Journal of Ethnopharmacology 199 (2017) 68–85

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Exploring the pharmacological mechanism of Yanghe Decoction on HER2-positive breast cancer by a network pharmacology approach

Liuting Zeng*,[1], Kailin Yang[1]

Hunan University of Chinese Medicine, Changsha 410208, Hunan Province, China

ARTICLE INFO

Keywords:
Yanghe Decoction
HER2-positive breast cancer
Network pharmacology
Traditional Chinese medicine
Chinese medicine formulae

ABSTRACT

Ethnopharmacological relevance: Certain Chinese medicine formulae from traditional Chinese Medicine (TCM) are effective for treating and preventing diseases in clinical practice. Yanghe Decoction (YHD) is a Chinese medicine formula that is used to treat breast cancer, especially HER-positive breast cancer; however, the active compounds, potential targets, and pharmacological and molecular mechanism of its action against cancer remain unclear. Therefore, further investigation is required.

Methods: A network pharmacology approach comprising drug-likeness evaluation, oral bioavailability prediction, Caco-2 permeability prediction, multiple compound target prediction, multiple know target collection, breast cancer genes collection, and network analysis has been used in this study.

Results: Four networks are set up, including HER2-positive breast cancer network, compound-compound target network of YHD, YHD-HER2-positive breast cancer network and compound-known target-HER2-positive breast cancer network, and some HER2-positive breast cancer and YHD related targets, clusters, biological processes and pathways are found. We also found some potential anti-cancer compounds.

Conclusion: Our works successfully predict, illuminate and confirm the molecular synergy of YHD for HER2-positive breast cancer and found the potential HER2-positive breast cancer associated targets, cluster, biological processes and pathways. This study not only provide clues to the researcher who explores pharmacological and molecular mechanism of YHD acting on HER2-positive breast cancer, but also demonstrates a feasible method for discovering potential drugs from Chinese medicine formulae.

1. Introduction

Breast cancer is the most common malignancy and the leading cause of cancer mortality in women worldwide (Torre et al., 2015). In China, breast cancer has become one of the fastest growing cancers in the past 30 years, the rising rate is close to 96%, only slightly lower than lung cancer (You et al., 2013). American Cancer Society (ACS) estimates that there will be 246,660 cases of women diagnosed with breast cancer in US and 40,450 women die of the disease during 2016 (Siegel et al., 2016). The prognosis of newly diagnosed breast cancer patients is determined by the classification of breast cancer. There are at least four main subtypes of breast cancer according to different patterns of gene expression: luminal A, luminal B, the HER2 over-expressing subtype and the triple-negative subtype. Luminal A tends to have the best prognosis and luminal B includes estrogen receptor (ER) + and/or progesterone receptor (PGR) +, and human epidermal growth factor receptor 2 (HER2) + or HER2−, while the other 2 subtypes confer bad prognosis (Sotiriou et al., 2003; Sørlie et al., 2001). HER2-positive breast cancer includes luminal B (ER+ and/or PR+ and HER2+) and HER2 overexpressing subtype (ER-, PR- and HER2+). Studies have shown that 20–30% of breast cancers are Her2/neu-overexpressing subtypes. Also, some people think that the incidence of HER-2 overexpressing breast cancer is about 10% (Perou et al., 2000). Luminal B breast cancer incidence is about 8% of all breast cancers (Kim et al., 2006).

Currently, more than breast cancer treatment is the use of surgery, radiotherapy, chemotherapy and endocrine therapy. Chemotherapeutic drugs on HER-2 positive breast cancer are mainly: 1) anthracycline

Abbreviations: **ER**, estrogen receptor; **PGR**, progesterone receptor; **HER**, human epidermal growth factor receptor; **CAM**, complementary and alternative medicine; **TCM**, traditional Chinese medicine; **YHD**, Yanghe Decoction; **OB**, oral bioavailability; **DL**, drug-likeness; **PPI**, protein-protein interaction; **GO**, Gene Ontology; **IGFR**, insulin-like growth factor receptor; **HB-EGF**, heparin-binding growth factor; **EGFR**, epidermal growth factor receptor; **PRE**, progesterone response element; **TGF**, transforming growth factor; **HDAC**, histone deacetylase; **CDK**, cyclin dependent kinases; **EMT**, epithelial-mesenchymal transition; **IGFBP**, IGF-binding protein; **FGFR**, fibroblast growth factor receptor

* Corresponding author.
E-mail addresses: 2057188405@qq.com (L. Zeng), yklab2014@hotmail.com (K. Yang).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jep.2017.01.045
Received 5 November 2016; Received in revised form 5 January 2017; Accepted 23 January 2017
Available online 24 January 2017
0378-8741/

and paclitaxel combination (Schneeweiss et al., 2015); 2) endocrine therapy, such as aromatase inhibitors (Ellis et al., 2001); 3) molecular targeted therapy, such as Herceptin (transtumab) and Tykerb (lapatinib) (Piccart Gebhart et al., 2005; Joensuu et al., 2006).

However, the adverse effects of chemotherapy, such as paclitaxel-induced gastrointestinal reactions, bone marrow suppression, immune dysfunction and organ damage, osteoporosis caused by endocrine therapy, and molecular targeted therapy-induced cardiac toxicity and its high costs, makes a portion of the patients eventually interrupt chemotherapy (Tao et al., 2015a; Gonzalez-Angulo et al., 2007). With the development of medicine, breast cancer treatment has entered a stage of comprehensive treatment of diversification. Complementary and alternative medicine (CAM) becomes patient selection. Traditional Chinese medicine (TCM), as an important part of CAM, may play a key role in breast cancer treatment. In countries around the world, including Western countries, TCM has been increasingly used in the past few decades, which has a significant effect in the prevention and treatment of cancer (Zhu et al., 2016; Sun et al., 2016). According to TCM theory, we think that TCM as an adjuvant therapy has the advantage of reducing the symptoms of advanced cancer, while western medicine cannot provide any other treatment. However, recent research shows that herbal formula, a type of TCM, play an important role in the whole process of anti-cancer, which can promote the recovery of patients after surgery, radiotherapy or chemotherapy (J. Zhou et al., 2014, F.Y. Zhou et al., 2014, R. Zhou et al., 2014). Herbal formula is able to reduce the complications, increase the sensitivity of chemotherapy drugs or reduce the side effects of conventional chemotherapy, and improve quality of life (Liang et al., 2014).

Yanghe Decoction (YHD) was first recorded in *the Life-saving Manual of Diagnosis and Treatment of External Diseases (Waike Zhengzhi Quansheng Ji)* wrote by Wang Hongxu, and is a famous formula for the treatment of breast cancer. It consists of six herbs and one gelatin: *Rehmanniae Radix Praeparata* (Shu Di Huang), *Cinnanmomi Cortex* (Rou Gui), *Ephedra Herba* (Ma Huang), *Colla Cornus Cervi* (Lu Jiao Jiao), *Sinapis Semen* (Jie Zi), *Licorice* (Gan Cao), *Zingiber officinale Roscoe* (Sheng Jiang or Jiang Tan). According to TCM theory, YHD can warm Yang and nourish blood, disperse cold and activate stagnancy, and is mainly used for Ruyan (breast cancer) and so on (Dou et al., 2015a). Also, a number of clinical and basic research found that YHD with some herbal formula have an effect on breast cancer and its precancerous lesions (Li et al., 2014; Dou et al., 2015b; Liu and Tian, 2009; Kang et al., 2011; Tian, 2008), in particular HER2-positive subtype (Qiu, 2013). Therefore, YHD, alone or with other drugs (herbal formulae or western medicine), has the potential to be a drug for HER2-positive breast cancer. However, its pharmacological mechanism has not been clarified completely.

In ancient China, Chinese medicine formula is a combination that composed of herb and so on guided by principles of formulating prescription. In hundred years, the formula constantly modified based on experience and results, and eventually become one of the best and widely used prescriptions. Therefore, this formula that selected through time and a large number of cases coincides with the concept of modern combination therapy. In addition to good effect such as anti-tumor brought by multi-target, multi-biological process and multi-pathway, its toxicity and side effect are smaller. In order to uncover the mechanism of formula on disease, we need a method that is suitable for its characteristics. Due to the rapid development of bioinformatics, the network pharmacology approach has become a new means to reveal Chinese medicine formulae's molecular mechanism efficiently and systemically (Hopkins, 2008; Tang et al., 2016). Network pharmacology through systematic idea studies the relationships between drugs, targets, and diseases, and shows the network of drug-targets by a visual way. Still, it abstracts the interaction relationship into a network model and studies the effect of drugs on biological network from a holistic perspective. Therefore, we use the network pharmacology method to explore the impact of YHD on HER2-positive breast cancer so as to clear its medicinal value.

2. Materials and methods

2.1. Data preparation

2.1.1. Composite compounds of YHD

To collect the compounds of YHD, we used the TCM Database@ Taiwan (Chen et al., 2014) (http://tcm.cmu.edu.tw/zh-tw/, updated in March 2014), which is the most comprehensive TCM database in the world; The Traditional Chinese Medicine Systems Pharmacology Database (Ru et al., 2014) (TcmSPTM, http://lsp.nwsuaf.edu.cn, updated on May 31, 2014), a unique system pharmacology platform designed for Chinese herbal medicines. 383 herbal compounds were found: 100 in *Cinnanmomi Cortex*, 363 in *Ephedra Herba*, 280 in *Licorice*, 76 in *Rehmanniae Radix Praeparata*, 51 in *Sinapis Semen*, 265 in *Zingiber officinale Roscoe*. Cervil Cornus Colla consists of macromolecular proteins, small molecule amino acid and so on, which can prove by our early stage research (J. Zhou et al., 2014, F.Y. Zhou et al., 2014, R. Zhou et al., 2014); so it plays a Supplementary material supporting role. Hence Cervil Cornus Colla is not suitable for this network pharmacological research, we decide to exclude it. The details are described in Table S1 (see Supplementary material).

2.1.2. Pharmacokinetic prediction

Due to the disadvantages of biological experiments as time-consuming and high-cost, identification of ADME (absorption, distribution, metabolism and excretion) properties by in silico tools has now become an inevitable paradigm in pharmaceutical research. In this study, three ADME-related models, including the evaluation of oral bioavailability (OB), Caco-2 permeability and drug-likeness (DL), are employed to identify the potential bioactive compounds of YHD.

Oral bioavailability. OB prescreening is used to determine the fraction of the oral dose of bioactive compound which reaches systemic circulation in the TCM remedy. Here, a reliable in silico model OBioavail 1.1 (Xu et al., 2012) which integrates the metabolism (P450 3A4) and transport (P-glycoprotein) information was employed to calculate the OB values of herbal ingredients.

Caco-2 permeability. Caco-2 cell monolayers are widely applied as standard permeability-screening assay for prediction of the compound's intestinal absorption and fraction of oral dose absorbed in humans (Ano et al., 2004). The Caco-2 cell permeation values of all molecules are calculated by in silico model using the VolSurf approach (Hu et al., 2009).

Drug-likeness evaluation. Drug-likeness is a qualitative profile used in drug design to evaluate whether a compound is chemically suitable for drug, and how drug-like a molecule is with respect to parameters affecting its pharmacodynamic and pharmacokinetic profiles which ultimately impact its ADME properties (Walters and Murcko, 2002). In order to identify drug-like compounds, we apply a database-dependent model using the Tanimoto coefficient to calculate the DL (see Eq. (1)) of each compound in YHD.

$$\mathrm{f}(x, y) = \frac{xy}{|x|^2 + |y|^2 - xy} \tag{1}$$

x represents the new ingredient, and y is the average molecular properties of all ingredients in Drug-Bank database (available online: http://www.drugbank.ca).

In this work, the compounds of OB≥30%, Caco-2 > −0.4 and DL ≥0.18 are selected for subsequent research, others are excluded.

According to these indexes and some references (Gao et al., 2009; Tian et al., 2006), several compounds are included: stigmasterol, (+)-catechin, 24-Ethylcholest-4-en-3-one, beta-sitosterol, campest-5-en-3beta-ol (campesterol), delphinidin, diosmetin, eriodictyol, genkwanin, herbacetin, kaempferol, leucopelargonidin, luteolin, Mandenol, naringenin, Pectolinarigenin, poriferast-5-en-3beta-ol (83-47-6,