Marine Natural Products as a Source of Potential Pancreatic Cancer Therapeutics

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ABSTRACT

Pancreatic cancer has the lowest survival rate of any form of cancer and is an increasingly significant concern for global health. The death rates for pancreatic cancer have changed little over time; even with recent improvements to first-line drug combinations to treat pancreatic cancer there has been little improvement in the patient prognosis. Greater attention must be paid to the identification and development of novel chemotherapeutic strategies with unique mechanisms of action. The marine environment, with its exceptional biodiversity, is a complex chemical space and a rich source of novel compounds with significant therapeutic promise. Here we review the marine natural products reported from 2006 to 2017 with compelling activity and potential for the control of pancreatic cancer based on in vitro and in vivo results. A key goal of this review is to draw attention to substances that warrant additional preclinical developmental studies.

INTRODUCTION

Distinguished by an extremely low 5-year survival rate of 8% in the United States, pancreatic cancer (PC) is a significant public health issue rife with challenges \[1\]. Due to the poor prognoses of its patients relative to other cancers, PC is the seventh highest cause of cancer-related deaths worldwide and the fifth highest cause of cancer-related deaths in the United States, based on statistics of the Centers for Disease Control and Prevention (CDC), despite being a relatively uncommon form of cancer in incidence. PC caused over 330,000 deaths worldwide in 2012 \[2-3\]. The efficacy of PC treatments has changed little over time as a result of difficulties in the diagnosis of the disease and a poor response to chemotherapies and radiation therapies. This disparity, compared with other cancer types, has led to the projection that PC will become the second deadliest cancer by 2030, surpassed only by lung cancer \[4\]. Any improvements in treatment will be a much-needed reprieve for patients diagnosed with this uniquely challenging, lethal disease.

As with other types of cancer, the best prognoses are often associated with early tumor detection. Due in part to the physiology of the pancreas, however, there are inherent limitations with the predominant diagnostic tool, computed tomography (CT), in the detection of tumors smaller than 10 mm, a size that is a critical threshold for survival times. Improved diagnostic methods such as endoscopic ultrasonography and PC biomarker detection show promise but require additional specialized training for technicians and are not as widely available \[5\] as CT scanning.

One method of disease tracking that has become more useful is genomic screening. As of 2018, tracking of the BRCA1, BRCA2, PALB2, PRSS1, SPINK1, STK11, CFTR, and ABO \[6\] genes, as well as recent identification of the ATM, p16/CDKN2A, hMLH1, TP53 \[7\] genes and others, may reveal red flags for individuals with a heightened risk of developing PC. Although any or all of these genes could develop mutations due to environmental exposure or aging, some of their mutations are strongly heritable and could predispose entire families to PC. For example, mutations in p16/CDKN2A, which are most notable for their strong association with melanoma, are often characterized as mutations of the germline, and this is one of the highest-risk gene mutations for PC \[6-7\]. Like p16/CDKN2A, many of the genes found in association with PC are also associated with other diseases \[6-7\], such as breast cancer (ATM, BRCA1, and BRCA2), Li-Fraumeni syndrome (TP53), Lynch syndrome (hMLH1), cystic fibrosis (CFTR) \[8-9\], and Peutz-Jeghers syndrome (STK11). With so many genes identified as having links to PC, further exploration of the effects of mutations is needed to characterize drugs that can specifically target PC.

Treatment options for PC, as for most cancers, are highly dependent on the stage at the moment of diagnosis. For PC patients, surgery is currently the only potentially curative treatment, but it is appropriate for only 15% to 20% of patients, largely due to the high rate of metastasis \[10\]. As a result, chemotherapy and radiation therapy are important in PC treatment. Some of the current PC chemotherapeutics, such as gemcitabine, were developed to target some of the very gene mutations used in tracking the disease, such as BRCA1 and BRCA2 \[11\]. For patients suitable for surgery, these treatments can be used before or after an operation to reduce the risk of further tumor growth, further spread, or recurrence. For patients not suitable for surgery, chemotherapy and radiation therapy are the primary options for extending the survival time and improving the quality of life. The use of radiation therapy in PC patients has been controversial; it is most efficacious when performed in tandem with chemotherapy (chemotherapy alone has also been shown to improve patient outcomes) \[12-13\]. This finding lends credence to the assertion that increasing the chemotherapeutic options for PC is a critical strategy in addressing this disease globally.

Currently, there are four major first-line drugs approved for the treatment of PC: fluorouracil, erlotinib, gemcitabine, and mitomycin C (Fig. 1). Fluorouracil and gemcitabine act by replacing pyrimidine and cytidine,
respectively, during the biosynthesis of nucleic acids and DNA replication. As a result, tumor growth is arrested by false nucleoside incorporation–induced cell death. Mitomycin C is a DNA cross-linking agent, which can react with two different positions in DNA, halt DNA synthesis, and lead to apoptosis. Erlotinib is a small-molecule human epidermal growth factor receptor 1/epidermal growth factor receptor (HER1/EGFR) tyrosine kinase inhibitor, preventing the growth factor and receptor from being phosphorylated and effectively disrupting the signaling pathway\textsuperscript{[14]}. Regardless of whether these drugs are used alone or in combination, none have been effective in significantly improving the overall survival rates for PC.

\textbf{Figure 1.} \textit{First-line drugs used for the treatment of pancreatic cancer}\textsuperscript{[4]}.

The marine environment provides a rich source of structurally unique natural products with promising biological activity and has been important in the discovery and development of novel therapeutics. Due to the nature of PC and the limited treatment options for it, marine natural products represent a valuable resource worthy of further investigation and development, with the end goal of improving the treatment options and prognosis for patients diagnosed with this extremely deadly disease. In this review, we summarize active marine compounds identified with activity against PC \textit{in vivo} or \textit{in vitro} and reported between 2006 and 2017.

\section{2. Active marine natural products against pancreatic cancer}

\subsection{2.1 Alkaloids}

Axistatins 1 to 3, formamides 4 and 5, and agelasine F (Fig. 2) were isolated from the sponge Agelas axifera. They have exhibited encouraging cytotoxicity to BxPC-3 cells, murine lymphocytic leukemia (P388), breast adenocarcinoma (MCF-7), central nervous system glioblastoma (SF-268), lung large-cell carcinoma (NCI-H460), colon adenocarcinoma (KM20L2), and prostate carcinoma (DU-145). Their IC50 range is 1.6 to 8.7 μM\textsuperscript{[15]}.

Secobatzelline A (Fig. 2) and isobatzellines A, C, and D, isolated from the Caribbean sponge Batzella sp., exhibited cytotoxicity for AsPC-1, PANC-1, BxPC-3, and MIA PaCa-2 (human PC cell lines), with an IC50 of less than 10 μM. Notably, these compounds are more potent than 5-fluorouracil\textsuperscript{[16-17]}. Isobatzellines A, C, and D were also found to exhibit in vitro cytotoxicity against P388 leukemia cells, with an IC50 of 0.42, 2.6, and 12.6 μg/mL, respectively\textsuperscript{[17]}.
The mechanism of action of isobatzellines A, C, and D is DNA intercalation. Isobatzelline E acts mainly through topoisomerase II inhibition\[18]. Secobatzelline A also showed cytotoxicity against the murine P388 tumor cell line and human lung carcinoma A-549 cell line, with IC50 values of 0.06 µg/mL and 0.04 µg/mL, respectively\[16].

Manzamine A (Fig. 2) was first isolated from a sponge from Okinawa\[19]. Manzamine A exerts cytotoxicity to AsPC-1 cells, with an IC50 of 4.2 µM after 3 days of treatment\[20]. In addition, manzamine A showed antimetastastic activity through inhibition of migration in a collagen matrix and abrogation of cell dissociation\[20]. Finally, manzamine A inhibited autophagy in AsPC-1 and PANC-1 PC cells by uncoupling vacuolar ATPases\[21]. Autophagy has been shown to be essential for pancreatic tumor growth\[22-23]. Manzamine A was also reported to show inhibition of P388 mouse leukemia cells having an IC50 of 0.07 µg/mL\[19].

Brocazine E (Fig. 2) was isolated from Penicillium brocae MA-231, a fungus obtained from the salt-tolerant white mangrove (Avicennia marina); it showed promising cytotoxic activities against the human PC cell line SW1990, with an IC50 of 2.1 µM. Its effects were more potent than those of the positive control, gemcitabine, a common first-line drug for PC\[24].

\(N\)-methylniphatyne A (Fig. 2), isolated from an Indonesian sponge, \textit{Xestospongia} sp., showed cytotoxic activity against PANC-1 cells, with an IC50 value of 16 µM\[25].

\[
\begin{align*}
R_1 &= a-H, R_2 = b-CH_3 \text{ Axistatin 1} \\
R_1 &= b-H, R_2 = a-CH_3 \text{ Axistatin 2} \\
R &= CH(CH_3)_2 \text{ Axistatin Formamide } R=H \\
R &= SCH_3 \text{ Isobatzelline A } R=SCH_3 \\
R &= H \text{ Isobatzelline B } R=H \\
R &= SCH_3, \Delta^3 \text{ Isobatzelline C } R=SCH_3, \Delta^3
\end{align*}
\]
2.2 Peptides

Sansalvamide A (Fig. 3), a cyclic depsipeptide isolated from a marine fungus of the genus Fusarium\[^{26}\], is an inhibitor of topoisomerase I\[^{27}\]. A sansalvamide analog was found to inhibit 23% of the DNA synthesis measured by [3H] methyl thymidine incorporation (p < .05). Treatment of AsPC-1 cells with 10 μM sansalvamide led to 70% inhibition of proliferation (both p < .01) caused by G0/G1 arrest and induction of apoptosis\[^{27}\].

The cyclic peptide microsclerodermin A (Fig. 3), isolated from the sponge Amphibleptula sp.\[^{28}\], exhibited cytotoxic activity against AsPC-1 and PANC-1, with an IC50 of 2.3 ± 0.5 μM and 4.0 ± 0.4 μM, respectively. It induced apoptosis in AsPC-1 and PANC-1 cells. Microsclerodermin A may inhibit NFκB through inactivation of the GSK3β signaling pathway\[^{29}\].

Apratoxins (Fig. 3) are a class of cyclic depsipeptides isolated from the marine cyanobacteria Lyngbya majuscula (now called Moorea producens)\[^{30}\]. In vitro experiments revealed that apratoxin A inhibited breast, ovarian, lung, and pancreatic cancer cell lines, with an IC50 range of 0.002 to 0.02 μM. In vivo potency of apratoxin A was evaluated in a human cancer xenograft model, BxPC3-T1, for PC at 1 mg/kg and 0.75 mg/kg, which exceeded the maximum tolerated dose. The treated versus vehicle-control effect on tumor size (T/C ratio) was 53% and 41%, respectively\[^{31}\].
2.3 Terpenes

Spongiatriol (Fig. 4), first isolated from Great Barrier Reef sponges of the genus Spongia in the 1970s, can induce apoptosis in the AsPC-1 and PANC-1 cell lines and showed cytotoxicity to the AsPC-1, PANC-1, BxPC3, and MIA PaCa-2 cell lines, with an IC50 of 13 µM, 8 µM, 6 µM, and 13 µM, respectively. It was also shown to inhibit NFκB transcriptional activity in the A549 NFκB-luc reporter cell line, with an IC50 of 3.4 µM. This might be the target of spongiatriol’s inhibition of PC cell proliferation.

Phorbaketals E and I and phorbin A (Fig. 4), isolated from the Korean marine sponge Monanchora sp., showed moderate cytotoxicity against MIA PaCa-2 and PANC-1, with an IC50 range of 5.2 to 25.3 µM, respectively, and thus it was more potent than the positive control 5-fluorouracil. They also showed moderate cytotoxicity against the human renal cancer cell lines A498 and ACHN, with an IC50 range of 4.9 to 18.0 µM.

Neopetrosiquinones A and B (Fig. 4), isolated from sponges of the Petrosiidae family, showed activity against PANC-1, with an IC50 of 6.1 and 13.8 µM, respectively. Neopetrosiquinone A also showed inhibition of the proliferation of AsPC-1 cells, with an IC50 of 6.1 µM. Additionally, the A and B compounds inhibited the proliferation of the human colorectal adenocarcinoma cell line, with an IC50 value of 3.7 and 9.8 µM, respectively.

Figure 3: Chemical structures of modified peptides active against pancreatic cancer
12-deacetoxy-23-hydroxysclaradial, 12-O-acetyl-16-deacetoxy-23-acetoxyescalarfuran, 12-deacetoxy-23-hydroxyheteronemin, 12-deacetoxy-23-acetoxy-19-O-acetylscalarin, 12-deacetoxy-23-O-acetoxyheteronemin, and 12-deacetoxyescalradial (Fig. 4) were isolated from a Korean sponge, Psammocinia sp. They exhibited cytotoxicity against human A498, ACHN, and PANC-1, with an IC50 range of 0.4 to 48 μM\(^{36}\).

Sarcophytol A (Sarc A) (Fig. 4), isolated from the soft coral Sarcophyton glaucum, shows in vivo antitumor activity\(^{37}\). In a mouse xenograft model of PC, the tumor volume was reduced in mice fed with a diet containing 0.01% Sarc A (1,759 + 310 mm\(^3\) vs. 2,364 + 467 mm\(^3\)) (p < .05) compared with the controls. Mice treated with Sarc A also showed a lower incidence of tumors (42.8% in the Sarc A group vs. 90.0% in the control group at 25 to 27 weeks). This suggests that PC developed more slowly in mice treated with Sarc A\(^{38}\). These characteristics make Sarc A an exceptionally promising starting point for the treatment of PC\(^{38}\).

**Figure 4.** Chemical structures of terpenoids active against pancreatic cancer.
2.4 Polyketides

Lasonolide A (Fig. 5) was isolated from the deep-water marine sponge *Forcepia* sp.\(^{[39]}\) and prepared through synthesis\(^{[40]}\). Lasonolide A showed cytotoxicity to PANC-1 cells, with an IC\(_{50}\) of 68 ± 10 nM\(^{[39]}\); it was also discovered to inhibit the A-549 human lung carcinoma cells and P388 murine leukemia cell line, with an IC\(_{50}\) of 19 ng/mL and 2 ng/mL, respectively\(^{[41]}\).

Leiodermatolide (Fig. 5), isolated from the lithistid sponge *Leiodermatium* sp., showed potent cytotoxicity towards AsPC-1, PANC-1, BxPC-3, and MIA PaCa-2, with an IC\(_{50}\) range of 3.3 to 50 nM, making it much more potent than gemcitabine, with an IC\(_{50}\) range of 12 to 240 nM\(^{[42]}\). *In vivo*, leiodermatolide reduced the tumor weight (1.0 g vs. 1.8 g) and increased the survival time (9 weeks vs. 8 weeks) compared with gemcitabine as a positive control\(^{[43]}\). The mechanism of action is hypothesized to be an effector of microtubule dynamics\(^{[43]}\). Leiodermatolide was also discovered to inhibit human A549 lung adenocarcinoma, DLD-1 colorectal carcinoma, NCI/ADR-Res ovarian adenocarcinoma, and P388 murine leukemia, with an IC\(_{50}\) of 5.0, 8.3, 233, and 3.3 nM, respectively\(^{[42]}\).

(+)-Spongistatin 1 (Fig. 5) is a macrocyclic lactone isolated from the sponges *Trachycladus spinispirulifer* and *Hyrtios* sp.\(^{[44]}\). (+)-Spongistatin 1 induced apoptosis in L3.6pl cells in a time- and dose-dependent manner at a concentration of 0.5 nM. In an *in vivo* experiment, (+)-spongistatin 1 significantly reduced the pancreatic tumor volume and weight without influencing the mouse body weight. The mechanism of action affects the invasion and migration involved in inhibiting cell adhesion, inducing apoptosis in anoikis-resistant tumor cells, as well as affecting the antiapoptotic protein Bcl-2\(^{[45]}\).

Mayamycin (Fig. 5) isolated from cultured *Streptomyces* sp. strain HB202 from the marine sponge *Halichondria panicea*, showed cytotoxic activity against PAXF1657L cells, with an IC\(_{50}\) of 0.15 µM\(^{[46]}\).

Comazaphilones D to F (Fig. 5) isolated from the marine fungus *Penicillium commute* QSD-17, showed activity against the human pancreatic tumor cell line SW 1990, with an IC\(_{50}\) value of 51, 26, and 53 µM, respectively\(^{[47]}\); this activity was stronger than that of 5-fluorouracil, with an IC\(_{50}\) value of 120 µM. Comazaphilones A to C showed weak or no activity against SW 1990 cells\(^{[47]}\). The double bond at C-10 and the location of the orsellinic acid unit at C-6 were found to be important for cytotoxicity\(^{[47]}\).
Figure 5. Chemical structures of polyketides active against pancreatic cancer.

DISCUSSION

Underscored by the fact that PC survival rates have been stagnant over the past few decades, the limited pipeline of current drug leads specific to PC must be expanded to improve future treatment options. This review has addressed several marine natural products that provide encouraging opportunities for the control of PC and therefore warrant further investigation and development with modern techniques. Notably, leiodermatolide is more potent than gemcitabine in vitro and in vivo. It shows improved cytotoxicity (with an $IC_{50}$ range of 3.3 to 50 nM vs. 12 to 240 nM) towards AsPC-1, PANC-1, BxPC-3, and MIA PaCa-2. Additionally, it reduces the tumor weight (1.0 g vs. 1.8 g) and increases the survival time (9 weeks vs. 8 weeks)\(^{42-43}\). Spongistatin 1 (10 µg/kg/day), compared with a vehicle-only control, led to a reduced pancreatic tumor volume (100 mm$^3$ vs. 350 mm$^3$) without influencing the mouse body weight after 22 days. Interestingly, spongistatin 1 induced a dose-dependent decrease in the invasion of L3.6pl cells (vehicle control: 100%; 0.25 mM: not significant; 0.5 mM: 35%; 1 mM: < 10%), suggesting that spongistatin 1 may have potential for controlling metastasis\(^{45}\). In a Balb/c nude mouse feeding experiment with transplanted MIA PaCa-2 cells, sarcophytol A, compared with a standard diet, led to a reduced tumor size (1,759 + 310 mm$^3$ vs. 2,364 + 467 mm$^3$) after 21 days. In hamsters treated with $N$-nitrobis-(2-hydroxypropyl) amine (BHP), Sarc A supplementation resulted in a lower incidence of pancreatic tumor development (42.8% in the Sarc A group vs. 90.0% in the standard diet control group) after 25 weeks. In both of these experiments, Sarc A supplementation did not influence the body weight or cause any abnormal histological changes. These results suggest that Sarc A may be a relatively safe option for stalling PC progression and extending patient survival times, which would provide additional time for medical intervention\(^{38}\).

Along with these promising potential therapeutics, earlier diagnosis and screening approaches are needed to improve the efficacy of treatment options for PC, such as those described in this review. In using such approaches, the lifespan and quality of life are increased for those afflicted. One hope is to utilize the known genetic mutations associated with PC. Mutations in genes such as BRCA1, BRCA2, PALB2, PRSS1, SPINK1, STK11, CFTR, and ABO\(^6\), as well as ATM, p16/CDKN2A, hMLH1, TP53\(^7\), and others, may be used to calculate the probabilities of developing PC and other cancers. More epidemiological work could then be done to identify populations with these mutations. The mutations could also serve as more potential drug targets specific to PC. With advances in PC models and screening methods, old compounds should be rescreened to uncover any missed bioactivity.
CONCLUSION

The focus of research moving forward should be to integrate cancer genomics with novel therapeutics, such as the marine natural products outlined in this paper. Previous work has shown that these compounds are effective against PC and offer options for the development of therapeutics aimed at increasing the survival rate and quality of life for PC patients.

DECLARATION OF CONFLICTING INTEREST

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