Toxicon 148 (2018) 165–171

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Anti-neoplastic activity of Amblyomma sculptum, Amblyomma parvum and Rhipicephalus sanguineus tick saliva on breast tumor cell lines

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ARTICLE INFO

Article history:
Received 16 December 2017
Received in revised form
22 April 2018
Accepted 25 April 2018
Available online 27 April 2018

Keywords:
Cancer
Tick
Saliva
Anti-neoplastic activity

ABSTRACT

Cancer is one of the most troubling diseases and is becoming increasingly common. Breast cancer has a high cure rate when diagnosed early, but when diagnosed late, treatment is frequently painful, devastating and unsuccessful. The search for new treatments that are more effective and less harmful has led to several substances and biomolecules from plants and animals with potential anti-tumor activity. Within this context, ticks have emerged as an excellent source of new molecules with a wide array of therapeutic properties. Various molecules in tick saliva have immunomodulatory, anticoagulant, anti-inflammatory and anti-tumor effects across different tumor cell lines. Our study evaluates the effect of saliva from three widespread and important tick species in Brazil (Amblyomma sculptum, Amblyomma parvum and Rhipicephalus sanguineus) on MCF-7, MDA-MB-231 breast cancer cell lines and on the non-neoplastic MCF-10A cell line. We found that tick saliva from all three tick species showed cytotoxicity to tumor cells (MCF-7, MDA-MB-231) but not to the non-tumor cells (MCF-10A). Morphological changes on the surface of MCF-7 and MDA-MB-231 tumor cells did not occur on the MCF-10A cells. We also demonstrated that tumor cells die by apoptosis induced by caspase-3 and caspase 7 activity, suggesting that intrinsic pathway apoptosis may be triggered by tick saliva. These changes were not observed in MCF10A cells, which remained broadly unchanged even after exposure to diverse types of saliva. These results suggest that tick saliva from these tick species is a source of molecules, or biomolecules, useful for the potential source for the development of new breast cancer drugs.

1. Introduction

Breast cancer is the second most common type of cancer in women and one of the leading causes of death worldwide (INCA, 2011). Men may also develop breast cancer, but the prevalence is low, making up less than 1% of breast cancer cases. If diagnosed during initial stages, breast cancer has a good chance of cure, with a 5-year survival rate in 97% of cases (INCA, 2015). Even when the diagnosis is delayed, new therapies have increased patient survival rates, enabling a decent quality of life (INCA, 2015). On the other hand, late-stage breast cancer diagnosis worsens prognoses and available treatments become more painful, long-lasting and have lower cure rates. Thus, given the importance of breast cancer in public health, new therapeutic alternatives are needed, especially for patients with late diagnosis.

Ticks are hematophagous ectoparasites that remain fixed to their hosts as they feed for hours or days, necessitating a diverse and effective pharmacological arsenal of bioactive molecules to block host defenses (Steen et al., 2006). Of these, inhibitors of platelet aggregation and blood coagulation, anti-inflammatory and immunosuppressive compounds have been extensively studied (Francischetti et al., 2009). In addition to these immunosuppressive molecules that allow feeding and fixation to hosts, recent studies have shown that salivary molecules have excellent antitumor potential. For example, inhibitory proteins in tick saliva, such as

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https://doi.org/10.1016/j.toxicon.2018.04.024
0041-0101/

Ixolaris - a protein derived from the salivary glands of the tick Ixodes scapularis, displayed antiangiogenic efficiency against the U87-MG cell line, a primary model of glioblastoma (Carneiro-Lobo et al., 2009). A protein derived from the salivary glands of the Amblyomma cajennense tick (Amblyomin-X) reduces tumor mass and produces some metastatic effects in murine models of melanoma (Chudzinski-Tavassi et al., 2010) and induces dose-dependent apoptosis in murine renal cancer cells (Akagi et al., 2012). The effects of Dermacentor variabilis tick saliva on migration, invasion and signaling activities of osteosarcoma cells, Saos-2, and breast tumor cells, MDA-MB-231, have also been evaluated. The constituents of this saliva modulated the invasive and migratory activity of host cells and tumor cells (Poole et al., 2013).

Given the severity and prevalence of cancer, obtaining molecules from plant extracts and animal secretions that combat various forms of this disease is an important objective of health research (Shafi et al., 2009). New research that suggests the use of biomolecules from natural elements as antitumor substances appears as probable solutions to this dilemma. Some discoveries such as the inhibition of metastasis and angiogenesis from natural products, in addition to the suppression of miRNA expression in cancer cells are important findings. A good example presented by Park et al. (2017) is the acceleration of the factors that influence the pathways of apoptosis by the main substances present in *Angelica sinensis*, which has antitumor characteristics influencing the amount of reactive oxygen species (ROS). *Serratia marcescens* and patupilone (EPO 906) have been screened through clinical trials in inhibiting the progression of gliobastoma multiforme. (Park et al., 2017).

In view of the possibilities exposed, our work used the tick saliva of three different species of Brazilian fauna in different breast cancer cell lines to verify its antineoplastic effect.

2. Material and methods

2.1. Tumor cell lines

The following human breast cell lines were used: MCF-10A a non-invasive and non-neoplastic mammary gland cell line; MCF-7, a non-invasive breast adenocarcinoma cell line; and MDA-MB-231, an invasive ductal breast carcinoma cell line. The MCF-7 cells were grown in RPMI-1640 medium (Sigma, St. Louis, MI) enriched with inactivated fetal bovine serum at a concentration of 10% and maintained in a CO^2 oven at 37 °C with 5% CO^2. The MDA-MB-231 cells were cultured in the same manner as above; however, they were kept in an incubator at 37 °C without CO^2, according to the recommendations of the ATCC (American Type Culture Collection). The MCF-10A cells were grown in DMEM medium (Sigma, St. Louis, MI) enriched with fetal bovine serum at a final concentration of 5%, human insulin at a final concentration of 10 ?g/mL, epidermal growth factor at a final concentration of 10 ng/mL and maintained in a CO^2 oven at 37 °C with 5% CO^2, according to the recommendations of the ATCC. MCF-10A cells were grown in DMEM/F12 medium (Sigma, St. Louis, MI) with sodium bicarbonate and penicillin/streptomycin, supplemented with EGF (20 ng/ml), bovine insulin (10 μg/ml), hydrocortisone (0.5 μg/ml) and 5% bovine fetal serum (BFS) at 37 °C with 5% CO2, in a humid chamber.

2.2. Ticks

Laboratory tick colonies of Amblyomma sculptum, Amblyomma parvum and Rhipicephalus sanguineus were kept in the Laboratory of Ixodology of the Faculty of Veterinary Medicine, Federal University of Uberlandia (LABIX-UFU). The ticks were fed inside feeding chambers glued to the shaved back of New Zealand white rabbits (Szabó et al., 1995). Unfed ticks of each species were released inside the feeding chamber for both colony maintenance and for saliva harvest. Unfed ticks and those that had already fed were kept in a humid incubator at 27 °C and 80% humidity.

2.3. Tick saliva collection

For saliva collection, unfed adult ticks, 25 females and 10 males per chamber, were released as described above. Rabbits were always tick-bite naïve and different hosts were used for each tick species. Partially engorged and engorged females were collected, usually 7–10 days after entering the feeding chamber, and cleaned with 10% PBS at room temperature (25 °C). The ticks were then fixed to double-sided tape on a flat, rigid surface and cleaned with 70% alcohol. To stimulate salivation, 10 to 20 ?l of a 0.2% dopamine solution was inoculated into the hemocele of each tick, using a 1.0 syringe and a 12.5 × 0.33 mm needle. Secreted saliva was collected with an automatic pipette, stored in 2.0 mL eppendorfs and kept in ice during the collection period. Afterwards the saliva was filtered (22 ?m pores) and stored at −80 °C until use. Protein concentration was quantified by the Bradford method. Several collections were carried out during the two years of the study.

2.4. Saliva induced cytotoxicity

MTT (Microculture Tetrazolium) is a colorimetric assay for assessing cell viability. In our study, 96-well plates with 2 × 10? cells/well were used. 150 ?L of cells from each of the three cell lines were placed in different plates enriched with RPMI medium and 50 ?L of pure saliva per well (35 ?g/mL), and arranged in triplicates. The plates were then kept in a 5% CO^2 oven at 37 °C for 24 h. Afterwards, the medium was removed from the wells and 190 ?L of RPMI and 10 ?L of MTT (5 mg/mL) were added to each well. The plates were then wrapped in laminated paper to avoid light exposure and conditioned for an additional 4 h in the oven under the same conditions as described previously. Next, the medium was removed from the oven and 100 ?L of detergent solution (Sodium Dodecyl Sulfate - SDS/50% DDF) was added to dissolve the crystals formed by the MTT. Finally, the plates were read in a spectrophotometer at an absorbance of 540 nm.

2.5. Saliva induced neoplastic cell morphological changes

Morphological changes and cellular damage were qualitatively investigated using an inverted digital and contrast phase microscope (EVOSfl-AMG, Seattle, USA). For this purpose, the MCF-7, MDA-MB-231 and MCF-10A cell lines were cultured in a 48-well plate with 1 × 10? cells/well. The cells were treated with pure saliva at a dose (100 ?L or 35 ?g/mL) standardized in previous experiments on morphological cellular alterations, and then incubated in an oven at 37 °C for 24 h.

2.6. Saliva induced apoptosis and necrosis

A FITC Annexin V/7AAD Apoptosis Detection Kit (BD PharmigenTM, San Jose, California, USA) was used to evaluate apoptosis and necrosis in neoplastic cells after exposure to the saliva of the different tick species. After incubating 1 × 10? cells/well in 48-well plates with 100 ?l of saliva/well (35 ?g/ml) for 24 h, the tumor cells were detached from the plate with 50 ?L trypsin/well and incubated for 20 min in a CO^2 oven at 37 °C. Afterwards, the medium was removed, and the cells were washed twice with cold PBS and then resuspended in a binding buffer at a concentration of 1 × 10? cells/ml. Then, 100 ?L of the solution was transferred to a 5-mL culture tube, to which 5 ?l FITC Annexin V and 5?l 7AAD were added. Next, the cells were gently agitated and incubated for 15 min