Description of the Annual Reproductive Cycle of Wreckfish *Polyprion americanus* in Captivity

Volume 3 • Issue 4 | December 2018
Review

Potential Human Health Applications from Marine Biomedical Research with Elasmobranch Fishes

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Received: 31 October 2018; Accepted: 20 November 2018; Published: 6 December 2018

Abstract: Members of the subclass of fishes collectively known as elasmobranchs (Class Chondrichthyes, Subclass Elasmobranchii) include sharks, skates, rays, guitarfish, and sawfish. Having diverged from the main line of vertebrate evolution some 400 million years ago, these fishes have continued to be successful in our ever-changing oceans. Much of their success must be attributed to their uncanny ability to remain healthy. Based on decades of basic research, some of their secrets may be very close to benefitting man. In this short review, some of the molecular and cellular biological areas that show promise for potential human applications are presented. With a brief background and current status of relevant research, these topics include development of new antibiotics and novel treatments for cancer, macular degeneration, viral pathogens, and Parkinson’s disease; potentially useful genomic information from shark transcriptomes; shark antibody-derived drug delivery systems; and immune cell-derived compounds as potential cancer therapeutic agents.

Keywords: elasmobranch; shark; marine biomedicine; antibiotics; squalamine; transcriptomes; antibody; nanobodies; immune modulators; tumor cell apoptosis

1. Introduction

For thousands of years, cultures around the world have envisioned the sea as a potential treasure for remedies to human ailments. The mystique surrounding medicinal secrets from the oceans has continued into modern times, with the quest to discover “drugs from the sea” (for a recent review, see Malve, 2016 [1]). While a handful of drugs have been developed from marine invertebrates, marine vertebrates have remained underutilized as a potential source for new therapeutic agents. Sharks and their skate and ray relatives, collectively termed elasmobranchs (Class Chondrichthyes, Subclass Elasmobranchii), have successfully evolved from descendants that existed during the Devonian period some 400 million years ago. While much of their success can be attributed to their numerous sensory systems (some of which are the most sensitive in the animal kingdom) [2] and their reproductive strategies (more similar to birds and mammals than to the bony fishes) [3], their uncanny ability to remain relatively disease-free remains poorly understood.

It is rare to find a “sick” shark in the wild, with the principal causes of death attributed to anthropogenic interaction (namely over-fishing) [4,5], predation [6], and natural senescence (old age) [7]. It is the intent of this short review to present the applicability of elasmobranch research to human health issues by updating some areas of research that have shown promise in the past, as well as to introduce some novel approaches to potential therapies based on recent discoveries.

2. Novel Antibiotics against Human Pathogens

That human pathogens are adapting to existing antibiotic drugs and becoming increasingly resistant to treatment is no secret [8]. Unless new and improved antibiotics are discovered, effective treatment of bacterial, fungal, parasitic, and viral infections, as well as chronic diseases including...
cancer, will continue to be a challenging task. While interest from the United States Department of Defense to develop new antibiotic compounds to combat wound infection pathogens was the impetus for studying antimicrobial properties of stingray epidermal mucus [9], treatment of nosocomial, or hospital-acquired (HA), and community-acquired (CA) pathogens would also provide benefits. With the recurring observation of infection-free healing of wounds among elasmobranchs [10–12], these fishes might be a surprisingly rich source of novel antibiotics.

In 2017, the isolation of 1860 bacterial symbionts from the epidermal mucus of three stingray and one skate species was described [9]. When screened for their abilities to produce antibacterial compounds with inhibitory activity against a range of pathogenic test strains, 311 (16.7%) of the isolates demonstrated activity against one or more of the pathogens, 57 of which produced either broad-spectrum antibiotics or activities against methicillin-resistant Staphylococcus aureus (MRSA) or vancomycin-resistant Enterococcus (VRE) only.

The decision to explore stingray mucus for antibiotic compounds instead of their shark relatives was driven by the relative ease of mucus collection from rays compared with sharks. Sharks also produce epidermal mucus, but because of the characteristic presence of superficial dermal denticles on shark skin, their mucus is not as accessible. As is often the case, there are exceptions, as two recent studies have demonstrated that mucus-associated bacteria from six species of shark possess antibiotic activity. In one study, antibiotic activity was detected in 41% of bacterial associates from blacktip sharks, Carcharhinus limbatis; 29% from tiger sharks, Galeocerdo cuvier; 13% from bull sharks, Carcharhinus leucas; 10% from lemon sharks, Negaprion brevirostris; and 7% from blacknose sharks, Carcharhinus acronotus [13]. In the second study, as much as 20% of the culturable bacterial isolates from the mucus of white sharks, Carcharodon carcharias, was shown to produce antibacterial activity [14]. Such a growing database of antibiotic-producing marine bacteria has implications for host-microbe associations among the elasmobranch fishes and may reveal promising candidates for future drug discovery initiatives.

3. Squalamine: Revisiting a Novel Compound with the Potential to Treat a Variety of Human Diseases

In 1993, the isolation and purification of an aminosterol from shark stomach tissue with broad-spectrum antifungal, antibacterial, and antiprotzoal activity was described [15]. This compound (a 7,24-dihydroxylated 24-sulfated cholestane steroid conjugated to spermidine at C-3) was named squalamine, because it was isolated from the spiny dogfish, Squalus acanthias. Also present in other shark tissues including liver and gall bladder, squalamine can now be synthesized [16]. This is a significant development in that the natural population of sharks will not be relied upon as a constant source of this product. Squalamine has also been found to be antiangiogenic, a property useful in inhibiting growth of solid tumors, because solid tumors depend upon the recruitment of blood vessels to thrive. Squalamine has been shown to have antitumor activity in rodent models of human brain, breast, lung, and ovarian cancers [17–22]. Several phase I and phase II human trials using squalamine have been conducted [23–25], although to date, none of these studies have resulted in successful phase III trials. Squalamine does not appear to be related in chemical structure or mechanism of action to any chemotherapeutic substance currently in use.

More recently, clinical tests have been initiated with squalamine in the form of squalamine lactate [26] to investigate its antiangiogenic properties to treat the eye disease known as age-related macular degeneration (AMD) [27,28], a leading cause of blindness in older people. While ongoing AMD studies have been inconclusive, future applications of squalamine may take advantage of another of its properties, namely antiviral activity. In fact, squalamine has been tested against a broad spectrum of human viral pathogens, including single positive-stranded RNA viruses associated with dengue, yellow fever, and equine encephalitis, and the double stranded DNA Hepatitis B virus [29]. Squalamine, with its net positive charge by virtue of its spermidine moiety, displays a high affinity for anionic phospholipids. When it enters a cell, squalamine binds to the intracellular membrane.
phospholipids, neutralizing the negative charge and displacing any positively charged proteins bound
to the membrane [30]; this impacts the cell’s ability to support virus replication. Although it has been
hypothesized that squalamine might also serve an antiviral function within the shark [29], the role of
squalamine in sharks, skates, and rays remains unclear.

Squalamine also shows tremendous promise with Parkinson’s disease, a disease characterized by
the presence in brain tissue aggregates primarily formed by the protein α-synuclein [31]. Squalamine
suppresses the formation of α-synuclein aggregates and their associated toxicity in neuronal cells by
competing with α-synuclein for binding to lipid membranes [32].

4. Genomic Information from Shark Transcriptomes

The transcriptome is defined by Wang et al. as “the complete set of genomic transcripts in a
cell, and their quantity, for a specific developmental stage or physiological condition” [33]. Cellular
genetic information is transcribed into RNA, with the resulting readouts of all the genes of a given
cell are referred to as its transcriptome. While the human genome has been studied for many years,
the first high-quality genome sequence generated from a cartilaginous elasmobranch relative was from
the elephant shark, *Callorhinchus milii* (Class Chondrichthyes, Subclass Holocephali) [34], followed
closely thereafter by a complete mitochondrial genome of the white shark, *Carcharodon carcharias* [35].
Subsequently, additional elasmobranch genome projects have been initiated [36], with transcriptomes
now available for the whale shark, *Rhincodon typus* [37]; white shark, *C. carcharias* [38]; catshark,
*Scyliorhinus canicula* [39]; spiny dogfish, *S. acanthias* [40]; and little skate, *Leucoraja erinacea* [41]. Recently,
Hara and co-workers [42] provided complete genome analyses of brownbanded bamboo shark,
*Chiloscyllium punctatum*, and cloudy catshark, *Scyliorhinus torazame*, plus an improved assembly of the
whale shark genome that revealed important discoveries with regard to Hox genes, antibody genes,
and opsin and olfactory receptor genes.

Genomics and transcriptomics studies provide a basis for molecular exploration of phenotypes
unique to elasmobranchs, as well as insight into evolutionary origins of vertebrates. Genomic analysis
of their established traits of morphology, reproduction, sensory capabilities, and longevity, combined
with their slow rate of DNA evolution [43], has the potential to elucidate specific molecular mechanisms
underlying these unique features. Elasmobranch transcriptomes may be more useful for human
biomedical applications than initially thought. A recent comparison of gene transcripts between white
shark, *C. carcharias*, and zebrafish, *Danio rerio*, revealed the surprising result that white shark gene
products associated with metabolism, molecular functions, and the cellular locations of these functions
were more similar to human than to zebrafish [38]. In fact, these same shark transcriptome gene
expression studies have identified positive selection for genes, such as legumain, that play important
roles in immune system responses to certain cancers, including colorectal cancer [44]. An interesting
feature of the shark genome is the high proportion of dinucleotide microsatellite repeats, with a lower
abundance than other vertebrates of repeating trinucleotide DNA sequences [38]. This observation is
worthy of note as abnormally higher numbers of trinucleotide repeats in humans have been linked
to a variety of neurological disorders, including spinobulbar muscular atrophy, myotonic dystrophy,
and Huntington’s disease, as well as certain types of cancer (i.e., hereditary nonpolyposis colon
carcinoma and sporadic bladder carcinoma) [44–49]. While it is difficult to assess neurological disease
in elasmobranchs, the relatively lower proportion of trinucleotide microsatellite repeats in the white
shark genome may provide a genetic mechanism for the relatively low incidence of malignant neoplasia
among elasmobranchs [38]. As transcriptomes from more species become available, the transcriptome
assemblies and the derived gene transcripts will be invaluable as new molecular tools in support of
ongoing research with elasmobranch models [50].

5. Shark Antibody-Derived Drug Delivery Systems

Our basic understanding of the elasmobranch immune system has received considerable attention
since the early work by comparative immunology pioneers of the 1960s [51–53]. Based on these
and other classic studies, advancements in characterizing the structural and functional organization of cellular and molecular components of the elasmobranch immune system have established that, in addition to utilizing basic nonspecific mechanisms of innate immunity, the elasmobranch fishes are the earliest jawed vertebrates to possess all the components necessary to perform the specific responses associated with adaptive immunity [54–56]. Much of our current understanding has been chronicled during the past decade through several timely reviews [57–60], and nicely organized into a recently published comprehensive reference volume addressing numerous aspects of elasmobranch immunobiology [61].

Even though translation of this knowledge to clinical therapies remains a challenge, some potential applications are worthy of note. One particularly exciting approach is based on the unique structural properties of some of the elasmobranch immunoglobulins. While it was initially believed that pentameric and monomeric IgM were the only immunoglobulins circulating in elasmobranch blood, several monomeric immunoglobulins unrelated to IgM, namely, IgX, IgR, and IgW, are now known to exist [62–66]. Another monomeric form, called IgNAR, is unlike other immunoglobulins in that it is a homodimer of heavy chains without the characteristic dimerization with corresponding light chains [67,68]. In the absence of covalent linkage to light chains, the variable regions are relatively unrestricted and potentially more flexible [69]. Recently, IgNAR fragments containing only the single domain variable regions (VNARs) have been shown to bind tightly to a variety of antigens, creating the possibility of adapting these molecules to future diagnostic work or drug delivery systems [70–75].

Although existing traditional monoclonal antibody approaches hold therapeutic promise, they are complicated by the large size of the molecules. IgNAR fragments are considerably smaller (~12 kDa vs. ~150 kDa) than traditional monoclonal antibodies, and thus are referred to as ‘nanobodies’; nanobodies have also been identified in camalid species [76]. Their significantly smaller size carries the advantage of diminishing steric hindrance that might prevent larger antibodies from accessing and recognizing certain epitopes [77] and, consequently, reducing their potential utility in disease therapy. In addition to the ability to circumvent complications related to accessibility, elasmobranch nanobodies have favorable attributes such as high affinity and antigen specificity, extraordinary thermal stability and resistance to denaturation, and the potential to complement classical antibodies [78]. Combined with the propensity to bind epitopes that are considered inaccessible to conventional monoclonal antibodies and their ability to resist denaturation, VNARs represent an emerging prospect for use in therapeutic, diagnostic, and biotechnological applications [78].

Much remains to be understood about the role of IgNAR in the immune system of cartilaginous fishes. It is obvious, however, that the unique singular molecular framework of VNARs holds many advantages in developing reagents for scientific research, disease diagnosis, and potentially therapeutic interventions [78]. The large evolutionary distance between sharks and mammals facilitates successful IgNAR responses against antigen targets that are refractory to conventional methods of generating polyclonal and monoclonal antibodies. VNARs may also be useful in developing therapeutic agents for treating acute and inflammatory diseases. Although testing in relevant disease models has not yet occurred, elasmobranch nanobodies have significant potential to improve current understanding and eventual treatment of human diseases. Additional research to establish the full repertoire of VNAR domains, as well as potential immunogenicity in different mammalian species, is necessary in order for the successful application of VNARs in clinical therapeutics.

6. Elasmobranch Immune Cell-Derived Compounds

While designing drug delivery systems based on unique structures of shark immunoglobulins holds tremendous promise, another potential source of novel immune modulators for development into therapeutic agents is short-term cultures of elasmobranch immune cells. Historically, in vitro culture of any type of elasmobranch cell has been challenging [79], primarily because of the cellular environment consisting of high osmolarity (typically 940–1000 mOsm) and retention of high amounts of urea and trimethylamine oxide (approximately 400 mM and 70–100 mM, respectively) [80].
Although two continuously proliferating cell lines have been derived from spiny dogfish shark, S. acanthias, and little skate, L. erinacea, embryonic somatic tissue [81–83], the only success with cells of immune tissue origin has been in studies demonstrating phagocytic activity and induction of apoptosis with short-term cultures of lymphomyeloid tissue cells [84,85]. A lymphomyeloid tissue unique to sharks, skates, and rays, called the epigonal organ, also has the potential to lead to therapeutic agents with human benefit. Recent studies have demonstrated that in 72–96 h culture of bonnethead shark, Sphyra na tiburo, epigonal cells secrete compounds into the surrounding culture medium, termed ‘epigonal conditioned medium’, that inhibit the growth of several tumor cell lines [86]. Specifically, compounds in this epigonal cell conditioned medium have been shown to induce apoptosis in a T-cell leukemia cell line (Jurkat) through binding to the TRAIL receptor and activating the mitochondrial caspase-mediated pathway [87]. Moreover, the compounds in this conditioned medium preferentially target transformed cells as opposed to normal cells [87]. Efforts are underway to identify bioactive components for further evaluation as potential therapeutic agents.

7. Summary/Conclusions

Potential biomedical applications of elasmobranch research are starting to receive favorable attention with advances in the understanding of elasmobranch physiology, especially the immune system. Recent advances are leading to development of new genomics tools, and discovery of novel antimicrobials and antibody structures, as well as compounds produced by unique immune tissues of elasmobranch fishes. With the generation of new tools and new approaches, scientists researching the biomedical potential of elasmobranch fishes are increasing. Such efforts can only lead to greater insight and awareness of the unique features of elasmobranch physiology with the potential to benefit human health.

Funding: No external funding was provided to write this review.

Conflicts of Interest: The authors declare no conflict of interest.

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