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Original article

The defensin from avocado (*Persea americana* var. *drymifolia*) PaDef induces apoptosis in the human breast cancer cell line MCF-7

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ABSTRACT

Antimicrobial peptides (AMPs) are cytotoxic to cancer cells; however, mainly the effects of AMPs from animals have been evaluated. In this work, we assessed the cytotoxicity of PaDef defensin from avocado (*Persea americana* var. drymifolia) on the MCF-7 cancer cell line (a breast cancer cell line) and evaluated its mechanism of action. PaDef inhibited the viability of MCF-7 cells in a concentration-dependent manner, with an IC_{50} = 141.62 μg/ml. The viability of normal peripheral blood mononuclear cells was unaffected by this AMP. Additionally, PaDef induced apoptosis in MCF-7 cells in a time-dependent manner, but did not affect the membrane potential or calcium flow. In addition, PaDef IC_{50} induced the expression of *cytochrome c*, *Apaf-1*, and the *caspase 7* and *9* genes. Likewise, this defensin induced the loss of mitochondrial $\Delta\psi m$ and increased the phosphorylation of MAPK p38, which may lead to MCF-7 apoptosis by the intrinsic pathway. This is the first report of an avocado defensin inducing intrinsic apoptosis in cancer cells, which suggests that it could be a potential therapeutic molecule in the treatment of cancer.

1. Introduction

Cancer is a major public health concern worldwide. In 2012, cancer caused 8.2 million deaths, with breast cancer being one of the most prevalent cancers [1]. Cancer treatment requires conventional approaches, such as surgery, radiotherapy or chemotherapy; however, these approaches have a low therapeutic index and severe side effects [2]. These limitations have led to the search for new anticancer therapies. An attractive alternative is the use of antimicrobial peptides, or AMPs, which represent a novel family of anticancer agents that avoid the limitations of conventional treatments [3].

The AMPs are biologically active molecules, which are mainly cationic and amphipathic, and are produced by a wide variety of organisms as essential components of their innate immune response [4]. The primary role of AMPs is to defend the host against pathogenic microorganisms [4]; however, these peptides exhibit a wide range of properties, including modulation of the innate immune response and anticancer activity [5]. Currently, over 2500 AMPs are reported in The Antimicrobial Peptide Database (URL http://aps.unmc.edu/AP/main.php) [6], and 7% of them have shown cytotoxicity to cells from different types of cancer. The cytotoxic mechanisms of AMPs include necrosis (e.g., magainin 2) [7], apoptosis (e.g., lactoferricin and cecropin) [8,9], and alternative mechanisms, such as cell cycle arrest (e.g., beta-defensin-2) [10] and autophagy induction (e.g., FK-16) [11]. For the most part, these effects have been evaluated using AMPs from animals, and very little is known about AMPs from plants.

Plants produce small cysteine-rich AMPs as a mechanism of natural defense, and these AMPs are abundantly expressed in the majority of species [12]. Plant AMPs have a molecular weight between 2 and 10 kDa, are basic, and contain 4, 6, 8, or 12 cysteines that form disulfide bonds conferring structural and thermodynamic stability [13]. Numerous sequences of AMPs have been reported (≈320) [6], and twelve families have been described based on the identities of their amino acid sequences and the

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Table 1
Sequence of primers used in this study.

Gene	Sequence	Tm (°C)	Product size (bp)	Reference
Fas	5′ GTGAGGGAAGCGGTTTACGAGTGA 3′ 5′ TGGGCATTAACACTTTTGGACGAT 3′	66	256	This study
Fas L	5′ TGTGATCAATGAAACTGGGCTGTA 3′ 5′ ATCATCTTCCCCTCCATCATCACC 3′	57	233	This study
Caspase 8	5′ AGATCTGGCCTCCCTCAAGTTCCT 3′ 5′ AAATTTGAGCCCTGCCTGGTGTCT 3′	66	244	This study
Caspase 7	5′ AACCCAAACTCTTCTTCATTCAGG 3′ 5′ TAATAGCCTGGAACCGTGGAATAG 3′	57	145	This study
Cyt c	5′ TCAGCACCATGGCGGAAGACA 3′ 5′ TCCTTTAGCGGTCATTGCCTTCTG 3′	66	151	This study
Apaf-1	5′ AAATGGACACCTTCTTGGACGACA 3′ 5′ CAGAAAAGCAGGCATGGTAAACAG 3′	58	223	This study
Caspase 9	5′ AGGACATGCTGGCTTCGTTTCTG 3′ 5′ CCAAATCCTCCAGAACCAATGTCC 3′	66	257	This study
β-actin	5′ AAAACCTAACTTGCGCAGAAAACA 3′ 5′ TGTCACCTTCACCGTTCCACTTT 3′	57	317	This study

number and position of cysteines forming disulfide bonds. Recent reports have demonstrated the anticancer properties of three families: thionins, cyclotides and defensins [14]. Several studies have demonstrated that plant defensins inhibit the proliferation of cancer cells, including breast, colon and cervical cancer cells, without exerting side effects on normal cells [15–17]. However, the mechanism of action of plant defensins against cancer cells, as well as their selectivity, is poorly understood. In a previous study, we isolated the cDNA of PaDef defensin from avocado fruit (*Persea americana* var. drymifolia) and expressed it as a fusion protein in the endothelial cell line BVE-E6E7. We showed that the conditioned media from these cells have antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, important pathogens affecting animals and humans [18]. However, its cytotoxic effect has not been explored. In this study, we demonstrated that PaDef defensin from avocado induces the intrinsic apoptosis pathway in the breast cancer cell line MCF-7, which is a novel property for a plant defensin.

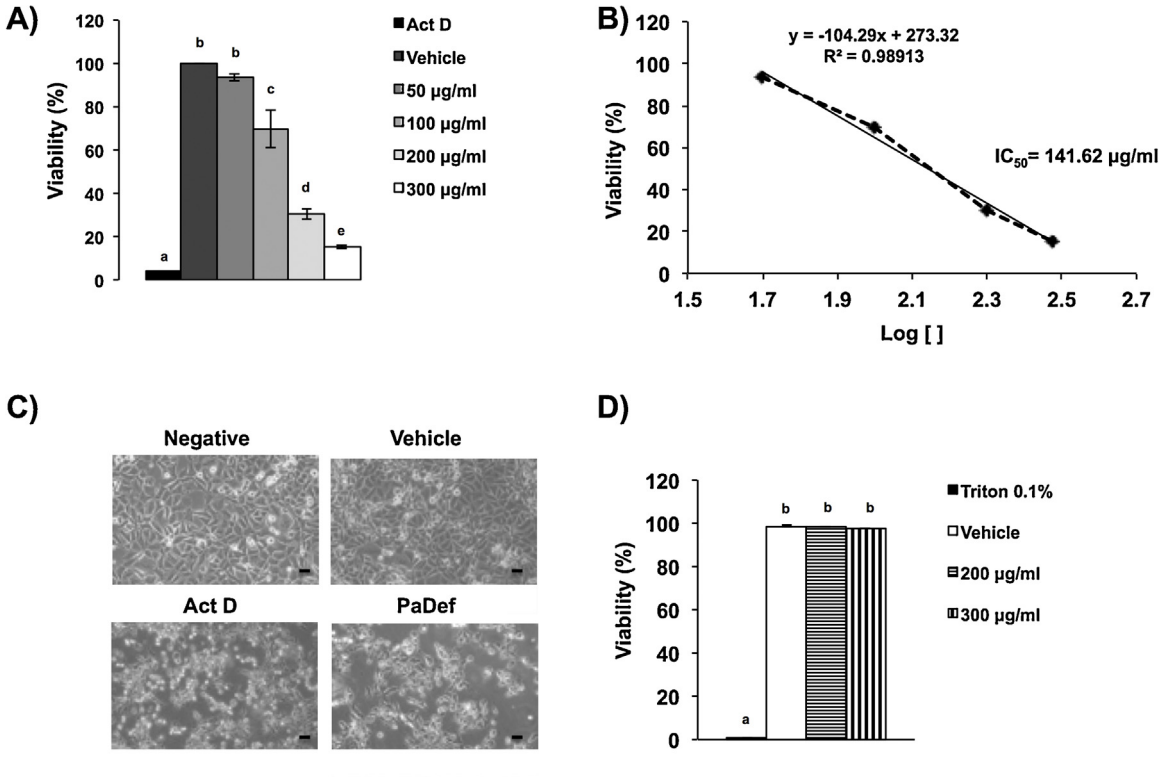

Fig. 1. PaDef defensin from avocado is cytotoxic to breast cancer MCF-7 cells. (A) Effect of PaDef on the viability of MCF-7 cells. Cells were treated with defensin (50, 100, 200 and 300 µg/ml), and viability was evaluated by trypan blue assays at 48 h. Cell viability is shown as the percentage of viable cells with respect to cells treated with vehicle (DMSO 0.4%). Data represent the mean of three independent experiments performed in triplicate. (B) Linear regression analysis of the concentration-response to calculate the half maximal inhibitory concentration (IC_{50}) of PaDef on MCF-7 cells; IC_{50} = 141.62 µg/ml; R^2 = 0.9891. (C) MCF-7 cell morphology after different treatments. Photographs are representative of at least two independent experiments and were taken with bright field microscopy. Scale bars: 10 µm. Act D (Actinomycin D), PaDef IC_{50} = 141.62 µg/ml. (D) Effect of PaDef on the viability of human peripheral blood mononuclear cells. Cells were treated with defensin (200 and 300 µg/ml), and viability was evaluated by trypan blue assays at 48 h. Cell viability is shown as the percentage of viable cells with respect to cells treated with vehicle (DMSO 0.4%). Data represent the mean of three independent experiments performed in triplicate. Different letters denote significant differences (same letter denotes no difference) in all values compared with each other (one-way ANOVA and Tukey's pairwise comparison, $P < 0.05$).