

# Benefits of a special sea cucumber extract in anti-angiogenic therapy and RTK inhibition for cancer

## Introduction

Angiogenesis, or the growth of new blood vessels, is involved in such vital processes as wound healing, restoration of blood flow to tissues after injury, and menstruation<sup>1-2</sup>. However, when the body is unable to control angiogenesis, diseases such as age-related macular degeneration, rheumatoid arthritis, or psoriasis can result<sup>3</sup>. But more importantly, angiogenesis is central to tumor growth, proliferation, invasion, and metastasis.

When normal tissues are diseased or injured, such as in tumors, different growth factors or proteins are released into the nearby tissues to stimulate angiogenesis. The growth factors subsequently bind to their corresponding receptor tyrosine kinases (RTKs) within endothelial cells in the blood vessels. This receptor binding results in the activation of the otherwise dormant endothelial cells, causing them to divide and migrate towards the diseased tissues or, in this case, the tumor cells. Adhesion molecules help the growing new blood vessels to sprout forward. These sprouting endothelial cells roll up to form new blood vessel tubes. Ultimately these tubes form a network of new blood vessels that can circulate blood<sup>4</sup>. With the new blood vessels now feeding it, the tumor can continually grow in size, invade other tissues and facilitate the spread of the cancer to other organs.

Inhibiting angiogenesis at some point in this process is obviously central to stopping tumor growth. Normally, the body has naturally-occurring angiogenesis inhibitors that try to counter the potentially abnormal effect of growth factors. However, when a tumor reaches a size of about 2 mm in diameter, growth factors are produced in overwhelming amounts and the effect of natural angiogenesis inhibitors is overpowered<sup>4</sup>. As a result, a cascading process of new blood vessel formation is initiated. One aspect of cancer therapy research, called RTK inhibition, concentrates not necessarily on stopping the overproduction of angiogenesis-related growth factors but on blocking these from binding to their receptors in order to stop the signaling that launches the process of new blood vessel formation.

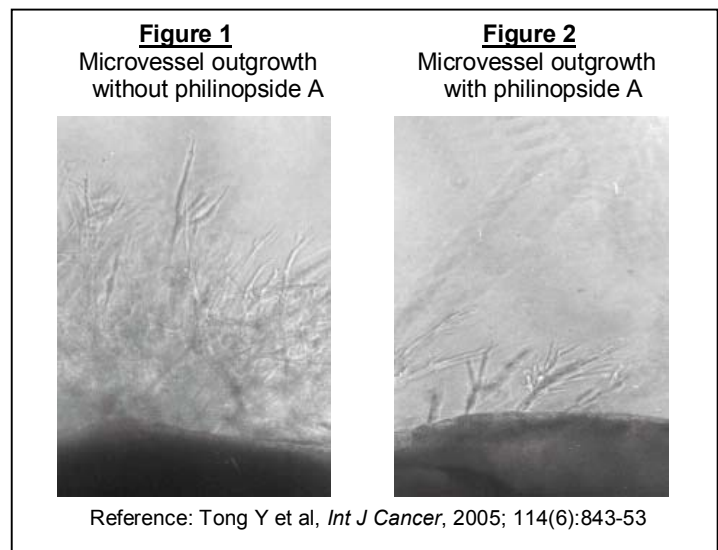
For more than three decades now, cancer therapy research has tried to find resources-- natural or otherwise-- that can block the angiogenesis process. Researchers have since developed synthetic angiogenesis inhibitors, but they have also found potential angiogenesis inhibitors in natural marine resources, most especially sea cucumbers. Sea cucumbers are delicacies in the South China seas and are known to be rich in nutritional compounds such as polysaccharides<sup>5</sup> and lactones<sup>6</sup>. In the 1990s, scientists have uncovered that sea cucumbers are active inhibitors of angiogenesis as well (U.S. Patent No. 5,985,330). This comes as no surprise to people living in the South China seas. For centuries, sea cucumbers have been considered as an integral part in Chinese folk medicine, primarily used as treatment for stomach ulcer and stomach cancer. The discovery that these natural resources are angiogenesis inhibitors only reinforces their centuries-old history of traditional use. More recently, a research team in China published more concrete evidence that philinopside A (chemical formula  $C_{55}O_{22}H_{85}SO_3Na$ ), extracted from a special sea cucumber species, is not only able to suppress new blood vessel formation but also inhibit RTK binding of growth factors<sup>7</sup>.

## The anti-angiogenic and RTK inhibitory functions of Philinopside A

Results of studies by the research team in China revealed that due to philinopside A's significant inhibition of three important stages of angiogenesis (endothelial cell proliferation, migration and tube formation), the formation of new blood vessels was greatly reduced. It was observed that, at various doses, philinopside A inhibited proliferation of human microvascular endothelial cells (HMECs) at rates of up to 98.7%. Using the same doses, HMEC migration was also inhibited by as much as 94.1%. Figures 1 and 2 illustrate the sprouting of microvessels in cultured rat aortas before and after treatment with philinopside A. After addition of philinopside A, the outgrowth is visibly reduced, indicating that it did block blood vessel formation. In more specific tests, philinopside A was found to inhibit proliferation in several cancer cell lines, including MKN-28 (gastric cancer), SPC-4A (colorectal cancer), HL-60 (leukemia), A-549 (lung tumor), BEL-7402 (liver cancer), MCF-7 (breast cancer), HCT-116 (colorectal cancer) and HO-8910 (ovarian cancer)<sup>7</sup>.

In subsequent studies, philinopside A produced a more potent anti-angiogenic effect than Suramin, a synthetic angiogenesis inhibitor manufactured by Parke-Davis, requiring only a minimal dosage to produce the same effect as Suramin. A comparable result, in terms of dosages, was obtained when philinopside A was compared against 5-Fluorouracil (5-FU), a popular chemotherapeutic agent<sup>7</sup>.

Further studies revealed that philinopside A is able to shrink mouse sarcoma 180 tumor tissues by inducing apoptosis, or cell death. It produced a 10-fold increase in apoptotic endothelial cells and a 9-fold increase in apoptotic tumor cells compared to the untreated tissues. A similar degree of tumor shrinkage was observed using 5-FU but a much higher concentration (at least 12 times more than philinopside A) was needed. In addition, both compounds operate through different modalities: philinopside A through apoptosis of tumor and endothelial cells and 5-FU through cytotoxicity of tumor cells<sup>7</sup>. The method by which it reduces tumor size is what sets philinopside A, and other angiogenesis inhibitors, apart from other cancer treatments. By inducing apoptosis of tumor and endothelial cells, treatment is localized and damage to the surrounding healthy cells is minimized. Chemotherapeutic agents and/or radiation, on the other hand, directly kills both healthy and cancer cells (cytotoxicity) and generates more physiological side effects.



Perhaps the most novel function of philinopside A is its ability to inhibit RTK binding. It has been previously mentioned that shutting off the switch close to the source can effectively stop the angiogenesis process. To achieve this, growth factors must be prevented from attaching to their receptors.

There are at least 20 known growth factors associated with angiogenesis, each with its own corresponding receptor(s)<sup>8</sup>. Some of these growth factors include the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). The type of growth factors produced and their degree of responsiveness to receptors can vary from tumor to tumor. For example, VEGF is highly expressed in solid tumors-- such as in breast cancer, gliomas, and gastrointestinal cancer-- and attaches to the receptor fetal liver kinase-1 (Flk-1), among other receptors. By interfering with the stimuli at the receptor site, VEGF-RTK inhibitors can stop further tumor growth in these types of cancer.

The studies on Philinopside A illustrate that it is able to stop the signaling caused by the binding of VEGF, FGF, PDGF and EGF to their corresponding receptors (Flk-1, FGFR-1, PDGFR- $\beta$ , and EGFR, respectively). Two other angiogenesis inhibitors, PD153035 and SU5416, were also tested. Results indicate that PD153035 inhibits the EGF receptor (EGFR) alone. Similarly, SU5416, manufactured by Pfizer, only inhibits the Flk-1 tyrosine kinase. It was concluded that because growth factors tend to critically overlap, inhibition of one RTK alone might not be enough to sufficiently block RTK signaling. Philinopside A, with its inhibition of all four tested RTKs, might possibly prove to be an effective RTK inhibitor with a lethal dose (LD<sub>50</sub>) of only 625 mg/kg orally in mice<sup>7</sup>.

## Discussion

The inhibition of angiogenesis now seems like the most logical and valuable tool in the fight against cancer. Yet when it was first hypothesized in 1971 by Dr. Judah Folkman that tumor growth depends on new blood vessel formation, it was considered heresy by scientists<sup>9</sup>. Through continuing research by him and others who believed in his hypothesis, angiogenesis inhibition has since been firmly established as an innovative cancer therapy and has led to the development of angiogenesis and RTK inhibitor drugs-- collectively known as targeted therapies-- by various pharmaceutical companies worldwide.

In 2004, the first angiogenesis inhibitor drug was approved for use in the United States. Although not the first targeted therapy drug approved, Avastin (bevacizumab), is the first drug proven to actually delay tumor growth by targeting the VEGF tyrosine kinase, which is mainly responsible for vascular growth. Manufactured by Genentech, Avastin works in combination with standard chemotherapy drugs like the Saltz regimen (a combination of three drugs: irinotecan, 5-FU and leucovorin). Patients who received both Avastin and the Saltz regimen were found to survive 5 months longer than those who received the Saltz regimen alone<sup>10</sup>.

Although Avastin is technically the first angiogenesis inhibitor, there are other drugs that belong to a class called tyrosine kinase inhibitors (or RTK inhibitors). Unlike Avastin, these drugs have not been shown to starve tumors and induce apoptosis. Rather they interfere with the growth factor-to-receptor signaling that facilitates the proliferation of the cancer. In a way, they indirectly influence angiogenesis inhibition. Gleevec (imatinib mesylate) is a drug manufactured by Novartis used to treat chronic myeloid leukemia (CML) by blocking the Bcr-Abl tyrosine. In studies, patients have a 76% response rate to Gleevec compared to interferon, the standard CML treatment, which has a 12% response rate. Gleevec also works

as a PDGFR inhibitor for the treatment of gastrointestinal stromal tumors (GIST), a rare form of stomach cancer. Additional studies have found that within two years of using Gleevec, GIST patients develop a 75% resistance to it<sup>11</sup>.

Three other RTK inhibitor drugs, Tarceva (erlotinib), Iressa (gefitinib) and Erbitux (cetuximab), block the EGF tyrosine kinase. The first two are used to treat non small cell lung carcinoma (NSCLC) while Erbitux is indicated for advanced colorectal cancer. Tarceva, manufactured by OSI Pharmaceuticals, was found to have an overall survival of 6.7 months<sup>12</sup>. Both Iressa, manufactured by AstraZeneca, and Erbitux, manufactured by Imclone Systems, have not been shown to prolong survival although some patients have responded to the treatment. In clinical trials, Iressa caused significant tumor shrinkage in about 10% of lung cancer patients<sup>13</sup>. Erbitux produced a 10.8% tumor response rate among colorectal cancer patients<sup>14</sup>.

These are just examples of a growing class of drugs that can be collectively called “smart bombs” -- a reference to the selective way these drugs target cancer cells and minimize damage to healthy cells<sup>15</sup>. And now this term not only refers to synthetic drugs but to naturally occurring compounds like philinopside A in special sea cucumbers as well. Based on the evidence presented, philinopside A shows great potential as a relevant cancer therapeutic agent because of its dual role as an anti-angiogenic agent and RTK inhibitor.

In its function as an angiogenesis inhibitor, philinopside A has been shown to inhibit cell proliferation, cell migration and tube formation -- three of the most important stages in angiogenesis. Evidence also suggests that treatment with philinopside A caused tumor shrinkage and apoptosis without any effect on normal cells. This is the very essence of targeted therapy: confining the treatment to the diseased area.

Philinopside A's broad range of RTK inhibitory effect is an important factor that makes it potentially more effective than other agents that target only a specific RTK. Angiogenesis is a complex process that likely involves multiple RTK signaling pathways. Inhibition of one RTK, therefore, may not be able to fully suppress angiogenesis. A concrete example to illustrate this point is the inhibitory effect of Gleevec on GIST patients. In approximately 10% of GIST cases that are attributed to abnormal PDGF tyrosine kinase signaling, Gleevec has been shown to have favorable results. More recent studies, however, have found that after a year or two of treatment, these GIST patients develop resistance to it. There may be other factors that lead to the drug resistance but a prevailing theory is that inhibiting PDGFR alone may not be enough to stop the tumor from growing. A Pfizer drug, Sutent (also known as SU11248), currently in clinical trials, has been shown to benefit 65% of GIST patients resistant to Gleevec. Although Gleevec and Sutent are both RTK inhibitors, Sutent targets several more RTKs than Gleevec: VEGFR, PDGFR- $\alpha$  and PDGFR- $\beta$ , Flt3 and C-kit<sup>16</sup>. This suggests that therapies that target a wide range of RTKs will provide more effective and long-term anti-angiogenic effects than those that target a limited number of growth factor receptors.

Preliminary studies have certainly shown philinopside A's potential as an angiogenesis and RTK inhibitor; however, further investigational studies including human clinical trials are warranted in order to firmly establish it as a viable anti-cancer therapeutic agent.

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