Medicinal Plant Research 2023, Vol.13, No.1, 1-12
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Review and Prospect Open Access

Targeted Editing of the Ginsenoside Biosynthesis Pathway in Ginseng Using CRISPR/Cas9 Technology and Its Implications

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Medicinal Plant Research, 2023, Vol.13, No.3 doi: 10.5376/mpr.2023.13.0003
Received: 31 Aug., 2023
Accepted: 12 Oct., 2023
Published: 20 Oct., 2023

Preferred citation for this article:
Liu C.C., 2023, Targeted editing of the ginsenoside biosynthesis pathway in ginseng using CRISPR/Cas9 technology and its implications, Medicinal Plant Research, 13(3): 1-12 (doi: 10.5376/mpr.2023.13.0003)

Abstract CRISPR/Cas9 technology, as a revolutionary gene editing tool, has garnered widespread attention in the field of plant biology. This review focuses on ginseng (*Panax ginseng*) and explores the application of CRISPR/Cas9 technology in targeted editing of the ginsenoside biosynthesis pathway, as well as its potential impact on medicinal value. *Panax ginseng*, a significant traditional medicinal herb, contains bioactive compounds known as ginsenoside, which possess various medicinal properties such as immunomodulation, anti-fatigue, and anti-cancer effects. To enhance ginsenoside production, the precise regulation of its biosynthesis pathway through CRISPR/Cas9 gene editing has emerged as an effective strategy. This review elaborates on the ginsenoside biosynthesis pathway, the advancements in CRISPR/Cas9 technology, and the latest research progress in manipulating the ginsenoside biosynthesis pathway using this technique. Through this review, we delve into the application of CRISPR/Cas9 technology in the ginsenoside biosynthesis pathway of ginseng, providing a theoretical basis and technical reference for further exploring the medicinal potential of ginseng.

Keywords CRISPR/Cas9 technology; Ginsenoside; Biosynthesis pathway; Site-specific gene editing; Medicinal value

Ginseng (*Panax ginseng*), a perennial herbaceous plant belonging to the *Panax* genus in the family of Araliaceae, stands as one of the oldest and most valuable traditional Chinese medicines with a long history of use in China. It is renowned as the "King of Herbs" and its medicinal value has been globally recognized (Yu and Luo, 2009, Modern Chinese Medicine, 11(7): 45-45). The active components of ginseng root, known as ginsenoside, have been extensively studied for their pharmacological effects, including immune regulation, anti-fatigue, antioxidant, and anti-tumor properties (Leung and Wong, 2010). As the aging population increases and lifestyles change, the demand for ginseng preparations continues to rise. However, the lengthy cultivation period and susceptibility to environmental factors hinder the production of ginseng, making it unable to meet market demands. Consequently, seeking novel approaches to enhance the production of ginsenoside, the active medicinal compounds in ginseng, holds significant scientific and practical value.

The biosynthesis pathway of ginsenoside is extremely complex, involving the regulation of multiple key enzymes. In past studies, conventional breeding and genetic improvement methods have been attempted to increase the yield of ginsenoside, but progress has been limited. In recent years, the rapid development of gene editing technology has provided new avenues for the improvement and optimization of ginseng. Among them, CRISPR/Cas9 technology, as an efficient and precise gene editing method, has been widely applied in the research and improvement of plant genomes (Li et al., 2013; Xu et al., 2015). By precisely editing the genes related to the synthesis of ginsenoside in ginseng, precise control of the ginsenoside synthesis pathway can be achieved, thereby enhancing its medicinal value (Kim et al., 2017).

This review will focus on the application of CRISPR/Cas9 technology in the ginsenoside biosynthetic pathway of ginseng, as well as its impact on the medicinal potential of ginseng. We will introduce the medicinal value of ginseng and the importance of ginsenosides, explore the basic principles of CRISPR/Cas9 technology and its application in plant gene editing, particularly in the case of ginseng. Furthermore, we will delve into the precise

editing of the ginsenoside biosynthetic pathway using CRISPR/Cas9 technology, analyzing the editing efficiency and outcomes, and discussing the potential influence of these edits on the medicinal value of ginseng. By comprehensively discussing the application of CRISPR/Cas9 technology in the ginsenoside biosynthetic pathway of ginseng, we can better understand the revolutionary potential of this technique in improving the yield and quality of medicinal plants, providing new insights and methods for genetic improvement and medicinal development of ginseng.

1 Biosynthetic Pathway of Ginsenoside

The biosynthetic pathway of ginsenosides is a complex molecular network involving the conversion of multiple key enzymes and intermediate products. The elucidation of this pathway contributes to the understanding of the medicinal value and biosynthetic mechanism of ginseng.

1.1 Medicinal properties and classification of ginsenosides

Ginsenosides, the main components of ginseng medicinal value, are crucial to understanding their pharmacological properties. Ginsenosides exhibit a wide range of biological activities in medicine, including immune regulation, antioxidant, anti-inflammatory, and anti-tumor effects. Ginsenosides can be classified into two major categories based on the glycoside framework: dammarane-type (Dammarane) and oleanane-type (Oleanane). The dammarane-type includes two classes: Panaxadiol and Panaxotriol (Figure 1).

Panaxadiol-type are one of the most common types of triterpene saponins in ginseng. They are believed to possess various pharmacological activities, such as anti-inflammatory, antioxidant, and immune-modulating effects. Among them, Rb1, Rb2, Rc, and Rd are representative compounds of panaxadiol-type. Panaxatriol-type are also commonly found in ginseng and exhibit similar pharmacological effects. In which, Re, Rg1, Rg2, and Rh1 have been extensively studied for their anti-inflammatory, antioxidant, and anticancer activities, attracting much attention. Oleanane, a less studied but equally important category of triterpene saponins, such as Ro, have shown some potential in pharmacological research despite their lower content in ginseng.

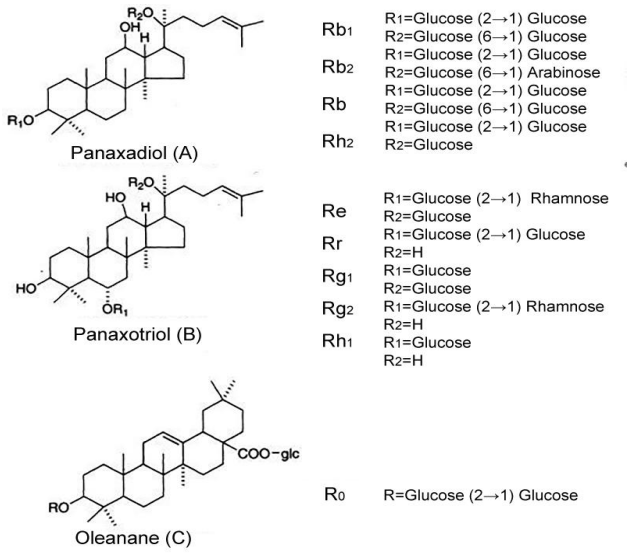

Figure 1 Classification of ginsenosides in ginseng
Notes: A: Panaxadiol; B: Panaxotriol; C: Oleanane

These different types of ginsenosides possess diverse pharmacological properties, thus understanding the synthesis mechanisms and regulation of different types of ginsenosides in the research of ginseng synthesis pathway has become crucial. Through in-depth study of the biosynthesis of different types of triterpenoid saponins, it is possible to selectively optimize the production of specific types of ginsenosides, thereby enhancing the medicinal effects of ginseng.

1.2 Biosynthetic pathway of ginsenosides

Ginsenosides, highly oxidized tetracyclic triterpenoid compounds, are synthesized through the mevalonate pathway and squalene pathway (Ming et al., 2010). In ginseng, these reaction steps are catalyzed by a series of specific enzymes, and the subcellular localization and interactions of these enzymes constitute the biosynthetic pathway of ginsenosides (Zhang et al., 2016).

1.2.1 Mevalonate pathway

The mevalonate pathway (MVA) is one of the precursors in the formation of squalene. In this process, acetyl-CoA undergoes catalysis by a series of enzymes to eventually form mevalonic acid (Figure 2). Among these enzymes, 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) plays a crucial role in catalyzing the formation of mevalonic acid from HMG-CoA.

1.2.2 Squalene pathway

The squalene pathway is a pivotal step in the formation of the ginsenoside framework. Mevalonic acid is catalyzed by farnesyl pyrophosphate synthase (FPS) to produce farnesyl pyrophosphate (FPP). Under the catalysis of terpene cyclase, FPP transforms into squalene. Squalene, in turn, is transformed into 2,3-oxidosqualene through the action of squalene epoxidase (Figure 3). Through a series of complex enzymatic reactions, 2,3-oxidosqualene undergoes multiple steps to generate the carbon-ring framework of dammarane and oleanolic-type ginsenosides, which constitute the ginsenoside skeleton.

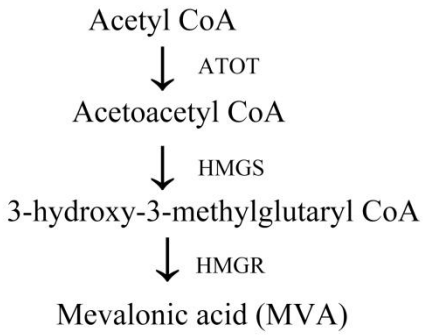

Figure 2 Mevalonate pathway biosynthesis

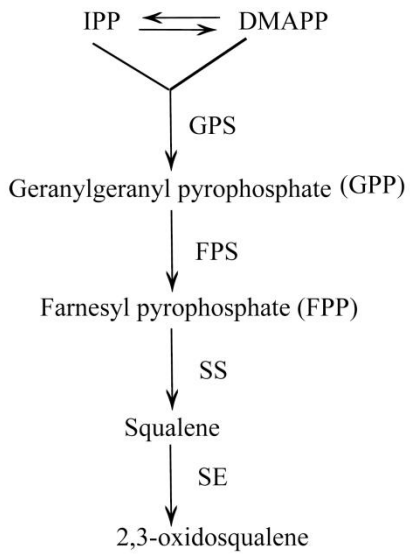

Figure 3 Biosynthesis of 2,3-oxidosqualene

1.2.3 Glycosylation reaction

Glycosylation reaction refers to a chemical reaction where a sugar molecule is transferred onto another molecule (such as a protein, lipid, or another sugar molecule) under the catalysis of glycosyltransferases. This reaction is widely present in biological systems and holds significant biological significance.

In the biosynthesis of ginsenosides, glycosylation reaction is a pivotal step where sugar molecules are attached to the carbon skeleton. Specifically, glycosyltransferases selectively recognize sugar molecules and the carbon skeleton, transferring sugar molecules onto the carbon skeleton to form glycosidic bonds. These glycosylation reactions are catalyzed by a series of glycosyltransferases.

The biosynthetic pathway of ginsenosides involves a complex enzymatic reaction process, with the participation of multiple key enzymes. Understanding the biosynthetic pathway of ginsenosides contributes to uncovering the pharmacological effects and biological activities of ginsenosides. Additionally, it provides a theoretical basis for the development of ginseng resources. In the future, methods such as genetic engineering and metabolic engineering can be employed to further optimize the biosynthetic pathway of ginsenosides, enhancing the utilization value of ginseng resources.

1.3 Complexity of regulatory mechanisms and pathways

The biosynthetic pathway of ginsenosides is intricately regulated within the cell by complex mechanisms, which directly influence the production, accumulation, and proportions of different types of ginsenosides. This regulation plays a crucial role in the cross-regulation of transcription, translation, and metabolic pathways, yet many mysteries remain unresolved at present.

Transcriptional regulation: Transcriptional regulation of key enzyme genes is a crucial mechanism governing the biosynthesis of ginsenosides. Transcription factors and regulatory elements exert positive or negative control over the expression of key enzyme genes by recognizing specific DNA sequences. These transcription factors can be influenced by external environmental factors, hormones, and developmental stages, thereby modulating ginsenoside synthesis under different conditions.

Substrate feedback regulation: Substrate concentration exerts negative feedback regulation over the activity and expression of key enzymes. High substrate concentrations can inhibit enzyme activity, thereby constraining the rate of ginsenoside synthesis. This substrate feedback regulation aids in maintaining ginsenoside levels at appropriate levels and prevents excessive accumulation.

Signal transduction network: Intracellular signal transduction networks also play a significant role in ginsenoside biosynthesis. These networks can be regulated by external biological stresses, endogenous hormones, and other metabolic products. Through these signaling pathways, cells can adjust ginsenoside synthesis to accommodate environmental and physiological demands.

2 Application of CRISPR/Cas9 Technology in Ginseng Gene Editing

2.1 Basic principles and applications of CRISPR/Cas9 technology

CRISPR/Cas9 technology is a gene editing tool based on the natural immune system of bacteria (Figure 4). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a unique DNA sequence, and bacteria use it to incorporate viral genetic information into the CRISPR region to defend against viral infections. The Cas9 protein is an endonuclease capable of recognizing and cleaving foreign DNA that matches the DNA sequence stored in the CRISPR region. By designing specific guide RNAs, the CRISPR/Cas9 system can target specific positions within the target gene, guiding the Cas9 protein to perform cleavage (Li et al., 2015).

The broad applications of CRISPR/Cas9 technology encompass genome editing, functional studies, and biomedical research. In the context of ginseng gene editing, CRISPR/Cas9 technology empowers scientists with the precise ability to edit the ginseng genome, thereby facilitating the exploration and manipulation of the ginsenoside biosynthetic pathway to enhance the production of medicinal components (Hsu et al., 2014).

Through CRISPR/Cas9 technology, researchers can selectively edit key genes related to the biosynthesis of ginsenosides, thereby enhancing the production of specific types of ginsenosides. For instance, by inhibiting competing metabolic pathways or enhancing the expression of crucial enzymes, the synthetic pathways of specific ginsenosides can be altered. Furthermore, CRISPR/Cas9 technology enables the achievement of balanced yields of different types of ginsenosides, better catering to the medicinal requirements of herbal medicine.

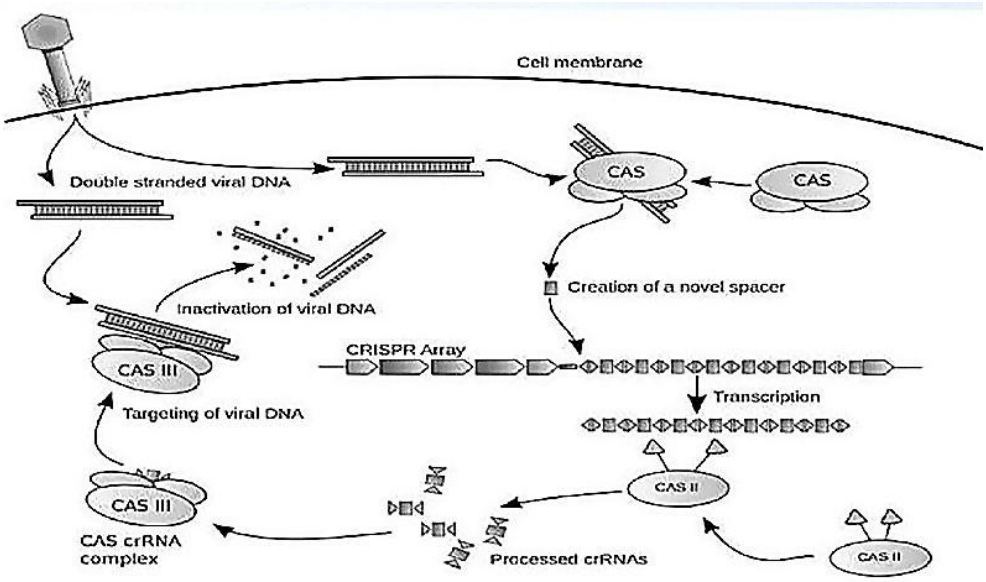

Figure 4 Mechanism of of the CRISPR/Cas9 system (Image Source: Free Document Center)

2.2 Characteristics and challenges of the ginseng genome

The ginseng genome exhibits distinctive features within the plant kingdom. It boasts a relatively large genome size, harboring a multitude of genes and repetitive sequences. Moreover, the ginseng genome demonstrates polyploid characteristics, with varying levels of ploidy among different ginseng types. This complexity poses challenges for the application of gene editing tech-nology in ginseng, as the efficiency and stability of editing must account for the influence of different ploidy backgrounds.

Assembling the ginseng genome is a challenging endeavor. The presence of complexity and repetitive sequences renders the high-quality assembly of the ginseng genome more challenging. The incompleteness of genome sequences might impact the precision of gene editing technology. Therefore, further improvements in genome assembly remain necessary to enhance the accuracy of gene editing techniques.

Gene functional identification within the ginseng genome is a complex issue. Despite the availability of genome sequence information, precise functional identification of genes is still required. The functions of different genes might vary among different types of ginseng, necessitating in-depth research to ascertain their roles in ginsenoside biosynthesis (Xu et al., 2017).

The complexity of ginsenoside biosynthesis pathway poses a challenge. Different types of biosynthetic pathways for ginsenosides have interconnections and mutual regulations. Therefore, comprehending the functions of different enzymes and regulatory networks within the pathway becomes crucial. Gene editing technology can assist in unraveling the intricacies of this pathway, but it also requires extensive support from molecular biology and metabolic studies.

2.3 Precise editing and pathway optimization

One significant advantage of the CRISPR/Cas9 technology is the ability to achieve precise gene editing, that is, accurately modifying specific genes at specific locations within the genome. In ginseng gene editing, precise

editing can be used to modify key genes related to ginsenoside synthesis, thereby optimizing the synthesis pathway and yield (Qi et al., 2011). This method is more precise than traditional breeding methods, and can increase or decrease specific medicinal components.

Pathway optimization refers to enhancing the production of target metabolites by editing key genes and adjusting metabolic pathways. In ginseng, the goal of pathway optimization is to increase the production of specific types of triterpene saponins. Through CRISPR/Cas9 technology, researchers can selectively edit key genes in the biosynthetic pathway, such as cycloartenol synthase and glycosyltransferase, to alter the synthesis route of specific triterpene saponins, thus achieving an increase in yield.

In ginseng genome editing, researchers can design specific guide RNAs to direct the Cas9 protein to cut at specific locations within the target gene. Through this cleavage, the cell activates its self-repair mechanism, which may introduce deletions or insertions mutations. This can be used for targeted editing of specific genes, such as restricting competing metabolic pathways or enhancing the expression of key enzymes, thereby optimizing the production of specific ginsenosides.

While targeted editing and pathway optimization techniques hold tremendous potential for enhancing ginsenoside synthesis, the potential outcomes and risks need to be approached with caution. Mutations introduced through editing could adversely affect other metabolic pathways and growth development. Furthermore, the edited genome might exhibit different effects under diverse environmental conditions. Hence, comprehensive molecular, physiological, and metabolic analyses are essential throughout the gene editing process to ensure the efficacy and stability of the edits.

2.4 Realization of high-quality yields and potential risks

Through gene editing technology, achieving high-quality yields of ginseng is a significant objective in ginseng research. High-quality yields encompass not only enhancing the synthesis of specific types of ginsenosides but also elevating the purity and activity of medicinal components. This can be accomplished by modifying synthesis pathways, enhancing the expression of key enzymes, and altering metabolic routes.

Using gene editing techniques, researchers can selectively enhance or suppress the expression of specific key enzymes to achieve the goal of high-quality production. For instance, by upregulating the expression of oleanolic acid synthase (OAS), the synthesis rate of precursor triterpenoids can be increased, consequently elevating the production of specific ginsenosides. Conversely, inhibiting the expression of key enzymes in competitive pathways can reduce the synthesis of other metabolites, thereby increasing the proportion of the target ginsenoside.

The achievement of high-quality yields requires a comprehensive consideration of the impact of introduced mutations on the overall metabolic pathways. Editing may lead to unforeseen side effects, such as disruption of other metabolic pathways or abnormalities in growth and development. Additionally, the post-edited genome might exhibit varying effects under different growth conditions, necessitating verification across diverse environments. To ensure the genetic stability of edits, long-term metabolic and expression analyses are imperative.

During gene editing, especially in polyploid plants, phenomena like gene loss and gene flow may occur. Gene loss refers to the unintended cleavage or deletion of the target gene during the editing process, resulting in its loss of function. Gene flow means the spread of introduced exogenous DNA fragments among different genomes during editing. These phenomena can potentially impact the editing outcome and the stability of target products, warranting careful consideration and management.

3 Key Enzymes and Their Functions in the Ginsenoside Biosynthetic Pathway

3.1 Oleanolic acid biosynthetic pathway and key enzymes

Oleanolic acid serves as a pivotal intermediate in the biosynthesis of ginsenosides, acting as a precursor for

multiple triterpene saponins. The synthetic pathway of oleanolic acid constitutes a complex biosynthetic route, involving a series of consecutive reactions catalyzed by key enzymes. The conversion from protopanaxadiol glycosides to oleanolic acid is of paramount importance within this pathway.

3.1.1 Key enzyme genes and their functions

β-amyrin synthase (β-ASD) is the first critical enzyme in the oleanolic acid biosynthesis pathway, catalyzing the oxidation of β-amyrin to oleanolic acid. The expression level of the β-ASD gene influences the accumulation of oleanolic acid, and regulating this gene can affect the yield and proportion of different types of ginsenosides.

Oleanolic acid synthase is another key enzyme in the oleanolic acid biosynthetic pathway, catalyzing the synthesis of oleanolic acid. The expression of the oleanolic acid synthase gene is positively correlated with the accumulation of oleanolic acid. Hence, by modulating the expression levels of the oleanolic acid synthase gene, the synthesis of oleanolic acid can be enhanced, consequently impacting the production of specific ginsenosides.

3.1.2 Application of gene editing in key enzyme genes

Gene editing technology can precisely edit key enzyme genes, thereby modulating the oleanolic acid synthesis pathway. For instance, by enhancing the expression of the DDS gene or reducing the expression of β-ASD, regulation of oleanolic acid accumulation and yield can be achieved. This targeted editing approach aids in optimizing the oleanolic acid synthesis pathway, consequently leading to an increase in the production of specific ginsenosides.

3.2 Glycosyltransferase genes and triterpenoid saponin glycosylation

Glycosyltransferases play a pivotal role in the triterpenoid saponin biosynthetic pathway, being responsible for coupling sugar moieties to precursor compounds like oleanolic acid, thus forming the structural framework of triterpenoid saponins. This enzyme class significantly contributes to determining the medicinal value, as it facilitates the modulation of yields and proportions of different types of triterpenoid saponins by regulating the expression of various glycosyltransferase genes (Gao et al., 2017).

3.2.1 Key glycosyltransferase genes and their functions

Glycosyltransferases are typically classified within the UDP-glycosyltransferase family (UGT). UGT gene family plays a pivotal role in the glycosylation reactions of triterpenoid saponins. Different UGT genes exhibit selective glycosylation activities towards distinct substrates, thus modulating the structure and diversity of triterpenoid saponins.

Glycosyltransferase genes encompass diverse functionalities, encompassing glycosylation reactions with a range of substrates. For instance, specific UGT genes may be responsible for adding various sugar moieties such as glucose, glucuronic acid, and xylose at specific positions on oleanolic acid, thus generating different types of triterpenoid saponins.

3.2.2 Application of gene editing in glycosyltransferase genes

The application of gene editing technology in glycosyltransferase genes enables the acidification control of ginsenosides. By editing specific UGT genes, it becomes possible to enhance or suppress the glycosylation reactions of specific ginsenosides, thereby influencing their types and yields. This targeted editing approach facilitates the optimization of specific medicinal components, enabling ginseng to better cater to diverse pharmacological requirements.

3.3 Cytochrome P450 genes and oxidation reactions

Cytochrome P450 (CYP) enzymes are a vital class of enzymes involved in oxidation reactions and are widely distributed in plants. In the biosynthetic pathway of ginsenosides, cytochrome P450 enzymes play a pivotal role by catalyzing oxidation reactions that regulate the structure and function of ginsenosides (Han et al., 2012). The oxidation reactions catalyzed by these enzymes can modify functional groups of dammarane-type and its derivatives, impacting the activity and pharmacological properties of ginsenosides.

3.3.1 P450 genes and their functions

Cytochrome P450 genes constitute an extensive family, with each gene encoding an enzyme that may possess distinct substrate specificities. In the synthesis of ginsenosides, cytochrome P450 enzymes encoded by different genes can catalyze diverse oxidation reactions of oleanolic acid, yielding various products.

Cytochrome P450 enzymes can introduce oxygen atoms into oleanolic acid molecules, resulting in functional groups such as hydroxyl and carboxyl groups. These oxidative reactions alter the molecular conformation and chemical properties, thus impacting the activity of ginsenosides. For instance, hydroxylation reactions may enhance the hydrophilicity of ginsenosides, affecting their pharmacological activity and bioavailability.

3.3.2 Application of gene editing in cytochrome P450 genes

The application of gene editing technology in cytochrome P450 genes enables precise control over oxidation reactions. By editing specific cytochrome P450 genes, it is possible to enhance or suppress specific oxidation reactions, thereby modulating the functional groups and activities of ginsenosides. This approach aids in optimizing the pharmacological properties of specific types of ginsenosides, rendering them better suited for clinical requirements.

3.4 Regulation genes and metabolic pathway regulation

3.4.1 Role of regulatory genes in ginsenoside biosynthesis

Regulatory genes play a pivotal role in the ginsenoside biosynthetic pathway, as they regulate the expression of key enzyme genes within the pathway, consequently impacting the yield and diversity of ginsenosides. These regulatory genes encompass transcription factors, signal transduction molecules, etc. They respond to internal and external signals, governing the initiation and cessation of ginsenoside synthesis, as well as the relative proportions of different types of ginsenosides.

3.4.2 Regulation genes and optimization of medicinal components

Transcription factors can bind to the promoter regions of genes, regulating the transcription levels of target genes. In the ginsenoside synthesis pathway, specific transcription factors can activate or inhibit the expression of key enzyme genes, thus affecting the yield and proportions of ginsenosides. Through editing or regulating these transcription factor genes, the optimized synthesis of specific types of ginsenosides can be achieved.

Both intracellular and extracellular signaling molecules can activate or inhibit the expression of regulatory genes. For instance, plant hormones such as gibberellins and abscisic acid can modulate the expression of key genes in the ginsenoside synthesis pathway, influencing yield and quality. Regulating the synthesis, degradation, or receptor expression of these signaling molecules can modulate the synthesis of medicinal components in ginsenosides.

3.4.3 Optimization of metabolic pathway regulation

Intermediate metabolites in the ginsenoside biosynthetic pathway can also influence the overall synthesis process. By modulating key enzyme genes, the accumulation or flux of intermediate metabolites can be controlled, thereby affecting the production of specific ginsenosides. Through gene editing and metabolic engineering approaches, the regulation of these pathway intermediates can be optimized to achieve desired yield adjustments.

In the ginsenoside biosynthetic pathway, feedback inhibition mechanisms may exist, where synthesis products negatively regulate the expression of precursor enzyme genes in the pathway. Editing genes associated with these inhibition mechanisms can alleviate such feedback inhibition, consequently enhancing the yield of specific ginsenosides.

4 CRISPR/Cas9 Technology for Targeted Editing of Key Genes and Its Impact on Ginsenoside Biosynthesis

4.1 Application of CRISPR/Cas9 technology in plant gene editing

CRISPR/Cas9 technology is a precise gene editing tool that has found extensive application across various

organisms, including the field of plants (Jiang et al., 2013). This technique involves the formation of a complex between the Cas9 protein and a designed single guide RNA (sgRNA) molecule, which, upon specific pairing with the target DNA sequence, leads to DNA double-strand breaks. Cells can repair these breaks, potentially resulting in various types of mutations, such as insertions, deletions, or replacement edits, thereby achieving targeted gene editing.

In the biosynthetic pathway of ginsenosides, editing key genes can impact both the yield and types of ginsenosides produced. Selecting appropriate target genes is a pivotal aspect of successful editing. For different enzyme genes, analysis of their position and function in the synthesis pathway can guide the selection of genes that significantly influence ginsenoside production. Designing suitable sgRNAs ensures the specific binding of the Cas9 protein to the precise site of the target gene, enabling accurate editing.

To apply CRISPR/Cas9 technology in plants, it is necessary to construct suitable editing vectors. Editing vectors typically encompass the Cas9 protein and corresponding sgRNA sequences, along with a selected marker gene for screening transformed plants. Once constructed, vectors can be introduced into plant cells using methods such as Agrobacterium-mediated transformation, thereby facilitating the delivery of the editing payload.

4.2 Impact of targeted editing on ginsenoside biosynthesis

The application of targeted editing techniques can lead to alterations in the expression of target genes, subsequently affecting the expression levels of key enzymes in the ginsenoside biosynthesis pathway. Post-editing changes in gene expression could encompass upregulation, downregulation, or complete elimination of target gene expression. Through gene expression analysis, the effects of editing can be determined, along with their implications for the biosynthetic pathway.

Targeted editing of key genes can directly impact the accumulation of intermediates and final products within the ginsenoside biosynthetic pathway. For instance, editing the β-ASD gene might result in increased accumulation of oleanolic acid, thereby affecting the synthesis of different types of ginsenosides (Sun et al., 2013). Editing genes involved in oleanolic acid synthesis could alter the yield and proportion of different oleanolic acid derivatives. Changes in metabolite production could influence the activity and pharmacological effects of ginsenosides.

Ginsenosides are the primary bioactive constituents of ginseng, and their types and proportions directly influence the medicinal effects of ginseng. The effects of targeted editing on key genes in the ginsenoside biosynthetic pathway could lead to potential changes in the medicinal properties of ginseng. Edited ginseng varieties might exhibit distinct pharmacological activities, bioavailability, and clinical effects. Therefore, assessing the changes in medicinal effects resulting from editing requires thorough pharmacological research to ensure the preservation or enhancement of ginseng's medicinal properties.

4.3 Application of targeted editing in medicinal component optimization

As targeted editing technology matures for plant genomes, its potential in optimizing the ginsenoside biosynthetic pathway becomes increasingly intriguing. Precise editing of key genes offers the prospect of adjusting the yield and proportions of specific ginsenoside types, thus achieving the optimization of medicinal components. However, this optimization entails not only the editing of individual genes but also necessitates a comprehensive consideration of the entire synthetic pathway's regulation. Therefore, in future research, by integrating gene editing techniques with metabolic engineering approaches and employing systems biology methods, achieving more precise synthesis regulation of ginsenosides becomes feasible, ultimately enabling the synthesis of personalized medicinal components.

With the continuous advancement of gene editing technology, achieving more precise genome targeted editing is possible in the future, enabling finer optimization of medicinal components. Additionally, by integrating gene editing with metabolomics analysis, a deeper understanding of the impacts of different edits on the entire synthesis pathway can be gained, thereby better predicting and optimizing the yield and activity of medicinal

components. This comprehensive approach holds the potential to enhance the pharmacological effects of ginseng, positioning it to play a more significant role in the field of personalized medicine.

However, despite the immense potential of targeted editing techniques, they still encounter certain challenges in practical application. Issues such as editing efficiency, editing specificity for different genes, and the genetic stability of edited cells need further in-depth investigation. Additionally, the assessment of the safety and potential changes in pharmacological effects of edited products is crucial to ensure the safety and efficacy of edited ginseng for medicinal purposes.

5 Challenges and Prospects of CRISPR/Cas9 Technology in Editing the Ginsenoside Biosynthetic Pathway

CRISPR/Cas9 technology, as a powerful gene editing tool, holds immense potential in the field of ginseng gene editing. However, its application is still in a rapidly evolving phase, with numerous possibilities and directions for further optimization and expansion in the future.

One of these challenges involves enhancing editing efficiency and specificity. Future research can be directed towards developing more efficient and precise Cas9 proteins or alternative editing tools, aiming to increase the success rate of gene editing and reduce occurrences of nonspecific editing. Moreover, unintended mutations that may arise from gene editing are also a concern. Researchers can employ more advanced sequencing techniques, such as single-cell sequencing, to comprehensively analyze mutations in edited plants. This approach will help identify any inadvertent mutation events, enabling a better assessment of the safety and stability of the editing process.

The application of gene editing technology not only enables the specific editing of target genes but also facilitates the profound integration of genomics and metabolomics. Combining genomics and metabolomics allows for a more comprehensive understanding of the impact of changes in gene expression on metabolite accumulation after editing. Through high-throughput sequencing techniques, information about edited genomes and transcriptomes can be obtained, thereby predicting the effects of editing on the ginsenoside biosynthetic pathway.

Future research can be oriented towards achieving personalized medicinal goals. Gene editing technology offers the potential for tailored medicinal components, enabling the adjustment of the production and types of specific ginsenosides according to individual needs. By editing specific key genes, ginseng varieties that better align with specific pharmacological requirements can be obtained, thereby paving the way for the prospect of personalized medicine.

Apart from gene editing, other plant genome engineering technologies are also evolving. Various techniques, including other tools within the CRISPR/Cas system (such as gene activation and inhibition technologies) and RNA interference, can be synergistically employed to achieve more precise regulation and optimization of plant metabolic pathways. As gene editing technology continues to advance, ethical and legal considerations also warrant attention. When applying gene editing technology to ginseng gene editing, careful consideration of relevant ethical and legal regulations is essential to ensure the legitimacy and compliance of research and applications.

6 Conclusion

This review systematically explores the targeted editing of the ginsenoside biosynthetic pathway using CRISPR/Cas9 technology and its potential implications. Through analyses of aspects including ginsenoside biosynthetic pathways, key enzymes and their roles, as well as intermediate product conversions, a comprehensive understanding of the intricacies of ginsenoside synthesis is achieved. Subsequently, the fundamental principles and applications of CRISPR/Cas9 technology are elaborated upon, along with specific application cases in ginseng gene editing.

Regarding the impact of targeted editing on ginsenoside synthesis, the discussion encompasses changes in gene expression, alterations in the accumulation of metabolic products, and the potential implications for ginseng's medicinal efficacy. These changes directly influence the pharmacological properties and applications of ginseng. Furthermore, possibilities for further optimizing CRISPR/Cas9 technology are explored, including the integration of genomics and metabolomics, as well as advancements in the field of genomic engineering.

Despite significant advancements in the field of ginseng gene editing using CRISPR/Cas9 technology, several challenges and limitations persist. In future research, it is imperative to enhance editing efficiency and specificity, deepen the understanding of the impact of edits on ginsenoside biosynthesis, and holistically consider ethical and legal factors.

In conclusion, gene editing technology offers a robust tool for optimizing ginsenoside biosynthetic pathways, enabling personalized medicinal applications, and addressing challenges within the ginseng industry. With the ongoing advancement of this technology, there is a reason to believe that ginseng gene editing research will continue to drive advancements in the field of medicinal plants, contributing to a healthier world.

Acknowledgments

The author expresses her heartfelt gratitude to Livia Han for her thorough review of this manuscript and for providing meticulous suggestions for improvement. Some images in this review are sourced from the internet; should there be any concerns, please feel free to reach out to the author. The author hold the utmost respect for the rights of image owners. Once again, thank you for your understanding and support.

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