancer cells. Importantly, Mastoparan enhanced etoposide-induced cell death in vitro. Our data also suggests that Mastoparan worked synergistically with gemcitabine in a mouse model of mammary carcinoma. To our knowledge, this is the first study to show that C-terminal amidation affects both ACP potency as well as the mechanism of cancer cell killing. Moreover, this study also shows that ACPs work synergistically with chemotherapeutic agents in vivo. Collectively, these findings demonstrate that Mastoparan warrants consideration as a novel therapeutic agent for the treatment of several different cancers.

2. Materials and methods

2.1. Cell culture and conditions

Jurkat and THP-1 human leukemia cells, and HOPC murine myeloma cells were purchased from American Type Culture Collection (Manassas, VA), and were maintained in RPMI 1640 medium (Fisher Scientific, Ottawa, ON) supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Invitrogen, Burlington, ON, Canada). Cells were incubated at 37 °C in a 5% CO_2 humidified environment for a maximum of three months (passaged as required). While all cell lines were originally obtained from ATCC, MDA-MB-231 breast cancer cells were provided by Dr. S. Drover (Memorial University of Newfound, St. John's, NL, Canada). T47D breast cancer cells were a gift from Dr. Jonathan Blay (University of Waterloo, Waterloo, ON, Canada). MDA-MB-468, 4T1, and SKBR3 breast carcinoma cells were kindly provided by Drs. Patrick Lee, David Waisman, and Graham Dellaire, respectively, and Dr. Kerry Goralski provided MCF7 and paclitaxel-resistant MCF7-TX400 breast cancer cells (Dalhousie University, Halifax, NS, Canada). All breast carcinoma cells were maintained in DMEM (Fisher) supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine, and were incubated at 37 °C in a 10% CO_2 humidified environment for a maximum of 30 passages. All cell lines were passaged as needed to maintain optimal cell growth, and were routinely tested for mycoplasma contamination using the MycoProbe Mycoplasma Detection Kit (R&D Systems Inc., Minneapolis, MN). IMPACT III testing was performed on 4T1 mouse mammary carcinoma cells by IDEXX BioResearch (Columbia, CO) prior to use in animals. Human mammary epithelial cells (HMECs) were purchased from Lonza Inc. (Mississauga, ON, Canada), and were maintained in Clonetics MEGM (Lonza). HMEC cultures were maintained at 37 °C in a 5% CO_2 humidified environment for a maximum of six passages. Venous blood was collected from healthy consenting volunteers according to protocols approved by the University of British Columbia Research Ethics Committee. Red blood cells were collected by centrifugation, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation over LymphoPrep (Stemcell Technologies, Vancouver, BC, Canada) as previously described [16].

2.2. Reagents

Mastoparan (INLKALAALAKKIL-NH_2) and unamidated Mastoparan (INLKALAALAKKIL-COOH) were purchased from Peptide 2.0 Inc. (Chantilly, VA). Unless otherwise indicated, all experiments were conducted using Mastoparan-NH_2, equivalent to natural wasp Mastoparan. Peptide stocks were prepared in sterile water (2 mM) or in saline (1 mg/ml) for in vitro and in vivo experiments, respectively. Paclitaxel, Triton X-100, calcein, dimethylsulfoxide (DMSO), gemcitabine, saline, etoposide, and vinblastine were all purchased from Sigma-Aldrich Canada (Oakville, ON, Canada). Propidium iodide (PI) was from Invitrogen. BOC-D-FMK (pancaspase inhibitor) was purchased from EMD Biosciences (San Diego, CA), and VECTASHIELD (with DAPI) was purchased from Cedarlane Labs (Burlington, ON, Canada).

2.3. MTT assay

MTT assays were used to assess peptide-mediated cytotoxicity. All adherent cells were seeded (2×10^5 cells/ml) 24 h prior to initiating the experiment to promote cellular adhesion. Leukemia and myeloma cells were seeded (5×10^5 cells/ml) immediately before treatment. All experiments were conducted in flat-well tissue culture plates (Corning, Corning, NY) at a final FBS concentration of 2.5%. Cells were cultured at 37 °C in a 5% (leukemia cells, myeloma cells, and PBMCs) or 10% (breast cancer cells and primary mammary epithelial cells) CO_2 humidified environment under the indicated conditions for the indicated periods of time. Two hours before the end of the assay, MTT was added to a final concentration of 0.5 μg/ml. Triton X-100 (1% [v/v]) was used as a positive control for 100% cell death, and was added concomitantly with MTT. At the end of the assay, the plates were centrifuged (1400 rpm for 5 min), supernatants were discarded, and the formazan crystals were solubilized in DMSO. Optical density (490 nm) was measured using a microtiter plate reader (Bio-Tek Instruments, Winooski, VT). Percent cytotoxicity was calculated using the following formula: $((E\text{-}N)/(P\text{-}N)*100)$, where E, N, and P denote experimental optical density (OD), average OD for the negative control (vehicle), and average OD for the positive control (triton), respectively. Paclitaxel-resistant MCF7-TX400 cells express 2.6-fold more P-glycoprotein than MCF7 cells. P-glycoprotein interferes with the reduction of MTT [8,17]. Thus, trypan blue (Sigma-Aldrich) exclusion counts were used to assess Mastoparan-induced killing of MCF7-TX400 cells.