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Review Article

Rare ginsenosides: A unique perspective of ginseng research

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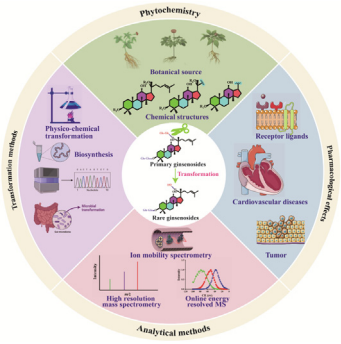
HIGHLIGHTS

- Rare ginsenosides (RGs), as the shining components, possessed great potential in drugs and nutraceuticals.
- A total of 144 rare ginsenosides with diverse skeletons and bioactivities were isolated from *Panax* species.
- RGs showed superior bioactivity for CVDs and cancer, and they acted as natural ligands for some specific receptors.
- The stereochemistry properties of RGs brought out diverse bioactivities and novel analytical strategies.
- Physico-chemical transformation, biotransformation, and biosynthesis for RGs are summarized.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Rare ginsenosides (Rg3, Rh2, C-K, *etc.*) refer to a group of dammarane triterpenoids that exist in low natural abundance, mostly produced by deglycosylation or side chain modification *via* physico-chemical processing or metabolic transformation in gut, and last but not least, exhibited potent biological activity comparing to the primary ginsenosides, which lead to a high concern in both the research and development of ginseng and ginsenoside-related nutraceutical and natural products. Nevertheless, a comprehensive review on these promising compounds is not available yet.

Aim of review: In this review, recent advances of Rare ginsenosides (RGs) were summarized dealing with the structurally diverse characteristics, traditional usage, drug discovery situation, clinical application, pharmacological effects and the underlying mechanisms, structure–activity relationship, toxicity, the stereochemistry properties, and production strategies.

Key scientific concepts of review: A total of 144 RGs with diverse skeletons and bioactivities were isolated from *Panax* species. RGs acted as natural ligands on some specific receptors, such as bile acid receptors, steroid hormone receptors, and adenosine diphosphate (ADP) receptors. The RGs showed promising bioactivities including immunoregulatory and adaptogen-like effect, anti-aging effect, anti-tumor effect, as well as their effects on cardiovascular and cerebrovascular system, central nervous system, obesity and diabetes, and interaction with gut microbiota. Clinical trials indicated the potential of RGs, while high quality data remains inadequate, and no obvious side effects was found. The stereochemistry properties induced by deglycosylation at C (20) were also addressed including pharmacodynamics behaviors, together with the state-of-art analytical strategies for the identification of saponin stereoisomers.

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Finally, the batch preparation of targeted RGs by designated strategies including heating or acid/ alkaline-assisted processes, and enzymatic biotransformation and biosynthesis were discussed. Hopefully, the present review can provide more clues for the extensive understanding and future in-depth research and development of RGs, originated from the worldwide well recognized ginseng plants.

Contents

Introduction

Ginsenosides mainly represent a group of dammarane type triterpenoids identified in *Panax* herbs including *Panax ginseng* C. A. Meyer, (*P. ginseng*), *Panax notoginseng* (Burk) F.H. Chen (*P. notoginseng*) and *Panax quinquefolius* L. (*P. quinquefolius*) and constitute the predominant chemical and pharmacological profiles of those plants [1]. According to their natural abundance, ginsenosides are usually divided into macro (primary) saponins (ginsenoside Rb1, Rg1, Re, Rd, *etc.*) and rare (secondary) ginsenosides (Rg5, Rk1, Rg3, *etc*). Rare ginsenosides (RGs) exist in extremely low natural concentration (normally less than 0.1 percent) and actually produced by partial hydrolysis of the macro or primary glycosides *via* steaming, acid/alkali treatment, or microbial metabolic transformation [2]. In addition, the natural ginsenosides occur generally in 20(*S*)-configuration, yet the deglycosylation of the C-(20)-hydroxyl group may result in an epimerization to produce the 20(*R*)-form of RGs, like 20(*R*)-Rh2 and 20(*R*)-Rg3 [3]. In some cases, this process along with side chain modification such as dehydrogenation, dehydration, or oxidation, and even cyclization to produce the panaxadiol type RGs. In red ginseng (a steamed product of the raw ginseng), a series isomerized RGs including 20(*S*/*R*)-Rg3, 20(*S*/*R*)-Rh1, and 20(*S*/*R*)-Rh2 were produced [4].

In recent years, RGs have attracted increased attention due to their more potent and diverse health benefits in treatment or prevention of cancer [4], cardiovascular and cerebrovascular [5],

inflammation [6], aging and nervous diseases [7], as well. Meanwhile, the notable discrepancy between the superior pharmacological effects and inferior bioavailability of ginsenosides were universally explained by the intestinal microbiome-mediated transformation from macro primary ginsenosides into the secondary RGs, demonstrating enhanced bioavailability than their substrates. Studies on the chemistry, bioactivities [8], biosynthesis [9], and analytical methods [10] were reviewed majorly for the primary ginsenosides, yet for the rare ginsenosides, only the bioactivity of some individual ones were summarized [11–13]. In the light of a rapid concern especially on the biological activities of RGs in the last decade, as shown in Fig. 1, this review summarized the studies published from 2001 to 2021 (Web of Science core collection), focusing on the structure diversity, pharmacological activities and their underlying mechanisms, microbiota-mediated interaction, traditional usage, drug discovery situation, clinical application, structure–activity relationship, toxicity, stereochemistry properties, and strategy for bulk preparation (physical/chemical conversion and microbial transformation). Furthermore, challenges and new insights into further development of rare ginsenosides are also discussed.

RGs identified in *Panax* species

With the advancement of natural products chemistry, more than 500 saponins were isolated from *Panax* genus, among them, majority of new compounds isolated recently were RGs, obtained not only by direct isolation from *ginseng* plants, but rather from steaming process, chemical transformation, and biotransformation [14]. The contents of RGs in roots of *Panax* species were in an order of *P. notoginseng, P. quinquefolius*, and *P. ginseng*, whereas for stem-leaves, it is in a rank of *P. ginseng, P. notoginseng* and *P. quinquefolius*. Considering the high contents of primary ginsenosides in stem-leaves with lower price, the stem-leaves of *Panax* plants are ideal raw materials for producing RGs [15].

Red ginseng that generated from steaming of raw ginseng is well reputed in China and Korea for distinctive effects comparing to the crude products, and a series of RGs such as ginsenoside Rk1, Rg5, Rg6, F4, 20(*R/S*)-Rs3, Rh4, Rs5, 20(*R/S*)-Rg3, Rk3, and Rs4 were identified in red ginseng, which were proven to be produced during the high temperature steaming process by hydrolysis and side chain modification of the primary ginsenosides. Moreover, the yield of RGs influenced by temperature and time of steaming, and epimerization at C-20 also occurs during that process [16].

Chemical transformation of RGs is a simple, economic and widely used strategy by hydrolysis under acidic or alkaline conditions. Strong acid hydrolysis shows a higher conversion efficiency, leading to not only deglycocylation of ginsenosides, but also the side chain derivatization such as dehydration, cyclization and double bond displacement, especially configuration inversion at C-20 of aglycones [17–19]. In contrast, alkali hydrolysis has the advantages of high conversion, mild reaction conditions which cause no epimerization and no cyclization of the side chain [20]. The enzymatic biotransformation by microorganisms or heterologously expressed enzymes becomes a new trend for preparation of RGs in the consideration of hydrolysis specificity and environmental friendliness, and a series of deglycosylated RGs, such as ginsenosides CK, Rh2, Mc, F2, and F1 as well as the aglycones were produced in this way [21].

RGs was firstly isolated from steamed ginseng, which enlarged the resources of ginsenosides, and further chemical reaction elucidated the transformation pathway that make it feasible to the rapid preparation of RGs. Importantly, the rapid development of

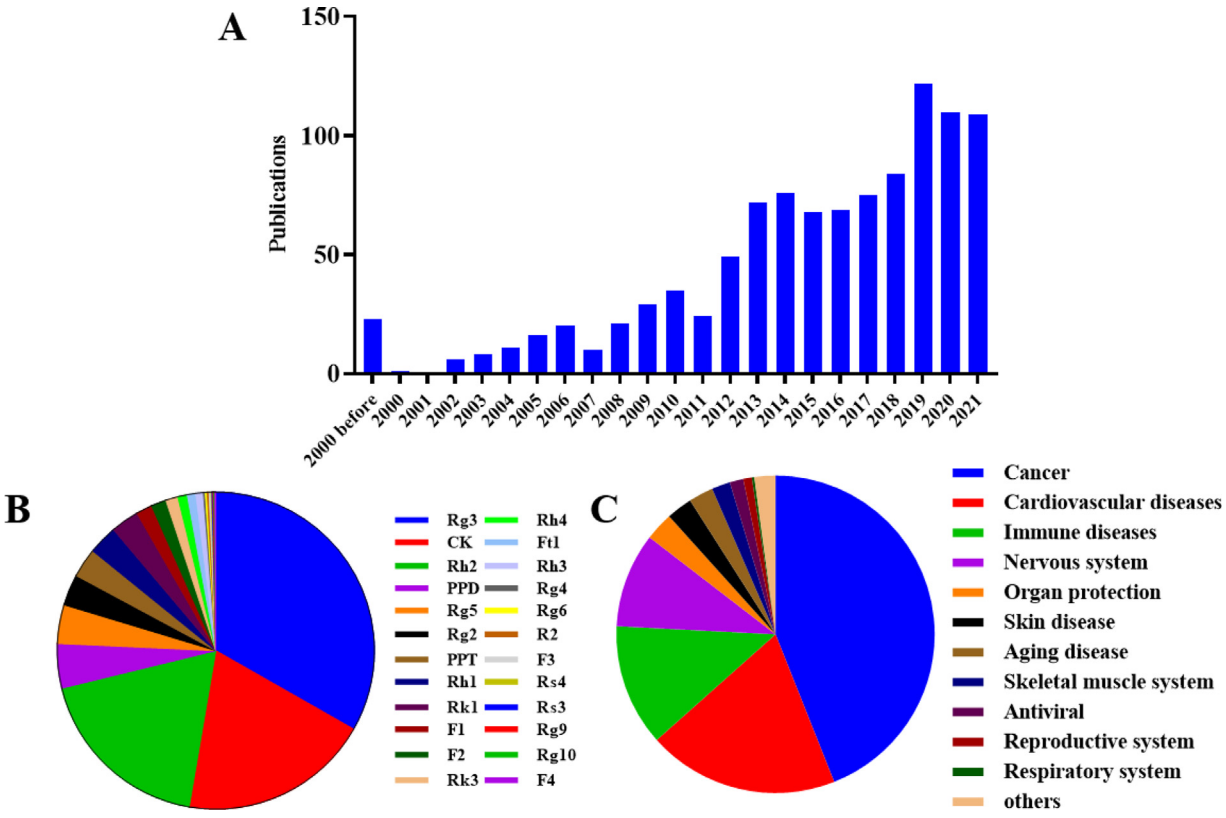

Fig. 1. An overview of the research trends of rare ginsenosides based on publications up to 2021. (A) The annual numbers of publication; (B) Publications of individual rare ginsenosides; (C) Types of diseases involved.

enzyme engineering has made it possible to selectively convert rare saponins.

RGs can be classified into protopanaxadiol (PPD) and protopanaxatriol (PPT) types and Va and Vb subtypes according to their steroid skeletons [22 23]. Briefly, the classification rule can be summarized as follows, PPD-type RGs possessed glycosylated modifications at C-3 and C-6 hydroxyls, while for PPT-type RGs, glycosylated modifications occurred at C-6 and C-20 hydroxyls. Furthermore, when C-17 side chain undergone variation including oxidation, dehydrogenation, dehydration, and cyclization, the subtypes of RGs depended on whether the hydroxyl group at the C-20 position participating in the reaction. When the hydroxyl groups at the C-20 position are retained, they are classified as Va type, conversely, if C-20 hydroxyl groups participated in the reaction, forming double bonds or rings, they are classified as vb type. The representative structures of RGs identified in *Panax* species were summarized in Fig. 2 and Table 1.

Traditional usage, drug discovery situation, and clinical application of RGs

The red ginseng, which is rich in RGs produced by ginseng steaming, has a long history of medicinal use. The first record of red ginseng can be traced back to *Bencao Mengquan*, which is the famous monograph of traditional Chinese medicine (TCM) in the Ming dynasty. In addition to sharing similar nourishing and tonify-

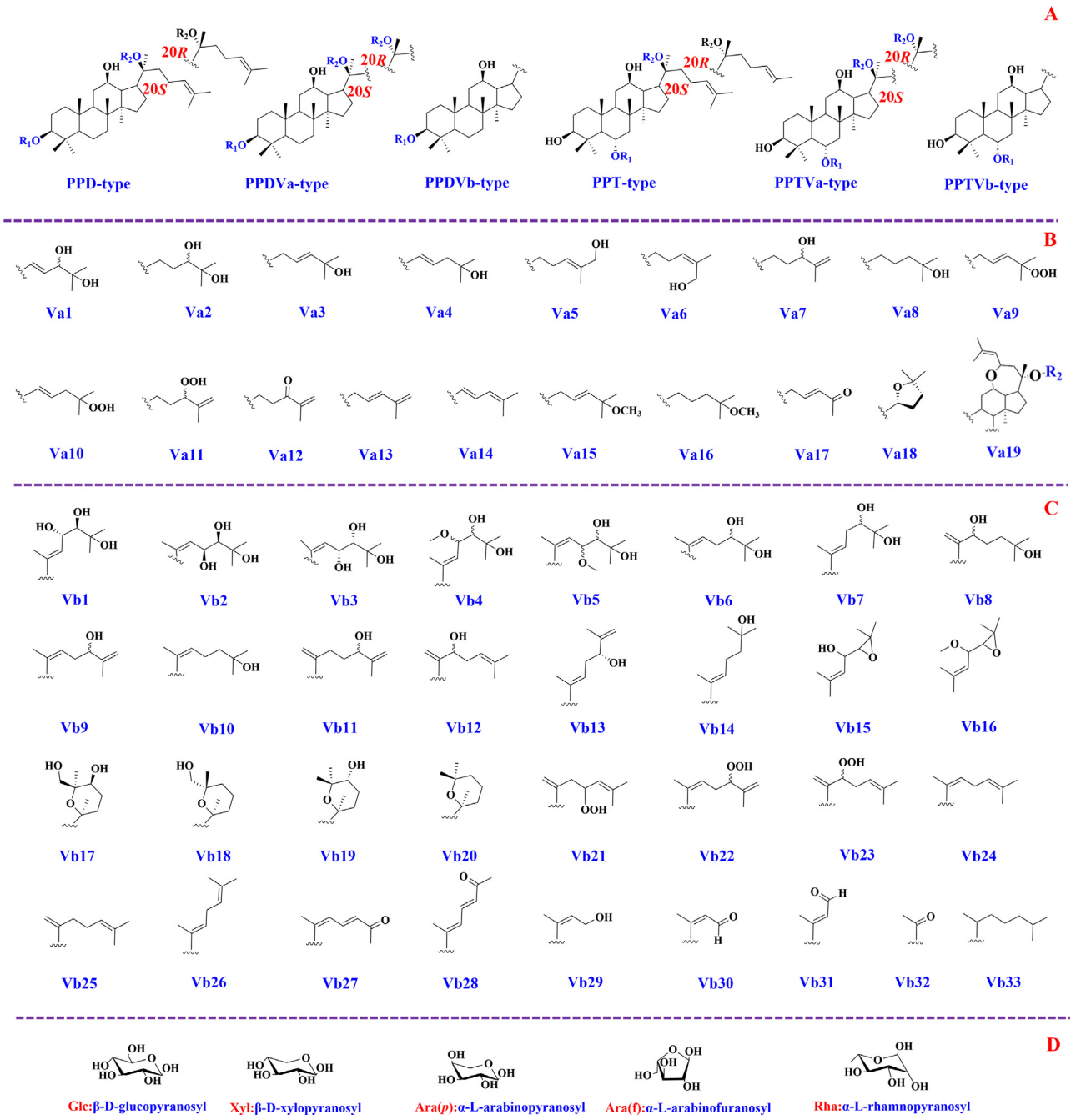

Fig. 2. Structures of RGs identified in *Panax* species. (A) Representative skeletons of RGs with different side-chain variation; (B) Moiety of Va-type side-chain variation; (C) Moiety of Vb-type side-chain variation; (D) Glycosyl substitution.

Table 1
Rare ginsenosides identified in *Panax* species.

No.	Compounds	Side-chain variation	R1	R2	M.F.	Source	Ref.
	PPD-type						
1	20(*S*)-Protopanaxadiol	–	H	H	$C_{30}H_{52}O_3$	bc	[166,261]
2	20(*R*)-Protopanaxadiol	–	H	H	$C_{30}H_{52}O_3$	bc	[162,230]
3	Compound K	–	H	Glc	$C_{36}H_{62}O_8$	abc	[166,262,263]
4	20(*S*)-Rh2	–	Glc	H	$C_{36}H_{62}O_8$	abc	[166,210,264]
5	20(*R*)-Rh2	–	Glc	H	$C_{36}H_{62}O_8$	abc	[265–267]
6	Ginsenoside F2	–	Glc	Glc	$C_{42}H_{72}O_{13}$	ab	[166,263,268]
7	Ginsenoside Mc	–	H	Glc(6,1)Ara(f)	$C_{41}H_{70}O_{12}$	ab	[166,269]
8	20(*S*)-Rg3	–	Glc(2,1)Glc	H	$C_{42}H_{72}O_{13}$	bc	[166,270]
9	20(*R*)-Rg3	–	Glc(2,1)Glc	H	$C_{42}H_{72}O_{13}$	abc	[266,267,271]
10	20(*S*)-Rs3	–	Glc(2, 1)Glc-6-Ac	H	$C_{44}H_{74}O_{14}$	c	[272]
11	20(*R*)-Rs3	–	Glc(2, 1)Glc-6-Ac	H	$C_{44}H_{74}O_{14}$	c	[272]
12	Notoginsenoside Ft1 (20*R*)	–	Glc(2,1)Glc (2,1)Xyl	H	$C_{47}H_{80}O_{17}$	c	[273]
13	Notoginsenoside St4 (20*S*)	–	Glc(2,1)Glc (2,1)Xyl	H	$C_{47}H_{80}O_{17}$	c	[274]
	PPDVa-type						
14	Notoginsenoside SFt2	Va2	Glc	H	$C_{36}H_{64}O_{10}$	c	[275]
15	Ginsenoside Rh12	Va2	H	Glc	$C_{36}H_{64}O_{10}$	a	[276]
16	Ginsenoside Rh13	Va3	H	Glc	$C_{36}H_{62}O_9$	a	[276]
17	Majoroside F4	Va3	Glc	Glc	$C_{42}H_{72}O_{14}$	a	[277]
18	Floralginsenoside E	Va4	Glc(2, 1)Glc	H	$C_{42}H_{72}O_{15}$	a	[278]
19	Notoginsenoside SFt1	Va7	Glc	H	$C_{36}H_{62}O_9$	c	[275]
20	Ginsenoside Rg7	Va7	Glc	Glc	$C_{42}H_{72}O_{14}$	a	[279]
21	25-OH-Ginsenoside Rg3(20*S*)	Va8	Glc(2, 1)Glc	H	$C_{42}H_{74}O_{14}$	c	[280]
22	25-OH-Ginsenoside Rg3(20*R*)	Va8	Glc(2, 1)Glc	H	$C_{42}H_{74}O_{14}$	c	[280]
23	Ginsenoside Rh6	Va9	H	Glc	$C_{36}H_{62}O_{11}$	a	[279]
24	Floralginsenoside F	Va9	Glc	Glc	$C_{42}H_{72}O_{15}$	a	[278]
25	Ginsenoside Rg12	Va10	Glc(2, 1)Glc	H	$C_{42}H_{72}O_{15}$	a	[281]
26	Floralquinquenoside D	Va11	Glc	Glc	$C_{42}H_{72}O_{15}$	a	[282]
27	Notoginsenoside SY4	Va15	Glc(2, 1)Glc	H	$C_{43}H_{74}O_{14}$	c	[283]
28	20(*S*)-25-OCH3-PPD	Va16	H	H	$C_{31}H_{56}O_4$	a	[284]
29	Notoginsenoside SY3	Va17	Glc(2, 1)Glc	H	$C_{41}H_{68}O_{14}$	c	[283]
30	Ginsengenin	Va18	H	H	$C_{30}H_{52}O_4$	c	[285]
31	Ginsenoside La	Va19	Glc	Glc	$C_{42}H_{70}O_{13}$	a	[286]
32	3β, 20(S)-dihydroxydammar-24-en-12β, 23β-epoxy-20-*O*-β-D-glucopyranoside	Va19	H	Glc	$C_{36}H_{60}O_8$	a	[287]
33	Notoginsenoside LY	Va19	H	Glc(6,1)Ara(f)	$C_{41}H_{68}O_{12}$	a	[288]
	PPDVb-type						
34	Notoginsenoside SP11	Vb1	Glc(2, 1)Glc	–	$C_{42}H_{72}O_{15}$	c	[289]
35	Notoginsenoside ST2	Vb4	Glc(2, 1)Glc	–	$C_{43}H_{74}O_{15}$	c	[270]
36	Notoginsenoside ST3	Vb5	Glc(2, 1)Glc	–	$C_{43}H_{74}O_{15}$	c	[270]
37	Ginsenoside Rh15	Vb9	Glc(2, 1)Glc	–	$C_{42}H_{70}O_{13}$	a	[290]
38	Ginsenoslaloside I	Vb13	Glc	–	$C_{36}H_{60}O_8$	c	[20]
39	Ginsenoside Rh10	Vb14	Glc	–	$C_{36}H_{62}O_8$	a	[291]
40	Ginsenoside Rg11	Vb15	Glc(2, 1)Glc	–	$C_{42}H_{70}O_{14}$	c	[17]
41	23-*O*-methylginsenoside-Rg11	Vb16	Glc(2, 1)Glc	–	$C_{43}H_{72}O_{14}$	c	[292]
42	24, 26-dihydroxy-panaxdiol	Vb17	H	–	$C_{30}H_{52}O_5$	c	[293]
43	26-hydroxy-panaxdiol	Vb18	H	–	$C_{30}H_{52}O_4$	a	[294]
44	24-hydroxy-panaxdiol	Vb19	H	–	$C_{30}H_{52}O_4$	c	[293]
45	Notoginsenoside R7	Vb20	Glc	–	$C_{36}H_{62}O_8$	a	[295]
46	20(*S*)-Panaxadiol	Vb20	H	–	$C_{30}H_{52}O_3$	c	[18]
47	20(*R*)-Panaxadiol	Vb20	H	–	$C_{30}H_{52}O_3$	c	[18]
48	Ginsenoside Rg5	Vb24	Glc(2, 1)Glc	–	$C_{42}H_{70}O_{12}$	c	[296]
49	Ginsenoside Rh3	Vb24	Glc	–	$C_{36}H_{60}O_7$	a	[265]
50	Ginsenoside Rs4	Vb24	Glc(2, 1)Glc-6-Ac	–	$C_{44}H_{72}O_{13}$	a	[346]
51	Ginsenoside Rs5	Vb25	Glc(2, 1)Glc-6-Ac	–	$C_{44}H_{72}O_{13}$	c	[298]
52	Ginsenoside Rk1	Vb25	Glc(2, 1)Glc	–	$C_{42}H_{70}O_{12}$	c	[297]
53	Ginsenoside Rk2	Vb25	Glc	–	$C_{36}H_{60}O_7$	c	[298]
54	Notoginsenoside ST12	Vb25	Glc(2, 1)Xyl	–	$C_{41}H_{68}O_{11}$	c	[299]
55	Isoginsenoside-Rh3	Vb26	Glc	–	$C_{36}H_{60}O_7$	a	[300]
56	Notoginsenoside ST11	Vb26	Glc(2, 1)Xyl	–	$C_{41}H_{68}O_{11}$	c	[299]
57	3-O-β-D-glucopyranoside-3β,l2β, 23β-triol-20-ene-dammar	Vb29	Glc	–	$C_{32}H_{54}O_8$	a	[301]
58	Notoginsenoside ST10	Vb31	Glc(2, 1)Glc	–	$C_{38}H_{62}O_{13}$	c	[299]
	PPT-type						
59	20(*S*)-Protopanaxatriol	–	H	H	$C_{30}H_{52}O_4$	bc	[267,302]
60	20(*R*)-Protopanaxatriol	–	H	H	$C_{30}H_{52}O_4$	c	[303]

(*continued on next page*)

Table 1 (*continued*)

No.	Compounds	Side-chain variation	R1	R2	M.F.	Source	Ref.
61	Ginsenoside F1	–	H	Glc	$C_{36}H_{62}O_9$	ab	[166,268,304]
62	20(*S*)-ginsenoside Rh1	–	Glc	H	$C_{36}H_{62}O_9$	ab	[166,305,306]
63	20(*R*)-ginsenoside Rh1	–	Glc	H	$C_{36}H_{62}O_9$	bc	[266,307]
64	Ginsenoside F3	–	H	Glc(6, 1)Ara(p)	$C_{41}H_{70}O_{13}$	a	[268]
65	Ginsenoside F5	–	H	Glc(6, 1)Ara(f)	$C_{41}H_{70}O_{13}$	a	[308]
66	20(*S*)-Ginsenoside Rg2		Glc(2, 1)Rha	H	$C_{42}H_{72}O_{13}$	abc	[309–311]
67	20(*R*)-Ginsenoside Rg2	–	Glc(2, 1)Rha	H	$C_{42}H_{72}O_{13}$	bc	[266,307]
68	20(*S*)-Notoginsenoside R2	–	Glc(2, 1)Xyl	H	$C_{41}H_{70}O_{13}$	ab	[312–314]
69	20(*R*)-Notoginsenoside R2	–	Glc(2, 1)Xyl	H	$C_{41}H_{70}O_{13}$	b	[314,315]
	PPTVa-type						
70	Notoginsenoside SP20 (20*R*)	Va1	Glc	CH3	$C_{37}H_{64}O_{11}$	c	[289]
71	Notoginsenoside T4	Va1	Glc	H	$C_{36}H_{62}O_{11}$	c	[316]
72	Vinaginsenoside R12	Va2	Glc	H	$C_{36}H_{64}O_{11}$	a	[18]
73	Notoginsenoside J	Va2	Glc	Glc	$C_{42}H_{74}O_{16}$	a	[317]
74	Quinquenoside L9	Va2	Glc(2,1)Rha	H	$C_{42}H_{74}O_{15}$	a	[318]
75	6-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-dammar-3β,6α,12β,20S,24R,25-hexaol	Va2	Glc(2,1)Glc	H	$C_{42}H_{74}O_{16}$	a	[319]
76	Ginsenoside ST2	Va3	Glc	H	$C_{36}H_{62}O_{10}$	c	[320]
77	Vinaginsenoside R15	Va3	Glc	Glc	$C_{42}H_{72}O_{15}$	a	[321]
78	Notoginsenoside R9	Va4	Glc	H	$C_{36}H_{62}O_{10}$	c	[322]
79	Notoginsenoside Rw2	Va4	Glc(2,1)Xyl	H	$C_{41}H_{70}O_{14}$	a	[323]
80	Floralginsenoside B	Va9	Glc	Glc	$C_{42}H_{72}O_{16}$	a	[278]
81	Floralginsenoside D	Va9	H	Glc(6,1)Ara(f)	$C_{41}H_{70}O_{15}$	a	[278]
82	Ginsenoside Rh20	Va4	Glc(2,1)Rha	H	$C_{42}H_{72}O_{14}$	a	[324]
83	Ginsenoside Km	Va5	H	Glc	$C_{36}H_{62}O_{10}$	a	[325]
84	Yesanchinoside R3	Va5	Glc	H	$C_{36}H_{62}O_{10}$	a	[326]
85	Ginsenoside Re5	Va5	Glc(2,1)Glc	H	$C_{42}H_{72}O_{15}$	a	[327]
86	Ginsenoside Ki	Va6	H	Glc	$C_{36}H_{62}O_{10}$	a	[325]
87	Yesanchinoside R1	Va6	Glc	H	$C_{36}H_{62}O_{10}$	a	[328]
88	Panajaponol A	Va6	Glc(2,1)Glc	H	$C_{42}H_{72}O_{15}$	a	[329]
89	Yesanchinoside R2	Va6	Glc(2,1)Xyl	H	$C_{41}H_{70}O_{14}$	a	[328]
90	Notopanaxoside A	Va7	Glc	H	$C_{36}H_{62}O_{10}$	a	[330]
91	Ginsenoside M7ed	Va7	H	Glc	$C_{36}H_{62}O_{10}$	a	[305]
92	6-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-dammar-25(26)-ene-3β,6α,12β,20S,24R-pentaol	Va7	Glc(2,1)Glc	H	$C_{42}H_{72}O_{15}$	a	[319]
93	Ginsenoside Rh21	Va7	Glc	Glc	$C_{42}H_{72}O_{15}$	a	[331]
94	Ginsenoside Rf2 (20*R*)	Va8	Glc(2,1)Rha	H	$C_{42}H_{74}O_{14}$	c	[332]
95	Floralquinquenoside A	Va9	Glc	H	$C_{36}H_{62}O_{11}$	a	[282]
96	Floralquinquenoside C	Va9	Glc(2,1)Rha	H	$C_{42}H_{72}O_{15}$	a	[282]
97	Ginsenoside SL1	Va11	Glc	H	$C_{36}H_{62}O_{11}$	c	[333]
98	Floralginsenoside Ka	Va11	H	Glc	$C_{36}H_{62}O_{11}$	a	[334]
99	Floralginsenoside C	Va11	H	Glc(6,1)Ara(p)	$C_{41}H_{70}O_{15}$	a	[278]
100	Floralquinquenoside B	Va11	Glc(2,1)Rha	H	$C_{42}H_{72}O_{15}$	a	[282]
101	Floralginsenoside A	Va11	Glc	Glc	$C_{42}H_{72}O_{16}$	a	[278]
102	Ginsenoside Rh11	Va12	H	Glc	$C_{36}H_{60}O_{10}$	a	[276]
103	Vinaginsenoside R25	Va12	Glc	Glc	$C_{42}H_{70}O_{15}$	a	[335]
104	Ginsenoside LS1	Va13	H	Glc	$C_{36}H_{60}O_9$	a	[336]
105	(20*S*,22*E*)-6-*O*-β-D-glucopyranosyl-dammar-22(23),24-diene-3β,6α,12β-triol	Va14	Glc	H	$C_{36}H_{60}O_8$	c	[337]
106	(20*R*,22*E*)-6-*O*-β-D-glucopyranosyl-dammar-22(23),24-diene-3β,6α,12β-triol	Va14	Glc	H	$C_{36}H_{60}O_8$	c	[337]
107	Ginsenoside Rh9	Va19	H	Glc	$C_{36}H_{60}O_9$	a	[279]
108	12, 23-Eproxyginsenoside Rg1	Va19	Glc	Glc	$C_{42}H_{70}O_{14}$	a	[338]
	PPTVb-type						
109	Notoginsenoside SP7	Vb2	Glc	–	$C_{36}H_{62}O_{11}$	c	[339]
110	Notoginsenoside SP8	Vb3	Glc	–	$C_{36}H_{62}O_{11}$	c	[339]
111	Notoginsenoside St1	Vb7	Glc	–	$C_{36}H_{62}O_{10}$	c	[270]
112	Notoginsenoside SP21	Vb8	Glc	–	$C_{36}H_{62}O_{10}$	c	[289]
113	Ginsenoside Rh14	Vb9	Glc(2,1)Rha	–	$C_{42}H_{70}O_{13}$	a	[290]
114	Sanchinoside B1	Vb10	Glc	–	$C_{36}H_{62}O_9$	a	[340]
115	(20Z), 25(OH)-ginsenoside Rg9	Vb24	Glc(2,1)Glc	–	$C_{42}H_{72}O_{14}$	c	[352]
116	Notoginsenoside Ab3	Vb11	Glc	–	$C_{36}H_{60}O_9$	a	[342]
117	Notoginsenoside Ab1	Vb12	Glc	–	$C_{36}H_{60}O_9$	a	[342]
118	(20E), 25(OH)-ginsenoside Rg9	Vb26	Glc(2,1)Glc	–	$C_{42}H_{72}O_{14}$	c	[352]
119	Notoginsenoside T1	Vb15	Glc	–	$C_{36}H_{60}O_{10}$	c	[316]
120	Ginsenoside Rg8	Vb15	Glc(2,1)Rha	–	$C_{42}H_{70}O_{14}$	a	[343]
121	Notoginsenoside T2	Vb16	Glc	–	$C_{37}H_{62}O_{10}$	c	[316]
122	Vinaginsenoside R10	Vb19	Glc	–	$C_{36}H_{62}O_{10}$	a	[18]
123	Vinaginsenoside R11	Vb19	Glc(2,1)Xyl	–	$C_{41}H_{70}O_{14}$	a	[18]
124	(20S)-6-O-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-dammar-20,25-epoxy-3β,6α,12β,24α-tetriol	Vb19	Glc(2,1)Glc	–	$C_{42}H_{72}O_{15}$	a	[319]
125	Ginsenoside SL3	Vb21	Glc(2,1)Rha	–	$C_{42}H_{70}O_{14}$	c	[333]
126	Ginsenoside ST1	Vb22	Glc	–	$C_{36}H_{60}O_{10}$	c	[320]

Table 1 (*continued*)

No.	Compounds	Side-chain variation	R1	R2	M.F.	Source	Ref.
127	Ginsenoside SL2	Vb22	Glc(2,1)Rha	–	$C_{42}H_{70}O_{14}$	c	[333]
128	Notoginsenoside Ab2	Vb23	Glc	–	$C_{36}H_{60}O_{10}$	a	[342]
129	Ginsenoside Rh4	Vb24	Glc	–	$C_{36}H_{60}O_{8}$	bc	[310,344]
130	Ginsenoside Rg9	Vb24	Glc(2,1)Glc	–	$C_{42}H_{70}O_{13}$	c	[341]
131	Ginsenoside F4	Vb24	Glc(2,1)Rha	–	$C_{42}H_{70}O_{12}$	ab	[310,345]
132	Ginsenoside Rg10	Vb25	Glc(2,1)Glc	–	$C_{42}H_{70}O_{13}$	c	[341]
133	Ginsenoside Rk3	Vb25	Glc	–	$C_{36}H_{60}O_{8}$	bc	[299,310]
134	Notoginsenoside T5	Vb25	Glc(3,1)Xyl	–	$C_{41}H_{68}O_{12}$	c	[316]
135	Ginsenoside Rg6	Vb25	Glc(2,1)Rha	–	$C_{42}H_{70}O_{12}$	bc	[310,347]
136	(20E)-Ginsenoside F4	Vb26	Glc(2,1)Rha	–	$C_{42}H_{70}O_{12}$	c	[348]
137	Notoginsenoside St7	Vb27	Glc	–	$C_{35}H_{56}O$	c	[299]
138	27-demethyl-(*E,E*)-20(22),23-dien-3β,6α,12β-trihydr-oxydammar-25-one	Vb28	H	–	$C_{29}H_{46}O_{4}$	a	[287]
139	Notoginsenoside St6	Vb28	Glc	–	$C_{35}H_{56}O_{9}$	c	[299]
140	Notoginsenoside St9	Vb30	Glc	–	$C_{32}H_{52}O_{9}$	c	[299]
141	Notoginsenoside St8	Vb31	Glc	–	$C_{32}H_{52}O_{9}$	c	[299]
142	Notoginsenoside R10	Vb32	Glc	–	$C_{30}H_{50}O_{9}$	a	[349]
143	Ginsenoside Rp4	Vb33	Glc	–	$C_{36}H_{64}O_{8}$	c	[71]
144	Ginsenoside Rp3	Vb33	Glc(2,1)Glc	–	$C_{42}H_{74}O_{13}$	c	[72]

[a]Directly isolated from *Panax* species; [b] Identified as biotransformation metabolites *in vivo*; [c] Prepared by physical and chemical transformation.

ing effects with ginseng, red ginseng showed beneficial effects in immunity improvement, fatigue relief, memory improvement [24]. In order to meet clinical needs better, more than one hundred prescription forms containing red ginseng are developed in China, which were primarily applied for cardiovascular diseases.

With the widespread use as herb medicine, adjuvant and dietary supplement and increasing attention worldwide, the research focusing on efficacy and safety of red ginseng and related RGs products experienced boom development. The clinical trials included but are not limited to cancer, cardiovascular diseases, cognitive & behavior diseases, which are being conducted by different country and institution such as China, South Korea, America [25].

For the cancer treatment, ginsenoside Rg3, as one of the representative RGs, showed significant anti-tumor effect. Previous literature indicated rare ginsenoside Rg3 could serve as a potential inhibitor for angiogenesis by suppressing vascular endothelial growth factor (VEGF) [26]. A clinical trial that recruited 228 hepatocellular carcinoma (HCC) patients indicated the combination therapy using transcatheter arterial chemoembolization (TACE) and Rg3 prolonged overall survival from 10.1 months to 13.2 months comparing with control group, and existed manageable side-effects [27]. Zhu *et al.* executed a comprehensive *meta*-analysis to evaluate the efficacy and safety of TACE with RGs in HCC treatment, and recommend Rg3 as the prior choice for clinical combination therapy [28]. Administration of epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) with Rg3 showed improvement on the progression-free survival and overall survival for non-small cell lung cancer (NSCLC) patients [29].

The red ginseng has a long medicinal history in the treatment of cardiovascular diseases (CVDs), clinical study found red ginseng improved coronary flow reserve (CFR) and increased absolute numbers of circulating angiogenic cells in first ST-elevation acute myocardial infarction (AMI) patients [30]. Another study showed supplementation with Rg3-rich red ginseng is a viable way to prevent cardiovascular disease risk [31]. Unfortunately, in some cases, the RGs showed barely function for the CVDs, and the largest and longest clinical trial (401 participants for 6 months) found administration of compound K did not significantly reduce total cholesterol and fasting plasma glucose [32].

As for the cognitive and behavior diseases, treatment with red ginseng remarkably improved Alzheimer's disease assessment scale (ADAS) and clinical dementia rating (CDR) [33]. Rare ginsenosides also showed clinical potential for other diseases such as liver dysfunction [34], physical performance deficiency [35]. In addition, the clinical pharmacokinetics study provided the evidence for drug administration, for instance, the plasma concentration of ginsenoside Rh2 reached steady state after oral administration of Rh2 twice daily for 5 days, which supported the twice-a-day dosing regimen [36].

Despite that large numbers of research reported the multiple efficacy and molecular mechanism of RGs at the animal and cellular levels, the clinical efficacy of RGs remains to be evaluated strictly. Furthermore, some of the previous clinical data showed inconsistent conclusion, which might resulted from insufficient reliable clinical data, for example, the heterogeneity of dose, duration, and study subjects [37]. Until now, only Shenyi Capsule (containing Rg3) was approved by China FDA for the treatment of lung and liver cancer.

Pharmacological activities of RGs

Potential targets of RGs

Acting on bile acid receptors

Bile acid (BA) receptors include cell surface G protein-coupled receptors (GPCRs), specifically the G protein-coupled BA receptor (TGR5) and nuclear receptors such as the farnesoid X receptor (FXR) [38]. TGR5 plays important roles in regulating energy metabolism, relaxing and refilling gallbladder, maintaining BA, lipid and glucose homeostasis [39]. FXR participates in regulating liver inflammation and regeneration, and counteracting pro-inflammatory and pro-atherogenic responses in cardiovascular diseases [40]. Both TGR5 and FXR have become promising targets for drug discovery and development.

Wu *et al.* reported that 20(*S*) PPT ameliorated thioacetamide (TAA)-induced hepatic fibrosis in mice *via* up-regulating expression levels of FXR while suppressing the expression of P2X7 receptor (P2X7r) and NOD-like receptor thermal protein domain associated protein 3 (NLRP3), confirmed by using a FXR deficiency HepG-2 cell line [41]. Ginsenoside Rh4 restored antibiotic-induced intestinal inflammation *via* upregulating the protein expression of CYP7A1 and increasing bile acids level, which was mediated by blocking the FXR-Fibroblast Growth Factor 15 (FGF15) pathway [42]. Ginsenoside C-K, acted as a TGR5

agonist, increased the glucagon-like peptide-1 (GLP-1) secretion, cyclic adenosine monophosphate (cAMP) levels, and intracellular Ca^{2+} in human enteroendocrine L-cells, verified by using human TGR5 transiently transfected CHO-K1 cells [43]. In our recent study, notoginsenoside Ft1 was identified as a novel agonist of TGR5 but an antagonist of FXR to ameliorate high fat diet-induced obesity and insulin resistance in mice by regulating glucose metabolism, increasing GLP-1 secretion and energy expenditure, particularly, these pharmacological effects could be abolished in a TGR5 knockout (KO) mice [44], and the corresponding bile acid signaling pathway regulated by RGs is showed in Fig. 3A.

Acting on steroid hormone receptors

Estrogen receptors (ER), glucocorticoid receptor (GR) and androgen receptor (AR), as ligand-inducible transcription factors, belonged to steroid hormone receptors (SHRs) superfamily [45]. These receptors participated in the regulation process of sex differentiation, reproduction, immune functions, and metabolism [46]. Ginsenosides showed beneficial hormone-like effects, which were presumably attributed to their structural similarity with the steroid hormones [47]. The effects of ginsenosides on the steroid hormones were shown in Fig. 3B.

Acting on estrogen receptor. Estrogen receptors are recently regarded as pivotal targets in the modulation of reproductive, cardiovascular, and immune physiology, and two subtypes of ERs (ERα and ERβ) have been identified [48]. Ginsenoside Rh1 was reported as an ER agonist in cultured human breast carcinoma MCF-7 cells, which up-regulated the mRNA expression of c-fos and pS2 [49]. The PPD and PPT as two metabolites of ginsenosides increased $[Ca^{2+}]_i$, endothelial nitric oxide synthase (eNOS) phosphorylation and nitric oxide (NO) production in HUVECs, and these effects were abolished by the GR antagonist and ER antagonist [50]. In our previous research, notoginsenoside Ft1 induced endothelium-dependent, NO-mediated relaxations in rat mesenteric arteries by activating ER and GR non-genomic signaling cascade, and phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (Akt) and extracellular signal-regulated kinase (ERK1/2) pathways were also activated in this process [51]. Ginsenoside Rg3 up-regulated nitric oxide production and eNOS phosphorylation *via* estrogen receptor-dependent PI3K and adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) pathway [52]. In some cases, RGs exerted their activities by block ER. PPD alleviated endometriosis by suppressing endometrial stromal cell autophagy and natural killer (NK) cell cytotoxicity, which completed by inhibiting ER [53]. Ginsenoside Rh2 was found to suppress growth of uterine leiomyoma *via* reducing ERα expression and c-Src, along with the increased p38 mitogen-activated protein kinase (MAPK) activity [54].

Acting on glucocorticoid receptor. Glucocorticoid receptor (GR) participated in modulating gene networks, inflammatory and

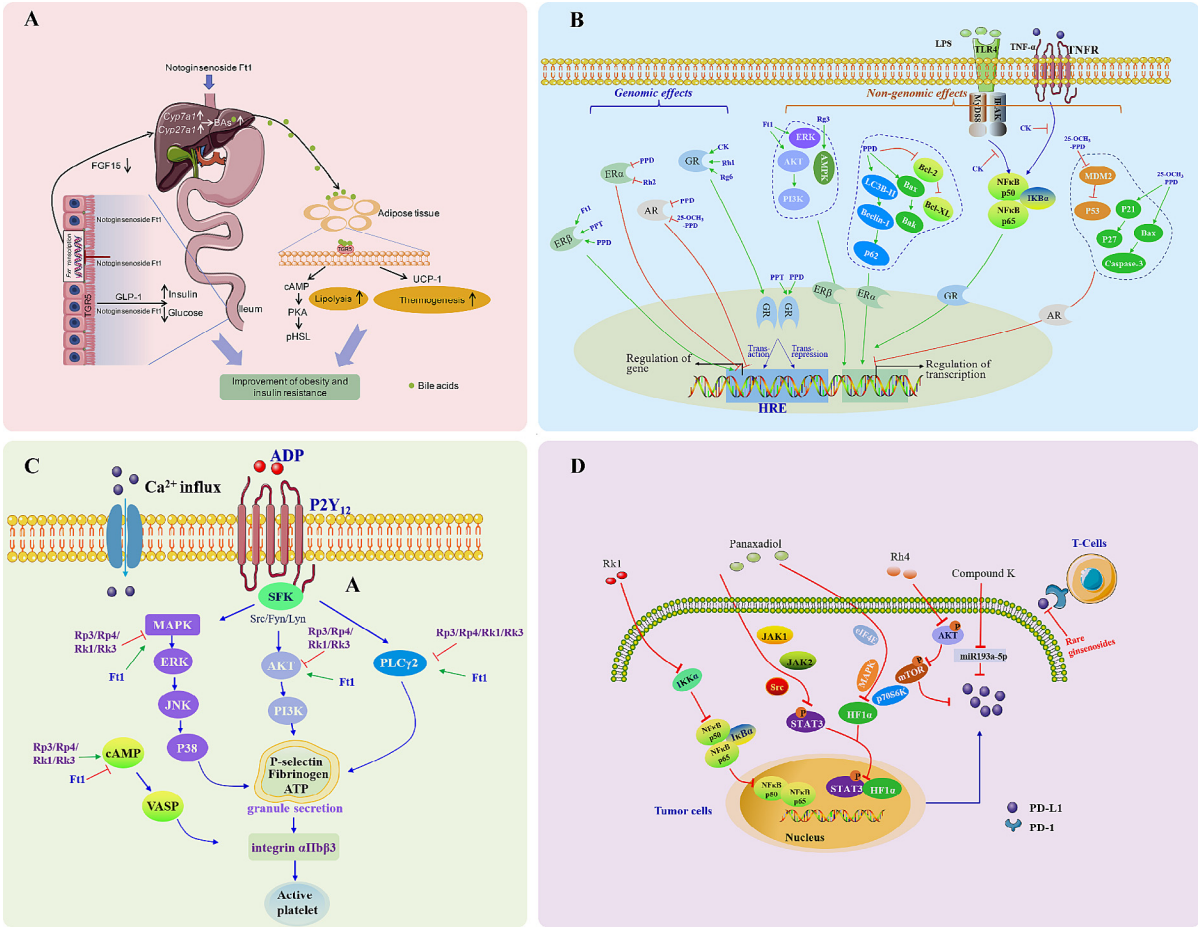

Fig. 3. Bioactivities of representative RGs from Panax species. (A) Targeting the FXR/TGR5 bile acids signaling pathways by RGs in cardiovascular diseases[44]; (B) Mechanism of RGs on steroid hormone receptors; (C) Mechanism of RGs on ADP P2Y12 receptors; (D) Targeting the PD-1/PD-L1 signaling pathways by RGs in cancers.

immune responses, metabolic homeostasis, and some other biological processes [55]. Ginsenoside C-K exhibited superior anti-inflammatory activities by competitively binding to GR with the dexamethasone (a synthetic glucocorticoid) and down-regulating TLR4/LPS-induced nuclear factor kappa-B (NF-κB) and MAPKs [56]. As a GR agonist, C-K could inhibit the reactive oxygen species and thus modulate Dectin-1-dependent inflammatory signaling [57], and protect joint by interfering with TNF-α and TNFR2 mediated synoviocyte function [58]. Ginsenoside Rh1 alleviated the dexamethasone-induced resistance, and enhanced anti-inflammatory effects *via* increasing the expression and binding of GR, while had no effect on the transactivation of glucocorticoid-responsive elements (GRE) driven genes (G6P and PEPCK) that might cause metabolic side-effects [59]. Ginsenoside Rg6 showed protect effect against acute kidney injury and cancer through modulating GR [60,61].

The side-effects of synthetic glucocorticoids predominantly attributed to GR transactivation activity, and it is of importance to develop selective GR agonist dissociating transrepression from transactivation activity. PPD and PPT might be the potent selective GR agonist, which exerted *trans*-repression activity, and suppressed activation of NF-κB, while without transactivation of GR [62].

Acting on androgen receptor. Androgen receptor (AR) plays vital role in modulating series of bioprocess including male sexual development and maintenance, bone density, strength, muscle mass, hematopoiesis, and metabolism [63]. Prostate cancers as the malignant tumor of male genitourinary system developed depending on the transcriptional activity of the AR. Androgen deprivation therapies is now regarded as the classic treatment strategy, which, unfortunately, may lead to deadly castration-resistant prostate cancer (CRPC) caused by amplification, mutations, and variations of AR gene/enhancer [64]. Aiming at developing selective AR modulators, Dong and co-workers revealed that 20(*S*)-PPD suppressed LNCaP xenograft tumor growth by down-regulating both the full-length AR and AR splice variants [65], and further proved that the inhibitory efficacy of 20(*S*)-PPD on castration-resistant regrowth of tumors after androgen deprivation is androgen-independent [66]. Furthermore, 20(*S*)-PPD exhibited synergistical effect with calcitriol on inhibiting growth and inducing apoptosis in human prostate cancer cells [67]. Rare ginsenoside 25-OCH_3-PPD showed significant inhibitory effects on prostate cancer whether it is androgen dependent or androgen independent [68].

Acting on platelet ADP receptors

ADP receptor mediated platelet aggregation plays an important part in regulating hemostasis and preventing blood loss, yet abnormal platelet aggregation can induce stroke and myocardial infarction [69]. Ginsenosides have been reported as candidates for cardiovascular diseases treatment, and their anti-platelet aggregation effects were investigated comprehensively [70]. Ginsenoside Rp4 inhibited ADP-induced platelet aggregation, together with P-selectin expression and weakened binding capacity between fibrinogen and integrin αIIbβ3, *via* down-regulating the phosphorylation of extracellular regulated protein kinases (ERK), p38, c-Jun N-terminal kinase (JNK), PI3K/AKT, and phospholipase $C_{\gamma 2}$ ($PLC_{\gamma 2}$) [71]. Ginsenoside Rp3 showed anti-platelet aggregation effect by increasing cAMP levels and vasodilator-stimulated phosphoprotein (VASP) phosphorylation [72]. In our continuation research on *P. notoginseng*, notoginsenoside Ft1 was identified as $P2Y_{12}$ receptor agonist, showing promotion effect on platelet aggregation induced by ADP, and downstream signaling regulatory such as PI3K and Akt involved [73]. Other RGs such as Rk1 and Rk3 also showed anti-platelet aggregation effect induced by ADP [74,75]. ADP receptor ($P2Y_{12}$)-mediated anti-platelet pathway is illustrated in Fig. 3C.

Pharmacological effects of RGs

Immunoregulatory and adaptogen-like effect

The "adaptogen-like effect" is considered as the characteristic property of ginseng, and means that it can maintain the homeostasis by up- / down-regulating the unbalance induced by internal and/or external factors [76]. The genus name of *Panax* derived from "panacea" (heal-all diseases) may represent the ancient cognition of those herbs. The bidirectional regulation of immune system was considered undoubtedly as the peculiar way of ginseng and ginseng-related products participated in complex pharmacological network [77].

RGs indicated regulatory potency in the innate immunity through activating the effector cells, leading to the up-regulated production of NO and increased cytokines level (IL-6, TNF-α, *etc.*), and enhanced activity of surface co-stimulatory molecules [78]. Red ginseng that rich in RGs could activate the macrophage in RAW264.7 cells [79]. Ginsenoside Rg3 improved cyclophosphamide-induced immunosuppression in Balb/c mice [80], and enhanced the adaptive immunity by increasing the release of IgG and upregulating the CD_3^+ and CD_4^+ expression levels [81]. Nevertheless, excessive immune activation under various stimuli can induce acute or chronic inflammation, and the latter will develop to autoimmune diseases. It was reported that ginsenoside C-K was capable of decreasing proinflammatory cytokines and increasing protective cytokines levels in serum and macrophage culture supernatants, whereas without effecting on IL-4 levels in collagen induced arthritis (CIA) mice and adjuvant-induced arthritis rats [82,83]. Furthermore, ginsenoside Rg3 exhibited remarkable anti-hepatitis B activity by decreasing pro-inflammatory cytokines levels *via* stimulating TNF receptor-associated factor 6 (TRAF6) and transforming growth factor β activated kinase-1 (TAK1) degradation and inhibiting JNK/activator protein-1 (AP-1) signaling pathway [84]. In fact, RGs demonstrated broad spectral bioactivities in the intervention of cancer, osteoporosis, and diabetes *via* immune-modulation effect [76].

Anti-aging effect

Aging refers to physiological degradation process after maturity, or change caused by various external factors including various diseases. Aging has traditionally been considered a "natural" and inevitable process. Nevertheless, recent researches indicated slow aging and increase the healthy lifespan of organisms by interventions with lifestyle changes and pharmaceuticals and natural health products [85].

In Eastern medical theory, ginseng is believed to keep the body light and prolong life, and used for anti-aging by maintaining vigor and vitality. In aging mice model induced by D-Gal, red ginseng exhibited anti-aging effects by decreasing the levels of acetylcholinesterase (AChE) and malondialdehyde (MDA), increasing superoxide dismutase (SOD) and catalase (CAT) expression [86]. Ginsenoside Rh2 alleviated the doxorubicin-induced cellular senescence *via* reducing NF-κB activation, decreasing ROS level and promoting mitophagy, thereby attenuating aging diseases [87]. Ginsenoside F1 and Rg3 were found to suppress astrocytic senescence-associated secretory phenotype in glioblastoma cells [88,89]. Skeletal and cardiac muscle disorders occurred during the aging process, and 20(*R*)-ginsenoside Rh2 was discovered to increase the viability in myoblasts and cardiomyocytes by upregulating Akt1/PKB phosphorylation at serine 473 [90]. Rg3 enhanced the telomerase activity, an important anti-senescence marker, in human osteoarthritic chondrocytes, and decreased the number of SA-beta-Gal-positive cells [91].

Effect on cardiovascular and cerebrovascular system

Cardiovascular and cerebrovascular diseases have become the leading cause of human death, and development of novel drugs and therapies with high potency but minor side effects from herbal medicines have always been concerned [92]. Studies showed rare ginsenosides Rg3, Rk1, Rg5, Rk3, and Rh4 inhibited platelet aggregation and promoted blood circulation through inhibiting thromboxane A2 synthesis, attenuating glycoprotein IIb/IIIa (αIIb/β3) activation, and suppressing MAPKs [70]. Ginsenoside C-K showed great improvement for atherosclerosis through promoting autophagy and regulating NF-κB, p38, JNK, and MAPK signaling [93]. Heart failure commonly accompanied with abnormal Ca^{2+} cycle that was mediated by SUMOylation of SERCA2a, and ginsenoside Rg3 showed protective effect on transverse aortic constriction in mice by regulating the SUMOylation of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a), which could be abolished in SUMO1 KO mice [94]. The insulin-like growth factor 1 (IGF-1)/insulin-like growth factor 1 receptor (IGF1R) axis is of significance for cerebral angiogenesis and neurogenesis in ischemic stroke. Ginsenoside F1 has been revealed as a bioactive compound for improving cerebrovascular function and promoting recovery from ischemic stroke *via* activation of the IGF-1/IGF1R pathway to promote angiogenesis [95].

Effect on central nervous system

Central nervous system diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), and depression are seriously threatening people's life and health. It was found that ginsenoside Rh2 improved performance of memory-related behavior in scopolamine (Scop)-induced memory deficits mice, suggesting Rh2 can be a prospective candidate for Alzheimer's disease [96]. Ginsenoside Rg5 improved the memory and cognitive impairment caused by thermal stress *via* modulating heme-oxygenase-1 (HO-1)/nuclear factor E2-related factor2 (Nrf2) signaling pathway [97]. Ginsenosides Rg3, Rg5 and 20(*S*)-PPD showed antidepressant-like effects in chronic unpredictable mild stress (CUMS) induced- and LPS-induced depression-like model [98–100]. Ginsenoside F2 exhibited notable activity against two glioblastoma cell lines, U373 and Hs683, by inducing mitochondrial impairment *via* disturbing the intracellular redox balance and activating AMPK signaling [101].

Anti-tumor effects

Red ginseng as a steamed product of *P. ginseng* showed a striking anti-cancer potential, of which rare ginsenosides including Rg3, Rh2, Rk1, Rg5, Rk3, Rh4, and aglycones (PPD and PPT) were considered to be the predominant bioactive components [4]. Approved by China FDA in 2003, the Shenyi capsule containing Rg3 was clinically applied to treat various types of cancers [102]. The Rh2 is intestinal metabolite of Rg3, which also exhibited remarkable anti-cancer effect [13], and a phase II clinical trial of Rh2 on the non-small cell lung cancer has been conducted in USA (NCT02714608). As summarized in previous excellent reviews, rare ginsenosides exert the anti-cancer effects *via* some signaling pathways such as MAPK, PI3K/Akt/ mechanistic target of rapamycin (mTOR), wingless (Wnt)/β-catenin, and NF-κB [11,13,103–106]. Recently, immunotherapy against tumor by rare ginsenosides achieved great advancement, while few review have spotlight on it. We herein discussed the programmed death 1 (PD1)/programmed cell death-ligand 1 (PD-L1) immune checkpoint as a potential new target for the cancer immunotherapy of rare ginsenosides (Fig. 3D).

In the tumor microenvironment (TME), multiple oncogenic pathways modulated the transcription of PD-1 and PD-L1 and induce immune evasion. The ginsenoside panaxadiol inhibited PD-L1 expression and tumor proliferation in human colon cancer cells *via* suppressing hypoxia-inducible factor 1-alpha (HIF-1α) and signal transducer and activator of transcription 3 (STAT3) pathway cooperatively. Furthermore, the PD-1/PD-L1 interaction induced killing activity suppression of T cell was restored by panaxadiol [107]. The ginsenoside C-K showed remarkable inhibitory effect on prostate cancer, and the activation of miR193a-5p was primarily responsible for its anti-cancer effect [108]. Ginsenoside Rk1 down-regulated the PD-L1 expression in lung adenocarcinoma cells *via* blocking NF-κB, therefore activating T-cell and inhibiting tumor immune escape [109]. In esophageal cancer, ginsenoside Rh4 suppressed PD-L1 expression by blocking the AKT/mTOR pathway [110]. However, the undesired efficacy of checkpoint inhibitor immunotherapy was partially attributed to the immunosuppressive feature of TME. Reported by Guo and co-workers, ginsenoside Rg3 remodeled the immunosuppressive TME in orthotopic CRC mice *via* inducing immunogenic cell death (ICD), and the effect was enhanced by combination use of anti-PD-L1 and co-formulation (Rg3 + quercetin). In addition, the combined administration strategy up-regulated the immunostimulatory cells and activated dendritic cells [111].

Effects on obesity and diabetes

Obesity has reached epidemic proportions in the past few years and causes several metabolic complications, type 2 diabetes as the primary complication, which accompanied with serious morbidity and increased mortality [112]. It is urgent to develop effective pharmaceuticals with minor side effect for the prevention and treatment of obesity [113].

Kim and co-workers found that fermented *P. notoginseng* (rich in rare ginsenosides) rather than *P. notoginseng* significantly decreased the food and calorie intake, body weight, and total fats in high-fat diet (HFD)-fed mice, and the involved mechanism was suppressing appetite and energy intake related signaling pathways [114]. Ginsenoside Rg2 apparently alleviated obesity phenotype in HFD mice and inhibited adipocyte differentiation in 3 T3-L1 preadipocytes *via* activating AMPK pathway and suppressing the expression of peroxisome proliferator-activated receptor γ (PPARγ), CCAAT enhancer binding protein α (C/EBPα), and sterol regulatory element binding protein-1c (SREBP-1c) [115]. Similarly, ginsenoside F2 and Rg3 inhibited adipogenesis or induced browning of adipocytes through activating AMPK pathway [116,117]. The inflammation participated in obesity development, and ginsenoside C-K decreased the macrophage M1-type inflammatory factor expression in obese mice [118].

Diabetes is a complex chronic systemic disease with the hallmark of hyperglycemia which was induced by a defect in insulin secretion and/or insulin action [119]. Ginseng was traditionally applied to treat "Xiao-ke" (emaciation and thirst) in China that refers to diabetes in modern medicine [120]. Ginsenoside Rk3 improved glucose tolerance and insulin resistance, and ameliorated hepatic gluconeogenesis and lipid accumulation in HFD/streptozotocin (STZ) induced type 2 diabetes mellitus mice by activating AMPK/Akt pathway [121]. In another report, Rg5 significantly attenuated diabetes mellitus type 2 (T2DM) symptoms through enhancing the liver mitochondrial biogenesis and insulin sensitivity [122]. Ginsenoside C-K stimulated GLP1 (the pivotal incretin) secretion in NCIeH716 cells and maintained the capacity of intestinal differentiation [123]. Ginsenoside Rg5 exerted remarkable protective effect against diabetic renal injury through reducing oxidative stress and NLRP3 inflammasome *via* blocking NF-κB and MAPK pathway [124].

Other pharmacological effects

RGs show broad spectral pharmacological activities in addition to the above mentioned. Recently, Fu and co-workers found Rg3 alleviated pulmonary fibrosis caused by bleomycin, and blocked

migration and proliferation of fibroblasts and the epithelial mesenchymal transition (EMT) [125]. Rg3 protected the reproductive toxicity of triptolide (TP) by downregulating miR-26a and alleviating TP-induced apoptosis [126]. In another study, by inhibiting NorA-mediated efflux, co-administration with 20(*S*)-Rh2 enhanced the antibacterial effects of ciprofloxacin [127]. Ginsenoside Rk1 regulated protein expression of Nrf2/HO 1 to alleviate H_2O_2 induced oxidative stress, thereby protecting melanocytes and preventing the development of Vitiligo [128]. Ginsenoside C-K ameliorated auditory functional injury in mice by reducing threshold shifts, central auditory function damage, and morphological deficits [129].

Multiple ingredients, multiple targets and multiple ways were considered as the characteristic of traditional Chinese medicine, and the above-mentioned function of rare ginsenosides strongly supported this theory. We should note that the regulation effect of rare ginsenosides is bi-directional like the activation and inhibition role on immune system. Most of studies focused on single target and ignored the interactions, which need a systems biology method to investigate the holistic effect.

Structure-activity relationship of rare ginsenosides

Modern pharmacological research reported the diverse function of RGs, which showed relation with their distinct chemical structure. We herein summarized the structure – activity relationships (SAR) of RGs, hoping to provide useful information for modifying ginsenoside structures that could increase the bioactivity of RGs. The crucial factors that contribute to the efficacy of RGs included i) number of sugar molecules; ii) sugar linkage; iii) double bonds within C-17 side chain.

The representative SAR of RGs were firstly found in anti-tumor activity, and results showed the naturally occurring ginsenosides (e.g., Rb1 and Rc) possessed negligible anti-tumor effect, while the rare ginsenosides (e.g., Rg3 and Rh2) exhibited potent efficacy. Importantly, the anti-tumor effect increased as the number of sugar moieties in a ginsenoside decreased, and among these ginsenosides, the PPD (aglycone) showed remarkable effect, which could be candidate for tumor treatment [130]. Consistent SAR were also observed for Angiotensin-I converting enzyme (ACE) inhibition [131], P2X7 ligand affinity assay [132], muscle-type creatine kinase test [133]. It is now generally accepted that increasing sugar moieties are prone to attenuate efficacy of ginsenosides. The possible reason was associated with hydrophobic character of ginsenosides that sugar moieties reduced the lipophilicity of the whole ginsenosides, making them difficult to penetrate cell membranes, in addition, the primary transport mechanism of ginsenosides was passive diffusion [70]. Nevertheless, in some cases, the aglycone of ginsenosides without sugar moieties displayed weaken bioactivity. Advanced glycation end products (AGEs) served as the important marker of diabetic complications, and aglycone PPD and PPT exhibited inferior effect with IC_{50} values of 451.45 and 449.23 μM compared with glycosylated rare ginsenosides, indicating that the sugar moiety at C-6 or C-20 position likely acts an critical role in ginsenoside pharmacological effect [134].

Sugar linkage is another factor that influence the bioactivity of ginsenosides. For anti-tumor effect, most of the potential compounds are PPD-type rather than PPT-type, of which sugar moiety connected at C-3 and C-6, respectively. Study found Rh2 (PPD-type) exhibited strong anti-tumor effect than Rh1 (PPT-type) [135]. Chen *et al.* showed C-3 sugar moiety contributed to the longer circulation in vivo and enhanced the tumor active targeting ability of ginsenosides [136]. Compared with PPT-type ginsenosides, the PPD-type ginsenosides exhibited potent function in the field of anti-glycation [134], inhibition of ACE [131], anti-androgen-independent prostate cancer [137]. By applying molecular docking analysis, the underlying mechanism could be explained as the sugar moiety at C-6 increases the steric hindrance of these molecules to target proteins [138].

Apart from the difference of sugar moiety, the modification of C-17 side chain of ginsenosides is indispensable for the efficacy. For the treatment of Alzheimer' s disease, ginsenosides Rk1, Rg5, Rk3, and Rh4 displayed remarkable anti-inflammatory potential than Rh1 and Rg3, which could be attributed to the double bond in carbon-20, 21 or carbon-20,22 [139]. Ma's group found rare ginsenosides with double bond in C-20,22 showed strong anti-tumor effect against HL-60 and Hep-G2 cell lines with the IC_{50} values of 10.32 and 24.33 μM, respectively. In addition, the acetylation at the sugar group enhanced the anti-proliferative effect [20]. Similarly, the function-promotion effect induced by C-20 double bonds was also discovered in the LPS-stimulated bone marrow-derived dendritic cells [140]. It was reported the insertional orientation of ginsenosides into membranes is influenced by the number and site of polar hydroxyl groups, and in some cases, the elimination of the double bonds at C-24/25 could increase bioactivity [141]. By reduction of the double bond at C-24/25 from Rg3, the chemical stable dihydroginsenoside Rg3 (2H-Rg3) was obtained, and it displayed significant anti-platelet aggregation effect than Rg3 [142]. Moreover, addition of hydroxyl at C-25 increased anticancer effect, and 20(*S*)-25-OH-PPD showed strong anti-proliferative effect than PPD [143]. The schematic diagram of structure–activity relationship was shown in Fig. 4.

Superiority in pharmacological activity of RGs than primary ginsenosides

The steamed *P. ginseng*, *P. notoginseng*, and *P. quinquefolius* showed superior efficacy in the treatment of cancer, CVDs, and cognitive disorders than raw ones, which were attributed to the production of rare ginsenosides [1]. In order to validate these results, the bioactivity comparison between natural occurring ginsenosides (primary ginsenosides) and rare ginsenosides were conducted in different research groups. Kim *et al.* found the thermal degradation product of Rd (Rg3, Rk1, and Rg5) rather than itself showed significant inhibitory effect on tumor cell proliferation [144]. For the PPT-type ginsenoside Re, similar enhanced anti-tumor effect was observed for its thermal degradation products that consisted of Rg2, Rg6, and F4 [145]. Compared with the Rb1, microbiota metabolite C-K effectively inhibited cell proliferation and induce cell apoptosis in HGC-27 cells [146]. For the cognitive function improvement, study reported ginsenoside Rh1 and PPT exhibited more potent effect than their precursor ginsenoside Rg1 in improving memory and hippocampal excitability [147]. Ginsenoside Rh1 and 20(*S*)-PPT rather than their precursor Rg1 indicated remarkable anti-inflammatory effects in mice with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. The underlying mechanism included inhibiting TNBS-induced NF-κB activation and restoring TNBS-induced Th17/Treg imbalance [148]. In another study, rare ginsenosides showed better anti-atherogenic activity by blocking leukocyte endothelial interaction and transmigration *via* downregulating NF-κB signaling [149]. In conclusion, the rare ginsenosides showed superior bioactivity in certain disease, which was associated with the bioavailability and membrane permeability, and the accurate mechanism need further investigation.

Toxicity of RGs

Ginseng has a long history of medicinal use, and in China, it is also consumed as food, which showed good safety. Until now, several research have evaluated the safety of rare ginsenosides. The

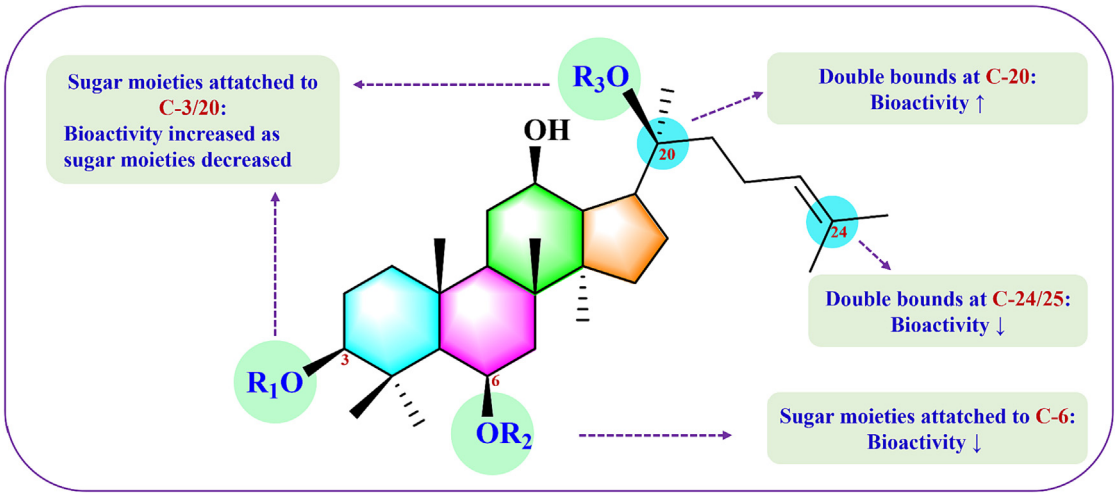

Fig. 4. Structure-activity relationship of rare ginsenosides.

acute and repeated dose toxicity study of 20(*S*)-Rg3 showed LD_{50} in acute toxicity is above 1600 mg/kg and 800 mg/kg in mice and rats, respectively, and the no-observed-adverse-effect level (NOAEL) for female and male SD rats was 180 mg/kg, indicating it possessed well safety [150]. For ginsenoside 25-OCH_3-PPD, the NOAEL was found to be 240 mg/kg/day in beagle dogs [151].

In some studies, the high dose of ginsenosides showed minimal side-effects. Gao *et al*. conducted the subchronic toxicity studies with ginsenoside compound K delivered to dogs *via* intravenous administration. Result showed after administration of C-K, hepatotoxicity was observed in group administrated with 20 and 60 mg/kg/day, and this hepatoxicity might be reversible in the recovery periods [152]. In another toxicity study, 26-week intramuscular repeated administration with 20(*S*)-Rg3 in rats caused increases in the spleen and kidney weights, white blood cell (WBC) counts and in the percentage of neutrophils, but a decrease in the percentage of lymphocytes, with doses of 10.0 or 20.0 mg/kg/day. The NOAEL for rats was considered to be 4.2 mg/kg/day [153]. In conclusions, there is no significant toxicity of ginsenosides at conventional doses, and long usage at high doses could induce minimal reversible side-effects, which should be rigorously investigated in drug development.

Interaction between ginsenosides and gut microbiota

The interaction between drugs including natural products and gut microbiota is complex and bidirectional: the gut microbiota metabolize enzymatically the drugs to modify their structures and concurrently alter their bioavailability, bioactivity or toxicity, and meanwhile, the drugs may impact on the intestinal microecology to influence the physiological function of the host [154]. Ginsenosides have been considered as the prodrugs that undergo bio-transformation in gut after oral administration to produce the lipophilic RGs, such as ginsenosides Rg3, Rh2, C-K, *etc*., showing more potent activity, better membrane permeability and enhanced bioavailability comparing to their precursor saponin [155]. Conversely, ginsenosides including RGs can regulate and reconstitute the homeostasis of the gut ecosystem that impaired in the cases of subhealth or illness [156]. The representative interactions between microbiota and ginsenosides is summarized in Fig. 5.

Metabolic activation of ginsenosides in gut microbiota

It was found that ginsenosides were primarily metabolized to produce RGs by gut microorganisms in colon rather than hydrolyzed under the physiologically gastric acidic conditions [157]. Akao and co-workers reported the first biotransformation of ginsenoside in gut microbiota and ginsenoside C-K was identified after oral administration of ginsenoside Rb1. The transformation rate of ginsenosides in human subjects are dependent on the vitality of the strain in microbial flora [158]. Kim *et al*. found certain types of gut microbiota, such as *Ruminococcus* spp., *Bacteroides* spp. and *Bifidobacterium* spp are critical for the metabolism of Rb1 [159]. Ginsenoside C-K, but not its parent ginsenoside Rb1, exhibited remarkable anti-proliferative and pro-apoptotic activity in colorectal cancer cells [160]. Gut microbiota also mediated the production of the other bioactive RGs including ginsenosides Rg3 [161], Rh2 [162], the aglycons 20(*S*)-PPT [163] and 20(*S*)-PPD [164], which demonstrated superior bioactivity compared with their precursor ginsenosides [165]. The intestinal bacteria participated in the hydrolysis of ginsenosides by the hydrolases they generated, including *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *etc*. [166]. It was reported that prebiotics supplement promoted the abundance of *Prevotella* with glycoside hydrolysis capacity, thus increasing the biotransformation and bioavailability of primary ginsenosides [167].

The impact of RGs on gut microbiota

The healthy adult gut microbiota is dominated by *Bacteroidetes* and *Firmicutes*, and *Actinobacteria*, *Proteobacteria*, and *Micrococcus verrucosa*, as well as methanogenic archaea, eukaryotes, and various bacteriophages [168], which maintains a dynamic balance under normal physiological condition, whereas a disorder of microbiota occurs in the onset and development of multiple diseases such as cardiovascular and immune diseases [169,170]. Ginsenosides exert promotion or inhibition effects on gut microbiota, thereby mediating their beneficial role on host. Oral administration of ginsenoside Rh4 regained the abundance of beneficial bacteria phyla *Bacteroides* and ameliorated the antibiotic-induced intestinal inflammation [42]. Ginsenoside Rk3 restore the decrease of the prebiotic *Bacteroidetes*, *Lachnospiraceae*, *Bifidobacteriaceae, Akkermansia, and Lactobacillus* in cancer diseases. By targeting the gut-liver axis, ginsenoside Rk3 suppressed hepatocellular carcinoma development [171].

Recently, the correlation between gut microbiota and immune regulatory factors including immunoglobulin A (IgA) and antimicrobial peptides in homeostasis and pathology have been highly concerned [172,173]. The 20(*S*)-ginsenoside Rh2 could ameliorate T-cell acute lymphoblastic leukemia associated with gut microbiota

and immune system. It could improve intestinal homeostasis through up-regulating tight junction proteins levels, antimicrobial peptides and IgA, and *Bacteroidetes*, *Verrucomicrobia*, *Akkermansia*, and *Lactobacillus* [174]. Ginsenoside Rh4 considerably ameliorated the colonic tissue damage induced by antibiotics intake through up-regulating the tight junction proteins expression of zonula occludens-1 (ZO-1), Occludin, and Claudin-1, thereby rectifying the abnormal increased ratio of Firmicutes/Bacteroidetes [42].

It was found that the increased microbiota by ginsenosides administration acted a beneficial role in human health. For instance, the abundance of genus *Bacteroides* is lower in patients with coronary artery disease, and treatment with live *Bacteroides vulgatus* significantly attenuated atherosclerotic lesion formation in atherosclerosis-prone mice and inhibited proinflammatory immune responses [175]. The *Bacteroides* participated in stimulation, development, and homeostasis of the immune system and prevention of bacterial and viral infections [176]. In recent year, *Akkermansia muciniphila* has been recognized as the next-generation beneficial microorganisms. After two decades of research, the critical role of *Akkermansia muciniphila* in different diseases including obesity, diabetes and liver steatosis has moved from correlations to causality [177]. In a representative study, higher gene richness and *Akkermansia muciniphila* abundance was found in subjects with the healthiest metabolic status, particularly in fasting plasma glucose, plasma triglycerides and body fat distribution. In order to validate these correlations, mice and humans with obesity was administrated with this type strain, and the high-fat diet-induced metabolic disorders were reversed after treatment [178]. Taken together, some typical microbiotas have been validated as the beneficial supplements in the treatment of various diseases, and ginsenosides could restore the decreased abundance of such microbiota, and eventually ameliorated symptoms.

Stereoisomeric characteristics of RGs

Different bioactivities of stereoisomers

As mentioned above, the steaming- or acidic hydrolysis-induced deglycosylation of ginsenoside at C-(20)-hydroxyl group

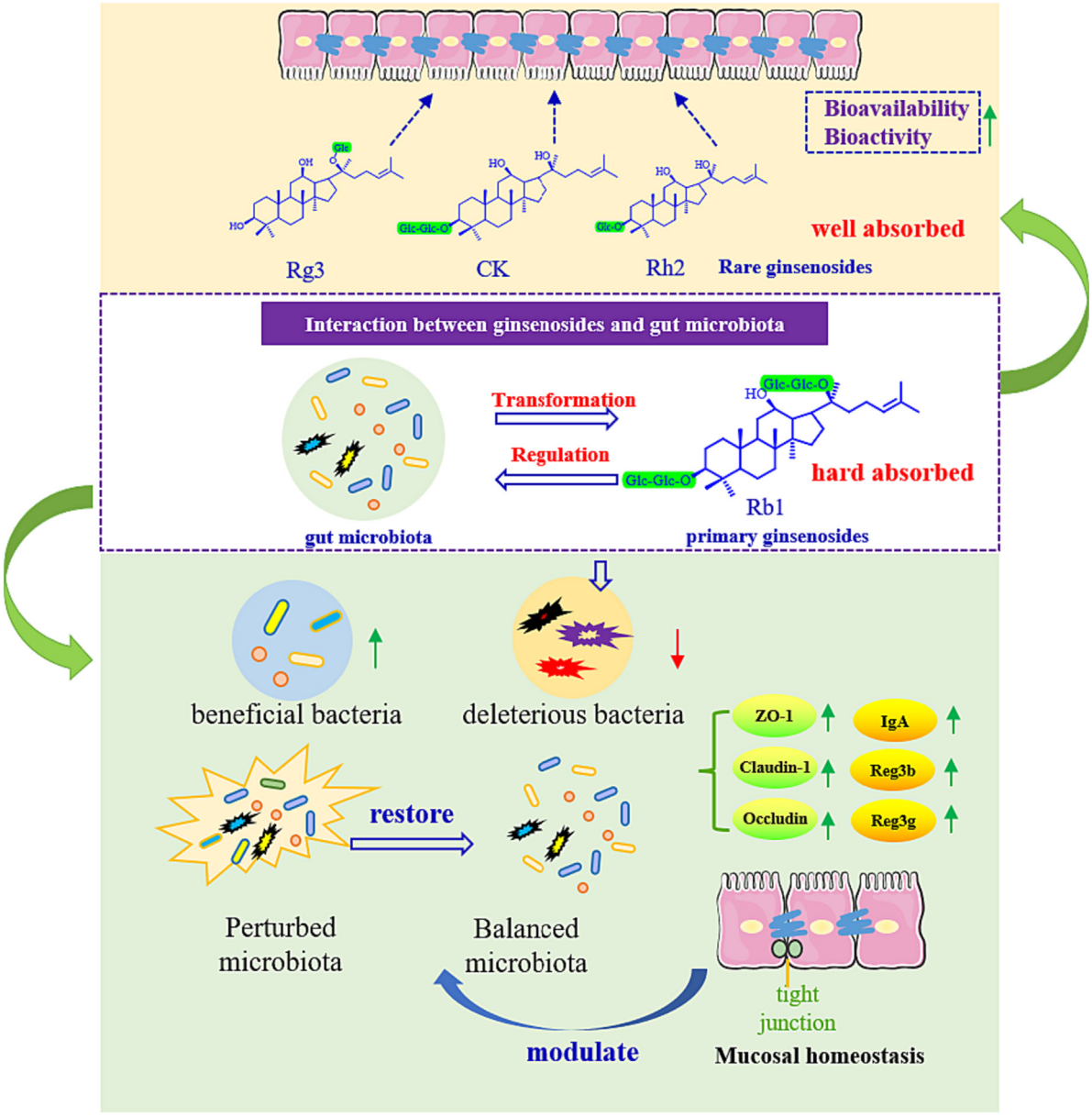

Fig. 5. The interaction pattern between ginsenosides and gut microbiota.

normally lead to the epimerization from 20(*S*)-form to the 20(*R*)-form of RGs, such as 20(*R*)-Rg3, 20(*R*)-Rh2, and 20(*R*)-Rs3 [3]. The stereochemistry-driven difference in bioactivities is mostly focused on the 20(*S*/*R*)-Rg3 and 20(*S*/*R*)-Rh2 epimers.

A stereoisomer-specific antitumor assay revealed the 20(*S*)-forms rather than 20(*R*)-forms of Rg3 and Rh2 possessed more potent antitumor effect in hepatic carcinoma HepG2 cells [179]. 20(*S*)-Rh2 rather than 20(*R*)-Rh2 suppressed colorectal cancer cell invasion through targeting Janus kinase 2 (JAK2)/STAT3 pathway [180]. Nevertheless, some opposite results demonstrated the 20(*R*)-ginsenosides rather than 20(*S*)-epimers showed stronger antitumor effect. For instance, 20(*R*)-Rg3, but not 20(*S*)-Rg3, significantly inhibited the tumor growth rate and enhanced cellular immunity [181]. 20(*R*)-Rh2 and 20(*R*)-Rg3 exhibited more potential effect in cancer treatment than their 20(*S*) forms [182,183].

In an angiogenesis inducing activity experiment, 20(*S*)-Rg3 exhibited 10-fold higher the PPARγ agonist activity than its 20(*R*) forms [184]. 20(*S*)-Rg3 also displayed stronger effect than 20(*R*)-Rg3 in inhibiting vascular smooth muscle cell proliferation and migration, and reversing replicative senescence of human diploid fibroblasts [185,186]. 20(*S*)-Rg3, but not 20(*R*)-Rg3, was found to possess the ion channel regulatory [187,188], antiviral [189], hydroxyl radical-scavenging activities [190]. On the contrary, 20(*R*)-Rg3 rather than 20(*S*)-Rg3 exhibited more potent immune promoting activity [191] and inhibition ability of oxidative stress [192]. Interestingly, the 20(*S*/*R*)-epimers even possessed the opposite bioactivity. 20(*R*)-Rh2 was found acting as an inhibitor, whereas its 20(*S*)-isomer as an inducer, of the ADP-induced platelet aggregation [193].

The stereoisomeric structure–activity relationship of RGs and the underlying mechanism was proposed. The tight hydrophobic packing near the chiral center could be critical factor that influenced the stereospecific function of 20(*S*)-Rg3 in the modulation of voltage-dependent Na^+ channel activity [187]. A docking analysis found that the chiral center of 20(*S*)-Rg3 forms a critical hydrogen bond with Tyr473 of PPARγ ligand binding domain, while 20(*R*)-Rg3 cannot interact optimally with Tyr473 for its sterically strained binding pocket [184]. For the converse effect of 20(*S*)- and 20(*R*)-Rh2 epimers on ADP induced platelet aggregation, the inducing effect of 20(*S*)-Rh2 is attributed to the hydrogen bonding with Asp266 *via* the C-20 hydroxyl, whereas interactions with Tyr105 is responsible for the inhibitory effect of 20(*R*)-Rh2 [193]. Taken together, binding site with amino acid residues and hydrophobic interactions might contribute to the favorable target affinity of 20(*S*)-ginsenosides. Although 20(*R*)-ginsenosides possessed prefer bioactivities in some researches, no stereospecific molecular mechanism have been reported for their phenotype. The configuration dependent differences of RGs in bioactivities displayed in Table 2.

Approaches for separation and identification of RGs

The production process of RGs will bring out isomers, and the similar molecular structures and fragment ions make it burdensome to conduct a delicate phytochemical analysis for rare ginsenosides isomers. Benefitting from the rapid development of analytical technique and strategy, great breakthrough have been achieved for separation and identification of isomers [194]. Nuclear Magnetic Resonance (NMR) spectroscopy could distinguish structures of stereoisomers of ginsenosides based on characteristic chemical shifts of the target peaks. It must be note, however, that only suiting for analysis of pure compounds not mixtures limited its application in ginsenosides characterization [195]. By equipping High Performance Liquid Chromatography (HPLC) with diode array detector (DAD), rare ginsenosides 20(*S*/*R*)-Rh1, 20(*S*/*R*)-Rg3, Rk1, Rg5, Rg6, F4, and Rk3 were successfully quantitatively analyzed in 70 min [196]. In the black ginseng samples, 19 rare ginsenosides were simultaneously quantified by HPLC–Evaporative Light Scattering Detector (ELSD) by adding acetic acid as modifier [16]. To address the dilemma of isomers separation, Ning *et al.* established the multiple heart-cutting (MHC) 2D-LC platform for the separation of ginsenosides isomers [197].

Mass spectrometry (MS)-based analytical approaches including Quadrupole- Time-of-flight (Q-TOF), Ion trap-Time-of-flight (IT-TOF), Quadrupole-Orbitrap (Q-Orbitrap), and Quadrupole-Trap (Q-Trap) acted important part in the multi-component quantification and characterization of ginsenosides [14]. To investigate the pharmacokinetic manner of red ginseng, a specific and reliable method for simultaneous quantification of twenty-four ginsenosides was established using Ultra-Fast Liquid Chromatography-Tandem Mass Spectrometry (UFLC–MS/MS), and ginsenoside isomers were well separated by multiple reaction monitoring (MRM) scan mode [198]. The UPLC-Q-TOF-MS/MS-based metabolomic method was applied to reveal chemical profile change of notoginseng during steaming, and the identified rare ginsenosides profile (Rg3, Rh1, Rh2, *etc.*) was beneficial for the quality evaluation of steamed notoginseng [199].

Accurate structural identification of ginsenosides is a sophisticated task because of their numerous isomers. Song and coworkers interpreted the fragmentation pathways by assigning the accurate mass of each fragment ion after collision induced dissociation (CID), which greatly facilitated the structure deduction

Table 2
The C-20 configuration dependent differences of RGs in bioactivities.

Pharmacological difference between two stereospecific forms	Dominant configuration	*In vivo*/*In vitro*	Ref.
Inhibiting the growth of HepG2 hepatocarcinoma cells. LD_{50}: 20(*S*)-Rg3 (45 μM), 20(*R*)-Rg3 (invalid)	20(*S*)	in vitro	[179]
Inhibiting the growth of SKOV3 and 3AO ovarian cancer cells. IC_{50}: 20(*S*)-Rg3 (146.8 and 242.6 mg/mL, respectively), 20(*R*)-Rg3 (higher than 640 mg/mL)	20(*S*)	in vitro	[350]
The PPARγ agonist activity. 20(*S*)-Rg3 was 10-fold higher than 20(*R*)-Rg3	20(*S*)	in vitro	[184]
The antiviral activity against MHV-68. IC_{50}: 20(*S*)-Rg3(10.82 μM), 20(*R*)-Rg3 (25.24 μM)	20(*S*)	in vitro	[189]
Hydroxyl radical-scavenging activity. IC_{50}: 20(*S*)-Rg3 (0.51 mM), 20(*R*)-Rg3 (invalid)	20(*S*)	in vitro	[190]
Regulating voltage-dependent Na^+ channel activity. IC_{50}: 20(*S*)-Rg3 (6.1 μM), 20(*R*)-Rg3 (invalid)	20(*S*)	in vitro	[187]
Inhibiting oxidative stress induced by cyclophosphamide in mice. 20(*R*)-Rg3 was more potent than 20(*S*)-Rg3	20(*R*)	in vivo	[192]
Inhibiting H22 tumor growth. Inhibition rate: 20(*S*)-Rg3 (23.6 %), 20(*R*)-Rg3 (40.9 %)	20(*R*)	in vivo	[181]
The adjuvant effect on OVA-induced immune responses. 20(*R*)-Rg3 promoted higher response for IgG, IFN-g and IL-5 than 20(*S*)-Rg3	20(*R*)	in vivo	[191]
Inhibiting the growth of HepG2 cells. LD_{50}: 20(*S*)-Rh2 (10 μM) 20(*R*)-Rh2 (invalid)	20(*S*)	in vitro	[179]
Inhibiting the growth of HCT116 CRC cells. IC_{50}: 20(*S*)-Rh2 (19.6 μM), 20(*R*)-Rh2 (higher than 30 μM)	20(*S*)	in vitro	[180]
Regulating platelets aggregation induced by ADP. 20(*S*)-Rh2 (promotion effect), 20(*R*)-Rh2 (inhibition effect)	20(*R*)	in vitro	[193]
The antiviral activity against MHV-68. IC_{50}: 20(*R*)-Rh2(2.77 μM), 20(*S*)-Rh2 (invalid)	20(*R*)	in vitro	[351]

[200]. Silver ion as the newly alternative transition metal ion could modify the fragmentation pathways of ginsenosides, and the ion intensity of fragment ions $[M + Ag\text{-}H_2O]^+$ generated in enantiomer-selective manner, and therefore these enantiomeric ginsenosides could be unambiguously identified in this way [201]. Electron-induced dissociation (EID) could activate ions by electronic excitation that provides more structural fragment ions of ginsenosides such as glycosidic and cross-ring cleavages, which accelerated the structure recognition [202]. Furthermore, energy-resolved mass spectrometry (ERMS) was another novel method for isomer discrimination, which explicated the linkage manners among those substructures [203]. Guided by this strategy, ginsenoside isomers such as Rg3 and F2 were successfully identify according to their different intensity of the fragment ions [204].

In addition to MS, ion mobility spectrometry (IMS) could capture structural heterogeneity of analyte populations according to their specific collision cross section (CCS) value [205]. Characterization methods of the multi-components in *Panax* species by LC-IM-MS were established recently by different research groups [206–209]. Particularly, the four-dimensional separations strategy (2D-LC, IMS, and MS) was built to efficiently and reliably identify the components in white ginseng and red ginseng, and the isomers including optical and geometric stereoisomers were apparently differentiated by their distinct Collision Cross-Section (CCS) values [207].

Transformation of RGs

Physically and chemically induced transformation

Steaming-induced transformation

Steaming after harvest of the crude drugs has been an age-old field processing technology in traditional Chinese medicine aimed at improving or modifying the drug properties, and widely applied in processing of ginseng plants including *P. ginseng* [4], *P. notoginseng* [210], and *P. quinquefolius* [211], as well as their leaves [15], flowers [212], and berries [213]. It has been proven that steaming could induce the transformation of primary ginsenosides such as Rb1, Rg1, Rd, Re, *etc.* in the crude ginseng into the rare saponins such as 20(*S*/*R*)-Rg3, Rk1, Rg5, *etc.* by deglycosylation, deoxidation and dehydrogenation [214,215]. Baking is also used for processing the crude ginsengs but with lower transformation efficiency of ginsenosides compared with steaming [212]. In fact, the steaming is a dual process of high temperature and humidity which is more conducive to the hydrolysis of ginsenosides, for instance, the fresh *p. notoginseng* rather than the dried one showed higher transformation efficiency of ginsenosides at the initial steaming stage [216].

Kim and co-workers explored the influence of temperature on transformation of ginsenosides, and the result showed that the RGs including Rg3, Rg2, Rh1, and Rh2 were less generated when being steamed under the temperature of 80 °C or 90 °C for 6 h, yet those RGs markedly increased at 100 °C [217]. In another study, the change of the primary ginsenosides Rf, Re, Rg1, and Rb1 was not obvious at 100 °C–3h steaming of ginseng, whereas ginsenosides Rg1 and Re could not be detected at a 120 °C–3h process, indicating that the conversion reaction is temperature dependent [214].

The duration time of steaming is another factor impacting the conversion of ginsenosides. In a steaming process of *P. notoginseng* at 100 °C from 0 to 48 h, the concentration of the rare ginsenosides 20(*S*/*R*)-Rh1, Rk3, Rh4, 20(*S*/*R*)-Rg3, Rk1, and Rg5 increased gradually [216]. The proliferation inhibition of colorectal cancer cells by an extract of steamed *P. notoginseng* root could be enhanced with the steaming time increased, which was explained by the increased production of Rh1, 20(*S*/*R*)-Rg2, Rg3 and Rh2 during steaming [210].

pH-dependent transformation

Acid or alkali catalyzed hydrolysis has been applied to improve the conversion efficiency of rare ginsenosides. Organic acids, such as citric acid, succinic acid and formic acid were commonly used and possessed different conversion ratio of ginsenosides [218]. Formic acid could transform ginsenosides Re, Rg2, and Rf into rare ginsenosides F1, 20(*R*/*S*)-Rh1, Rf2, Rg6, F4, and Rg9 [219]. Heteropolyacids, novel catalytic agents, exhibited efficient performance in transforming ginsenosides, and the catalytic efficiency of $H_4SiW_{12}O_{40}$ is ca. 410 times higher than that of formic acid when using Rg1 as substrate [220]. Amino acids, as the bioactive components in *Panax* genus, also have remarkable impact on ginsenosides transformation, and acidic amino acids possessed a higher transformation yield than neutral amino acids and basic amino acids [15,221].

Alkali catalyzed hydrolysis is also used to yield RGs and has advantage of not changing the configuration of the aglycone skeletons at C-20. It was reported that primary ginsenosides were configuratively transformed into 20(*S*)-ginsenosides including 20(*S*)-Rh1, 20(*S*)-Rh2, 20(*S*)-PPT, and 20(*S*)-PPD under the reaction condition by setting the temperature at 200 °C for 40 min, with addition of NaOH 2.0 g [222].

Apparently, the steaming and acid or alkali catalyzed hydrolysis of ginsenosides demonstrated low cost and high yield production of RGs with rich structural diversity including a series of epimers and side chain modifiers, which is beneficial for new lead compounds discovery and drug candidates development [210,223]. Of course, their disadvantages are also obvious in unstable process, complex products and environmental pollution as well. The proposed transformation pathway of PPD- and PPT-type ginsenosides by physico-chemical methods were shown in Fig. 6.

Biotransformation

Biotransformation is now considered as the most prospective strategy for RGs production for the high specificity, mild reaction conditions, and remarkable conversion efficiency [224]. Isolated from ginseng roots soil, the microbial strain GS514 exhibited remarkable capacity to transform ginsenoside Rb1 or Rd into Rg3 [161]. Endophytes living in the healthy plants possessed the ability to produce secondary metabolites [225]. In one study, the endophytic bacteria strain PDA-2 isolated from ginseng showed potential activity to produce Rg3 and Rh2 [226]. The human intestinal bacteria were widely applied to produce rare ginsenosides including *Lactococcus lactis* (producing Rg3, C-K) [227], *Bifidobacterium sp.* (producing Rh2, C-K) [228]. In addition, Mushroom *Schizophyllum commune* (producing C-K, C-Mc, C-Y) [229], *Aspergillus niger* (producing 20(*R*/*S*)-PPD) [230], and *Lactobacillus paralimentarius* (producing C-K) [231], attracted extensive attention in recent years.

Aiming at specific transformation of the individual RGs, glycosidases from different sources were screened for selectively hydrolyzing sugars at different positions to yield single rare ginsenosides [232]. The targeted transformation of Ginsenoside C-K, a potent anti-tumor molecule identified from the in vivo metabolite of PPD-type ginsenosides caused great interest recently [165]. Ginsenoside Rb1 with high natural abundance might be the most ideal substrate for production of ginsenoside C-K by two classical pathways: Rb1 → Rd → F2 → CK, and Rb1 → gypenoside XVII → F2 → CK [233]. Recently, a novel and thermostable glycosidase identified and purified from *Caldicellulosiruptor bescii* shows high capacity of completely converting all PPD-type ginsenosides into ginsenoside C-K in a transformation pathway: Rb2/Rb3/Rc → Rd → F2 → CK [233]. It was found that the outer glucose linked

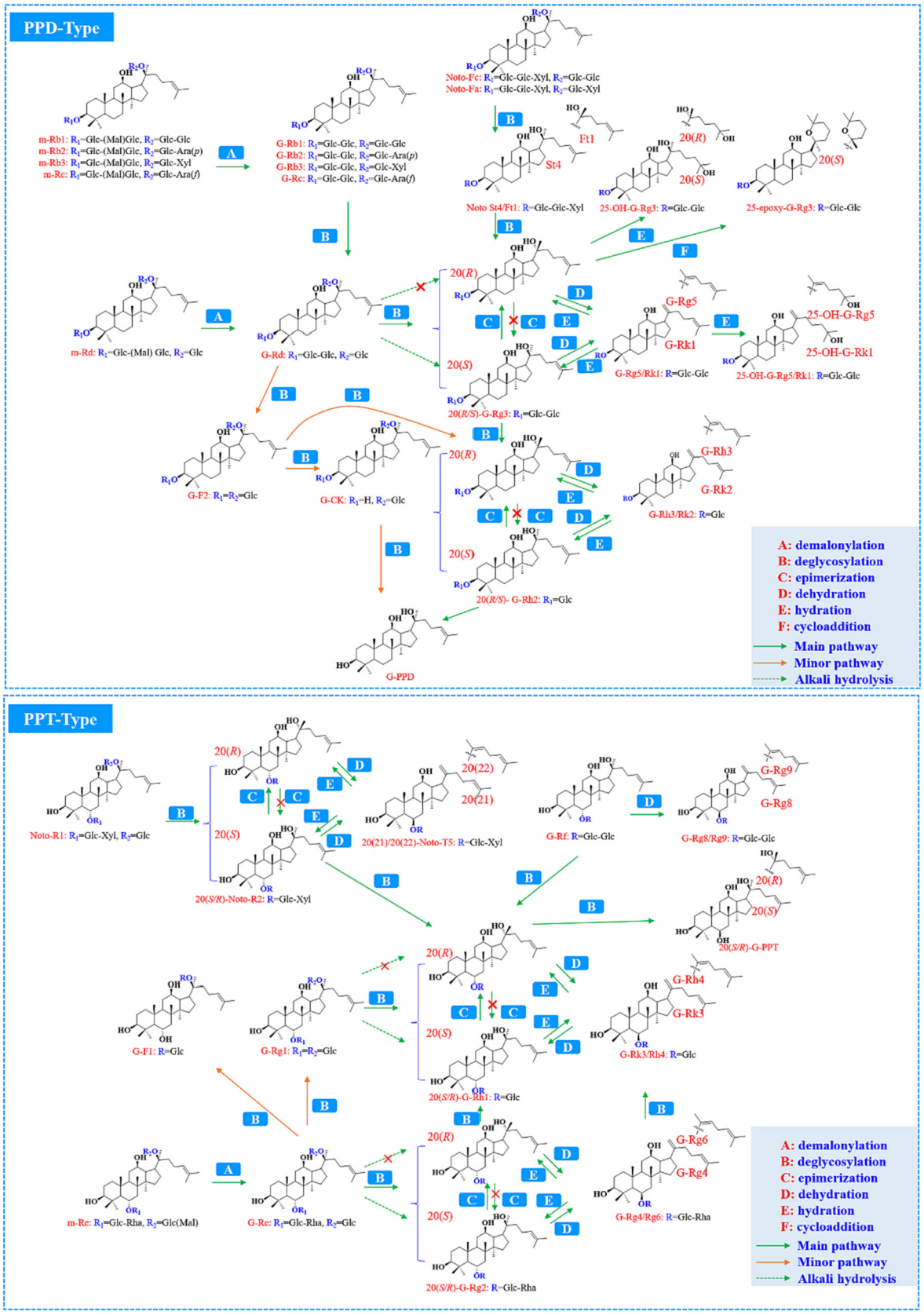

Fig. 6. The potential transformation pathway of ginsenosides during physical and chemical processing.

to C-3, but not C-20, are more prone to be hydrolyzed by the following new transformation pathways such as Rb3 → CMx1 → CMx → CK, Rc → CMc1 → CMc → CK, and Rb2 → CO → CY → CK [234]. The directional biotransformation of ginsenoside Rh2 (another anti-tumor candidate) are also attracted wide attention, yet the scale-up production of Rh2 is still a challenge [235].

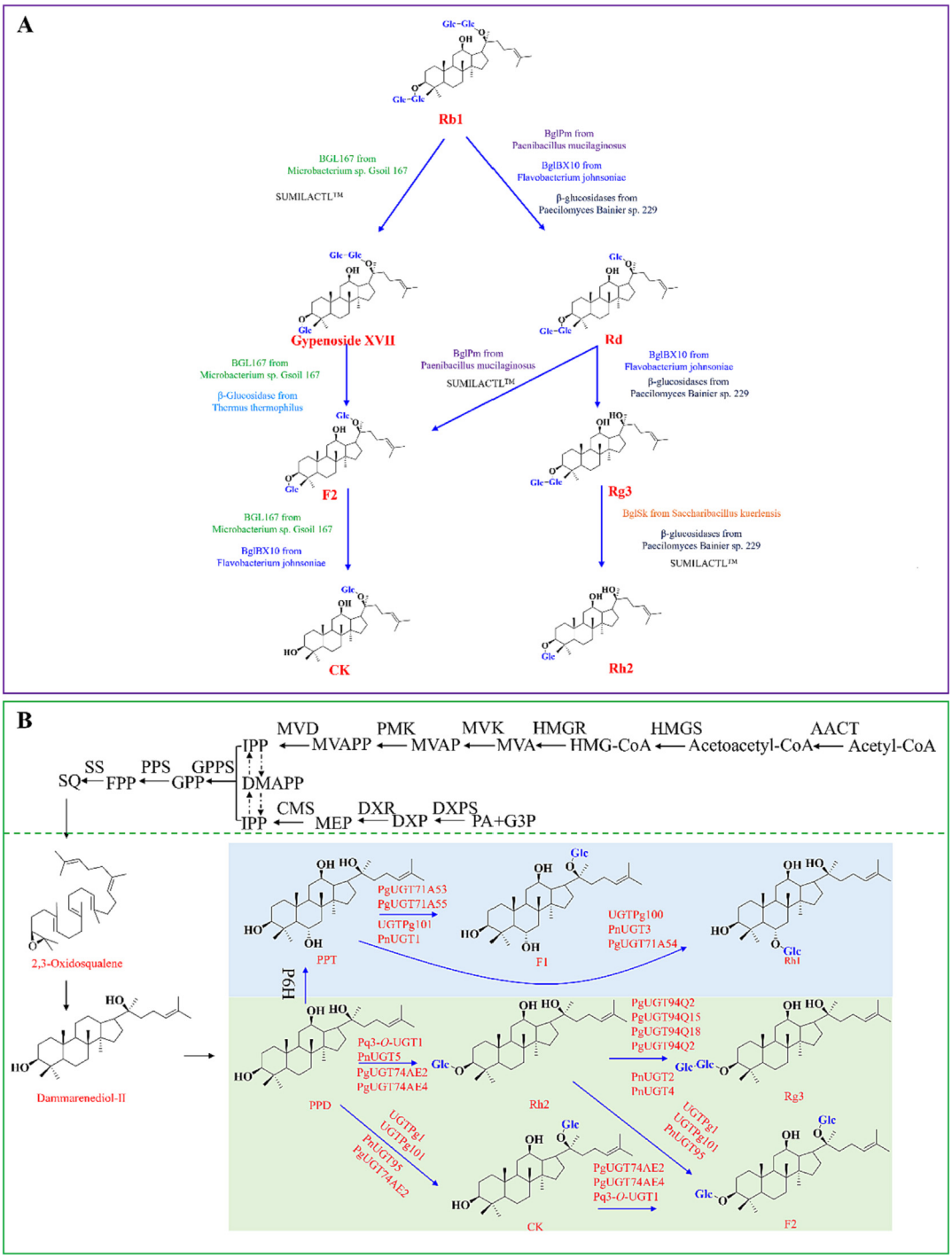

Fig. 7. The potential transformation pathway of rare ginsenosides mediated by biotransformation (A) and biosynthesis (B).

In one representative study, the glycoside hydrolases Abf22-3 and BglBX10 were found to convert Rb1 to Rg3, and then Rg3 was completely transformed into Rh2 by BglSk [236]. The aglycon protopanaxadiol could be produced by hydrolyzing the remained glucose of CK or Rh2 [230,237]. Our group recombinated a new glycoside hydrolase isolated from *Herpetosiphon aurantiacus* that was capable of transforming vina-ginsenoside R_7 into the rare notoginsenoside ST4 [238]. For the production of PPT-type RGs, one recombinant glycoside hydrolase (BglPC28) is capable of producing Rg2 using Re as the substrate [239]. Moreover, a gram-scale production of PPT from Re and Rg1 was achieved by using recombinant β-glucosidase (BgpA) [240]. The potential transformation pathway of the representative RGs (C-K and Rh2) by microorganisms and glycosidases were shown in Fig. 7A.

Biosynthesis

The above-mentioned transformation approaches of RGs are actually the glycoside hydrolysis, called "top-down", the biggest disadvantage is that the substrate still needs to be obtained naturally. In past decade, synthetic biology achieved great progress in the manufacture of some medicinal natural products owing to its

Table 3
The comparison of different production strategies of rare ginsenosides.

Methods	Advantage	Limitation	Reaction characteristics	Main production pathway
Physical method	Low cost, easy-to-operate, enriching the structure diversity, keeping the morphological characteristics	Low conversion yields, poor product uniformity	*R/S* epimerization; Preferentially hydrolyzing the sugars at C-20	Rb1 → Rd → 20(*R/S*)-Rg3 → Rk1/ Rg5 → Rk2/Rh3; Rg1 → 20(*S/R*)-Rh1 → Rk3/Rh4
Chemical method	Fast reaction rate, production of *R*-configured ginsenosides	Safety concern of side reactions, environmental pollution	Acid hydrolysis: *R/S* epimerization; Alkaline hydrolysis: Retaining stereo configuration; Preferentially hydrolyzing the sugars at C-20	Acid hydrolysis: Rb1 → Rd → 20(*R/S*)-Rg3 → Rk1/ Rg5 → Rk2/Rh3; Rg1 → 20(*R/S*)-Rh1 → Rk3/Rh4 Alkaline hydrolysis: Rb1 → Rd → 20(*S*)-Rg3 → 20(*S*)-Rh2 → 20(*S*)-PPD; Rg1 → 20(*S*)-Rh1 → 20(*S*)-PPT
Biotransformation	High specificity, high conversion yields	High cost, strict requirements for food grade microbe, difficult purification procedures	Retaining stereo configuration; Selectivity for the position of hydrolysis of sugars	Rb1 → Rd → F2 → CK; Rb1 → Rd → 20(*S*)-Rg3 → 20(*S*)-Rh2; Re → 20(*S*)-Rg2; Rg1 → 20(*S*)-Rh1
Biosynthesis	High specificity, *de novo* synthesis, environmentally-friendly	Identified and characterized UGTs are limited, low catalytic activity	Retaining stereo configuration; Selectively transferring sugar moieties to the linking position of sapogenins	PPD → 20(*S*)-Rh2 → 20(*S*)-Rg3; PPD → CK → F2; PPT → 20(*S*)-Rh2; PPT → F1

de novo synthesis capacity, referred to as "down-top" [241]. More than 20 consecutive enzymatic reactions have been identified for the biosynthesis of ginsenosides, which have been summarized in our previous reviews [2,242]. Herein the biosynthesis of hot rare ginsenosides is briefly discussed. By using a one-pot reaction, the biosynthesis of C-K was successfully achieved by Zhou and co-workers, and UGTPg1, the first characterized UGT for glycosylation of tetracyclic triterpenoid substrates from plants, was the key enzyme for this process [243]. Subsequently, Zhou's group discovered four novel UGT-encoding genes (UGTPg100, UGTPg101, UGTPg102, and UGTPg103) from *P. ginseng*, among them UGTPg100 specifically glycosylates the C6-OH of PPT to generate 20(*S*)-Rh1, while UGTPg101 catalyzes PPT to produce F1 [244]. By sequencing and assembling the ginseng transcriptome *de novo*, two new UDP-glycosyltransferases were characterized, of which the PgUGT74AE2 catalyzed the C3 hydroxyl groups of PPD and compound K to produce 20(*S*)-Rh2 and F2, whereas PgUGT94Q2 catalyzed 20(*S*)-Rh2 to produce 20(*S*)-Rg3 [245]. Our group found the GT95^{syn} was capable of catalyzing 20(*R*)-PPD and 20(*R*)-PPT to generated 20(*R*)-CK and 20(*R*)-F1, which could meet the demands for the production of 20(*R*)-form ginsenosides [246]. The biosynthesis pathways of RGs were shown in Fig. 7B. Importantly, the comparison of different production strategies of RGs was summarized in Table 3.

Strategy for batch preparation of RGs

To prepare the desired rare ginsenosides, the transformation conditions were optimized by Sun's group, and eleven single ginsenosides were treated for nine steaming cycles and ginsenosides 20(*R/S*)-Rg3, Rk1, Rg5, Rh2, Rk2, and Rh3 were produced from Rb1/ Rc/ Rb2/Rd, whereas Rk3, Rh4, Rg6, and F4 were degraded from Rg2 [215]. The transformation mechanism of ginsenosides by physico-chemical could be concluded as deglycosylation, dehydration, epimerization, and hydration reactions. It should be note that carbocation acted as a critical role in these process, which is the rate-determining step in the generation of 20(*R*)-ginsenosides which are nonexistent naturally [247]. Meanwhile, the relationship between transformation efficiency and saponin structures was also discussed, indicating that C-20 sugar moiety exhibited most thermally unstable, followed by C-6 and then C-3 sugar moiety [248]. Vo *et al.* conducted kinetic study for the Rg3 production from Rb1, and results showed the degradation rate constants of Rb1 and Rg3 were 0.013 h^{-1} and 0.073 h^{-1} at 80 °C, and the corresponding rate constants were 0.045 h^{-1} and 0.155 h^{-1} at 100 °C, demonstrating an optimum reaction parameter as temperature of 180 °C and lasting for 30 min [249]. Assisted by the response surface methodology, the maximum 20(*R*)-Rg3 de% was achieved to be 94.52 % under the condition of D,L-tartaric acid concentration 1.19 mol/L, temperature 107.9 °C and time 2.79 h using the PPT-type saponins as raw material [250].

The enzymes catalytic activity is imperative to produce rare ginsenoside in a more effective and environment-friendly way [251]. Fan *et al.* established a substrate fed-batch strategy to biocatalytic production of ginsenoside C-K in a deep eutectic solvent based on choline chloride, results showed the C-K conversion increased by 29.1 %, and the method achieved a C-K productivity of 142 mg/L·h^{-1} [252]. Temperature acts as a crucial role on enzyme activity, and some thermostable enzymes such as β-glucosidase Tpebgl1 and β-glucosidase BGL3T have been identified and purified for producing Rg3 [253,254]. Additionally, Ca^{2+} was capable of improving the thermostability of the enzyme, and under the optimal reaction condition (pH 5.0 and 90 °C), the transformation from Rb1 to Rg3 achieved a molar conversion of 97.9 %, and Rg3 productivity of 4620 mg/L/h [255]. In order to produce Rg3 in the functional food and pharmaceutical industries, an efficient novel recombinant enzyme from a GRAS (generally regarded as safe) host, *Lactobacillus ginsenosidimutans* EMML 3041, was explored, and results showed that 30 g of Rg3 could be generated from 50 g of Rb1 in one step [256].

Synthetic biology is now serving as an ideal strategy to bulk preparation of rare ginsenosides. Wang *et al.* elucidated the complete biosynthetic pathway of primary triterpene glycosylation products of *P. notoginseng*, and based on this, a final CK titer of 1.17 g/L was produced [257]. Encouragingly, large-scale production of other ginsenosides such as PPD titer of 11.02 g/L [241], Rh2 titer of 2.25 g/L [241], and Rg3 titer of 1.3 × 10^3 g/L were achieved [245]. Furthermore, other biotechnological approaches like tissue culture [258], chemical elicitors [259], transgenic plants, have been advanced for large-scale production of RGs [260].

Conclusion and future prospective

Recent years have witnessed tremendous progress in the field of rare ginsenosides researches, and this review is strongly indicative of the notion that rare ginsenosides, as the shining components,

show great possibility for drugs and nutraceuticals development. To our knowledge, this article is the first systematic review of rare ginsenosides. The rare ginsenosides acted an important part in the treatment of cardiovascular and cerebrovascular diseases and cancer. Particularly, the cross-talking with gut microbiota is beneficial to decipher the mystery of the discrepancy between better bioactivity and low bioavailability of ginsenosides. Owing to the long history of medicinal use, the clinical trials and drug development of rare ginsenosides are ongoing, and no obvious side events were reported. We summarized the stereospecific bioactivities and pharmacokinetics, and advanced analysis apparatus and strategies for the separation and recognition of isomers generated in the production process, which enhances the quality control of rare ginsenosides contained products. Finally, both ancient and state-of-art approaches for the production of rare ginsenosides get rapid development, making it feasible to meet the increasing demand for rare ginsenosides in the pharmaceutical health-care industry.

The great advancement of rare ginsenosides studies makes them prospective to be developed as new drugs, but some bottlenecks, nevertheless, are urgent to be overcame. (1) The definite protein targets of rare ginsenosides in the treatment of tumors and cardiovascular diseases remains unclear, and solid clinical data is imperative for drug development. (2) It is crucial to transform the knowledge of gut microbiota to practice in ginsenosides therapy. (3) The identification of rare ginsenosides isomers is still challenging. (4) The further scale production of rare ginsenosides is required.

To discover the primary targets of natural products, one straightforward approach is target-based strategies that identify small molecules that bind to target proteins by high-throughput screening (HTS) of large compounds libraries, but is time-consuming and laborious. The mass spectrometry-based proteomics is emerging as robust method for target identification and validation. Direct Screening strategies based on protein stability of natural product targets including stability of proteins from rates of oxidation (SPROX), drug affinity responsive target stability (DARTS), cellular thermal shift assay (CETSA), thermal proteome profiling (TPP), and Limited proteolysis (LIP). In addition, HTS technologies such as DNA-encoded library screening and genome-wide gene editing screens (e.g., CRISPR–Cas9) could indirectly identify targets. For the clinical trials, the randomized, multi-center, double-blind, placebo-controlled study are required to obtain high quality data. Importantly, clinical trials should be analyzed *via* a set of standardized ways and an unbiased assessment of their qualities. The Consolidated Standards of Reporting Trials (CONSORT) statement is a useful standardization for the design of clinical trials. Discovering the microorganisms and bioactive ginsenosides responsible for therapeutic effects is necessary, and integration of metabolomics and gut microbiota enable us unravel the intricate interaction between hosts and gut microbiota at system level. Subsequently, aiding by delicate methods, the gut microbiota species and corresponding enzymes influencing the ginsenosides transformation could be identified, thereby guiding clinical rational use of ginseng and ginsenosides. For instance, since the microbiota-mediate ginsenosides transformation is achieved by glycosidase, combination use of ginsenosides and glycosidase agonists is able to improve the efficacy. Recently, based on the CID approach, the integration of quantitative structure-retention relationship (QSRR) and optimal collision energy (OCE) advances structural annotation and isomers recognition, which could facilitate the precise analysis of rare ginsenosides. More importantly, the emerging controllable electron activated dissociation (EAD) technology offered multiple structural fragment information that extremely boost the development of isomers structure analysis. Increased production could be achieved by following approaches, identifying and characterizing the unknown UGTs of key rare ginsenosides (e.g., Ft1); improving the catalytic efficiency and stability of enzymes by uncovering the molecular mechanism of catalysis *via* homologous modeling and molecular docking; adopting combinatorial catalytic strategies (combination physiochemical transformation with biotechnical methods).

CRediT authorship contribution statement

Wenxiang Fan: Methodology, Investigation, Validation, Visualization, Writing – original draft. **Linhong Fan:** Investigation. **Ziying Wang:** Resources. **Yuqi Mei:** Investigation. **Longchan Liu:** Investigation. **Linnan Li:** Investigation. **Li Yang:** Conceptualization, Supervision. **Zhengtao Wang:** Conceptualization, Writing–review & editing, Supervision.

Compliance with Ethics Requirement

This is a review manuscript and does not contain any studies with human or animal subjects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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