Towards efficient bioproduction of anti-tumor ganoderic acids (GAs): genome editing and synthetic biology approaches

Han Xiao

State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic & Developmental Sciences, and Laboratory of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dong-chuan Road, Shanghai, 200240, China

Ganoderic acid (GA), a triterpenoid from the traditional Chinese medicinal mushroom *Ganoderma lucidum* (Ling-zhi), possesses antitumor and other significant pharmacological activities. However, there is a lack of methods of genetic manipulation in mushroom, such as gene disruption, which hinders the studies on biosynthesis of these useful natural products in *G. lucidum*. Here, a modern genome editing tool of the CRISPR-Cas9 assisted gene disruption was attempted and we thus established this technology for the first time in mushroom by taking *Ganoderma* species as typical examples. It may help to provide metabolic engineering approaches for hyper production of GAs in *G. lucidum*. In our parallel efforts, owing to the immaturity in genetic manipulation of mushrooms as well as their much slow growth compared to other microorganism, biosynthesis of GAs in a heterologous host is therefore looked at as an attractive alternative. Using *Saccharomyces cerevisiae* as a host, we did a systematic screening of 72 candidates of cytochrome P450 monooxygenase (CYP450) genes from *G. lucidum*, which are considered responsible for the GA biosynthesis from lanosterol. As a result, overexpression of *cyp5150l8* led to the production of an antitumor GA, 3-hydroxy-lanosta-8, 24-dien-26 oic acid (HLDOA) in *S. cerevisiae*, as confirmed by HPLC, LC-MS and NMR. A titer of 14.5 mg/L of HLDOA was obtained at 120 h of the yeast fermentation. Our *in vitro* enzymatic experiments indicate that CYP5150L8 catalyzes a three-step biotransformation of lanosterol at C-26 to synthesize HLDOA. Furthermore, we constructed a dual tunable system for balancing the expression of CYP5150L8 and a *Ganoderma* P450 reductase iGLCPR, and performed a comprehensive optimization of CYP5150L8 expression, iGLCPR expression and glycerol usage. The best strain in optimized condition was able to produce 154.45 mg/L HLDOA. The results will be helpful to the GA biosynthetic pathway elucidation as well as to future optimization of heterologous cell factories for GA production.

Reference

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