Probiotic microbes: Are their anti-melanogenicity and longevity promoting activities closely linked through the major "pathogenic" kinase PAK1?

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SUMMARY
PK1-deficient mutant of C. elegans lives 60% longer than the wild-type. Interestingly, PK1-deficient mutant of melanocytes produces less melanin (only a half compared with the wild-type) in the presence of either serum (PDGF) or α-MSH (alpha-melanocyte stimulating hormone). These observations indicate that the major "pathogenic" kinase PK1 is responsible for both shortening the healthy lifespan, and PDGF/α-MSH-dependent melanogenesis. For screening of PK1-blocking probiotic bacteria or their products, their anti-melanogenic as well as longevity promoting properties were examined. Recently it was found that C. elegans fed with Lactobacillus rhamnosus in Xinjiang cheese lives 40% longer than the worm fed with the standard E. coli. Interestingly, a Chinese traditional medicine called "ChiBai" fermented with the Lactobacillus rhamnosus also inhibited the α-MSH-induced melanogenesis, and this bacteria itself produces butyric acid that blocks the oncogenic HDAC (histone deacetylase)-PK1 signaling pathway. These findings strongly suggest, if not proven, that anti-melanogenic activity of Lactobacillus and many other probiotic bacteria might serve as a reliable indicator for their longevity promoting activity. In this context, a popular Japanese Lactobacillus-fermented milk drink called "Calpis", developed a century ago, and recently proven to inhibit the melanogenesis by suppressing the PK1-dependent tyrosinase gene expression, may potentially prolong our healthy lifespan.

Keywords
PK1, Lactobacillus, melanogenesis, longevity, C. elegans, Bacillus, COVID

1. Introduction

In 1908, a Russian/Ukrainianphysiologist, Ilya Mechnikov (1845-1916), shared a Nobel Prize in Medicine with Paul Ehrlich (1854-1915), a German/Jewish pathologist who developed the first chemotherapy called "Salvarsan" (or 606) against Syphilis in 1909. The former was an expert in phagocytes called macrophages, and published a rather sensational book entitled "Prolongation of life: Optimistic Studies" in 1907. In this book he proposed a theory that Lactobacillus from stomach from long-living Bulgarians who ingest routinely the Bulgarian local yogurts fermented with Lactobacillus could potentially be useful for our longevity. More than a century later, there are increasing biochemical evidences, provided mainly from the Far-East research groups, supporting this theory in principle.

In this century, the majority of scientists studying on the longevity opt for testing the potential longevity-promoting (so-called elixir) effect of given chemicals or organisms on a tiny worm called C. elegans, mainly because its lifespan is the shortest among aminal kingdom, around 15 days at 20°C. Not surprisingly among the genes responsible for shortening the life-span of this organism shared with mammals are "oncogenic" genes encoding PI-3 kinase (AGE), PK1, ILK, AKT and TOR (1-4). PI-3 kinase deficient mutant of this worm lives 100% longer than the wild-type (1), and PK1-deficient mutant lives 60% longer than the wild-type (2). Interestingly in both cases, these mutants show a very low fertility (less than 14% of the wild-type) (1,2), indicating that these oncogenes are essential for their fertility. In other words, longevity trades fertility (1,2).

Unfortunately, however, any chemical compounds such as LY3023414 which block the oncogenic PI-3 kinase-AKT signalling cannot be used clinically for promoting the longevity, simply because this pathway is essential for heart development/function as well (2,5). Tiny "experimental" invertebrates such as C. elegans and
Drosophila have no cardiovascular system. Fortunately, in 2021, PAK1-deficient mutant of mice was proven to live significantly longer than the wild-type without any complication on either heart or brain (6). In addition, mice treated with rapamycin, a TOR-inhibitor, have been shown to live longer than the control mice (7). However, this drug has been used mainly to suppress the immune response against grafted organs (2). Therefore it may be rather risky for ordinary people, in particular during pandemics of COVID and other deadly viruses.

2. Natural chemicals, that prolong the lifespan of C. elegans, inhibit melanogenesis by blocking PAK1

Using C. elegans as a target, a number of natural longevity promoters have been identified. Among are curcumin (CC), caffeic acid (CA), caffeic acid phenethyl ester (CAPE), and melatonin (8-11). Interestingly, all these longevity promoters are known to inhibit melanogenesis, without affecting directly the enzymatic activity of tyrosinase which is responsible for biosynthesis of melanin from tyrosine (12-15). Since the first three chemicals (CC, CA and CAPE) at least have been known to block PAK1, during 2015-2017 we examined whether melanogenesis of melanoma (B16F10) requires PAK1 or not. We found that treatment of melanoma with si-RNA specific for PAK1 (silencing PAK1 gene) clearly reduces the melanin synthesis to a half of the control cells only when cells are activated with either serum (PDGF) or alpha-MSH (16), indicating that the "induced" melanogenesis depends on PAK1, although the "basic" melanogenesis without PDGF or alpha-MSH does not (Figure 1A).

3. Lactobacillus rhamnosus extends the lifespan of C. elegans and inhibits melanogenesis

In 2016, to our great surprise, researchers found that C. elegans fed with Lactobacillus rhamnosus which is used for fermentation of Xinjiang cheese lives 40% longer than the worm fed with the standard E. coli. In 2020, other researchers found that an extract from a Chinese traditional herb mixture called "ChiBai" fermented with Lactobacillus rhamnosus inhibits alpha-MSH-induced melanogenesis of B16F10 melanoma by suppressing the tyrosinase gene expression (18) which depends on PAK1 (Figure 1B, 16). These two independent findings altogether indicate that both longevity-promoting and anti-melanogenic activities of this bacterium closely link to each other, and perhaps suggesting its PAK1-blocking activity. Incidentally, in 2021, another group found that Lactobacillus rhamnosus inhibits COVID fibrosis in part by producing butyric acid (19), which is known to inhibit HDAC (histone de-acetylase), thereby blocking PAK1 (20,21) that is responsible for inflammation, melanogenesis, oncogenesis and so many other diseases (for a review. 22).

4. Bacillus subtilis also extends the lifespan of C. elegans and inhibits melanogenesis.

It is well known that vitamin D3, a PAK1-blocker, is also anti-melanogenic and extends the healthy lifespan of C. elegans by 40% at 1 mg/mL (23,24). Interestingly, another vitamin called K2 or menaquinone 7 (Figure 2 left), derived from a traditional Japanese soybean product called "Natto" (fermented by Bacillus subtilis natto), also blocks PAK1 and is anti-melanogenic (25), although its longevity-promoting activity has not been tested as yet. In 2019, however, it was found that C. elegans fed with Bacillus subtilis, instead of the standard E. coli, at 20°C has 30% lesser size (number of eggs laid) than the E. coli-fed, and far more resistant to heat-shock at 34°C than

Figure 1. (A), “Melanogenic” signaling pathway (PDGF-PAK1). (B), Blocking melanin synthesis. PAK1-blockers do not inhibit directly tyrosinase, but suppress its gene expression.
the *E. coli*-fed, while a half of the latter die within 6 h (26). Since the litter size is reciprocal to the lifespan, and heat-resistance is proportional to the lifespan (1, 2), it is most likely that *Bacillus subtilis* is a longevity promoter, just like *Lactobacillus*. Interestingly a traditional Korean soybean paste (or cake) called "Doenjang" fermented with *Bacillus subtilis* is mainly produced in the Southern west region (Sunchang) of Korea, which is well known as the "longevity" town.

According to two Korean groups, "Doenjang" contains a PAK-blocker called "ortho-dihydroxyisoflavone" (Figure 2 right) that suppresses cancer growth, angiogenesis and melanogenesis (27, 28). More interestingly, in 2015, another Korean group found that Genistein ([4',5,7-trihydroxyisoflavone]), which is often produced by yeast fermentation, indeed extends the lifespan of *C. elegans* significantly, and increases its heat resistance by boosting *HSP16* gene expression at 50 μM (29). More recently genistein was found to boost the tumor suppressor p21 (CDK inhibitor) by blocking the JAK-PAK1 signaling pathway (30, 31).

5. Anti-melanogenic activity might be used as a reliable indicator for both PAK1-blocking and longevity-promoting activities

Indeed, it has been shown in 2005 that HDAC inhibitors such as butyrate and TSA (trichostatin A), which eventually block PAK1 (21), extend the healthy lifespan of *Drosophila* (32). Thus, if a given bacterium or chemical (natural or synthetic) inhibits alpha-MSH/PDGF-induced melanogenesis of B16F10 melanoma by suppressing tyrosinase gene expression, instead of inhibiting tyrosinase activity itself, it is hypothesized that this bacterium or chemical would be a PAK1-blocker, and therefore might extend the healthy lifespan. In other words, the inhibition of the inducible melanogenesis (without any inhibition of cell growth per se) might serve as an indicator for screening any PAK1-blocking probiotic bacteria, foods or chemicals/drugs that contribute to the longevity.

In this context, it would be worth noting that two independent Chinese and Japanese groups in 2016 and 2020, respectively found that an old Japanese *Lactobacillus* fermented milk drink called "Calpis", which was developed a century ago by a Japanese monk (Kaiun Mishima) using *L. helveticus*, inhibits the inducible melanogenesis of B16F10 melanoma by suppressing PAK1-dependent tyrosinase gene expression (33, 34). Thus, it is quite possible that this popular fermented milk could contribute to both COVID prevention/therapy and the longevity eventually (for review, 35).

**Funding:** None.

**Conflict of Interest:** The authors have no conflicts of interest to disclose.

**References**

1. Johnson TE, Lithgow GJ. The search for the genetic basis of aging: the identification of gerontogenes in the nematode *Caenorhabditis elegans*. J Am Geriatr Soc. 1992; 40:936-945.
2. Yanase S, Luo Y, Maruta H. PAK1-deficiency/down-regulation reduces brood size, activates *HSP16.2* gene and extends lifespan in *Caenorhabditis elegans*. Drug Discov Ther. 2013; 7:29-35.
3. Kumsta C, Ching TT, Nishimura M, Davis AE, Gelino S, Catan HH, Yu X, Chu CC, Ong B, Panowski SH, Baird N, Bodmer R, Hsu AL, Hansen M. Integrin-linked kinase modulates longevity and thermotolerance in *C. elegans* through neuronal control of HSF-1. Aging Cell. 2014; 13:419-430.
4. Blackwell TK, Sewell AK, Wu Z, Han M. TOR signaling in *Caenorhabditis elegans* development, metabolism, and aging. Genetics. 2019; 213:329-360.
5. Huddleston H, Tan B, Yang FC, White H, Wenning MJ, Orazi A, Yoder MC, Kapur R, Ingram DA. Functional *p85alpha* gene is required for normal murine fetal erythropoiesis. Blood. 2003; 102:142-145.
6. Hawley E, Gehlhausen J, Karchugina S, et al. PAK1 inhibition reduces tumor size and extends the lifespan of mice in a genetically engineered mouse model of neurofibromatosis type 2 (NF2). Hum Mol Genet. 2021; 30:1607-1617.
7. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Frenkel K, Carter CS, Paehr M, Javors MA, Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature. 2009; 460:392-395.
9. Pietsch K, Saul N, Chakrabarti S, Stürzenbaum SR, Menzel R, Steinberg CE. Hormetins, antioxidants and pro-oxidants: defining quercetin-, caffeic acid- and rosmarinic acid-mediated life extension in *Caenorhabditis elegans*. Biogerontology. 2011; 12:329-347.

10. Havermann S, Chovolou Y, Humphf HU, Wätjen W. Caffeic acid phenethyl-ester increases stress resistance and enhances lifespan in *Caenorhabditis elegans* by modulation of the insulin-like DAF-16 signalling pathway. PLoS One. 2014; 9:e100256.

11. Karadas O, Ozpinar N, Bilgic E, Ozcelik F, Karadas S. The physiological and lifespan alterations in *Caenorhabditis elegans* exposed to different dosages of melatonin. Pak J Pharm Sci. 2019; 32: 625-630.

12. Reiter R; Robinson J. Melatonin: your body's natural wonder drug, Bantam Book, New York, 1995.

13. Park SY, Jin ML, Kim YH, Kim Y, Lee SJ. Aromatic-turmerone inhibits α-MSH and IBMX-induced melanogenesis by inactivating CREB and MITF signaling pathways. Arch Dermatol Res. 2011; 303:737-744.

14. Lee JY, Choi HJ, Chung TW, Kim CH, Jeong HS, Ha KT. Caffeic acid phenethyl ester inhibits alpha-melanocyte stimulating hormone-induced melanin synthesis through suppressing transactivation activity of microphthalmia-associated transcription factor. J Nat Prod. 2013; 76:1399-1405.

15. Seo YK, Kim SJ, Boo YC, Baek JH, Lee SH, Koh JS. Effects of p-coumaric acid on erythema and pigmentation of human skin exposed to ultraviolet radiation. Clin Exp Dermatol. 2011; 36:260-266.

16. Be Tu PT, Nguyen BCQ, Tawata S, Yun CY, Kim EG, Maruta H. The serum/PDGF-dependent "melanogenic" role of the minute level of the oncogenic kinase PAK1 in melanoma cells proven by the highly sensitive kinase assay. Drug Discov Ther. 2017; 10:314-322.

17. Azat R, Liu Y, Li W, Kayir A, Lin DB, Zhou WW, Zheng XD. Probiotic properties of lactic acid bacteria isolated from traditionally fermented Xinjiang cheese. J Zhejiang Univ Sci B. 2016; 17:597-609.

18. Ho CC, Ng SC, Chuang HL, Chen YY, Wen SY, Kuo CH, Mahalakshmi B, Le QV, Huang CY, Kuo WW. Seven traditional Chinese herbal extracts (ChiBai) fermented by *Lactobacillus rhamnosus* attenuates PDE4B-mediated interleukin-6 induced by SARS-CoV-2 membrane glycoprotein. J Nutr Biochem. 2021; 98:10821.

19. Koyama Y, Adachi M, Sekiya M, Takekawa M, Imai K. Histone deacetylase inhibitors suppress IL-2-mediated gene expression prior to induction of apoptosis. Blood. 2000; 96:1400-1405.

20. Hirokawa Y, Arnold M, Nakajima H, Zalberg J, Maruta H. Signal therapy of breast cancers by the HDAC inhibitor FK228 that blocks the activation of PAK1 and abrogates the tamoxifen-resistance. Cancer Biol Ther. 2005; 4:956-960.

21. Maruta H, Ahn MR. From bench (laboratory) to bed (hospital/home): How to explore effective natural and synthetic PAK1-blockers/longevity-promoters for cancer therapy. Eur J Med Chem. 2017; 142:229-243.

22. Messing JA, Heuberger R, Schisa JA. Effect of vitamin D3 on lifespan in *Caenorhabditis elegans*. Curr Aging Sci. 2013; 6:220-224.

23. Maruta H, Kittaka A. Chemical evolution for taming the 'pathogenic kinase' PAK1. Drug Discov Today. 2020; 25:959-964.

24. Xia Y, Midoun SZ, Xu Z, Hong L. Heixuedian (heix), a potential melanotic tumor suppressor gene, exhibits specific spatial and temporal expression pattern during *Drosophila* hematopoiesis. Dev Biol. 2015; 398:218-230.

25. Hoang KL, Gerardo NM, Morran LT. The effects of *Bacillus subtilis* on *Caenorhabditis elegans* fitness after heat stress. Ecol Evol. 2019; 9:3491-3499.

26. Choi YH, Choi BT, Lee WH, Rhee SH, Park KY. Doenjang hexane fraction-induced G1 arrest is associated with the inhibition of pRB phosphorylation and induction of Cdk inhibitor p21 in human breast carcinoma MCF-7 cells. Oncol Rep. 2001; 8:1091-1096.

27. Choi YH, Choi BT, Lee WH, Rhee SH, Park KY. Signal therapy of breast cancers by the HDAC inhibitor **Vigna angularis** extends lifespan in *Caenorhabditis elegans*. Biomol Ther (Seoul). 2015; 23:77-83.

28. Ono M, Takeshima M, Nishi A, Higuchi T, Nakano S. Genistin suppresses v-Src-driven proliferative activity by arresting the cell-cycle at G2/M through increasing p21 level in Src-activated human gallbladder carcinoma cells. Nutr Cancer. 2021; 73:1471-1479.

29. Xu J, Xiong H, Zhao Z, Luo M, Ju Y, Yang G, Mei Z. Genistin suppresses allergic contact dermatitis through regulating the MAP2K2/ERK pathway. Food Funct. 2021; 12:4556-4569.

30. Zhuo Y, Sun H, Lu J, Li X, Chen X, Tao D, Huang W, Huang B. Lifespan extension and elevated *hsp* gene expression in *Drosophila* caused by histone deacetylase inhibitors. J Exp Biol. 2005; 208:697-705.

31. Song J, Shan C, Liu S, Zheng H, Liu M, Jin F, Wang L. Skin resistance to UVB-induced oxidative stress and hyperpigmentation by the topical use of *Lactobacillus helveticus* NS8-fermented milk supernatant. *J Appl Microbiol*. 2017; 123:511-523.

32. Ikarashi N, Fukuda N, Ochiai M, Sasaki M, Kon R, Matsumoto R, Ohira M, Koyama Y, Adachi M, Sekiya M, Takekawa M, Imai K. Histone deacetylase inhibitors suppress IL-2-mediated gene expression prior to induction of apoptosis. Blood. 2000; 96:1400-1405.

33. Pham MT, Yang AJ, Kao MS, Gankhuyag U, Zayabaatar E, Jin SC, Huang CM. Gut probiotic *Lactobacillus rhamnosus* attenuates PDE4B-mediated interleukin-6 induced by SARS-CoV-2 membrane glycoprotein. *J Nutr Biochem*. 2021; 98:10821.

34. Phi KM, Hyun DH, Song J, Kim Y, Lee S, Park J, Song Y, Park T, Kim JS, Kim SJ, Kim SJ, Park JH. Genistein suppresses allergic contact dermatitis through regulating the MAP2K2/ERK pathway. *Food Funct*. 2021; 12:4556-4569.

35. Maruta H, He H. PAK1-blockers: Potential therapeutics against COVID-19. *Med Drug Discov*. 2020; 6:100039.

Received February 9, 2022; Revised February 24, 2022; Accepted February 27, 2022.

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