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Review Article

Biomedicine in 21st century by Huaier: The practical health maintenance and successful recovery from cancer

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Preface

Cell is full of signals and networks, which link intensively each other. In **Figure 1**, the symbolic network, intra-cellular multi-signal trafficking on metabolic pathways were shown with a comparison to integrated circuit (IC) chart. These panels showed strong similarity between integrated signal traffics in the cell and the superconductor substrate.

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In the term “molecular analysis” in medical science, we should investigate characteristics of each molecule together with links and traffics intra/inter the cell, to prove the functional meaning in healthy condition as well as under disrupted or impaired conditions. It might be a matter of course, the cellular function is similar, or beyond single IC chip. In fact, we should remember that even the laboratory complex with huge buildings cannot reproduce all the systems existing in a single cell.

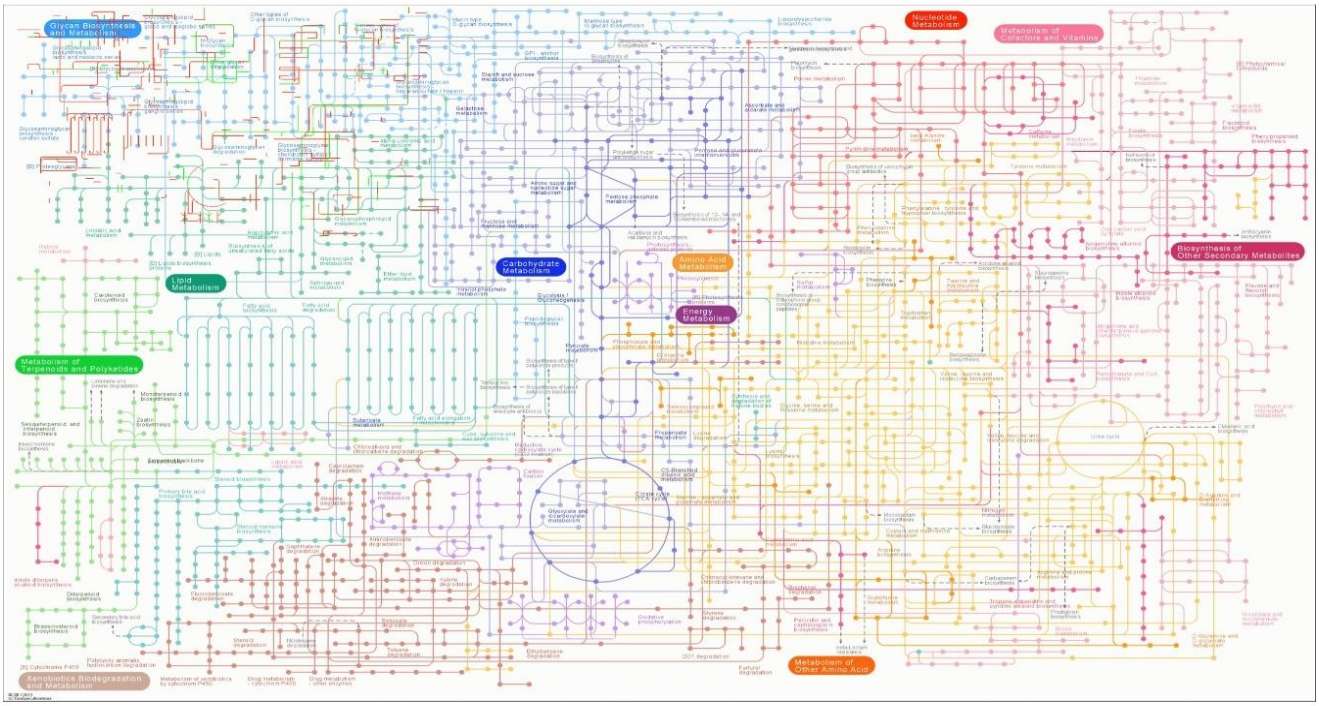
In 2017, we dared to challenge to have thorough information to clarify and identify the molecular mechanisms for recovery from cancer (genome scope project). Main information of cancer researches was derived from the results obtained in the process of carcinogenesis. We initiated clinical research to obtain the molecular evidences in the process of recovery. This approach could not be accomplished without effective medicine for cancer recovery, and the answer was Huaier. Here we present the genome scope view to show IC chart on a mode of action in successful Huaier therapy for cancer recovery.

The results obtained from the recovery process demonstrated significant difference from those obtained from the process of carcinogenesis. In addition, molecular aspects observed as clinical evidence were significantly different from those from *in vitro* researches. Typical differences were well observed in the genetic changes in regeneration systems, especially related to transcription regulation of pluripotency in induced pluripotent stem (iPS) / embryonic stem (ES) cells. The most remarkable changes were quantity of influenced signaling molecules together with the influenced networks, cascades, or pathways. In the present study, we present the figures for overviewing and for total understanding of those drastic changes, especially in quantity.

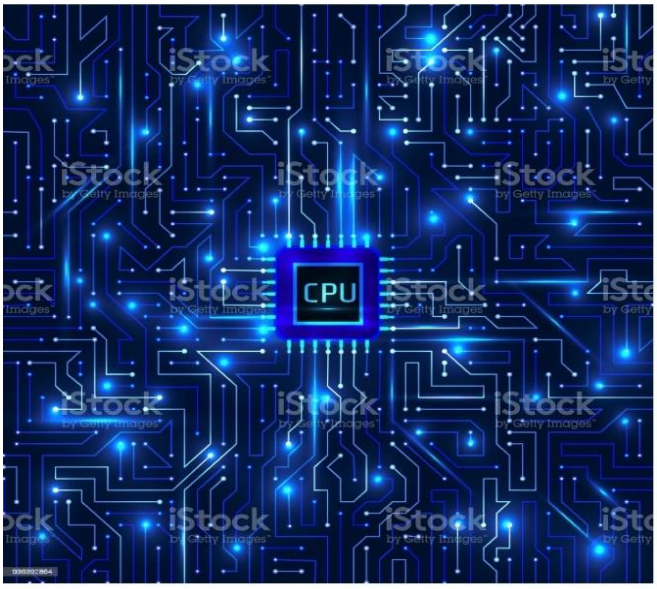
Fig. 1. A strong similarity between signaling network in a cell to integrated circuit chart for superconductor substrate. Panel A; Intra-Cellular multi-signal trafficking on metabolic pathways with a comparison to Panel B; Integrated Circuit (IC) chart.

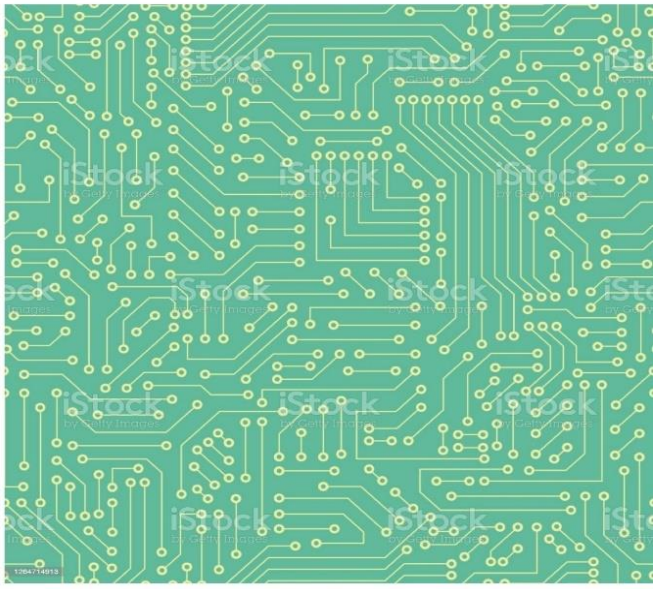
Fig.1

A. Intra-Cellular multi-signal trafficking on metabolic pathways



B. Integrated Circuit (IC) chart





Keywords: Huaier (*Trametes robiniophila murr*); cancer recovery; intra/inter cell communication; transcriptional control; microRNA misregulation; tissue regeneration; neurodegenerative diseases

1. What is Huaier (*Trametes robiniophila murr*) ?

To begin with, we should introduce Huaier (*Trametes robiniophila murr*). Medicinal herbs have recently attracted much attention among world-wide audiences, especially as a supplement therapy to the diseases and disorders, and also to maintain and improve human health. Among historical nature remedies in Asia, Huaier (*Trametes robiniophila murr*) has long been reported for significant efficacy on longevity, health maintenance, and cancer [1-3].

The difficulty in the herbal medicine exists in a lack of quantitative preparation with standardized quality, which long prevents the common use and distribution to public. Recent advancement in technology enabled to cultivate those useful species in quantity, and quality-controlled distribution in the conventional granule forms has begun since 1970's, and currently Huaier is recognized widely as the patent anti-cancer drug in China (Chinese administration license No. Z-20000109) [4, 5].

The effective ingredient of Huaier was proteoglycan, which consists of 41.53% polysaccharides, 12.93% amino acids and 8.72% water. The proteoglycan is the most effective anticancer element among all of the isolated ingredients of Huaier extract, which was confirmed on breast cancer MCF-7, liver cancer H22, lung cancer Lewis and sarcoma murine S180 cells (unpublished data) [6-15]. More importantly, Huaier has no toxicity or side effects ($LD_{50}=\infty$), and promises a broad spectrum of usage to any health problems at any situation without any disturbance to the conventional therapy.

At the beginning of 21st century, Huaier therapy was introduced in Japan at Ariake Hospital attached to the National Cancer Institute, to the severe advanced stage cancer patients who could not have more conventional chemotherapy or surgical dissection. Surprisingly, the significant improvement of prognosis and Quality of Life (QOL) assessments were observed by a dose-dependent manner, especially on hepatocellular carcinoma and breast cancer. Thereafter, accumulated successful results were enough plausible to the medical doctors as many patients together, to recognize anti-cancer effects of Huaier. We ourselves have confirmed the drastic improvements not only in cancer patients, but also in the other disorders in skin, liver, kidney diseases and disorders, and more importantly, neurodegenerative diseases such as Parkinson's disease [16]. In China, the basic investigations revealed the effects by *in vitro* experiments and using both mice and rats inoculated with cancer cells, in which. Enormous numbers of

reports, mainly from China, indicated as much molecular mechanisms responsible for cancer-specific apoptosis, autophagic cell death, and efficacy even on the metastatic lesions with specific immunomodulation by using *in vitro* cultured cancer cells [2-15]. However, the reality of Huaier effects remained unsolved even after the decades of sequential research works. We had to wait until we launched into the era of the systematic MEGA-DATA analysis with enough specialists to handle human bioinformatics [17-20].

It is extremely interesting to live and experience the fruitful successes in the new epoque for cancer therapy in 21st century with Huaier treatment, which was the result from the expedition in search for the natural products (herbs, fruit, flowers, and etc.) for immortality organized by Qin Shi Huang, the first Emperor in Qin dynasty, at B.C. 247−210.

2. Integrated Circuit Of Efficient Signal Transfer For Inside And Cell To Cell Communications.

Not only in cancer cases, we observed multi-dimensional efficacies of Huaier, especially on liver dysfunction, nephritis, bronchiectasis, constipation and skin lesions in a dose-dependent manner [16]. At first, we searched for the hypothetical candidate pathway which may explain these multiple events observed in the clinical features. It should have a character to integrate multiple signal cascades to modulate tissue- and organ-homeostasis.

We made a hypothesis that Huaier efficacy should be related to the multi-dimensional oncogenic potential such as Hippo signaling pathway [21, 22], and proved this hypothesis by a simple experiment using mutant Drosophila strain with disrupted Hippo signaling pathway. The Huaier treatment successfully rescued disrupted function in the mutant fly, with a dose-dependent manner, just the same as shown in cancer patients [16]. This rescue of Hippo signaling pathway function was derived from the rescue in disrupted transcriptional control, and accompanied with a strong shift of the metabolome profile to the early embryonic pattern to “rejuvenescence” form, as predicted in B. C. 220.

With this success, we considered that Huaier should possibly influence cell fate, not only in cancer, but also in many diseases by the Hippo pathway control. However, examinations using experimental models had significant differences and limitations compared with the reality in human biosystems, which urged us to consider clinical research for the next step.

In contrast, many *in vitro* investigations followed to our experiments in order to confirm the results, to provide the evidence that dysregulation in Hippo signaling pathway, disruption of transcription control is the major course of carcinogenesis [23-29]. However, without the observations to recover from the disease by Hippo signaling rescue, these reports were mostly the repetition of the past history. Although we sincerely hoped to have the additional successful drug candidates for cancer recovery, we could not yet detect any success similar to Huaier. In addition, no additional findings rather than Hippo signaling pathway rescue for the possible mechanisms for cancer recovery.

3. Huaier Effects on Cancer Siganling Cascade

Here we need two years and a half to report fruitful results from our clinical research, genome scope project [30-37]. As a first step, we analysed 92 blood samples from 31 patients, average generating over 7.4 Giga bases per sample to start with (currently reaches over 120). We would like to emphasize that, this analysis could be performed with 1) accumulated information of human genes and their molecular aspects, 2) tremendous efforts of the researchers to organize the quantitative information in order, and most importantly, 3) distinguished specialists and experts in BGI Kobe and Shenzhen to accomplish the task.

In **Fig. 2** demonstrated IC chart of signaling pathways related to cancer. The results of each patient were shown by KEGG pathway characterization [38]. Here we present 2 cancer patients [30, 32-34, 36] and 2 age-matched normal controls (70 years' old) [30, 33, 36], with 3 month's Huaier administration of 20g/day. As for the patient with stage IV pancreatic cancer with both lung metastasis with both lung metastasis, we used 60 g/day Huaier [32]. This patient has been successfully dissected original cancer lesion (caput pancreatis) after 6 months' Huaier treatment, and identified significant reduction of cancer cells, decrease of cancer lesion, and that without any metastasis in lungs and even in the adjacent lymph nodes.

In order to recover from cancer, such a drastic integration of altered signaling molecules and the control on signal trafficking is required. The molecules, signaling pathways, together with a functional link each other, contained up to 85% alteration and modification of total transcriptomes [30]. It is far to be enough to comprehend total evidence by *in vitro* experiments using cultured cancer cells. It is next to impossible to imagine those patterns of alteration varied among individuals according to genomic

potential. Detailed information has been described in the previous reports [30-37], and here we emphasize the reality of molecular basis of cancer recovery.

We recognized the similarity of Huaier effects on integrated signal transfer to the function of DYRK1A (dual-specificity tyrosine-regulated kinase 1A [39]) since 2017, from the starting time of our genome scope project. In October 2020 (2.5 years later), we finally realized the reality of striking similarity of the effects defined between DYRK1A and Huaier. Interestingly, we did not identify any alterations in DYRK1A, whereas many mutations in the mutated EGFR and other receptor tyrosine kinases (c-MET/erbB-2) [33]. There might be hierarchy of tyrosine kinases according to the importance of function, and that of the most importance remained intact for survival.

Huaier influenced almost all the molecules essential for living and survival [30-37]. The typical findings for the rescue and compensation of disrupted signaling pathways were derived from transcriptional control, via miRNA- and piRNA-mediated control as reported previously, through the attachment of the transcriptional factors (RNA editing events), and all these from the alteration and modification of the genome-wide transcription and translation. At first, high ratio of SNP variants as A-G transitions (241,896 in total, 37.0%) and A-C transversions (43,213 in total, 6.5 %), with skipped-exon (51%), which influenced significant changes in transcriptional factors and corresponding gene expression in multiple signal transduction pathways [30].

Huaier administration contributed the rescue of defected cell communication systems by massive down-regulation in a wide variety of signal transferring pathways [32]. In addition, Huaier seemed to take part in the epigenetic post-transcriptional control for the prevention in carcinogenesis and tumorigenesis for whole family members [30, 33].

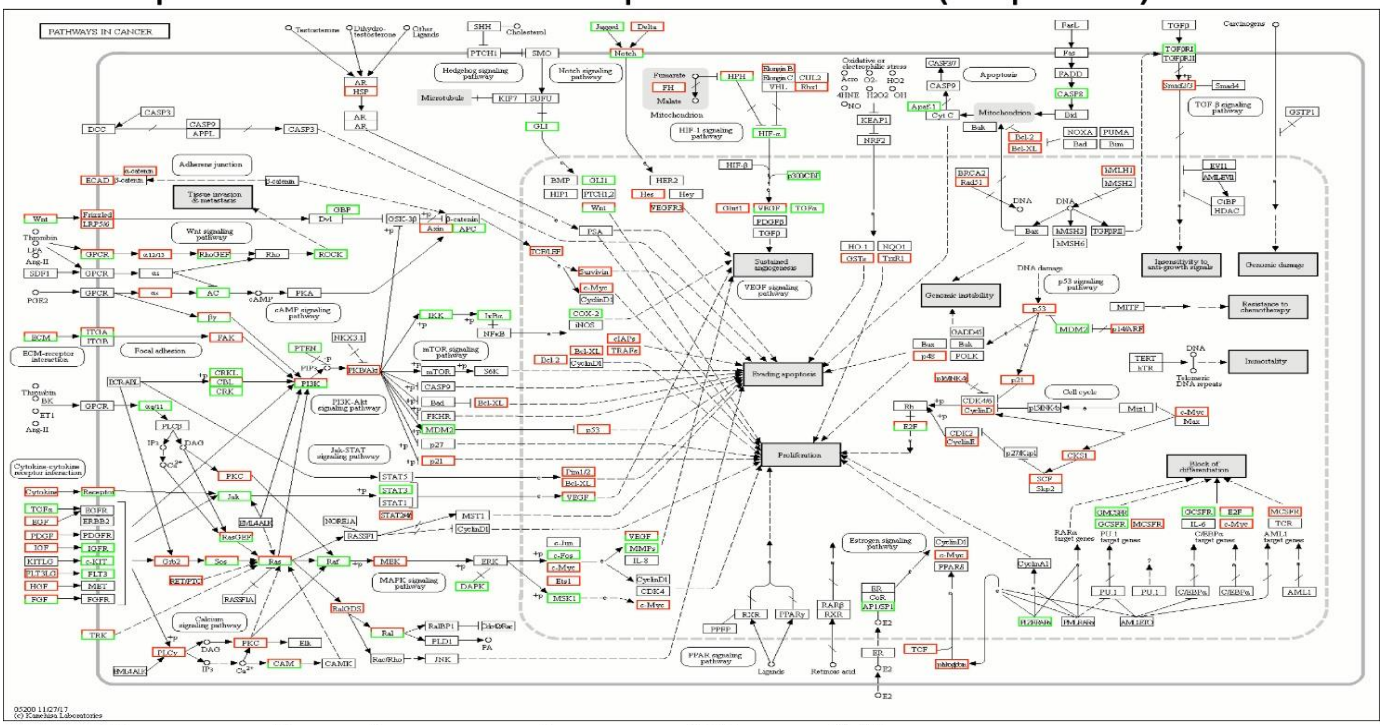
Which cancer strategy or conventional chemotherapy could cover all these required genomic and genetic alterations in any of cancer patients?

Fig. 2 KEGG pathway characterization on schematic summary of signaling pathways related to cancer. Each panel represents the results from cancer patients with 3 month's Huaier treatment with 20g/day. Panel A, Hepatocellular carcinoma + pancreas cancer (suspected); Panel B, Pancreatic adenocarcinoma (Stage IV), with 60g/day Huaier administration; Panel C, Normal control (70 years' old male); Panel D,

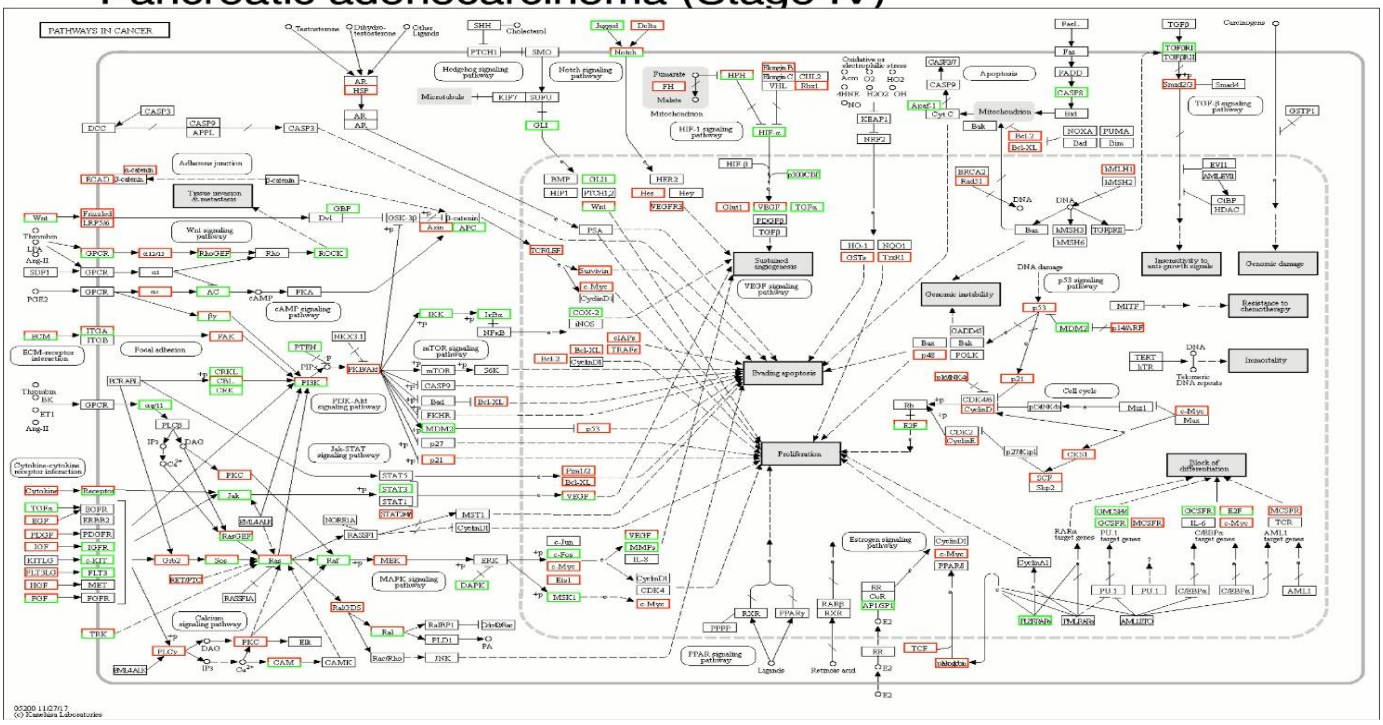
normal control (71 years' old female). Red box indicates up-regulation of transcriptomes, and down-regulation by green box. Each name of signaling cascade was written besides a representative molecule.

Fig. 2

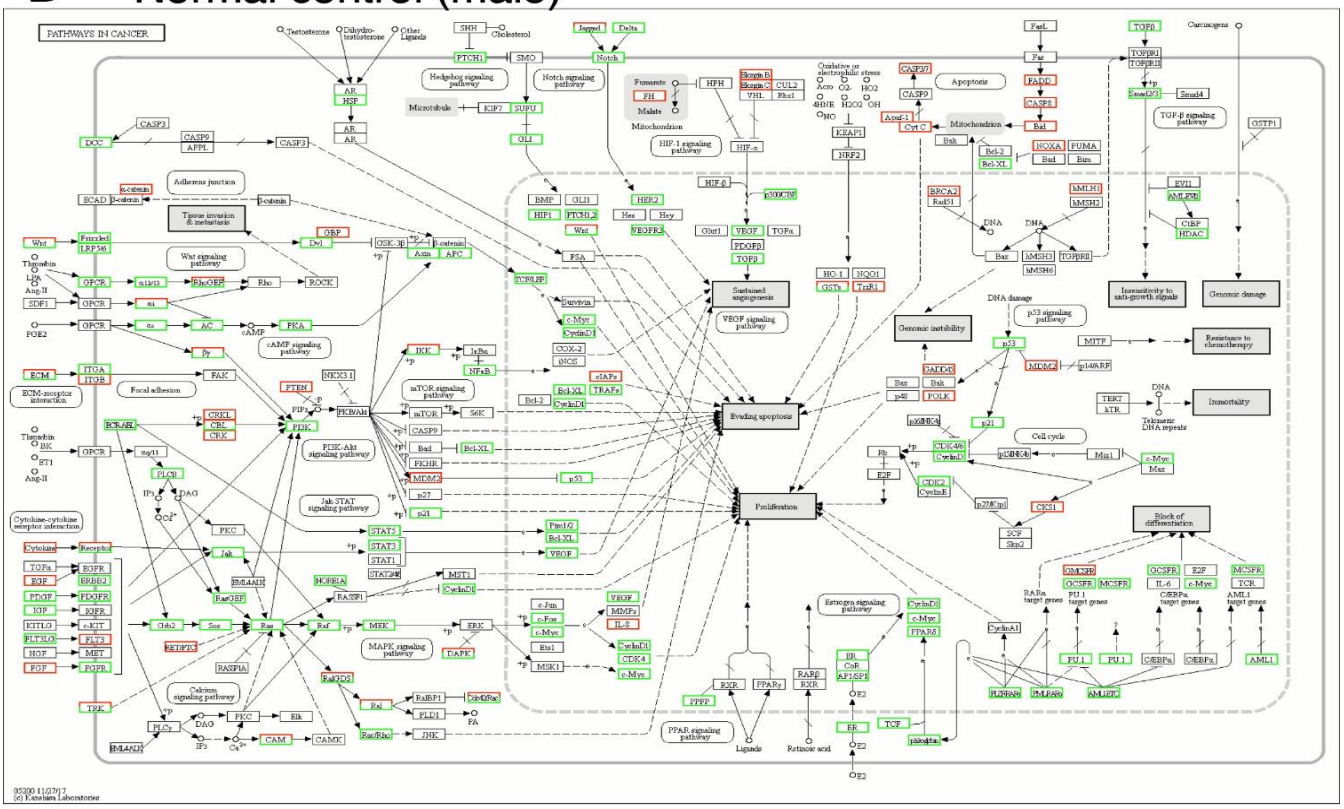
A Hepatocellular carcinoma + pancreas cancer (suspected)



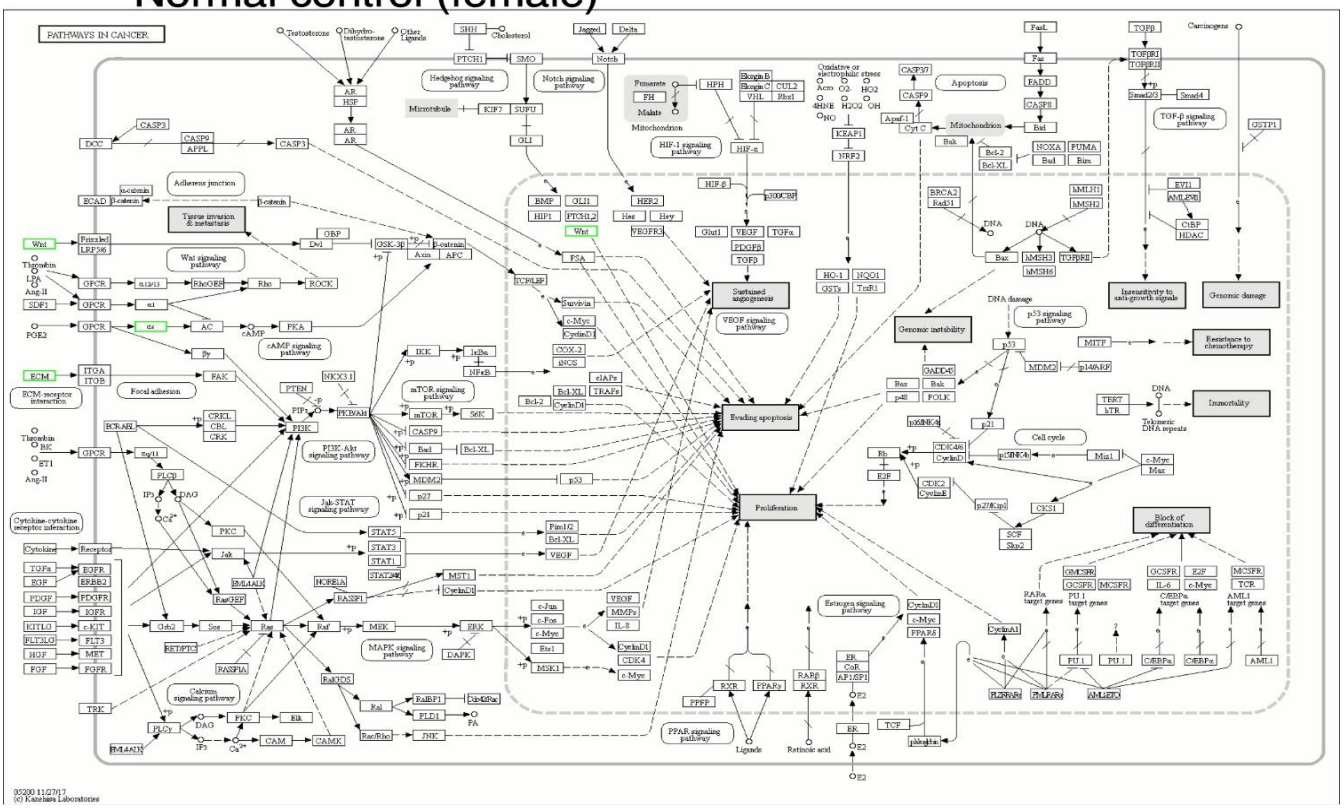
Pancreatic adenocarcinoma (Stage IV)



B Normal control (male)



Normal control (female)



4. Huaier Rescue on Misregulated Transcriptional Control in Cancer

Significant impact of Huaier administration exists in its transcriptional control. **Fig. 3** demonstrates a quantitative, and a wide variety pattern of the molecular rescue by Huaier on transcriptional misregulation in cancer.

This is a special feature of molecular basis of Huaier. Usually, medicine which alters or modifies transcriptional factors inevitably caused strong toxicity to the recipients. Transcriptional misregulation itself is toxic, but the artificial alteration and modification to the molecule or system is even more toxic. We should repeat the fact that Huaier has no toxicity and side effects, since the changes in vivo are occurred as spontaneous reaction by Huaier administration. We have not found any other compounds or natural products like Huaier yet. Of course, there might be mimics in smaller scale in efficacy, but not enough to be used widely in public.

Thus, we should have a sky-high point of view to see the reality, to accomplish the aim of cancer therapy to any patients, at any stage and any origin. The results obtained was striking and beyond any expectations and imaginations we have had, and only a few researchers could comprehend the real meaning of them. The sequential publications [16, 30-37] demonstrated the reality of the meaning of "cancer recovery" in genetic code language. Thanks to the KEGG signaling pathway characterization by Kyoto University, Japan [39], the altered genes and functions were clearly shown in these publications.

We should remember that it is obvious that mono-targeted strategy could not be enough to treat cancer [40]. We should reconsider cancer strategy from the fact that human biology can go through this much dynamic alterations during the long process of complete recovery from cancer, with compensation of the readjustment in multiple signaling pathways. As described, mono-targeted agents, such as FOLFOX, FOLFIRINOX, and cisplatin, commonly used in the conventional chemotherapy, should have certain limitations against diseases originated from multiple factors and environmental stresses throughout a life-long time [32, 40].

We completely agree with the surgical dissection of cancer lesion, tumor mass should be the first choice, if applicable [30-32].

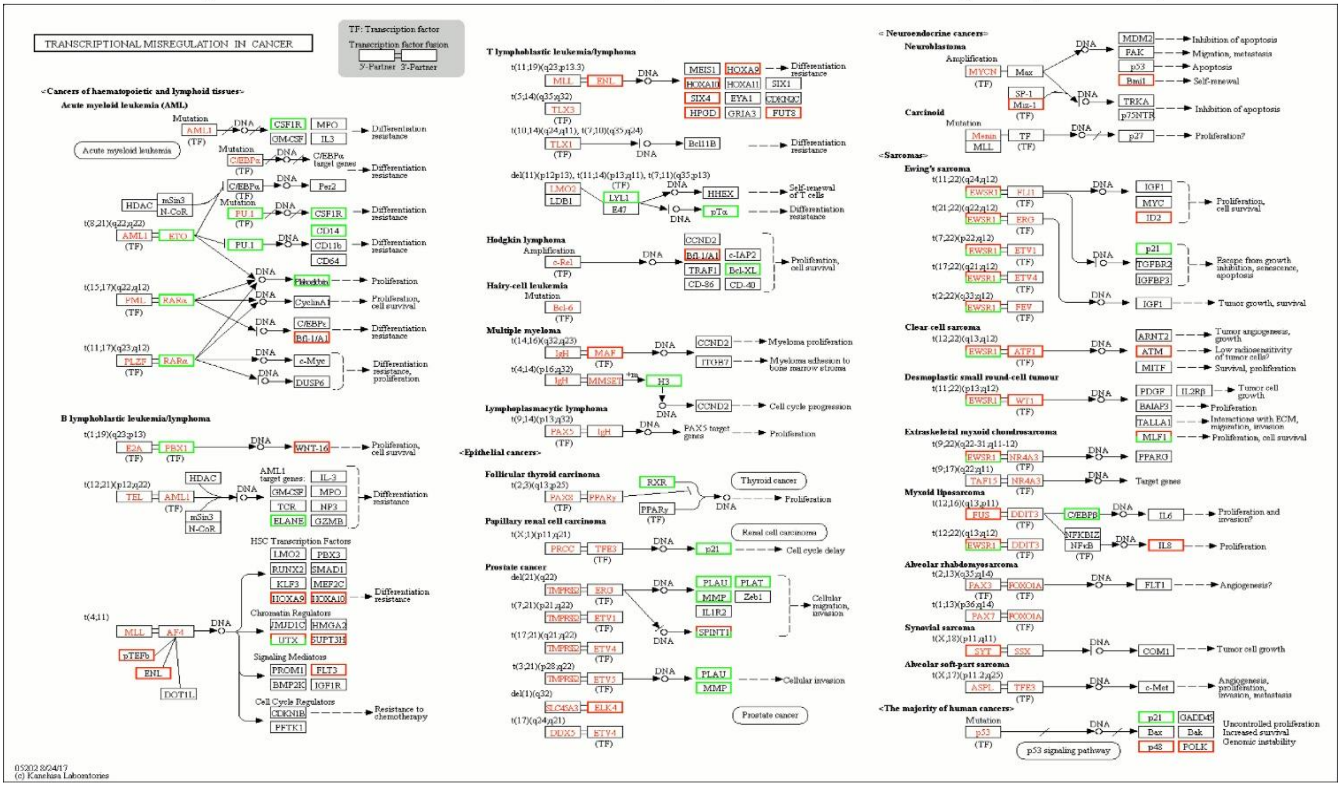
However, even with the successful dissection of the lesion, it still remains possibility of occult metastasis, and more importantly, (cancer) stem cell control is required for long years to prevent relapse or recurrence [33, 34]. At longest, we experienced the case of recurrence of breast cancer at 39 years after surgical dissection.

It became more popular to use this kind of MEGA-DATA analysis among all over the world, and recently involving SARS-COV2 and other virus infections as emphasized before [30-34]. Our data detected multi-virus infections such as papilloma virus, HIV-1 infection, and other DNA virus infection, too.

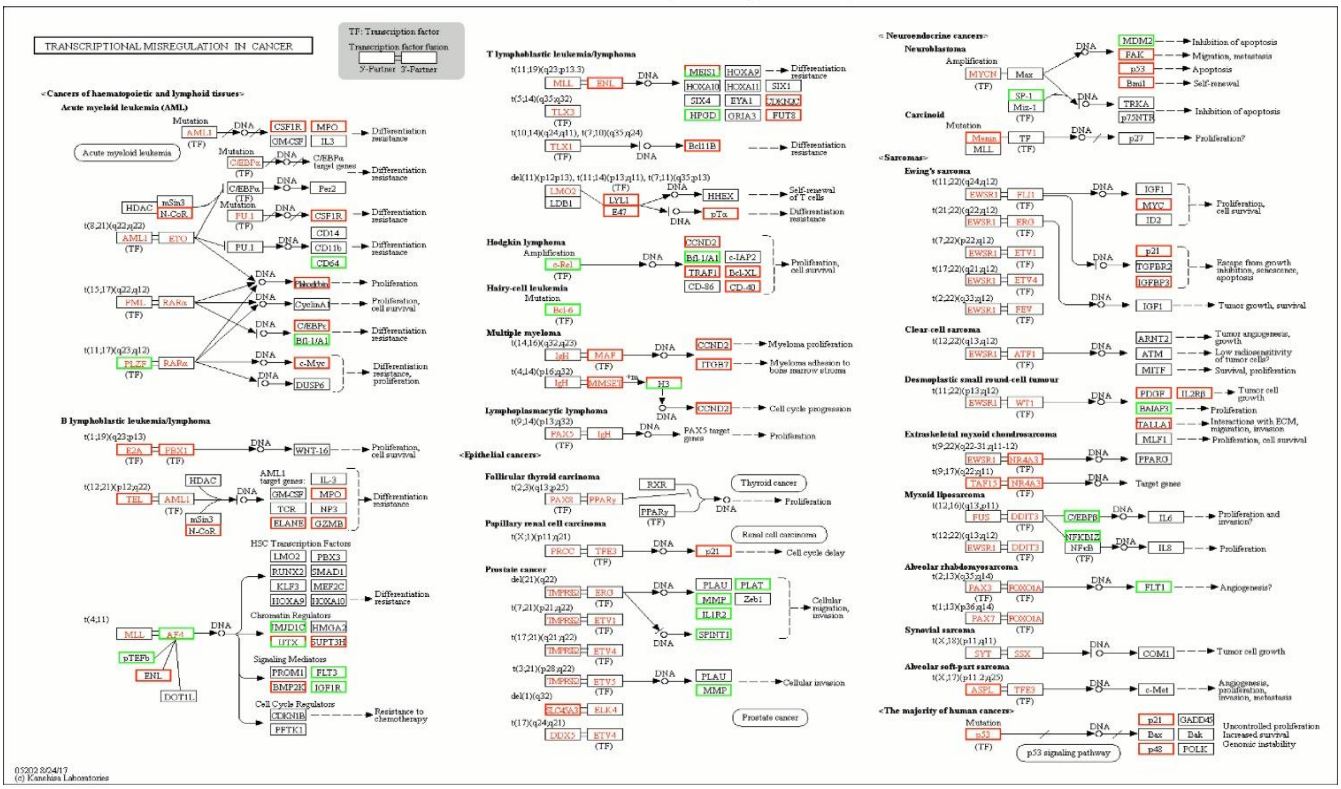
In contrast, as compared to conventional chemotherapy using platinum (II) complex caused significant down-regulation of the most of the transcripts in every signaling pathway, especially in signal transfer in central and peripheral nervous systems [37].

Fig. 3 KEGG pathway characterization on schematic summary of signal transfer in transcriptional misregulation in cancer. Each panel represents the results from cancer patients with 3 month's Huaier treatment with 20g/day. Panel A, Hepatocellular carcinoma + pancreas cancer (suspected); Panel B, Pancreatic adenocarcinoma (Stage IV), with 60g/day Huaier administration; Panel C, Normal control (70 years' old male); Panel D, normal control (71 years' old female). Red box indicates up-regulation of transcriptomes, and down-regulation by green box. Each name of related cancer was written on the top of each cluster for specified cancer. Red letters in the box indicates mutations in the expressed genes.

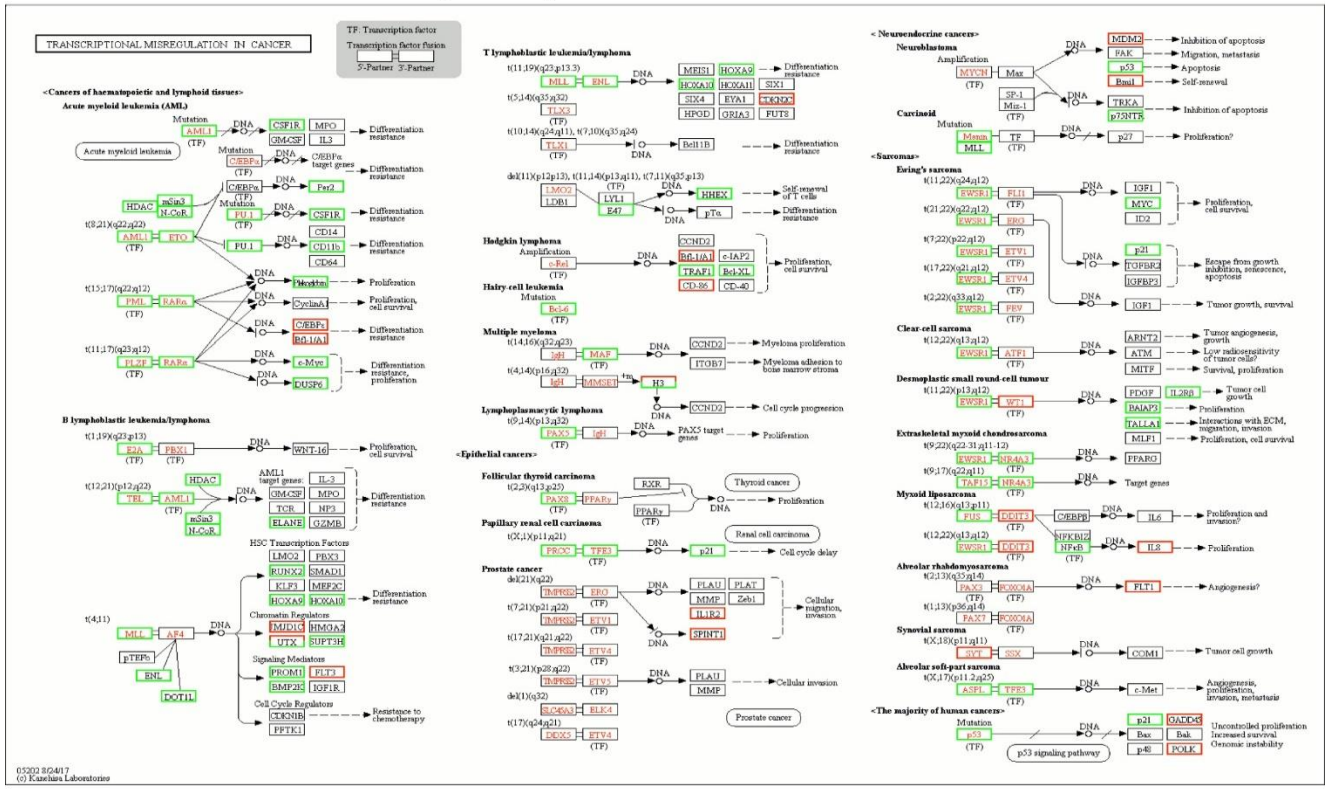
A Hepatocellular carcinoma + pancreas cancer (suspected)



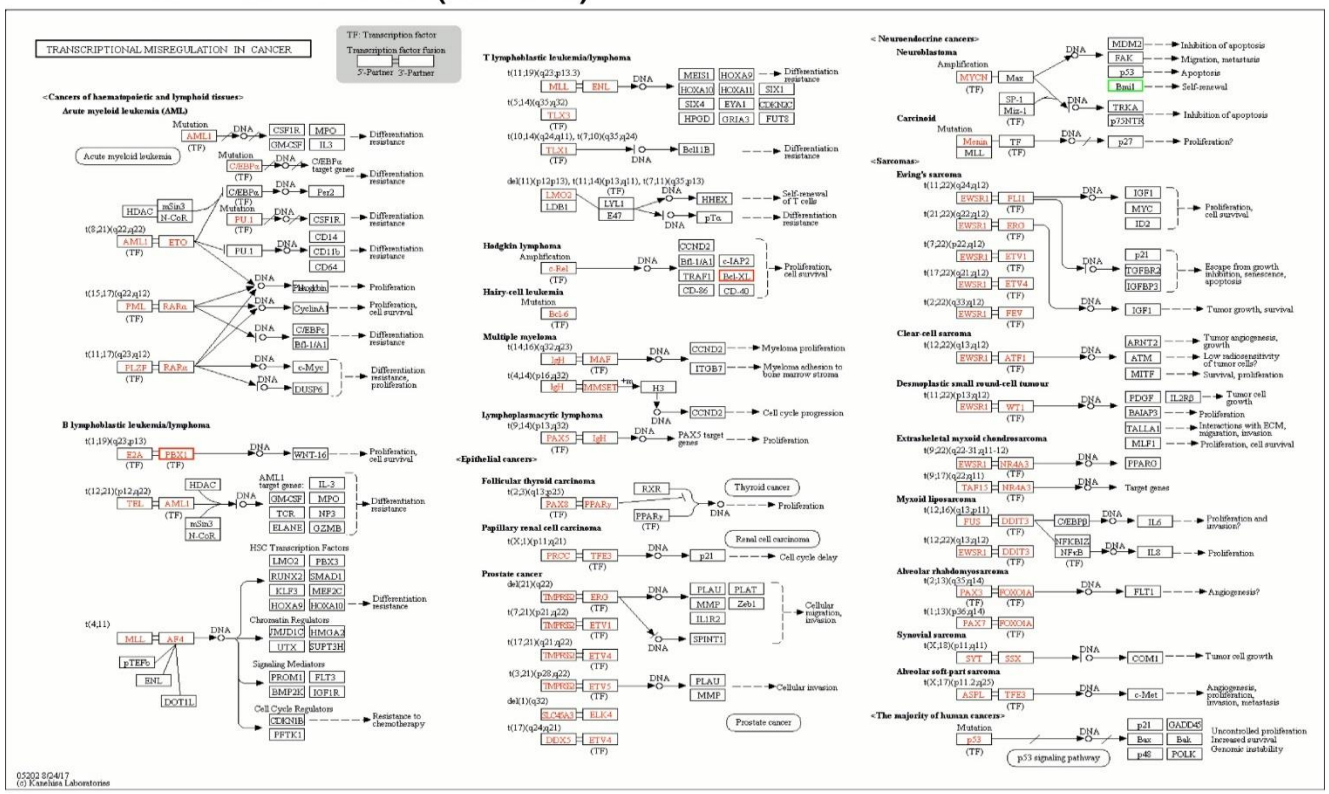
Pancreatic adenocarcinoma (Stage IV)



B Normal control (male)



Normal control (female)



5. Efficacy of Huaier influenced by MicroRNA Control

In **Fig. 4** panels demonstrate Huaier effects on microRNAs so far known to correlated with carcinogenesis. There are limited numbers of microRNA in cancer reported independently, however, in fact, the reality of microRNA function was still remained unknown [41, 42]. In addition, many microRNAs were not identified yet. We have detected numerous novel microRNAs, together with novel non-coding small RNAs (siRNA, miRNA, and pi RNA), function unknown, and those sequences have been deposited to The NCBI GEO (GSE157086), and continuously up-loaded with newly identified sequences throughout the project period.

At this point, we should ask a question, "what is normal"? According to genome scope view, the normal condition to each individual was demonstrated as no change in gene expression level. This directly indicates the condition with no up-regulation or down regulation in transcriptomes, especially in transcription factors, silence of genetic alterations. In Figs. 2-4, female normal control showed such a silence situation, but except in drastic up-regulation to rescue the transcriptional misregulation.

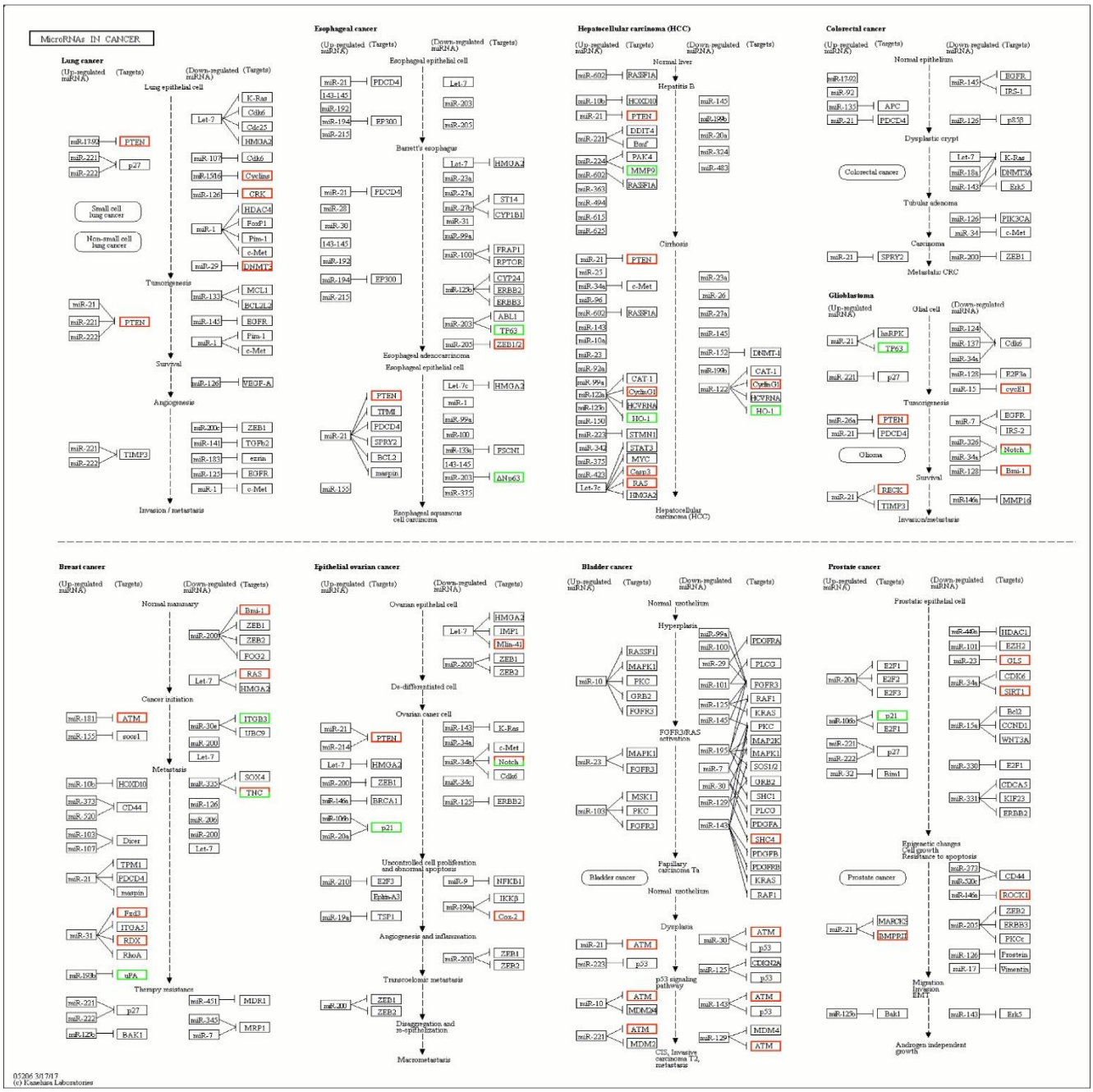
These results well demonstrate that cancer recovery process requires 1) transcriptional control first, and 2) resulting transcriptome alterations next. We should not be trapped into the specified molecule analysis, but should have a sky-high view of the generated biosystems to comprehend the drastic movements of the total genome and gene expression processes [36].

Fig. 4 KEGG pathway characterization on schematic summary of microRNA correlated with the transcriptomes in the process of carcinogenesis. Each panel represents the results from cancer patients with 3 month's Huaier treatment with 20g/day. Panel A, Hepatocellular carcinoma + pancreas cancer (suspected);Panel B, Pancreatic adenocarcinoma (Stage IV), with 60g/day Huaier administration; Panel C, Normal control (70 years' old male); Panel D, normal control (71 years' old female). Red box indicates up-regulation of transcriptomes, and down-regulation by green box. Each name of related cancer was written on the top of each cluster for specified cancer.

Fig.4

A

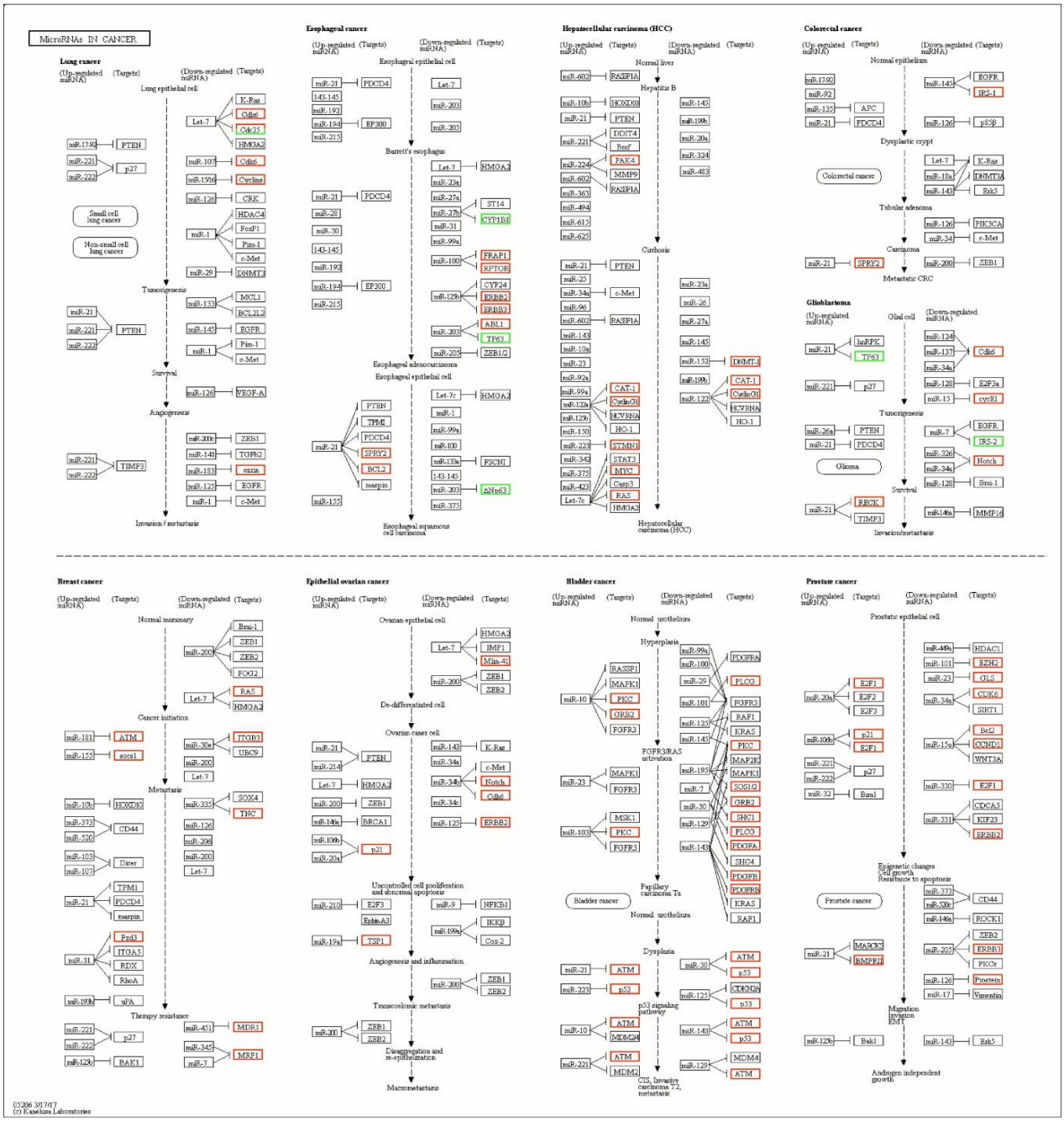
Hepatocellular carcinoma + pancreas cancer (suspected)



B

Fig.4 (continued)

Pancreatic adenocarcinoma (Stage IV)



c

Normal control (male)

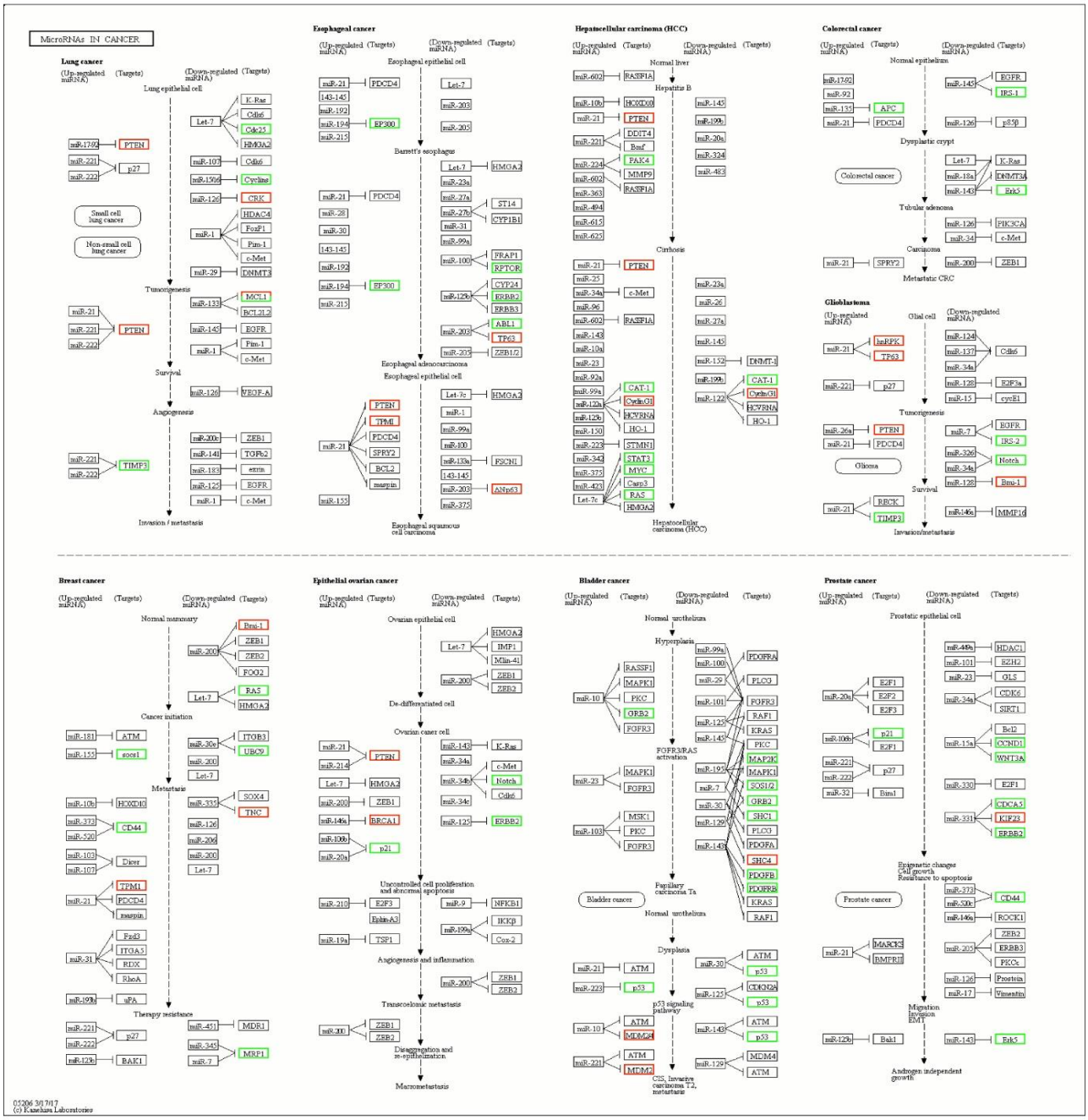
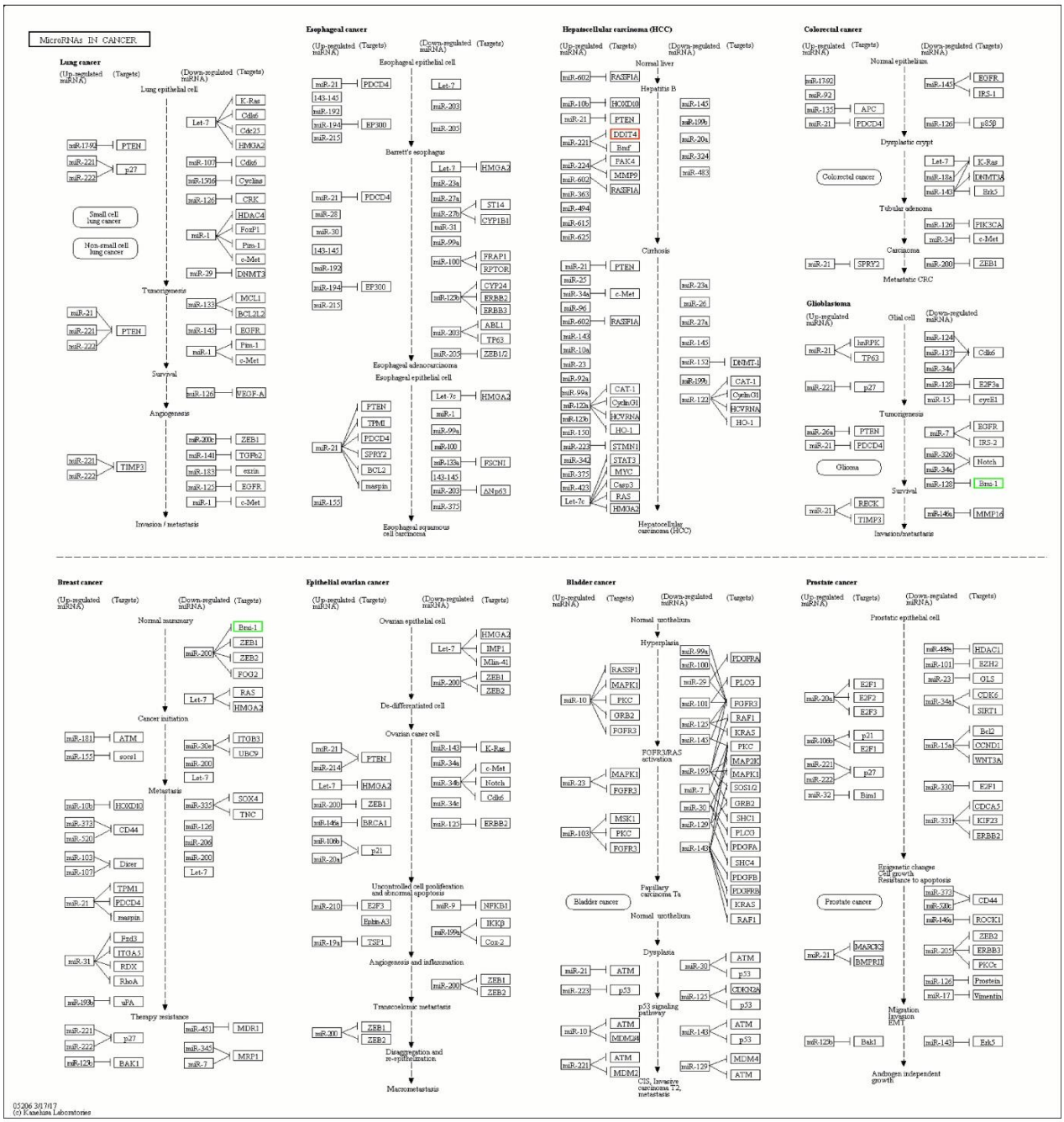


Fig.4 (continued)

D

Normal control (female)



6. Recommended Huaier therapy

Anti-cancer effects of Huaier are;

1) to promote cancer specific cell death inside the pathogenic lesion [16, 34]
2) to discard the resulting, damaged cell debris [16, 34]
3) to repair damaged and/or dissected tissues with normal cells (tissue regeneration; transcription regulation of pluripotency in induced pluripotent stem (iPS) / embryonic stem (ES) cells) [33, 34]
4) to prevent the relapse and recurrence together with adjacent and distinct metastasis [30-34]

It is obvious that surgical dissection of tumor mass is the first choice [1, 2], if applicable, however, it is just the beginning to overcome the disease like cancer.

Our genome scope project proved that many "normal" individuals were not normal from a point of view of molecular and biophysiological functions [16, 30-35] (see **Figs. 2-4**). Many normal persons showed drastic genomic and genetic alterations for the rescue of the latent, invisible disruptions of multiple signaling pathways after Huaier administration. Even with no symptoms, there was an enormous daily risk of cancer caused by accumulation of environmental stresses and ageing process. Early diagnostic technology has some limitations for correct and precise evaluation of the risk, and excessive, frequent medical examinations using endoscopy and radiographic measures in contrast increase risks for carcinogenesis.

Huaier quantity for cancer patients are;

1. 60g/day for pancreatic cancer, breast cancer, malignant melanoma for at least 1 year to 2 years
2. 20g/day for oesophageal cancer, colorectal cancer for at least 1 year to 2 years
3. 3g/day for atopic dermatitis, liver dysfunction by drug allergy, and swift recovery from accidental injuries

If surgical dissection of cancer lesions is applicable, it is desired to have Huaier 20g/day prior to the operation. Stage 0 breast cancer (atypical cell detection by biopsy) can be treated with 3g/day Huaier. The quantity varies among the stage of cancer and individual characteristics (hereditary situations). If any hereditary mutations in the family, epigenetic consideration should be considered, and should pay special attention to nutrition and daily life style.

Even before the diagnosis of cancer, Huaier has its potential to re-adjust the functional disruption in many kinds of signaling network and its links, and to compensate the impaired signal transfer even in neurons, and in the neurodegenerative diseases such as Parkinson's disease [35]. In total, this effects result in maintenance of homeostasis for a long-range of life.

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Conflict of interest

The authors have no competing interest to declare.

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