Post-Print of Research on Synthetic Biology of Plant Natural Products

Authors: Dai Zhubo, Wang Yong, Zhi-Hua Zhou, Shengying Li, Zhang Xueli

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Abstract

Plant natural products have extensive applications in the pharmaceutical and healthcare sectors. Direct extraction from plants is currently the predominant method for producing plant-derived natural products; however, this approach entails numerous issues concerning environmental impact, safety, and efficiency. Grounded in the principles of synthetic biology, the construction of artificial cell factories for fermentative production of plant-derived natural products constitutes a novel paradigm for resource acquisition. This article will survey the current research status of producing plant-derived natural products using artificial synthetic cells, approaching from research trajectories and exemplified through case studies of production applications for compounds such as terpenoids, phenylpropanoids, and alkaloids.

Full Text

Synthetic Biology for Production of Plant-Derived Natural Products

DAI Zhubo[1], WANG Yong[2], ZHOU Zhihua[2], LI Shengying[3], ZHANG Xueli[1]*

[1] Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China
[2] Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China
[3] Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China

Abstract
Plant-derived natural products (PNPs) have extensive applications in pharmaceutical and nutraceutical fields. Currently, direct extraction from plants remains the primary production method, yet this approach suffers from signifi-

cant environmental, safety, and efficiency issues. Based on synthetic biology principles, constructing artificial cell factories for fermentative production of PNPs represents a novel resource acquisition paradigm. This review examines the research progress in microbial production of PNPs, focusing on case studies involving terpenoids, phenylpropanoids, and alkaloids to illustrate the current state of artificial cell synthesis.

Keywords: plant-derived natural products, synthetic biology, microbial cell factories, terpenoids

Plants synthesize trace amounts of secondary metabolites that play crucial roles in biological defense and signal transduction. Due to their strong physiological activities in humans, these plant natural products have been widely applied in medicine and healthcare. According to the Chinese Academy of Social Sciences' Pharmaceutical Blue Book "China Pharmaceutical Market Report (2012)," China' s pharmaceutical market reached 926.1 billion RMB in 2012 and is projected to hit 2.3 trillion RMB by 2020 [**?**]. Plant natural products have long served as essential sources for drugs, health supplements, and cosmetics, with highly effective medications such as morphine, artemisinin, ephedrine, and paclitaxel all derived from plants. The annual sales of steroid drugs exceed $40 billion, with production currently based on plant saponins including diosgenin, hecogenin, and sisalagenin [**?**, **?**]. Additionally, plant natural products like berberine alkaloids from *Coptis chinensis* demonstrate excellent safety as antibiotic alternatives in livestock farming. High-quality plant natural products including ginsenosides, rose essential oil, lycopene, anthocyanins, and novel sweeteners such as mogrosides and steviol glycosides constitute primary ingredients for cosmetics, health supplements, and flavoring agents, offering broad market prospects [**?**].

Plant extraction remains the dominant production method for plant natural products, yet this traditional approach suffers from numerous drawbacks: low and variable natural product content, long plant growth cycles, complex analogs that hinder purification, and severe destruction of biological resources, particularly wild plants [**?**]. As market demand grows, wild medicinal plants like *Ganoderma lucidum*, *Panax ginseng*, and *Panax notoginseng* have become endangered or extinct in the wild, making current resource supply unsustainable [**?**]. Chemical synthesis faces challenges as most natural products have complex structures with multiple chiral centers, leading to formation of inactive or toxic optical isomers that are difficult to separate. Moreover, chemical synthesis involves cumbersome steps, low conversion rates, high energy consumption, and organic solvents that cause pollution, making it unsuitable for industrial-scale production [**?**]. Plant tissue culture methods are operationally complex, time-consuming, and economically unviable for industrialization due to high production costs. In contrast, fermentative production of plant natural products—similar to brewing beer with *Saccharomyces cerevisiae*—offers advantages including short production cycles, independence from seasonal and raw material constraints, relatively pure fermentation products, and ease of separation and

purification, facilitating large-scale industrial production.

Based on synthetic biology principles, designing and constructing artificial synthetic cells for fermentative production of plant natural products can effectively control raw material supply while protecting natural resources and the environment. This green and efficient production model has gained recognition from both scientific and industrial communities [**?**]. In recent years, rapid advances in synthetic biology have continuously expanded the variety of microbially synthesized plant-derived natural products, with yields increasing annually [**?**]. This review examines the research landscape of artificial cell production of plant natural products, presenting case studies of terpenoids, phenylpropanoids, and alkaloid compounds.

Research Route for Plant Natural Product Synthetic Biology

The process of achieving fermentative production of plant natural products involves three fundamental components: characteristic gene element mining and optimization, biosynthetic pathway optimization, and cell factory performance enhancement.

Characteristic Element Mining and Optimization Beyond promoters and terminators that primarily control gene expression, identifying and optimizing key gene elements in plant natural product biosynthetic pathways constitutes the core and foundation for applying synthetic biology to revolutionize natural product production.

Current pathway elucidation primarily employs genome or transcriptome-based heterologous reconstruction methods. Recent advances in gene sequencing and bioinformatics have enabled breakthroughs in deciphering biosynthetic pathways for important plant natural products including morphine [**?**], glycyrrhizin [**?**], ginsenosides [**?**], tanshinones [**?**, **?**], cucurbitacins [**?**], mogrosides [**?**], etoposide [**?**], and vinblastine [**?**]. A batch of critical gene functional elements has been identified and characterized, with the catalytic mechanisms of cytochrome P450 enzymes—known as "universal biocatalysts" —being intensively studied [**?**]. However, while nature contains tens of thousands of valuable plant natural products (such as artemisinin and morphine), only a tiny fraction of their biosynthetic mechanisms have been resolved due to various constraints. Therefore, developing efficient, reliable, and cost-effective platforms for large-scale elucidation of molecular bases and process mechanisms of plant natural product synthesis holds strategic significance for systematic conservation and effective exploitation of this natural treasure trove.

For enzymes with clear crystal structures and catalytic mechanisms, rational design approaches can be employed. For instance, based on the structure of *Candida tenuis* xylose reductase (XR), mutations at amino acid sites such as Lys274 and Asn276 enhanced XR' s preference for the cofactor NADPH by 170-fold [**?**, **?**]. However, functional enzymes from plant natural product syn-

thesis pathways are typically complex, with the vast majority lacking resolved crystal structures. For such enzymes, random mutagenesis is generally used to enrich positive mutations, substantially improving enzyme activity, thermal stability, substrate affinity, and specificity [**?**]. For example, directed evolution of *Rhodobacter sphaeroides* phytoene desaturase through random mutagenesis nearly doubled lycopene production in engineered strains [**?**].

Additionally, functional enzyme fusion and protein scaffold construction can form controllable enzyme complexes in metabolic pathways, increasing effective substrate concentration and reducing accumulation of toxic intermediates, thereby improving substrate conversion rates [**?**]. Fusing yeast endogenous farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase significantly enhanced geranylgeranyl diphosphate synthesis capacity in *S. cerevisiae* [**?**]. Through protein scaffold optimization of the spatial arrangement and stoichiometry of three mevalonate synthesis enzymes, mevalonate concentration was increased 77-fold [**?**].

Biosynthetic Pathway Optimization Heterologous biosynthetic pathway reconstruction in host cells requires consideration of multiple factors affecting pathway efficiency, including material and energy balance, impact of heterologous metabolites on host cell physiology (toxicity), and compatibility between heterologous metabolites, functional enzymes, biosynthetic pathways, and host cells. Several optimization strategies have been developed:

(1) Material flux distribution control. Regulating expression of key node genes controls the allocation ratio of target compounds within the material supply network. In constructing artemisinic acid-producing strains, increasing upstream gene expression to enhance precursor supply while suppressing branch pathway gene expression to reduce substrate competition significantly improved fermentation production [**?**, **?**].

(2) Precise pathway control. Establishing promoter libraries to precisely control gene expression levels enables coordinated expression of pathway genes, reduces intermediate metabolite accumulation, decreases cellular burden, and ultimately improves fermentation performance [**?**]. Using promoters of varying strengths to coordinately express fatty acid synthesis and heterologous betulinic acid synthesis pathways revealed that engineered strain betulinic acid production could vary across a 200-fold range [**?**].

(3) Dynamic pathway control. Dynamic quantitative monitoring of pathway metabolites represents a crucial optimization step. Biosensors can convert metabolite changes into real-time signals through multiple outputs, enabling dynamic monitoring and feedback. This property allows construction of dynamic metabolite regulation circuits to increase target compound production [**?**]. For example, a biosensor responsive to propionyl-CoA was used to monitor intracellular propionyl-CoA accumulation, feeding back to downregulate acetyl-CoA carboxylase expression and reduce cellular toxicity [**?**]. An RNA microarray-

identified farnesyl diphosphate biosensor was used to balance metabolic flux and increase amorphadiene production [**?**].

Cell Factory Performance Enhancement Comprehensive efficiency improvement of cell factories involves multiple factors including efficient substrate utilization, product storage capacity, and excellent fermentation performance. Recent advances have developed new strategies in subcellular compartment utilization, product storage, enzyme carriers, and strain contamination resistance.

(1) Full utilization of organelle space. Mitochondria, Golgi apparatus, and endoplasmic reticulum provide catalytic environments for bioreactions. Targeting farnesyl diphosphate synthase and valencene synthase to yeast mitochondria using leader peptides increased natural fragrance valencene synthesis [**?**]. Anchoring relevant enzymes from the morphine biosynthetic pathway to the endoplasmic reticulum increased morphine production rates [**?**].

(2) Cell membrane engineering. Many plant natural products are hydrophobic compounds that generally remain within cells, but limited cellular space affects target product production. Scientists at Tianjin Institute of Industrial Biotechnology recently investigated the effect of *E. coli* membrane modification on terpenoid synthesis capacity. Regulating genes related to membrane synthesis increased membrane mass and inward stacking, significantly enhancing storage space for -carotene and increasing its production in engineered strains [**?**].

(3) Endoplasmic reticulum engineering. Providing more catalytic sites for functional enzymes represents a recently developed approach. Knocking out the *PHA1* gene in *S. cerevisiae* increased endoplasmic reticulum content, substantially expanding attachment space for endoplasmic reticulum-localized cytochrome P450 enzymes, increasing enzyme content and catalytic product yields [**?**].

(4) Utilization of atypical nutrients. Contamination by foreign microorganisms is common during large-scale fermentation of engineered strains, severely affecting production efficiency. Scientists from Novogy and MIT first modified nutrient utilization pathways in *S. cerevisiae*, *Yarrowia lipolytica*, and *E. coli*. Using nutrients that contaminants cannot utilize during fermentation significantly improved contamination resistance of engineered microbial cells [**?**].

Production Case Studies

Over the past decade, plant natural product synthetic biology has advanced rapidly, with artificial cell factories successfully created for series of terpenoids, phenylpropanoids, and alkaloids.

2.1.1 Terpenoid Fragrances The global fragrance and flavor market is vast, with terpenoid essential oils such as santalol, patchoulol, nerolidol, and -elemene widely used in daily chemicals, food, and pharmaceuticals. -elemene,

a sesquiterpene extracted from medicinal plants *Curcuma wenyujin* and *Curcuma zedoaria*, constitutes the active ingredient of a national Class I anticancer drug. However, low natural content and complex chemical analogs make separation costs prohibitively high. Collaborating with the Chinese Academy of Chinese Medical Sciences, Tianjin Institute of Industrial Biotechnology used metabolic engineering and synthetic biology to enhance terpenoid biosynthetic flux and product compatibility in *S. cerevisiae*. Through protein engineering of germacrene A synthase and construction of high-yield strains, coupled with thermal conversion of germacrene A to -elemene, the cost of high-purity -elemene was reduced to 0.15% of plant extraction costs [**?**].

2.1.2 Tanshinones Recent years have witnessed significant progress in artificial cell research for tanshinones in China. Tanshinones are abietane-type diterpenoids and the main active components of traditional Chinese medicine *Salvia miltiorrhiza*, exhibiting antioxidant, antibacterial, anti-inflammatory, and antitumor activities. A collaborative effort among the Chinese Academy of Chinese Medical Sciences, Dalian Institute of Chemical Physics, Iowa State University, Institute of Genetics and Developmental Biology, Shanghai Chenshan Plant Science Research Center, and Tianjin Institute of Industrial Biotechnology identified two functional enzymes catalyzing formation of the tanshinone basic skeleton—miltiradiene—and constructed high-yield yeast strains [**?**, **?**]. Using C-13 isotopic labeling, they determined the carbon skeleton' s role in tanshinone biosynthesis. Subsequently, the key enzyme gene *CYP76AH1* catalyzing conversion of miltiradiene to the metabolic intermediate ferruginol was discovered, enabling construction of high-yield ferruginol-producing yeast strains [**?**]. Further identification of P450 genes catalyzing C-7, C-11, and C-20 positions yielded yeast cell factories capable of simultaneously producing multiple tanshinone compounds [**?**].

2.1.3 Steviol Glycosides Steviol glycosides represent next-generation healthy natural sweeteners. The Shanghai Institute of Plant Physiology and Ecology constructed a functional gene database for *Stevia rebaudiana*, thoroughly mining key enzymes in steviol glycoside biosynthesis. They successfully identified critical enzymes and reconstructed a non-natural synthetic pathway for de novo steviol glycoside synthesis in *E. coli*. Through rational design and optimization, they substantially increased production of key intermediates and obtained the main component RA [**?**]. Building on this, they elucidated rubusoside biosynthesis from *Rubus suavissimus* and *Angelica keiskei*, reported six new diterpene glycosyltransferases, and investigated their substrate recognition mechanisms. Through orthogonal combination of glycosyltransferases from different species, they achieved efficient whole-cell conversion of rubusoside in microbial cells [**?**], laying the foundation for synthetic biological manufacturing of important diterpene glycoside compounds and providing a successful example of engineering *E. coli* as a chassis for complex terpenoid heterologous synthesis.

2.1.4 Ginsenosides Ginsenosides are active components of precious medicinal herbs *Panax ginseng* and *Panax quinquefolius*, comprising mixtures of protopanaxadiol, protopanaxatriol, and oleanolic acid aglycones glycosylated by glycosyltransferases. They exhibit antitumor [**?**, **?**], hypoglycemic [**?**], and immune-promoting functions. Collaborating with the Chinese Academy of Chinese Medical Sciences, Tianjin Institute of Industrial Biotechnology successfully constructed the protopanaxadiol biosynthetic pathway in *S. cerevisiae* and discovered that squalene epoxidase plays a key role in controlling triterpenoid biosynthesis [**?**]. By enhancing key gene expression, protopanaxadiol production was increased 262-fold. Two-phase fermentation optimization further elevated yields to 1 g/L [**?**]. Recently, the collaborative team published a construction scheme achieving triterpenoid synthesis flux at the 10 g/L level, creating an efficient yeast cell factory producing ginsenoside precursors at 15 g/L [**?**]. Additionally, introducing three functional modules (oleanolic acid, protopanaxadiol, and protopanaxatriol) into the same chassis yielded the first-generation "ginseng yeast" cell factory capable of simultaneously synthesizing three ginsenoside aglycones [**?**].

The Shanghai Institute of Plant Physiology and Ecology, collaborating with the Shanghai Institute of Materia Medica, first cloned and identified key glycosyltransferases and P450 reductase PgCPR1 required for synthesizing rare ginsenosides CK, Rh2, Rg3, Rh1, and F1 from *Panax ginseng*. Through cell factory construction and optimization, they achieved fermentative production of rare ginsenosides from simple sugars, with current yields exceeding 2 g/L [**?**, **?**, **?**]. Recently, the team further mined over 20 glycosyltransferases from *Panax ginseng* and *Panax notoginseng*, comprehensively elucidating ginsenoside biosynthetic pathways. Related glycosyltransferase patents have entered examination in six countries and regions, with grants already obtained in China and Japan (PCT/CN2013/088819).

2.1.5 Triterpenoid Acids Fruit cuticular waxes from apple, hawthorn, loquat, jujube, and pear contain trace amounts of high-value triterpenoid acids including corosolic acid, maslinic acid, alphitolic acid, and asiatic acid. These compounds have broad applications in antiviral therapy [**?**], diabetes control [**?**], and skin repair [**?**], representing important dietary supplements. Corosolic acid demonstrates significant anti-diabetic effects [**?**] and is considered a natural plant insulin. Current production relies primarily on direct plant extraction. To create efficient microbial cell factories for fermentative production of these pharmacologically active compounds, Tianjin Institute of Industrial Biotechnology developed a "plug-and-play" rapid biosynthetic pathway elucidation platform integrating plant tissue culture, differential transcriptome sequencing, metabolite analysis, candidate gene characterization in chassis cells, and compound identification. Using this platform, they first screened from hawthorn' s P450 library the functional P450 enzyme MAA45 capable of catalyzing 2 -hydroxylation of oleanolic acid and ursolic acid to produce maslinic acid and corosolic acid. Based on this, they created efficient *S. cerevisiae* cell factories producing maslinic acid

(384 mg/L) and corosolic acid (141 mg/L) [**?**, **?**].

2.1.6 Carotenoids Carotenoids have important applications in pharmaceuticals, nutraceuticals, cosmetics, and food. Representative carotenoids include -carotene, lycopene, and astaxanthin. Tianjin Institute of Industrial Biotechnology systematically investigated regulatory mechanisms for efficient microbial terpenoid synthesis from three perspectives: material metabolism, energy metabolism, and cellular physiology [**?**, **?**]. (1) In material metabolism, IspG and IspH were identified as critical rate-limiting steps requiring coordinated expression—individual IspG overexpression caused toxic intermediate HMBPP accumulation, severely inhibiting cell growth [**?**]. (2) In energy metabolism, multiscale modular regulation of central metabolic pathways (pentose phosphate and TCA) revealed that TCA flux represents the primary limiting factor for aerobic NADPH synthesis in *E. coli*, resolving redox imbalance in terpenoid synthesis [**?**]. (3) In cellular physiology, systematic investigation of membrane storage capacity limitations identified membrane morphology and glycerophospholipid synthesis capacity as key constraints. Introducing exogenous membrane-folding proteins altered *E. coli* membrane morphology, forming inward membrane folds. Enhancing glycerophospholipid synthesis further increased these folds, significantly improving membrane storage capacity and terpenoid production [**?**]. Based on these findings, a series of microbial cell factories for efficient carotenoid production were constructed, with lycopene pilot validation in 200 L fermenters achieving 7 g/L after 48 hours.

Phenylpropanoids and Shikimate Pathway-Derived Products

Phenylpropanoids are natural compounds featuring a benzene ring connected to three straight-chain carbons, including anthocyanins, resveratrol, and caffeic acid. They exhibit significant pharmacological activities in antioxidant effects, cardiovascular health, antiviral properties, and coagulation. Recent breakthroughs in fermentative production include Evolva' s resveratrol technology, which has entered industrialization. Chinese scientists have made pioneering progress in gastrodin, scutellarin, salidroside, and danshensu.

2.2.1 Gastrodin *Gastrodia elata* is a precious traditional Chinese medicinal herb, with gastrodin as its main active component widely used clinically for treating neurasthenia and related syndromes. However, *Gastrodia* resources are scarce with low gastrodin content (only 0.4%), making plant extraction expensive. Chemical synthesis suffers from high costs and severe pollution, while the biosynthetic pathway remains unelucidated. Tianjin Institute of Industrial Biotechnology used the shikimate pathway' s chorismic acid as precursor in *E. coli*, overexpressing key genes from *E. coli*, *Nocardia*, and *Bacillus subtilis* while introducing plant-derived glycosyltransferase UGT73B6 to create the first gastrodin synthesis pathway in *E. coli*. Further regulation of the shikimate pathway, UDP-glucose pathway, NADPH reducing power, glycosyltransferase mutagenesis and screening, and fermentation optimization significantly increased

gastrodin yield. Current production costs from glucose are projected below 500 RMB/kg, merely 1/200 of plant extraction and half of chemical synthesis costs [**?**]. Recently, Qingdao Institute of Bioenergy and Bioprocess Technology and Hunan Normal University investigated biotransformation using the aromatic precursor 4-cresol degradation pathway, achieving 433.3 mg/L gastrodin in shake flasks with 5 mmol/L 4-cresol precursor [**?**, **?**, **?**].

2.2.2 Scutellarin Scutellarin-based medicines are essential for cardiovascular and cerebrovascular diseases, occupying approximately 7% market share in China' s cardiovascular field. Tianjin Institute of Industrial Biotechnology, collaborating with Yunnan Agricultural University, used synthetic biology and bioinformatics to successfully identify key genes in scutellarin biosynthesis from *Erigeron breviscapus* genome and constructed scutellarin-producing cell factories in *S. cerevisiae*. Through metabolic engineering and fermentation optimization, current production has reached the hundred-milligram level [**?**].

2.2.3 Danshensu Danshensu is a polyphenolic drug from *Salvia miltiorrhiza*. Tianjin University exploited structural similarity between danshensu and the native metabolite 4-hydroxyphenylpyruvate to create a novel artificial biosynthetic pathway. Rational engineering of artificial elements lactate dehydrogenase and hydroxylase enhanced specificity toward non-natural substrates, enabling efficient microbial synthesis of danshensu with fermentation yields reaching 7 g/L [**?**].

2.2.4 Salidroside Salidroside is a promising adaptogenic drug. *Rhodiola* grows in alpine environments with scarce resources, difficult cultivation, low medicinal component content, and toxic impurities. Tianjin Institute of Industrial Biotechnology constructed a salidroside synthesis pathway in *E. coli* using the shikimate pathway. Through enzyme directed evolution and metabolic regulation, they obtained a high-yield strain. Further fermentation optimization reduced production costs to merely 1/40 of plant extraction [**?**].

Conclusion

In recent years, synthetic biology-based design of artificial cell factories for fermentative production of plant-derived natural products has achieved remarkable successes, including successful construction of cell factories for artemisinin, -elemene, lycopene, ginsenosides, and morphine [**?**]. Compared with traditional methods, this novel resource acquisition strategy offers significant advantages in sustainable resource utilization and economic benefits, emerging as an innovative paradigm.

Statistics indicate China alone possesses over 10,000 medicinal plants (traditional Chinese medicines) containing bioactive components, providing a rich precursor drug repository for modern drug development and a diverse natural

enzyme library from their biosynthetic pathways. However, effective exploitation of this "dual treasure trove" represents a highly interdisciplinary frontier requiring collaborative efforts from biology, informatics, chemistry, traditional Chinese medicine, and pharmacology.

Current theoretical and technical challenges remain: large-scale biosynthetic pathway elucidation and element library construction, high-throughput pathway assembly and optimization, and artificial system debugging are still in early development stages with limited AI automation. Some compound classes lack sufficient research foundation, and heterologous synthesis efficiency in engineered cells remains relatively low, making fermentation costs economically uncompetitive compared to traditional routes. Further breakthroughs are needed. Nevertheless, with maturing AI technology, low-cost plant genome sequencing, improved high-throughput gene synthesis, and advanced metabolic network-based optimization concepts, humanity will ultimately usher in a new era of artificial synthesis for plant-derived natural products.

DAI Zhubo is an Associate Professor at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, and a member of the Youth Innovation Promotion Association. His research focuses on molecular pharmacognosy and synthetic biology, establishing green production models for natural medicines and nutraceuticals based on efficient microbial cell factories. His work includes creating "ginseng yeast" and "essential oil yeast" cell factories. He has published in *Biotechnology and Bioengineering*, *Metabolic Engineering*, and other journals, applied for 12 patents (including 1 PCT), and received the First Prize of the Chinese Pharmaceutical Association Science and Technology Award.

ZHANG Xueli is a Professor at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, and Director of the CAS Key Laboratory of Systems Microbial Biotechnology. He is a leading talent in the National Ten Thousand Talents Program, recipient of the NSFC Excellent Young Scientists Fund, and an expert in the CAS Hundred Talents Program. His research applies microbial metabolic engineering and synthetic biology to construct efficient cell factories for bulk chemicals and plant natural products, successfully creating strains producing L-alanine, succinic acid, D-lactic acid, lycopene, and -elemene. He has published over 50 SCI papers cited more than 1,600 times, holds 24 issued patents in China, US, Europe, and other countries, and received the Chinese Patent Excellence Award and provincial/ministerial science and technology awards.

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Note: Figure translations are in progress. See original paper for figures.