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Featured Topic: Breast Cancer

Adipokines Toward the Molecular Dissection of Interactions Between Stromal Adipocytes and Breast Cancer Cells

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Using association rules mining to explore pattern of Chinese medicinal formulae (prescription) in treating and preventing breast cancer recurrence and metastasis

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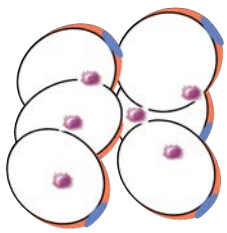

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A Word from the Editor in Chief

Prof. Jack Wong, Editor in Chief
Director, Regulatory Affairs, Asia Pacific,
Terumo BCT (Asia Pacific) Ltd (Singapore Branch)
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Dear Readers,

Wish you and your loved ones Happy and Healthy 2014!

Asia Health Care Journal has published the fourth edition, it is one of the publications of the Asia Regulatory Professional Association (ARPA). We gathered experts from academy, industry and government to share new findings and valuable experience.

Some key updates in 2013
1. The RA Alumni Asia Pacific was formed and created an award to Regulatory Affairs(RA) students
2. Our ARPA Hong Kong chapter chairman, Professor Raymond Tong was awarded **JCI Hong Kong Ten Outstanding Young Persons in 2013**
Read more: http://www.jcihk.org/toyp/2010_toyp_awardees/

Hope you enjoy the journal! ■

Prof. Jack Wong
Asia Regulatory Professional Association

Key activities

ARPA has five key areas of activity:
- *Education: This includes regulatory training in universities and government officials. The RA Alumni Asia Pacific was formed in 2013 with representatives from Hong Kong, Taiwan and Singapore.*
- *Publishing: Prof. Raymond Tong and Prof. Jack Wong published the Handbook of Medical Device Regulatory Affairs in Asia.*
- *Regulatory networking forum: ARPA organizes regular quarterly meetings with government and industry representatives to share regulatory updates face to face in university premises.*
- *Guideline/standards creation: The new body is considering set up a working group to create standards and guidelines that are appropriate for regulatory affairs professionals in Asia, and more specific than the general international quality system standard and quality system for medical devices standard, ISO 9000 and ISO 13485, respectively.*
- *Regulatory award scheme: To create a judging panel and a two-part award scheme: for motivating students performed well by recognizing them on high regulatory affairs exam marks and to create a mechanism to celebrating and sharing good practice within industry. The conception is also to display particular examples of good regulatory affairs professionals and their work.*

ARPA
Asia Regulatory Professional Association

The Asia Regulatory Professional Association (ARPA) is an organization of healthcare regulatory affairs professionals in Asia. ARPA aims to raise the standard and social recognition of regulatory professionals as part of healthcare team.

Details of ARPA can be found in
http://www.healthcare.org.hk/Content.aspx?t1=22&t2=79

Values of Asia Regulatory Professional Association (ARPA)

To uphold and enhance standards among regulatory affairs professionals in Asia and to encourage the creation of better educated regulatory teams in the area, regardless of the background and regulatory situation of their countries. A new body, the Asia Regulatory Professional Association (ARPA), was established in 2010 with more than 2000 members today.

Structure

ARPA strives to be neutral. There is a good balance of key individuals from different countries as well as from academic and regulatory bodies.
- The ARPA chairman is Dr. Saleh S. Al-Tayyar from Saudi FDA and co-chairman is Madam Liu Li-Ling from Taiwan FDA. Dr. Saleh and Madam Liu are also the chairman and co-chairman in Asia Harmonisation Working Party (AHWP) to help avoiding duplication with relevant work that is ongoing within that organization which aims to work towards greater harmonization in medical device regulations in Asia.
- Prof. Rosanna Peeling is our advisor (ex-WHO staff, now working in London University).

Hong Kong Regulatory Affairs Academy

Prof. Raymond Tong
Department of Health Technology and Informatics
Hong Kong Polytechnic University

Singapore Regulatory Affairs Academy

Prof. Teoh Swee-Hin
Division of BioEngineering
School of Chemical and Biomedical Engineering
Nanyang Technological University

Taiwan Regulatory Affairs Academy

Madam Liu Li-Ling
Director
Division of Medical Devices and Cosmetics
Taiwan Food and Drug Administration (Taiwan FDA)

Dr. Chiou Chi-Ming
Medical and Pharmaceutical Industry Technology and Development Center (PITDC)

Vietnam Regulatory Affairs Academy

Mr. Nguyen Minh-Tuan
Director General
Department of Medical Equipment and Construction
Ministry of Health

14 winners were named for EY Entrepreneur Of The Year 2013 China

Jacky Kwan, Chairman of Bamboos Professional Nursing Services Ltd. was awarded in the Emerging Entrepreneur Category

About EY Entrepreneur Of The Year

EY Entrepreneur Of The Year is the world's most prestigious business award for entrepreneurs. The unique award makes a difference through the way it encourages entrepreneurial activity among those with potential, and recognizes the contribution of people who inspire others with their vision, leadership and achievement. As the first and only truly global award of its kind, Entrepreneur Of The Year celebrates those who are building and leading successful, growing and dynamic businesses, recognizing them through regional, national and global awards programs in more than 145 cities in more than 60 countries.

China awards honor bold entrepreneurs in the mainland China and Hong Kong / Macau regions who have achieved success by combining innovation and capabilities with opportunity, and recognize the benefits that entrepreneurs and their entrepreneurial spirit bring to the broader good of China's economy.

This year's winners are drawn from a diverse range of industries, comprising emerging industries, real estate, life sciences, culture and technology, consumer products, industrial products, and services. Their achievements through innovation have transformed their business and industry, and created a long-lasting positive impact on their market and society. ■

The EY Entrepreneur Of The Year China 2013 Award Winners

Consumer Products
- Jimmy Tang, Chairman and CEO, Prince Jewellery and Watch Company
- Terry Sio, President, Rainbow Group

Culture and Technology
- Liang Guangwei, Chairman and President, Shenzhen Huaqiang Holdings Limited

Emerging Enterpreneur
- Jacky Kwan, Chairman, Bamboos Professional Nursing Services Limited
- Terry Tsang, Founder and Chairman, Mad Head Limited
- Chen Haibin, Board Chairman and President, Zhejiang DIAN Diagnostics Co., Ltd.
- Zhang Bangxin, Chairman and Chief Executive Officer, TAL Education Group

Life Sciences
- Yan Xijun, Chairman, Tasly Pharmaceutical Group Co. Ltd.
- Wang Zhaoming, Chairman, Inner Mongolia Hotision & Monsod Drought-Resistance Greening Co. Ltd.

Industrial Products
- Simon Suen, Chairman, SML Group

Real Estate
- Hui Wing Mau, Chairman, Shimao Property Holdings Limited

Services
- Chen Feng, Chairman, HNA Group
- Chen Miaolin, Chairman, New Century Tourism Group Co., Ltd.
- Yang Guoping, Chairman, Dazhong Transportation (Group) Co., Ltd.

Hong Kong Regulatory Affairs Academy Chair, Professor Raymond Tong Kai-yu of the Interdisciplinary Division Biomedical Engineering at The Hong Kong Polytechnic University was awarded the "Ten Outstanding Young Persons"2013

Professor Tong is recognized for his passion and distinguished accomplishment in innovation and applied scientific research in neuro-rehabilitation for stroke patients and the elderly. His commitment for extending the frontiers of knowledge has sparked off in the secondary school years when he served organized many community service programmes in elderly centres and saw many people suffering from stroke. "During the sharing session with them, I realize that the elderly and persons after stroke wish to regain their independency in daily activities and to maintain good quality of life," said Professor Tong, who was inspired by their wish to embark on an academic career that will bring research and invention to benefit the elderly and stroke patients.

Raymond completed his BEng in Computer Engineering with first-class honour at the University of Hong Kong in 1995 and furthered his studies abroad in the UK. He finished PhD in Bioengineering from the University of Strathclyde, Glasgow, in 1998 and joined The Hong Kong Polytechnic University in the following year. Over the years, he has made great strides in developing a wide range of rehabilitation devices. His innovative work on rehabilitation robot system "Hand of Hope" was the first Hong Kong invention to have received the grand prize in the 40-year history of the International Exhibition of Inventions of Geneva, making Hong Kong internationally visible in this emerging area in healthcare technology.

His creativity also proved to work on the e-platform with the development of KineLabs 3D motion software (www.polyu.edu.hk/kinelabs), which has received Winner Award (e-Health) in the Asia Pacific Information and communications Technology Award in 2012 in Brunei. More importantly, the systems developed by Professor Tong have already reached hospitals and elderly centers to facilitate stroke rehabilitation and elderly exercise - with fun.

Professor Tong has a roll of honour for his ground-breaking innovation. He was also the recipient of the Grand Award of the innovation awards for young members from the Hong Kong Institute of Engineers in 2008. Professor Tong is keen to impart his knowledge to the next generations through teaching and supervision of research students. He is also the Editor for two published books "Biomechatronics in Medicine and Health Care" 2011 and "Handbook of Medical Device Regulatory Affairs in Asia" 2013. Prof. Tong contributes significantly to professional bodies. He is presently the Chairman, Asia Regulatory Professional Association (ARPA)-Hong Kong Academy. He is a senior Member of the Engineering in Medicine and Biology Society of the IEEE and Member of the Hong Kong Institute of Engineers (HKIE). He has developed new regulatory courses in the Hong Kong Polytechnic University on "Intellectual Property, Standards & Regulation of Medical Devices" and "Medical Devices Regulatory and Risk Management" since 2004. Prof. Tong has been collaborated with Regulatory Professionals and Companies in strengthening and fostering Medical & Healthcare Devices Industry. ■

Mr. Aaron Kwok
Vice-President, BioMedical Engineering Alumni Association of The Hong Kong Polytechnic University (BMEAA of HKPU)
Email: albmeaa@polyu.edu.hk

RA Alumni Asia Pacific is established on 18 June, 2013 !

It is my pleasure on behalf of RA Alumni Asia Pacific to introduce you who we are.

With the support and help, driven by Prof. Jack Wong, the **RA Alumni Asia Pacific** was created on 18 June, 2013 with different RA professional from Singapore, Taiwan and Hong Kong including Universities (Nanyang Technological University, the Hong Kong Polytechnic University and National Taipei University of Technology), Hospital (KK Woman's and Children Hospital), and Consultant companies (Qualtech Consulting Corporation).*

RA Alumni is part of Asia Regulatory Professional Association which aims to raise the standard and social recognition of Regulatory Professionals as part of healthcare team.

In Asia Pacific region, Regulatory standards are diversified among countries. We have about 23 different countries within Asia Pacific, within that they have their own standards such as CFDA from China, KFDA from Korea, TFDA from Taiwan, TGA from Australia, etc.

Therefore, **RA Alumni Asia Pacific** is a platform for students who completed the RA course. We offered linkage for Regulatory related professional and expertise together, by providing field updated knowledge, networking, working and learning opportunity. Besides internal activities, we will also organize external activities such as participation in International RA meeting, and Student Awards to appreciate students who work hard and put their effort on learning Regulatory in University.

This is especially important for students who just completed the RA course and are looking for interest in RA field.

Our memberships includes Basic member who completed ARPA RA courses and Certified member who completed ARPA RA courses and passed the ARPA exam. This is completely free of charge to join us right now and we will keep you updated information by providing updated articles.

For details, please visit our website: http://www.healthcare.org.hk/Content.aspx?t1=22&t2=80

Our RA Alumni will continue expanding with more different RA Professional joining from all over Asia Pacific Region. If your institute is interested in it, please kindly contact us by visiting our website or simply send us email.

First Activities:

This is our pleasure to announce our first activities would be Student Awards in the coming June - July 2014, where we would like to encourage existing students to put their effort or interest on Regulatory field, our Award Panel will be from University, Hospital and RA expertise.

We will announce more detailed application method, deadline, etc via Universities RA module conducted by Prof. Jack Wong, our website and LinkedIn.

RA Alumni Asia Pacific is an organization of health regulatory affairs professionals in Asia Pacific region.
Our aims to raise the awareness of Regulatory Professionals as well as building up RA network within Asia Pacific.
Our contact email address is: raalumniapac@gmail.com. If you are interested in joining us, please kindly contact us by email.
*Special Thank you to all committee members who dedicated their time and effort in helping the RA Alumni Asia Pacific set up.

Dr. Jianping Chen
Dr. Jianping Chen is currently an Associate Professor at The University of Hong Kong. Dr. Chen is an expert, researcher, author in the field(s) of Prevention and treatment on breast cancer. After graduating from the Bachelor Degree in Chengdu University, she joined the Chengdu University of Traditional Chinese Medicine as physician and Sun Yat-sen University as professor. After few years, Dr. Chen came to Hong Kong to pursue her research work.
Dr. Chen has long been engaged in study, teaching and clinical experiences of Chinese Medicine both traditional and nowadays. She is experienced in pharmacology, prescription compatibility and health care. She is strong in treatment patterns study and new medicines development on diseases such as cardiovascular and cerebrovascular diseases, fever and digestive system diseases. She is also a specialist in cardiovascular and cerebrovascular diseases, tumor (especially on gynecological and digestive system) as well as health care by Chinese Medicine.

The other authors: Yanhua He[1], Xiao Zheng[1], Cindy Sit[2], Wings TY Loo[1,6], ZhiYu Wang[1], Ting Xie[3], Bo Jia[4], Qiaobo Ye[4], Kamchuen Tsui[5], Louis WC Chow[6]

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Using association rules mining to explore pattern of Chinese medicinal formulae (prescription) in treating and preventing breast cancer recurrence and metastasis

Abstract

Background

Chinese herbal medicine is increasingly widely used as a complementary approach for control of breast cancer recurrence and metastasis. In this paper, we examined the implicit prescription patterns behind the Chinese medicinal formulae, so as to explore the Chinese medicinal compatibility patterns or rules in the treatment or control of breast cancer recurrence and metastasis.

Methods

This study was based on the herbs recorded in Pharmacopoeia of the People's Republic of China, and the literature sources from Chinese Journal Net and China Master Dissertations Full-text Database (1990 – 2010) to analyze the compatibility rule of the prescription. Each Chinese herb was listed according to the selected medicinal formulae and the added information was organized to establish a database. The frequency and the association rules of the prescription patterns were analyzed using the SPSS Clenmentine Data Mining System. An initial statistical analysis was carried out to categorize the herbs according to their medicinal types and dosage, natures, flavors, channel tropism, and functions. Based on the categorization, the frequencies of occurrence were computed.

Results

The main prescriptive features from the selected formulae of the mining data are:
(1) warm or cold herbs in the Five Properties category; sweet or bitter herbs in the Five Flavors category and with affinity to the liver meridian are the most frequently prescribed in the 96 medicinal formulae;
(2) herbs with tonifying and replenishing, blood-activating and stasis-resolving, spleen-strengthening and dampness-resolving or heat-clearing and detoxicating functions that are frequently prescribed;
(3) herbs with blood-tonifying, yin-tonifying, spleenstrengthening and dampness-resolving, heat-clearing and detoxicating, and blood-activating with stasis-resolving functions that are interrelated and prescribed in combination with qi-tonifying herbs.

Conclusions

The results indicate that there is a close relationship between recurrence and metastasis of breast cancer with liver dysfunctions. These prescriptions focus on the herbs for nourishing the yin-blood, and emolliating and regulating the liver which seems to be the key element in the treatment process. Meanwhile, the use of qitonifying and spleen-strengthening herbs also forms the basis of prescription patterns.

Background

Breast cancer is one of the most common malignant tumors among women, and the incidence increases every year in both developed and developing countries [1]. Every year, among the 1.2 million women diagnosed with breast cancer worldwide, 500 thousand cases die of the disease. Along with a sharp increase in life expectancy, expansion of urbanization and adaptation of western lifestyle, the increase in incidence rates is even more obvious in developing countries [2-5]. In China, the number of cases increased by 38.5% from 2000 to 2005. Compared with the early surveys in the 1990s, breast cancer accounted for the largest increase in mortality rates in 2005 [6].

Today, the standard therapies for breast cancer include surgery, chemotherapy, radiation therapy, and hormonal therapy. However, even though patients receive systemic treatment, there is still 10% to 30% chance of recurrence and metastasis. Among the patients with local recurrence, 75% to 93% will eventually develop distant metastasis with an extremely low 5-year survival rate [7,8]. Visceral metastasis is the main reason for treatment failure and cause of death.

Lung, bone, liver and brain are the most common sites of distant spread of breast cancer [9,10]. Since metastasis is the main reason for cancer treatment failure, management of metastasis is the key factor for determining the prognosis of the patients [11].

Recently, the use of natural Chinese herbal medicine with anti-tumor effects is receiving more and more attention from the public [12]. In traditional Chinese medicine (TCM), the treatment and prevention of breast cancer recurrence and metastasis is a holistic approach through multi-level, multi-target and multi-channel control. TCM differs from Western medicine, which adopts ways to block a single transfer in a particular process. In comparison, Chinese medicine adopts an overall therapeutic approach to treat and prevent recurrence and metastasis, to improve the immune system of patients, and to strengthen the body's susceptibility to diseases. Meanwhile, Chinese medicine also aims at reducing the side effects of radiotherapy and chemotherapy, reversing drug resistance and improving quality of life and survival for patients. Therefore, these unique advantages have gradually made the Chinese medicinal approach in combating breast cancer recurrence and metastasis the research focus of both the local and overseas scholars [13,14].

In Chinese medicinal therapy, experienced Chinese medical practitioners prescribe a medicinal formula—a combination of various single herbs—for the treatment of ailments. According to TCM theories, pharmacological and pharmacodynamic relationship exists among herbs, which is deemed as Chinese medicinal compatibility. The compatibility of Chinese herbal medicine has particular rules and patterns. In Chinese medicinal database, there are over ten thousand medicinal formulae which enclose complicated information. However, a well-established and orderly system for organizing the information of Chinese medicinal formulae does not exist. This implies that a large amount of implicit prescription patterns behind the formulae have not been fully disclosed [15,16].

Association rules mining is one of the methods for discovering meaningful associations or correlations between variables in large databases. It identifies frequent item sets from the data sets, and then uses these frequent item sets to form their association rules. To select meaningful rules from the set of all possible rules, minimum thresholds on support and confidence are the two important constraints. An association rule has the form *LHS⇒RHS*, where LHS and RHS are sets of items, and the RHS set is likely to occur whenever the LHS set occurs. One of the applications of association rules mining is to mine association rules in medical record data [17,18]. Since association rules mining is a popular and well-researched method, it can be used to investigate the Chinese herbal medicine compatibility patterns, and to reflect the interdependence and relationship between the variables. Therefore, it can provide scientific evidence for clinical applications of Chinese medicine, and thereby offer an implication for the integration of Chinese medicinal therapy with modern Western medical therapies to better treatment or prevention of breast cancer recurrence and metastasis [19]. The support supp(X) of an item set X is defined as the proportion of transactions in the data set containing the item set. It is a function used for evaluation of the potential usefulness of the rules. The confidence of a rule is defined as conf(X => Y), which can be interpreted as an estimate of the probability P (Y|X) [20].

Methods

Sources of literature

This study was based on Pharmacopoeia of the People's Republic of China[21] recorded to investigate the prescription patterns of using Chinese medicine for treatment and prevention of breast cancer recurrence and metastasis. The sources of literature included the Chinese Journal Net and the China Master Dissertations Full-text Database (1990 – 2010) (Table 1). The name of each herb was used as a keyword to obtain the relevant literature, and only the literature which focused on "breast cancer", "advanced stage of breast cancer" and/or "post-operation of breast cancer" was eligible for inclusion. According to the following inclusion and exclusion criteria, a total of 131 papers describing various medicinal formulae for clinical applications were included (96 medicinal formulae with a total of 180 Chinese herbal medicines (herbs); the total cumulative occurrences of 180 herbs appearing in 96 formulae were 1001 times). The terminologies used in this article refer to 'WHO International Standard Terminologies on Traditional Medicine in the Western Pacific Region', which has documented the common technical terms used in traditional medicine.

Table 1 Data source of the literature (1990-2010)

Database	Source	Keyword(s)	Number of literature
Chinese Journal Net Database	The herbs recorded in Chinese Materia Medica and Pharmacopoeia of the People's Republic of China (2005 Edition) Volume I	"breast cancer" and/or "advanced stage" and/or "postoperation"" clinical research " ""TCM", "prevention and treatment of breast cancer recurrence and metastasis" and be eligible for selection criteria	121
China Master Dissertations Fulltext Database			8
China PhD Dissertations Fulltext Database			2

Among the 131 papers searched from the databases, 96 medicinal formulae were included in the study according to the inclusion and exclusion criteria.
According to the inclusion and exclusion criteria, a total of 131 papers which described various Chinese medicinal formulae for clinical applications were included 96 medicinal formulae with a total of 180 herbs. Total cumulative occurrences of 180 herbs appearing in 96 formulae is 1001 times.

Inclusion criteria

There were five types of literature included, including literature: (1) related to clinical research on using Chinese medicine for the prevention and treatment of breast cancer recurrence and metastasis; (2) related to clinical research on using Chinese medicine for thetreatment of advanced stage breast cancer; (3) related to clinical research on using Chinese medicine for the prevention of postoperative breast cancer recurrence and metastasis (especially at stage III or later when metastasis had occurred); (4) with randomized controlled trials as the study design; and (5) where the clinical study aims to prove the efficacy of experimental group with Chinese medicinal treatment over control group.

Exclusion criteria

Literature with the following criteria were excluded: (1) small-sample-sized studies with less than 20 cases; (2) studies which primarily aimed to treat complications of operations or to reduce the side effects of chemotherapy; (3) studies without investigation into the use of Chinese medicine for the treatment and prevention of breast cancer recurrence and metastasis; (4) studies which provided only the names of formulae but without descriptions of herbal ingredients; (5) duplicate publications reporting the same group of participants; and (6) literature in which the clinical trial received a Jadad score of less than 2.

Statistical analysis

Association rules mining is a popular and wellresearched method for dis-

covering interesting relations between variables in large databases [22]. We used the following definition for item sets and association rules. An association rule has the form *LHS⇒RHS*, where LHS and RHS are sets of items and the RHS set is likely to occur whenever the LHS set occurs [23].

Two parameters (support factor and confidence factors) were essential in association rules mining. With regard to support and confidence in discovering the association rules, the user shall set the minimum support (min-sup) and the minimum confidence (min-conf) as critical values providing the baselines for discovery. Only the combinations that satisfy the minimum thresholds on support and confidence were considered to mine meaningful rules. The selection of thresholds (support and confidence) was
always an issue. If the minimum confidence is set too high, a lot of useful data will be missed. To find an effective drug compatibility mode, we discovered central tendency of association rules to be more obvious at the
support of 0.1 and confidence of 0.6 in the two correlation analysis of these herbs (used pairs of couplet herbs) and the pairs of herbal functions. So the minimum support of 0.1 and the minimum confidence of 0.6 were specified in this study.

Based on Pharmacopoeia of the People's Republic of China, the ingredients of Chinese medicine were listed according to the selected medicinal formulae and were organized to establish a database. The computing software Microsoft ACCESS was used as a storage tool, and then the SPSS Clenmentine Data Mining System was used as a platform to analyze the frequency and the association rules of the prescription patterns. An initial statistical analysis of the database was carried out to categorize the herbs according to their medicinal types and dosage, natures, flavors, channel tropism, and functions. The frequencies of occurrence and use were then computed based on the categorization. In additionthe associations between different functions of Chinese herbs from the formulae were also examined using the association rules mining.

Results

Associations between Five Properties and Five Flavors from 180 herbs prescribed in 96 formulae

The 180 herbs were categorized according to the Five Properties and Five Flavors (Table 2 & Table 3). Based on the Five properties and Five flavors theory of TCM, herbs with a warm (67 herbs, 37.22%) or cold (60 herbs, 33.33%) nature were most frequently prescribed in terms of the occurrence frequency, while herbs with a warm (appeared 90 times, 93.75%), cold (appeared 71 times, 73.96%) and neutral (appeared 82 times, 85.42%) nature were the top three prescribed herbs in terms of the frequency of use. According to the Five Flavors (Table 3), herbs that were sweet (94 herbs, appeared 93 times, 96.88%) or bitter (87 herbs, appeared 86 times, 89.58%) were the top two prescribed herbs in terms of both the frequencies of occurrence and use.

Association between Channel tropism theory from 180 herbs prescribed in 96 formulae

The results of the association analysis based on channel tropism theory among 180 herbs (Table 4) showed that the top two most frequently prescribed herbs have high affinity to the liver and spleen. The herbs that have high affinity to the liver channel were used most frequently (frequency of use = 44.79%).

Frequency distribution of a single herb prescribed in Chinese medicinal formulae

There are 96 medicinal formulae with a total of 180 herbs included in this study. The total cumulative occurrence of 180 herbs appearing in 96 formulae was 1001 times. The following 13 herbs were frequently prescribed (over 20 times):

[a] Principal function in qi-tonifying, including : Huang Qi (Radix Astragali), Bai Zhu (Rhizoma Atractylodis Macrocephalae), Gan Cao (Radix Glycyrrhizae Uralensis), Tai Zi Shen (Radix Pseudostellariae), Dang Shen (Radix Codonopsitis Pilosulae),

[b] Principal function in spleen-fortifying and dampnessresolving, including : Fu Ling (Sclerotium Poriae Cocos), Yi Yi Ren (Semen Coicis),

[c] Principal function in heat-clearing and detoxicating, including : Shan Ci Gu (Pseudobulbus Shancigu), Bai Hua She She Cao (Herba Hedyotidis Diffusae),

[d] Principal function in blood-tonifying, including: Dang Gui (Radix Angelicae Sinensis),

[e] Principal function in yin-tonifying, including: Gou Qi Zi (Fructus Lycii),

[f] Principal function in blood-activating and stasisresolving, including: E Zhu (Rhizoma Curcuma Phaeocaulis), and

[g] Principal function in qi-regulating, including: Chen Pi (Pericarpium Citri Reticulatae) (Table 5).

Frequency distribution of categorized herbs according to their functions

Herbs with tonifying and replenishing (qi-tonifying, blood-tonifying, yin-tonifying and yang-tonifying), blood-activating and stasis-resolving, spleen-fortifying and dampness-resolving or heat-clearing and detoxicating functions appeared to be most frequently prescribed for the treatment and prevention of breast cancer recurrence and metastasis (Table 6). The top three functions included herbs with qi-tonifying, heat clearing and detoxicating, and blooad-activiating and stasis-resolving functions.

Associations between pairs of herbs functions from the formulae

Association rules mining was applied to investigate the associations between pairs of herb functions from the formulae, and to examine the Chinese medicinal compatibility patterns (Table 7). The minimum support of 0.1 and the minimum confidence of 0.6 were specified. The top three pairs of herbal functions with the highest confidence included the blood-tonifying paired with qi-tonifying functions (93.18%), the qi-regulating paired with qi-tonifying functions (93.10%) and the yin-tonifying paired with qi-tonifying functions (92.50%).

Table 2 Association of the herbs from 96 formulae with Five Properties (N = 180 in 96 formulae)

Five properties	Number of herbs	Occurrence frequency (%)	Number of formulae	Frequency of use (%)
Warm	67	37.22	90	93.75
Cold	60	33.33	71	73.96
Neutral	40	22.22	82	85.42
Cool	8	4.44	44	45.83
Hot	5	2.78	8	8.33

Occurrence frequency = number of herbs belonging to each category / total number of recorded herbs (i.e. 180); Frequency of use = number of formulae recording the use of the herbs / total number of selected formulae (i.e.: 96).

Table 3 Association of the herbs from 96 formulae with Five Flavors (N = 180 in 96 formulae)

Five flavors	Number of herbs	Occurrence frequency (%)	Number of formulae	Frequency of use (%)
Sweet	94	52.22	93	96.88
Bitter	87	48.33	86	89.58
Pungent	64	35.56	80	83.33
Salty	21	11.67	37	38.54
Sour	18	10	40	41.67

Occurrence frequency = number of herbs belonging to each category / total number of recorded herbs (i.e. 180); Frequency of use = number of formulae recording the use of the herbs / total number of selected formulae (i.e.: 96).

Table 4 Association of the herbs from 96 formulae with Channel tropism (N = 180 in 96 formulae)

Channel Tropism	Number of herbs	Occurrence frequency (%)	Number of formulae	Frequency of use (%)
Liver (Gall bladder)	120(13)	27.09	49(9)	44.79
Spleen (Stomach)	126(59)	28.44	42(35)	43.75
Lung (Large intestine)	77(14)	17.38	36(13)	37.50
Kidney (Urinary bladder)	64(12)	14.45	28(9)	29.17
Heart (Small intestine)	51(4)	11.51	26(4)	27.08
Triple energizers (Pericardium)	3(2)	1.13	3(2)	3.13

Occurrence frequency = number of herbs belonging to each category / total number of recorded herbs (i.e.: 180); Frequency of use = number of formulae recording the use of the herbs / total number of selected formulae (i.e.: 96).

Table 5 The top 13 herbs being used among the 180 herbs of the formulae in frequency

No.	Herbs (Pharmaceutical name)	Number of occurrences	Frequency of use (%)
1	Huang Qia (Radix Astragali)	60	62.50
2	Bai Zhua (Rhizoma Atractylodis Macrocephalae)	45	46.88
3	Fu Lingb (Sclerotium Poriae Cocos)	39	40,63
4	Shan Ci Guc (Pseudobulbus Shancigu)	27	28.13
5	Dang Guid (Radix Angelicae Sinensis)	26	27.08
6	Yi Yi Renb (Semen Coicis)	25	26.04
7	Bai Hua She She Caoc (Herba Hedyotidis Diffusae)	25	26.04
8	Gan Caoa (Radix Glycyrrhizae Uralensis)	23	23.96
9	Tai Zi Shena (Radix Pseudostellariae)	22	22.92
10	Gou Qi Zie (Fructus Lycii)	22	22.92
11	Dang Shena (Radix Codonopsitis Pilosulae)	21	21.88
12	E Zhuf (Rhizoma Curcuma Phaeocaulis)	20	20.83
13	Chen Pig (Pericarpium Citri Reticulatae)	20	20.83

Frequency of use = number of formulae recording the use of the herbs / total number of selected formulae.

Table 6 The top 10 kinds of function herb among the 180 herbs of the formulae in frequency

No.	Herbs (Pharmaceutical name)	Number of herbs occurrences in various formulae	Occurrence frequency (%)	Number of formulae use of the herbs	Frequency of use (%)
1	Qi-tonifying	213	21.28	79	82.29
2	Heat-clearing and detoxicating	113	11.29	53	55.21
3	Blood-activating and stasisresolving	104	10.39	56	58.33
4	Yang-tonifying	79	7.89	36	37.5
5	Spleen-fortifying anddampness-resolving	77	7.69	51	53.13
6	Yin-tonifying	70	6.99	40	41.67
7	Blood-tonifying	67	6.69	44	45.83
8	Phlegm-resolving	50	05.	37	38.54
9	Qi-regulating	48	4.8	29	30.21
10	Liver-soothing	21	2.1	19	19.79

Occurrence frequency = number of occurrences for the herbs appearing in various formulae / total cumulative occurrences for 180herbs appearing in 96 formulae (i.e.: 1001); Frequency of use = number of formulae recording the use of the herbs / total number of selected formulae.

Table 7 The pairs of herbal functions being used among the formulae

Herb functions (LHS, X)	Number of formulae		Herb functions (RHS, Y)	Number of formulae	Support (X) (%)	Confidence (X =>Y) (%)
Blood-tonifying	44	→	Qi-tonifying	41	42.71	93.18
Yin-tonifying	40	→	Qi-tonifying	37	38.54	92.50
Phlegm-resolving	37	→	Qi-tonifying	34	35.42	91.89
Spleen-fortifying and dampnessresolving	51	→	Qi-tonifying	46	47.92	90.20
Qi-regulating	29	→	Qi-tonifying	27	28.13	93.10
Yang-tonifying	36	→	Qi-tonifying	32	33.33	88.89
Heat-clearing and detoxicating	53	→	Qi-tonifying	46	47.92	86.79
Blood-activating and stasisresolving	56	→	Qi-tonifying	47	48.96	83.93
Blood-activating and stasisresolving	56	→ →	Heat-clearing and detoxicating	35	36.46	62.50
Heat-clearing and detoxicating	53	→	Spleen-fortifying and dampnessresolving	32	33.33	60.38
Blood-tonifying	44	→	Yin-tonifying	27	28.13	61.36

Occurrence frequency = number of occurrences for the herbs appearing in various formulae / total cumulative occurrences for 180herbs appearing in 96 formulae (i.e.: 1001); Frequency of use = number of formulae recording the use of the herbs / total number of selected formulae.

Associations between pairs of couplet herbs from the formulae

Couplet herbs are two herbs used in pair to increase the therapeutic effect or reduce the toxic effect. To further examine the compatibility patterns of coupletmedicinal prescriptions, we targeted the herbs for healthy-qi reinforcement (including qi-tonifying, yintonifying, blood-tonifying, yang-tonifying and spleenfortifying and dampness-resolving), and the herbs for pathogenic-factor elimination (including heat-clearing and detoxicating, blood-activating and stasis-resolving, and qi-regulating), which were frequently prescribed for the treatment and prevention of breast cancer recurrence and metastasis (Table 8). The minimum support of 0.1 and the minimum confidence of 0.6 were specified. The top three pairs of couplet herbs with the highest confidence included the Tai Zi Shen paired with Bai Zhu (86.36%), the Bai Zhu paired with Huang Qi (84.44%), and the Bai Zhu paired with Fu ling (77.78%).

Discussion

From the herbal perspective, breast cancer is the local manifestation of a whole-body disease, referred to as an intrinsically deficient but extrinsically excessive syndrome. Based on TCM theories, deficiency of spleen qi, inadequate source of engendering transformation, deficiency of qi and blood, and excess of phlegm-dampness are believed to be the main mechanism responsible for development of breast cancer [24,25].

Medicinal formulae often include herbs that are sweet or bitter The 180 herbs were classified according to the Five Flavors, and herbs that were sweet or bitter were the top two most frequently prescribed herbs in the formulae. In TCM theories, herbs that taste sweet can be used for supplementation, moderation and harmonization, referred to as tonifying and replenishing herbs. Herbs that taste bitter can be used for discharging and downbearing, referred to as heat-clearing and detoxicating herbs. However, sweet tasting herbs with spleenstrengthening functions were prescribed and used more frequently than herbs with a bitter taste for clearing heat.

There is a close relationship between recurrence and metastasis of breast cancer and liver, and herbs for nourishing the yin-blood, emolliating and soothing the liver, and smoothing the meridians are the keys of breast cancer treatment

Breast cancer is different from the other cancer types, as the onset of this disease usually peaks at menopausal [26]. The pathological characteristic of this period is marked by exhaustion of heavenly tenth. During this period, the body suffers from yin-blood deficiency, and liver-kidney depletion. Liver is the organ for storing blood. Liver functions in free coursing, and its functions are based on sufficiency of yin-blood. In other words, the free coursing relies on the sufficiency of yin-blood stored in the liver. Therefore, not only herbs for soothing the liver and regulating qi are needed, but also the herbs for emolliating the liver blood are essential for the treatment and prevention of breast cancer recurrence and metastasis. From the association rule mining, the herbs, such as Shao Yao, Wu Wei Zi, Ji Xue Teng, Sheng Shu Di, Gou Qi Zi, Nu Zhen Zi, and Dang Gui, are used directly for blood-tonifying and liver-emolliating in treatment of breast cancer. In general, herbs for nourishing the yin-blood, emolliating the liver, soothing the liver and smoothing the meridians play a key role in breast cancer treatment.

Ample clinical research of Chinese formulae reinforces the spleen to regulate qi and soothe the liver to alleviate pain. Thus, they do not only resist tumor and strengthen the body, but also have anti-cancer effects on metastatic breast cancer [27,28].

The use of herbs for reinforcement of healthy qi and elimination of pathogenic factors is a common Chinese medicinal combination

From the TCM perspective, the etiology of breast cancer is due to deficiency of the healthy qi, which is related to spleen qi deficiency, and liver-kidney depletion. This deficiency will result in malfunctioning of spleen, liver and kidney for transportation and transformation, and free coursing. Without the proper functioning, stagnation and obstruction of the breast collaterals will ultimately be developed and transformed into breast cancer [29].

The use of qi-tonifying and spleen-fortifying herbs is the basis of prescription patterns for preventing breast cancer recurrence and metastasis

Restoration of healthy qi is an effective way to treat diseases and to prevent further progression. The use of qitonifying and spleen-fortifying herbs is to replenish the source of engendering transformation for qi and blood, and to achieve qi-tonifying, blood-replenishing and harmony of the five visceral functions. This is particularly essential for nourishing the liver and smoothing the qi movement. At the same time, spleen-strengthening and qi-replenishing herbs also have the functions for resolving dampness and dispelling phlegm. Therefore, the formulae prescribed herbs such as Huang Qi, Bai Zhu, and Fu Ling, among others.. From the association rules mining, the results showed that the combination of the herbs should also focus on the functions for qi-tonifying. The use of couplet herbs involving Huang Qi and Bai Zhu is to achieve the effects of spleen-strengthening and qi-replenishing, and dampness-drying and water-draining; the use of couplet herbs involving Bai Zhu and Tai Zi Shen is to achieve the effects of fluid-engendering and lung-moistening; the use of couplet herbs involving Bai Zhu and Fu Ling is to achieve the effects of dampness-resolving. The effectiveness of these tonifying and replenishing herbs on tumor resistance and immunity enhancement has also been proven by clinical studies [30,31].

Table 8 The commonly used pairs of couplet herbs in the formulae

Herbs (LHS, X)	Number of formulae		Herb (RHS, Y)	Number of formulae	Support (X) (%)	Confidence (X => Y) (%)
Tai Zi Shen	22	→	Bai Zhu	19	19.79	86.36
Bai Zhu	45	→	Huang Qi	38	39.58	84.44
Bai Zhu	45	→	Fu Ling	35	36.46	77.78
Bai Hua She She Cao	25	→	Fu Ling	19	19.79	76.00
Bai Hua She She Cao	25	→	Yi Yi Ren	17	17.71	68.00
Yi Yi Ren	25	→	Fu Ling	17	17.71	68.00
E Zhu	20	→	Shan Ci Gu	12	12.50	60.00

Conclusions

The results showed that recurrence and metastasis of breast cancer is considered to have a close relationship with liver dysfunctions. These prescriptions focus on the herbs for nourishing the yin-blood, and emolliating and regulating the liver. Strengthening of liver function seems to be the key to successful treatment. Meanwhile, the use of qi-tonifying and spleen-strengthening herbs also forms the basis of prescription patterns. It is also noteworthy that liver function is promoted by strengthening the spleen. ■

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Authors' contributions

YH performed the study; JC was in charge of the study work, advice in the study design and modified in manuscript writing.

XZ, CS, ZW, TX, BJ, QY and KT equally conducted and performed the study.

LC and WTYL gave expert advice in the study design and participated in manuscript writing.

Competing interests

The authors declare that they have no potential and competing interests.

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Adipokines – Toward the Molecular Dissection of Interactions Between Stromal Adipocytes and Breast Cancer Cells

Introduction

After more than a half century of efforts, cancer remains the leading cause of death globally, second only to cardiovascular diseases. The World Health Organization estimates that 84 million people will die from cancer in the next ten years if no action is taken(http://www.who.int/cancer). Obesity appears to play important roles not only in cardiovascular and metabolic diseases, but also in cancer etiology (Bray 2004). For example, overweight and obesity account for 25% of the patients with breast cancer, the most frequent cancer and the second leading cause of cancer death among women (Calle et al. 2003; McTiernan 2003). Excess adiposity over the pre- and post-menopausal years is an independent risk factor for breast cancer and its relapse (Alokail et al. 2009; Katoh et al. 1994; McTiernan 2005; Saxe et al. 1999), and is associated with late-stage disease and poor prognosis (Lorincz and Sukumar 2006). On the other hand, information is limited on why excess body fat increases cancer risks and how obesity affects the prognosis and therapy of cancer.

Dysfunctional adipose tissue, characterized by aberrant production of adipokines, is believed to be a key player in obesity-related mammary carcinogenesis. Adipokines are a family of molecules selectively secreted by fat tissue (Deng and Scherer 2010). In obese subjects, the production of adipokines is dysregulated, which in turn contributes to medical conditions associated with obesity (Galic et al. 2010). Evidence from clinical, epidemiological and experimental studies suggest that adipokines are key pathological mediators in obesityrelated cancer diseases, although the underlying mechanisms remain to be uncovered and may vary from site to site (Prieto-Hontoria et al. 2010; van Kruijsdijk et al. 2009). The present review is to provide a systemic update on how adipokines affect breast cancer cell function and mammary tumor initiation and development. Specifically, the detailed roles of three adipokines (adiponectin, lipocalin-2 and leptin) in mammary carcinogenesis will be discussed by integrating the information derived from cellular, animal and clinical studies.

The mechanistic links for each adipokine will be assembled to model the process of breast cancer development under obesity conditions.

Stromal adipocytes in obesity-associated mammary carcinogenesis

Mammary gland comprises of epithelial and stromal cells. Stromal tissue regulates the development and differentiation of breast epithelial cells (Creydt et al. 2010; Polyak and Kalluri 2010). Adipocyte is one of the predominant stromal cell types in the microenvironment of mammary tissue. Proper function of adipose tissue plays an important role in mammary gland development and lactation process (Couldrey et al. 2002; Wiseman and Werb 2002). The differentiation/redifferentiation of fat cells apparently regulates epithelial cell cycles and contributes to the maintenance of the mammary epithelial "niche" (Arendt et al. 2010; Hovey and Aimo 2010). The close relationship between adipose tissue and mammary tumor growth has been demonstrated by many in vitro and in vivo experimental studies (Elliott et al. 1992; Miller et al. 1981; Sheffield and Welsch 1988). Mature adipocytes can promote the growth of breast carcinoma cells in a collagen gel matrix culture (Manabe et al. 2003). Cotransplantation of tumor cells with adipocytes into mice results in increased tumor growth and metastasis (Iyengar et al. 2005). On the other hand, factors derived from mammary tumor cells stimulate the reversion of mammary adipose phenotype and promote the differentiation of adipose stem cells into carcinoma-associated fibroblast (Guerrero et al. 2010; Jotzu et al. 2010). Conditioned media from breast cancer cells facilitates the accumulation of pre-adipocyte cells in the cancer tissue (Meng et al. 2001).

Multiple mechanisms are implicated in linking abnormal adipose tissue with breast cancer development (Fig. 1). First, adipocyte is the predominant stromal cell type in mammary tissue responsible for local estrogen production, thus contributing to the development of estrogen-dependent breast cancer in postmenopausal women (Sinicrope and Dannenberg 2011). Obese women are at increased risk of developing estrogen receptor (ER)-positive breast cancer (Cleary and Grossmann 2009). Under obese condition, adipose tissue becomes "inflamed" to produce inflammatory mediators, such as tumor necrosis factor alpha (TNFα) and interleukin (IL)-1β, which promote the expression of cytochrome P450 aromatase, an enzyme responsible for the synthesis of estrogen from androgen, in adipocytes (Subbaramaiah et al. 2011). Second, increased fat mass in obese condition is associated with altered energy metabolism (McTiernan 2005). The concept of a relationship between dysregulated metabolism and carcinogenesis was first enunciated

by Otto Warburg more than 80 years ago (Davison and Schafer 2010). There is now a large body of evidence supporting a link between obesity, metabolic syndrome, insulin resistance with increased risk of cancers (Vona-Davis et al. 2007; Wysocki and Wierusz-Wysocka 2010). Type 2 diabetes and high level of circulating blood glucose have been shown to be positively correlated with increased breast cancer mortality (Bjorge et al. 2010; Wolf et al. 2005). Recent studies show that the use of metformin, an oral antidiabetic drug that has been used for many years, is associated with decreased cancer risk (Dowling et al. 2011). Additionally, the increased fat mass is associated with aberrant insulin signaling (insulin resistance) and increased insulin levels, which directly stimulate mammary carcinogenesis (Vona-Davis et al. 2007). During breast cancer progression, the composition of the extracellular matrix is dynamically altered and adipose tissue is critically participated in this process (Erler et al. 2006; Fata et al. 2004). Adipocyte-derived collagen VI could activate the pro-survival and proliferation pathways to promote tumor growth and development (Iyengar et al. 2003). More recently, fat tissue has been recognized as an important secretory organ that can produce various hormones, cytokines and growth factors, collectively called adipokines (Galic et al. 2010). Dys-regulated expression and function of these adipokines play significant roles in the pathogenesis of obesity-related breast cancer diseases (Deng and Scherer 2010; Paz-Filho et al. 2011; Schaffler et al. 2007) (Fig. 2). A number of them, including leptin and lipocalin-2, promote breast cancer cell survival, proliferation and tumor development, whereas adiponectin, the anti-inflammatory adipokine, has opposite effects (Jarde et al. 2011; Leng et al. 2011; Wang et al. 2007b; Yang and Moses 2009). Obese women with reduced serum adiponectin levels and low serum adiponectin levels are associated with an increased risk for breast cancer development and mortality (Duggan et al. 2011; Mantzoros et al. 2004). Women with higher adiponectin levels have a reduced risk of breast cancer (Korner et al. 2007; Miyoshi et al. 2003). Moreover, tumors in women with low serum adiponectin levels are more likely to show a biologically aggressive phenotype with poor prognosis (Miyoshi et al. 2003). The level of leptin increases in serum with increasing adiposity. In women diagnosed with breast cancer, the balance of adiponectin and leptin has been indicated to correlate with the disease development (Grossmann et al. 2008b). Serum leptin to adiponectin ratio is increased significantly in breast cancer patients and positively correlated with tumor size (Chen et al. 2006). Adiponectin levels are negatively correlated with leptin, and patients with higher levels of leptin are at increased risk for late stage tumors (Cust et al. 2009). The reduced levels of adiponectin and elevated leptin are associated with lymph node metastasis (Hou et al. 2007). Another adipokine, lipocalin-2, is found to be associated with aggressive types of breast cancers and poor prognosis (Leng etal. 2011).

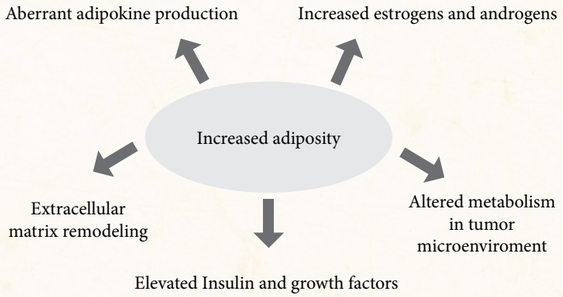

Fig. 1. Multiple mechanisms are implicated in linking increased adiposity with breast cancer development.

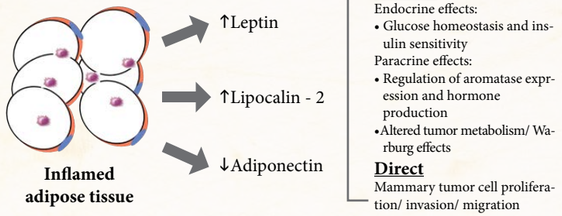

Fig. 2. Dysregulated production of adipokines, such as leptin, lipocalin-2 and adiponectin, from inflamed adipose tissue, contributes to mammary tumor development through both indirect and direct mechanisms.

Taken together, the above experimental and epidemiological evidences suggest that adipose tissue play an important role in breast cancer development and adipokines are key mediators linking obesity with breast cancer disease. The following sections of this chapter will elucidate the detailed role of adipokines, with special focus on the three adipokines, adiponectin, leptin and lipocalin-2, in mediating the stromal-epithelial interactions, in turn influencing the growth and proliferation of breast cancer cells.

Adipokines as key stromal factors in regulating mammary carcinogenesis

Adiponectin

Adiponectin is a 30-kDa glycoprotein exclusively secreted from adipocytes (Scherer et al. 1995). Human adiponectin gene is located on chromosome 3q27 and encodes a 244 aminoacids polypeptide (Wang et al. 2008). Circulating concentrations of adiponectin range from 3-30 μg/mL, accounting for ~0.05 % of total human blood proteins (Ryan et al. 2003). Unlike many other adipokines that are up-regulated in obesity, circulating levels of adiponectin are inversely associated with obesity-related disorders (Cnop et al. 2003; Pajvani and Scherer 2003; Wang et al. 2009).

Endogenous adiponectin is predominantly present as several characteristic oligomeric complexes (Wang et al. 2008). The basic building block of the adiponectin complex is a trimer or low molecular weight (LMW) oligomer, which is formed via hydrophobic interactions within its globular domain. Two trimers self-associate to form a disulfide-linked hexamer or middle molecular weight (MMW) oligomer, which further assembles into a bouquet-like high molecular weight (HMW) multimeric complex that consists of 12-18 monomers (Radjainia et al. 2008). Post-translational modifications, including disulfide bond formation at a conserved cysteine residue and glycosylations occurred on several hydroxylated lysine residues within the collagenous domain, are involved in the assembly and stabilization of the oligomeric structures (Wang et al. 2006b; Wang et al. 2005a; Wang et al. 2002). Different oligomeric complexes of adiponectin activate distinct signalling pathways and possess different biological functions. Two putative adiponectin receptors, termed AdipoR1 and AdipoR2, have been identified. Both receptors are integral membrane proteins containing seven transmembrane spanning domains (Yamauchi et al. 2003). They show unique distributions in various tissues and different affinities for the distinctive forms of circulating adiponectin. T-cadherin, which is highly expressed in endothelium and smooth muscle, has been identified as an adiponectin co-receptor with preference for hexameric and HMW adiponectin multimers (Hug et al. 2004).

Unlike most of the adipokines that are causally linked to obesity-related diseases, adiponectin has potent insulin-sensitizing, anti-inflammatory, anti-atherogenic and antitumorigenic activities (Kadowaki et al. 2006; Wang et al. 2007b; Wang et al. 2008; Wang et al. 2009). Notably, adiponectin potently inhibits the proliferation of various types of cells, including aortic smooth muscle cells, myelomonocytic cells, hepatic stellate cells and several types of cancer cells (Arita et al. 2002; Ding et al. 2005; Wang et al. 2005b; Yokota et al. 2000). It selectively binds to various carcinogenic growth factor and prevent the interactions of these growth factors to their respective receptors (Wang et al. 2005a). In addition, adiponectin inhibits the growth and migration of vascular endothelial cells, prevents new blood vessel formation, and attenuates the growth of transplanted fibrosarcoma cell tumors in mice (Brakenhielm et al. 2004).

The stomal effects of adiponectin have been nicely presented in mouse models with spontaneous mammary tumor development. Study by Lam et al demonstrates that insufficient production of adiponectin in adipocyte per se promotes tumor onset and development in MMTV-polyomavirus middle T antigen (MMTV-PyVT) transgenic mice (Lam et al. 2009; Landskroner-Eiger et al. 2009). A distinctive basal-like subtype of tumors, characterized by high proliferative activity and unfavorable prognosis, is derived from adiponectin haplodeficient MMTV-PyVT mice (Lam et al. 2009). Histological analysis demonstrated typical morphologic features including markedly elevated geographic tumor necrosis, ribbon-like architecture associated with central necrosis, pushing margin of invasion, and stromal lymphocytic response in tumors (Livasy et al. 2007). In contrast, the original MMTV-PyVT mice showed a well-structured and organized morphology. In more advanced malignant stages, mice lacking adiponectin give rise to a larger tumor burden, an increase in the mobilization of circulating endothelial progenitor cells, and a gene expression fingerprint indicative of more aggressive tumor cells. The potent angio-mimetic properties of adiponectin modulate tumor vascularization and deficiency of this hormone creates a chronically hypoxic microenvironment (Landskroner-Eiger et al. 2009). Breast cancer consists of a heterogeneous group of tumors classified into five types, in which the HER2/neu positive and the basal type (most are ER and HER2 negative) have the worst clinical prognosis. Tumors derived from adiponectin haplodeficient MMTV-PyVT mice show a triple-negative genotype (Lam et al. 2009), which may be aroused from a different origin or subgroups of stem cells that develop tumor more aggressively. The origin of this subtype tumor is unclear, but suggested to be the basal/myoepithelial cells, derived from epithelial-to-mesenchymal transition as a result of dedifferentiation, or from stem cells (Livasy et al. 2007).

In human mammary tumor tissue, adiponectin mRNA expression was observed only in the adipose tissues. On the other hand, AdipoR1 and AdipoR2 mRNA expression was observed in breast cancer cells, adipose tissues and normal breast epithelial cells (Takahata et al. 2007). In breast cancer specimen, a strong positive correlation between insulin as well as IGF1 receptor and AdipoR1 expression, but not AdipoR2 expression, could be observed. AdipoR1 is significantly higher in invasive breast cancer compared to preinvasive DCIS and inversely correlated with tumor size (Pfeiler et al. 2011). AdipoR2 expression is significantly correlated with vascular and lymphovascular invasion of breast cancer (Pfeiler et al. 2009). These results suggest a possibility that adiponectin might modulate the growth of normal breast epithelial cells and breast cancer cells directly through AdipoR1 and AdipoR2 receptors, and that the association of low serum adiponectin levels with a high breast cancer risk might be explained, at least in part, by the direct effect of adiponectin on the breast epithelial cells. The altered expression of AdipoR1 in invasive breast cancer also suggests that adiponectin might exert inhibitory effects on the transformation of preinvasive to invasive breast cancer. Further studies are warranted to investigate the prospective association between the mammary adiponectin levels and the risk of obesity-related breast cancers in humans.

Leptin

Leptin is a 16-kDa protein hormone abundantly expressed in white adipose tissue (Jarde et al. 2011). The circulating level of leptin is in the range of 5-50 ng/ml (Garofalo and Surmacz 2006). Obese individuals show a much higher plasma level (over 100 ng/ml) (Oksanen et al. 1997). Leptin was originally discovered by positional cloning of the obese (ob) gene, which is mutated in the massively obese ob/ob mice (Zhang et al. 1994). Leptin acts in the brain to regulate food intake and energy expenditure (Kelesidis et al. 2011). Treatment with leptin significantly reduces the body weight and food intake of the ob/ob mice. The leptin receptor mutant db/db mice, which are phenotypically similar to ob/ob mice, do not respond to leptin treatment (Campfield et al. 1995). The biological activity of leptin is mediated through the transmembrane leptin receptor ObR, which is expressed as at least six different subtypes in numerous tissues and cell types. Primarily the long isoform (ObRb) is responsible for activating leptin signaling pathways (Ahima and Osei 2004).

In general, higher body weight and/or obesity has been associated with shortened mammary tumor latency and increased incidence for development of spontaneous and carcinogen-induced tumors in animals (Dogan et al. 2007). In two sequential studies, MMTV-transforming growth factor (TGF)-α mice were crossed to genetically obese ob/ob and db/db mice. Surprisingly, neither type of these mice developed mammary tumors, suggesting that an intact leptin axis is essential for mammary tumorigenesis (Cleary et al. 2004). On the other hand, obesity induced by high fat diet significantly increases the number of tumors and reduces the tumor latency in MMTV-TGF-α mice (Cleary et al. 2010). The involvement of leptin signaling in mammary tumorigenesis was further confirmed by a study using obese Zucker rats, a rat model of genetic leptin receptor deficiency. Administration of chemical carcinogen methylnitrosourea could only induce a smaller number of Zucker rats to develop mammary tumor compared to lean controls (Lee et al. 2001). These findings demonstrate that leptin is a growth factor to support breast cancer development.

Both normal and malignant mammary tissues have been shown to produce leptin and express leptin receptors (Sheffield 2008). Leptin and its receptor are overexpressed in human breast tumor tissues (Garofalo et al. 2006). Expression of ErbB2 promotes high level expression of long-form leptin receptor and response to leptin. In general, the leptin/ObR correlates with higher tumor grade and worse prognosis (Surmacz 2007). Ishikawa et al observed that overexpression of both leptin and leptin receptors in breast cancer tissue are associated with distant metastasis (Ishikawa et al. 2004). The expression of leptin receptor showed a significant positive correlation with the level of leptin expression, suggesting an autocrine regulation of leptin expression in mammary tumor cells (Fiorio et al. 2008; Ishikawa et al. 2004; Revillion et al. 2006). The mRNA levels of leptin and leptin receptor are correlated positively with estrogen (ER) and progesterone receptors (PR), suggesting a possible interaction between leptin and oestrogen systems to promote breast carcinogenesis (Jarde et al. 2008b; Revillion et al. 2006). Analysis of human breast tumor tissues has also suggested an inverse relationship between leptin and adiponectin in breast cancer development (Jarde et al. 2008b). While leptin was expressed in a similar manner in invasive ductal carcinoma and in situ lesions, no tissue from in situ ductal carcinoma exhibited adiponectin expression. Moreover, myoepithelial cells of normal tissue adjacent to breast cancer exhibited 65% positivity for adiponectin while no cells in this group were positive for leptin expression, suggesting a possible leptin–adiponectin interaction on myoepithelial cells (Jarde et al. 2008b).

Lipocalin-2

Lipocalin-2, a 25-kDa secretory glycoprotein originally purified from human neutrophils, is constitutively expressed in adipose tissue (Esteve et al. 2009; Law et al. 2010). This protein structurally belongs to the lipocalin superfamily that shares the highly conserved structure of an 8-stranded antiparallel beta-barrel (Goetz et al. 2002). Circulating level of lipocalin-2 is elevated in obese animals and humans (Auguet et al. 2011; Hoo et al. 2008; Wang et al. 2007a; Yan et al. 2007; Zhang et al. 2008). Clinical, animal and cellular studies demonstrate the causal involvement of lipocalin-2 in obesity-associated medical complications (Auguet et al. 2011; Catalan et al. 2009; Esteve et al. 2009; Jin et al. 2010; Kanaka-Gantenbein et al. 2008; Law

et al. 2010; Moreno-Navarrete et al. 2010; Sommer et al. 2009; van Dam and Hu 2007; Yan et al. 2007; Zhang et al. 2008). In humans, the serum concentration of lipocalin-2 is associated closely with obesity-related anthropometric and biochemical variables, and represents an independent risk factor for metabolic and cardiovascular disorders (Catalan et al. 2009; Choi et al. 2008; Ding et al. 2010; Esteve et al. 2009; Hemdahl et al. 2006; Lee et al. 2010; Wang et al. 2007a; Yndestad et al. 2009). Role of lipocalin-2 in regulation of cell proliferation, differentiation and apoptosis has been demonstrated (Devireddy et al. 2001). Lipocalin-2 may sequester the intracellular iron causing cell death.

Lipocalins function to transport and present ligands to cell surface receptors and to form macromolecular complexes (Flower 1995). The first identified ligand of lipocalin-2 was bacterial catecholate-type ferric siderophores, such as enterobactin (Goetz et al. 2002). Thus this protein was originally considered as a potent bacteriostatic agent (Berger et al. 2006). A number of studies have reported that lipocalin-2 weakly binds to the tripeptide N-formyl-Met-Leu-Phe (fMLF), a potent neutrophil chemoattractant, and possibly other lipophilic mediators of inflammation, including platelet activating factor and leukotriene B4 (Strong et al. 1998). Recently, chemical screens combined with crystallography and fluorescence detection reveal a complex of lipocalin-2 that binds iron together with a small metabolic product called catechol (Bao et al. 2010). The formation of the complex blocks the reactivity of iron, permits its transport in the circulation and facilitates recycling in endosomes. The lipocalin-2-catechol-Fe(III) complex represents an unforeseen endogenous siderophore for iron traffic in aseptic tissues. This mammalian siderophore plays a critical role in both cytoplasmic and mitochondrial iron homeostasis. Lacking this siderophore results in the accumulation of abnormally high amounts of cytoplasmic iron and elevated levels of reactive oxygen species (Devireddy et al. 2010).

The promoting effects of lipocalin-2 on mammary tumor development have been signified by two independent studies using MMTV-ErbB2 (V664E) and MMTV-PyVT mouse models (Berger et al. 2010; Leng et al. 2009). Leng et al found that the initiation time of the mammary tumor in MMTV-ErbB2 (V664E) mice complete lacking lipocalin-2 expression was dramatically delayed by ~100 days compared to the mice with two copies of lipocalin-2 alleles (Leng et al. 2009). Furthermore, the tumor burden, the number of tumors per mouse as well as the lung metastasis were dramatically reduced. Another study also showed reduced tumor weight and number of tumors per mouse in MMTV-PyVT mice lacking lipocalin-2 expression (Berger et al. 2010). However, there was no difference observed during early mammary tumorigenesis between the wild type and lipocalin-2 knockout group. Based on this, they concluded that lipocalin-2 played a more important role in the later stage of tumor development in MMTV-PyVT model, which shows a more aggressive phenotype with much shorter tumor latency (Berger et al. 2010).

Positive correlations between the circulating level of lipocalin-2 and the invasive and metastatic status of breast cancer have been reported (Yang and Moses 2009). The expression patterns of lipocalin-2 in mammary tumor samples have been analyzed by a number of studies (Bauer et al. 2008; Stoesz et al. 1998; Yang et al. 2009). Lipocalin-2 positive cells can be identified in the infiltrating carcinomas but not in normal mammary tissues (Bauer et al. 2008). High expression of lipocalin-2 correlates with low ER and PR expression, highhistologic grade, lymph nodes metastasis, high-proliferation index and poor disease-free survival (Leng et al. 2011). The induced expression of lipocalin-2 staining in either the tumor or the stroma area is correlated with the advanced stages and the metastatic status. Orthotopic studies demonstrated that lipocalin-2-expressing breast tumors displayed a poorly differentiated phenotype and showed increased local tumor invasion and lymph node metastasis (Yang et al. 2009).

In summary, animal models have provided unique tools to dissect the roles of individual adipokine in mammary tumor development and to elucidate the multiple pathways responsible for the dialogue between adipocytes and breast cancer cells. The information obtained from the mammary tumor models with deficient adipokine expressions demonstrate that in general, adipokines elicit their activities on tumor progression through regulating a) cancer cell transformation, proliferation and migration; b) local and systemic inflammation; and c) pathological angiogenesis. In addition, the role of adipokines to regulate systematic energy metabolism also impacts the behaviors of breast cancer cells and tumor development.

Signaling mechanisms responsible for the regulation of breast cancer cell function by adiponectin, leptin and lipocalin-2

Although adipokines are the key players in obesity-related mammary carcinogenesis, the underlying mechanisms remain largely uncharacterized. Individual adipokines affect mammary tumor development in different manners through distinctive signalling pathways, with concomitant influences on proliferative, inflammatory, and metastatic properties of the tumor cells (Schaffler et al. 2007; Vona-Davis and Rose 2007). Moreover, the mechanistic networks of adipokines in mammary tumor development are usually intertwined with their role in regulating inflammation and angiogenesis (Lorincz and Sukumar 2006; Wang et al. 2007b). Here, the specific signaling mechanisms that are directly involved in regulating the breast cancer cell functions will be discussed and linked with animal and clinical presentations.

Diversified signaling mechanisms of adiponectin: cross-talking with Wnt/β-catenin pathway

Adiponectin acts as an inhibitory factor for the proliferation of human breast carcinoma cells and mammary tumor development (Arditi et al. 2007; Dieudonne et al. 2006; Grossmann et al. 2008a; Hebbard et al. 2008; Jarde et al. 2008a; Kang et al. 2005; Nakayama et al. 2008; Pfeiler et al. 2008; Wang et al. 2006a). *In vitro* treatment with adiponectin at physiological concentrations attenuates the growth of an ER-negative human breast carcinoma MDA-MB-231 cells by inhibiting cell proliferation and inducing apoptosis (Kang et al. 2005; Wang et al. 2006a). It also inhibits insulin- and growth factors-stimulated proliferation in ER-positive human breast cancer cells (Li et al. 2011; Wang et al. 2006a). These in vitro data are supported by animal study demonstrating that adiponectin supplement therapy suppresses the MDAMB-231 breast tumor development in nude mice (Wang et al. 2006a).

Cell-type dependent signalling mechanisms have been suggested to mediate the growth inhibitory effects of adiponectin (Grossmann et al. 2008a) (Fig. 3). In MCF-7 cells, adiponectin induces AMP-activated protein kinase (AMPK) phosphorylation and inactivates p42/p44 MAPkinase (ERK1/2) (Dieudonne et al. 2006). By contrast, the inhibitory effects of adiponectin on T47D cell growth are associated with inactivation of ERK1/2 but not AMPK or p38 MAPK (Korner et al. 2007; Wang et al. 2006a). In MDA-MB- 231 cells with ectopic ER over-expression, globular adiponectin inhibits cell proliferation by blocking JNK2 signaling (Grossmann et al. 2008a). A cross-talk between adiponectin and ER signaling exists in breast cancer cells and that adiponectin effects on the growth and apoptosis of breast cancer cells in vitro are partly dependent on the presence of 17-beta estradiol (Pfeiler et al. 2008). In ER-negative MDA-MB-231 cells, adiponectin could modulate the glycogen synthase kinase-3beta (GSK3β)/β-catenin signaling pathway (Wang et al. 2006a). Prolonged treatment with adiponectin markedly reduces serum-induced phosphorylation of Akt and GSK3β, decreases intracellular accumulation and nuclear translocation of β-catenin, and suppresses cyclin D1 expression (Wang et al. 2006a). An increase of protein phosphatase 2A activity has been implicated in the dephosphorylation of Akt by adiponectin treatment in MDA-MB-231 cells (Kim et al. 2009). Although the effects of adiponectin on tumor metastasis are not conclusive, it is suggested that LKB1 is required for adiponectin-mediated inhibition of adhesion, migration and invasion of breast cancer cells (Taliaferro-Smith et al. 2009).

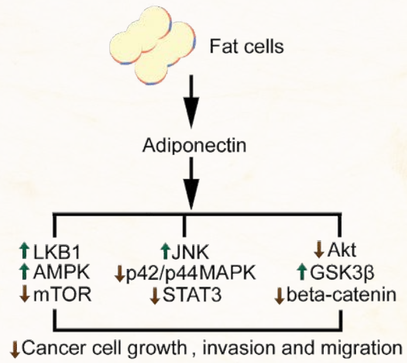

Fig. 3. Signaling pathways that mediate the anti-tumor activities of adiponectin.

Hyperactivation of the canonical Wnt/β-catenin pathway is one of the most frequent signal abnormalities in many types of cancers (Brown 2001; Howe and Brown 2004; Prosperi and Goss 2010). The central event in this pathway is the stabilization and nuclear translocation of β-catenin, where it binds to the transcription factor TCF/LEF and consequently activates a cluster of genes that ultimately establish the oncogenic phenotype (Jin et al. 2008). Stabilization of β-catenin protein and over-expression of cyclin D1 have been observed in over 50% of human breast tumors and increased β-catenin activity was found to be significantly correlated with the poor prognosis of breast cancer patients (Brown 2001). Given the close proximity between mammary gland cells and adipocytes, decreased adiponectin production might be causally linked to increased β-catenin accumulation and cyclin D1 overexpression observed in breast cancer patients. This possibility is supported by animal studies. The isolated mammary tumor cells from adiponectin haplodeficient MMTVPyVT mice are presented with hyperactivated phosphatidylinositol-3-kinase (PI3K)/Akt/β- catenin signaling, which at least partly attributes to the decreased phosphatase and tensin homolog (PTEN) activities (Lam et al. 2009). PTEN is one of the most frequently mutated tumor suppressors that can prevent the activation of the cell survival PI3K/Akt signaling pathway (Carnero et al. 2008). In MMTV-PyVT animals with reduced production of adiponectin, PTEN is inactivated by a redox-regulated mechanism involving thioredoxin and thioredoxin reductase. Specificity protein 1, a redox-regulated transcription factor, is involved in mediating the effects of adiponectin to stimulate the expression of Wnt inhibitory factor-1, a Wnt antagonist frequently silenced in human breast tumors (Liu et al. 2008). In summary, these findings have not only suggested a cross-talk between adiponectin and Wnt signaling pathway, but also provided a novel mechanistic insight to explain how metabolic alterations in adiponectin haplodeficient tumor may gain a survival advantage.

Leptin-mediated signaling in breast cancer cells: in relation to other mitogenic receptors

Leptin acts as a mitogen and survival factor for human breast cancer cells (Markowska et al. 2004). Leptin receptors are expressed in various human breast cancer cell lines and in human primary breast carcinoma (Frankenberry et al. 2006; Garofalo et al. 2006; Hu et al. 2002; Laud et al. 2002; Sheffield 2008). Leptin acts through multifaceted signaling pathways,including Jak2/STAT3 (Janus kinase 2/signal transducer and activator of transcription 3), PI3K/Akt, ERK1/2 and SOCS3 (Fusco et al. 2010; Palianopoulou et al. 2011; Saxena et al. 2007; Yin et al. 2004). Different sensitivities to recombinant leptin treatment have been found in distinctive breast carcinoma cell lines. For example, in MCF 7 cells, leptin induces a strong phosphorylation of STAT3 and ERK1/2, leading to an increased cell viability and proliferation (Fusco et al. 2010). This response is not present in MDA-MB 231 cells, in which leptin potentiates the anti-proliferative action of cAMP elevating agents by concurring to cell cycle arrest at G1 phase and inducing apoptosis (Naviglio et al. 2009).

Leptin induces the expression of vascular endothelial growth factor (VEGF) in both human and mouse mammary tumor cells, and promotes angiogenesis, which is related to the worse prognosis of breast cancer (Zhou et al. 2011). HIF-1alpha and NFkappaB are implicated in leptin-regulated VEGF expression through both canonic (MAPK, PI-3K) and non-canonic (PKC, JNK and p38 MAP) signalling pathways (Gonzalez-Perez et al. 2010). Leptin contributes to the elevated circulating estrogen levels in obese women. It stimulates aromatase activity in adipose stromal cells at high concentrations (Magoffin et al. 1999). The action of leptin to enhance the promoter activity of aromatase is mediated by AP-1 in MCF-7 cells (Catalano et al. 2003). These evidence suggest that elevated leptin concentrations may cause locally augmented VEGF and estrogen in the breast and thereby promote tumor formation.

Leptin exerts its activity not only through its own receptors, but also through crosstalks with other signaling systems implicated in tumorigenesis (Ozbay and Nahta 2008). Co-treatment of leptin and insulin-like growth factor (IGF)-I significantly increases proliferation as well as invasion and migration of breast cancer cells (Saxena et al. 2008). A bidirectional crosstalk between leptin and IGF-I signaling exists to synergistically activate the downstream effectors, Akt and ERK1/2. Moreover, leptin and IGF-I treatment transactivates epidermal growth factor receptor (EGFR) to induce invasion and migration of breast cancer cells. In breast cancer cell lines, HER2 and ObR are coexpressed and physically interacted (Fiorio et al. 2008; Ray et al. 2007). Leptin treatment increases HER2 phosphorylation on Tyr 1248 (Fiorio et al. 2008). Coexpression of HER2 and the leptin/ObR system might contribute to enhanced HER2 activity and reduced sensitivity to anti-HER2 treatments. These data suggest indicate the possibility of using EGFR inhibitors to counter the pro-cancerous effects of leptin and IGF-I in breast cancers. Exogenous leptin induces tyrosine phosphorylation of HER2 in SKBR3 cells, which showed marked overexpression of HER2. Leptin-induced HER2 phosphorylation was partially reduced by an EGFR inhibitor, AG1478, or a Jakinhibitor, AG490. Moreover, leptin-induced phosphorylation of ERK1/2 could be abrogated by a HER2 tyrosine kinase inhibitor, AG825 (Soma et al. 2008). In fact, the influence of leptin on breast cancer development not only relates to the presence or absence of HER2 but also depends on ER status (Ray et al. 2007). Knocking down of ERalpha attenuates leptininduced activation of STAT3, whereas the enhancement of leptin-mediated STAT3 activity is independent of ERalpha ligands. ERalpha binding to STAT3 and Jak2 might lead to an increased ERalpha-dependent cell viability(Binai et al. 2010). Leptin plays important role in enhancing in situ estradiol production and promoting estrogen-dependent breast cancer progression. The ability of leptin to transactivate ERalpha and mimic the classic features of ERalpha signaling has been observed in MCF-7 breast cancer cell line. MAPK pathway is found to be involved in this process. Moreover, estradiol-induced activation of ERalpha can be potentiated by leptin exposure (Catalano et al. 2004).

Taken together, these findings suggest that the leptin system plays an important role in breast cancer pathogenesis and progression, and that it represents a novel target for therapeutic intervention in breast cancer disease (Cirillo et al. 2008).

Lipocalin-2: Controversies and role in epithelial to mesenchymal transition

Lipocalin-2 is a putative in vivo estrogen target gene and paracrine factor that mediates the growth regulatory effects of estrogen in normal breast epithelium (Seth et al. 2002). It contains an ER response element in its promoter. On the other hand, in T47D breast cancer cells, hormone treatment

decreases the mRNA expression of lipocalin-2 (Mrusek et al. 2005), suggesting that normal and cancerous estrogen receptor-positive cells are distinct at the molecular level. Elevated lipocalin-2 may influence the steroid status of the mammary epithelial cells. When ectopically introducing lipocalin-2 into MCF-7 cells, their ERdependent tumor growth in the xenografted mice is lost and the tumor cells become ERnegative (Yang et al. 2009). These data imply that modulation of lipocalin-2 expression may enable the breast cancer cells to become sensitive to ER therapy, a result that might be translated into clinical usage for ER-targeted therapy.

Both human and mouse mammary tumor cell lines have been used to examine the importance of lipocalin-2 in mammary tumor formation. Overexpression of lipocalin-2 in mouse 4T1 and human MDA-MB-468 cells greatly promoted their ability in cell migration and invasion (Leng et al. 2009; Shi et al. 2008). Moreover, implantation of 4T1 or MDA-MB- 468 cells ectopically expressing lipocalin-2 generated a significant more number of lung metastatic nodules compared to those implanted with the unmodified cells. The lung metastasis could be blocked by injection of a polyclonal antibody against lipocalin-2 (Leng et al. 2009). In HER2-positive human breast cancer cell line SKBR3, knocking down lipocalin-2 expression reduced the migration and in and ER-positive MCF-7 cells, These findings are consistent with the study by Fougere et al suggesting that the anti-migration activity of NFAT3 is through inhibition of lipocalin-2 gene expression (Fougere et al. 2010). In human tissue and urine samples, lipocalin-2 levels are consistently associated with invasive breast cancer (Yang et al. 2009).

Lipocalin-2 has been shown to induce the epithelial to mesenchymal transition (EMT) in breast cancer cells (Leng et al. 2011). Cells undergone EMT show increased motility and invasiveness as well as elevated lipocalin-2 expression. When ectopically expressed in MCF- 7 cells, lipocalin-2 induces a typical EMT change of the cell morphology, accompanied by a loss of epithelial marker (E-cadherin) and an increased expression of the mesenchymal markers (vimentin and fibronectin) (Yang et al. 2009). Lipocalin-2 silencing in aggressive breast cancer cells inhibits cell migration and the mesenchymal phenotype. Increased secretion of lipocalin-2 from the tumor cells might directly affect MMP-9 activity to promote cell motility or the transition to a more mesenchymal/aggressive phenotype (Leng et al. 2011). Much higher blood gelatinase activities are found in the tumor-bearing MMTVErbB mice with normal lipocalin-2 expression than those deficient of lipocalin-2 expression (Leng et al. 2009). ERalpha is also suggested to participate in lipocalin-2-induced EMT. By contrast, in 4T1 cells lipocalin-2 appears to reverse the EMT process induced by Ras expression (Hanai et al. 2005). Different phases or sites of lipocalin-2 treatment have been suggested for these controversial findings. During EMT, the initial increased lipocalin-2 stimulates epithelial migration and the elevated exogenous lipocalin-2 may facilitate the recovery (Mori et al. 2005; Yang et al. 2002).

In summary, although lipocalin-2 regulates EMT, one of the key processes involved in tumor progression and metastasis, the underlying mechanisms remain to be further elucidated.

Concluding remarks

The prevalence of obesity and its associated diseases has posed a huge healthcare impact on our society. During the past two decades, a panel of adipokines critically involved in pathological processes of obesity-associated breast cancer diseases has been discovered. Their contributions to the development of breast cancer and the underlying mechanisms are divergent. For example, adiponectin deficiency is associated with an accelerated mammary tumor development and altered Wnt/β-catenin signaling. On the other hand, the tumors of mice without lipocalin-2 are less metastatic and show slower rate of growth. Clearly, individual adipokines are able to modulate specific oncogenic and metabolic pathways, which synergistically promote or antagonize the development of breast cancer disease under obese conditions. The three adipokines discussed in this chapter not only represent potential therapeutic targets for breast cancer, but can also serve as biomarkers for early diagnosis and disease prevention. Compounds related to leptin that may have therapeutic use are currently being investigated in pre-clinical studies (Gonzalez et al. 2006; Ray and Cleary 2010; Rene Gonzalez et al. 2009; Surmacz 2007). Continued reearch will undoubtedly provide more insights into the relationship between adipose tissue-derived factors and breast cancer development, as well as the ways to intervene these interactions. ■

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Mitochondrial decay in aging: 'Qi-invigorating' schisandrin B as a hormetic agent for mitigating age-related diseases

Summary

The 'Mitochondrial Free Radical Theory of Aging' (MFRTA) proposes a primary role of mitochondrial reactive oxygen species (ROS) in the aging process. The 'Reductive Hotspot Hypothesis of Mammalian Aging' serves as a supplement to the MFRTA by explaining how the relatively few cells that have lost oxidative phosphorylation capacity due to mitochondrial DNA mutations can be toxic to the rest of the body and result in the development of age-related diseases.

Schisandrin B (Sch B), which can induce a glutathione antioxidant response and a heat shock response via the redox-sensitive signaling pathways, is a hormetic agent potentially useful for increasing the resistance of tissues to oxidative damage. The enhancement in cellular/mitochondrial antioxidant status as well as the heat shock response afforded by Sch B can preserve the structural and functional integrity of mitochondria, suggesting a potential role in ameliorating age-related diseases.

Future studies will be focused on investigating whether or not Sch B can produce the hormetic response in human subjects.

Keywords: schisandrin B; glutathione antioxidant response; heat shock response, mitochondria; hormesis

Mitochondrial Free Radical Theory of Aging

Aging is defined as a progressive degeneration in tissue/organ homeostasis as well as an increase in the likelihood of death[1]. Over past few decades, reactive oxygen species (ROS) are believed to be a determinant genotoxin in causing DNA damage, with resultant mutagenic and cytotoxic effects. DNA damage is also associated with a decrease in the number of vi-

able cells and impairment in cellular functional capacity, both of which are manifestations of the aging process. With regard to the role of ROS in aging, Denham Harman causally correlated free radical-induced tissue oxidative damage with the aging process and proposed the 'Free Radical Theory of Aging' [2,3]. During aerobic respiration, mitochondrial ROS (mtROS) are unavoidably generated as a result of electron transport chain activity. Given that mitochondrial DNA (mtDNA) molecules are located in proximity to the sites of mtROS generation (eg. complex I and III[4]), mtDNA is more prone to oxidative damage than genomic DNA[5]. Harman later refined his free radical aging theory to become the 'Mitochondrial Free Radical Theory of Aging' (MFRTA), which proposed mtROS as the primary cause of aging in humans and other multicellular organisms. mtROS can disrupt the structural and functional integrity of mitochondrial membranes and thereby trigger a vicious cycle of mtROS production and mitochondrial dysfunction, with resultant progressive systemic functional degeneration. As promising as it seemed to be, the "vicious cycle" theory has been shown to be irreconcilable with current experimental findings in gerontology. Aged mitochondria with membrane lesions are tagged for degradation in a process called mitophagy, which constantly degrades and recycles the materials of aged mitochondria. Furthermore, mitochondria are periodically rejuvenated by mitochondrial fission, so that any given mitochondrion in the cell should possess relatively intact membranes and proteins. According to MFRTA, mtDNA mutations which accumulate during aging occur randomly. However, it has been found that all of the mutant mitochondria in a given cell contain the same array of mutations in mtDNA, i.e. a deletion of all 13 mtDNA-encoded proteins, which are involved in the assembly of complex I[6] and complex III[7]. To address this anomaly, Aubrey de Grey proposed the 'Reductive Hotspot Hypothesis of Mammalian Aging' as a supplement of the MFRTA[8]. In fact, mitochondria with the 13 mtDNA mutations linked with complex I and III assembly can escape mitophagy. As mitochondria with mutated mtDNA are defective in oxidative phosphorylation (OXPHOS), they do not produce any mtROS and therefore are protected from mitophagy because of the absence of oxidative damage in membranes. While OXPHOS-negative mitochondria are clonally expanded by mitochondrial fission, OXPHOS-negative cells have to utilize an alternative electron transport system to reoxidize NADH and generate NAD^+ in order to sustain ATP production by glycolysis. In so doing, the plasma membrane redox system (PMRS)[9], which is located on the plasma membrane, is utilized to transport electrons from NADH inside the cell to the outside of the cell, thereby regenerating NAD^+ from NADH. Like the mitochondrial electron transport, the PMRS is imperfect and generates superoxide during the process. The high activity of PMRS creates a highly reductive "hotspot" around the plasma membrane, leading to a burst of superoxide production. The superoxide generated outside the cell can potentially initiate free radical reactions, including lipid peroxidation chain reactions involving circulating materials such as low density lipoprotein (LDL), a major carrier of cholesterol in the blood. The resultant oxidized LDL not only increases the risk of atherosclerosis[10], but also distributes its potentially toxic oxidized cholesterol, presumably through the intermediacy of oxidized LDL, throughout the entire body[11]. Thus the relatively few cells that have lost OXPHOS capacity as a result of mtDNA mutations may be toxic to the rest of the body and contribute the development of age-related diseases.

Based on experimental findings that do not support the notion that mitochondrial ROS may be important in the process of aging[12], the MFRTA has been regarded by some gerontologists as an "out-dated" aging theory that is no longer correct[13]. One such observation involves *Mclk1*$^{+/-}$ mutant mice, which showed increased mitochondrial oxidative stress arising from a deficit in NADPH-mediated mechanisms of ROS detoxification, but have a long-lived phenotype - which would appear to be irreconcilable with the MFRTA[14,15]. Although the fundamental question regarding whether or not mitochondrial oxidative stress plays a causal role in the aging process remains unresolved, the situation with the *Mclk1*$^{+/-}$ mutant mice, which suffer from enhanced mitochondrial oxidative stress at young ages but proceed to show paradoxical improvement in biomarkers of aging, could be viewed as hormesis, a phenomenon that will be discussed in the later section.

The role of mitochondrial oxidative stress in the development of age-related diseases

Despite the aforementioned controversy, the generation of ROS within the mitochondria remains the currently most accepted cause of aging. Increased levels of ROS within mitochondria are the principal trigger, not only for mitochondrial dysfunction, but also for diseases associated with aging in general[16,17]. The mitochondrion has been considered as "the gate of life and death" by virtue of its important role in both bioenergetics and apoptosis (or programmed cell death)[18]. With regard to the role of the mitochondrion in bioenergetics, ROS are unavoidably generated during the electron transport process, particularly from complex I and complex III[19], and the mitochondrion is an immediate target of these ROS. An excessive ROS production can damage mitochondrial membranes, regulatory proteins and DNA, with resultant disruption in mitochondrial structural and functional integrity. Mitochondrial decay in structure and function is believed to be one of the primary causal factors in the process of aging and in age-related diseases.

Several studies have revealed a complex network of signaling pathways modulated by nutrients, such as insulin-like growth factor-1 (IGF-1), target of rapamycin (TOR), sirtuins (SIRT), AMP kinase and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) that are connected and then converge to inhibit oxidative stress within the mitochondria[20]. Animal models in which components of these signaling pathways were modulated (such as the induction of SIRT1, AMPK and PGC1-α or the silence of IGF-1 and TOR) displayed a generalized phenotype characteristic of decelerated aging. It has been shown that calorie restriction affects mitochondria through SIRT1, which, being part of the IGF-1 pathway, is able to deacetylate and thereby activate forkhead boxO (FoxO) transcription factors, which in turn activate stress response genes and increase longevity 16, [21-27]. In addition, SIRT1 can also activate PGC-1α, which has emerged as a master key regulator of mitochondrial biogenesis [28,29]. Taken together, the various signal pathways, converging on the regulation of mitochondrial redox status, are tightly interconnected, indicating the existence of a complex and highly regulated machinery for controlling age-related diseases and lifespan. In the following section, paradigms relevant to cancer, cardiovascular diseases (CVD) and neurodegenerative diseases are used to illustrate the pathological role of oxidative stress in these age-related diseases.

Carcinogenesis

Cancer is a disease that involves a multistep process of mutations and preferential clonal expansion of highly neoplastic mutated cells. Recently, mitochondrial defects have been shown to be associated with genomic instability, which predisposes to carcinogenesis. For instance, mitochondrial decay causes a reduction in the synthesis of ATP, which is important for driving many ATP-dependent reactions including transcription, DNA replication as well as DNA repair. In other words, dysfunctional mitochondria may be mutagenic due to the genotoxic effect of ROS[30]. In addition, it has been demonstrated that the depletion in mtDNA affects the cellular availability of deoxyribose nucleoside triphosphates (dNTP), presumably due to the reduced ATP supply for the ATP-dependent dNTP synthesis processes in mtDNA-depleted cells[31,32], and an imbalanced dNTP pool may be carcinogenic[33]. Mutations of mitochondrial complex I subunit 5 gene, which cause an increase in mtROS production, were found to be associated with cancers such as breast cancer, leukemia and lung cancer[30]. Epidemiological studies also showed a close correlation between the incidence of cancer[34]and the elevated ROS production from aged cells[35], implying that ROS may play an important role in tumor initiation, promotion and progression.

Cardiovascular diseases (CVD)

CVD are the leading cause of morbidity and mortality in developed countries. In particular, over 3.8 million men and 3.4 million women die of myocardial infarction (MI) every year in the world[36]. Age-associated changes in vascular system, including arterial thickening and stiffening, are known to increase the susceptibility to CVD[37]. With regard to the changes in vascular system, atherosclerosis, the ROS-promoted accumulation of LDL (in the form of oxidized LDL) in arterial walls, is a risk factor for MI. It has now been well established that ROS lead to atherosclerosis through causing oxidative modification of LDL, inflammation and endothelial injury in blood vessels[38]. The blockage of coronary arteries can result in myocardial ischemic injury and subsequent necrotic cell death if the supply of nutrients and oxygen is not resumed early enough. To terminate myocardial ischemia by restoring the blood supply, the re-introduction of oxygen to the previously ischemic cells results in a burst of ROS production, with subsequent oxidative and irreversible damage to the cardiac tissue, which is referred to as "ischemia/reperfusion injury". In this regard, the reperfusion-induced ROS production is at least in part attributed to the increased electron leakage in Ca^{2+} overloaded mitochondria due to the stimulatory effect on TCA cycle and OXPHOS[39].

Neurodegenerative diseases

A large and compelling body of evidence has shown that accumulation of unfolded or misfolded proteins in neurons is closely associated with various neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Friedreich's ataxia. The pathogenic, dysfunctional, mis/un-folded protein aggregates were found to be associated with the derangement of mitochondrial bioenergetics and excessive ROS production, with a resultant pathogenesis of neurological disorders[40-42].

AD is a neurodegenerative disorder of cognitive and memory decline, speech loss and dementia. The hallmark of AD is a progressive accumulation of sensile plaques which consist of amyloid β-peptide (Aβ). It has been shown that the formation of Aβ is partially induced by oxidative stress[40]. Interestingly, Aβ aggregates were found to produce a hormetic effect, with antioxidant activity at low concentrations but prooxidant activity at high concentrations[43]. A high concentration of Aβ can cause H_2O_2 production, which in turn oxidizes mitochondrion-relevant proteins such as voltage-dependent anion channels, aconitase, glyceraldehyde phosphate dehydrogenase and lactate dehydrogenase[44]. It is well documented that the activity of mitochondrial α-ketoglutarate dehydrogenase[45]and complex IV[46] are reduced in AD patients, presumably due to the direct inhibitory effect of Aβ[47], with a resultant impairment in mitochondrial energy production and hence the neuronal cell death.

PD, a neurodegenerative disease of bradykinesia, tremor, gait difficulty, postural instability and rigidity[48], is characterized by Lewy body formation and dopaminergic neuron loss in the substantia nigra. It has been shown that mitochondrial complex I activity is decreased in the substantia nigra of PD patients[49], presumably as a result of an increase in oxidative modification (protein carbonylation) of complex I, which results in the misassembly and dysfunction of the protein complex 41. The oxidative metabolism of dopamine and the relatively high concentration of ferrous ion in dopaminergic neurons further increase their susceptibility to oxidative stress-induced functional impairment[42], which in turn results in mitochondrial bioenergetic decay and ultimately cell death. Another hallmark of PD is the formation of Lewy bodies, which contain α-synuclein. α-Synuclein is predominantly localized in the cytosol. The aggregation of α-synuclein (as α-synuclein protofibrils) is promoted by oxidative stress and dopamine adducts[50], and in this form was found to interact with various mitochondrial components. As such, α-synuclein, which contains a cryptic mitochondrial targeting signal, was imported into mitochondria and then associated with the inner membrane. The import of α-synuclein into mitochondria was correlated with the reduced activity of mitochondrial complex I[51]. The impaired function of respiratory complex I as well as permeabilization of the outer mitochondrial membrane[52]led to the loss of mitochondrial membrane potential, cytochrome c release and apoptosis[53], with a resultant destruction of the dopaminergic neuron.

What is hormesis?

The term hormesis has long been used in the field of toxicology to describe a biphasic dose-response phenomenon in which a chemical has a beneficial effect at low doses, but causes a toxic effect at high doses. For example, a low concentration of vitamin A is essential for eye function while a high concentration of vitamin A results in anorexia, headaches and drowsiness[54]. Similarly, glutamate promotes neuronal survival and adaptive plasticity at low concentrations, whereas it causes excitotoxicity in the nervous system at high concentrations[55]. In this connection, the concept of hormesis is now being increasingly considered in the context of aging research. It has recently been suggested that a single or multiple exposure to low doses of otherwise detrimental agents, such as ionizing radiation, heat stress and ROS generators, might produce a variety of effects on age-related diseases and longevity[56,57]. In recent decades, accumulating evidence has demonstrated that a mild stress arising from prooxidant exposure, heat shock, ischemic preconditioning, calorie restriction, radiation or physical exercise induces the expression of an array of stress resistance proteins such as heat shock proteins, antioxidant enzymes and growth factors. The increased protein expression is mediated by the activation of various signaling pathways/transcription factors, including SIRT/FOXO[58], electrophile response element (EpRE)/nuclear factor erythroid 2-related factor 2 (Nrf2)[59], cAMP responsive element-binding protein (CREB)[60] or nuclear factor kappaB (NFκB)[61]. As such, tolerance is developed to a subsequent and more severe stress in various biological systems[62]. Recent findings have shown that a small amount of ROS production arising from an increased mitochondrial respiration promotes longevity and metabolic health, thus establishing the basis of mitochondrial hormesis or "mitohormesis". For instance, calorie restriction, reduction of glucose metabolism by pharmacological means as well as physical exercise were all found to induce mitochondrial metabolism, with an associated modest increase in mitochondrial ROS production, which in turn elicits a mitohormetic response in preventing age-related disease and retarding the aging process[16]. In addition, much research effort has recently focused on the hormetic response induced by dietary phytochemicals, which are derived from plants as bacterial toxins. Phytochemicals, such as curcumin, isothiocyanates and resveratrol, have been found to increase antioxidant capacity, as do chemopreventive agents and anti-inflammatory agents at low concentrations, supporting their potential usage as nutraceuticals for counteracting the deleterious effect of ROS. Taken together, investigations on the induction of hormetic responses, particularly in mitochondria, may provide new insights into the prevention of age-related diseases and retardation of the aging process.

Hormetic signaling pathways

While a high level of ROS production can cause age-related diseases and accelerate the aging process, a low level of ROS production induced by a hormetic agent can trigger various redox-sensitive signal transduction pathways, with the eliciting of protective cellular responses.

Nrf2/EpRE pathway

ROS regulate cell survival and death by triggering redox signaling, wherein mitogen-activated protein kinases (MAPK) play an essential role[63,64]. The stimulation of adaptive responses to oxidative stress requires one or more members of the MAPK cascade. The ultimate effects of MAPK activation depend on their ability to phosphorylate downstream signaling molecules, with the subsequent expression of appropriate genes that govern cellular redox homeostasis. Among the three distinct MAPK pathways, extracellular signal-regulated protein kinase (ERK) is activated by mitogens

and growth factors[65], whereas C-Jun-NH2-terminal kinases (JNK) and p38 MAPK (p38) are regulated by extracellular stresses such as UV and oxidative stress [66,67]. Upon their activation, ERK, JNK, and p38 can phosphorylate a range of transcription factors[63], which in turn change the profiles of gene expression that result in a variety of biological responses. Nrf2 is a redox-sensitive transcription factor that binds to EpRE, also earlier termed as "antioxidant response element" (ARE)[68]. It has been shown that the translocation of Nrf2 from the cytosol to the nucleus is facilitated by phosphorylation[69], with subsequent enhancement in the expression of antioxidant defense genes, including the catalytic and modulatory subunit of γ-glutamate cysteine ligase, glutathione reductase (GR), glutathione peroxidase and glutathione transferases (GST)[68,70], which are also collectively referred to as the "glutathione antioxidant response". Given that the dysregulation of reduced glutathione (GSH) levels was found to be associated with the pathogenesis of various age-related diseases[71], the induction of a glutathione-dependent antioxidant response may prevent or attenuate age-related diseases. This postulation was supported by the observation that the extension of longevity in rodents was correlated with the elevated levels of GSH and GST in various tissues, with the latter being causally related to the activation of Nrf2/EpRE pathway[72].

Heat shock factor-1 (HSF1)

The "heat shock response" (HSR), which involves the induction of heat shock protein expression, is a highly conserved protective mechanism against a range of environmental stressors, such as oxidative stress and endoplasmic reticulum (ER) stress. The key players in the HSR are heat shock proteins (Hsps) which facilitate the correct folding of newly-synthesized proteins, re-folding of un/mis-folded proteins, protein trafficking, and directing misfolded proteins to proteasomal or lysosomal degradation. In addition to being molecular "chaperones", a family of Hsps, namely Hsp70, was found to regulate the activity of HSF1, a transcription factor that binds to heat shock element (HSE) in the promoter region of heat shock genes[73], and also possesses an indirect antioxidant activity by up-regulating antioxidant defense mechanism[74]. In addition, small Hsps (such as Hsp25, Hsp27) were found to increase GSH levels by enhancing glutathione redox cycling by elevating the activity of glucose-6-phosphate dehydrogenase – a crucial enzyme in the generation of NADPH, which is a cofactor for GR[75,76]. The crosstalk between Hsps and the glutathione antioxidant system may produce a synergistic effect on cytoprotection against oxidative stress. During the normal aging process, the expression of Hsps was found to increase, presumably due to an elevation in oxidative stress in aged organisms[77,78]. In this connection, a sustained induction of HSR by hormetic agents may prevent (or at least delay) the development of age-related diseases and thus retard the aging process.

NF-κB system

NF-κB is a pleiotropic transcription factor that regulates a range of biological functions, including those involved in development, in the immune system and in cell survival. Recently, it has been shown that the activation of the NF-κB system, which is associated with the aging process, is a manifestation of a prolonged inflammatory response, a suppression of autophagic clearance of cellular wastes and a reduction in apoptotic clearance of senescent cells[79-81]. The NF-κB system is further found to be functionally antagonistic to the Nrf2/EpRE[82] and FoxO signaling pathways[83], both of which are closely related to longevity. Furthermore, the NF-κB-induced expression of a range of inflammatory cytokines has been postulated to promote the senescent phenotype in cells[84]. In this connection, the suppression of the aging-induced activation of the NF-κB system may retard the aging process.

Schisandrin B as a hormetic agent

Schisandrin B (Sch B) is a dibenzocyclooctadiene derivative (lignan) abundantly found in Fructus Schisandrae (FS), the fruit of Schisandra chinensis (Turcz.) Baillon. There are three stereoisomers of Sch B found in FS, namely (−)Sch B (or gominsin N), (+)γ-schisandrin and (−)γ-schisandrin. According to traditional Chinese medicine (TCM) theory, FS is regarded as a 'Qi-invigorating' herb, in particular, nourishing the Qi of five major organs (i.e., heart, kidney, liver, lung and spleen). In the realm of TCM, Qi is regarded as a vital substance, which is fundamental to life and provides energy for the human body. In this regard, FS can invigorate the Qi of the human body, with a resultant efficient energy utilization and longevity. Interestingly, an extensive body of experimental evidence suggests that the mitochondrion, which is the "gate of life and death", appears to be the main subcellular target for Sch B[85]. In this connection, Sch B may be a potential hormetic agent that could prevent age-related diseases and thus retard the aging process.

Pharmacological actions of Sch B

Sch B acts like a hormetic agent in cultured cells, with the cytoprotective effect predominating at low concentrations[86] and the cytotoxic effect occurring at high concentrations[87]. No detectable toxic effects of Sch B treatment were observed following a single oral dose (0.8 g/kg), multiple doses (200 mg/kg × 30) or dietary supplementation (0.012%, w/w, starting from 9 months of age until death) in mice[88-90]. Sch B treatment is therefore generally regarded as safe. Previous investigations in our laboratory have shown that Sch B treatment can protect against oxidant-induced injury in the brain[91], heart[92], kidney[93], liver[94]and skin[95]of rodents. In these studies, the tissue protection afforded by Sch B was invariably associated with an enhancement of mitochondrial glutathione antioxidant status and was likely mediated by an increase in the resistance of mitochondria to Ca^{2+}-induced permeability transition[96,97]. The ability of Sch B to protect against oxidant-induced tissue injury suggests its potential in preventing age-related diseases such as coronary heart disease and neurodegenerative disorders. In this regard, the tissue/cytoprotection afforded by Sch B treatment against myocardial ischemia/reperfusion injury in rats[92], $A\beta_{1-42}$-induced cytotoxicity in primary cortical neurons[98] and paraquat toxicity in differentiated PC12 cells[99]may have clinical implications in the prevention/treatment of myocardial infarction, Alzheimer's disease and Parkinson's disease. Sch B also induced apoptosis in human hepatic carcinoma cells and leukemia cells[87,100,101], and also decreased the viability of adenocarcinoma cells following UV exposure[102]. Dietary Sch B was found to ameliorate the mitochondrial decay associated with aging, which was paralleled by increased longevity in aging mice[90]. In addition to oxidative stress, chronic inflammation and protein misfolding may also be important causes of both age-related diseases and the aging process in general. With respect to these observations, Sch B was found to suppress inflammation[103]as well as induce the expression of the molecular chaperones, Hsp25 and Hsp70[85].

Biochemical mechanism underlying the Sch B-induced antioxidant response

The ability of Sch B to enhance cellular/mitochondrial glutathione-dependent antioxidant capacity is not tissue-specific, suggesting the involvement of a biochemical reaction which is universally found in all cell types in its mode of action. Given that the cytochrome P-450 system (CYP) is ubiquitously found in various cell types[104]and methylenedioxy group-containing compounds are metabolized by CYP[105], Sch B, which is a known substrate for CYP, can produce ROS through CYP-catalyzed biotransformation in various cell types (unpublished data). The concomitant ROS production associated with Sch B metabolism in cells or tissues was paralleled by changes in the cellular/tissue protection afforded by Sch B *in vitro* and *in vivo*[106,107]. We therefore propose that Sch B may act as a hormetic agent that produces a low concentration of ROS, which one might term "signaling ROS"[108], with a resultant triggering of redox-sensitive signaling pathway(s) and eliciting of a glutathione antioxidant response. Recently, our laboratory has shown that (−)Sch B caused a dose-dependent and sustained increase in ROS production as well as a time-dependent activation of MAPK, particularly ERK1/2. The MAPK activation was followed by an enhancement in Nrf2 nuclear translocation and the eliciting of a glutathione-dependent antioxidant response in cultured hepatocytes and cardiomyocytes[109,110]. In addition, long-term Sch B treatment by oral route increased the expression of Hsp25 and Hsp70 in various rat tissues[85]. A recent study showed that gomisin N [or namely (−)Sch B] enhanced TNFα-induced apoptosis in Hela cells by suppressing NF-κB signaling[111]. As noted earlier, the activation of Nrf2/EpRE and HSF1 signaling pathways promote longevity, whereas activation of the NF-κB pathway accelerates the aging process.

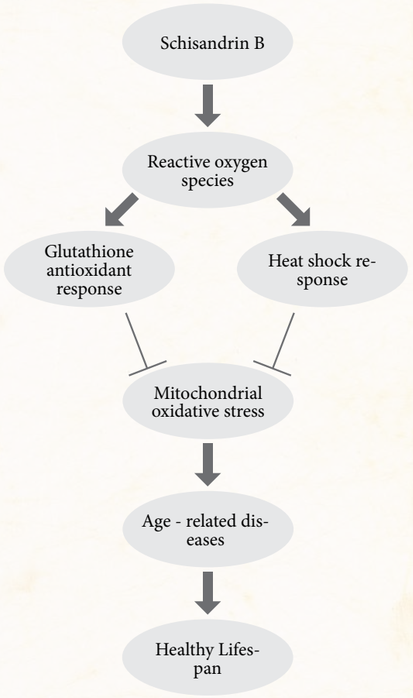

Fig. 1. The schisandrin B-induced hormetic response in prolonging a healthy lifespan.

Conclusions

The MFRTA proposes that a vicious cycle of mtROS production can cause the disruption of mitochondrial structural and functional integrity, which is manifest as an aging phenotype. At odds with the MFRTA is the observation that the significant loss-of-function mtDNA mutations accumulate only to low levels in most tissues, even at very advanced age. To address this anomaly, the 'Reductive Hotspot Hypothesis of Mammalian Aging' serves as a supplement to the MFRTA. This theory attempts to explain how the relatively few cells that have lost oxidative phosphorylation capacity due to mtDNA mutations may be toxic to the rest of the body and result in the development of age-related diseases. Given the crucial involvement of oxidative stress in age-related diseases, the enhancement of cellular/mitochondrial antioxidant capacity may be beneficial in promoting health and prolonging lifespan. In addition, experimental evidence has shown that the activation of Nrf2/EpRE and HSF1/HSE signaling pathways promotes longevity, whereas the activation of NF-κB pathway accelerated the aging process. A number of dietary interventions with phytochemicals have been shown to elicit hormetic responses via the Nrf2/EpRE pathway[112]. Sch B, which can enhance glutathione-dependent antioxidant capacity and heat shock protein production via the redox-sensitive ERK/Nrf2/EpRE and HSF1/HSE pathways, respectively, is a hormetic agent that may be potentially usefully for increasing the resistance of tissues to oxidative damage. The enhancement in cellular/mitochondrial antioxidant status as well as the heat shock protein induction afforded by Sch B can preserve the structural and functional integrity of mitochondria, suggesting that it may show potential in ameliorating age-related diseases (Fig. 1). Future studies will be focused on investigating whether or not Sch B can produce such a hormetic response in human subjects. ■

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澳門成年冠心病患者運動自我效能與運動鍛鍊調查

【摘要】目的

了解成年冠心病患者運動鍛煉情況與運動自我效能水平，探討兩者之間的關係，為提高和改善其運動鍛鍊狀況提供理論依據。方法便利抽樣抽取澳門冠心病患者130例，應用運動自我效能量表測量患者的運動自我效能，應用運動鍛鍊狀況表評估患者的運動鍛鍊狀況。結果澳門成年冠心病患者做適量運動鍛鍊有46.9%，運動的自我效能得分為45.46±16.72.。將運動鍛練狀況分為低量、適量及過量運動，三者存著顯著性差異，P<0.01，並以運動適量的組別運動自我效能得分最高。而影響運動的因素為患病年齡、身體因素及社會環境因素。結論在健康教育中，應重視提高冠心病患者的患病年齡、身體及社會環境自我效能感，教導正確的運動方法，改善運動鍛鍊狀況。

WHO估計到2030年，幾乎有2360萬人將死於心血管病，預計它們將繼續成為死亡的一個主要原因[1]。中國的冠心病死亡人數已列世界第二位[2]。澳門死於循環系統疾病（包括心臟病），排名第二，佔總死亡率25.8~27%[3]。有研究表明中國冠心病發病率呈上升趨勢，特別是隨著人民物質生活水準的提高，年齡在45~59歲的中年人冠心病的發病率也在不斷上升[4]。缺乏體力活動和青年冠心病的發生密切相關[5]，但冠心病患者對運動依從性不理想及認知不足；沒有認知到堅持運動對控制冠心病的危險因素的重要性；甚至將冠心病患者不宜做激烈運動的建議理解成少運動、 不做運動[6]；在澳門，老年人比年輕人更積極做運動[7]。根據WHO冠心病的身體活動建議，已診斷為CHD，出現復發性心血管事件的風險極高，應採二級預防，建議所有正從重大CHD事件（包括冠狀動脈血運重建術）中恢復者參加規律（週三次或以上，每天累計30分鐘）的輕至中等強度的有氧運動[1]。目的是通過降低他們的心血管風險，預防復發性心血管事件。國外多項研究表明，運動自我效能與規律鍛煉顯著正相關；運動自我效能是一種特殊狀態的自信心形式，是一種人們在各種困難情境中對自我組織和執行運動行為的能力判斷，可以預測人們的動機和行為；運動自我效能高者，參與運動的堅持性更好[8]，能更有效地克服外界阻力[9]。本研究旨在了解澳門成年人冠心病患者運動鍛鍊和運動自我效能水平，分析兩者之間的關係，從而為提高和改善冠心病患者合適運動鍛鍊提供理論依據。

對象與方法

調查對象

2012年12月至2013年1月以便利抽樣取得冠心病患者130例。納入組標準：①年齡18-64.9歲；②經確診為冠心病進入康復期患者（病者自訴的診斷）；③可以自我進行運動鍛鍊者；④自願參加本次研究並簽署知情同意書。排除標準：①有嚴重軀體疾病；②視力及聽力障礙；③精神及認知功能障礙。

研究工具

一、運動自我效能量表

自我效能（self-efficacy）的定義是指個體對自己的行為能力及行為能否產生預期結果所抱的信念[9]。自我效能量表分四維度：身體因素、心理因素、物理環境因素及社會環境因素；量表由15個條目組成，每條目計分1~5分，被試者根據各自的情況回答，總分75分，得分越高自我效能感越高，依據標準分為過低、中等、良好三級[10]。運動自我效能度的劃分標準：≦30分，即過低或等於總分的40％，為運動自我效能度；31-59分，即位於總分的40％~80％之間，為中等；>60分，即高於或等於總分80％，為良好[11]。是次研究對量表進行重測信度及內容效度分析；總量表重測信度Cronbach’Sα系數0.997，各維度Cronbach’sα則在0.992-0.997之間，信度良好。總量表內容效度為0.94，各條目為0.83-1.00。

二、身體鍛鍊狀況調查

身體鍛練有氧運動是指長時間（15分鐘以上）有節奏、會令心率上升的肌肉運動，每週三次或以上，每天累計30分鐘[12]。身體鍛鍊狀況調查共9題，按運動鍛練的量、頻率、強度的調查而自設問卷。對量表進行重測信度及內容效度分析；各條目重測信度k系數0.67-1.00。

資料收集方法

研究前獲得倫理審議會批准（HSEARS20120906003），在心臟科門診部就診前30分鐘進行問卷調查，向每受調查對象說明本次調查目的，並簽署知情同意書，無論參與研究與否，所接受的醫療服務同等不變。若不願意可不參加或中途覺得不舒適時可隨時退出，並不會影響他接受服務之權利。一般由調查員詢問填寫，個別年輕要求自我填寫，遇有不明向調查員查詢。共發問卷130份，收回問卷130份，回收率100%。

統計學方法

所有資料輸入SPSS16.0統計軟件包進行分析，採用頻數、百分率、構成比、均數、標準差對成人冠心病患者的一般人口學特徵、身體鍛鍊狀況，以及成年人的運動自我效能總體及各個維度的得分進行描述；以卡方檢驗比較適量運動鍛鍊及不適量運動鍛鍊人群一般狀況、運動自我效能總體的差異；以單因素方差分析檢驗運動鍛鍊與運動自我效能各個維度得分是否有差異；對存在差異的因素經Logistic多變數邏輯進行回歸分析方法提取最有影響力的因素。

研究結果

研究對象的基本資料

本研究130例成人冠心病患者中，男性75人（57.7%），女55人（42.3%）。年齡以55~64.9歲數組最多，有79人（60.8%），以25~34.9歲數組最少，有4人（3.1%）。婚姻狀況為已婚108人（83.1%），未婚16人（12.3%），其他6人（4.6%）。職業以退休為最多，有39人（30%），餐飲服務業最少，有6人（4.6%）。個人月收入以<10000澳門幣收入最多，有60人（46.2%），以澳門幣25001~30000人數最少，有4人（3.1%）。教育程 度方面，以中學程度為最多，有62人（47.7%），未受正式教育人數最少，有4人（3.1%）。患上冠心病時間以<2年最多，有53人（40.8%），<5年最少，有19人（14.6%）（表1）。

研究對象的運動鍛鍊狀況

適量運動鍛鍊有60人（47.6%），低量的有55人（43.7%），過量的有11人（8.7%）。獲取運動鍛鍊訊息的主要途徑從醫護人員90人（69.2%）及電視23人（17.7%）。最能影響做運動的人是其他（自己），有59人（45.4%）；伴侶影響最少，只有19人（14.6%）。家居附近運動資源的有108人（83.1%）。而最常做的運動為散步有45人（34.6%）、其次為跑步9人（6.9%）、不能堅持運動原因以無時間為多，39人（30%）。堅持運動原因以對身體好為最多，有73人次（56.2%）、運動後心情好8人次（6.2%）。以年長（55-64.9歲，53.1%）、退休人群（77%）、低於澳門幣10000圓個人收入（72.7%）、患病年齡以≧10年（78.6%）、中學教育程度（56.5%）的人群做合適運動鍛練的比率較多；年齡越年青（35-44.9歲，94.7%）、高收入人士則較少（25%）、大專或大學上較少年（25.7%）的人群做合適運動鍛練的比率較少。

不同人口學特徵影響冠心病運動鍛鍊情況

對不同人口學特徵的成年冠心病患者進行了比較，結果在年齡、職業、個人月收入、教育程度、患上冠心病時間等方面存在顯著性差異。（表2）

成人冠心病患者運動自我效能與運動鍛鍊的關係

從表3看出三組運動不同的人，以運動適量的人群的運動自我效能總體及各維度得分均高於低量運動及運動過量的人群，而以低量運動的人群在總體及各維度得分最低，三者存在著顯著性差異（P<0.001），運動的自我效能度越高，執行適量運動鍛鍊的能力越高。（表3）將影響運動的各因子及運動自我效能總得分進行LOGISTIC回歸分析的結果。結果顯示自我效能總分每多一分，做合適運動的機會將會增加13.5%（p<0.001）。而患上冠心病多於10年的病人做適量運動的機會，則分別是患病少於5年及10年的病人的6.17倍及7.04倍（p<0.05）。（表4）而當自我效能總得分被替換為運動自我效能各維度的得分後，LOGISTIC素得分每多一分時，做合適運動的機會分別增加22.2%（p<0.05）和回歸分析結果顯示自我效能的身體因素和社會環境因28.4%（p<0.01）。而患冠心病時間多於10年的病人做適量運動的機會分別是患病少於2年及5年的病人的5.62和 7.46倍 （p<0.05）。（表5）

討論

年齡

患病少於2年的人較多有不適量運動，有38人（77.7%），較國內與李丹2008年所做的老年人規律鍛鍊調查結果（79.47%）為低[10]，但符合古勤等在2006年所做的澳門健康調查及2010澳門市民體質監測中年青人積極做運動的比例較年長為低結論[6][7]。這可能與調查對象的不同使結果存在差異，如成年人可能在工作居多，較老年人少時間去做運動有關。

表1 澳門成年冠心病患者基本資料(n=130)

項目	例數(n)	百分比(%)
性別		
女	55	42.3
男	75	57.7
年齡		
18-24.9	0	0
25-34.9	4	3.1
35-44.9	19	14.6
45-54.9	28	21.5
55-64.9	79	60.8
婚姻狀況		
未婚	16	12.3
已婚	108	83.1
其他	6	4.6
職業		
餐飲服務業	6	4.6
紀律部隊	7	5.4
博彩及娛樂業	12	9.2
醫療	13	10
文職	16	12.3
退休	39	30.0
其他	37	28.5
個人每月收入(澳門幣)		
<10,000	60	46.2
10001~15000	24	18.5
15001~20000	15	11.5
20001~25000	7	5.4
25001~30000	4	3.1
>30000	20	15.4
教育程度		
未受正式教育	4	3.1
小學	29	22.3
中學	62	47.7
大專	7	5.4
大學或大學以上	28	21.5
患上冠心病的時間		
<2年	53	40.8
2-4.9年	19	14.6
5-9.9年	30	23.1
≥10年	28	21.5

職業

做合適運動以退休人較多（77%），說明退休人群較多時間堅持合適的運動鍛鍊，從不能夠堅持做運動鍛練的原因分析，主要原因是「沒有時間」，反映出需要工作的人群會由於工作因素以致沒有時間進行合適的運動鍛練。從表4可見醫療/紀律部隊進行合適的運動鍛鍊的比例最低（10%），這可能與工作忙碌、需要輪值工作有關。

個人月收入

本研究結果是低收入的人群較多做適量的運動；考慮這結果可能本研究以退休人士佔多數（77%）有關，退休人士運動鍛練狀況較其他人群理想，而他們的個人月收入會較低。本研究結果是低收入、退休、年齡層較大組別（55~64.9）的人群較多做適量的運動，學歷高及高收入的較多人做不合適的運動，從不能夠堅持做運動鍛練的原因分析，主要原因是「沒有時間」，反映出需要工作的人群會由於工作因素以致沒有時間進行合適的運動鍛練；而本澳2006年的健康調查亦發現年長者比年輕者更積極做運動[7]，上述結果與國外的研究表示會運動的人是年輕、教育程度良好、過去參與運動等的結果不相同[11]；在這方 面初步看來，本澳市民在年青時會因為工作、忙碌等問題而忽略運動鍛練， 縱使有較高的教育程度、甚至是醫護人員，已有相當的知識，但運動鍛練狀況亦未如理想，這點應值得重視。

運動自我效能對成年冠心病患者的影響

自我效能總分每多一分做合適運動的機會增加22.5%。說明成年冠心病人群運動的自我效能分數與做適量運動鍛練機會越高，與其他因素對比，最具影響力。表5可知，而身體因素及社會環境因素每多一分做合適運動的機會增加22.2%及28.4%，這說明身體因素及社會環境因素與其他客觀因素相

表2 不同人口學特徵成年冠心病患者運動鍛練狀況比較

項目	不適量運動	運動適量	總計 n=130	χ值	P值
年齡#					
35-44.9	18(94.7%)	1(5.3%)	19(100%)	18.386	0.000***
45-54.9	16(57.1%)	12(42.9%)	28(100%)		
55-64.9	32(40.5%)	47(59.5%)	79(100%)		
職業					
餐飲博彩服務業	13(72.2%)	5(27.8%)	18(100%)	31.534	0.000***
醫療/紀律部隊	18(90.0%)	2(10.0%)	20(100%)		
文職	12(75.0%)	4(25.0%)	16(100%)		
退休	9(23.0%)	30(77.0%)	39(100%)		
其他	15(55.6%)	22(44.4%)	37(100%)		
個人每月收入(澳門幣)					
<10,000	20(33.3%)	40(72.5%)	60(100%)	17.721	0.000***
10001-30000	34(68.0%)	16(32.0%)	50(100%)		
>30000	15(75.0%)	5(25.0%)	20(100%)		
教育程度					
未受正式教育/小學	16(48.5%)	17(51.5%)	33(100%)	31.534	0.012*
中學	27(43.5%)	35(56.5%)	62(100%)		
大專/大學或以上	26(74.3%)	9(25.7%)	35(100%)		
患上冠心病的時間					
<2年	38(77.7%)	15(28.0%)	53(100%)	18.755	0.000***
≥2-5年	10(52.6%)	9(47.4%)	19(100%)		
≥5-10年	15(50.0%)	15(50.0%)	30(100%)		
≥10年	6(21.4%)	22(78.6%)	28(100%)		
鍛練的時間					
日間	14(22.2%)	49(77.8%)	63(100%)	52.922	0.000***
黃昏/晚上	27(69%)	12(31%)	39(100%)		
沒有	28(100%)	0(0%)	28(100%)		

#4個缺失數據，*p<0.05;***p<0.001

表3 運動鍛練與運動自我效能總分和各維度得分結果（均值±標準差）(n=130)

項目	低量運動	運動適量	運動過量	F	P值
身體因素	9.2586±4.828	14.3443±4.198	12.3636±3.354	19.599	0.000*
心理因素	7.7586±4.160	12.1148±3.077	9.4545±2.544	22.234	0.000*
物理環境因素	6.0517±3.347	10.0000±3.785	8.6364±3.585	18.208	0.000*
社會環境因素	11.5517±5.598	19.3770±4.163	14.7273±3.849	39.012	0.000*
自我效能總分	34.6207±15.875	55.8361±11.250	45.1818±8.611	37.434	0.000*

*p<0.001

表4 適量運動鍛練之影響因素、自我效能總分進行Logistic回歸分析

變量值	P 值	Exp值	95% CI
患上冠心病的時間 (對照組:≧10年)			
<5年	0.044	0.162	0.028-0.952
<10年	0.028	0.142	0.025-0.810
自我效能總分	0.001	1.135	1.051-1.227

表5 適量運動鍛練之影響因素、自我效能各維度進行Logistic回歸分析

變量值	P 值	Exp值	95% CI
患上冠心病的時間 (對照組:≧10年)			
<2年	0.041	0.178	0.034-0.932
<5年	0.036	0.134	0.021-0.877
自我效能(身體因素)	0.021	1.222	1.031-1.449
自我效能(社會環境因素)	0.005	1.284	1.078-1.529

比，作用強大，如身體及社會環境因素覺得適合時，就能克服其他阻碍因素，繼續做適量運動鍛練。本研究結果顯示，運動效能良好的人，即使他們在遇到困難的阻礙，也能很好堅持適量的運動鍛練。運動自我效能的高低與參與運動的動機成正比。運動自我效能是心理學中的一種特殊狀態的自信心形式，是一種人們在各種困難情境中對自我組織和執行運動行為的能力判斷，可以預測人們的動機和行為[12]。這與張媛媛在2010年及李丹在2008年所研究結果運動自我效能高者，參與運動的堅持性更好的結果一致[8，10]。

建議

心血管疾病是世界上最大殺手，在2008年奪走1730萬人的生命；其中，估計有730萬人死於冠心病，佔全球死亡的30%[13]。應用自我效能理論，增强冠心病患者的自我效能度，從而改善冠心病患者的適量運動。自我效能對個人的運動行為有很大的影響，提升運動自我效能就成為促進個人運動行為的策略之一。醫護人員或相關工作人員，應利用自我效能的四個信息來提升冠心病患者的運動自我效能，改善這些人群的運動鍛煉現狀。

建立運動信心，增强個人對成功的體驗

運動自我效能可稱做是特殊情境的運動信心，而運動信心是影響個體運動學習及表現相當重要而關鍵的一個心理因素[14]。當個體運動信心較强時，其本身對運動的各種條件（如場地的適應、本身的技術等）的瞭解較能掌控，也較有面對挑戰的能力，其自我效能也較强。

可編制冠心病運動小冊子派予他們，指引其做合適的運動。而在心臟科門診可播放有關冠心病運動的影片而增强其知識，建立其做運動的信心。

健康教育可以通過行為矯正，通過有計劃、有系統的教育活動來促進個體自願執行有利健康的活動，當局可設立心臟康復中心，醫護人員可以針對病患的個體情況、運動條件及對運動項目的興趣訂定適宜的運動目標制定運動處方，可增加個體運動的自我效能。使其享受到運動後帶來的愉快及好處。對一些年青在職的、高學歷、低量運動或過量的人群，亦可通過此方法來增加運動。對於身體不適時，可根據運動處方減少運動或改變運動的方式，而天氣不好時，可進行適當的室內鍛練。對于女性患者，以低自我效能及低量運動居多，應改變她們以家務代替鍛煉的觀念，讓她們運動鍛煉可以使身體更全面均衡的鍛煉，有助冠心病的康復及改善心血管的功能。

加强合作，減少浪費資源，增加成效

經制定運動處方後，醫院及衛生中心或其他醫療機構可以資源共享來共同監督冠心病患的運動成效，設立電話咨詢制度，給予鍛煉的指導。衛生中心可定期組織冠心病患運動小組，讓他們可以分享運動鍛鍊的樂趣及運動鍛鍊受到其他事情阻礙時給予鼓勵支持，增加其成功的愉快的經驗，以增加自我效能。

結論

澳門成年冠心病患者運動自我效能處於中度，有待進一步加强。低量運動及低自我效能的成年冠心病人群為數不少，極需進一步改善運動鍛鍊的狀況以達至適量的運動鍛鍊；在對成人冠心病患者合適運動鍛鍊影響因素分析中，以患病時間、個人月收入、自我效能最具影響力的因素，衛生當局有關人員應通過自我效能的四個信息來源，以及配合制定或修改的到位健康教育內容及運動鍛鍊的資源，提高成人冠心病患者的自我效能度，進而改善合適鍛鍊的現況，達到改善冠心病患者心臟的功能能力，以運動鍛鍊降低冠狀動脈疾病之血管危險因素，以獲得最佳的身心健康。■

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