公益財団法人 セコム科学技術振興財団
研究成果報告書

研究課題名

腸内細菌由来新規大腸がんリスク要因、コリバクチンの発がん機序解明と予防法の確立

Understanding a mechanism of an onset of colorectal cancer by colibactin and its cancer prevention

研究期間

平成 31 年　10 月　～　令和 5 年　9 月

報告年月

令和 5 年　9 月

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Abstract

Recent studies have shown that *Escherichia coli* often carries a biosynthetic gene cluster referred to as either the *pks* island or the *clb* cluster that allows the production of a genotoxic polyketide–nonribosomal peptide hybrid secondary metabolite called colibactin. While the gene cluster is not always expressed, when the strain that resides in the colon produces the genotoxin, it is suspected to become a risk factor for colorectal cancer. Therefore, there is a strong interest in devising a simple method for colibactin-producing strain detection and understanding the detailed mechanism of how colibactin can induce oncogenesis, in order to develop convenient early screening methods and possible preventive treatments against colorectal cancer. However, the definitive chemical structure of colibactin remained elusive until recently, primarily due to its low yield and instability. In this review, we will briefly trace the recent studies leading to the identification of the structure of the active intact colibactin. We also designed an activity-based fluorogenic probe for detecting colibactin-producing strain that could discern colibactin production levels among the *E. coli* strains screened. Using the probe, we isolated a wild-type high-colibactin-producing strain from a colorectal cancer tissue sample that proved valuable in identifying new colibactin metabolites and structurally characterizing them by NMR.

It has been indicated that strains of *Escherichia coli* that produce DNA-crosslinking natural product colibactin are one of the possible etiological agents of colorectal cancer. However, how people acquire those strains and how prevalent the infection is among the general populous still remain poorly understood. It could make a significant contribution toward preventing the onset of colorectal cancer if we can develop agents that can specifically suppress the production of colibactin by such *E. coli* strains. Toward achieving this goal, a screening method aiming at discovering ClbP-specific inhibitors was established. ClbP is the peptidase responsible for maturation of biologically inactive precolibactin to genotoxic colibactin by removing the prodrug scaffold *N*-myristoyl-D-asparagine (*N*-myr-Asn). By inhibiting ClbP, colibactin-producing *E. coli* strains will no longer be able to produce the genotoxin, suppressing potentially carcinogenic events in the colorectal tract.

Our screening procedure was designed to be comprised of three steps, combining an activity-based probe assay and an LC–MS-based assay to ensure overall efficiency and accuracy. A library containing 67,965 compounds was screened with this method, and a candidate compound 6 with a mild inhibitory activity was identified. Our preliminary screening work testified the reliability of the screening method we developed. The simple protocols applied to this screening method can be adopted to an automated high-throughput screening method to further improve the efficiency of identifying potential candidate compounds. A following research is in progress that targets to expand the search space of our screening efforts and examine the mechanism of inhibition by the compounds identified. Through those efforts, we are aiming to discover potent ClbP inhibitors that can act as chemoprophylactic agents against colorectal cancer.