Toxicon 102 (2015) 69–73

Contents lists available at ScienceDirect

Toxicon

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

Review

Exploring the anti-tumoral effects of tick saliva and derived components

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ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form
29 April 2015
Accepted 11 June 2015
Available online 13 June 2015

Keywords:
Tick saliva
Angiogenesis
Cytotoxicity
Antitumor activity

ABSTRACT

Ticks are blood-feeding arthropods with an outstanding ability to remain attached to its host for considerable periods while blood-feeding and remaining unnoticed. Their success results from the ability to modulate hemostatic and host immune responses. The ability to “bypass” a host's defenses, prevent blood clotting and wound healing makes ticks utterly interesting animals for the development of new drugs. Studies worldwide on various tick species have shown that tick saliva possesses a wide array of lipidic and proteic biomolecules with useful properties. These include not only immunomodulatory, anti-inflammatory, anti-platelet and anti-clotting properties, but also cytotoxic and cytolitic properties that act against various cell types, and anti-angiogenic properties, which have gained increasing prominence. We searched PubMed, Science Direct, Elsevier and other sites for publications regarding tick saliva and its effects on cancer cells and angiogenesis. Our aim was to compile a list of molecules with potential for host adaptation and for the development of new cancer treatment drugs.

1. Introduction

Cancer is a major cause of morbidity and mortality worldwide. The incidence of cancer is increasing due to aging populations, genetic loads, life habits, environmental factors and industrialization. Despite the importance of cancer to public health, current treatment options are insufficient. Therefore, therapeutic alternatives are needed to add to or replace existing treatments with limited effectiveness and/or with a variety of side effects.

Ticks are obligatory hematophagous ectoparasites that depend on a host's blood to survive (Ribeiro, 1995). This process relies on biomolecules with anti-hemostatic and/or immunomodulator properties that are inoculated into the host with saliva (Kazimirova and Stibraniova, 2013; Steen et al., 2006; Ribeiro et al., 2006; Decrem et al., 2008; Francischetti et al., 2009). In fact, the constituents of tick saliva interfere with various components of the immune system and at various levels including modulating the activity of enzymes, antibodies, vasoactive amines, adhesion molecules, proteins of the complement system, cell signaling components, chemokines and cytokines (Francischetti et al., 2009; Chmelar et al., 2011). Components of tick saliva also modulate various cell types including macrophages, dendritic cells, neutrophils, mast cells, natural killer (NK) cells, and B and T lymphocytes (Steen et al., 2006; Francischetti et al., 2009; Ribeiro et al., 1985). Additionally, tick saliva contains bioactive molecules with anti-angiogenic properties and cytotoxic and cytolitic properties that act against various cell types (Ferreira et al., 2002) (Table 1).

There are over 800 tick species throughout the world living in highly diverse environments and feeding on various hosts. Hence, the quantity and diversity of bioactive molecules in tick saliva may vary considerably (Gern, 2005) and can be considered an important source of molecules with different therapeutic properties including some with anti-tumoral effects (Simons et al., 2011). Additionally, these molecules have a low risk of microbial resistance, toxicity and immunogenicity in humans and domestic animals (Simons et al., 2011), which are essential characteristics of safe products.

We review the main experimental evidence showing the relevance of tick saliva components as potential alternative cancer treatments.

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http://dx.doi.org/10.1016/j.toxicon.2015.06.001
0041-0101/

Table 1
Primary substances obtained from tick saliva and their biological functions.

Tick	Biomolecules	Biologic effect	References
Rhipicephalus appendiculatus and *Amblyomma variegatum*	Salivary Gland Extract (SGE)	Apoptosis induction.	Kazimirova et al., 2006
Amblyomma cajennense	Amblyomin-X -recombinant protein	Inhibitors of factor Xa; antitumor effect.	Chudzinski-Tavassi et al., 2010
Amblyomma variegatum	Calreticulin	Inhibitor of angiogenesis.	Valenzuela et al., 2002
Amblyomma cajennense	Saliva	Cytotoxic effects.	Simons et al., 2011
Dermacentor reticulates *Ixodes ricinus* *Rhipicephalus appendiculatus* *Amblyomma variegatum*	Salivary Gland Extract (SGE)	Binding of growth factors, inhibition of cell proliferation.	Hajnická et al., 2011
Dermacentor variabilis	Saliva	Inhibition of migratory and invasive activities.	Poole et al., 2013a,b
Ixodes scapularis *Ixodes ricinus*	Metalloprotease	Inhibitor of angiogenesis.	Francischetti et al., 2005; Decrem et al., 2008
Ixodes scapularis	Troponin I-like	Inhibitor of angiogenesis.	Fukumoto et al., 2006
Ixodes scapularis	Ixolaris	Inhibition of angiogenesis; antitumor effect.	Francischetti et al., 2002; Carneiro-Lobo et al., 2009
Haemaphysalis longicornis	Haemangin	Inhibitor of angiogenesis.	Islam et al., 2009
Haemaphysalis longicornis	HLTnI; troponin I-like molecule	Inhibitor of angiogenesis.	Fukumoto et al., 2006

2. Review

2.1. General properties of tick saliva and their potential anti-tumoral effects

Most tick species remain attached to their hosts for several days. To continue blood-feeding, ticks need to deal with reactive mechanisms from the host, such as hemostatic, inflammatory and healing processes. Because tick infestations are recurring processes, host defenses also include adaptive immune responses. Nonetheless, ticks are evolutionary survivors that cope with all such processes by repeatedly injecting saliva into the feeding site during extended feeding periods. Tick saliva is produced by a pair of highly developed salivary glands and is so effective at controlling the host's reactive mechanisms that ticks are considered smart pharmacologists (Ribeiro, 1995). Some properties of tick saliva have more obvious potential anti-tumoral properties. Some components of tick saliva have already been identified and characterized. For example, the recombinant protein TAP (RTAP) of the *Ornithodoros moubata* tick has been tested in a variety of animal models for arterial and venous thrombosis. RTAP has shown greater efficacy without prolonged bleeding relative to traditional anticoagulants (Stoll et al., 2007; Fioravanti et al., 1993). The amblyomin-X protein from the saliva of *Amblyomma cajennense* ticks is an important candidate for anticancer therapy because of its cytotoxic effect on various tumors including pancreatic tumors and melanomas (which will be discussed later) (Simons et al., 2011).

Cell proliferation, migration and invasion, a hallmark of many reactive processes but also of neoplastic cells, is inhibited by the saliva of several tick species (Carvalho-Costa et al., 2015). Tick saliva affects angiogenesis, which is another essential feature of neoplastic growth. Additionally, recent, striking data have shown that tick saliva and its isolated components have significant and selective cytotoxic activity on tumor cells. These processes are detailed bellow.

2.2. Effects of tick saliva and derived components on different types of cancer cells

Kazimirova et al. (2006) demonstrated that the salivary gland extract (SGE) from ticks suppressed proliferation of HeLa cells in a dose dependent manner and induced apoptosis. These authors also demonstrated that the anti-proliferative effects of SGE are species-specific given that extracts from female *Rhipicephalus appendiculatus* and *Amblyomma variegatum* were more potent than the same extracts from other tick species. This suggests that the amount of active compounds in the salivary glands of ticks changes during feeding and explains how the tick can circumvent the host's immune response and remain fixed at the same feeding site for days. The study showed that tumor cell growth suppressor(s) and/or apoptotic triggers are present in tick salivary glands; however, further research into the characteristics and modes of action of these natural substances is necessary.

These results are remarkable because they imply that SGE and tick saliva have components that can suppress tumor growth and trigger apoptosis and necrosis pathways in neoplasic cells. However, more research on the properties and function of these natural substances is necessary.

Inhibiting angiogenesis is another strategy that reduces the growth of several types of tumors. Carneiro-Lobo et al. (2009) reported the anti-angiogenic efficiency of Ixolaris protein isolated from the salivary glands of the *Ixodes scapularis* tick. They subcutaneously inoculated U87-MG cells (models of glioblastoma – a primary brain tumor) into the flanks of 6 week-old, male Balb/C nude mice. The treatment was performed daily for 17 days. They observed that the vessel numbers in the tumors of mice treated with Ixolaris were lower relative to a control. Ixolaris also acted as an anti-coagulant by inhibiting the complex. The formation of tumor vessels *in vivo* was effectively suppressed. Therefore, this protein may attenuate the procoagulant status of cancer patients and prevent angiogenesis, thereby interfering with two important components that contribute to tumor growth and metastasis. The study concluded that Ixolaris may block primary tumor growth and angiogenesis in models of melanoma and glioblastoma.

Other study demonstrated that the protein Ixolaris can act on melanoma cells, a type of highly metastatic cancer, decreasing the growth of primary tumor and its metastatic potential, even as tumor angiogenesis (de Oliveira Ada et al., 2012). According to this research, ixolaris can successfully block the experimental metastasis of B1610 cells (murine model of melanoma) *in vivo* at low concentrations and can inhibits TF (tissue factor) activity in vitro. Enzymatic assays made with B16F10 and human U87-MG tumor cells as the TF source demonstrated that ixolaris inhibits the generation of FX in either murine, human or hybrid FVIIa/TF complexes. Immunohistochemical analyses showed that inhibition of melanoma growth by ixolaris is following by a downregulation of