Review

DOI: 10.5582/ddt.2011.v5.6.266

Effective neurofibromatosis therapeutics blocking the oncogenic kinase PAK1

Hiroshi Maruta

NF/TSC Cure Org., Melbourne, Australia.

ABSTRACT: Neurofibromatosis (NF) is a family of genetic diseases which are caused by dysfunction of either *NF1* gene or *NF2* gene. One in 3,000 people suffer from this tumor-carrying NF. *NF1* gene product is a RAS GTPase activating protein (GAP) of 2,818 amino acids, which normally attenuates the GTP-dependent signal transducing activity of the G protein RAS. Dysfunction of this GAP leads to the abnormal activation of RAS, and eventually an oncogenic kinase called PAK1 as well. *NF2* gene product is "Merlin" which directly inactivates PAK1. Thus, dysfunction of Merlin causes the abnormal activation of PAK1. In other words, dysfunction of *NF1* gene (causing type 1 NF) is basically the same as dysfunction of *NF2* gene (causing type 2 NF). In fact the growth of both NF1 and NF2 tumors requires PAK1, and all PAK1 blockers, synthetic chemicals or natural products, suppress the growth of these NF tumor cells both *in vitro* (cell culture) and *in vivo* (mice).

However, until recently, no FDA-approved effective NF therapeutics is available on the market. Here a series of anti-PAK1 products shall be introduced, which would be potentially useful for the life-long treatment of NF patients in the future. These include the most potent HDAC (histone deacetylase) inhibitor FK228 (IC_{50}: around 1 nM), that eventually blocks PAK1, the direct PAK1 inhibitor PF3758309 (IC_{50}: around 10 nM), a CAPE (caffeic acid phenethyl ester)-based propolis extract called "Bio 30" from NZ (New Zealand), and an ARC (artepillin C)-based green propolis extract (GPE) from Brazil. Although the first two drugs are potent, none of them is available on the market as yet. The last two natural (bee-made) products are available on the market, and have been used for the therapy of NF and tuberous sclerosis (TSC) as well as many PAK1-dependent solid cancers such as breast and pancreatic cancers as well as glioma, which altogether represent more than 70% of all human cancers. Since PAK1 is not essential for the normal cell growth, propolis extracts cause no side effects.

Address correspondence to:
Dr. Hiroshi Maruta, NF/TSC Cure Org., 14 Curtin Avenue, Brunswick West, Australia 3055.
e-mail: maruta20420@yahoo.co.jp

Keywords: NF, cancer, propolis, AMPK, FOXO, lifespan, TSC

1. Introduction

The genetic disease or disorder now called neurofibromatosis (NF in short) was first recognized in 1882 by a German pathologist at Strasbourg University, Friedrich Von Recklinghausen (1883-1910). Later, by the detailed genetic analysis, NF was revealed to have two distinct types, type 1 (NF1) and type 2 (NF2). Dysfunction of *NF1* gene causes NF1, and that of *NF2* gene does NF2. *NF1* gene product has two distinct functional domains. The C-terminal half is a RAS GTPase activating protein (GAP), which attenuates the GTP-dependent signal transducing activity of RAS, a G protein, whose mutation causes malignant transformation (*1*). The N-terminal half is an inactivator of another G protein called RAC (*2*), which is activated by RAS through the PI3-kinase, a unique phospholipid kinase that phosphorylates PIP2 to produce the oncogenic PIP3. Dysfunction of either N or C-terminal half leads to the abnormal activation of the kinase PAK1, a RAC/CDC42-dependent Ser/Thr kinase (*1-4*) (for detail, see Figure 1). *NF2* gene product is Merlin, which turned out to be a direct inhibitor of PAK1 (*4*). Thus, dysfunction of *NF2* gene also leads to the abnormal activation of PAK1. In other words, although NF1 and NF2 are genetically distinguished, phenotypically at the molecular levels, the outcome of these two diseases is basically the same. In fact, both NF1 and NF2 require PAK1 for their growth (*3,4*). NF1 tumors are roughly divided into three types: malignant peripheral nerve sheath tumor (MPNST), plexi-form and dermal neurofibroma (wart-like skin tumor). MPNST is a cancer, but the remaining two are benign, and represent 90% of NF1 tumors. NF2 tumors are divided into two types, meningioma and schwannoma, and both are benign tumors developed in brain and along spine, and lead to loss of eye sight or hearing or both, and in the worst cases cause the total paralysis. Unlike most of cancers, both NF1 and NF2 develop even in the very early stage of life such as 6 months after the birth, and last for the rest of life, increasingly getting worse, without a proper treatment.

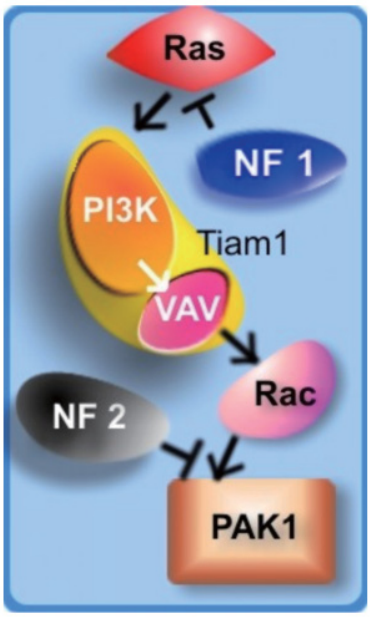

Figure 1. Oncogenic RAS pathways leading to the activation of PAK1. Oncogenic RAS mutants activate the kinase PAK1 through PI3-kinase and VAV or Tiam1 that activate the GTPases RAC/CDC42. This oncogenic signaling is blocked by two tumor suppressors, *NF1* gene product (RAS GAP) and *NF2* gene product (Merlin).

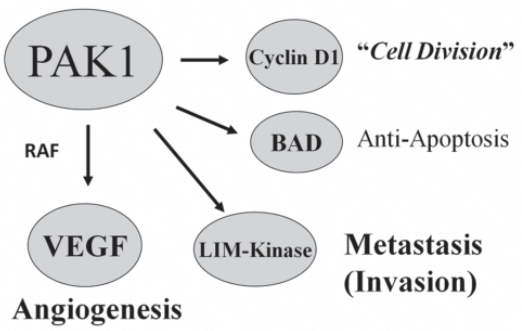

Figure 2. Oncogenic roles of the kinase PAK1.

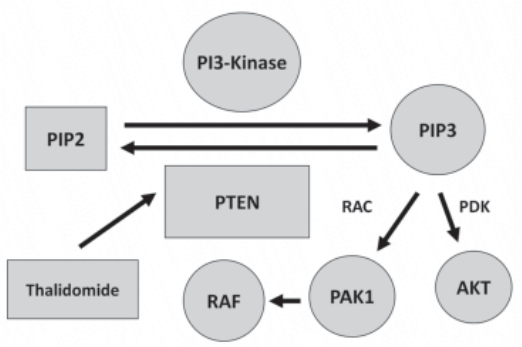

Figure 3. PTEN reverses the oncogenc PI3-kinase signaling.

Thus, unlike the cancer therapy, the systemic treatment of both NF1 and NF2 has to last life-long, and would be certainly desirable to be both safe and inexpensive. Besides, none of conventional anti-cancer drugs such as DNA/RNA/MT (microtubule) poisons is effective on either NF1 or NF2 tumors. Currently the only effective treatment of both NF1 and NF2 are physical treatments, such as surgical removal and gamma knife (a pin-point Cobalt radiation). However, these invasive treatments often cause significant damage on a variety of nerves in the vicinity of tumors, leading to the irreversible loss of the corresponding functions. Furthermore, in the case of NF1, around a half tends to suffer from a learning deficit (LD), although its extent varies from one person to another. This LD cannot be cured by any physical treatment, because its cause is not tumor *per se*, but the abnormal activation of PAK1 in brain which impairs the learning process. In addition, as mentioned before, NF1 patients, in particular NF1 children, have a fragile bone structure, mainly due to vitamin D3 deficiency (*5*). However, this deficiency cannot be eliminated by any physical treatment. Thus, in principle, the ultimate cure of both NF1 and NF2 should owe to either gene therapy using the functional *NF1* or *NF2* gene, or chemotherapy using chemical compounds or natural products which selectively inactivate PAK1. However, in reality, the expression of *NF1* or *NF2* gene in the whole body of each NF patient cannot be achieved by the current level of gene therapy technology. Thus, the most realistic possibility would be the chemotherapy using specific PAK1 blockers.

During past several years since *NF2* gene product "Merlin" was identified as the direct PAK1 inhibitor (*4*), a series of PAK1 blockers were developed or identified by several groups including our own team. In the following sections some of them will be introduced for NF patients and clinicians, which would be useful for the systemic NF therapy at present or in near future.

The oncogenic kinase PAK1 is involved in the following several aspects of oncogenesis (malignant transformation of normal cells) by activating several kinases such as RAF, ERK, and LIM-kinase as well as growth factor genes encoding TGFα and VEGF (see Figure 2): anchorage-independent cell growth, blocking apoptosis (programmed cell death), metastasis/invasion and tumor-induced angiogenesis (blood vessel formation) which is essential for the growth of solid tumors such as NF tumors (*6*,*7*). Thus, unlike the conventional anti-cancer drugs which fail to block both metastasis and angiogenesis of tumors, PAK1 blockers could suppress these malignant aspects of tumors, in addition to killing directly the tumor cells.

By the same token, however, PAK1 blockers cannot be used for the treatment of "pregnant" patients, because like thalidomide these PAK1 blockers might cause the abnormal child birth (teratogenesis) by harming the angiogenesis essential for the normal development of embryos. For thalidomide is now well known to block the PAK1-dependent angiogenesis, and in fact inactivates both PAK1 and another oncogenic kinase called AKT by up-regulating the tumor suppressing phosphatase "PTEN" (*8*), the major antagonist of the oncogenic PI3-kinase, reconverting the oncogenic PIP3 to the inactive PIP2 (see Figure 3). However, it is now clear that the

anti-angiogenesis alone is not sufficient for causing the teratogenesis, because a few thalidomide derivatives such as "CC-5013", which are still anti-angiogenic, are not teratogenic at all (*9*).

In my opinion, AKT blockers including thalidomide and its non-teratogenic derivatives would not be suitable for the life-long treatment of NF, because unlike PAK1, AKT is essential for the growth or survival of normal fast growing cells such as those in bone marrow, and the GLUT-4 dependent glucose uptake by normal cells. In other words, AKT inhibitors cause a series of side effects including immuno-suppression and insulin-resistant diabetes (type 2). PAK1 blockers alone cause basically no side effect.

2. Natural products

2.1. *Propolis*

Propolis is a bee product which has been used for four thousand years since the ancient Egyptian era to treat wounds, infectious and inflammatory diseases, because it has at least anti-bacterial, anti-fungal and anti-viral activities, and boosts the immune system. In other words, it is a traditional multi-functional antibiotic produced by honey bees. Originally back to a million years ago, bees created this resinous material from young buds of poplar trees and a few other plants to protect their larva from any harmful microorganisms or stresses. The wall and bottom of each hexagonal honey comb consists of propolis and fat/wax. In general ethanol-soluble extract (tincture) of propolis is used for topical treatment of wounds. Its long-lasting strong anti-biotic effect is clearly revealed by the fact that propolis was used to preserve the deceased royal family's bodies as mummies under pyramids from the ancient Egyptian era till today. Hippocrates (460-370 BC), the father of medicine in the ancient Greece, recognized its potent healing power, and coined it "Propolis", meaning the protection of city ("polis") or comb. Since he survived around 90 years in the ancient Greece where the average life span of human beings was only 50 years, I tend to believe that he also took propolis regularly, which extends the life span as well, as we shall discuss later.

There is an old German saying: no beekeeper catches cancer. More precisely, only one in 3,000 beekeepers get a cancer, while one in three ordinary people (non-beekeepers) once suffers from a cancer during their life, according to a study by the German Beekeepers' Association (DBB). So the most intrigue question would be why beekeepers are a thousand times more resistant than the ordinary people to cancers. Since neither honey nor royal jelly has any significant anti-cancer activity, it is most likely that a third bee product, namely "propolis", is the major source which makes beekeepers so resistant to cancers. Yes, in 1988, a Jewish-American oncologist at Columbia University, Drizer Grunberger and his colleagues found that propolis from Israel contains an anti-cancer polyphenol called CAPE (caffeic acid phenethyl ester) (see Figure 4) and his colleague Koji Nakanishi succeeded in the chemical synthesis of CAPE (*10*). Since then, propolis became a popular alternative cancer therapeutic, partly because it is rather inexpensive and causes little side effect.

However, it turned out that the CAPE content of propolis vastly varies from one sample to another, depending on where it is produced or harvested. For instance, propolis samples from Far-East, Europe/US, and Oceania are largely the CAPE-based, but those from Egypt, India and Brazil contain no CAPE. Nevertheless, Brazilian green and red propolis showed a strong anti-cancer property. In 1994, a group at Hayashibara Institute in Japan found that the major anti-cancer ingredient in Brazilian green propolis extract (GPE) is another polyphenol called ARC (artepillin C) (*11*), and Hitoshi Hori and his colleagues at Tokushima University in Japan succeeded in the chemical synthesis of ARC in 2002 (*12*) (see Figure 5). Brazilian red propolis extract (RPE) has neither CAPE nor ARC, but contains a triterpene, and suppresses the growth of pancreatic cancer cells *in vitro* (*13*). So an interesting question has arisen: is there any common mechanism underlying the anti-cancer property among these three distinct propolis samples? In 2005, the first clue to the molecular mechanism of CAPE to suppress the growth of cancers was revealed: caffeic acid (CA) down-regulates a G protein called RAC which is the direct activator of the oncogenic kinase PAK1 (*14*). Since CAPE is a hydrophobic derivative of CA, it is most likely that CAPE also down-regulates RAC, and eventually inactivates PAK1. Yes, we confirmed that CAPE indeed blocks PAK1 in a tiny nematode called

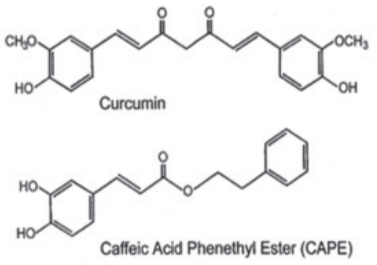

Figure 4. CAPE and curcumin.

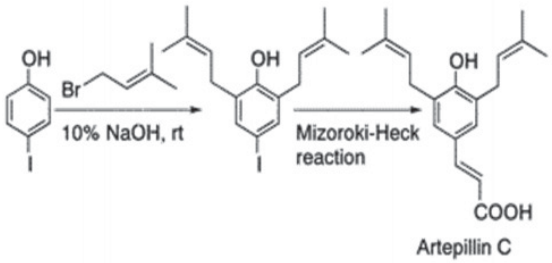

Figure 5. Chemical synthesis of artepillin C.

C. elegans, and activates the *HSP16* gene which is normally inactivated by PAK1 (*6*). Interestingly, ARC also activates the *HSP16* gene in this worm, strongly indicating that ARC also inactivates PAK1 (*6*). In 2007, we confirmed that ARC also indeed inactivates PAK1 selectively, without affecting another oncogenic kinase AKT (*15*). Thus, during 2006-2007, we examined if a CAPE-based propolis extract from NZ (New Zealand) called "Bio 30", and the ARC-based GPE suppress the growth of both NF1 and NF2 tumor xenografts in mice. Yes, both "Bio 30" (see Figure 6) and GPE almost completely block the growth of *NF1*-deficient MPNST and *NF2*-deficient tumor (schwannoma) *in vivo* (*15,16*). Although ARC is the sole anti-cancer ingredient in the GPE, representing 8% of dry weight of this extract, "Bio 30" contains not only CAPE but also several other anti-cancer polyphenols such as pinocembrin, galangin, chrysin, apigenin and CA (*17-21*), representing more than 24% of dry weight of this extract (for detail, see Table 1), and these polyphenols work synergistically with CAPE, and boost the anti-cancer/NF activity of CAPE alone by 600 times *in vitro* (*16*).

For 3-4 years since mid-2007, we have been conducting human trials of "Bio 30" (alcohol-free liquid) containing 250 mg of extract/mL, mainly for NF patients world-wide, and the effective minimum daily dose of "Bio 30" (25 mg/kg = 1 mL/10 kg) has stopped the growth of their tumors in most cases of both NF1 and NF2 patients as well as glioma and pancreatic cancer patients, without any side effect (*22*). Furthermore, in three NF1 (dermal neurofibroma) cases, tumors completely disappeared in a month. Also at least in three cases of NF2 (both schwannoma and meningioma) and one glioma case, these brain tumors shrank by more than 50% in 2-3 years. In two pancreatic cancer cases, both early and metastasized "terminal" cancers completely disappeared in one year (*22*). These preliminary clinical trial data strongly suggest that at least "Bio 30" is effective in suppressing the growth of both NF1 and NF2 tumors as well as glioma and pancreatic cancers for which the conventional anti-cancer drugs are basically useless. However, it should be worth noting that unlike the "Bio 30"-sensitivity of NF/cancer-carrying "cloned" nude mice which are genetically identical, that of these NF/cancer patients turned out to vastly vary from one person to another unsurprisingly, not only depending on the huge difference in their symptom *per se*, but also that in their genetic background. For instance, to cure an early pancreatic cancer needs only 25 mg/kg of "Bio 30" daily for a year, to cure a terminal metastasized pancreatic cancer needs 200 mg/kg of "Bio 30" for a year. The great advantage in "Bio 30" is that even such a massive daily dose causes no side effect. Likewise, shrinking a few wart-like dermal NF1 tumors with 25 mg/kg daily needs only a few weeks, while shrinking either large plexi-form NF1 tumors or NF2-associated brain tumors with the same minimum daily dose needs 2-3 years.

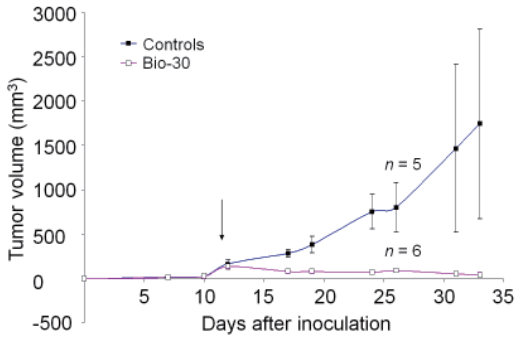

Figure 6. "Bio 30" shrinks the NF2 tumor (NF2-deficient Schwannoma xenograft) in mice.

Table 1. Polyphenol content (mg/g) in "Bio 30" *versus* Chinese red propolis extract (RPE)

Polyphenols	"Bio 30" (*16*)	Chinese RPE (*23*)
CAPE	12	17
CA (caffeic acid)	12	13
Pinocembrin	110	84
Galangin	60	37
Chrysin	30	50
Apigenin	12	ND*

* Not Determined.

Although we have not done any systematic clinical trials of GPE for NF patients, at least in two NF1 (dermal neurofibroma) cases, GPE (8 mg/kg, daily) was sufficient to shrink these skin tumors completely in a month or so, suggesting that GPE is also useful for NF patients. For the last decade, GPE has been used for human trials for a variety of PAK1-dependent cancers such as pancreatic, colon, gastric, breast, prostate, lung, liver, ovarian, cervical, and thyroid cancers as well as glioma, melanoma, and multiple myeloma (MM) which represent more than 70% of cancers, and in many cases, GPE has been proven to be effective in either stopping their growth or shrinking these solid cancers. Thus, I trust that GPE would be useful for both NF1 and NF2 patients as well. The only minor problem with GPE is that it costs several times as much as "Bio 30" which costs only a dollar daily, for a life-long treatment of NF. On the other hand, like any other CAPE-based propolis, "Bio 30" causes an allergic skin reaction only in 1% of population, while GPE causes no allergic reaction.

There are at least two distinct red propolis extracts (RPEs). One is Brazilian RPE, which contains the anti-cancer di-terpene and tri-terpene, and blocks the PAK1-dependent growth of human pancreatic cancer cells with the IC_{50} around 10 μg/mL (*13*), strongly suggesting that these terpenes also block PAK1, and would be potentially useful for NF therapy in the future. However, it remained unavailable on the market until a US company called "JuneBees" recently started selling 30 mL bottles of RPE on-line (junebees.com). Like GPE, this Brazilian RPE is rather expensive, currently costing US$ 70 per bottle. The other RPE is a Chinese brand

from Shandong province, and just like "Bio 30" contains CAPE and several other anti-cancer polyphenols (for detail, see Table 1). This Chinese RPE has the IC_{50} (around 4 μg/mL), similar to that of "Bio 30", to inhibit the PAK1-dependent angiogenesis (*23*), suggesting that the Chinese RPE might be useful for the treatment of NF.

GPE is generally provided in capsules, but "Bio 30" is currently provided as a water-miscible PG (propylene glycol) solution or tincture (75% ethanol solution). Thus, "Bio 30" tastes rather bitter, and when diluted with water, it tends to form a sticky precipitate. In an attempt to improve both the taste and absorption through the gastrointestinal membranes, Manuka Health in NZ and Cyclochem in Japan have jointly developed a new "Bio 30" product which is encapsulated in a natural ring oligo sugar called gamma cyclodextrin (CD). This water-soluble CD (see Figure 7), enzymatically synthesized from starch, has a hydrophobic cavity inside of this nano particle, which is able to capture any hydrophobic small molecules such as those in propolis (*24*). Since the "bitter" polyphenols are inside of CD, propolis extract would be no longer bitter, being suitable in particular for young children, and this CD complex, a mixture of "Bio 30" and CD (1:3 by dry weight), forms a stable and homogeneous emulsion which no longer precipitates. "Bio 30" in this complex could be more efficiently absorbed by intestinal membranes, because bile acids, stored in gallbladder and are secreted into intestine, then replace CD to form a nano micelle of "Bio 30" (*24*). This CD complex of "Bio 30" is currently being tested for its therapeutic efficacy on human cancer xenograft in mice, before the commercialization. However, it is possible to prepare a home-made CD complex, simply by mixing "Bio 30" (alcohol-free liquid) and 20% CD solution at the ratio of 1:5 by volume on a kitchen table of NF patients, and according to a preliminary human trial, the CD complex appears to be more effective than "Bio 30" alone to shrink at least dermal neurofibromas of a few NF1 patients.

The initial reason why we began to focus our research effort on the NZ propolis extract "Bio 30" was a 2002 poster by a University of Sydney group claiming that a NZ propolis shows the highest CAPE content (6-7% of dry extract) among propolis samples around the world. However, we realized that "Bio 30" contains only 12 mg of CAPE per g of dry extract (= 1.2% of extract), and have never found such a CAPE-rich sample in NZ propolis. A few propolis experts suspect that the 2002 report based on a rather low-tech TLC analysis of CAPE content might over-estimate the CAPE content, because often CAPE and a much more abundant polyphenol called pinobanskin 3-acetate overlap each other. Thus, in an attempt to create the "legendary" CAPE-rich propolis extract called "CAPE 60" (containing 60 mg of CAPE per g of extract), we added an extra CAPE (chemically synthesized) to "Bio 30", and compared the anti-cancer efficacy with "Bio 30" alone *in vivo*. As expected, "CAPE 60" is clearly more effective in suppressing both the growth and metastasis of MPNST in mice (*16*), confirming that the higher CAPE content, the more effective the propolis extract. Recently, enzymatic synthesis of CAPE was developed and further improved by a group in Taiwan (*25,26*). A mixture of CA and phenethyl alcohol (PA) can be converted almost 100% to CAPE by the immobilized lipase B called "Novozym 435" (see Figure 8). Thus, in near future, it would be possible for us to create the "CAPE 60" simply by adding a "natural" (enzymatically synthesized) CAPE to "Bio 30" or any other propolis extracts to boost their therapeutic effect for NF patients. Interestingly, coffee beans are rich in CA, while PA is a major fragrance of roses.

2.2. *Chinese (Sichuan) peppepr extract*

Around an early 2005, we started exploring a natural anti-PAK1 source(s) available inexpensively on the market, which would be suitable for a life-long treatment of NF, before "Bio 30" became available on the market.

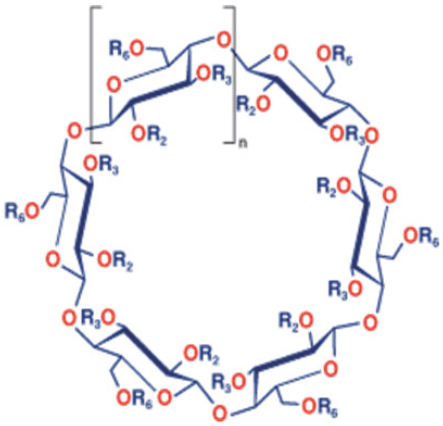

Figure 7. Cyclodextrins (CDs). N = 6, alpha-CD; N = 7, beta-CD; N = 8, gamma CD.

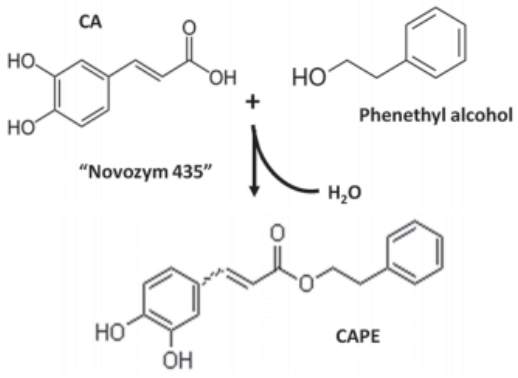

Figure 8. "Enzymatic" synthesis of CAPE.

One of them turned out to be an extract of Chinese (Sichuan) peppercorns, which has been used for thousand years in China as a seasoning for the preparation of a Sichuan cuisine called "Mapo-Tofu", a spicy bean curd dish. This reddish peppercorn is called "Hua Jiao" in Chinese or "Kasho" in Japanese, and related to a Japanese greenish peppercorn called "Sansho" which has been used as a seasoning for "Kabayaki", a grilled eel dish, and a traditional medicine for removing/killing intestinal parasitic nematodes.

We found that 70% ethanol extract of "Hua Jiao" contains an anti-cancer ingredient called "Pepperin" that selectively inactivates PAK1, without affecting another oncogenic kinase AKT (*27*). This extract blocks the growth of MPNST cells with the IC_{50} around 10 μg/mL, without affecting normal cell growth, and at the dose of around 100 mg/kg, twice a week, ***i.p.***, strongly suppresses the growth of *NF1*-deficient breast cancer xenograft in mice (*27*), suggesting that this extract would be useful for NF treatment. However, for the oral administration, ethanol has to be removed from the extract, so that patients would not suffer from the alcoholism. We found that a warm water (around 45°C) can replace 70% ethanol for the extraction of peppercorns. However, this water extract often tends to precipitate the anti-cancer ingredient "Pepperin" when the extract is cooled down to around room temperature. Furthermore, this extract is rather "bitter", although it is not "spicy" at all (the major pungent ingredient "Sanshool" is not water-soluble). Thus, just like the CAPE-based propolis extract, we are currently developing the CD complex of this extract called "Kasui" (water-extract of "Hua Jiao") by adding CD (20-30 g per 200 mL extract) to eliminate the bitter taste, and capture the "Pepperin" inside of CD molecule. According to an NF2 patient who prepared a home-made CD complex of "Kasui", this CD complex is no longer bitter, but apparently "sweet". This CD complex costs only around a dollar daily, and would be suitable for a life-long treatment of NF patients, without any side effect. For a few NF2 patients who carry the "Bio 30"-resistant tumors, we recently started a human trial of this CD complex alone or in combination of CD complex of "Bio 30", in an attempt to overcome this "Bio 30"-resistance.

2.3. *Bitter melon from China or Okinawa*

"Good medicines are bitter." That is an old saying that parents often use to encourage their young children to swallow a bitter medicine. Clearly good NF medicines such as propolis and "Kasui" are not its exceptions. In Okinawa, the southwest island of Japan, people used to live much longer than the rest of Japan, until their eating habit was gradually changed to the so-called American style. Besides Okinawa people are slimmer, less diabetic and more resistant to the summer heat. In an attempt to find the secret recipe of the Okinawa people's longevity and better health, back to 1983, Doroles Takemoto's group at Kansas State University examined the anti-cancer effect of a water-extract from an Okinawa's bitter melon called "Goya". They found that this bitter extract suppresses the development of a chemical induced cancer in mice (*28*). However, the molecular mechanism underlying this anti-cancer property remained unknown. 25 years later, in 2008, a Chinese group in Beijing and Australian group in Sydney jointly found that a similar Chinese bitter melon extract suppresses the diabetes in mice, lowering the blood sugar level, and identified the anti-diabetic tri-terpene in this extract (*29*). Interestingly, this tri-terpene activates the tumor suppressing kinase AMPK, suggesting that this tri-terpene is responsible for suppressing both the insulin-resistant diabetes (type 2) and tumor development. As discussed in detail later, AMPK activates a glucose transport protein called GLUT-4 which is essential for cellular uptake of glucose from blood stream (*30*). Furthermore, another tumor suppressing kinase called "LKB1" activates AMPK, and inactivates PAK1, directly (*31*). Thus, it is most likely that this tri-terpene blocks PAK1 as well as activates AMPK. For more than a dozen anti-PAK1 compounds such as CAPE and curcumin almost invariably activate AMPK (*32*,*33*). In other words, the "bitter" melon extract from Okinawa or China would be a potentially good NF therapeutic, although nobody knows as yet how much we should take daily this bitter extract for NF/cancer therapy.

2.4. *Curcumin (Turmeric)*

As shown in Figure 4, CAPE in propolis and curcumin, the spicy yellow ingredient in Indian curry (Turmeric powder) are structurally very similar, and as expected, both polyphenols were found to block PAK1 and activate AMPK (*6*,*32-34*). Like CAPE, curcumin suppresses the growth of many PAK1-dependent cancer cells *in vitro*, but either CAPE or curcumin alone has never been used clinically, mainly because of their poor bioavailability (water-insolubility). However, around 2005, Razella Kurzrock's group at MD Anderson Cancer Center solubilized curcumin by encapsulating curcumin in liposomes, and successfully started demonstrating its high efficacy in suppressing the PAK1-dependent growth of human colon and pancreatic cancer xenografts in mice: Curcumin in liposomes (20-40 mg/kg) suppresses the growth of these cancers by 50% (*35*,*36*). Furthermore, in 2009 an Indian group led by Sarasija Suresh at Nootan Dental College in Bangalore found that a chemically synthesized beta-dextrin derivative called hydroxypropyl-beta-CD would be the best among CDs to solubilize curcumin effectively and showed both its anti-angiogenic and anti-inflammatory effects *in vivo* (*37*). Thus, in the future (probably in a decade or so) curcumin in either liposomes or CDs would become available on the market for the therapy of these cancers and NF tumors as well.

2.5. *Berberine*

An old Chinese or American Indian herb called "Goldenseal" (or "Yellow Root") contains an anti-cancer and anti-inflammatory yellow-colored bitter-tasting alkaloid called Berberine. Unlike the majority of anti-cancer products, Berberine is relatively water-soluble, and its tannic acid salt (Berberine Tannate) is tasteless and used clinically world-wide as an antibiotic for the therapy of infectious diseases such as malaria. In 1990, an NCI group found that Berberine (0.1 mg/mL) induces a morphological differentiation of RAS-transformed teratocarcinoma cells *in vitro* (*38*). However, the molecular mechanism underlying its anti-cancer action remained unknown until recently. In 2006 a French group of GlaxoSmith Kline (GSK) found that Berberine (100 mg/kg, daily) blocks both the synthesis and accumulation of fats such as cholesterol in mice by activating the tumor suppressive kinase AMPK (*39*). In 2009 a Chinese group at Hong Kong University found that Berberine inactivates both RAC and CDC42, thereby inactivating PAK1 (*40*). Thus, like propolis and other anti-PAK1 products, this alkaloid would be useful for the treatment of PAK1-dependent cancers, NF and inflammatory diseases such as arthritis and asthma as well as type 2 diabetes and obesity. However, its minimum effective daily dose for cancer/NF patients still remains to be determined.

3. Anti-PAK1 drugs

3.1. *The ring peptide FK228*

Around 1993, the most potent anti-cancer antibiotic was developed by a Japanese group of Fujisawa Pharmaceuticals (later renamed "Astellas"). This antibiotic is a unique ring peptide called FK228 which suppresses the growth of RAS cancers such as pancreatic and colon cancer cells with the IC_{50} around 1 nM (*41*). Since these solid cancers require PAK1 for their growth, it has been suspected that FK228 might block the oncogenic PAK1 signaling somehow. In 1998, the direct target of this compound was identified by Minoru Yoshima at Tokyo University and the Fujisawa group jointly. FK228 inhibits histone deacetylase (HDAC) whose zinc metal in the catalytic center is covalently bound to FK228, only when FK228 is reduced by the intracellular glutathione (GSH) which breaks the di-sulfide bond of FK228 molecule (*42*). In other words, FK228 is a prodrug which is activated by GSH. Once histones on chromatin are acetylated, owing FK228, a specific group of genes would be activated. One of them is *p21/WAF1* gene encoding an inhibitor of cyclin-dependent kinases (CDKs). How does FK228 block the oncogenic RAS signaling by activating this gene? Well, RAS activates cyclin D1, an activator of CDKs, through PI3-kinase and PAK1, shifting cell cycle from G1 to S phase. In other words, FK228 blocks the oncogenic RAS-PAK1 signaling by activating *p21* gene which produces the antagonist of RAS-activated cyclin D1. This also means that FK228 blocks downstream of PAK1. However, we suspected another mechanism must be involved in the anti-RAS action of FK228, because we found that FK228 suppresses completely the growth of both *NF1*-deficient (MPNST) and *NF2*-deficient cancer cells even at 100 pM (*43*). In the case of breast cancer cells, the IC_{50} of FK228 is around 5 pM. Around 2005, we found that FK228 inactivates PAK1 with IC_{50} below 1 nM (*44*), indicating that FK228 blocks both upstream and downstream of PAK1. Having been encouraged with these findings, we examined whether FK228 suppresses the growth of human MPNST xenograft in mice. Yes, it shrank MPNST completely at the dose of 2.5 mg/kg, twice a week, *i.p.*, for two months (*43*). Unfortunately, however, FK228 turned out not to pass the blood brain barrier (BBB), so that it would not be effective on brain tumors associated with NF1 or NF2. Nevertheless, in principle, it would be effective in suppressing MPNST, plexi-form and dermal neurofibromas of NF1 patients, when this drug becomes available some days in the future. Currently, a US company called "Celgene" is taking the full responsibility for the on-going clinical trials (phase 2) of this compound mainly for CTCL (cutaneous T-cell lymphoma), a rare cancer which represents less than 0.1% of all cancers.

3.2. *UnPAK309 (PF3758309)*

In 2010, Brion Murray's team at Pfizer Oncology in San Diego developed a new compound called PF3758309 (in short UnPAK 309) that selectively inhibits PAK family kinases including PAK1 with the IC_{50} between 5-15 nM in cells (*45*). *In vivo* (cancer xenografts in nude mice), this compound suppresses the growth of several PAK1-depepndent human cancers such as breast, colon and lung cancers as well as melanoma at daily doses around 20 mg/kg. Since its derivatives pass the BBB, it is likely that UnPAK309 could be potentially useful for the treatment of brain tumors such as glioma, and tuberous sclerosis (NF/TSC) tumors in the future. However, as UnPAK309 clinical trials have just started, in realty it would take several years until this potent PAK inhibitor becomes available on the market for cancer/NF patients.

Another thing worth being pointed out here would be that unlike CAPE and other anti-PAK1 compounds, UnPAK309 directly inhibits AMPK (the IC_{50} in cells around 40 nM) as well, instead of activating AMPK (*45*). Thus, this drug would be the first clear exception for the rule that all anti-PAK1 drugs are AMPK activators.

3.3. *Combination of two Tyr-kinase inhibitors, PP1 and AG 879/GL-2003*

For the following two reasons, we have recently developed our own anti-PAK1 drugs which pass the BBB. FK228 tends to develop the multi-drug resistance

(MDR) by activating MDR genes at least in pancreatic and breast cancers. As mentioned earlier, FK228 fails to pass the BBB. Our best choice was the combination of two distinct Tyr-kinase inhibitors, PP1 and GL-2003 (a water soluble hexylamine derivative of AG 879). This combination blocks two distinct Tyr-kinases, namely SRC family kinases and ETK, essential for the full activation of PAK1 (*46,47*). At dose (20 mg/kg, twice a week, *i.p.*) of each drug, the growth of both FK228-resistant human pancreatic and breast cancer xenografts in mice was almost completely suppressed without any adverse effect (*48*). Since the target kinases of these two drugs are clearly distinct, the chance for cancers to develop the MDR to both drugs would be mathematically null. Furthermore, recently a water-soluble (hexylamine) derivative of PP1 called GL-2010 has also been developed by my former colleagues at WEHI, and a clinical trial of this new combination (GL-2003 and GL-2010) is expected to start in a not-too-distant future.

3.4. *Ivermectin*

In 2004 a Russian group in Moscow reported that an old, safe and inexpensive antibiotic called "Ivermectin" has an anti-cancer property *in vivo* (human cancer xenografts in mice): Ivermectin (3-5 mg/kg, daily) almost completely suppresses the growth of a few distinct human cancers such as melanoma (*49*). However, its anti-cancer mechanism at molecular levels remained entirely unknown. This antibiotic was developed during 1980s jointly by Merck in US and Kitasato Institute in Tokyo as an anti-parasitic drug that kills selectively intestinal nematodes by blocking GABA receptor, but not mammalian counterparts (*50*). A very low dose such as 0.2 mg/kg (taken orally once or twice) is sufficient to eliminate these parasites completely. Since the anti-cancer action works on mammalian cancer cells, it is obvious that its target is not mammalian GABA receptor. Then one day I noticed that this drug has been known for some years to reduce dramatically (by around 90%) the litter size (the number of eggs laid) of *C. elegans*, a non-pathogenic tiny nematode living in nature (woods). This effect happened to be the precisely same as what CAPE or ARC does on the same worm, as discussed later in detail. So I was prompted to examine whether Ivermectin blocks the oncogenic PAK1 signaling or not. Besides it has been known that melanomas require PAK1 for their growth, as the anti-PAK drug FK228 blocks the growth of melanoma. In collaboration with Tamotsu Sudo's group at Hyogo Cancer Center in Japan, we have confirmed that Ivermectin indeed inactivates PAK1, and blocks the PAK1-dependent growth of both human ovarian cancer and NF2 tumor (Schwannoma) cells with the IC_{50} around 5 μM (*51,52*). Thus, this inexpensive old drug could be used as an alternative if cancer/NF patients happen to be among 1% of population who show an allergic skin reaction to the CAPE-based propolis such as "Bio 30", or resistant to any propolis.

4. PAK1 blockers = AMPK activators

As briefly mentioned before, more than a dozen of anti-PAK1 compounds such as CAPE, curcumin, apigenin, berberine, resveratrol, emodin, salidroside, capsaicin/capsiate, OSU-03012, GW2974, metformin, *etc.* are known to activate the tumor suppressing kinase AMPK (see Table 2). AMPK is an AMP-activated kinase that is activated when the AMP/ATP ratio increases. This ratio rises when the cellular glucose/ATP level drops, due to either calorie restriction (CR), fast or extensive physical exercise. Thus, AMPK serves as a sensor of the cellular glucose/ATP level, and is activated in an AMPK-dependent manner. In most cases, these PAK1 blockers activate AMPK through another tumor suppressing kinase called LKB1 which phosphorylates Thr 172 of AMPK (*31*). AMPK phosphorylates several distinct proteins. As mentioned before, one of them is the glucose transporter protein "GLUT-4", which is essential for the cellular uptake of glucose from blood stream. AMPK activates GLUT-4, leading to a transient rise of the cellular glucose level by lowering the blood glucose level. Another target of AMPK is the tumor suppressing transcription factor "FOXO" which is essential for the longevity (*53*). In mammals, AMPK activates FOXO, while PAK1 inactivates FOXO (*54,55*). Thus, most of PAK1 blockers such as CAPE and curcumin activate FOXO through these two distinct routes in a concerted manner. At least two PAK1 blockers = AMPK activators, salidroside and curcumin, were shown to extend the life span of *C. elegans* and the fruit fly Drosophila, respectively (*56,57*). Among the major target genes of FOXO is *HSP16* gene encoding a small heat shock protein, and this "FOXO-HSP16" signaling pathway extends the life span of this

Table 2. Anti-PAK1 and AMPK activating activity of anti-PAK1 products

Products	PAK1 inactivation	AMPK activation	Ref.
CAPE	+	+	*6, 32*
Curcumin	+	+	*33, 34*
Resveratrol	+	+	*95, 96*
Berberine	+	+	*37, 40*
Salidroside	+	+	*31, 94*
Metformin	+	+	*31, 93*
Emodin	+	+	*91, 92*
Capsaicin	+	+	*89, 90*
Apigenin	+	+	*82, 83*
OSU-03012	+	+	*84, 85*
GW2974	+	+	*61*
AG 879	+	+	*86, 87*
AICAR	?	+	*88*
UnPAK309	+	-	*45*

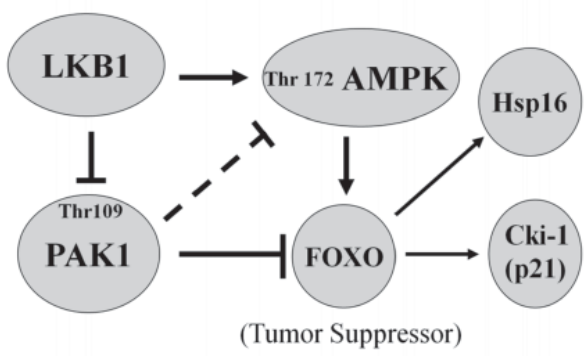

Figure 9. "LKB1" controls both AMPK and PAK1.

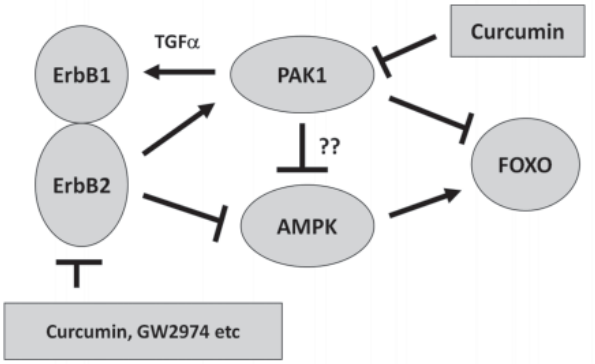

Figure 10. "PAK1-ErbB2" cycle that blocks AMPK.

worm by 50% (*58*). We found that PAK1 blockers from propolis, CAPE and ARC, strongly activate *HSP16* gene in *C. elegans* shortly after the heat shock, and make this worm heat resistant as does the *PAK1*-deficient mutation (*6*). For heat shock proteins in general protect the essential cellular proteins from their denaturation caused by heat (*59*). Thus, it is most likely that both CAPE-based propolis and ARC-based propolis extracts would extend the life span of this worm, and probably all human beings including cancer/NF patients as well, by activating this longevity (FOXO-HSP16) signaling pathway. The higher this signaling, the longer we live.

Why do most of PAK1 blockers turn out to be AMPK activators, or *vice versa*? One of the mechanisms underlying this "mysterious" formula/equation, PAK1 blockers = AMPK activators, is that LKB1 inactivates PAK1 by phosphorylating Thr 109, as well as activating AMPK simultaneously (*31*) (see Figure 9). A second mechanism is based on the vicious oncogenic cycle that PAK1 and the Tyr-kinase ErbB2 form: PAK1 activates another Tyr-kinase called ErbB1 by up-regulating its ligands such as TGFα, which activate ErbB1, and in turn ErbB1 activates ErbB2 by forming a hetero-dimer (*46,60*). Both ErbB1 and ErbB2 are essential for the full activation of PAK1 (*46*). Furthermore, ErbB2 somehow inactivates AMPK (*61*). Thus, if either PAK1 or ErbB2 is blocked, this oncogenic cycle would stop, and AMPK would be reactivated (Figure 10).

5. A "fluorescent" *in vivo* screening for NF therapeutics

Generally speaking, to get the FDA-approval for clinical trials of a given anti-cancer/NF drug under the development, one has to prove its therapeutic effect as well as its safety (non-toxicity) *in vivo*, using a human cancer (or NF1/NF2 tumor) grafted in mice or rats. However, it would cost both time and money to screen for the most effective and safe drug(s) from a huge library of thousands test samples. Here I shall introduce a quick and inexpensive *in vivo* screening system, using a tiny transparent nematode (*C. elegans*) strain called CL2070 which carries the transgenic fusion gene "*HSP16-GFP*" (*62*), in order to screen for a series of PAK1 blockers = AMPK activators which would be useful for NF therapy.

This fusion gene would mass-produce the S65T mutant of green fluorescent protein (GFP) from a jelly fish when the promoter of *C. elegans HSP16* gene is activated by the heat shock treatment (at 35ºC for 2 h) (*62,63*). However, the expression of GFP takes place rather slowly, and reaches the maximum during 12-24 h after the heat shock, in the absence of a PAK1 blocker = AMPK activator, because normally PAK1 suppresses the *HSP16* gene by inactivating the "FOXO" in this worm as in mammals (*6,55*). When this worm is treated with PAK1 blockers such as CAPE, ARC and Ivermectin overnight, the expression of GFP reaches the maximum in a few hours after the heat shock, and the whole transparent worm glows greenly under the blue light (*6*). Of course these PAK1 blockers cause no side (adverse) effect. Instead they make this worm much more heat-tolerant by over-production of heat shock proteins such as HSP16 (*6*). Furthermore, these PAK1 blockers reduce the litter size (the number of eggs laid) of this worm by 85-90%, although all eggs are hatchable (*6*). These phenotypes are precisely the same as those of the *PAK1*-deficient mutant (RB689) of this worm (*6*). Since the more HSP16 is activated, the longer this worm lives (*63*), it is most likely that like salidroside and curcumin (*56,57*), all these PAK1 blockers would extend significantly the life span of this worm. Furthermore, as described earlier, CA is a precursor of CAPE, and blocks PAK1 by down-regulating its activator RAC (*14*). During 2010-2011 a German group at Humboldt University in Berlin found that CA and its covalently-linked dimer called Rosmarinic acid (RA) significantly extend the life span of this worm through "FOXO", and activate heat shock genes such as *HSP12*, inducing the heat-tolerance (*64*). Since RA is a CAPE derivative (3,4-dihydroxyl, 8-carboxyl) as well as CA dimer, it is most likely that CAPE and many other PAK1 blockers would execute such a life-prolonging (so-called 'elixir') effect.

Interestingly, more than two decades ago, Tom Johnson's group at University of Colorado showed that dysfunction of PI3-kinase/Age-1 in this worm lengthens

the life span by 100% and reduces significantly the litter size (*65*). Furthermore, dysfunction of AKT is also known to lengthen the life span of this worm, but just by 50% (*66*). Since both AKT and PAK1 are downstream of PI3-kinase, inactivating "FOXO", it is most likely that dysfunction of PAK1 would contribute to the remaining 50% extension of life span. As described earlier, the "FOXO-HSP16" signaling pathway indeed extends the life span of this worm by 50% (*58*). However, we have not confirmed directly this notion as yet, because unlike the HSP16-GFP/litter size/thermal endurance tests which take only a few days, the longevity test would take 15-30 days, and is technically far more laborious. Of course, if one tries to compare the life span between the *PAK1*-deficient healthy mice (*67*) and the control, the test would take 3-6 years, clearly more than 50 times longer than this worm (RB689 mutant *versus* the control).

In my opinion, the above "fluorescent" GFP assay would be the best approach to the systematic screen for PAK1 blockers (AMPK activators), as this system could be automated by linking a number of 96-well plates containing CL2070 treated with a variety of test compounds to a fluorescence reader for the quantitative analysis. The more fluorescence, the stronger anti-cancer property. Once a few "best" (the most potent) candidates are selected out through this system to make a short list of cancer/NF therapeutics, their anti-cancer/NF property should be re-confirmed by means of the "conventional" xenograft in nude mice, to convince the FDA or cancer/NF patients for their final approval.

6. PAK1-dependent non-cancerous diseases

So far at least 70% of all human cancers, in particular solid tumors, have been identified as PAK1-dependent tumors: Among them are pancreatic, colon, breast, prostate, gastric, lung, cervical, ovarian, and thyroid cancers as well as glioma, melanoma, hepatoma, *NF2*-deficient mesothelioma, MM and MPNST, a malignant NF1 tumor. In addition, the remaining 90% of NF1 tumors, NF2 tumors (meningioma and schwannoma) and TSC tumors also require PAK1 for their growth. TSC is caused by dysfunction of the tumor suppressors TSC1 or TSC2, which leads to the abnormal activation of the oncogenic kinase "TOR" (*68*). Although the "TOR" could be blocked by Rapamycin and its derivatives such as "Afinitor" (*68-71*), they are extremely expensive and cause at least a few rather serious side effects such as immuno-suppression and hypertension. Thus, these TOR inhibitors would not be so suitable for a life-long treatment of TSC. However, since TOR requires PAK1 for the full activation, as curcumin blocks the oncogenic TOR-Raptor interaction (*72*), PAK1 blockers such as propolis could be used for the TSC therapy. In fact, the CAPE-based propolis "Bio 30" stops even the drug-resistant epilepsy often associated with TSC children in our human trials.

In addition, there are several other non-cancerous diseases which also require PAK1 (*67,68,73-81*): Among them are AIDS (HIV-infection), malaria, Alzheimer's (AD), Huntington's (HD), inflammatory diseases (asthma and arthritis), insulin-resistant diabetes (type 2), obesity, hypertension, seizures such as epilepsy, autistic diseases such as Fragile X syndrome, and learning deficit (LD). Thus, propolis and other PAK1 blockers would serve as the effective (and safe) therapeutics for these diseases as well. So it would not be a big surprise if these anti-PAK1 products such as propolis and the "bitter" melons extend our life span in good health by either curing or delaying some of these PAK1-dependent diseases including cancers and NF throughout our entire life.

Acknowledgements

The author is very grateful to the following funding sources for their generous support of his NF research: Ludwig Institute for Cancer Research, DFG (Deutche Forschungs Gemeinschaft), Denise Terrill Classic Award/Texas NF Foundation, NF Cure Japan, Peterson Foundation (Philadelphia), Yamada Bee Farm and Neurofibromatosis Inc. Also the author is in debt of the following colleagues of his: Drs. Tony Burgess, Yumiko Hirokawa, Hong He, Anjali Tikoo, Shanta Messerli, Maria Demestre, Lan Kluwe, Victor Mautner, Ken Hashimoto, Keiji Terao and Yuan Luo for their great team work. Finally, this review is dedicated to two young NF patients, Ms. Denise Terrill (1976-2009) and Mr. Louis Lee (1994-2009) who prompted the author in 2002 to start his research effort for developing the first effective NF therapeutics, but unfortunately died of the complications associated with their NF2 in the summer of 2009.

References

1. Maruta H, Burgess AW. Regulation of the Ras signalling network. Bioessays. 1994; 16:489-496.
2. Starinsky-Elbaz S, Faigenbloom L, Friedman E, Stein R, Kloog Y. The pre-GAP-related domain of neurofibromin regulates the cell migration through the LIM kinase/cofillin pathway. Mol Cell Neurosci. 2009; 42:278-287.
3. Tang Y, Marwaha S, Rutkowski JL, Tennekoon GI, Phillips PC, Field J. A role for Pak protein kinases in Schwann cell transformation. Proc Natl Acad Sci U S A. 1998; 95:5139-5144.
4. Hirokawa Y, Tikoo A, Huynh J, Utermark T, Hanemann CO, Giovannini M, Xiao GH, Testa JR, Wood J, Maruta H. A clue to the therapy of neurofibromatosis type 2: NF2/merlin is a PAK1 inhibitor. Cancer J. 2004; 10:20-26.
5. Lammert M, Friedman JM, Roth HJ, Friedrich RE, Kluwe L, Atkins D, Schooler T, Mautner VF. Vitamin D deficiency associated with number of neurofibromas in neurofibromatosis 1. J Med Genet. 2006; 43:810-813.
6. Maruta, H. An innovated approach to *in vivo* screening for the major anti-cancer drugs. In: Horizons in Cancer

Research. Nova Science Publishers, Melbourne, Australia, 2010; pp. 249-259.
7. Kiosses WB, Hood J, Yang S, Gerritsen ME, Cheresh DA, Alderson N, Schwartz MA. A dominant-negative p65 PAK peptide inhibits angiogenesis. Circ Res. 2002; 90:697-702.
8. Knobloch J, Schmitz I, Götz K, Schulze-Osthoff K, Rüther U. Thalidomide induces limb anomalies by PTEN stabilization, Akt suppression and stimulation of caspase-dependent cell death. Mol Cell Biol. 2008; 28:529-538.
9. Richardson PG, Schlossman RL, Weller E, *et al*. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. Blood. 2002; 100:3063-3067.
10. Grunberger D, Banerjee R, Eisinger K, Oltz EM, Efros L, Caldwell M, Estevez V, Nakanishi K. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Experientia. 1988; 44:230-232.
11. Aga M, Shibuya T, Sugimoto T, Kurimoto M, Nakajima SH. Isolation and identification of anti-microbial compounds in Brazilian propolis. Biosci Biotechnol Biochem. 1994; 58:945-946.
12. Uto Y, Hirata A, Fujita T, Takubo S, Nagasawa H, Hori H. First total synthesis of artepillin C established by *O*,*O*'-diprenylation of *p*-halophenols in water. J Org Chem. 2002; 67:2355-2357.
13. Awale S, Li F, Onozuka H, Esumi H, Tezuka Y, Kadota S. Constituents of Brazilian red propolis and their preferential cytotoxic activity against human pancreatic PANC-1 cancer cell line in nutrient-deprived condition. Bioorg Med Chem. 2008; 16:181-189.
14. Xu JW, Ikeda K, Kobayakawa A, Ikami T, Kayano Y, Mitani T, Yamori Y. Downregulation of Rac1 activation by caffeic acid in aortic smooth muscle cells. Life Sci. 2005; 76:2861-2872.
15. Messerli SM, Ahn MR, Kunimasa K, Yanagihara M, Tatefuji T, Hashimoto K, Mautner V, Uto Y, Hori H, Kumazawa S, Kaji K, Ohta T, Maruta H. Artepillin C (ARC) in Brazilian green propolis selectively blocks the oncogenic PAK1 signaling and suppresses the growth of NF tumors in mice. Phytother Res. 2009; 23:423-427.
16. Demestre M, Messerli SM, Celli N, Shahhossini M, Kluwe L, Mautner V, Maruta H. CAPE (caffeic acid phenethyl ester)-based propolis extract (Bio 30) suppresses the growth of human neurofibromatosis (NF) tumor xenografts in mice. Phytother Res. 2009; 23:226-230.
17. Kumar MA, Nair M, Hema PS, Mohan J, Santhoshkumar TR. Pinocembrin triggers Bax-dependent mitochondrial apoptosis in colon cancer cells. Mol Carcinog. 2007; 46:231-241.
18. Bestwick CS, Milne L. Influence of galangin on HL-60 cell proliferation and survival. Cancer Lett. 2006; 243:80-89.
19. Miyamoto S, Kohno H, Suzuki R, Sugie S, Murakami A, Ohigashi H, Tanaka T. Preventive effects of chrysin on the development of azoxymethane-induced colonic aberrant crypt foci in rats. Oncol Rep. 2006; 15:1169-1173.
20. Kuo ML, Lin JK, Huang TS, Yang NC. Reversion of the transformed phenotypes of v-H-*ras* NIH3T3 cells by flavonoids through attenuating the content of phosphotyrosine. Cancer Lett. 1994; 87:91-97.
21. Huang MT, Smart RC, Wong CQ, Conney AH. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res. 1988; 48:5941-5946.
22. Maruta H, Messerli S. In: PAK and its blockers that control our life (Maruta H, ed.). Cell Logistic, 2012. (in press)
23. Izuta H, Shimazawa M, Tsuruma K, Araki Y, Mishima S, Hara H. Bee products prevent VEGF-induced angiogenesis in human umbilical vein endothelial cells. BMC Complement Altern Med. 2009; 9:45.
24. Terao, K, Maruta H. In: CD complex of NZ propolis for improving its anti-cancer property. Heart Publishers, Tokyo, Japan, 2010.
25. Widjaja A, Yeh TH, Ju YH. Enzymatic synthesis of caffeic acid phenethyl ester. J Chin Inst Chem Engrs. 2008; 39:413-418.
26. Chen HC, Ju HY, Twu YK, Chen JH, Chang CM, Liu YC, Chang C, Shieh CJ. Optimized enzymatic synthesis of CAPE by RSM. N Biotechnol. 2010; 27:89-93.
27. Hirokawa Y, Nheu T, Grimm K, Mautner V, Maeda S, Yoshida M, Komiyama K, Maruta H. Sichuan pepper extracts block the PAK1/cyclin D1 pathway and the growth of *NF1*-deficient cancer xenograft in mice. Cancer Biol Ther. 2006; 5:305-309.
28. Jilka C, Strifler B, Fortner GW, Hays EF, Takemoto DJ. *In vivo* anti-tumor activity of the bitter melon (*Momordica charantia*). Cancer Res. 1983; 43:5151-5155.
29. Tan MJ, Ye JM, Turner N, Hohnen-Behrens C, Ke CQ, Tang CP, Chen T, Weiss HC, Gesing ER, Rowland A, James DE, Ye Y. Anti-diabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. Chem Biol. 2008; 15:263-273.
30. Russell RR 3rd, Bergeron R, Shulman GI, Young LH. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. Am J Physiol. 1999; 277:H643-H649.
31. Deguchi A, Miyoshi H, Kojima Y, Okawa K, Aoki M, Taketo MM. LKB1 suppresses p21-activated kinase-1 (PAK1) by phosphorylation of Thr109 in the p21-binding domain. J Biol Chem. 2010; 285:18283-18290.
32. Lee ES, Uhm KO, Lee YM, Han M, Lee M, Park JM, Suh PG, Park SH, Kim HS. CAPE (caffeic acid phenethyl ester) stimulates glucose uptake through AMPK (AMP-activated protein kinase) activation in skeletal muscle cells. Biochem Biophys Res Commun. 2007; 361:854-858.
33. Pan W, Yang H, Cao C, Song X, Wallin B, Kivlin R, Lu S, Hu G, Di W, Wan Y. AMPK mediates curcumin-induced cell death of CaOV3 ovarian cancer cells. Oncol Rep. 2008; 20:1553-1559.
34. Cai XZ, Wang J, Li XD, Wang GL, Liu FN, Cheng MS, Li F. Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. Cancer Biol Ther. 2009; 8:1360-1368.
35. Li L, Braiteh FS, Kurzrock R. Liposome-encapsulated curcumin: *In vitro* and *in vivo* effects on proliferation, apoptosis, signaling, and angiogenesis. Cancer. 2005; 104:1322-1331.
36. Mach CM, Mathew L, Mosley SA, Kurzrock R, Smith JA. Determination of minimum effective dose and optimal dosing schedule for liposomal curcumin in a xenograft human pancreatic cancer model. Anticancer Res. 2009; 29:1895-1899.
37. Yadav VR, Suresh S, Devi K, Yadav S. Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech. 2009; 10:752-762.
38. Chang KS, Gao C, Wang LC. Berberine-induced morphologic differentiation and down-regulation of c-Ki-*ras*2 protooncogene expression in human teratocarcinoma

cells. Cancer Lett. 1990; 55:103-108.
39. Brusq JM, Ancellin N, Grondin P, Guillard R, Martin S, Saintillan Y, Issandou M. Inhibition of lipid synthesis through activation of AMP kinase: An additional mechanism for the hypolipidemic effects of berberine. J Lipid Res. 2006; 47:1281-1288.
40. Tsang CM, Lau EP, Di K, Cheung PY, Hau PM, Ching YP, Wong YC, Cheung AL, Wan TS, Tong Y, Tsao SW, Feng Y. Berberine inhibits Rho GTPases and cell migration at low doses but induces G2 arrest and apoptosis at high doses in human cancer cells. Int J Mol Med. 2009; 24:131-138.
41. Ueda H, Manda T, Matsumoto S, Mukumoto S, Nishigaki F, Kawamura I, Shimomura K. FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* No. 968. III. Antitumor activities on experimental tumors in mice. J Antibiot (Tokyo). 1994; 47:315-323.
42. Nakajima H, Kim YB, Terano H, Yoshida M, Horinouchi S. FR901228, a potent antitumor antibiotic, is a novel histone deacetylase inhibitor. Exp Cell Res. 1998; 241:126-133.
43. Hirokawa Y, Nakajima H, Hanemann CO, Kurtz A, Frahm S, Mautner V, Maruta H. Signal therapy of *NF1*-deficient tumor xenograft in mice by the anti-PAK1 drug FK228. Cancer Biol Ther. 2005; 4:379-381.
44. Hirokawa Y, Arnold M, Nakajima H, Zalcberg J, Maruta H. Signal therapy of breast cancer xenograft in mice by the HDAC inhibitor FK228 that blocks the activation of PAK1 and abrogates the tamoxifen-resistance. Cancer Biol Ther. 2005; 4:956-960.
45. Murray BW, Guo C, Piraino J, *et al.* Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. Proc Natl Acad Sci U S A. 2010; 107:9446-9451.
46. He H, Hirokawa Y, Manser E, Lim L, Levitzki A, Maruta H. Signal therapy for RAS induced cancers in combination of AG 879 and PP1, specific inhibitors for ErbB2 and Src family kinases, that block PAK activation. Cancer J. 2001; 7:191-202.
47. He H, Hirokawa Y, Gazit A, Yamashita Y, Mano H, Kawakami Y, Kawakami T, Hsieh CY, Kung HJ, Lessene G, Baell J, Levitzki A, Maruta H. The Tyr-kinase inhibitor AG879, that blocks the ETK-PAK1 interaction, suppresses the RAS-induced PAK1activation and malignant transformation. Cancer Biol Ther. 2004; 3:96-101.
48. Hirokawa Y, Levitzki A, Lessene G, Baell J, Xiao Y, Zhu H, Maruta H. Signal therapy of human pancreatic cancer and *NF1*-deficient breast cancer xenograft in mice by a combination of PP1 and GL-2003, Anti-PAK1 Drugs (Tyr-kinase Inhibitors). Cancer Lett. 2007; 245:242-251.
49. Drinyaev VA, Mosin VA, Kruglyak EB, Novik TS, Sterlina TS, Ermakova NV, Kublik LN, Levitman MKh, Shaposhnikova VV, Korystov YN. Antitumor effect of avermectin. Eur J Pharmacol. 2004; 501:19-23.
50. Omura S, Crump A. The life and times of ivermectin – a success story. Nature Rev Microbiol. 2004; 2:984-989.
51. Hashimoto H, Messerli S, Sudo T, Maruta H. Ivermectin inactivates the kinase PAK1 and block the PAK1-dependent growth of human ovarian cancer and NF2 tumor cell lines. Drug Discov Ther. 2009; 3:243-246.
52. Hashimoto H, Sudo T, Maruta H, Nishimura R. The direct PAK1 inhibitor, TAT-PAK18, blocks preferentially the growth of human ovarian cancer cell lines in which PAK1 is abnormally activated by autophosphorylation at Thr 423. Drug Discov Ther. 2010; 4:1-4.
53. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. Nature. 1993; 366:461-464.
54. Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, Gygi SP, Brunet A. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. Curr Biol. 2007; 17:1646-1656.
55. Vadlamudi R, Kumar R. p21-activated kinase 1: An emerging therapeutic target. Cancer Treat Res. 2004; 119:77-88.
56. Wiegant FA, Surinova S, Ytsma E, Langelaar-Makkinje M, Wikman G, Post JA. Plant adaptogens increase lifespan and stress resistance in *C. elegans*. Biogerontology. 2009; 10:27-42.
57. Lee KS, Lee BS, Semnani S, Avanesian A, Um CY, Jeon HJ, Seong KM, Yu K, Min KJ, Jafari M. Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in *Drosophila melanogaster*. Rejuven Res. 2010; 13:561-570.
58. Walker GA, White TM, McColl G, Jenkins NL, Babich S, Candido EP, Johnson TE, Lithgow GJ. Heat shock protein accumulation is upregulated in a long-lived mutant of *C. elegans*. J Gerontol A Biol Sci Med Sci. 2001; 56: B281-B287.
59. Engelberg D, Zandi E, Parker CS, Karin M. The yeast and mammalian Ras pathways control transcription of heat shock genes independently of heat shock transcription factor. Mol Cell Biol. 1994; 14:4929-4937.
60. Goldman R, Levy RB, Peles E, Yarden Y. Heterodimerization of the erbB-1 and erbB-2 receptors in human breast carcinoma cells: a mechanism for receptor transregulation. Biochemistry. 1990; 29:11024-11028.
61. Spector NL, Yarden Y, Smith B, Lyass L, Trusk P, Pry K, Hill JE, Xia W, Seger R, Bacus SS. Activation of AMP-activated protein kinase by human EGF receptor 2/EGF receptor tyrosine kinase inhibitor protects cardiac cells. Proc Natl Acad Sci U S A. 2007; 104:10607-10612.
62. Link CD, Cypser JR, Johnson CJ, Johnson TE. Direct observation of stress response in *Caenorhabditis elegans* using a reporter transgene. Cell Stress Chaperones. 1999; 4:235-242.
63. Rea SL, Wu D, Cypser JR, Vaupel JW, Johnson TE. A stress-sensitive reporter predicts longevity in isogenic propoulations of *Caenorhabditis elegans*. Nat Genet. 2005; 37:894-898.
64. Pietsch K, Saul N, Chakrabarti S, Stürzenbaum SR, Menzel R, Steinberg CE. Hormetins, antioxidants and prooxidants: Defining quercetin-, caffeic acid- and rosmaniric acid-mediated life extension in *C. elegans*. Biogerontol. 2011; 12:329-347.
65. Friedman DB, Johnson TE. A mutation in the *age-1* Gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. Genetics. 1988; 118:75-86.
66. Vanfleteren JR, Braeckman BP. Mechanisms of life span determination in *Caenorhabditis elegans*. Neurobiol Aging. 1999; 20:487-502.
67. Allen JD, Jaffer ZM, Park SJ, *et al.* p21-activated kinase regulates mast cell degranulation *via* effects on calcium mobilization and cytoskeletal dynamics. Blood. 2009; 113:2695-2705.
68. Wong M. Mammalian target of rapamycin (mTOR) inhibition as a potential antiepileptogenic therapy: From tuberous sclerosis to common acquired epilepsies. Epilepsia. 2010; 51:27-36.
69. Manning BD, Cantley LC. Rheb fills a GAP between TSC

and TOR. Trends Biochem Sci. 2003; 28:573-576.
70. Kenerson H, Dundon TA, Yeung RS. Effects of rapamycin in the Eker rat model of tuberous sclerosis complex. Pediatr Res. 2005; 57:67-75.
71. Yalon M, Ben-Sira L, Constantini S, Toren A. Regression of subependymal giant cell astrocytomas with RAD001 (Everolimus) in tuberous sclerosis complex. Childs Nerv Syst. 2011; 27:179-181.
72. Beevers CS, Chen L, Liu L, Luo Y, Webster NJ, Huang S. Curcumin disrupts the mammalian target of rapamycin-raptor complex. Cancer Res. 2009; 69:1000-1008.
73. Nishida K, Komiyama T, Miyazawa S, Shen ZN, Furumatsu T, Doi H, Yoshida A, Yamana J, Yamamura M, Ninomiya Y, Inoue H, Asahara H. Histone deacetylase inhibitor suppression of autoantibody-mediated arthritis in mice *via* regulation of $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ expression. Arthritis Rheum. 2004; 50:3365-3376.
74. Hayashi ML, Rao BS, Seo JS, Choi HS, Dolan BM, Choi SY, Chattarji S, Tonegawa S. Inhibition of p21-activated kinase rescues symptoms of fragile X syndrome in mice. Proc Natl Acad Sci U S A. 2007; 104:11489-11494.
75. Nguyen DG, Wolff KC, Yin H, Caldwell JS, Kuhen KL. "UnPAKing" human immunodeficiency virus (HIV) replication: Using small interfering RNA screening to identify novel cofactors and elucidate the role of group I PAKs in HIV infection. J Virol. 2006; 80:130-137.
76. Luo S, Mizuta H, Rubinsztein DC. p21-activated kinase 1 promotes soluble mutant huntingtin self-interaction and enhances toxicity. Hum Mol Genet. 2008; 17:895-905.
77. Apostol BL, Simmons DA, Zuccato C, Illes K, Pallos J, Casale M, Conforti P, Ramos C, Roarke M, Kathuria S, Cattaneo E, Marsh JL, Thompson LM. CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice. Mol Cell Neurosci. 2008; 39:8-20.
78. Ma QL, Yang F, Calon F, Ubeda OJ, Hansen JE, Weisbart RH, Beech W, Frautschy SA, Cole GM. p21-activated kinase-aberrant activation and translocation in Alzheimer disease pathogenesis. J Biol Chem. 2008; 283:14132-14143.
79. Heredia L, Helguera P, de Olmos S, Kedikian G, Solá Vigo F, LaFerla F, Staufenbiel M, de Olmos J, Busciglio J, Cáceres A, Lorenzo A. Phosphorylation of actin-depolymerizing factor/cofilin by LIM-kinase mediates amyloid beta-induced degeneration: A potential mechanism of neuronal dystrophy in Alzheimer's disease. J Neurosci. 2006; 26:6533-6542.
80. Fonte V, Kipp DR, Yerg J 3rd, Merin D, Forrestal M, Wagner E, Roberts CM, Link CD. Suppression of *in vivo* beta-amyloid peptide toxicity by overexpression of the Hsp-16.2 small chaperon protein. J Biol Chem. 2008; 283:784-791.
81. Sicard A, Semblat JP, Doerig C, Hamelin R, Moniatte M, Dorin-Semblat D, Spicer JA, Srivastava A, Retzlaff S, Heussler V, Waters AP, Doerig C. Activation of PAK-MEK signaling pathway in malaria parasite-infected erythrocytes. Cell Microbiol. 2011; 13:836-845.
82. Way TD, Kao MC, Lin JK. Apigenin induces apoptosis through proteasomal degradation of HER2/*neu* in HER2/*neu*-overexpressing breast cancer cells *via* the phosphatidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem. 2004; 279:4479-4489.
83. Zang M, Xu S, Maitland-Toolan KA, Zuccollo A, Hou X, Jiang B, Wierzbicki M, Verbeuren TJ, Cohen RA. Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. Diabetes. 2006; 55:2180-2191.
84. Porchia LM, Guerra M, Wang YC, Zhang Y, Espinosa AV, Shinohara M, Kulp SK, Kirschner LS, Saji M, Chen CS, Ringel MD. 2-amino-*N*-{4-[5-(2-phenanthrenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]-phenyl} acetamide (OSU-03012), a celecoxib derivative, directly targets p21-activated kinase. Mol Pharmacol. 2007; 72:1124-1131.
85. Bai LY, Weng JR, Tsai CH, Sargeant A, Lin CW, Chiu CF. OSU-03012 sensitizes TIB-196 myeloma cells to imanitib mesylate *via* AMP-activated protein kinase and STAT3 pathways. Leuk Res. 2010; 34:816-820.
86. He H, Hirokawa Y, Manser E, Lim L, Levitzki A, Maruta H. Signal therapy for RAS-induced cancers in combination of AG 879 and PP1, specific inhibitors for ErbB2 and Src family kinases, that block PAK activation. Cancer J. 2001; 7:191-202.
87. He H, Hirokawa Y, Gazit A, Yamashita Y, Mano H, Kawakami Y, Kawakami T, Hsieh CY, Kung HJ, Lessene G, Baell J, Levitzki A, Maruta H. The Tyr-kinase inhibitor AG879, that blocks the ETK-PAK1 interaction, suppresses the RAS-induced PAK1activation and malignant transformation. Cancer Biol Ther. 2004; 3:96-101.
88. Sun Y, Connors K, Yang DQ. AICAR induces phosphorylation of AMPK in an ATM-dependent, LKB1-independent manner. Mol Cell Biochem. 2007; 306:239-245.
89. Shin DH, Kim OK, Jun HS, Kang MK. Inhibitory effect of capsaicin on B16-F10 melanoma cell migration *via* the phosphatidylinositol 3-kinase/Akt/Rac1 signal pathway. Exp Mol Med. 2008; 40:486-494.
90. Hwang JT, Park IJ, Shin JI, Lee YK, Lee SK, Baik HW, Ha J, Park OJ. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process *via* activating AMP-activated protein kinase. Biochem Biophys Res Commun. 2005; 338:694-699.
91. Huang Q, Shen HM, Ong CN. Emodin inhibits tumor cell migration through suppression of the phosphatidylinositol 3-kinase-Cdc42/Rac1 pathway. Cell Mol Life Sci. 2005; 62:1167-1175.
92. Xue J, Ding W, Liu Y. Anti-diabetic effects of emodin involved in the activation of PPARgamma on high-fat diet-fed and low dose of streptozotocin-induced diabetic mice. Fitoterapia. 2010; 81:173-177.
93. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001; 108:1167-1174.
94. Li HB, Ge YK, Zheng XX, Zhang L. Salidroside stimulated glucose uptake in skeletal muscle cells by activating AMP-activated protein kinase. Eur J Pharmacol. 2008; 588:165-169.
95. Waite KA, Sinden MR, Eng C. Phytoestrogen exposure elevates PTEN levels. Hum Mol Genet. 2005; 14:1457-1463.
96. Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. Proc Natl Acad Sci U S A. 2007; 104:7217-7222.

(Received September 30, 2011; Revised October 03, 2011; Accepted October 11, 2011)