PHYTOTHERAPY RESEARCH
Phytother. Res. **28**: 656–672 (2014)
Published online 14 August 2013 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5054

REVIEW

Herbal Therapeutics that Block the Oncogenic Kinase PAK1: A Practical Approach towards PAK1-dependent Diseases and Longevity

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Over 35 years research on PAKs, RAC/CDC42(p21)-activated kinases, comes of age, and in particular PAK1 has been well known to be responsible for a variety of diseases such as cancer (mainly solid tumors), Alzheimer's disease, acquired immune deficiency syndrome and other viral/bacterial infections, inflammatory diseases (asthma and arthritis), diabetes (type 2), neurofibromatosis, tuberous sclerosis, epilepsy, depression, schizophrenia, learning disability, autism, etc. Although several distinct synthetic PAK1-blockers have been recently developed, no FDA-approved PAK1 blockers are available on the market as yet. Thus, patients suffering from these PAK1-dependent diseases have to rely on solely a variety of herbal therapeutics such as propolis and curcumin that block PAK1 without affecting normal cell growth. Furthermore, several recent studies revealed that some of these herbal therapeutics significantly extend the lifespan of nematodes (*C. elegans*) and fruit flies (*Drosophila*), and PAK1-deficient worm lives longer than the wild type. Here, I outline mainly pathological phenotypes of hyper-activated PAK1 and a list of herbal therapeutics that block PAK1, but cause no side (harmful) effect on healthy people or animals. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: PAK1; cancer; neurofibromatosis; tuberous sclerosis; diabetes; Alzheimer's disease; AIDS; longevity; propolis; curcumin; King of Bitters; Thunder god vine.

INTRODUCTION

Since a team of Harold Varmus and Mike Bishop at UCSF found (Stehelin *et al*., 1976) that a constitutively activated mutant (v-SRC, found in an oncovirus called Rous Sarcoma Virus) of a normal cellular gene called c-SRC is the cause of chicken sarcoma, and its gene product was identified as a Tyr-kinase by two groups (Collett and Erikson, 1978; Sefton *et al*., 1981), a variety of Tyr-kinases have been identified as oncogenic kinases. However, these Tyr-kinases represent only less than 1% of the whole protein kinase family, and the remaining protein kinases are Ser/Thr-kinases such as PKA (cAMP-dependent kinase), PKB (AKT) and PKC.

More than 35 years ago, we found in a soil amoeba (*Acathamoeba castellanii*) a unique Ser/Thr kinase called MIHCK for myosin I heavy chain kinase (Maruta and Korn, 1977). Myosin I is an unconventional single-headed myosin and lacks the C-terminal tail that is essential for forming a bipolar filament. Unlike the conventional skeletal muscle myosin, which is double-headed myosin (Myosin II) and forms thick (bipolar) filaments, the Myosin I ATPase is not activated by actin filaments (F-actin), until the MIHCK phosphorylates its heavy chain. Myosin I is involved in membrane ruffling in the leading edge of amoeba, and the MIHCK triggers the amoeboid movement such as phagocytosis. Interestingly, like human platelet or smooth muscle myosin II light chain kinases (Adelstein & Conti, 1975, Chacko *et al*., 1977), MIHCK can phosphorylate smooth muscle myosin II light chain (MIILC) as well, and the MIILC phosphorylation triggers the actin activation of this myosin II ATPase, leading to smooth muscle contraction of blood vessel and stomach.

The first mammalian homologue of the amoeba MIHCK called PAK (for p21-activated kinase) was cloned by Ed Manser's group at Singapore National University (Manser *et al*., 1994), and they found that the RAS-related GTPases (p21) called RAC/CDC42 are its direct activators. Up to now, six members of PAK family have been cloned in mammals including human, and PAK1-3 belong to group 1, whereas PAK4-6 belong to group 2 (for review, see Knapp, 2013). Group 1 PAKs are activated by both RAC and CDC42, whereas group 2 PAKs are activated only by CDC42. Furthermore, only group 1 PAKs have a unique Pro-rich motif (residues 186–203 of PAK1) of 18 amino acids (called PAK18, see Fig. 1) in the N-terminal half, which binds the SH3 domain of PIX, and this PAK18-PIX interaction is essential for the full-activation of group 1 PAKs in cells (Manser *et al*., 1998). Interestingly, MIHCK is activated by both RAC and CDC42, but lacks the PIX-binding motif (PAK18). Thus, evolutionarily, the amoeboid MIHCK belongs to so-called 'group 1.5'

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Received 24 June 2013
Accepted 12 July 2013

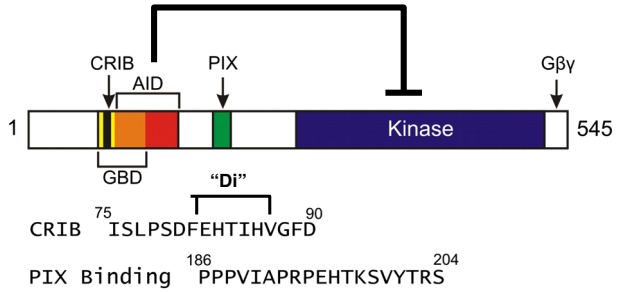


Figure 1. Human PAK1 domains. The catalytic (kinase) domain is in the C-terminal half (in blue). The N-terminal half contains a few regulatory domains such as GTPase-binding domain, auto-inhibitory domain and PIX-binding domain. CRIB domain contain 'Di' motif which is responsible for the formation of catalytically inactive homo-dimer (Lei *et al.*, 2000). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

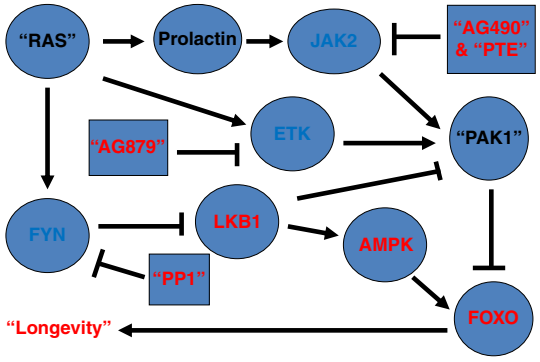


Figure 2. RAS-Tyr kinase pathways leading to activation of PAK1. Signal transducers are in circles, whereas chemical compounds are in squares. Tumor suppressors (chemicals or proteins) are in red, whereas oncogenic signal transducers are in black and oncogenic Tyr-kinases are blue. RAS activates PAK1 through three distinct Tyr-kinases (JAK2, ETK and FYN), and PAK1 in turn inactivates FOXO, shortening lifespan. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

(between groups 1 and 2). PAK family kinases were found in all eukaryotes of animal kingdom from yeast to human, but no PAK homologue is present in either bacteria or plants. Interestingly, however, some bacteria or plants produce a variety of PAK1-blockers (antibiotics or phyto-therapeutics) which are potentially useful for the therapy of PAK1-dependent diseases or disorders, leading to the extension of our healthy lifespan.

The first pathological phenotype of hyper-activated PAK1 was identified by Jeff Field's group at University of Pennsylvania (Tang *et al.*, 1997). In the early 1980s, shortly after the discovery of the oncogenic SRC gene, several groups independently found that constitutively activated mutants of another normal cellular gene called RAS (found in mouse oncoviruses) also cause malignant tumors such as pancreatic and colon cancers even in human (For review, see Maruta & Burgess, 1994). RAS is a GTPase or G protein (GTP-dependent transducer), which activates several kinases such as RAF and PI-3 kinase (a unique lipid kinase), leading to the activation of RAC and CDC42, which eventually activate PAKs and ACK, an oncogenic Tyr-kinase (Manser *et al.*, 1993, 1994). Jeff's group found that in RAS-transformed fibroblasts (cancer cells), PAK1 is hyper-activated, and expression of a kinase-dead mutant (dominant negative = DN) mutant of PAK1 in RAS cancer cell lines suppresses their malignant (anchorage-independent) growth *in vitro* (cell culture) or *in vivo* (grafted in mouse). This established the first link between PAK1 and cancers (in particular solid tumors such as pancreatic and colon cancers). Later, it was found that hyper-activated PAK4 is also responsible for a variety of solid tumors (Qu *et al.*, 2001).

Interestingly, for the full activation in cells, PAK1 requires at least three distinct non-receptor Tyr-kinases called ETK, FYN and JAK2, all of which are activated by RAS (see Fig. 2). Synthetic chemicals such as AG879 (ETK-specific inhibitor), PP1/PP2 (FYN-specific inhibitors) and AG490 (JAK2-specific inhibitor) independently block the activation of PAK1 in cancer cells (He *et al.*, 2000; He *et al.*, 2001; He *et al.*, 2004), and in particular the combination of PP1 and GL-2003 (the water-soluble derivative of AG879) suppressed almost completely the growth of human solid tumors such as pancreatic and breast cancer xenografts in mice (Hirokawa *et al.*, 2007). Thus, these specific Tyr-kinase inhibitors or their water-soluble derivatives could be potentially useful for the therapy of solid tumors in the future.

Furthermore, during the last decade, it became clear that PAK1 is responsible not only for these cancers, but also for a variety of other diseases or disorders such as infectious diseases (AIDS, malaria and flu), inflammatory diseases (asthma and arthritis), brain tumors (NF1, NF2 and TSC), neuronal diseases (AD, HD, PD, LD, epilepsy, depression, schizophrenia, etc.), insulin-resistant diabetes (type 2), hypertension, obesity, etc. Thus, for the therapy of these PAK1-dependent illnesses, several distinct synthetic chemicals that block PAK1 selectively have been developed. Furthermore, since PAK1 is not essential for the growth of normal cells, unlike the conventional chemos (DNA/RNA/microtubule poisons), these PAK1-blockers would not cause any side effect. Unfortunately, however, none of synthetic PAK1-blockers has been available on the market as the FDA-approved drugs for these patients as yet.

Thus, responding to the urgent needs of terminal cancer patients, suffering from pancreatic cancer or a variety of metastasized cancers, who are resistant to conventional chemos, several years ago, we began identifying PAK1-blockers among natural products such as antibiotics, propolis (a bee product), other herbal/phyto-therapeutics and even fruits/vegetables, which have been used for more than a thousand years as traditional medicines. Here, I present a short list of PAK1-dependent diseases/disorders and PAK1-blocking phyto-therapeutics, to give readers an overview of current PAK research in particular towards phyto-therapy of diseases/disorders (without any side effect), leading to the potential extension of our healthy lifespan or at least the improvement of our quality of life (QOL).

PAK1-DEPENDENT PATHOLOGICAL PHENOTYPES (DISEASES OR DISORDERS)

Cancers (in particular solid tumors)

Both PAK1 and PAK4 are hyper-activated or hyper-expressed in a variety of solid tumors and are essential for the malignant growth of these tumors (He and Maruta, 2013). Oncogenic mutants of three distinct RAS genes (Ki-RAS, Ha-RAS and N-RAS) present in

human (and other mammals) activate both PAK1 and PAK4 (see Fig. 3), causing a variety of human cancers including pancreatic and colon cancers. An oncogenic mutant of Ki-RAS is found in more than 90% of human pancreatic cancers, more than 50% of colon cancers and around 30% of lung cancers (Maruta and Burgess, 1994). PAK1 or PAK4 in turn activates several distinct effectors such as beta-catenin and the kinase RAF (Sun *et al*., 2000), which are responsible for malignant (anchorage-independent) growth of cells. Furthermore, all solid tumors require the VEGF-mediated angiogenesis for their continuous growth, and PAK1 (or PAK4) is essential for the activation of VEGF gene through beta-catenin (Kiosses *et al*., 2002; Easwaran *et al*., 2003). Therefore, blocking PAK1 or PAK4 is sufficient for suppressing the growth of all solid tumors. Metastasis/invasiveness of cancers, the ultimate cause of premature death of all cancer patients, requires LIM kinase (LIMK) which is activated by PAK1 or PAK4 (Edwards *et al*., 1999). Thus, blocking these PAKs could prevent cancers from their metastasis. For details, see a previous review (He and Maruta, 2013).

Regarding human leukemia cells, nobody has ever examined directly whether their fast growth requires PAK1 or not, probably because leukemia in general has been effectively treated by conventional chemos (DNA/RNA/microtubule poisons) in the past. However, since (PAK1-blocking) propolis can inhibit the growth of a leukemia cell line called HL-60 at 3 micro g/ml (Eom *et al*., 2010), it is most likely that leukemia could also be a sensitive therapeutic target of PAK1-blockers in general.

Neurofibromatosis (NF)

Back in 1882, a German pathologist Friedrich von Recklinghausen (1833–1910) at Strasbourg University first identified a rare neuronal disorder associated with multiple tumors in skin, spine and brain, which was coined NF. It is a genetic disease occurring around 1 in 3000 births. More than a century later, it was found that there are three (genetically) distinct types of NF. NF1 (type 1) is caused by dysfunction of *NF1* gene which encodes a RAS/RAC GAP of 2818 amino acids (Maruta, 2011), whereas NF2 (type 2) is caused by dysfunction of *NF2* gene which encodes a protein of 595 amino acids called Merlin, a direct PAK1 inhibitor (Hirokawa *et al*., 2004). A third type of NF is

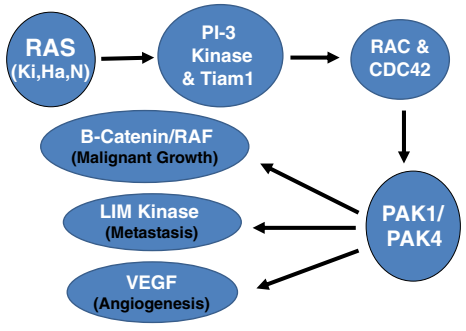


Figure 3. Oncogenic RAS-PAKs pathways. Oncogenic signal transducers are in circles. RAS activates PAKs through PI3 kinase/Tiam 1 and RAC/CDC42, and PAKs in turn activate beta-catenin/RAF, LIM kinase and VEGF, leading to the malignant growth, metastasis and angiogenesis for solid tumors. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

schwannomatosis, but the exact genetic defect in this NF is little known, although its phenotype *per se* is somewhat between NF1 and NF2. Both NF2 and schwannomatosis occur in around 1 in 30,000 births.

90% of NF1 tumors are benign and divided into two categories, dermal tumor called neuro-fibroma and plexi-form, while the remaining 10% tumors are malignant one called malignant peripheral nerve sheath tumor (MPNST). All NF1 tumors are caused by dysfunction of a RAS/RAC GAP (attenuator), and therefore in NF1 tumors RAS is hyper-activated, and as a consequence PAK1 is hyper-activated. Moreover, it was confirmed that like RAS cancers MPNST indeed requires PAK1 for their growth (Tang *et al*., 1998). Merlin is a PAK1 inhibitor, and we found that the growth of NF2 tumors developed in brain or along spine (both schwannoma and meningioma) requires PAK1 (Hirokawa *et al*., 2004). Thus, although NF1 and NF2 tumors are genetically distinct, effective therapy of these two NF tumors would be basically same, that is blocking the hyper-activated PAK1.

During 2006–2007, we have demonstrated for the first time that the growth of both NF1 and NF2 tumors of human origin grafted in nude mice was almost completely suppressed by two distinct propolis samples (Demestre *et al*., 2009; Messerli *et al*., 2009). One propolis from New Zealand is caffeic acid phenethyl ester (CAPE) based, and the other propolis from Brazil is artepillin C (ARC) based. Both propolis samples (as well as CAPE or ARC alone) block PAK1 (Demestre *et al*., 2009; Messerli *et al*., 2009) and cause no side effect on healthy animals. Thus, since mid-2007, we have conducted a human trial of these two propolis (called Bio 30 and GPE) mainly for NF1 and NF2 patients, because no FDA-approved NF therapeutics is available on the market until now. In most cases, the growth of both NF1 and NF2 tumors was strongly suppressed, and in many cases, the size of NF tumors was gradually reduced over a few years (Maruta, 2011).

Tuberous sclerosis (TSC)

TSC is also a rare genetic disease or disorder caused by dysfunction of either *TSC1* or *TSC2* genes (Hodges *et al*., 2001) which encode subunit1 and 2 of the TSC complex, a Rheb GAP (attenuator). Since the GTPase Rheb is an activator of the oncogenic kinase Target of Rapamycin (TOR), dysfunction of the TSC complex leads to the hyper-activation of both Rheb and TOR, and eventually causes TSC tumor mainly in brain, kidney and heart (Manning and Cantley, 2003). When TSC occurs in brain, it often causes epilepsy. Like NF, TSC develops in a very early stage of our life (within 6 months after birth), and its conditions progressively worsen if it is not properly treated. So far, no FDA-approved TSC therapeutics is available on the market as yet. In principle, TOR inhibitors such as Rapamycin and Affinitor could be potentially useful for the treatment of TSC, and in mouse or rat models, these drugs suppressed the growth of TSC tumors (Kenerson *et al*., 2005). However, both Rapamycin and Affinitor (from Novartis) are immuno-suppressors and too expensive, and therefore would not be suitable for a life-long treatment of TSC

patients. Besides, Affinitor has not been approved by FDA for TSC treatment as yet.

Interestingly, however, PAK1-blockers could be used for the therapy of TSC. The reason is as follows. The full activation of TOR requires its interaction with a protein called Raptor (Hara *et al*., 2002), and their interaction indirectly requires PAK1. Curcumin, which directly inhibits PAK1, eventually blocks this TOR–Raptor interaction (Beevers *et al*., 2009). How? Since PAK1 inactivates FOXO which inactivate Raptor gene, curcumin could down-regulate Raptor. In fact, in our human trials of CAPE-based propolis (Bio 30) for epilepsy of TSC children, the propolis suppressed the TSC-associated epilepsy (Maruta, 2011). It is also known that TOR could activate PAK1 through S6 kinase (S6K), the direct substrate of TOR, that activates PAK1, forming a vicious oncogenic (TOR-S6K-PAK1) cycle (see Fig. 4). Thus, inexpensive natural PAK1-blockers such as curcumin and propolis, which do not cause any side effect, would be far more suitable for a life-long TSC therapy than TOR inhibitors.

Infectious diseases (AIDS, malaria, flu)

Viral infection. *Acquired immune deficiency syndrome (AIDS).* AIDS is caused by human immunodeficiency virus (HIV) infection. This life-threatening infection takes place mainly in Sub-Saharan Africa and South-east Asia areas. HIV, a retrovirus, infects vital cells in our immune system such as helper T cells (in particular CD4+ T cells), macrophages and dendritic cells. When CD4+ T cell numbers decline below the critical threshold (around 200 cells per micro liter), the cell-mediated immunity is lost, exposing the patient to a wide variety of opportunistic microbial infections. Thus, the major cause of premature death of AIDS patients is deadly infection by a variety of pathogenic bacteria, fungi, and tumor viruses, mainly due to the loss of their immune system.

One of HIV proteins essential for viral infection or replication is NEF. Two decades ago, NEF was found to be associated with a Ser/Thr kinase called NAK (for NEF-associated kinase) which comes from host cells (Sawai *et al*., 1994). Later, NAK was identified PAK2 by a few groups (Sawai *et al*., 1996; Renkema *et al*., 1999). Interestingly, NEF activates PAK2 via lipid rafts (Krautkrämer *et al*., 2004). However, based on siRNA approach, it was found that PAK1, and not PAK2, is essential for HIV infection in multiple cell systems (Nguyen *et al*., 2006). It still remains to be clarified at molecular levels in detail how PAK1 contributes to the HIV infection, and whether PAK1 is also activated by NEF or not.

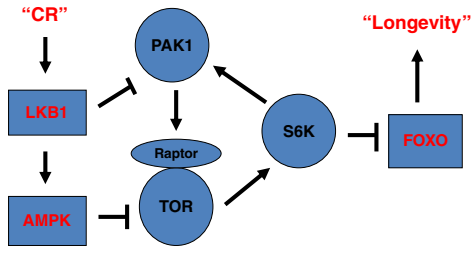


Figure 4. Cross-talk between PAK1 and TOR pathways. Tumor suppressors are in squares, whereas oncogenic signal transducers are in circles. PAK1 is essential for the oncogenic TOR–raptor interaction, while TOR activates PAK1 through S6K. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Flu (Influenza virus infection). A bee product called Propolis, an alcohol extract of honeycomb resin, has been used as a traditional medicine for the therapy of a variety of inflammatory diseases such as asthma and arthritis as well as viral infections such as flu, since the ancient Egyptian era. However, the molecular mechanism underlying the anti-viral action of propolis remained totally unknown until recently. The first clue to our understanding of the anti-viral action of propolis was the discovery of caffeic acid (CA), CAPE, apigenin and ARC in propolis. These polyphenols share a unique common biological property: blocking the oncogenic kinase PAK1 (Maruta, 2011).

Once PAK1 was implicated in the replication of several viruses including HIV as discussed above, a Korean group led by Young-Ki Choi first started examining whether PAK1 is activated (auto-phosphorylated) by the replication of influenza A virus or not and showed that this is indeed the case (Pascua *et al*., 2011). Moreover, transfection of a constitutively activate mutant (T423E) of PAK1 in A549 cells induced around 10-fold higher viral titers compared with the control. Furthermore, PAK1-specific siRNA reduced the virus yield by 10–100-fold, and treatment with TAT-PAK18, a cell-permeable anti-PAK1 peptide, suppressed both ERK1/2 phosphorylation and infectious viral production. These findings clearly indicate that like malaria and HIV, the influenza virus activates PAK1 in host cells during the infection, and PAK1 is essential for the robust replication of these pathogens.

Human papilloma virus (HPV). HPV is a necessary cause of cervical cancer and a few other genital cancers (zur Hausen, 1989). Interestingly, like host cells infected by HIV and flu viruses, papillomas caused by HPV infection were shown recently to carry hyper-activated PAK1 and PAK2 through the ErbB1-RAC signal pathway (Wu *et al*., 2010). In the case of HIV and flu virus infection, their infection and replication require PAK1-3 in host cells. Thus, it is quite conceivable that HPV infection also require PAK1/PAK2 in these genital cells. If that is the case, PAK blockers would be potentially far simpler therapeutics (than type-specific HPV vaccines) for these HPV-dependent cancers as well. In support of this notion, the estrogen-dependent growth of cervical cancers was shown to be strongly inhibited by a natural PAK1 blocker called curcumin (Singh and Singh, 2011). For the malignant growth of cervical cancers, the HPV-encoding oncoproteins (E6 and E7) and the female hormone (estrogen) are known to work synergistically (Singh and Singh, 2011), and as discussed earlier, estrogen receptor and PAK1 form a vicious oncogenic cycle.

Bacterial infection. *Helicobacter pylori* is a Graham-negative entero-bacterium found in the stomach, where it adheres to the surface of epithelial cells. More than 50% of world population harbors *H. pylori* in their upper gastrointestinal tract. However, over 80% of infected individuals show no symptom. *H. pylori* bacteria are present in patients with chronic gastritis, gastric ulcers and gastric carcinomas.

50–60% of *H. pylori* isolates carry a DNA segment called CagA pathogenicity island. Patients infected with CagA-positive strains show a stronger inflammatory

response in the stomach and are at a greater risk of developing peptic ulcers and gastric cancers than those with CagA-negative strains (Kusters *et al*., 2006).

After CagA-positive *H. pylori* adheres to the gastric epithelial cells, the protein CagA is translocated into host cells, Tyr-phosphorylated and activates several distinct signal pathways. One of them is the oncogenic RAC/CDC42-PAK1 pathway. Through a siRNA approach, a Korean group recently found that down-regulation of PIX, an SH3 adaptor protein, blocks the CagA-induced activation of PAK1 (Baek *et al*., 2007). Furthermore, PIX-siRNA also blocks the CagA-induced (proteolytic) activation of the cytokine IL-1, which requires the PAK1-activated caspase-1. *H. pylori* lipopolysaccharide (LPS) induces the PAK1–caspase-1 interaction. These data indicate that upon LPS induction, CagA-activated PAK1 stimulates IL-1 production and therefore plays a major role in the inflammatory response, which is one of hallmarks in *H. pylori* pathogenesis.

Malaria infection. Malaria still remains one of the most devastating infectious diseases, especially in Sub-Saharan Africa, where it claims the lives of more than 1 million persons every year, most of whom are young children. The agent responsible for the most severe form of human malaria is *Plasmodium palcifarum*, an intracellular parasite belonging to the phylum Apicomplexa. Transmitted to the human host through the bite of an infected Anopheles mosquito, the parasite quickly reaches the liver and invades a hepatocyte, where the first round of asymptomatic asexual multiplication (exo-erythrocytic schizogony) requires 7 days to reach the completion.

More than a decade ago, we started suspecting that malaria infection requires one of group 1 PAKs (PAK1-3), because we found that an old anti-malaria drug called quinidine suppresses the PAK1-dependent growth of RAS-transformants with the IC50 around 25 micro M (Hirokawa *et al*., 2004). However, quinidine is rather toxic to be used as an anti-cancer drug, and we began to identify a far more potent and safer alternative.

One of them was FK228, which eventually inactivates PAK1 with the IC50 below 1 nM by inhibiting the histone deacetylase (HDAC) and suppresses the growth of a variety of PAK1-dependent solid tumors such as pancreatic and breast cancers as well as mesothelioma, schwannoma and MPNST (Maruta, 2011)

One of the urgent tasks in malaria therapy is to develop or identify a new anti-malaria drug which is effective on malaria strains which have become resistant to widely used anti-malaria drugs such as Chloroquine. Around 2005, in collaboration with Alan Cowman's group at Walter and Eliza Hall Institute in Melbourne, we found that FK228 indeed blocks the chloroquine-resistant malaria infection at least in cell culture (Cowman & Marura, unpublished observation), strongly suggesting, if not yet proving, that PAK1-3 is essential for malaria infection. A few years later, a siRNA approach revealed that PAK3 inhibition leads to a reduction in parasite infection (Prudêncio *et al*., 2008), clearly indicating that at least PAK3 is essential for malaria infection.

Following hepatocyte rupture, free malaria parasites invade red blood cells and initiate cycle of asexual multiplication (erythrocytic schizogony), which is responsible for malaria pathogenesis. Making a long story short, a few years ago, while Christian Doerig (currently at Monash University in Melbourne) still worked in Lausanne, Switzerland, his group found that several distinct MEK inhibitors severely impaired parasite DNA replication in erythrocytes (Sicard *et al*., 2011). However, since the parasite genome contains no MEK homologue; it is most likely that a host erythrocyte MEK is essential for parasite infection. Later, it was revealed that human MEK1 is phosphorylated at Ser 298 in infected erythrocytes, but not in un-infected erythrocytes. Furthermore, IPA-3, a PAK1-3 inhibitor, blocks parasite maturation and multiplication as well as reduces phosphorylation of MEK1 at Ser 298 (Sicard *et al*., 2011). Thus, it is now clear that a host erythrocyte PAK-MEK pathway is activated by malaria infection and is required for its further propagation.

Inflammatory diseases

Propolis has been used for the treatment of a variety of inflammatory diseases since the ancient Egyptian era, and as discussed later in detail, a few distinct (CAPE- or ARC-based) propolis samples block PAK1. Thus, it was strongly suggested, if not proven as yet, that a variety of inflammatory diseases also require PAK1. A few years ago, it was found that the healthy PAK1-deficient mice are resistant to the LPS-induced degranulation (calcium release) of mast cells (Allen *et al*., 2009), which is the hall mark of allergic inflammatory reactions. Furthermore, FK228, which blocks PAK1 by inhibiting HDAC, also suppresses the asthma and arthritis in mouse or rat models (Nishida *et al*., 2004). Thus, it is now very clear that PAK1 is essential for a variety of inflammatory diseases such as asthma, arthritis and *H. pylori*-induced gastric ulcer.

Neuronal diseases (AD, HD, PD, LD, etc.)

Alzheimer's disease (AD). In addition to a variety of brain tumors such as gliomas, retinoblastoma, NF and TSC, several non-tumorigenic neuronal diseases also have been shown to require PAK1 for their progression. One of them is AD, which is the most common form of dementia, and so far no effective cure has been found for this aging-related disease. AD was first identified or recognized in 1906 by a German psychiatrist and neuropathologist Alois Alzheimer (1864–1915). Most often, AD is diagnosed in people over 65 years of age, although AD can occur much earlier, albeit far less frequently. This prevalence rate is strongly associated with age: of those 65–74 years old, only 3% is AD patients, compared with those over 85 years old, nearly 50% of which suffer from AD (Evans *et al*., 1989).

AD is a prevalent neurodegenerative disease characterized clinically by a progressive cognitive decline with aging, and pathologically by decades-long prodromal accumulation of neuritic (senile) plaques containing amyloid-beta (AB) peptide aggregates and neurofibrillary tangles (NFT) containing tau protein aggregates. Although the precise role of NFT in AD still remains to be clarified, AB aggregates clearly cause the neuronal cell death. Thus, the majority of current AD research has been focused on molecular biology/pathology of AB in AD patients or AD models of mice, rats or

nematodes such as *C. elegans* which carry or express the AB aggregates.

One of the first clues to the key role of PAK1 in AD pathogenesis or AB-induced neurotoxicity was provided by two Latin American groups (Heredia *et al*., 2006; Mendoza-Naranjo *et al*., 2007). They found that fibrillary AB peptide of 42 amino acids (fAB) activates LIMK somehow, and induces cofilin phosphorylation in cultured neurons (Heredia *et al*., 2006). Furthermore, in AD brain, the number of phospho-LIMK-positive neurons is significantly increased in those regions affected by AD pathogenesis. Moreover, the synthetic peptide S3, which blocks the phosphorylation of cofilin by LIMK, inhibited the fAB-induced neuronal degeneration, clearly indicating that LIMK-cofilin signal pathway (leading to stimulation of actin polymerization) is involved in the fAB-induced neuronal degeneration *in vitro* (Heredia *et al*., 2006).

Since LIMK is phosphorylated at the same residue and activated by two distinct kinases, PAK1 (RAC/CDC42-activated kinase) and Rho-activated kinase (ROCK), the next question would be: which kinase is actually required for the fAB-induced neurotoxicity? However, so far, there is no solid evidence suggesting that ROCK inhibitors such as Y-27632 and H-1152 block the fAB-induced neurotoxicity. On the other hand, several distinct evidences have been accumulated that fAB activates PAK1 through Tiam 1 in a Ca^{2+} -dependent manner, which activates RAC/CDC42 (Mendoza-Naranjo *et al*., 2007). Furthermore, curcumin, which directly inhibits PAK1, was shown to suppress the abnormal translocation (activation) of PAK1 in brain of AD mouse model carrying AB aggregates (Ma *et al*., 2012). However, the bioavailability of curcumin alone is very poor, and for its clinical application, it has to be capsulated with liposomes or cyclodextrin which solubilizes curcumin.

In this clinical context, it would be worth noting that a cofilin phosphatase called SSH, which antagonizes LIMK, suppresses the fAB-induced neurotoxicity (Mendoza-Naranjo *et al*., 2012), suggesting that an SSH activator (s) would be potentially useful for AD therapy. Interestingly, a new synthetic compound called C21 (see Fig. 5) turned out to be an SSH activator. Making a long story short, the C21, a potent angiotensin II type 2 (AT_2) receptor agonist, was recently shown to enhance the cognitive functions (in particular spatial learning) that were impaired in an AD mouse model in which AB42 had been injected intra-cerebro-ventricularly (Jing *et al*., 2012). How does this orally active AT_2 agonist block the neurotoxicity of AB42? Interestingly, this compound activates the SSH as does angiotensin II (see Fig. 6). A decade ago, this non-peptide AT_2 agonist (C21) was developed by a Swedish group of Anders Hallberg and Mathias Alterman at Uppsala University,

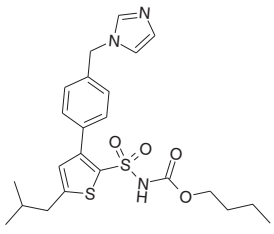


Figure 5. Compound 21 (C21).

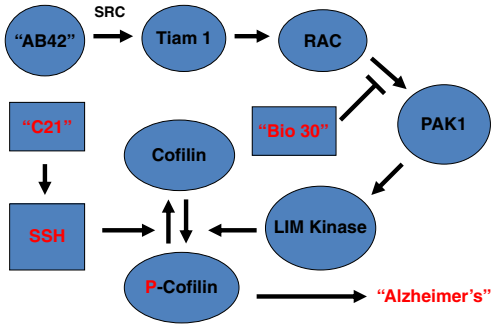


Figure 6. A pathway leading to AD (Alzheimer's). C21 activates SSH which suppresses the AB42-activated PAK/LIMK-dependent AD. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

and the Km of C21 for its receptor is below 1 nM (Wan *et al*., 2004). More interestingly, C21 (1 mg/kg daily) appears to suppress the LPS-induced inflammation in hyper-sensitive rats by inhibiting NF-κB, but without affecting blood pressure (Steckelings *et al*., 2011). Since PAK1 is involved in the activation of NF-κB, and essential for inflammation, it is quite possible that C21 blocks PAK1 as well. Clinical trials (phase 1) of C21 are currently conducted by a Swedish biotech company called Vicore Pharma.

Huntinton's diseases (HD). HD is an autosomal-dominant progressive neurodegenerative disorder that prominently affects basal ganglia leading to clinically significant motor function, cognitive and behavioural deficits. HD is caused by an expanded CAG repeat encoding poly-glutamine (poly Q) tract in exon 1 of the *HD* gene (Rubinsztein and Carmichael, 2003). Normal *HD* alleles have 37 or less CAG repeats in this polymorphic tract: having more than 37 CAG repeats causes HD. Normal *HD* gene product is a huge and aggregation-prone protein of 3148 amino acids called Huntingtin (Htt) that accumulates as aggregates in HD brain. The length of polyQ tract directly correlates with aggregation rate and disease onset, with the longer expansions leading to the earlier onset of HD.

The precise mechanism for mutant Htt (muHtt)-induced neuronal toxicity still remains to be poorly understood. However, according to a recent study by David Rubinsztein's group at Cambridge University, the wild-type (WT) Htt prevents cleavage of PAK2 by caspase-3 and caspase-8, a modification that constitutively activates PAK2, mediating neuronal cell death (Luo and Rubinsztein, 2009). Thus, muHtt aggregates fail to block this pro-apoptotic function of PAK2. Furthermore, the same group reported that both PAK1 and PIX contribute to aggregation of muHtt, and silencing PIX attenuates the aggregation of muHtt (Eriguchi *et al*., 2010). It was also recently found that CEP-1347, a direct PAK1 inhibitor, reduces the muHtt aggregation and neurotoxicity as well as restores motor function in HD mice (Apostol *et al*., 2008). These findings together strongly suggest that natural BBB-permeable PAK1 blockers such as propolis and curcumin could be useful for the treatment of both HD and AD in the future.

Parkinson's disease (PD). PD is a progressively degenerative neurological disorder which affects the control of body movements. Symptoms result from the degeneration of neuronal cells located in the substantia nigra of brain. This causes a deficiency in dopamine, a

chemical neurotransmitter (messenger) necessary for the smooth controlled movement of muscles. The PD symptoms appear when about 70% of dopamine producing cells are damaged. Symptoms develop slowly and gradually progress over years, but could be greatly helped if effective therapeutics become available in the future.

Although what triggers the death or damage of dopamine-producing neurons still remains unknown, the apoptosis of dopaminergic (DA) neurons is known to require a kinase called JNK. Thus, 15 years ago, Kyowa Hakko and Cephalon developed the potentially first PD therapeutic called CEP-1347 which blocks JNK somehow (Maroney *et al*., 1998). Later, we found that CEP-1347 directly inhibits PAK1, leading to the inactivation of JNK (Nheu *et al*., 2002). These findings strongly suggest that PAK1 is responsible for the progression of PD symptoms. Unfortunately, however, CEP-1347 failed to become available on the market.

Several years ago, a German group at Bonn University found that CAPE in propolis, which blocks PAK1 selectively, protects DA neuron from 6-hydroxydopamine-induced apoptosis (Noelker *et al*., 2005). Thus, it is now clear that PD is among PAK1-dependent diseases. Both CAPE-based and ARC-based propolis, which block PAK1 without causing any side effect, are available inexpensively on the market, and I trust these bee-made PAK1 blockers would be suitable for the life-long therapy of PD.

Learning deficits (LD). Mental retardation (MR) or LD is associated with either hyper-activation or dysfunction of group 1 PAKs. The first example was found during 1998–2000. It is a non-syndromic or non-specific X-linked MR which is caused by mutations in PAK3 that lead to a premature termination or missense mutation (R67C) of PAK3, disrupting actin dynamics in dendritic spines (Allen *et al*., 1998; Bienvenu *et al*., 2000). These findings indicate that PAK3 is essential for normal development of cognitive/memory function.

However, around half of NF1 children suffer from a low IQ (Hyman *et al*., 2006), and in these NF1 children, PAK1 is hyper-activated, due to dysfunction/lack of NF1 gene which encodes RAS/RAC GAPs. This finding suggests that hyper-activation of PAK1 also contributes to MR or LD. However, there is no MR/LD case among NF2 patients who also suffer from hyper-activation of PAK1, due to lack of merlin which is a PAK1 inhibitor (Hirokawa *et al*., 2004). These NF cases suggest that hyper-activation of PAK1 alone is required, but not sufficient, for the development of MR or LD.

Trisomy 21, an extra copy of chromosome 21 which carries an extra set of more than 300 distinct genes, results in Down Syndrome (DS), which is the most common genetic cause for cognitive impairment leading to MR/LD, occurring in a frequency of around 1 in 700 live births. Interestingly, a Spanish group recently found that in a DS mouse model, the oncogenic PI-3 kinase-AKT-TOR pathway is abnormally activated, and Rapamycin, a TOR inhibitor, blocks the abnormal activation of these kinases (Troca-Marín *et al*., 2011), suggesting that either TOR inhibitors or PAK1-blockers, which eventually block TOR, could be potentially useful for DS therapy in the future.

Several years ago, Susumu Tonegawa's group at MIT found that PAK1 is required for the genetic disorders (abnormalities) such as MR and autism associated with FXS (Hayashi *et al*., 2007). In a mouse model where the *fragile X MR 1 (FMR1)* gene is silenced (KO), FXS-associated abnormalities are rescued, at least partially at both cellular and behavioural levels, by inhibition of PAK1 through postnatal expression of a DN mutant of PAK1 in the forebrain. Finally, they demonstrated *in vitro* the direct interaction between PAK1 and FMR1 protein, indicating the possibility that like merlin (NF2 gene product), the FMR1 protein could be a PAK1-blocking tumor suppressor. These findings altogether strongly suggest that anti-PAK1 drugs will be among future therapeutics for FXS and autism.

Accordingly, shortly after this finding on FXS, Susumu Tonegawa (the 1987 Nobel laureate in physiology/medicine) co-founded a new biotech company called 'Afraxis' in San Diego to develop a series of potent PAK1-specific inhibitors, and around the end of 2011, they patented a few potent PAK1-specific inhibitors (IC_{50} around 10 nM). Since these PAK1-inhibitors pass blood–brain barrier (BBB), they would be potentially useful for the therapy of FXS and several other PAK1-dependent neuronal disorders/diseases in the future.

Depression. Depression is a common mental health condition that affects one in around 20 people every year. Although everybody experiences low mood from time to time, feeling sad, worthless or hopeless for a period of two weeks or more can be a clear sign of clinical depression. Alcoholic people are among chronically depressed people. Due to the very low sensitivity or number of their dopamine (DA) receptor, they cannot feel the basal level of this pleasure neuronal hormone (=DA), unless alcohol temporarily boosts the DA production or secretion. In the worst cases, prolonged depression often drives people to commit suicide, escaping from their miserable life for good…

Until recently, few scientists were aware that clinical depression also requires PAK1 as do cancers. However, I was recently informed that CAPE, the major anti-cancer/anti-PAK1 polyphenol in propolis, suppresses the depression at least in an animal model (Ichihara, K *et al*., personal communication). This rather unexpected news suddenly solved a mystery of centuries overnight, as to why honey bees have been so much addicted to collect a variety of anti-PAK1 products such as CAPE, apigenin, ARC from a variety of plants to prepare their propolis (honey combs) for 100 million years, wherever on this earth they live. We have no difficulty in imagining why they love to collect glucose or fructose to prepare honey, because they are among sweets that certainly make them happy. If anti-PAK1 products could make them feel so happy, or suppress their depression if any, we can guess why bees love to collect these anti-depressants… Naturally, I started looking for a few natural anti-depressants and found apigenin in propolis, curcumin in Indian curry, berberine in Chinese yellow root and salidroside in golden root among effective anti-depressants. What is more, they share the unique common property with CAPE, that is the ability to block the oncogenic kinase PAK1 and activate the tumor suppressing kinase AMPK simultaneously, eventually leading to an extension of healthy lifespan, as discussed later in detail.

According to a decade old paper (Nakazawa *et al*., 2003), apigenin (100 mg/kg) increases the levels of

dopamine and improved the impaired behaviour such as the duration of immobility caused by a forced swimming test (FST) in depressed mice. In other words, apigenin can make these depressed mice happy again by boosting DA production or secretion, as alcoholic drinks such as beer and wine would do for the people in whom the DA-sensitivity (or number) of DA receptor is rather low. Later, using FST, a very similar anti-depressant (DA boosting) effect of curcumin (10 mg/kg), berberine (20 mg/kg), the salidroside-based adaptogen *Rhodiola rosea* extract (20 mg/kg) was shown by others in mice or rats (Xu *et al*., 2005b; Peng *et al*., 2007; Perfumi and Mattioli, 2007). Furthermore, it was recently reported that the aromatic ring of salidroside and related compounds (aceteosides) could be biosynthesized from Tyr through DA on an olive leaf culture (Saimaru and Orihara, 2010). Thus, it is quite conceivable that a reverse metabolism could produce DA from salidroside in mammals, making them feel very happy!

These findings together strongly indicate that PAK1 normally suppresses DA production, making people depressed, although the detailed molecular mechanism underlying these complex phenomena still remains to be clarified further in the future.

Schizophrenia. Like depression, schizophrenia is a neuronal disorder which is not associated with any detectable impaired cognitive function. That is why even several highly ingenious scientists were well known to suffer from schizophrenia during a certain period of their long active career or life. As described in the biography '*A Beautiful Mind*', John Nash, a mathematical genius, at Princeton University suffered from schizophrenia for several decades of his career, but luckily recovered from it just before he shared the 1994 Nobel Prize in economics (Nasar, 1998). According to another book, '*The Life of Isaac Newton*', this great British mathematical genius also suffered from schizophrenia at age 51, although he recovered from it later (Westfall, 1993).

A recent report (Chen *et al*., 2011) drew my attention to schizophrenia, a neurodevelopmental disorder with a genetic predisposition, almost for the first time of my life. The reason is very simple: Using *C. elegans*, as the tiniest (and simplest) model, they discovered the specific role of the GTPase RAC and its effector PAK1 in schizophrenia. Schizophrenia is caused by dysfunction (loss-of-function mutation) of a gene called disrupted-in-schizophrenia 1 (*DISC1*), presumably among several other distinct genes. Since this worm lacks any gene homologous to the mammalian *DISC1* gene, they generated a transgenic worm expressing presumably a DN mutant of mammalian DISC1 that is linked to GFP, and they found that DISC1-GFP is localized in the growth cone, and this transgenic worm exhibits axon guidance defects. Furthermore, the DISC1 mutation activates the RAC-PAK1 signaling pathway in this worm.

In mammals, DISC1 interacts with TRIO, a RAC activator. In other words, DISC1 normally blocks the TRIO-RAC-PAK1 pathway in mammals. Thus, like merlin and LKB1, DISC1 could be among tumor suppressors that block the oncogenic kinase PAK1. Accordingly, it is presumed that PAK1 is abnormally activated in the brain of schizophrenia patients with DISC1 mutation, and that in principle, anti-PAK1 drugs could suppress schizophrenia as well. In fact, according to a recent review (Kulkarni and Dhir, 2010), among PAK1-blockers, berberine, at least, appears to suppress schizophrenia as well as other PAK1-dependent neuronal disorders such as depression. Thus, it would be worth testing the therapeutic effect on DISC1-induced schizopherenia of other natural PAK1-blockers such as propolis and curcumin.

Diabetes (type 2)

Diabetes is a disease which is caused by either insulin-deficiency (type 1) or insulin-resistance (type 2), eventually leading to high blood glucose levels, due to impaired function of cells to intake glucose from blood stream. Normally, insulin activates a glucose-transporter called GLUT-4 which is essential for glucose uptake by cells. Insufficient production of insulin alone causes diabetes (type 1). However, even in the presence of sufficient levels of insulin, if insulin fails to activate GLUT-4, diabetes (type 2) takes places. The major therapy of type 1 diabetes is daily injection of insulin. However, the therapy of type-2 diabetes would be more complicated, because insulin injection alone has no therapeutic effect on this type of diabetes.

How does insulin activate GLUT-4? Insulin binds its receptor (IR), which is a Tyr-kinase. Then, IR is self-activated and activates PI-3 kinase, which phosphorylates PIP2, producing PIP3. Then, PIP3 activates another kinase called AKT. AKT phosphorylates and activates GLUT-4, leading to the translocation of GLUT-4 to the plasma membrane. Interestingly, another kinase called AMPK also phosphorylates and activates GLUT-4 (see Fig. 7). AMPK is usually activated by the kinase LKB1, which is activated when cellular glucose level is reduced, by either exercise or fast (Shaw *et al*., 2004). In other words, exercise or fast/calorie restriction (CR) stimulates insulin sensitivity (through LKB1-AMPK cascade) in patients suffering from type 2 diabetes. How does PAK1 interfere with insulin-induced GLUT-4 activation? A cell surface Tyr-kinase called ErbB2 and PAK1 form a vicious oncogenic cycle, and ErbB2 somehow blocks the LKB1-AMPK cascade (see Fig. 8). Thus, curcumin which not only inhibits PAK1 directly, but also down-regulates ErbB2, reactivates the LKB1-AMPK cascade (Pan *et al*., 2008), leading to GLUT-4 activation and cellular glucose uptake. In other words, type 2 diabetes is among PAK1-dependent diseases.

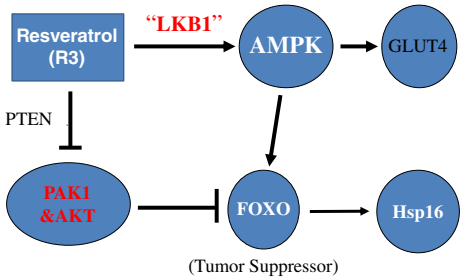


Figure 7. Three kinases (PAK1/AKT/AMPK) control FOXO. The tumor suppressor FOXO is controlled by three distinct kinases. AMPK activates FOXO, whereas PAK1 and AKT inactivate FOXO. FOXO extends lifespan by activating HSP16 gene and several other genes. AMPK also activates GLUT-4, a glucose-transporter essential for up-take of glucose by cells. R3 in grapes activates AMPK through LKB1 and inactivates PAK1/AKT by up-regulating PTEN, eventually extending lifespan. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

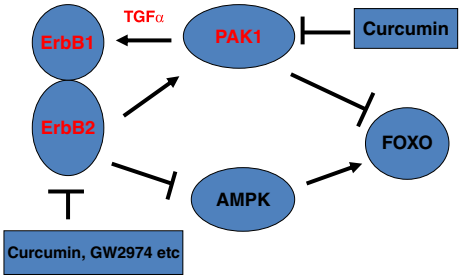


Figure 8. ErbB2 controls PAK1 and AMPK. Signal transducers are in circles, whereas chemicals are in squares. Oncogenic transducers are in red, whereas tumor suppressors are in black. The ErbB1/ErbB2 complex and PAK1 form a vicious oncogenic cycle, whereas this complex inactivates the tumor suppressor AMPK. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Interestingly, as discussed later in detail, most of natural PAK1 blockers such as curcumin and propolis (CAPE and ARC) activate the LKB1-AMPK cascade and therefore would be useful for the therapy of type 2 diabetes (Fuliang *et al.*, 2005) and several other PAK1-dependent diseases (Maruta *et al.*, 2013).

Short lifespan

Fast/CR or exercise or combination of both, which leads to a reduction of cellular glucose/ATP levels, significantly extends the lifespan of mice by activating the tumor suppressing LKB1-AMPK cascade (Carling, 2004; Viollet and Andreelli, 2011). Very interestingly, LKB1 not only activates AMPK by phosphorylating Thr 172, but also inactivates PAK1 by phosphorylating Thr 109 (Deguchi *et al.*, 2010). In other words fast/CR could inactivate the oncogenic kinase PAK1, contributing to the prevention of cancers, type 2 diabetes and several other PAK1-dependent diseases or disorders that have been discussed in detail earlier.

The extension of lifespan or longevity requires a tumor suppressing transcription factor called FOXO (Kenyon, 2010). In mammals, FOXO is phosphorylated and activated by AMPK (Greer *et al.*, 2007), while it is phosphorylated and inactivated by at least two distinct oncogenic kinases, AKT and PAK1 (Mazumdar and Kumar, 2003; Plas and Thompson, 2003). Thus, in mammals including mice, it is most likely that hyper-activation of PAK1 would shorten their lifespan. Currently, the lifespan of the healthy PAK1-deficient mice (Allen *et al.*, 2009) is indeed under investigation by a group at National Institute on Aging in Baltimore. If this mutant mouse lives longer than the WT (whose average lifespan is around 3 years), the outcome of this study would be revealed in 5 years or so.

Nevertheless, we recently found that PAK1-deficient mutant (RB689) of *C. elegans* lives significantly longer than the WT (whose average lifespan is only around 2 weeks), clearly indicating that PAK1 shortens the lifespan of this worm (Yanase *et al.*, 2013). In this worm, *PAK1* gene turned out to be fourth among the major life-shortening genes. In the past, dysfunction of three other oncogenic genes encoding either insulin-like signaling (ILS), AGE-1 (PI-3 kinase) or TOR has been shown to extend the healthy lifespan of this worm (Friedman and Johnson, 1988; Dorman *et al.*, 1995; Jia *et al.*, 2004). Interestingly, these three oncogenic kinases are closely linked to PAK1. ILS activates PI-3 kinase, and the product of PI-3 kinase (PIP3) eventually activates the GTPase RAC, which in turn activates PAK1. Furthermore, as discussed earlier, TOR and PAK1 form a vicious oncogenic cycle through raptor and S6 kinase. Furthermore, RAC-deficient mutant of *Drosophila*, in which PAK1 remains inactive, lives twice as long as the WT, indicating that PAK1 shortens the lifespan of fruit flies as well (Shuai *et al.*, 2011).

Interestingly, the phenotype of PAK1-deficient worm or fly is not only the longer lifespan, but also a reduced brood size (number of eggs laid) and increased resistance to a variety of stresses such as heat. These three phenotypes are shared by three other longevity mutants (ILS deficient, PI3-kinase deficient and TOR deficient) of this worm as well as longevity mutants of fruit fly (Methuselah and RAC-deficient) and mouse (TOR deficient and snell dwarf) (Lin *et al.*, 1998; Vergara *et al.*, 2004; Anisimov *et al.*, 2011; Shuai *et al.*, 2011). In other words, there is a clear 'trade-off' between fertility and longevity/stress resistance. Thus, it would be quite possible to screen for natural or synthetic PAK1-blockers which would extend the lifespan of this worm, fly or mouse, by screening simply for fertility reducers using this worm, just overnight, instead of testing their effect on the lifespan for 3–4 weeks. Alternatively, we could use a strain of *C. elegans* called CL2070 which carries an HSP16-GFP fusion gene (the promoter of HSP16 gene linked to GFP cDNA). Since it has been well established that the longevity is proportional to the FOXO-dependent expression of HSP16 gene which produces a small heat-shock protein (Lithgow *et al.*, 1994), we could screen for PAK1-blockers by testing their ability to activate the HSP16-GFP gene expression shortly after a heat-shock treatment (at 30 °C for 2 h). This test is also very quick and can be achieved overnight.

In fact, we have confirmed that CAPE and ARC from two distinct propolis samples, which block PAK1 selectively, indeed dramatically reduce the brood size (fertility) of this worm, as well as activate HSP16-GFP gene expression in CL2070 (Yanase *et al.*, 2013). Furthermore, we found that an old antibiotic called Ivermectin, which was developed as a pesticide by Merck and Kitasato Institute in an early 1980s, reduces dramatically the fertility of this worm at a sub-lethal dose and indeed blocks PAK1 in ovarian cancer cells as well as their PAK1-dependent growth (Hashimoto *et al.*, 2009). So far, a few herbal PAK1-blockers have been shown to extend the lifespan of this worm or fruit fly: polyphenols (proanthocyanidins or pterostilbene (PTE)) in blueberry, curcumin, salidroside, CA and rosmarinic acid (Wilson *et al.*, 2006; Wiegant *et al.*, 2009; Liao *et al.*, 2011; Pietsch *et al.*, 2011). Thus, I trust that a test of either fertility or HSP16-GFP expression of this worm, which can be done overnight, could be an excellent novel approach for a quick selection of PAK1-blockers from a large library of synthetic chemicals or natural products.

PAK1-BLOCKING (AMPK-ACTIVATING) HERBAL THERAPEUTICS

As shown in Fig. 4, the CR activates the tumor suppressing kinase LKB1, and this kinase in turn activates

AMPK and inactivates PAK1 simultaneously (Deguchi *et al*., 2010), whereas the vicious oncogenic cycle of PAK1 and ErbB2 blocks the anti-oncogenic LKB1-AMPK cascade (see Fig. 8). Accordingly, a variety of herbal PAK1-blockers such as CAPE and curcumin turned out to be LKB1-AMPK activators as well. Thus, these herbal PAK1-blockers (=AMPK activators) not only suppress the growth of all solid tumors, but also several other non-tumor PAK1-dependent diseases listed above, and eventually contribute to an extension of healthy lifespan, by improving our QOL through FOXO, the longevity/tumor suppressing transcription factor. Here, I briefly introduce the most common PAK1-blocking (LKB1/AMPK-activating) herbal therapeutics.

Propolis (CAPE and ARC)

For last 100 million years, honey bees kept preparing the hexagonal honey combs for the protection of their larvae from a variety of pathogenic foreign invaders such as bacteria, fungi and viruses. Each honey comb mainly consists of fats and an alcohol-soluble resin called propolis. Since the ancient Egyptian era, people used the propolis, an alcohol extract of honey comb, for the treatment of a variety of infectious and inflammatory diseases as well as the preparation of mummies from deceased royal families to be kept for good under pyramids. In other words, propolis has been used as a bee-made antibiotic. However, the detailed molecular mechanism as to how propolis works as an antibiotic remained almost totally unknown, until several years ago, when a variety of pathogens and inflammatory diseases require the oncogenic kinase PAK1, and propolis contains several distinct PAK1 blockers such as CAPE, CA, apigenin, ARC and triterpenes, depending on where the source of a given propolis is harvested by honey bees (Maruta, 2011). Propolis prepared in temperate zones such as Europe, Far East, North America and Oceania is based on CAPE (see Fig. 9), whereas Brazilian green and red propolis samples are based on ARC and triterpenes, respectively, and contain basically no CAPE. In terms of antibiotics, propolis is very unique, simply because unlike other antibiotics, propolis works on a variety of viruses such HIV, influenza virus and HPV. The target of propolis is a host mammalian enzyme (PAK1), instead of pathogenic viral or bacterial proteins. That is a part of the reason why molecular biology of propolis has been best studied mainly by oncologists, instead of microbiologists.

In late 1980s, Dezider Grunberger (1922–1999) at Columbia University began asking why bee-keepers are 1000 times resistant to cancers than the non-beekeeper population. While one in three non-beekeepers (ordinary people) suffer from a cancer during their whole life, only one in 3000 beekeepers suffers from cancers. There are three distinct bee products: honey, royal jelly and propolis. However, neither honey nor royal jelly has any anti-cancer property. Thus, he speculated that propolis must be the main source that makes beekeepers extremely resistant to cancers. Indeed, his group confirmed that propolis has a strong anti-cancer property, and the major ingredient in their propolis turned out to be CAPE (Grunberger *et al*., 1988). A decade later, the major anti-cancer ingredient in Brazilian green propolis was identified ARC (Kimoto *et al*., 1998). Around the turn of this century, we and others found that a variety of solid tumors such as pancreatic and colon cancers as well as NF tumors require PAK1 for their growth, as discussed earlier in detail. Then, in 2005, a Japanese group found that CA, one of the anticancer ingredients in propolis, down-regulates the GTPase RAC, an activator of PAK1 (Xu *et al*., 2005a). Since CAPE is a far stronger anti-cancer derivative of CA, it is most likely that CAPE also could block PAK1. Yes, we confirmed that both CAPE-treated and PAK1-deficient *C. elegans* share the exactly same phenotypes, markedly reduced fertility and very strong heat resistance through the activation of HSP16 gene (Yanase *et al*., 2013). Furthermore, we found that ARC also blocks PAK1 (Messerli *et al*., 2009). Moreover, another Japanese group found that triterpene-based Brazilian propolis also blocks the PAK1-dependent growth of human pancreatic cancers, strongly suggesting that these propolis triterpenes also block PAK1 (Awale *et al*., 2008).

Thus, it is not a big surprise that all of these three distinct propolis samples show basically the same therapeutic effects on cancers, infectious and inflammatory diseases, as well as several other PAK1-dependent neuronal diseases/disorders such as AD, HD, PD and LD. In addition, propolis suppresses diabetes (type 2) in mice or rats (Fuliang *et al*., 2005), through CAPE, apigenin and ARC that activate the LKB1-AMPK cascade (Zang *et al*., 2006; Lee *et al*., 2007; Ueda *et al*., 2013), as discussed earlier.

Among these three propolis samples, we have worked mainly on the CAPE-based propolis called Bio 30 for the following reasons: the least expensive (because of being the most abundant around the world), and so far the most effective. Unlike Brazilian green or red propolis which work through ARC or triterpenes alone, the CAPE-based propolis contains several other anti-cancer ingredients such as CA, apigenin, chrysin, galangin and pinocembrin, which work synergistically with CAPE, boosting the anti-cancer effect of CAPE around 600 times *in vitro* (Demestre *et al*., 2009). Moreover, our recent interest in PAK1 research is focused on the life-long therapy of NF and TSC, because they are genetic diseases/disorders and developed at a very early stage of patients' life. Nevertheless, so far, no FDA-approved drug is available on the market for these diseases. Thus, we have conducted the first human trials of Bio 30 for NF/TSC patients as well as pancreatic/colon cancers for several years since mid-July of 2007, and in most cases Bio 30 suppressed the growth of these solid tumors, and several cases it did shrink NF tumors by more than 50% over a few years, and cured completely pancreatic cancers in a year, even if it was at the terminal (metastasized) stage (Maruta, 2011). Furthermore, unlike conventional chemos (DNA/RNA/microtubule poisons), none of propolis samples causes any serious side effect. Thus, there is no doubt that they are very safe (and inexpensive) life-long therapeutics for NF/TSC and several other PAK1-dependent neuronal diseases.

Curcumin

Among the natural anti-malaria substances, CAPE in propolis and curcumin in turmeric powder (the spicy yellow ingredient in Indian curry) are structurally very

similar (see Fig. 9), and just like CAPE, curcumin blocks PAK1 and activates the LKB1-AMPK cascade (Pan *et al*., 2008; Cai *et al*., 2009). Like CAPE, curcumin suppresses the growth of many PAK1-dependent cancer cells *in vitro*, but neither CAPE nor curcumin alone has been used clinically, because of their poor bioavailability (water insolubility). However, several years ago, 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% (Li *et al*., 2005; Mach *et al*., 2009).

Berberine

A traditional Chinese and American Indian herb called golden seal (or yellow root) contains an anti-cancer, anti-inflammatory, yellow-colored and bitter-tasting alkaloid called berberine (see Fig. 10). Unlike most of anti-cancer products, berberine is relatively water soluble, and its tannic salt (berberine tannate) is tasteless, and used clinically worldwide as an antibiotic for therapy of a variety of infectious diseases such as malaria. More than 2 decades ago, an NCI group found that berberine (0.1 mg/ml) induces the morphological differentiation of RAS-transformed teratocarcinoma cells *in vitro* (Chang *et al*., 1990). However, the molecular mechanism underlying its anti-cancer action remained unknown until recently. A few years ago, it was found that berberine inactivates both RAC and CDC42, thereby inactivating their effectors including PAK1 and other PAKs (Tsang *et al*., 2009). Furthermore, it was also found that berberine (100 mg/kg, daily) activates AMPK, blocking both synthesis and accumulation of fats such as cholesterol in mice (Brusq *et al*., 2006). Thus, like propolis and other anti-PAK1 products, berberine would be useful for therapy of all solid tumors as well as other non-tumor PAK1-dependent diseases/disorders including a variety of infectious disease, type 2 diabetes and obesity.

King of Bitters (Andrographolide)

There is an old saying that parents often use in an attempt to encourage young children to take a good medicine: all good medicines taste very bitter. Berberine appears to be among these bitter medicines. Are there any other bitter herbal medicines that block PAK1?

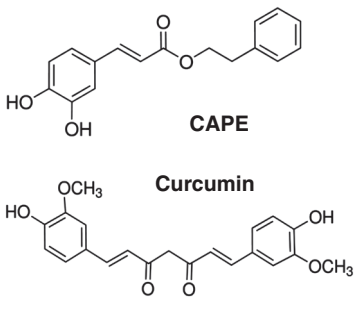


Figure 9. CAPE and curcumin.

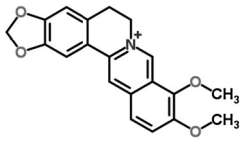


Figure 10. Berberine.

Yes, making a long story short, the Chinese herb called 'King of Bitter' is the most bitter one.

Recently Mesalamine (5-ASA), an Aspirin derivative, was found to block the oncogenic kinase PAK1 (Khare *et al*., 2012). This drug was developed around 1977 for the therapy of bowel ulcers. Since both bowel ulcers and cancers are known to require PAK1, as discussed earlier, I started looking for a series of anti-ulcer drugs, in particular natural and inexpensive products, which are used not only for ulcer, and but also for other PAK1-dependent diseases such as solid tumors, inflammatory diseases, a variety of infectious diseases, as well as AD and type 2 diabetes.

To my great surprise, the herb extract called HMPL-004, which is the 90% alcohol extract of leaves of a South-Asian plant called 'King of Bitters' (*Andrographis paniculata*) is more potent than Mesalamine to suppress the bowel ulcer, according to a recent clinical trial report from a group led by Bill Sandborn at UCSD in collaboration with a Chinese company (HMPL) in Shanghai (Tang *et al*., 2011). 1200 mg of HMPL-004 daily is as effective as 4500 mg of Mesalamine when they are orally administered. Thus, it is most likely that HMPL-004 blocks PAK1 as does Mesalamine. Indeed, Andrographolide, a diterpene lactone of MW 350 (See Fig. 11), the major anti-ulcer/anti-cancer ingredient in this extract has been shown to block the oncogenic PI-3 kinase, leading the inactivation of both PAK1 and AKT (Lee *et al*., 2010).

Is this bitter extract available on the market? Yes, several on-line companies are selling this extract in capsules very inexpensively. 400 mg capsule costs only 10 cents. Ulcer patients need three to four capsules daily (costing only 30–40 cents)! I have previously introduced an inexpensive propolis extract called 'Bio 30' for the life-long therapy of NF which requires PAK1. Currently, 25 ml bottle of Bio 30 costs around US$7, and since average adults (weighing around 60 kg) need 6–12 ml daily, their daily cost would be US$ 2–4. Thus, compared with Bio 30, this herb extract (called 'Chua Xin Lian' in China) is far cheaper. So far, at this dose, the herb extract causes no side effect. Thus, it would be worth testing the therapeutic effect of HMPL-004 on NF and several other brain diseases such as AD, because it passes the BBB.

Thunder god vine (Triptolide)

Triptolide, a diterpenoid triepoxide (see Fig. 12) from the traditional Chinese medicinal herb *Tripterygium wilfordii* (lei gong teng), inhibits the growth of PAK1-

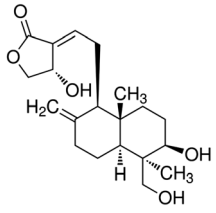


Figure 11. Andrographolide.

dependent cancers such as pancreatic and colorectal cancer cells *in vitro* and *in vivo* (Wang *et al*., 2009).

A few years ago, a Chinese group led by Qibing Mei at Xijing Hospital found that Triptolide (0.3 mg/kg) decreased the incidence of tumors in nude mice inoculated with human colon cancer cell lines. Triptolide inhibited the proliferation, migration and colony formation of colon cancer cells (with the IC_{50} at around 30 nM). Triptolide blocks the activation of RAC, an activator of PAK1, and eventually down-regulates cyclin D1 and CDK4 expression, leading to G1 arrest. Thus, like CAPE, Triptolide clearly inactivates PAK1. However, Triptolide causes a well-known side effect which reduces the sperm count in males. Thus, this herb extract is used as a male contraceptive as well.

However, Triptolide is poorly soluble in water, limiting its clinical use. In 2012, Ashok Saluja's group at University of Minnesota chemically synthesized its water-soluble analog (prodrug) called 'Minnelide' (Chugh *et al*., 2012). The efficacy of Minnelide was tested both *in vitro* and *in vivo* models of pancreatic cancer, including an orthotopic model of human pancreatic cancer grafted in nude mice. Minnelide was highly effective in reducing pancreatic tumor growth and spread, and improving survival.

Rhodiola rosea (Salidroside)

Extracts of plant adaptogens such as *Rhodiola rosea* (golden root) increase stress resistance in several models. These short plants grow on highlands in the Far East, over 3000 m above the sea level, such as Tibet and Japanese Alps, and reddish root extracts have been used as a traditional Chinese medicine. The major effective ingredient is a sugar called salidroside (see Fig. 13). A few years ago, salidroside was found to activate AMPK (Li *et al*., 2008). Shortly thereafter, it was discovered that an extract of these roots (10–25 micro g/ml) can extend the lifespan of *C. elegans* by activating FOXO, the longevity transcription factor, which eventually activates HSP16 gene encoding a heat shock protein (Wiegant *et al*., 2009). Since AMPK activates FOXO, it is most likely that the life-extending ingredient in this extract is salidroside. Interestingly, salidroside was found to suppress tumor-induced angiogenesis, which requires both PAK1 and AMPK (Skopińska-Rózewska *et al*., 2008). Since the activation of AMPK alone cannot block angiogenesis, these finding strongly suggest that salidroside blocks PAK1 as well as activates AMPK.

Natto (fermented soybean's vitamin K2)

Menaquinone 7 (MenaQ7) among vitamin K2 is the unique ingredient found only in a fermented sticky soy-bean called 'Natto'. Natto was originally developed in China, back in BC (before Christ), but further refined

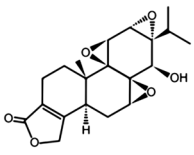
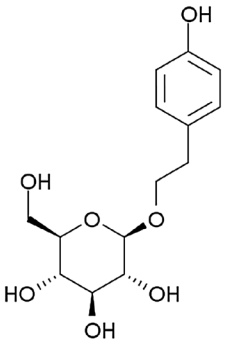


Figure 12. Triptolide.



Salidroside

Figure 13. Salidroside from *Rhodiola rosea*. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

in Japan, and became one of the most common Japanese traditional cuisines. However, it has been often recommended to be taken for the prevention or cure of osteoporosis and inflammatory diseases such as allergy. Natto is made by fermenting steamed soy beans with a fungus called 'natto' strain of *Bacillus subtilis*, which was first isolated by a Japanese scientist Shin Sawamura in 1906. Non-fermented soy beans contain no MenaQ7. Thus, it is clearly a fungal product (=antibiotic!).

MenaQ7 strongly suppresses the growth of human pancreatic and ovarian cancer cell lines (Shibayama-Imazu *et al*., 2003), both of which we found require PAK1 for their growth. As discussed elsewhere, two other antibiotics from bacteria/fungi, FK228 and ivermectin, which we found block PAK1, also suppress the growth of both pancreatic and ovarian cancer cells (Maruta, 2011). Interestingly, there are two types of ovarian cancer cell lines. The growth of one type depends on solely on PAK1, but the growth of the other type depends on two oncogenic kinases PAK1 and AKT. MenaQ7 blocks only the growth of the first type, and not the second type, clearly indicating that MenaQ7 blocks PAK1 selectively, without affecting AKT. Furthermore, it is well known that PAK1 down-regulates one of CDK inhibitors called p21, while AKT down-regulates another CDK inhibitor called p27 (Nheu *et al*., 2004). MenaQ7 activates only p21 gene (Liu *et al*., 2007).

Thus, it is now clear that MenaQ7 in Natto blocks PAK1 to suppress the PAK1-dependent growth of pancreatic and ovarian cancers as well as other solid tumors. Furthermore, it was recently found that Natto extract extends the lifespan of *C. elegans* (Ibe *et al*., 2013), which is shortened by PAK1 as discussed earlier.

Grapes (resveratrol)

Back in late 1920s, a small book of less than 100 pages entitled 'Grape Cure' (How to Conquer Cancers, Naturally) was published in US by Johanna Brandt from South Africa. In this book, she claimed that she succeeded in curing her own stomach/bowel cancer by eating 0.5–2.0 kg of grapes every day (Brandt, 1928). However, it took almost seven decades for a scientist (s) to identify the responsible anti-cancer ingredient in grapes. It is a polyphenol called resveratrol (or R3). Around 1997, John Pezzuto's group at University of Illinois in Chicago eventually found that resveratrol

from red skin of grapes suppresses the growth of cancer cells *in vitro*, suggesting that resveratrol-rich red wines could be beneficial for cancer prevention or therapy (Jang *et al*., 1997). Later, it was found that the major metabolite of resveratrol (R3) called piceatannol (PIC) is actually the principal anti-cancer compound (Potter *et al*., 2002). PIC inhibits a Tyr-kinase called SYK, leading to the inactivation of PI3 kinase. Since PI3 kinase is responsible for activation of two oncogenic kinases PAK1 and AKT, PIC could eventually inactivate PAK1. Furthermore, as shown in Fig. 3, R3 up-regulates PTEN, a PIP3 phosphatase (antagonist of PI3 kinase), leading to the inactivation of both PAK1 and AKT (Waite *et al*., 2005). In addition, R3 activates the LKB1-AMPK cascade somehow (Zang *et al*., 2006). A decade ago, David Sinclair's group at Harvard Medical School claimed that R3 activates directly Sir-2 in yeast, which is responsible for the longevity (Howitz *et al*., 2003). However, a few other groups dispute this 2003 report.

One of the clinical problems associated with R3 is its poor bioavailability, and several groups have tried to improve the bioavailability by chemically modifying R3.

Blueberry (PTE)

Blueberry is known to be rich in PTE, which is dimethoxyl derivative of resveratrol (see Fig. 14). The bioavailability of PTE is much higher than that of R3. Interestingly, this year, PTE was found to directly inhibit the Tyr-kinase JAK2, and the growth of a bone cancer called osteosarcoma (Liu *et al*., 2013).

Nearly two decades ago (around 1996), Alex Levitzki's group at Hebrew University in Israel developed a synthetic chemical called AG 490, which also directly inhibits JAK2. Interestingly, the over-all chemical structure of AG 490 is quite similar to PTE (see Fig. 14).

How does PTE or AG 490 block the growth of osteosarcoma? Several years ago, JAK2 was shown to activate PAK1 by phosphorylating three Tyr residues at 153, 201 and 285 (Rider *et al*., 2007). Furthermore, just like R3, PTE was shown to activate the LKB1-AMPK cascade and suppresses the growth of prostate cancers as well (Lin *et al*., 2012).

In other words, like many other solid tumors such as pancreatic, colon and breast cancers as well as NF, osteosarcoma and prostate cancer are among PAK1-dependent tumors, and natural PAK1-blockers (=LKB1/AMPK activators) such as Blueberry and Propolis extracts would be useful for the effective therapy of these solid tumors.

As discussed earlier, PAK1 shortens the lifespan of *C. elegans*, suggesting the possibility that natural PAK1-blockers (such as blueberry and propolis) could extend our healthy lifespan. Indeed, blueberry extract was shown to extend lifespan of both *C. elegans* and *Drosophila* (Wilson *et al*., 2006; Peng *et al*., 2012). Furthermore, the aging disease AD also depends on PAK1, and PTE was recently reported to improve the AD conditions in a mouse model (Chang *et al*., 2012).

Does prolactin that activate JAK2 (Rider *et al*., 2007) mediate RAS-induced PAK1 activation? YES, more than 2 decades ago, it was shown that RAS activates prolactin gene (Conrad and Gutierrez-Hartmann, 1992) and thereby JAK2 as well. Thus, at least three distinct Tyr-kinases (ETK, FYN and JAK2) are involved in RAS-induced PAK1 activation (see Fig. 2).

Capsaicin and capsiate

Red chili pepper contains an anti-cancer ingredient called capsacin. This pungent compound was found to inactivate both RAC and PI-3 kinase, thereby blocking their effectors, two oncogenic kinases (PAK1 and AKT) (Shin *et al*., 2008). It is also known that capsaicin activates the LKB1-AMPK cascade (Hwang *et al*., 2005). Thus, a Korean traditional cuisine called 'Kimchi', a cabbage pickle with Chili pepper and garlic, would be potentially beneficial for the therapy of a variety of cancers and other PAK1-dependent diseases. However, many western people are rather reluctant to eat this very spicy food everyday (perhaps except for Korean people!). Thus, more than 2 decades ago, Susumu Yazawa's group at Kyoto University in Japan managed to find a non-pungent derivative of capsaicin in a Thai variant of chili pepper called CH-19 Sweet (Yazawa *et al*., 1989). This derivative is called capsiate, and nitrogen atom in capsaicin is simply replaced with oxygen atom in capsiate (see Fig. 15). However, both compounds bind the same receptor TRPV1 (Iida *et al*., 2003) and activate the downstream signaling pathways, and eventually block PAK1 and activate the LKB1-AMPK cascade.

Sichuan peppercorns

A traditional Sichuan cuisine called 'Mapo-Tofu' is a spicy dish of beef and bean curd, and is prepared with two major seasonings, chili pepper and Sichuan peppercorns called 'Hua Jiao' in Chinese. Sichuan peppercorns (red) are closely related to Japanese green peppercorns called 'Sansho' in Japanese. Interestingly, several years ago, we found that 70% ethanol/warm (about 45 °C) water-soluble extracts of these Chinese/Japanese peppercorns (Zanthoxyli Fructus) from the plant

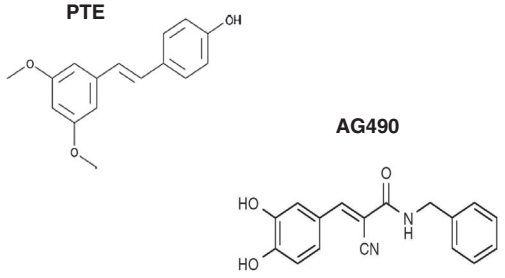


Figure 14. Pterostilben and AG 490.

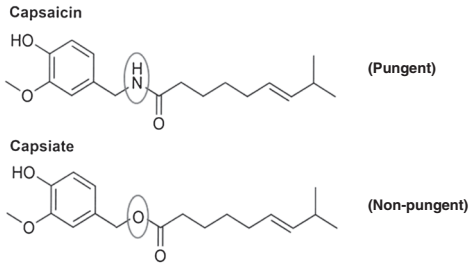


Figure 15. Capsiate and capsaicin.

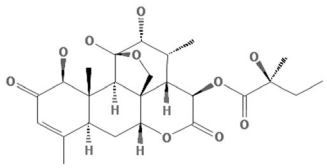


Figure 16. Glaucarubinone.

Zanthoxylum piperitum block selectively the oncogenic kinase PAK1, leading to the down-regulation of cyclin D1 (Hirokawa *et al*., 2006). Unlike capsaicin from chili pepper, these extracts are neither spicy nor inhibit AKT activation at the concentrations that block either cancer growth or PAK1 activation. The Chinese pepper extract, which is far more potent than the Japanese one, selectively inhibits the growth of NF1-deficient MPNST cells, without affecting the growth of normal fibroblasts and suppresses the growth of NF1-deficient human breast cancer (MDA-MB-231) xenograft in mice. Our data suggest that this peppercorn extract would be potentially useful for the treatment of PAK1-dependent solid tumors such as MPNST, pancreatic and breast cancers. Currently, John Beutler's group at NCI in Bethesda is isolating the major anti-PAK1 ingredient called 'Pepperin' in this extract to determine its chemical structure.

Simaroubaceae (Glaucarubinone)

As discussed earlier, infectious diseases such as malaria require PAK1, Thus, it is quite possible that some of the traditional anti-malaria herbal medicines could block PAK1. Back in 1947, it was shown that an extremely bitter extract from a bark of a tree family called Simaroubaceae administered at 1 mg/kg suppresses completely the growth of malaria in chickens. This extract has been used by native people in Amazon jungles to treat malaria for many centuries. More than 3 decades ago, the major anti-malaria ingredient in this extract was finally identified a triterpene/quassinoid called glaucarubinone (Trager and Polonsky, 1981). Interestingly, this compound (see Fig. 16) had been found earlier to inhibit the growth of murine leukemia cells at IC_{50} around 1 micro M (Ghosh *et al*., 1977). Furthermore, its therapeutic effect was demonstrated on a solid mammary tumor developed in mice (Valeriote *et al*., 1998). However, the molecular mechanism underlying its antimalaria/anticancer action remained unknown until recently. Finally a few years ago, John Beutler's group at NCI found that this compound blocks a transcription factor called AP1 with the IC_{50} around 20 nM (Beutler *et al*., 2009). Since the activation of AP1 requires either PAK1 or AKT, it is most likely that glaucarubinone blocks either PAK1 or AKT or both kinases. Finally, we confirmed directly that glaucarubinone blocks PAK1 and the growth of human schwannoma cells with the IC_{50} around 60 nM (Maruta *et al*., 2013). It is still unclear whether this compound blocks AKT or not, but since its more potent derivative called NBT-272 blocks both AKT and PAK1 with the IC_{50} around 10 nM (Castelletti *et al*., 2010), it is most likely that glaucabinone also affects both kinases.

CONCLUDING REMARKS

In short, PAK1 is responsible not only for cancers, but also for a variety of other diseases or disorders, and therefore both synthetic or natural products that block this kinase would be useful for the treatment of these illnesses in the future. However, none of synthetic PAK1-blockers is available on the market as yet. Thus, in this review, only natural PAK1-blockers inexpensively available on the market are discussed. Their potential pitfalls (side effects) are the reduction of both angiogenesis of embryos and fertility. Therefore, their usage should be avoided during pregnancy and IVF treatment.

In addition to these PAK1-blocking phyto-therapeutics from plants, there are a few natural PAK1-blockers called FK228 (Istodax) and Ivermectin from bacteria or fungi, which are currently available on the market as FDA-approved drugs. However, because of a limited space for this review, I omitted these antibiotics which have been discussed in detail previously (Maruta, 2011).

Conflict of Interest

The author has declared that there is no conflict of interest.

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