【要約】

Isolation and functional characterization of AP2/ERF transcription factors involved in the regulation of specialized metabolism in *Ophiorrhiza pumila*

チャボイナモリの二次代謝に関与する AP2/ERF 転写制御因子の単離と機能解明

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Abstract

Camptothecin (CPT), a monoterpenoid indole alkaloid (MIA), is well recognized for its antitumor activity through inhibition of DNA topoisomerase I. CPT is used as a precursor for the synthesis of clinically approved derivatives, topotecan and irinotecan. Plant extract as a main source of CPT has raised environmental concerns and the need to establish an alternative sustainable CPT producing system. Therefore, a thorough knowledge about CPT biosynthesis pathway and regulatory mechanism in producing plant is essential. The hairy roots (HR) system of *Ophiorrhiza pumila* producing feasible level of CPT has been established and used for the investigation of CPT biosynthesis. In this study, five genes that encode AP2/ERF transcription factor, namely *OpERF1* to *OpERF5*, were isolated from *O. pumila* HR. Phylogenetic analysis of AP2/ERF protein sequences suggested the close evolutionary relationship of *Op*ERF1 with stress-responsive ERFs in Arabidopsis and of *Op*ERF2 with ERFs that regulate alkaloid production, such as ORCA3 in *Catharanthus roseus*, *NIC2* locus ERF in tobacco, and JRE4 in tomato. We generated the transgenic HR lines of *O. pumila*, ERF1i and ERF2i, in which the expression of *OpERF1* and *OpERF2*, respectively, was suppressed using RNA interference technique. The transcriptome and metabolome of these suppressed HR were analyzed for functional characterization of *Op*ERF1 and *Op*ERF2. Although significant changes were not observed metabolome, including CPT and related compounds, the suppression of *OpERF2* resulted in reduced expression of genes in the 2-*C*-methyl-D-erythritol 4-phosphate and secologanin-strictosidine pathways, which supply strictosidine, a precursor for CPT and MIA biosynthesis. Furthermore, while it was not conclusive for *Op*ERF1, enrichment analysis of differentially expressed genes in the suppressed HR showed that the gene ontology terms for oxidation-reduction, presumably involved in secondary metabolism, were enriched in the ERF2i downregulated gene set. Expression of *OpERF2* is jasmonate inducible, as similar to other secondary metabolite-regulating group IX ERFs. These results suggest a positive role of *Op*ERF2 in regulating specialized metabolism in *O. pumila*.