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Molecular basis of longevity sustaining characteristics of Chinese medicine herbs

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ABSTRACT

The balancing between tumor suppression and cell viability is the key to balance aging and tumorigenesis, and reach tumor-free longevity, or healthy aging. We speculate that the balance of tumor suppression signaling and the cell growth sustaining mechanisms in molecular genetics might reflect the balance between Yin and Yang in Chinese medicine. Some Chinese medicine herbs are known to sustain longevity, and help to prevent aging related diseases, such as *Panax Ginseng, Ganoderma Lucidum* etc. Based on Chinese medicine databases and the molecular signature database, we try to apply *in silico* systems pharmacology to analyze the function of these herbs in modulating signaling pathways. We found that most of these herbs could enhance cellular mitochondrial function: oxidative phosphorylation. Surprisingly, the lipids metabolism pathways were found in all the herbs analyzed. Interestingly, the cell proliferation pathways, such as telomere maintenance pathway, Myc pathway, E2F pathway, as well as the cell quality control pathway (apoptosis pathway) were also found to be enhanced in most herbs, which added evidences to our balancing hypothesis. Thus, our data revealed the sustainable energy production, the sustainable cell proliferation, and the quality control build the life triangle for the mechanism of action of Chinese medicinal herbs with longevity sustaining property. This methodology might help to predict the molecular mechanism of Chinese medicine herbs, and further understand the formulation of Chinese medicine in preventing aging-related diseases. The limitations of this kind of analysis are also discussed.

Introduction

The balancing between tumor suppression and cell viability is the key to balance aging and tumorigenesis, and reach tumor-free longevity, or healthy aging.

As we previously reviewed, from the view of molecular genetics, the balance between aging and tumorigenesis means the balance of tumor suppressor signaling and the cell growth sustaining mechanism. These signals include the tumor suppressor signaling, such as p53 regulated pathways, apoptosis regulating pathways, immune response pathways, etc., the cell growth sustaining mechanism such as telomere maintenance mechanism, genetic material (DNA, RNA) fidelity maintenance mechanism, cell cycle progression pathways, as well as the function of energy producing machinery, mitochondria [1].

From the view of Chinese medicine, healthy aging means the balance between Yin and Yang, or between Yin and Qi (or Chi), which is speculated to be energy or mitochondrial function [2]. Some Chinese medicine herbs are known to sustain longevity, and help to prevent aging related diseases, such as *Panax Ginseng, Codonopsis Pilosula, Radix Astragali, Ganoderma Lucidum, Polygonatum sibiricum, Radix Ophiopogonis.* The *Panax Ginseng, Codonopsis Pilosula,* and *Radix Astragali* are known to enhance Qi in Chinese medicine. The *Ganoderma Lucidum, Polygonatum sibiricum. Radix Ophiopogonis* are known to nourish Yin in Chinese medicine, which was speculated as cell survival signaling [1]. However, due to the complicated composition of natural products in Chinese medical herbs, and the process of body absorption, biological availability, etc., it is extremely hard to study the mechanism of action for Chinese medical herbs with the concept of conventional pharmacological methodology.

In silico systems pharmacology was developed to predict and analyze the target genes or proteins for each natural compounds in Chinese medicinal herb based on the drug-target interactions [3–5]. Combined with the disease indications, *in silico* systems pharmacology could provide profound drug-target-disease network for further research or experimental validation, which greatly facilitate the dissection of molecular mechanism of Chinese medicine, and the precision application of Chinese medicine in clinical practice.

By combined the information from the Chinese Medicine database BATMAN-TCM [6], ETCM [7], TCM-ID [8], HERB [9], we collected the target gene information for each herb. Then we analyzed these targets by

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Table 1
Panax Ginseng Overlap Results.

Collection(s):	BIOCARTA, PID, HALLMARK				
# overlaps shown:	20				
# genesets in collections:	538				
# genes in comparison (n):	1319				
# genes in universe (N):	40,312				
Gene Set Name	# Genes in Gene Set (K) # Genes in Overlap (k) k/K	p-value	FDR q-value		
HALLMARK_XENOBIOTIC_METABOLISM	200	61	0.305	1.36E-41	7.29E-39
HALLMARK_FATTY_ACID_METABOLISM	158	46	0.2911	1.12E-30	3.02E-28
HALLMARK_BILE_ACID_METABOLISM	112	38	0.3393	2.41E-28	4.32E-26
BIOCARTA_NUCLEARRS_PATHWAY	34	23	0.6765	1.17E-26	1.57E-24
PID_RXR_VDR_PATHWAY	26	20	0.7692	3.28E-25	3.53E-23
HALLMARK_UV_RESPONSE_DN	144	39	0.2708	6.37E-25	5.71E-23
HALLMARK_ADIPOGENESIS	200	42	0.21	4.64E-22	3.56E-20
HALLMARK_MTORC1_SIGNALING	200	41	0.205	3.74E-21	2.01E-19
HALLMARK_MYOGENESIS	200	41	0.205	3.74E-21	2.01E-19
HALLMARK_OXIDATIVE_PHOSPHORYLATION	200	41	0.205	3.74E-21	2.01E-19
PID_HIF1_TFPATHWAY	66	25	0.3788	1.72E-20	8.43E-19
HALLMARK_GLYCOLYSIS	200	40	0.2	2.92E-20	1.31E-18
HALLMARK_APOPTOSIS	161	35	0.2174	3.94E-19	1.63E-17
PID_VEGFR1_2_PATHWAY	69	24	0.3478	1.04E-18	4.00E-17
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	200	38	0.19	1.62E-18	5.82E-17
HALLMARK_ALLOGRAFT_REJECTION	200	37	0.185	1.15E-17	3.87E-16
PID_AP1_PATHWAY	70	23	0.3286	2.37E-17	7.49E-16
HALLMARK_HYPOXIA	200	36	0.18	7.91E-17	2.36E-15
PID_SMAD2_3NUCLEAR_PATHWAY	82	24	0.2927	9.80E-17	2.78E-15
PID_TELOMERASE_PATHWAY	67	22	0.3284	1.19E-16	3.19E-15

investigating the overlap of gene sets in Molecular Signature Database (V7.4) [10]. By this way, we try to apply *in silico* systems Pharmacology to analyze the molecular mechanism of action of these herbs in modulating longevity related cellular functions.

Materials and methods

The Chinese Medicine database BATMAN-TCM [6] were used to predict the target genes for *Panax Ginseng, Codonopsis Pilosula, Radix Astragali, Ganoderma Lucidum, Polygonatum sibiricum, Radix Ophiopogonis.* The cutoff score for the target perdition was set as 20 (P<0.05). The ETCM [7], TCM-ID [8], and HERB [9]were used to confirm and add up the target genes predicted by BATMAN-TCM, the genes predicted were combined together for further analysis. Due to the lack of experimental target gene information in response to the treatment of Chinese medicine, at this stage, we tried to include as many target genes as possible, only the duplicate genes predicted from different databases were cleared. By this way, we could predict as many candidate pathways as possible, which might serve important role in further study.

To analyze the molecular signaling pathways conducted by these target genes of each herb, we computed the overlap of these targets with gene sets in Molecular Signature Database (V7.4) [10]. The Hallmark gene sets (composed of 50 gene sets that summarize well-defined biological states or processes and display coherent expression) [11], BioCarta gene sets (composed of 292 canonical pathways derived from the BioCarta pathway database)(www.BioCarta.com), and PID gene sets (composed of 196 canonical pathways derived from the PID pathway database) [12]were used to do overlap. For each herb, the top 20 overlap pathways were collected (FDR q-values<0.05).

Results

The gene targets and signaling pathways of several Chinese medicine herbs with longevity sustaining characteristics

Panax Ginseng

Panax Ginseng is one of the most popular Chinese medicine herbs with anti-aging property. The potential target genes were predicted based on the single compounds found in Ginseng, such as panasinsanol A, ginsenosides, ginsenoynes stigmasterol, etc. After removing the repeat genes, around 1319 target genes were collected and overlap with gene sets in Molecular Signature Database. The top 20 pathways were collected in Table 1. The top three overlapped pathways were HALLMARK XENOBIOTIC METABOLISM, HALLMARK FATTY ACID METABOLISM, and HALLMARK BILE ACID METABOLISM, suggesting the importance of lipid metabolism in *Panax Ginseng* action, which is related to mitochondrial function. We also found the direct mitochondria energy producing pathways HALLMARK OXIDATIVE PHOSPHORYLATION, which confirmed the impact of *Panax Ginseng* on mitochondrial function. We also found the hypoxia related pathways (PID HIF1 TFPATHWAY, HALLMARK HYPOXIA), suggesting the action of *Panax Ginseng* on hypoxia. Strikingly, we found both telomerase pathway (PID TELOMERASE PATHWAY) and death pathway (HALLMARK APOPTOSIS). These data suggested that *Panax Ginseng* itself already balanced in enhance both cell proliferation and tumor suppression function. In fact, in Chinese medicine, *Panax Ginseng* itself could be a formulation.

Codonopsis Pilosula

The slang for *Codonopsis Pilosula* is “economic ginseng”, which was applied similarly with *Panax Ginseng* in Chinese medicine practice. Based on the single compound components found in *Codonopsis Pilosula*, such as atractylenolides, stigmasterol, taraxerone, codonopsine, curcumene etc., the potential target genes were predicted. After removing the repeat genes, around 1341 target genes were collected and overlap with gene sets in Molecular Signature Database. The top 20 pathways were collected in Table 2. The top three overlapped pathways were HALLMARK XENOBIOTIC METABOLISM, HALLMARK FATTY ACID METABOLISM, and HALLMARK BILE ACID METABOLISM, same as in *Panax Ginseng.* Again, the mitochondria energy producing pathway (HALLMARK OXIDATIVE PHOSPHORYLATION) and hypoxia pathway (HALLMARK HYPOXIA) showed up. Together, these data suggested the similarity of *Codonopsis Pilosula* and *Panax Ginseng* in regulating mitochondrial function and hypoxia responses.

Radix Astragali

Radix Astragali is another popular Chinese medicine for enhancing Qi. Based on the single compound components found in *Radix Astragali*, such as astragalosides, chrysanthemaxanthin, quercetin, sitosterol,

Table 2
Codonopsis Pilosula Overlap Results.

Collection(s):	BIOCARTA, PID, HALLMARK				
# overlaps shown:	20				
# genesets in collections:	538				
# genes in comparison (n):	1341				
# genes in universe (N):	40,312				
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
HALLMARK_XENOBIOTIC_METABOLISM	200	69	0.345	1.59E-50	8.58E-48
HALLMARK_FATTY_ACID_METABOLISM	158	44	0.2785	3.32E-28	8.93E-26
HALLMARK_BILE_ACID_METABOLISM	112	35	0.3125	1.35E-24	1.94E-22
HALLMARK_MYOGENESIS	200	45	0.225	1.45E-24	1.94E-22
PID_VEGFR1_2_PATHWAY	69	28	0.4058	1.42E-23	1.53E-21
PID_REG_GR_PATHWAY	82	29	0.3537	2.36E-22	2.12E-20
HALLMARK_ADIPOGENESIS	200	41	0.205	6.82E-21	5.24E-19
PID_AP1_PATHWAY	70	26	0.3714	8.08E-21	5.44E-19
HALLMARK_ALLOGRAFT_REJECTION	200	40	0.2	5.24E-20	2.82E-18
HALLMARK_GLYCOLYSIS	200	40	0.2	5.24E-20	2.82E-18
HALLMARK_APOPTOSIS	161	36	0.2236	7.74E-20	3.79E-18
BIOCARTA_NUCLEARRS_PATHWAY	34	19	0.5588	8.44E-20	3.79E-18
HALLMARK_INFLAMMATORY_RESPONSE	200	38	0.19	2.81E-18	1.16E-16
PID_ATF2_PATHWAY	59	22	0.3729	7.13E-18	2.74E-16
PID_RXR_VDR_PATHWAY	26	16	0.6154	8.00E-18	2.87E-16
HALLMARK_OXIDATIVE_PHOSPHORYLATION	200	37	0.185	1.96E-17	6.59E-16
PID_ERBB2_ERBB3_PATHWAY	44	19	0.4318	4.67E-17	1.48E-15
BIOCARTA_NFAT_PATHWAY	51	20	0.3922	6.96E-17	2.08E-15
HALLMARK_UV_RESPONSE_DN	144	31	0.2153	8.83E-17	2.50E-15
PID_HIF1_TFPATHWAY	66	22	0.3333	1.16E-16	3.11E-15

Table 3
Radix Astragali Overlap Results.

Collection(s):	BIOCARTA, PID, HALLMARK				
# overlaps shown:	20				
# genesets in collections:	538				
# genes in comparison (n):	499				
# genes in universe (N):	40,312				
Gene Set Name	# Genes in Gene Set (K) # Genes in Overlap (k) k/K	p-value	FDR q-value		
HALLMARK_XENOBIOTIC_METABOLISM	200	37	0.185	3.17E-32	1.71E-29
HALLMARK_FATTY_ACID_METABOLISM	158	22	0.1392	6.58E-17	1.77E-14
BIOCARTA_NUCLEARRS_PATHWAY	34	12	0.3529	4.86E-15	8.72E-13
HALLMARK_UV_RESPONSE_UP	158	19	0.1203	1.33E-13	1.79E-11
BIOCARTA_PPARA_PATHWAY	52	12	0.2308	1.50E-12	1.61E-10
PID_ALPHA_SYNUCLEIN_PATHWAY	32	10	0.3125	3.90E-12	3.50E-10
PID_RXR_VDR_PATHWAY	26	9	0.3462	1.65E-11	1.27E-09
HALLMARK_APOPTOSIS	161	17	0.1056	2.15E-11	1.45E-09
HALLMARK_E2F_TARGETS	200	18	0.09	8.07E-11	3.94E-09
HALLMARK_MTORC1_SIGNALING	200	18	0.09	8.07E-11	3.94E-09
HALLMARK_OXIDATIVE_PHOSPHORYLATION	200	18	0.09	8.07E-11	3.94E-09
HALLMARK_BILE_ACID_METABOLISM	112	14	0.125	1.32E-10	5.90E-09
PID_ATF2_PATHWAY	59	11	0.1864	1.54E-10	6.36E-09
HALLMARK_DNA_REPAIR	150	15	0.1	7.02E-10	2.52E-08
BIOCARTA_FREE_PATHWAY	10	6	0.6	7.03E-10	2.52E-08
PID_AP1_PATHWAY	70	11	0.1571	1.05E-09	3.53E-08
BIOCARTA_ARENRF2_PATHWAY	20	7	0.35	2.88E-09	9.11E-08
HALLMARK_GLYCOLYSIS	200	16	0.08	5.03E-09	1.42E-07
HALLMARK_INFLAMMATORY_RESPONSE	200	16	0.08	5.03E-09	1.42E-07
PID_CMYB_PATHWAY	84	11	0.131	7.72E-09	2.08E-07

canavanine etc., the potential target genes were predicted. After removing the repeat genes, around 541 target genes were collected and overlap with gene sets in Molecular Signature Database. The top 20 pathways were collected in Table 3. The top three overlapped pathways were HALLMARK XENOBIOTIC METABOLISM, HALLMARK BILE ACID METABOLISM, and BIOCARTA NUCLEARRS PATHWAY, similar but different with *Panax Ginseng*. Again, the mitochondria energy producing pathways (HALLMARK OXIDATIVE PHOSPHORYLATION), mitochondrial and peroxisome related PPAR pathway (BIOCARTA PPARA PATHWAY) were found, confirming the connection between Qi and mitochondrial function. Strikingly, several cell quality control pathways (HALLMARK DNA REPAIR, HALLMARK APOPTOSIS, BIOCARTA FREE PATHWAY, BIOCARTA ARENRF2 PATHWAY) were found, as well as cell proliferation pathways, such as HALLMARK E2F TARGETS, HALLMARK MYC TARGETS, PID CMYB PATHWAY. These data suggested the action of *Radix Astragali* on enhancing mitochondrial function, anti-oxidation, and cell survival with quality control.

Ganoderma Lucidum

Ganoderma Lucidum is one of the most famous anti-aging herbs in Chinese medicine, known to nourish Yin and elongate the lifespan. Based on the single compound components found in *Ganoderma Lucidum*, such as ergosterol, ganosporelactones, ganoderiols, lucidones, ergotamine, ganodermanondiol etc., the potential target genes were predicted. After removing the repeat genes, around 597 target genes were collected and overlap with gene sets in Molecular Signature Database. The top 20 pathways were collected in Table 4. The top three over-

Table 4
Ganoderma Lucidum Overlap Results.

Collection(s):	BIOCARTA, PID, HALLMARK				
# overlaps shown:	20				
# genesets in collections:	538				
# genes in comparison (n):	597				
# genes in universe (N):	40,312				
Gene Set Name	# Genes in Gene Set (K) # Genes in Overlap (k) k/K	p-value	FDR q-value		
PID_RXR_VDR_PATHWAY	26	19	0.7308	7.81E-30	4.20E-27
BIOCARTA_NUCLEARRS_PATHWAY	34	19	0.5588	1.98E-26	5.31E-24
BIOCARTA_EICOSANOID_PATHWAY	22	14	0.6364	6.02E-21	1.08E-18
HALLMARK_XENOBIOTIC_METABOLISM	200	29	0.145	3.07E-20	4.12E-18
HALLMARK_UV_RESPONSE_DN	144	24	0.1667	2.01E-18	2.16E-16
PID_RETINOIC_ACID_PATHWAY	30	13	0.4333	1.38E-16	1.24E-14
PID_VEGFR1_2_PATHWAY	69	17	0.2464	1.85E-16	1.42E-14
HALLMARK_BILE_ACID_METABOLISM	112	20	0.1786	3.46E-16	2.33E-14
HALLMARK_APOPTOSIS	161	22	0.1366	3.99E-15	2.39E-13
HALLMARK_MYOGENESIS	200	23	0.115	4.19E-14	2.25E-12
BIOCARTA_KERATINOCYTE_PATHWAY	46	13	0.2826	9.43E-14	4.61E-12
PID_ATF2_PATHWAY	59	14	0.2373	1.51E-13	6.79E-12
HALLMARK_ESTROGEN_RESPONSE_LATE	200	22	0.11	3.74E-13	1.55E-11
BIOCARTA_PPARA_PATHWAY	52	13	0.25	5.42E-13	2.08E-11
PID_LYSOPHOSPHOLIPID_PATHWAY	65	14	0.2154	6.40E-13	2.30E-11
PID_AR_TF_PATHWAY	53	13	0.2453	7.09E-13	2.38E-11
PID_FGF_PATHWAY	55	13	0.2364	1.19E-12	3.63E-11
PID_REG_GR_PATHWAY	82	15	0.1829	1.21E-12	3.63E-11
PID_CERAMIDE_PATHWAY	44	12	0.2727	1.37E-12	3.87E-11
HALLMARK_PEROXISOME	104	16	0.1538	3.50E-12	9.42E-11

lapped pathways were PID RXR VDR PATHWAY, BIOCARTA NUCLEARRS PATHWAY and BIOCARTA EICOSANOID PATHWAY, suggesting the lipid metabolism as the major mechanism of action for *Ganoderma Lucidum*. Also, eicosanoid pathway is known to be involved in anti-aging mechanism. The mitochondrial and peroxisome related pathways (BIOCARTA PPARA PATHWAY, HALLMARK PEROXISOME) were also found. Interestingly, we observed pathways related to anti-skin aging, such as BIOCARTA KERATINOCYTE PATHWAY, HALLMARK ESTROGEN RESPONSE LATE, and PID CERAMIDE PATHWAY. These data showed that *Ganoderma Lucidum* could enhancing lipid metabolism and act on anti-skin aging.

Polygonatum sibiricum

Polygonatum sibiricum is called “immortal herb” in Chinese culture. The potential target genes were predicted based on the single compound components found in *Polygonatum sibiricum*, such as kinganone, isomucronulatol, isoliquiritigenin, etc. After removing the repeat genes, around 293 target genes were collected and overlap with gene sets in Molecular Signature Database. The top 20 pathways were collected in Table 5. The top three overlapped pathways were HALLMARK OXIDATIVE PHOSPHORYLATION, HALLMARK XENOBIOTIC METABOLISM, and HALLMARK GLYCOLYSIS, suggesting the action of *Polygonatum sibiricum* in regulating mitochondria and carbohydrate metabolism. The found of BIOCARTA ETC PATHWAY confirmed the function in mitochondria. Like in *Ganoderma Lucidum,* we also found pathways related to anti-skin aging, including PID CERAMIDE PATHWAY, BIOCARTA KERATINOCYTE PATHWAY, and HALLMARK ESTROGEN RESPONSE LATE. The cell survival pathway HALLMARK MYC TARGETS V1 showed up too. These data suggested the preference of *Polygonatum sibiricum* function in anti-aging, interestingly, mainly *via* regulating mitochondrial function and cell survival signals.

Radix Ophiopogonis

Based on the single compound components found in *Radix Ophiopogonis*, such as ophiopogonones, ophiopogonin, isoophipogonone etc., the potential target genes were predicted. After removing the repeat genes, around 272 target genes were collected and overlap with gene sets in Molecular Signature Database. The top 20 pathways were collected in Table 6. The top three overlapped pathways were HALLMARK XENOBIOTIC METABOLISM, HALLMARK FATTY ACID METABOLISM, and HALLMARK ADIPOGENESIS, suggesting the main function of *Radix Ophiopogonis* in lipid metabolism. The HALLMARK OXIDATIVE PHOSPHORYLATION, following significant pathways BIOCARTA NOS1 PATHWAY, HALLMARK ADIPOGENESIS, BIOCARTA NUCLEARRS_PATHWAY, and HALLMARK BILE ACID METABOLISM also confirmed this function. We found the cell death pathway HALLMARK APOPTOSIS in a significant rank (Table 6). Interestingly, the cell survival pathways, such as telomerase pathway (BIOCARTA TEL PATHWAY), BIOCARTA NOS1 PATHWAY, and PID ERBB1 RECEPTOR PROXIMAL PATHWAY were also regulated.

The signaling pathways shared by longevity sustaining herbs

To further analyze the molecular mechanism of action of above longevity sustaining herbs, we compare the top 20 pathways revealed from above herbs. Most significantly, we found lipid metabolism related pathways were enriched in all 6 herbs, including HALLMARK XENOBIOTIC METABOLISM, HALLMARK FATTY ACID METABOLISM, and HALLMARK BILE ACID METABOLISM, suggesting the importance of regulating lipid metabolism in longevity sustaining herbs. Interestingly, except *Ganoderma Lucidum*, we found the enhancement of mitochondrial function (HALLMARK OXIDATIVE PHOSPHORYLATION) in 5 herbs, not just in Qi enhancing herbs. On the other hand, we also found the cell proliferating pathways in 5 herbs, including telomerase pathway in *Panax Ginseng* (PID TELOMERASE PATHWAY), cell cycle pathways in *Radix Astragali* (HALLMARK E2F TARGETS, HALLMARK MYC TARGETS, PID CMYB PATHWAY), anti-skin aging pathways in *Ganoderma Lucidum* (BIOCARTA KERATINOCYTE PATHWAY, HALLMARK ESTROGEN RESPONSE LATE, and PID CERAMIDE PATHWAY), cell cycle pathways in *Polygonatum sibiricum* (HALLMARK MYC TARGETS), and telomerase pathway in *Radix Ophiopogonis* (BIOCARTA TEL PATHWAY). Interestingly, the BIOCARTA KERATINOCYTE PATHWAY was found in all 3 Yin nourishing herbs, which fit the concept of anti-skin aging function of Yin nourishing herbs.

Except *Polygonatum sibiricum*, apoptosis pathway (HALLMARK APOPTOSIS) was found in all other 5 herbs, suggesting the importance of clearance of damaged cells in longevity sustaining. Together, the sustainable energy production, the sustainable cell proliferation, and the quality control build the life triangle for the mechanism of action of Chinese medicinal herbs with longevity sustaining property. (Fig. 1).

Table 5
Polygonatum sibiricum Overlap Results.

Collection(s):	BIOCARTA, PID, HALLMARK				
# overlaps shown:	20				
# genesets in collections:	538				
# genes in comparison (n):	293				
# genes in universe (N):	40,312				
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
HALLMARK_OXIDATIVE_PHOSPHORYLATION	200	25	0.125	1.77E-23	9.53E-21
HALLMARK_XENOBIOTIC_METABOLISM	200	20	0.1	4.41E-17	1.18E-14
HALLMARK_GLYCOLYSIS	200	18	0.09	1.08E-14	1.93E-12
HALLMARK_FATTY_ACID_METABOLISM	158	16	0.1013	5.24E-14	7.05E-12
HALLMARK_ADIPOGENESIS	200	13	0.065	3.19E-09	3.43E-07
BIOCARTA_CERAMIDE_PATHWAY	21	6	0.2857	6.94E-09	6.22E-07
BIOCARTA_EXTRINSIC_PATHWAY	13	5	0.3846	2.41E-08	1.85E-06
HALLMARK_HEME_METABOLISM	200	12	0.06	3.15E-08	1.88E-06
HALLMARK_MTORC1_SIGNALING	200	12	0.06	3.15E-08	1.88E-06
BIOCARTA_KERATINOCYTE_PATHWAY	46	7	0.1522	4.19E-08	2.25E-06
HALLMARK_COAGULATION	138	10	0.0725	7.69E-08	3.60E-06
BIOCARTA_SPPA_PATHWAY	16	5	0.3125	8.02E-08	3.60E-06
PID_AVB3_OPN_PATHWAY	31	6	0.1935	8.85E-08	3.66E-06
BIOCARTA_AMI_PATHWAY	20	5	0.25	2.78E-07	1.07E-05
BIOCARTA_ETC_PATHWAY	10	4	0.4	5.55E-07	1.99E-05
BIOCARTA_INTRINSIC_PATHWAY	23	5	0.2174	5.93E-07	1.99E-05
PID_CERAMIDE_PATHWAY	44	6	0.1364	7.84E-07	2.48E-05
HALLMARK_BILE_ACID_METABOLISM	112	8	0.0714	1.75E-06	5.23E-05
HALLMARK_ESTROGEN_RESPONSE_EARLY	200	10	0.05	2.35E-06	6.10E-05
HALLMARK_MYC_TARGETS_V1	200	10	0.05	2.35E-06	6.10E-05

Table 6
Radix Ophiopogonis Overlap Results.

Collection(s):	BIOCARTA, PID, HALLMARK				
# overlaps shown:	20				
# genesets in collections:	538				
# genes in comparison (n):	272				
# genes in universe (N):	40,312				
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
HALLMARK_XENOBIOTIC_METABOLISM	200	24	0.12	6.34E-23	3.41E-20
HALLMARK_FATTY_ACID_METABOLISM	158	16	0.1013	1.66E-14	4.45E-12
HALLMARK_ADIPOGENESIS	200	15	0.075	8.93E-12	1.60E-09
HALLMARK_APOPTOSIS	161	13	0.0807	8.85E-11	1.19E-08
HALLMARK_PEROXISOME	104	11	0.1058	1.38E-10	1.49E-08
HALLMARK_BILE_ACID_METABOLISM	112	11	0.0982	3.10E-10	2.78E-08
PID_VEGFR1_2_PATHWAY	69	8	0.1159	2.27E-08	1.74E-06
BIOCARTA_TEL_PATHWAY	17	5	0.2941	7.81E-08	5.25E-06
BIOCARTA_ARENRF2_PATHWAY	20	5	0.25	1.92E-07	1.15E-05
BIOCARTA_NOS1_PATHWAY	21	5	0.2381	2.51E-07	1.35E-05
BIOCARTA_KERATINOCYTE_PATHWAY	46	6	0.1304	6.67E-07	3.26E-05
PID_VEGFR1_PATHWAY	26	5	0.1923	7.90E-07	3.54E-05
HALLMARK_ALLOGRAFT_REJECTION	200	10	0.05	1.21E-06	4.65E-05
HALLMARK_OXIDATIVE_PHOSPHORYLATION	200	10	0.05	1.21E-06	4.65E-05
BIOCARTA_BARR_MAPK_PATHWAY	14	4	0.2857	1.92E-06	6.90E-05
PID_ATF2_PATHWAY	59	6	0.1017	2.98E-06	9.84E-05
BIOCARTA_NUCLEARRS_PATHWAY	34	5	0.1471	3.20E-06	9.84E-05
PID_FCER1_PATHWAY	60	6	0.1	3.29E-06	9.84E-05
PID_AR_PATHWAY	61	6	0.0984	3.63E-06	9.97E-05
PID_ERBB1_RECEPTOR_PROXIMAL_PATHWAY	35	5	0.1429	3.71E-06	9.97E-05

Discussion

The artificial interference of aging process is the key to prevent or even treat aging-related diseases, and reach the goal of healthy aging. While the balancing between cell quality control pathways and cell survival signaling might be the key strategy for artificial interfering.

Traditional Chinese Medicine, as a balanced and integrated concept of medicine, possesses the great potential in promoting healthy aging.

As a proof of concept, we applied *in silico* systems pharmacology to analyze the function of 6 well known longevity sustaining herbs (3 Qi enhancing herbs and 3 Yin nourishing herbs) in modulating longevity-related signaling pathways. We have chosen the typical six Chinese medicines based on the popularity of these medicines in enhancing Qi or Nourishing Yin in clinical application, as well as the popularity in their application in aging intervention.

The mitochondrial function is tightly connected with energy production, apoptosis, as well as ROS production, the dysfunction of mitochondria has been established as one of the hallmarks of aging [13]. It has been shown in a meta-analysis of age-related gene expression profiles using 27 datasets from mice, rats and humans, that the age-related biological processes involved an underexpression of genes associated with energy metabolism, particularly mitochondrial genes [14]. Our data revealed that no matter the Qi enhancing herbs or the Yin nourishing herbs, they all acted in sustaining mitochondrial function, specifically, the oxidative phosphorylation. Other than this, the PPAR

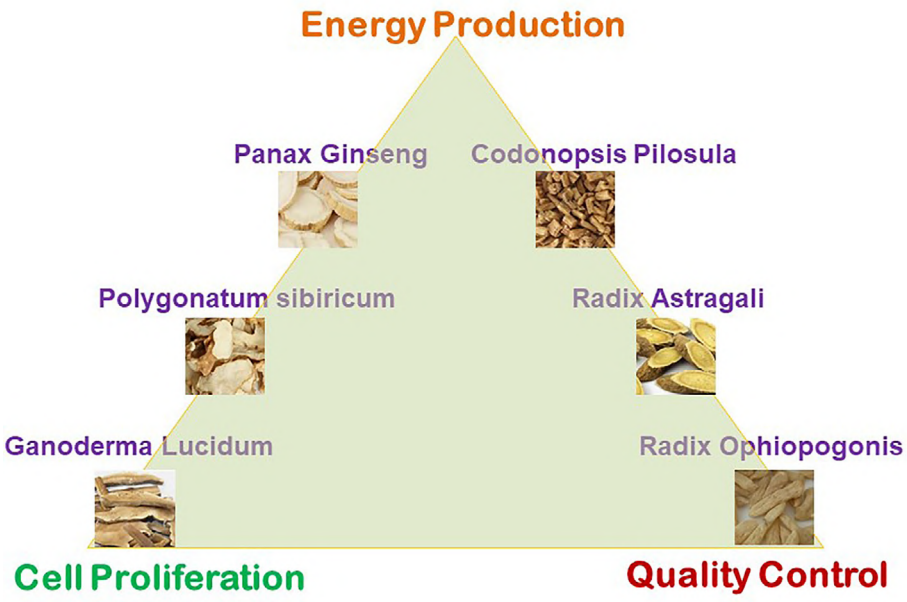

Fig. 1. The life triangle for longevity sustained by Chinese medicinal herbs.

pathway and/or NRF pathway were also revealed in *Radix Astragali, Ganoderma Lucidum,* and *Radix Ophiopogonis* (Table 3, 5 and 6), confirming the enhancement of mitochondrial function. We also found the lipid metabolism related pathways in all 6 herbs, including the pathways for fatty acid metabolism, bile acid metabolism, and PPAR regulating pathway. The role of lipid metabolism and its related pathways in aging related process could be involved in the energy production (cross-talking with mitochondrial function), cell survival, or cell communication, and in turn affects aging process [15]. Fatty acids are important source of energy *via* fatty acid oxidation in the liver during fasting, calorie restriction (CR) and high fat diet. It has been shown that mice lacking SIRT1 impaired the activation of PPARα and PGC1α, and thus the fatty acid oxidation, suggesting that SIRT1 plays as an important role in regulating fatty acid oxidation in the liver [16, 17]. However, whether the amount of lipid, such as triglycerides, cholesterol or lipoproteins could serve as the biomarker for aging process is still controversial, due to the complexity of the lipid metabolism regulation [15]. The systematic approaches are required to better understand the relationship between lipid metabolism and aging.

The systematic approaches could also be very powerful in dissecting the mechanism of action of Chinese medicine on lipid metabolism, and the impact on aging intervention. The systems pharmacology could serve the purpose. First, before design the wet lab experiments, we could apply the systems pharmacology on the Chinese medicinal herbs or formulation, to predict the possible pathways related to lipid metabolism. Then the systematic web lab systems, such as transcriptome or proteomics analysis could be performed to dissect the pathways related to lipid metabolism. This could be applied to the mixture of the formulation, the whole extract of the individual medicinal plant, the fraction, or the single compound. Finally, the web lab functional analysis could be applied to validate the systematic analysis. By this strategy, the complexity of both Chinese medicine and its target metabolism process could be better studied.

The maintenance of telomere is the key to sustain cell survival, the telomere attrition is another hallmark of aging [13]. The telomere/telomerase pathway was found in *Panax Ginseng* and *Radix Ophiopogonis*. Here we did not find the direct telomere regulating pathway in *Radix Astragali*, in which the effective component astragaloside could transform into cycloastragenol in vivo, and is known to activate telomerase and elongate telomere [18]. Our previous study also revealed that cycloastragenol could counteract the circadian rhythmic disorder in aged mice [19]. Instead of that, we revealed the cell cycle regulating pathways (E2F targets, cMyb pathway) in *Radix Astragali* (Table 3). The DNA repair pathway, free radical clearance pathway were also been found in *Radix Astragali* (Table 3). These pathways are known to be essential in quality control of cells, and are positively connected with longevity [20]. The cell cycle regulating pathway Myc targets was also been revealed in *Polygonatum sibiricum*, which is consistent with its potential in stem cell regulation [21].

The apoptosis pathway is essential for clearance of damaged or senescent cells. The senolytic drug discovery has become a promising strategy in anti-aging and aging-related disease [22]. We found the apoptosis pathway in 5 out of 6 longevity sustaining herbs, suggesting the potential of these herbs in senolytic drug discovery.

Together, our data revealed the sustainable energy production, the sustainable cell proliferation, and the quality control build the life triangle for longevity (Fig. 1), which could be fulfilled by the application of balanced Chinese medicinal herbs. This methodology might provide a way to predict the mechanism of action for Chinese medicine herbs.

The main drawback of this analysis is the limitation of the targets prediction information, unlike the experimental study, target prediction is lack of the up or down regulation information. Plus, the resources for characteristic natural compounds are limited. Also, we noticed that for pharmacological study of the above Chinese medicinal herbs, only *Panax Ginseng, Codonopsis Pilosula, Radix Astragali* were applied for transcriptome assay [23, 24]. Out of those, most studies were performed with cancer cell lines. With all the gene mutations and chromosome rearrangement in cancer cells, the understanding of the physiological action of these herbs is limited. The transcriptome analysis of the molecular regulation of Chinese medicinal herbs on cells or animals with wild type genetic background is expected.

Declaration of Competing Interest

None.

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