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Outline of Research Result

Structural diversity of fungal secondary metabolites induced by chemical epigenetic manipulation

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Research aims

Filamentous fungi produce a variety of secondary metabolites, many of which possess medically relevant biological activities. Since fungal genome sequencing has progressed, it has become apparent that fungi possess far more biosynthetic gene clusters than was evident from previous chemical studies. This observation suggests that a vast number of secondary metabolites have yet to be identified. A promising approach for identifying novel metabolites is to activate silenced genes by manipulating the chromatin-based epigenetic regulation of the genes involved in secondary metabolism.[1] Recently, the use of chemical epigenetic modifiers, such as histone deacetylase (HDAC) and/or DNA methyltransferase inhibitors, was introduced as a promising approach for identifying cryptic fungal secondary metabolites.[2] Here, we adopted this approach to examine the secondary metabolite profiles of a wide range of fungal species, and identified novel metabolites, encoded by silent biosynthetic gene clusters, in three fungal species.

Methods

To identify the optimal conditions for HDAC and/or DNA methyltransferase inhibitor treatment, several fungal species were cultivated under various inhibitor concentrations. Ethyl acetate extracts of culture media were harvested and HPLC was utilized to evaluate the efficacy of the treatments by assaying for the appearance of new peaks and/or an enhanced accumulation of constitutive secondary metabolites. Entomopathogenic fungi of the genus *Chaetomium*, and fungi isolated from medicinal plants and insects were used as microbial resources. The structures of new compounds were elucidated by spectral analyses. To analyze the histone acetylation state around the polyketide synthase gene *pksCH-2*, fungal cultures were treated with an HDAC inhibitor, and chromatin immunoprecipitation (ChIP), using an anti-acetyl histone 4 (H4Ac) antibody, was performed. To assess *pksCH-2* gene expression, treated and untreated cultures were analyzed by quantitative real-time PCR.

Results

Upon treatment with a number of HDAC and DNA methyltransferase inhibitors, suberoyl bis-hydroxamic acid (SBHA, 100–500 μM), a Zn^{2+} type HDAC inhibitor, and nicotinamide (NA, 10–100 μM), a NAD^{+} dependent HDAC inhibitor, were found to enhance production of fungal secondary metabolites in a wide range of fungal species. Specifically, treatment with SBHA or NA resulted in dramatic changes in the secondary metabolite profile in the culture medium from *Pestalotiopsis* sp. isolated from *Taxus cuspidata* var. *nana* (SBHA 500 μM), *Chaetomium indicum* (SBHA 500 μM) and *Chaetomium cancroideum* (NA 50 μM), compared to those of the negative controls (Fig. 1).

After scaling-up the fungal cultivations, we utilized spectral analyses to successfully identify a variety of novel natural products from the EtOAc extracts. These products included structurally diverse aromatic polyketides from *Pestalotiopsis* sp. and *C. indicum*, and unique branched fatty-acyl polyketides from *C. cancroideum* (panels 1–9, and 10–12 in Fig. 2, respectively).[3,4)]

To determine whether the induction of fungal secondary metabolite production by HDAC inhibitors is directly associated with chromatin-based epigenetic regulation through histone acetylation, we identified a non-reducing polyketide synthase (NR-PKS) gene, *pksCH-2*, encoding the common

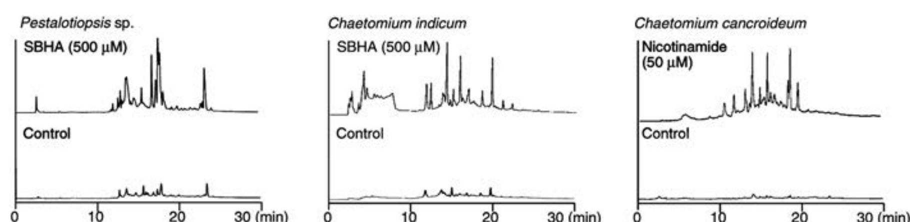

Fig. 1. Reverse-phase HPLC profile of culture media extracts from *Pestalotiopsis* sp., *Chaetomium indicum*, and *C. cancroideum*, cultivated in the presence or absence of inhibitors.

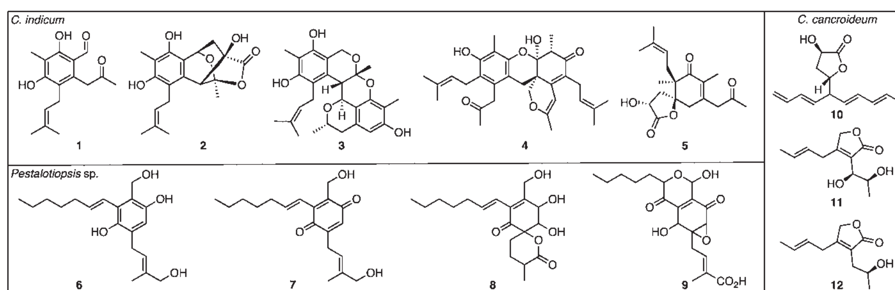

Fig. 2. Examples of novel secondary metabolites isolated in this study.

intermediate of **1–5** in the draft genome of *C. indicum*. RT-PCR analysis demonstrated that SBHA treatment resulted in an increase in *pksCH-2* gene expression. Meanwhile, ChIP analysis indicated that this increase in expression was accompanied by a change in the acetylation state of the NR-PKS locus from an inactive hypo-acetylated, to a transcriptionally active hyper-acetylated state. These results suggest that a chromatin-based epigenetic process regulated the expression of *pksCH-2* upon SBHA treatment, and that this regulation enhanced the production of a wide variety of polyketides in *C. indicum*.

Conclusion

We developed a chemical-based method for isolating novel secondary metabolites from fungi. Using the HDAC inhibitors, SBHA and NA, we induced epigenetic changes in *Pestalotiopsis* sp., *C. cancroideum* and *C. indicum*, and successfully isolated a variety of novel natural products. This study demonstrates the practicality of this chemical epigenetic method for identifying novel metabolites in a wide variety of fungi through the stimulation of silent gene clusters.

References

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