Biochemical and Biophysical Research Communications 434 (2013) 701–704

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Mini Review

Where do health benefits of flavonoids come from? Insights from flavonoid targets and their evolutionary history

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ARTICLE INFO

Article history:
Received 1 April 2013
Available online 23 April 2013

Keywords:
Flavonoids
Targets
Chronic diseases
Evolution
Gut microbiota

ABSTRACT

Flavonoid intake is negatively correlated with the incidence of some chronic diseases including cardiovascular diseases, type II diabetes, neurodegenerative diseases, and cancers. Thus, the molecular mechanisms underlying this correlation are of great interest. Although ample attention has been given to the free radical-scavenging potential of flavonoids, the poor bioavailability of exogenous flavonoids suggests that the direct antioxidant activity is unlikely responsible for their favorable effects. This study comprehensively analyzed flavonoid targets. The results show that the main functions of these targets are associated with cancers and cardiovascular and metabolic diseases. Moreover, evolutionary analysis of these targets showed that ~1000 of the targets have homologues in human gut bacterial metagenomes. Clusters of orthologous groups of proteins (COG) analysis indicated that most of these bacterial targets are associated with bacterial metabolism. Given that the metabolism of gut microbiota is coupled with the metabolism of the host, this finding implies that flavonoids exert their benefits by regulating gut microbes. Therefore, the health benefits of flavonoids are well explained by their targets rather than their direct antioxidant potential.

Considerable epidemiologic evidence has indicated that flavonoid-rich diets are associated with a low incidence of chronic diseases, such as cardiovascular diseases (CVDs), type II diabetes, neurodegenerative diseases, and possibly cancers [1]. Interest in elucidating the health benefits of flavonoids has been rapidly increasing over the past decade. A large number of studies on flavonoids focused on their free radical-scavenging potentials because reactive oxygen species (ROS) are critical participants in the pathogenesis of chronic diseases, and flavonoids are strong antioxidants *in vitro*. However, direct antioxidant activity is unlikely responsible for their favorable effects [2], primarily because of the poor bioavailability of exogenous flavonoids [3]. Our analysis of the biological functions of flavonoids provides further evidence that they have not evolved for scavenging intense ROS, but for filtering ultraviolet light and acting as signal molecules to attract rhizobia, to induce rhizobial nodulation genes, and to participate in allelopathic plant-plant interactions [2,4,5]. The evolutionary analysis also revealed that flavonoids have evolved excellent scaffolds with well-balanced rigidity and flexibility to adapt to diverse cavities of enzymes in the biosynthetic pipeline, which enables the compounds to bind various proteins [6]. Therefore, flavonoids are innate regulators of protein function, which likely underlies their beneficial effects. This speculation is reasonable, especially considering that flavonoids can express benefits *in vivo* through controlling cell signaling pathways with relatively low concentrations (nanomolar to low micromolar). In comparison, to act as direct antioxidants, they have to reach high concentrations (high micromolar) to outcompete *in vivo* antioxidants such as ascorbate [7]. This function suggests that obtaining a comprehensive insight into the health benefits of flavonoids requires a systematic analysis on flavonoid targets, despite that their benefits can be partially attributed to hormesis induced by Nrf2 activation [8].

Flavonoids are wide spread in plants and are classified into six types of scaffolds, namely, flavone, flavonol, flavanone, flavanol, isoflavone, and anthocyanidin [9] (Fig. 1). Thus, we searched flavonoids from PubChem (http://pubchem.ncbi.nlm.nih.gov/) using these scaffolds as substructures. Then, we collected targets for these flavonoids from STITCH (http://stitch.embl.de/), one of the most widely used databases for drug targets [10]. A total of 3713 targets for 1073 flavonoids were identified. The biological functions of these targets were initially analyzed using records in gene ontology (GO) and the Kyoto encyclopedia of genes and genomes (KEGG) pathways. The dominant functions of the targets are associated with regulation of cell proliferation and cell death, as well as cancers (Fig. 2). This finding is consistent with the likely negative

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http://dx.doi.org/10.1016/j.bbrc.2013.04.035

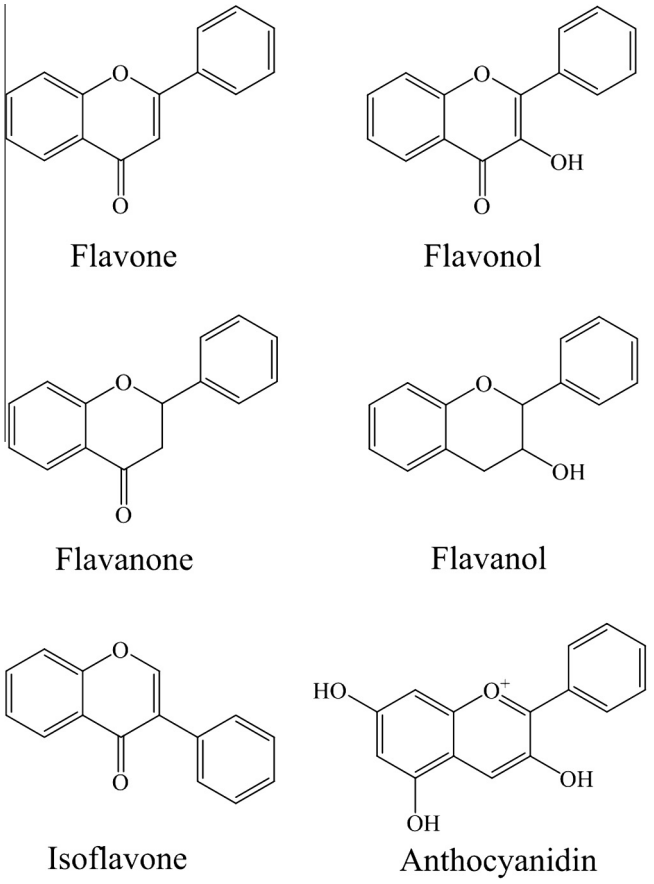

Fig. 1. Representative scaffolds of flavonoids.

correlation between flavonoid intake and cancer incidence. Since the therapeutic effects of drug targets are related to their potentials in causing genetic diseases [11], we searched the "GENETIC_ASSOCIATION_DB_DISEASE_CLASS" category in The Database for Annotation, Visualization, and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/) [12] to investigate the disease associations of flavonoid targets. Up to 1282 (34.5%) of the flavonoid targets were found to be linked to genetic diseases, which were statistically measured by Fisher Exact in the DAVID system. The most common diseases are cancer and cardiovascular and metabolic diseases (Table 1). This finding agrees well with the epidemiological observation that flavonoids are helpful in preventing these diseases.

In a previous study, Dančik et al. reported that natural product (NP) targets have more protein–protein interactions (PPIs) than human disease genes, which was explained in terms of the involvement of PPI hubs in drug therapy [13]. It is thus of great interest to explore whether the flavonoids also prefer to target PPI hubs. Using the high-quality human PPI data collected by Yu et al. [14], we observed an opposite trend in flavonoid targets that contain less PPI hubs than disease genes (Fig. 3). This trend holds for the 13403 targets of 4939 NPs derived from the Traditional Chinese Medicine Integrated Database (TCMID, http://www.megabionet.org/tcmid/) (Fig. 3) [15]. The present conclusion seems more robust because the present analysis depends on much more information on NPs and their targets than the previous study [13]. Since PPI hubs participate in diverse biological pathways, the less involvement of PPI hubs in NP targets has significant implications for explaining the relatively low side-effects of NPs.

In a recent effort to explore the evolutionary features of drug targets, we found that more than 90% of approved drug targets originated very early, that is, before the bilaterian radiation [16], prompting us to analyze the evolutionary characteristics of flavo-

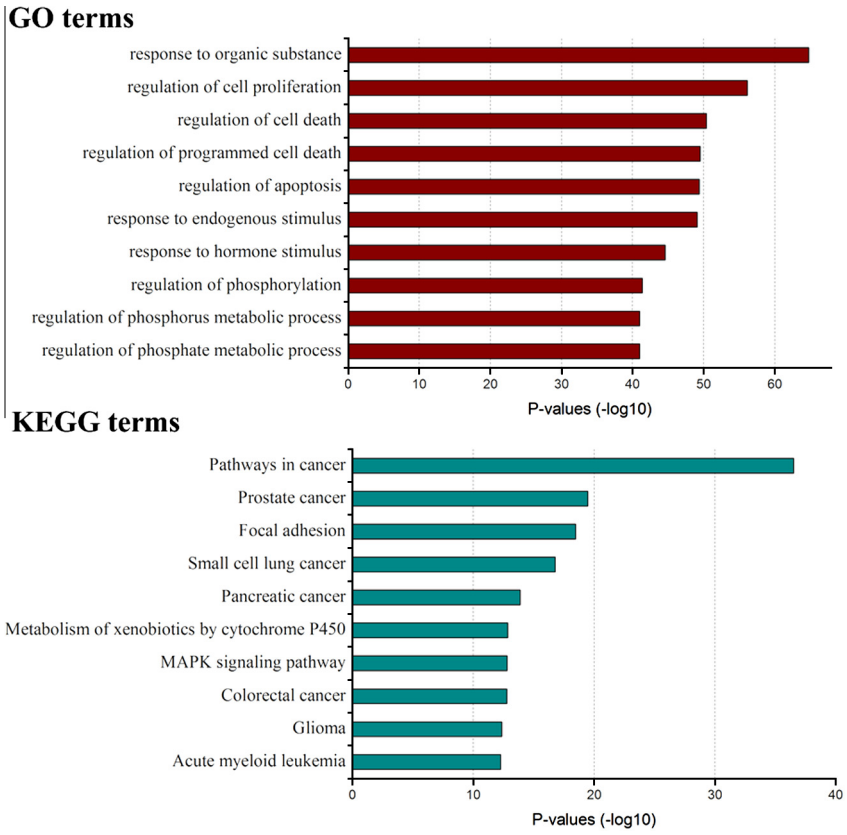

Fig. 2. Top 10 GO biological process terms and KEGG pathways significantly enriched among target genes of flavonoids. Horizontal axes show Benjamini–Hochberg corrected *p*-values.

Table 1
Disease associations of flavonoid targets.

Genetic disease class	Occurrence[a]	*p*-Value[b]
Cancer	431	1.72E−18
Cardiovascular	386	5.02E−18
Metabolic	453	1.11E−15
Pharmacogenomic	203	1.51E−13
Unknown	150	3.01E−12
Aging	79	2.67E−10
Normalvariation	176	8.00E−10
Other	392	2.51E−09
Renal	113	8.04E−09
Hematological	88	5.09E−06
Neurological	291	1.35E−05
Chemdependency	101	1.55E−05
Reproduction	135	6.13E−05
Infection	153	1.39E−03
Immune	371	3.61E−03
Vision	97	5.89E−03
Psych	259	1.70E−02

[a] Genes can appear in more than one cluster.
[b] *p*-Values were calculated using Fisher's exact test.

noid targets. Human genes have been separated into 19 phylogenetic classes (from early to late) using the phylostratigraphic method [17]. Class 1 genes originated from prokaryotes, whereas class 19 originated from primates. By assigning the flavonoid targets into a particular evolutionary class, we found that these targets are concentrated in classes 1 and 5, which corresponds to genes that originate from prokaryotes and metazoa, respectively (Fig. 4). Therefore, most of the flavonoid targets have very early origins.

Given that a large number of flavonoid targets originate from prokaryotes, they likely have homologues in human gut microbes. Based on the sequence alignment between flavonoid targets and human gut genome recently released by Human Microbiome Project (HMP) (http://www.hmpdacc.org/) (with an *E*-value threshold of 1×10^{-6} and a coverage of at least 50%), 934 flavonoid targets were found to have homologues in human gut bacterial metagenomes. Flavonoids have been recognized to regulate soil microbiota, and many soil bacteria are similar to those in the human gut [18]. Thus, the present finding suggests that gut microbiota modulation may be responsible for health benefits of flavonoids and these benefits have an evolutionary origin. By using clusters of orthologous groups of proteins (COGs) and the web server WebMGA [19], functional analysis of these bacterial targets were performed, indicating that most of these bacterial targets are associated with metabolism, such as energy production and conversion, as well as amino acid, carbohydrate, nucleotide, and lipid transport and metabolism (Table 2).

This finding implies that flavonoids may be beneficial via regulating the metabolism of gut microbiota, which directly participates in host metabolism through controlling expression of host genes and degrading plant polysaccharides into short-chain fatty acids [20–24]. In particular, the accumulating evidence indicates that gut microbiota may have an important role in the pathogenesis of metabolic diseases, including CVDs and type II diabetes by inducing low-grade, systemic and chronic inflammation [23,24]. Indeed, the health benefits of flavonoids have been preliminarily explained by some pioneers in terms of effects on gut and microbiota. First, flavonoids can be present in high amounts in the gastro-intestinal tract and exert antioxidant effects in the stomach and intestine [25]. Second, since gut microbiota is important for brain development and behavior, flavonoids may exert indirect effects on the brain through affecting the metabolism of gut microbiota [8].

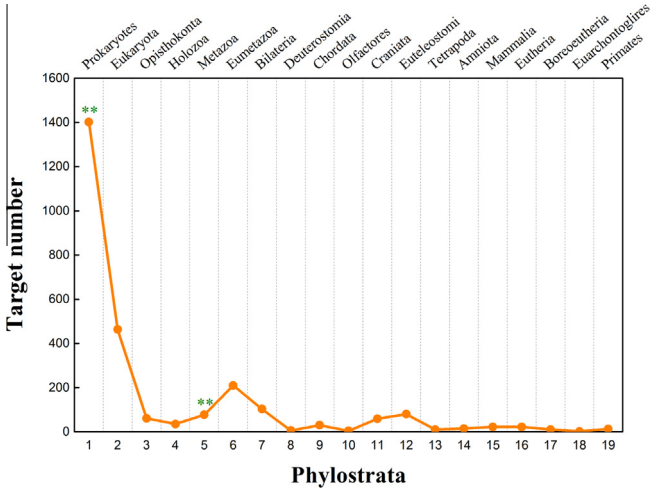

Fig. 4. Distribution patterns of flavonoid targets in 19 phylogenetic classes. The frequency of the targets in each phylogenetic class was compared with the frequency of targets in the complete genome (expected frequency). Deviations are shown by calculating log-odds ratios. The significance of the obtained deviations from the expected frequency was tested using two-tailed hypergeometric tests [17]. The obtained *p* values ($^{**}p < 0.001$) were corrected for multiple comparisons via false discovery rate at 0.05 level.

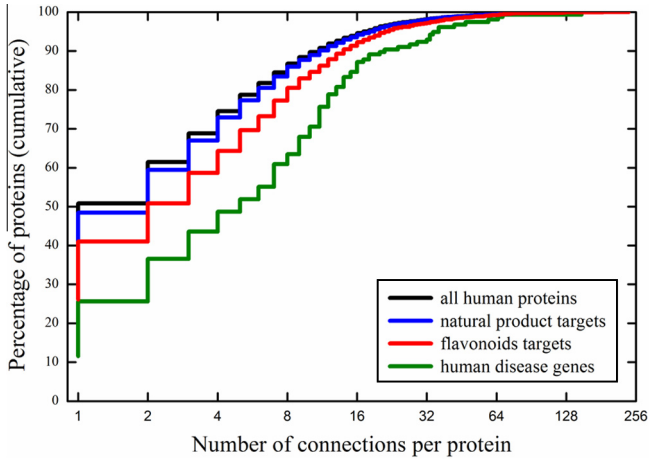

Fig. 3. Cumulative patterns of protein connectivity in protein–protein interaction (PPI) networks. The PPI and disease gene data were derived from http://www.yulab.org/DiseaseInt/. The Kolmogorov-Smirnov goodness-of-fit test indicates that the connectivity distribution differences between flavonoid targets, natural product targets and disease genes are statistically significant, with $p = 8.12E{-}5$ for natural product targets *vs* disease genes, and $p = 5.24E{-}3$ for flavonoid targets *vs* disease genes.

Table 2
Top 10 COG biological process terms significantly enriched among human gut microbial homologues of flavonoid targets.

COG category	Gene number
General function prediction only	16386
Amino acid transport and metabolism	15386
Energy production and conversion	13343
Carbohydrate transport and metabolism	12843
Nucleotide transport and metabolism	9949
Lipid transport and metabolism	8670
Posttranslational modification, protein turnover, chaperones	8525
Translation, ribosomal structure and biogenesis	8234
Coenzyme transport and metabolism	7115
Replication, recombination and repair	5507

In conclusion, analysis of the flavonoid targets reveals that the health benefits of flavonoids are explained by the biological functions of their targets. In addition, these benefits likely involve the regulation of gut microbes because ~1000 targets have homologues in human gut microbiota, which should be validated in further studies. The health benefits of flavonoids must extend beyond direct antioxidant activity and seem to have an evolutionary origin. Thus, this finding demonstrates the value of evolutionary consideration in medicine and pharmacology.

Acknowledgments

The authors thank Dr. Min Xiong and Bin Li for helpful assistance. This work was supported by the National Basic Research Program of China (973 project, grants 2010CB126100 and 2012CB721000), the National Natural Science Foundation of China (grant 21173092) and the Fundamental Research Funds for the Central Universities (grants 2011PY142 and 2011PY027).

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