（学位第３号様式）

学 位 論 文 要 旨

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題　目	Studies on the differentiation-inducing ability of the aroma compounds isolated from *Cucumis melo* var. *conomon* on RCM-1 colorectal cancer cells (カツラウリ由来の香気成分による大腸癌細胞の分化誘導に関する研究)

Cancer is the second leading cause of human death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018. Among over 100 types of cancers, colorectal cancer is very common regardless of gender. To date, different medical treatments, including surgery, chemotherapy, and radiation therapy, have been employed as cancer therapies, but the toxicity thereof has led to many patients suffering the side effects. Against this background, a new approach and anti-cancer agent with low side effects must be developed for cancer therapies. A human colorectal cancer cell line, RCM-1, was established from a colon cancer tissue diagnosed as a well-differentiated rectum adenocarcinoma. RCM-1 cells spontaneously form 'domes' resembling villiform structures. Two sulfur-containing compounds from *Cucumis melo* var. *conomon* (Katsura-uri), referred to as 3-methylthiopropionic acid ethyl ester (MTPE) and methylthioacetic acid ethyl ester (MTAE), can convert the unorganized cell mass into domes in the RCM-1 cell culture. However, the underlying molecular mechanisms and physiochemical property of such dome formation remain unclear. Herein, we performed a structure–activity relationship analysis and found that methylthioacetic acid (MTA) was the lowest molecular weight compound and the most potent dome-inducing activity among 37 MTPE and MTAE analogs, indicating that MTA is the minimum unit required to induce dome formation. According to our microarray analysis, MTA caused down-regulation of many genes involved in DNA replication and cell cycle control, with the *cell division cycle 25A (CDC25A)* and *cyclin E2 (CCNE2)* genes being the most significantly reduced. Pharmacological analysis showed that the administration of two cell-cycle inhibitors for inactivating CDC25A phosphatase (NSC95397) and the cyclin E2/cyclin-dependent kinase 2 complex (purvalanol A) increased the number of domes independently of MTA. Altogether, our results indicate that MTA induces dome formation via the downregulation of *CDC25A* and possibly *CCNE2* being important steps in this process. Dome formation in cell culture has been shown to partially mimic the differentiated function of colon and kidney and is recognized as a morphological differentiation marker. However, the domes developed in RCM-1 cell culture have not been validated as a reliable marker yet. Our results demonstrated that MTA (not more than 2 mM) differentiates the unorganized cell mass into the dome in RCM-1 cell culture by disclosing the correlation between dome formation and several intestinal differentiation markers such as alkaline phosphatase activity and the protein levels of dipeptidyl peptidase 4, villin, and Krüppel-like factor 4. Dome formation in RCM-1 cell culture was additively enhanced by the simultaneous administration of MTA and butyric acid (BA), suggesting that MTA directs the differentiation of RCM-1 cells, potentially through the same or similar pathway(s) shared with BA. Notably, a high dose of MTA (2 mM or more) elevated several apoptosis markers, such as DNA fragmentation, caspase-3/7 activity, and cleavage of poly(ADP-ribose) polymerase. Altogether, in addition to RCM-1 cell differentiation, MTA can trigger the apoptosis. These results indicate that MTA is a potential anticarcinogenic agent applicable in differentiation therapy and traditional chemotherapy against colorectal cancers.