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A cancer vaccine based on the marine antimicrobial peptide pardaxin (GE33) for control of bladder-associated tumors

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ABSTRACT

The marine antimicrobial peptide (AMP) GE33, also known as pardaxin, possesses antimicrobial and anticancer properties, and modulates host signaling. GE33 has cytotoxic effects on murine bladder carcinoma (MBT-2) cells. Here, we investigated the potential of GE33 combined with inactivated MBT-2 as a cancer vaccine. The presence of up to 12.5 μg of GE33 did not inhibit the proliferation or endogenous nitrous oxide (NO) levels of RAW264.7 cells. However, the secretion of MCP-1, IL-6, and IL-12 by RAW264.7 cells was affected by GE33. We proceeded to test the effectiveness of the vaccine by immunizing mice at 7, 14, and 21 days of age, and injecting live MBT-2 cells on the 28th day. Tumor growth by the 58th day was attenuated in mice treated with the vaccine, as compared to the control group. Induction of MBT-2 specific-tumor antigens was increased in mice immunized with our vaccine. Furthermore, activation of T-cell receptors, cytotoxic T-cells, and NK cells was enhanced, and these showed high specificity for targeting tumor cells. Finally, immunization controlled excess recruitment of monocytes, lymphocytes, T-helper cells, and NK cells, and decreased the expression of VEGF. This report provides empirical evidence that our GE33-based vaccine enhances antitumor immunity in mice.

1. Introduction

Antimicrobial peptides (AMP) are short amino acid chain molecules involved in the first line of defense against invading pathogens [1]. In addition to providing a host defense mechanism, they are also involved in the modulation of the immune response [2]. One such AMP is pardaxin, which is commonly used as shark repellent. It was originally isolated from the Red Sea flatfish *Pardachirus marmoratus*, and was characterized as early as 1980 [3]. Pardaxin is a 33 amino acid peptide that starts with glycine (G) and ends with glutamic acid (E); hence, it is also known as GE33 [4]. GE33 is a pore forming peptide with an α-helix structure, which exhibits selective cytolytic activity against bacteria through membrane disruption [5]. In addition it has been suggested that it stimulates an arachidonic acid cascade and a dopamine-releasing agent in the host, through extracellular signal-regulated kinase (ERK) and other signaling mechanisms [6–8]. Moreover, GE33 exerts antitumor activity through inducing apoptosis (observed in HeLa cells, and reported to be caspase- and ROS-dependent in HT-1080 cells) [9,10]. More recently, the antitumor activity of GE33 against murine fibrosarcoma has been studied *in vitro* and *in vivo* [11].

During cancer progression, uncontrolled proliferative cells invade various tissues through angiogenesis, where they form tumors [12]. Cases of bladder cancer have been increasing at an alarming rate in recent years, and about two per cent of cancer-associated mortality is due to bladder cancer [13]. Present cancer treatments, such as radiotherapy, surgery, chemotherapy, and other approaches, have specific benefits and drawbacks [14]. The use of chemotherapy is widespread, but is associated with various problems, including the development of drug resistance and biotransformation, improper biodistribution, poor drug clearance, and the inability to target drug delivery to tumor cells [15]. Hence, there remains a need for alternative anticancer drugs, and research into the ability of AMPs to induce anticancer immunity may facilitate their development [16].

Host cells produce nitric oxide (NO) in response to cancerous growth, as high NO concentrations are cytotoxic to tumors [17].

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Cancer immunity involves detection of cancer cells by the host machinery at an early stage, and the subsequent release of tumor associated antigens (TAA) to kill tumor cells [18]. In addition, tumor cell progression activates monocytes, macrophages, and/or dendrite cells. However, uncontrolled activation is harmful to the host [19]. The secretion of chemokine MCP1 plays an essential role in attracting monocytes or macrophages [19]. In response to the activation of immune cells, the pro-inflammatory cytokine IL-6 stimulates B-cell activation to produce tumor specific antibodies [20], and IL-12 enhances NK and T-cell activation and interferon (IFN)-γ production [21]. IFN-γ shifts T-cell differentiation towards a Th1-type response [21], and IL-10 down-regulates IFN-γ production [22]. In turn, IFN-γ production affects T-cell differentiation. T cell activated macrophages (TAM) are derived from peripheral blood monocytes, and are recruited to the tumor site [23]. TAM activation releases various growth factors, proteolytic enzymes, cytokines, and inflammatory mediators [24]. In addition, it has been reported that neutrophils mediate tumor progression, through matrix degradation, immunosculpting, tumor cell proliferation, increased metastasis, and enhanced angiogenesis [25].

The pro-inflammatory cytokine IL-10 regulates tumor incidence, growth, and metastasis [26]. Cancer vaccines enhance the cytotoxic

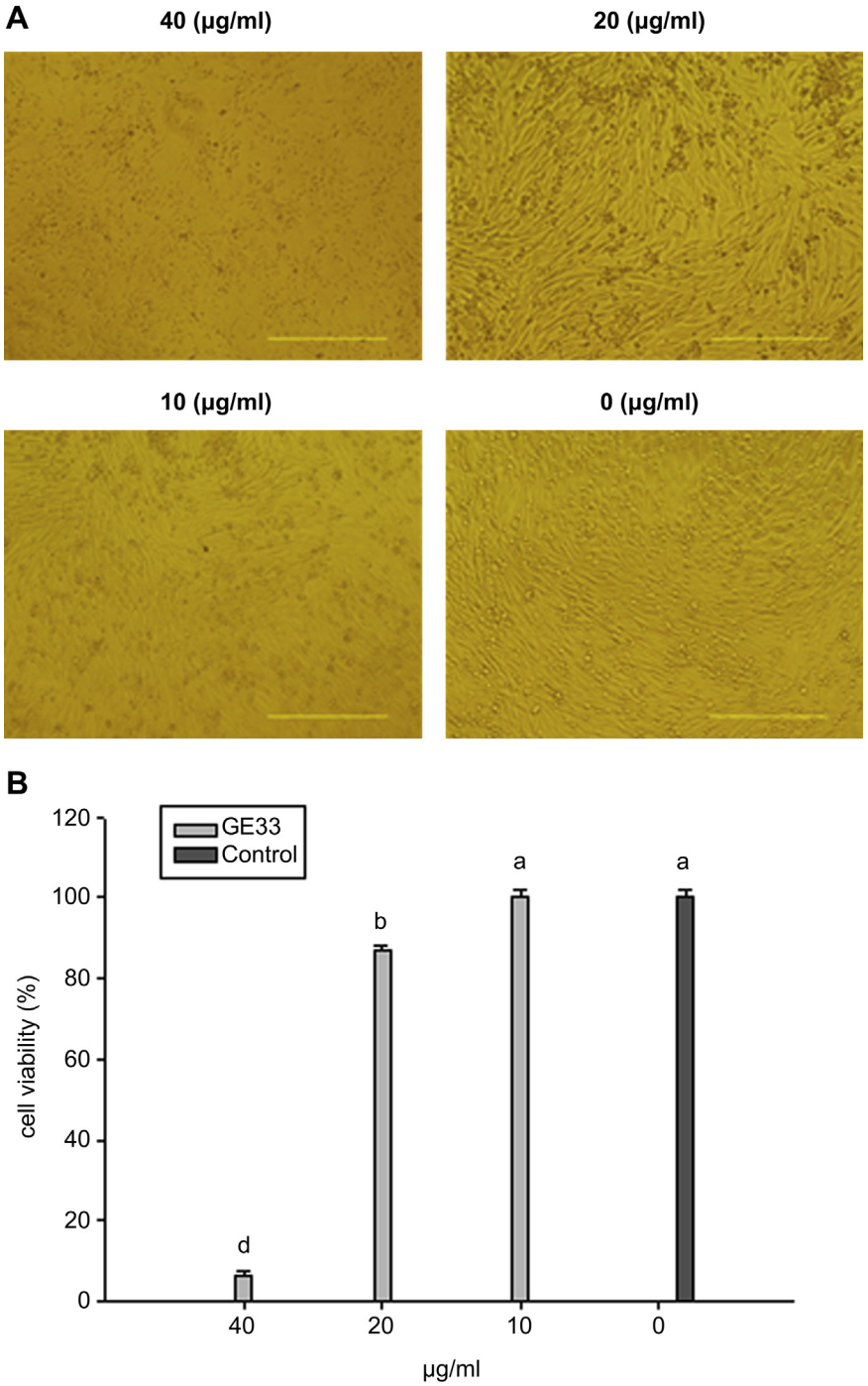

Fig. 1. GE33 is cytotoxic to mouse bladder tumor (MBT)-2 cells. MBT-2 cells were treated with 0, 10, 20, or 40 μg/ml of GE33 for 24 h. (A) Images showing cell morphology. (B) Cell viability measured by MTT assay ($r = 3$; $n = 3$).

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