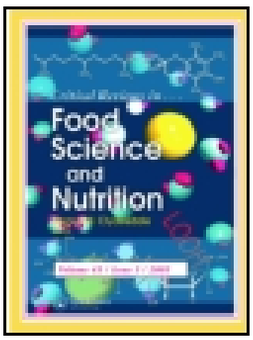

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REVIEW

Dietary polysaccharides exert biological functions via epigenetic regulations: Advance and prospectives

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ABSTRACT

Bioactive substances derived from natural products are valued for effective health-related activities. As extremely important component of plants, animal cell membrane and microbes cytoderm, polysaccharides have been applied as medications, foods and cosmetics stemming from their prominent biological functions and minor side-effects. Recent studies indicate that polysaccharides exert biological effects also through epigenetic mechanism. Through the intervention of DNA methylation, histone modification, and non-coding RNA, polysaccharides participatate in regulation of immunity/inflammation, glucose and lipid metabolism, antioxidant damage and anti-tumor, which presents novel mechanism of polysaccharide exerting various functions. In this review, the latest advances in the biological functions of dietary polysaccharides via epigenetic regulations were comprehensively summarized and discussed. From the view point of epigenetic regulation, investigating the relationship between polysaccharides and biological effects will enhance our understandings of polysaccharides and also means huge breakthrough of molecular mechanism in the polysaccharide research fields. The paper will provide important reference to these investigators of polysaccharide research and expand the applications of dietary polysaccharides in the functional food developments.

KEYWORDS

Polysaccharides; natural products; epigenetics; DNA-methylation; histone modifications; miRNA

Introduction

Polysaccharides are a group of macromolecular polymers composed of more than 10 homo/hetero monosaccharides, bound by glycosidic bonds in linear or branched chains, with molecular weights ranging from tens to millions (Zong, Cao, and Wang 2012). Together with proteins, nucleic acids and lipids (other macromolecules), polysaccharides are pivotal components for vital activities. And it is widely exist in the plants, microorganism, algae, and animals in nature. Polysaccharides isolated from this natural resources have attracted increasing attention due to their varieties of outstanding pharmacological functions (immune regulation, anti-tumor, antiviral, antioxidant and hypoglycemic), accessible feature and hypotoxicity (Yu et al. 2018). For instance, the polysaccharide extracted from *Astragalus* (APS) targeted macrophages, increased its phagocytosis, and activated the TLR4-mediated MyD88 pathway and then improved the body's immunity and anti-tumor abilities (Zhou et al. 2017). APS can also ameliorate obesity symptoms, fatty liver disease, neurological and cognitive dysfunction (Huang et al. 2017). The anti-infective ability of mice against lethal influenza A virus was seen to have been significantly increased by black ginseng polysaccharide (Kim et al. 2019). Lentinan (LEN) was proven to slow *Escherichia coli* lipopolysaccharide (LPS)-induced intestinal injury via regulating the compositions and metabolites of intestinal microbiota (Wang et al. 2019). The above are typical representative of naturally active polysaccharides with clinical importance.

Profound exploration of the mechanisms behind the variety of biological functions that polysaccharides exhibit is essential for further development and applications. Recent publications indicate that polysaccharides exert biological effect through epigenetic regulation, which is different from classical genetics. Epigenetic modulations mainly includes DNA methylation, histone modification, chromatin remodeling and non-coding RNA molecular mechanisms. These modifications can cause changes of gene transcription, affecting protein expression and eventually leading to phenotypic differences. It has been approved that many diseases, such as cancer, glucose or lipid metabolism, and angiocardiopathy are associated with epigenetics. Some parental damages caused by external stimuli (diet, toxins, alcohol, etc.) can even be transmitted into the next generation or beyond descendant through epigenetic pathways. Dietary polysaccharides also affect epigenetic modifications of organism and regulate DNA methylation, histone modification, and non-coding RNA to modulate gene expressions and exert different biological effects (see Figure 1). Based on epigenetic regulation mechanism, studing the relationship between polysaccharides and biological effects via epigenetic regulation will enhance our understandings of polysaccharides, which also means huge breakthrough of molecular mechanism in the polysaccharide research fields. In this review, the latest advances in the

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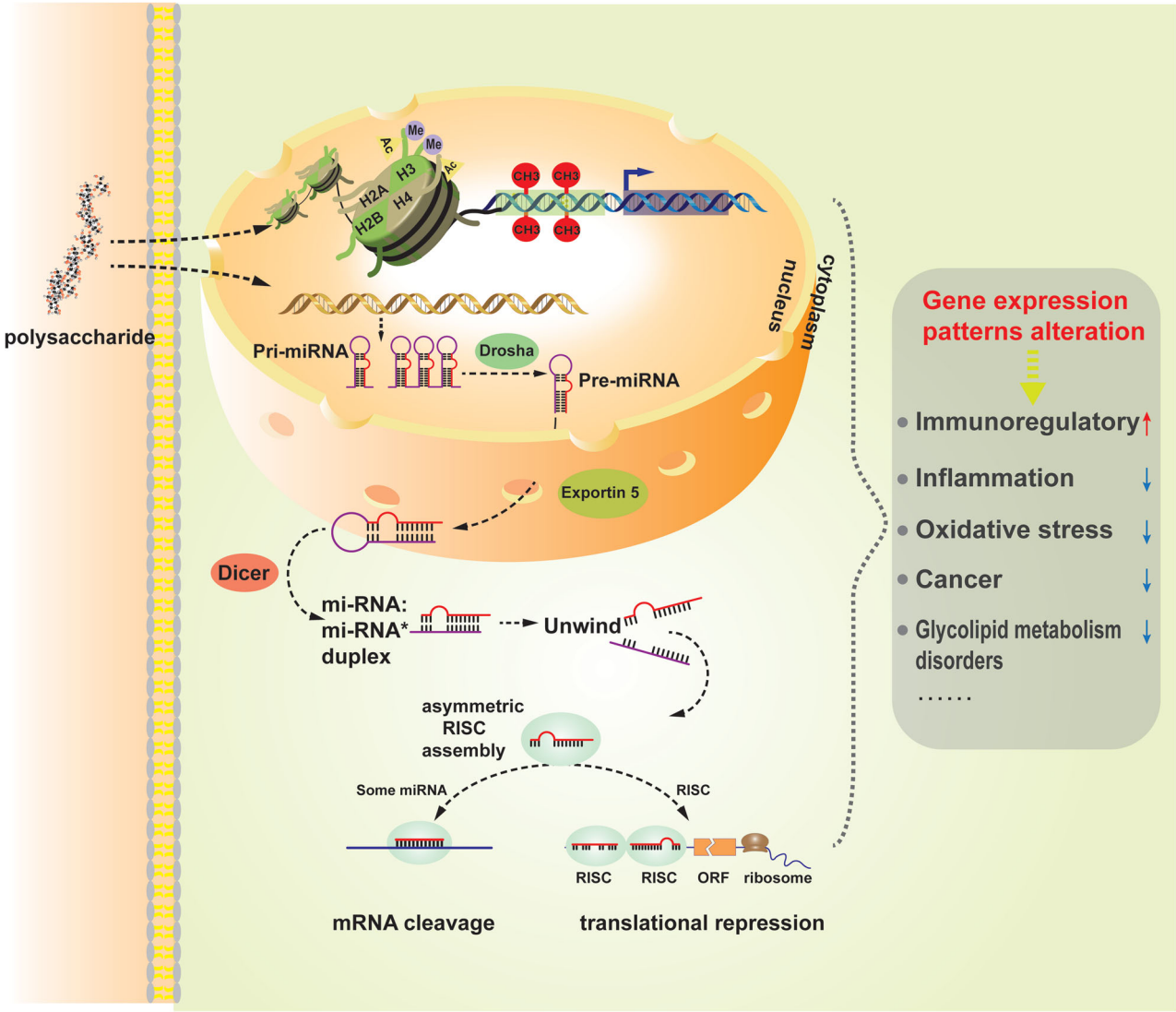

Figure 1. Polysaccharides regulate expression of related genes and exhibit corresponding biological effects by intervening epigenetic modification.
The binding affinity of transcription factor complexes to DNA, chromatin's "closed" or "open" transcription state controlled by DNA promoters methylation, chemical modification on amino-terminal of nucleosome histones, affects the expression patterns of genes in the course of disease development. After forming the RISC with corresponding protein, miRNA directly binds to the sequence of 3′ region of a complementary gene resulting in degradation of the bound mRNA or inhibiting its translation efficiency. Natural resource polysaccharides intervene in these epigenetic pathways, therefore restored some gene expression disorders caused by external stimulus, and ameliorate disease phenotypes.

biological functions of dietary polysaccharides via epigenetic regulations were comprehensively summarized and discussed, which will provide important reference to these investigators of polysaccharide research.

Primary epigenetic mechanisms

DNA methylation

DNA methylation refers to the addition of methyl group to the 5′ position of pyrimidine ring in DNA cytosine: guanine (CpG) dinucleotides catalyzed by DNA methyltransferases (DNMTs), which is modified to 5-methylcytosine (5mC). Another forms of DNA methylation include 6 mA which is present in lower organisms (e.g., bacteria, fungi, *Caenorhabditis elegans* and *Drosophila melanogaster*) (Sun et al. 2015). Regions containing high-density CpG dinucleotides of DNA are termed "CpG islands", half of which are located in the gene promoter, and about 56% of encoding genes contained this structure. The methylation status of CpG in "CpG islands" and "CpG island shores" determines the degree of gene expression. Methylated CpG site of gene regulatory elements (e.g. promoters) disrupts the binding affinity of transcription factor complexes to DNA, thus inhibiting promoter activity. Methylated DNA also directs transcriptional inhibition by recruiting methyl-CpG binding proteins (e.g. MeCP2), which in turn recruit histone-modifying enzymes to form "closed" chromatin conformations that attenuate transcription (Moore, Le, and Fan 2013). In brief, methylation of DNA is generally associated with gene silencing, conversely, demethylation activates it. For instance, promoters of housekeeping genes contain CpG islands remained demethylated. Related regions of proto-oncogenes are hypermethylated in contrast.

Histone modifications

The second epigenetic mechanism is the histone modification. Genomic DNA is packaged with specific protein forming the chromatin. It is tightly wrapped around the histone core and constitute the basic unit of the chromatin: nucleosome. Each nucleosome includes core histones H2A, H2B, H3, and H4, which are assembled doubled into an octamer together with 147 bp DNA around. As the linker histones, H1 family connects the surrounding DNA at the entrance and exit, keeping it correctly wrapped. This core histones have a positively charged central folding domain and an amino-terminal tail enriched basically with lysine and arginine residues protruding from core histones and could be covalently modified by acetylation, methylation, phosphorylation, ubiquitination, SUMO (small ubiquitin-related modifier), carbonylation and glycosylation. Various forms of these post-translational modifications (PTMs) regulate the electrostatic attraction between negatively charged DNA and histones to maintain the dynamic structure of the chromatin, therefore, and determine whether the chromatin is in a "closed" (inhibited) or "open" (more accessible to transcription machine) transcription state (Lawrence, Daujat, and Schneider 2016).

In specific terms, acetylation caused by activated histone acetyltransferases (HATs) or suppressed histone deacetylases (HDACs) reduces electrostatic attraction, resulting in a more "relaxed" phenomenon, that is, a more "open" structure to transcriptional factors. Acetylated histones recruit bromodomain-containing proteins that raise other transcription-activating proteins. Methylated histones regulated by histone demethylase (HDMs) and histone methyltransferase (HMTs) activate (H3K4me3) or repress (H3K27me3) transcription depending on the modification of these sites (Zhang, Cooper, and Brockdorff 2015). The modifications of histone affects chromosome structure ("loose" and "tight") and gene transcription, which is closely related to gene transcription initiation.

Non-Coding RNA

Another pivotal epigenetic markers is the non-coding RNAs (ncRNAs). Only 1-2% of genomic transcripts are translated into proteins. There are other types of RNAs namely rRNA, tRNA, snRNA, snoRNA, and microRNA that also have specific biological functions. MicroRNAs are small, 19-24 nucleotides in length non-coding RNAs. These are processed from larger pri-microRNAs. After forming an RNA-inducing silencing complex (RISC) with corresponding protein, through direct sequence-specific binding to the 3′ region of the complementary gene (perfect matching is not required) causing the degradation of bound mRNAs, or inhibition of its translational efficiency, miRNAs exert regulating gene expression post-transcriptionally. The human genome encodes about 1,000-2000 miRNAs, each of which can target hundreds of genes. They are estimated 74-92% coverage of protein-encoding mRNAs (Miranda et al. 2006). Alteration of miRNAs levels were identified in varieties of biological processes (cell growth, proliferation, organogenesis and metabolism) and disease development (obesity, cancer, and cardiovascular disease). Other typical small non-coding RNAs include siRNA, slightly larger piwi-RNA, and lncRNA all of which are critical components of RNA-related epigenetic pathways (Esteller 2011). Non-coding RNAs are an important form of epigenetics regulation at the posttranscriptional level.

Polysaccharides exert biological functions via epigenetic regulations

Numerous diseases, especially cancer, aging, diabetes, obesity, angiocardiopathy and neurodegenerative diseases are associated with epigenetic aberrations. "Eigenetic therapy" is termed as a new therapeutic method of modulating epigenetics by drugs. Recent studies have proven that natural ingredients possess great potential in changing epigenetic signatures, which means "epigenetic diet" (Hardy and Tollefsbol 2011). For instance, dietary polyphenolic phytochemicals exert a protective role against diseases and many biological functions. Common polyphenols including (−)-epigallocatechin-3-gallate (EGCG; green tea), curcumin (curry), resveratrol (grapes), isoflavone (soybeans) and isothiocyanates (cruciferous vegetables) reverse abnormal epigenetic modifications by regulating the DNMTs, HDACs, HMTs, HATs and HDMs activity, and restoring normal gene expression pattern and biological functions (Ayissi, Ebrahimi, and Schluesenner 2014). Moreover, other special substances also altering epigenetic processes, including DNA methylation, histone modification, chromatin remodeling, microRNA regulation acting at multiple targets, such as organosulfur compounds from cruciferous vegetables and garlic, triterpenoids from fruits and medicinal plants, ginsenosides from ginseng, folic acid, etc. (Huang et al. 2019). Now more and more phytochemicals were found to participate epigenetic regulations.

As a typical representative of natural products, polysaccharides isolated from natural resources also exhibit excited pharmacological functions (immunomodulatory, anti-tumor, antiviral, antioxidant and hypoglycemic). Interestingly, many natural polysaccharides can interfere with epigenetics (Table 1). These polysaccharides are broadly categorized according to their structure, source, solubility and chemical composition. Depending upon the origins, they are usually divided into: animals, plants (starch, pectin, inulin, xylan, arabinan, etc.), fungi and algae. In organisms, polysaccharides act as energy-storing compounds in the cytoplasm (starch, etc.) or as structural components of the membrane or cell wall (cellulose, etc.). Based on composition, they are characterized as homopolysaccharides (single type of monosaccharides, such as glycogen and cellulose composed of glucose molecules) and heteropolysaccharides (different types of monosaccharides). Structures of polysaccharides extracted from natural products are extremely complex and diverse, but, glucan, fructan, galactan, mannan, xylan, bhamnosan, etc. or a polymer of several types monosaccharides are the frequent compositions of their backbone chains. These various

Table 1. Polysaccharides from natural sources regulate epigenetic pathways.

Polysaccharide source	model	Epigenetic modification	molecular mechanisms	Reference
Sophora subprosrate	PCV2-infected swine alveolar macrophage 3D4/2 cells	histone acetylation	SSP→activity of HATs↓, HDACs↑→Ac-H3/H4↓→p-p65↓→inflammation↓	Yang et al. 2017
Sargassum	PCV2-infected RAW264.7 cells	histone acetylation	SP→HAT↓, HDAC↑→Ac-H3/H4↓→inflammation↓	Chen et al. 2018
	PCV2-infected mice	histone acetylation	PSW→HAT↓, HDAC↑→Ac-H3/H4↓→inflammatory cytokines↓	Chen et al. 2019
resistant starch (RS4)	Mice, human colon epithelial cells	Histone methylation	RS→H3K27me3 of NF-κB promoter↑→inflammation↓	Liu et al. 2019
	human rectal epithelium	miRNA	RS→miR-32↑→CRC and TRAF3	Malcomson et al. 2017
	HRM diet	miRNA	RS→miR17-92↓→colorectal cancer risk↓	Humphreys et al. 2014
	HRM diet	miRNA	RS→miR17-92↓→CRC↓	Nielsen et al. 2019
	rats	histone modification	HS→histone acetyltransferase↑, binding of GCN5 and Ac-H3/Ac-H4↑, H3K4me↑→SI and SGLT1↑	Inoue, Mochizuki, and Goda 2011; Inoue et al. 2015
	rats	histone modification	RS→Ac-H3 on the promoter/enhancer	Shimada et al. 2009a; Shimada et al. 2009b
	rats	histone modification	RS→Ac-H3/Ac-H4↓→Thrsp↓	Shimada, Mochizuki, and Goda. 2011
galacto-oligosaccharides	LPS-induced human colon epithelial FHC cells	miRNA	GOS→miR-19b, miR-590-5p and miR-495↑, miR29a, miR-31 and miR-142-5p↓, cell injury↓	Sun, Liang, et al. 2019
Tremella fuciformis	LPS-induced RAW264.7 cells	miRNA	TFPS→miR-155↓, Akt, p38MAPK, and NF-κB↓→inflammation↓	Ruan et al. 2018
Astragalus	LPS-induced H9c2 cells	miRNA	APS→miR-127↓→NF-κB↓, JNK↓, PI3K/AKT↑	Ren et al. 2018
	chickens	DNA methylation; histone modification	APS→promoter methylation of SOCS1↓, histone modification in promotor of TRIF change	Li Y, et al., 2018
	breeder cocks	miRNA	APS→16 miRNAs↑, 17 miRNAs↓	Wu et al. 2017
	hypoxia-induced rat NSCs	miRNA	APS→miR-138↑→cell injury↓	Zheng and Zhao 2018
	C. elegans.	miRNA	APS→miR-124↑→ATF-6↓→lifespan↑	Wang et al. 2015
	OS MG63 cells	miRNA	APS→miR-133a↑→JNK↓→apoptosis↑	Chu et al. 2018
	T2DM GK rats	miRNA	APS→miR-203a-3p↑→GRP78, CHOP, pJNK1 and caspase-12↓→IR in T2DM↓	Wei et al. 2018
Angelica sinensis	LPS-induced PC-12 cell	miRNA	ASP→miR-223↓→NF-κB↓	Li R, et al., 2018
	SH-SY5Y cells	miRNA	ASP→miR-675↓→PI3K/AKT and JAK/STAT↓	Yang et al. 2018
	hypoxia-induced H9c2 cell	miRNA	ASP→miR-22↓→PI3K/AKT↑, JAK1/STAT3↑	Pan and Zhu 2018
Actinidia eriantha	RAW264.7 cells	miRNA	AEPS→82 differentially miRNAs, miR-155↑→macrophage activation	Chen et al. 2019
Ganoderma lucidum	hepatoma-bearing mice	miRNA	GLPs→miR-125b↑→ Notch1 and FoxP3 ↓→Teffs/ Tregs↑	Li A, et al., 2015
	HepG2 cells	miRNA	GLPs→61 alterative miRNAs, hepatocarcinoma genes↓	Shen et al. 2014
	diabetic mice	miRNA	F31→mir324↓,	Xiao et al. 2018
Ginseng	sows	miRNA	GPS→10 miRNAs↑, 16 miRNAs↓→immune response↑	Sun, et al. 2019
Lentinan	hypoxia PNCM or H9c2 cells	miRNA	LEN→miR-22↓PI3K/AKT and β-catenin↑	Zhang and Zhao 2019
Lycium barbarum	hypoxia-injured H9c2 cells	miRNA	LBPs→miR-122↓→MEK/ERK, AMPK↑→cardiac function↑	Li Q, et al., 2019
	transgenic (Tg) mice	miRNA	LBPs→miR-1→CaM and cMLCK↑	Zhang et al. 2018
	H_2O_2-induced PC-12 cells	miRNA	LBPs→miR-194↓→p-PI3K and p-AKT↑, ROS and NO↓	Niu et al. 2018
	H_2O_2-induced HTM cells	miRNA	LBPs→miR-4295↑→PI3K/AKT and ERK↑→oxidative damage↓	Liu and Zhang 2019
Enteromorpha prolifera	C. elegans.	miRNA	EPP-1→miR-48, miR-51 and miR-186↓→SKN-1 and DAF-16↑→oxidative stress↓, lifespan↑	Lin, et al. 2020
Ocimum basilicum L.	HCC cells; HAL processed rat	histone modification	BPS→HIF-1α, G9a, LSD1, JMJD1A, JMJD2B, JARID1B and H3K9me2↓→tumor↓	Feng et al. 2018

(*continued*)

Table 1. Continued.

Polysaccharide source	model	Epigenetic modification	molecular mechanisms	Reference
Huaier	SMMC-7721 cells	miRNA	TP-1→miR-122↑, Cell growth, adhesion, migration, and motility↓	Li C, et al., 2015
green tea (Camellia sinensis)	PC-3 cells	miRNA	GTP→miR-93↓, DAB2↑, AKT and ERK1/2↓	Yang K. et al. 2019a; Yang K. et al. 2019b
Cyclocarya paliurus	hyperlipidemic rats	DNA methylation	CPP→DNA methylation in promoters↓→leptin and MTTP↓	Yang Z, et al. 2019
Vaccinium bracteatum Thunb.	mice	miRNA	VBTLP→miR-137↓→LKB1/AMPK↑→hepatic gluconeogenesis↓	Qian, Li, and Wang 2017
squid ink	CP-treated mice	histone modification	SIP→upstream regulators of Nrf2, keap-1, HDAC2, and PKC→Nrf2↑	Le et al. 2015
Polygonatum sibiricum	bone marrow-derived macrophages	miRNA	PSP→miR-1224↓→Limd1↑→osteoclastogenesis↓	Li B, et al., 2019
Pleurotus eryngii	HUMSCs	histone modification	CPS→H3K4me2, H3K4me3 and H3K4ac↑→iPSCs↑	Deng et al. 2016

polysaccharides perform a variety of physiological functions by regulating epigenetics.

Immunoregulatory & inflammatory activities

Immunoregulation is the most typical biological function of natural polysaccharides. Nowadays, major mechanisms underlaying polysaccharides activating diverse immune cells are generally thought to be associated with epigenetics. Representatively, macrophages exert phagocytosis on pathogens and cell fragments in vivo, which allows them to participate in nonspecific (innate immunity) and specific (cellular immunity) defenses and activation of lymphocytes or other immune cells. Porcine circovirus type 2 (PCV2) was used to infect swine alveolar macrophage 3D4/2 cells, toxic inflammatory response and oxidative stress were subsequently induced, accompanied by secretion of inflammatory cytokines (TNF-α, IL-1β, IL-6) and corresponding elevated HATs, HDACs activity, stimulative acetylation levels of H3 and H4. Pretreatment of cells with *Sophora subprostrata* polysaccharide (SSP) markedly decreased inflammatory cytokine levels while stimulating anti-inflammatory cytokine IL-10. Furthermore, SSP attenuated the activity of HATs, histone H3/H4 acetylation (Ac-H3/Ac-H4), down-regulating the phosphorylation of NF-κB p65, a classic inflammatory target (Yang et al. 2007). Similar efficacy was recorded in the same model using *Sargassum* polysaccharide (Chen H et al. 2018). Polysaccharide extracted of *Sargassum* weizhouense (PSW) (seaweed) successfully reduced inflammation in PCV2 infected mice, either by drastically inhibiting inflammatory cytokines, HATs or improving HDACs, which corresponds to subduing Ac-H3/Ac-H4 (Chen et al. 2019). These results indicate that polysaccharides might protect macrophages from viral-induced damages through the equilibrial maintenance of activities between HATs and HDACs, which contribute to the alterations of histone acetylation level. A macromolecular carbohydrate dietary fiber derived from wheat, with cross-links and chemically modified resistant starch (RS4) induced butyrate chow with epigenetic repression of pro-inflammatory genes in mice. Colon tissue of RS4-fed mice had higher cecal butyrate and increased tri-methylation of lysine 27 on histone 3 (H3K27me3) in the promoter of nuclear factor-kappa-B (NF-κB), with a concomitant attenuated expression of two additional inflammatory genes (Liu et al. 2016). This study links histone methylation to the biological activities of polysaccharides and their analogues.

The miRNA is also pivotal epigenetic regulation method of polysaccharides in inflammation . In two colitis model in vitro (FHC cells stimulated with LPS) and in vivo (injecting $Rag2^{-/-}$ SD rats with *Helicobacter hepaticus*), galactooligosaccharides (GOS) treatment significantly attenuated cell injury evidenced by increased cell viability, decreased apoptosis or suppressed inflammatory cytokines, up-regulated expressions of miR-19b, miR-590-5p and miR-495, inversely miR-29a, miR-31 and miR-142-5p. The above-mentioned effects were achieved by miR-19b adjustment (Sun, Liang et al. 2019). Typical inflammatory pathways are also correlated with miRNAs. *Tremella fuciformis* polysaccharides (TFPS) significantly inhibited the activation of Akt, p38MAPK, and NF-κB in LPS-induced RAW264.7 cells. One of the key small RNAs concerned with inflammation, miR-155, was observably degraded (Ruan et al. 2018). LPS-induced inflammation injury was alleviated by APS in H9c2 cells, additionally with down-regulation LPS-increased miR-127 and promoted the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathways (Ren et al. 2018). A polysaccharide isolated from the roots of *Angelica sinensis* (Oliv.) Diels deactivated the NF-κB pathway in spinal cord injury (SCI) model PC-12 cell, which is connected to the attenuation of LPS-induced miR-223 expression elevation (Li R et al. 2018). These results suggest that polysaccharides also reduce inflammation by regulating miRNA levels.

Alternatively, as immune adjuvants, some polysaccharides are capable of activating immune system. Using microarray assay and specific inhibitor, microRNA expression profiles in polysaccharide derived from the roots of *Actinidia eriantha* (AEPS)-activated RAW264.7 cells were investigated. It caused 82 differentially expressed miRNAs especially miR-155 verified by its inhibitor, and 62 target mRNAs predicted to be involved in the inactivation of RAW264.7 cell (Chen X et al. 2019). *Ganoderma lucidum* polysaccharides (GLPS) charactered by pharmaceutical applications in traditional

Chinese medicines and are known for excellent cancer-preventive properties through immunomodulation. In hepatoma-bearing mice, GLPS signally suppressed tumor growth associated with the rising ratio of effector T cell (Teffs) to regulatory T cell (Tregs). Moreover, relying on more secretion of IL-2, GLPS eliminated Treg suppression of Teff proliferation. Increased miR-125b content of GLPS treated T cells inhibited Notch1 and FoxP3, providing novel evidence for GLPS on the restriction of HCC via miR-125b downregulating Tregs accumulation and function (Li A et al. 2015). *Ginseng* polysaccharides (GPS) is well known as an immune modulator. 10 upregulated and 16 downregulated conserved miRNAs were identified in milk-derived exosomal shuttle RNAs (esRNAs) of sows supplemented with dietary GPS. Integrated analysis of each candidate miRNA and genes further revealed the presence of 51 highly conserved miRNA–gene interactions that were annotated to immunoregulatory functions. This work provided fundamental information on how GPS promotes immune response and healthy growth of the infant at molecular level (Sun, Xiong et al. 2019). Therefore, certain polysaccharides enhance the immune system concurrently through epigenetic pathways.

Notedly, numerous transgenerational effects of polysaccharides that based on epigenetics have been reported. Paternal dietary APS could transgenerationally promote growth performance and jejunal tissue morphology in chickens. In both jejunum and sperm, the promoter methylation level of SOCS1 was significantly reduced, histone modification in the promoter region of TRIF was also affected. APS supplementation reflects transgenerational endotoxin tolerance-like effect in jejunum mucosa of broiler chickens on account of epigenetic modifications (Li Y, et al. 2018). Protective efficacies of polysaccharides can also be observed in offsprings.

Oxidation and hypoxia injury protection

Natural polysaccharides exhibit prominent neuro- and cardio-protection by preventing hypoxia and oxidative injuries. The neural stem cells (NSCs) derived from the hippocampus of rat was subjected to hypoxia incubator, APS pretreatment alleviated cell injury-evidenced by increased cell viability, inhibited pro-apoptotic factors and enhanced antiapoptotic factors. High miR-138 expression was observed in APS group, while miR-138 inhibitor blocked its protective effect (Zheng and Zhao 2018). Both *Angelica sinensis* polysaccharide (ASP) and LEN exerted myocardial protection by decreasing miR-22. Simulating myocardial infarction (MI), hypoxia-induced H9c2 cell injury led to upregulated miR-22 content, phosphorylated PI3K, AKT, JAK1 and STAT3 were increased by ASP through mitigating miR-22 (Pan and Zhu 2018). LEN promoted cell viability and decreased apoptosis of hypoxia PNCM or H9c2 cells, accompanied by activation of PI3K/AKT and β-catenin pathways via down-regulation of miR-22 targeted to Sirt1 (Zhang and Zhao 2019). *Lycium barbarum* polysaccharides (LBPs) are the major ingredients of *Fructus lycii* and have multiple pharmacological activities. The protective actions of LBPs and its activation of MEK/ERK, AMPK signaling pathways in hypoxia-injured H9c2 cells were attenuated by miR-122 overexpression, which were accelerated by miR-122 suppression. Decreased infarct size and improved cardiac function were revealed in MI rats administrated with LBPs via the down-regulation of miR-122 (Li Q et al. 2019). miR-1 stands out as the most prominent miRNA in regulating cardiac function. LBPs improved the abnormal epicatechin gallate (ECG) and indexes of cardiac functions in P-V loop detection in transgenic (Tg) mice with miR-1 overexpression, and reversed reductions of CaM and cMLCK or other downstream effectors relevant to myocardial contractibility targeted by miR-1 (Zhang et al. 2018). These natural polysaccharides all have the potential for the adjunctive treatment of acute myocardial infarction (AMI) and heart failure.

In the oxidative stress-induced cell injury model, hydrogen peroxide (H_2O_2)-induced reduction of cell viability, augmentation of apoptosis and autophagy in PC-12 cells were mitigated by LBPs treatment. LBPs elevated the phosphorylation of PI3K and AKT meanwhile reducing the ROS and NO levels through miR-194 inhibition (Niu et al. 2018). In a glaucoma model of oxidative damage to human trabecular meshwork (HTM) cells induced with H_2O_2, LBPs significantly promoted their viability, reduced apoptosis and cleaved-Caspase-3/-9, ROS level, while these efficacies was reversed by miR-4295 (Liu and Zhang 2019). A water-soluble heteropolysaccharide isolated and purified from *Enteromorpha prolifera*, revealed ameliorative mean lifespan, ultraviolet-induced oxidative stress, and thermotolerance in *Caenorhabditis elegans* via the modulation of miR-48, miR-51 and miR-186 causing the up-regulation of SKN-1 and DAF-16 (Lin et al. 2020). miRNA is the important mediator of polysaccharides in the process of oxidation-induced injury protection.

Antitumor activities

The antitumor activities of polysaccharides has also gather increased interests. Hepatocellular carcinoma (HCC) is one of the most common and fatal cancers. Hypoxia facilitates epithelial-mesenchymal transition (EMT) and promotes HCC cells migration and invasion. Basil polysaccharide (BPS), an active ingredient isolated from Basil (*Ocimum basilicum L.*), inhibited progression and metastasis of tumor under hepatic artery ligation (HAL) processed intertumoral hypoxic rat model. The expression levels of HIF-1α, G9a, LSD1, JMJD1A, JMJD2B, JARID1B and H3K9me2 were reduced (Feng et al. 2018). Polysaccharides regulate the expression of histone modifying enzymes and exert anti-cancer effect. Histone modifying enzymes might be a new therapeutic target of polysaccharides.

Current pubilications indicates that miRNA is the main epigenetic regulation method of polysaccharides against tumor cells. High intakes of dietary fiber nondigestible carbohydrates (NDCs) are considered to be protective against colorectal cancer. Supplementing healthy individuals diets

with resistant starch (RS) alters the expression pattern of miRNA profiles in the macroscopically-normal human rectal epithelium. A total of 111 miRNAs were up- or down-regulated by at least two-fold, particularly miR-32, involved in the regulation of cell proliferation dysregulated in CRC and TRAF3 expression consequently NIK stabilization (Malcomson et al. 2017). RS consumption also reduced the risk of colorectal cancer associated with high ingestion of red meat. Supplementation with butylated resistant starch restored miR-17-92 to baseline levels, which corresponded with increased cell proliferation, and a decrease in target gene CDKN1A transcript (Humphreys et al. 2014). High-amylose potato starch (HAPS), high-amylose maize starch (HAMS), and butylated high-amylose maize starch (HAMSB) all altered the cecal microbial composition in a diet-specific manner exhibiting CRC (colorectal cancer) preventive effects by reducing colonic oncogenic miR-17-92 cluster miRNA expression (Nielsen et al. 2019). GLPs is commonly hypothesized to suppress tumor cells proliferation through immune effects. 61 differential expressed miRNAs in human hepatocarcinoma cells (HepG2) were observed (Shen et al. 2014). TP-1, a polysaccharide from one famous fungus *Huaier*. SMMC-7721 cell growth, adhesion, migration, and motility were significantly inhibited with TP-1 treatment accompanied by elevated miR-122 (Li et al. 2015). Neuroblastoma is the most common tumor diagnosed in children and infants. ASP has been identified to induce apoptosis of neuroblastoma SH-SY5Y cells. It might repress tumorigenesis through depressed miR-675-mediated inactivation of the PI3K/AKT and JAK/STAT pathways. It was thought that KIF1Bβ might be a target for miR-675 (Yang et al. 2018). Osteosarcoma (OS) has a high incidence, malignity, and frequency of recurrence and metastasis. ASP suppressed the proliferation, migration, and invasion of MG63 cells by up-regulating miR-133a which inactivated the JNK pathway (Chu et al. 2018). Homogeneous polysaccharide (GTP) composed of glucose units which is isolated from Green tea (*Camellia sinensis*) inhibited the growth of prostate cancer (PC)-3 cells via inducing apoptosis, raised tumor repressor disabled homolog 2 (DAB2) protein and inactivated AKT and ERK1/2 signallings (achieved by suppression of miR-93). miR-93 accelerated PC progression and metastasis by repressing DAB2 to activate Akt/ERK1/2 pathway (Yang, Gao, et al. 2019). Hence, the regulation of miRNAs is of great significance to the anti-tumor effect of polysaccharides.

Glycolipid metabolism

Epigenetics has close connections with energy metabolism. In rodents fed high-starch/low-fat (HS) diet when compared with those fed low starch/high-fat (LS) diet, the expression of genes in intestine involved in carbohydrate digestion and absorption, such as sucrase-isomaltase (SI) and sodium-dependent glucose cotransporter (SGLT1) was higher. HS diet intake induced jejunal expression of histone acetyltransferase. Concomitant induction of SI and SGLT1 was closely associated with the binding of GCN5 and Ac-H3/Ac-H4 and positively related to histone H3K4 methylation on the promoter/transcribed regions (Inoue et al. 2015; Inoue, Mochizuki, and Goda 2011). Using chromatin immunoprecipitation (ChIP) assay, Ac-H3 on the promoter/enhancer and transcriptional regions was reduced in the upper jejunum and elevated in the lower jejunum/upper ileum (Shimada, Mochizuki, and Goda 2009a). Inversely, Thrsp mRNA and protein were markedly decreased associated with a decline in the binding of ChREBP and Ac-H3/Ac-H4 (Shimada, Mochizuki, and Goda 2011). Jejunal reduction of the glucose-dependent insulinotropic polypeptide (GIP) by RS was also connected to decreases in Ac-H3/Ac-H4 on the promoter/enhancer region of it (Shimada, Mochizuki, and Goda 2009b). A recent study was conducted to evaluate the potential of *Cyclocarya paliurus* polysaccharide (CPP) to improve dyslipidemia and regulate the expression levels of lipid metabolism-related genes (leptin and MTTP) mRNA in hyperlipidemic rats' liver. Results showed that there was a considerable decrease in DNA methylation in corresponding promoters' regions (Yang, Gao, et al. 2019). This is currently one of the few evidence that polysaccharides regulate DNA methylation.

Ganoderma lucidum polysaccharides F31 have hypoglycemic effects on diabetic mice. Integrative analysis of transcriptomics and proteomics data demonstrated that three genes (Gck, G6pc, Pck) and three proteins (GCK, GLUT2, PK) about glycolysis and gluconeogenesis, the JAK2 protein in the insulin pathway, and the PPARs signaling pathway in lipid metabolism played important roles in the hypoglycemic effect of F31. Down-regulated miR-324 probably participate in the regulation of genes involving glucose metabolism. The top enrichment pathways for target genes were glycolysis/gluconeogenesis, PI3K-Akt, AMPK, biosynthesis NF-κB, JAK-STAT and mTOR, regulation of autophagy, and cAMP signaling pathway (Xiao et al. 2018). Insulin resistance (IR) is a common feature of type 2 diabetes mellitus (T2DM). APS attenuated IR in T2DM. miR-203a-3p was upregulated following APS treatment in T2DM Goto Kakizaki (GK) rats. It's target genes GRP78, CHOP, pJNK1 and caspase-12 were significantly decreased, regulating the protein expression of the ERS signaling pathway (Wei et al. 2018). VBT leaves' polysaccharide (VBTLP), extracted from *Vaccinium bracteatum Thunb.*, a traditional Chinese herb, alleviated hepatic gluconeogenesis to improve glucose metabolism via activation of LKB1/AMPK axis in vivo and in vitro, caused by miR-137 decline directly targeting AMPK and LKB1 (Qian, Li, and Wang 2017). The miRNA is equally a pivotal target of polysaccharides in regulating energy metabolism.

Other biological functions

Polysaccharides have protective effects on the reproductive system. Several enzymatic activities in testis and sperm samples from breeder cocks fed with APS were increased, 16 up-regulated and 17 down-regulated miRNAs appeared. Some of these miRNAs may be involved in testicular nutrient metabolisms and NK cell-mediated cytotoxicity pathway (Wu et al. 2017). APS also markedly extended the lifespan

of *C. elegans*. A highly conserved miRNA (miR-124) acting on three binding sites at ATF-6 gene 3'UTR was significantly upregulated, contributing to lifespan extension (Wang et al. 2015). Cyclophosphamide (CP) toxicity on the testis was hampered by squid ink polysaccharide (SIP) via restoration of antioxidant ability. SIP played preventive roles via elevating expressions of NQO-1 and HO-1 genes to activate Nrf2/ARE signal pathway and further discovered that upstream regulators of Nrf2, keap-1, HDAC2, and PKC, were concerned with it (Le et al. 2015). Polysaccharides are also beneficial to bone health. LBPs have been reported to promote the proliferation of osteoblasts. It fortified osteoblasts viability by raising miR-17 to target PTEN and consequently touched off PI3K/AKT pathway (Liang and Yue 2019). *Polygonatum sibiricum* polysaccharide (PSP) could inhibit expression of osteoclast related genes by Hippo signaling pathway based on 27 differentially expressed microRNAs especially miR-1224, and elevated its' target gene Limd1 level (Li B et al. 2019). Additionally, polysaccharide promotes pluripotent reprogramming. Cationized *Pleurotus eryngii* polysaccharide (CPS), hybridized with calcium phosphate (CP), was used to co-deliver plasmids (Oct4, Sox2, 5 Klf4, c-Myc) for generating induced pluripotent stem cells (iPSCs). Hybrid CPS facilitated epigenetic modification during the reprogramming. Histone H3K4 dimethylation (H3K4me2), tri-methylation (H3K4me3) and acetylation were positive in the iPSC colonies, which implied that CPS could exert a positive effect on histone modification (Deng et al. 2016). These reports demonstrate the diversity of physiological functions of polysaccharides through epigenetic regulation.

Interestingly, transgenerational effect of various polysaccharides through epigenetic regulation has been reported. Paternal dietary APS supplementation could transgenerationally promote growth performance and jejunal tissue morphology of chickens. In both jejunum and sperm, the promoter methylation level of SOCS1 significantly reduced, histone modification in the promoter region of TRIF was also affected. APS supplementation reflects transgenerational endotoxin tolerance-like effect in jejunum mucosa of broiler chickens on account of epigenetic modifications (Li et al. 2018). These interesting researches indicate that the functions of polysaccharides has potential intergenerational or transgenerational inheritance effects.

Conclusion and prospectives

Profiting from its excellent biological effects, polysaccharides have been widely used as protective compononets in clinical research. Classical studies generally focused on polysaccharides directly affecting phenotype changes, regulating related gene expressions and signaling pathways. Eepigenetic regulation provides a novel direction to explore the molecular mechanisms of different polysaccharides. There are still different view points about polysaccharides, some studies think that polysaccharides can enter cells directly, some studies think the opposite. How do polysaccharides regulate signal pathways in cells? Which transcription factors can be activated by polysaccharides? How do polysaccharides participate in gene transcriptions of miRNA, lncRNA, DNA methylation enzymes and histone modification enzymes? All of these questions are still in the initial stage of exploration.

In addition to enhancing immunity, regulating inflammation, reducing oxidative stress, anti-tumor, and improving glycolipid metabolism, polysaccharides also play a positive role in the regulation of obesity, diabetes and gut microbiota. Polysaccharide fraction of radish greens (PRG) and sporoderm-broken spores of *Ganoderma lucidum* (BSGLP) alleviated high fat diet-induced obesity through improving gut barrier function, modulating gut microbiota dysbiosis and regulating lipid metabolism (Do et al. 2021; Sang et al. 2021). These results demonstrate that dietary polysaccharides might be used as a prebiotic agent to restore the balance of gut microbiota, and thereby positively modulate the host's gene expression and metabolism (da Silva et al. 2021). Base on elevation of insulin sensitivity, amelioration of insulin resistance, increased glucose utilization and scavenging free radicals and lipid peroxidation, the konjac glucomannan, pumpkin polysaccharide, APS, GPS and other polysaccharides have been used as alternative medicine to treat diabetes mellitus in clinic (Wang et al. 2016). Notably, disturbances of DNA methylation, histone modifications, and RNA-mediated processes balance may cause several pathologies and contribute to obesity and T2DM (Ling and Rönn 2019). Moreover, gut microbiota and microbial metabolites can induce epigenetic modifications with potential implications susceptibility to obesity and diabetes mellitus (DM) (Cuevas-Sierra et al. 2019). However, there is still a scarcity of evidences that polysaccharides inhibit obesity through epigenetic regulations. Further exploring the frontier field of linking polysaccharides, microbiota and obesity, will enhance our understandings of polysaccharides, which will be benifited to the high efficient utilization of polysaccharides.

Diets can affect offsprings through epigenetic regulation (Guo, Luo, and Lin 2020). Whether do polysaccharides cause intergenerational or transgenerational inheritance effects via epigenetic regulations? APS has been shown the potential to transfer parental traits to offspring (Li Y et al. 2018). Under the intervention of factors such as toxins, alcohol, malnutrition or a high-fat diet, disorders of parental genes could repeat in progenies, even they were not directly exposed to the harmful environmental agents. These phenotypic traits are transmitted by epigenetic state changes in germ cells. If polysaccharides also affect the epigenetic patterns of gametes, their efficacies will also be equally beneficial to the offspring's health. Thus, further elucidation of the generational effects of polysaccharides is necessary. Overall, it is still a big task to explore dietary polysaccharides exerting biological functions via epigenetic regulations.

Disclosure statement

The authors declare that they have no competing financial interests.

Abbreviations

5mC	5-methylcytosine
APS	astragalus polysaccharide
Ac-H3/Ac-H4	histone H3/H4 acetylation
AEP	actinidia eriantha polysaccharide

AMI	acute myocardial infarction
AMPK	AMP-activated protein kinase
ASP	angelica sinensis polysaccharide
BPS	Basil polysaccharide
BSGLP	polysaccharide of sporoderm-broken spores of Ganoderma lucidum
ChIP	chromatin immunoprecipitation
CP	cyclophosphamide
CPP	cyclocarya paliurus polysaccharide
CPS	cationized Pleurotus eryngii polysaccharide
CRC	colorectal cancer
DM	diabetes mellitus
DNMTs	DNA methyltransferases
ECG	epicatechin gallate
EGCG	(−)-epigallocatechin-3-gallate
EMT	epithelial-mesenchymal transition
GIP	glucose-dependent insulinotropic polypeptide
GLP	ganoderma lucidum polysaccharide
GOS	galactooligosaccharides
GPS	ginseng polysaccharides
GTP	homogeneous polysaccharides from green tea
H3K27me3/H3K4me3	tri-methylation of lysine 27/4 on histone 3
H3K4me2/H3K4me3	histone H3K4 dimethylation/tri-methylation
HAL	hepatic artery ligation
HAMS	high-amylose maize starch
HAMSB	butylated high-amylose maize starch
HAPS	high-amylose potato starch
HATs	histone acetyltransferases
HCC	hepatocellular carcinoma
HDACs	histone deacetylases
HDMs	histone demethylases
HMTs	histone methyltransferases
HTM	human trabecular meshwork
iPSCs	induced pluripotent stem cells
IR	insulin resistance
JAK1	Janus kinase 1
LBP	lycium barbarum polysaccharide
LEN	lentinan
LPS	lipopolysaccharide
MeCP	methyl-CpG binding proteins
MEK/ERK	MAP kinse-ERK kinase
MI	myocardial infarction
miRNA	microRNA
ncRNAs	non-coding RNAs
NDCs	nondigestible carbohydrates
NSCs	neural stem cells
NF-κB	nuclear factor-kappa-B
PCV2	porcine circovirus type 2
PI3K	phosphoinositide 3-kinase
PSP	Polygonatum sibiricum polysaccharide
PTMs	post-translational modifications
RISC	RNA-inducing silencing complex
RS	resistant starch
SCI	spinal cord injury
SGLT	sodium-dependent glucose cotransporter
SI	sucrase-isomaltase
SIP	squid ink polysaccharide
SSP	Sophora subprostrata polysaccharide
SSP	Sargassum weizhouense polysaccharide
STAT3	signal transducer and activator of transcription 3
SUMO	small ubiquitin related modifier
T2DM	type 2 diabetes mellitus
Teffs	effector T cell
TFP	tremella fuciformis polysaccharide
Tg	transgenic Tregs regulatory T cell
VBTLP	VBT leaves polysaccharide

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