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RESEARCH ARTICLE

PEPPERMINT UP-REGULATED JMJD1B PROTEIN EXPRESSION IN DAUDI CULTURE CELLS

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ABSTRACT

JMJD1B is a promising molecule involved in tumor suppressor, whose gene loss or inactivation may lead to MDS and AML. The present study aimed to determine the effects of certain herbs and spices on JMJD1B expression. RT-PCR was used to quantify the expression level of the gene. Protein samples were then analyzed by Western blotting. Both RT-PCR and Western blotting revealed the up-regulation of JMJD1B expression in Daudi cells treated with ethanol extracts of Peppermint or Lemongrass. In conclusion, component(s) of certain herbs and spices may induce the up-regulation of JMJD1B.

INTRODUCTION

Since cytogenetic deletions involving chromosome 5 are frequently seen in hematopoietic malignancies including myelodysplasia (MDS) and acute myeloid leukemia (AML), JMJD1B (jumonji domain-containing 1b, also called as KDM3B or 5qNCA) has been suggested as the corresponding tumor suppressor gene. In support of this, constitutive expression of the JMJD1B protein has growth suppressive activity in a del (5q) cell line (Hu *et al.,* 2001). Furthermore, the JMJD1B had significantly reduced mRNA expression in del (5q) compared to non-del (5q) MDS patients and healthy controls (Graubert *et al.,* 2009). JMJD1B encodes a 191-kD protein that is localized primarily to the nucleus. As JMJD1B appears to be primarily a nuclear protein with a single zinc finger, it has been speculated that it functions as a nuclear co-activator. The relatively high level of expression of JMJD1B in leukemia cell lines and in normal bone marrow cells suggests that it may play an important role in the normal function of bone marrow cells. So far, many JMJ family proteins have been found and shown to be histone demethylases. However, JMJ family proteins can regulate gene expression by not only histone demethylation but also other histone modification. JMJ regulates cardiomyocyte proliferation via interaction with retinoblastoma protein (Jung *el al.,* 2005). JMJ also regulates the balance between self-renewal and differentiation of embryonic stem cells (Shen *et al.,* 2009). JMJD1A may affect spermatogenesis as well as embryonic development (Knebel *et al.,* 2006).

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In addition, the increased expression of JMJD1A might be associated with the progression of kidney cancer (Guo *et al.,* 2011). Thus, JMJ family proteins have interesting and important functions in a variety of cell types. Because it was well known that certain herbs were an alternative treatment for prevention against several diseases, we hypothesized that some herbs or spices could affect the expression of JMJD1B to expect for cancer prevention. Certain herbs have been touted to possess a lot of beneficial activities and herbal medications are currently being widespread for clinical use in therapy, as the herbs have relatively mild bioavailability and also low toxicity (Abad *et al.,* 2010). However, there are insufficient scientific data on the efficacy of precious herbal therapies. The precise mechanism for the effect of herbs is largely undefined, and limited data and a few convincing evidences have been provided at the molecular level (Okumura *et al.,* 2010, Nishimura *et al.,* 2011). Therefore, basic research and development pointed at elucidating the mechanism of action underlying the herbal effects should have very high priority. Therefore, we elected to test the in vitro effect of several herbs on the expression of JMJD1B in cultured cells.

MATERIALS AND METHODS

Cell culture

The human cell lines Daudi and Jurkat were maintained in RPMI1640 supplemented with 10 % fetal bovine serum (FBS), penicillin and streptomycin at 37°C in a humidified atmosphere containing 5 % CO2.

Extracts preparation

Herb and spice powders were purchased at food market in Japan. The powders were dissolved in 80 % ethanol and subsequently diluted in 40 % ethanol at a stock concentration of 50 mg/ml. The mixtures were vortexed rigorously for 3 min followed by 3 min sonication. After centrifugation (1500 g, 5 min), the supernatants were collected and stored at -20°C until use. For the cell treatments, a range of 0.5-10.0 µl was added into 1 ml of cell culture medium.

Reverse transcriptase polymerase chain reaction (RT-PCR)

JMJD1B, Rb1 and GAPDH mRNAs were analyzed by semi-quantitative RT-PCR. Total RNA was extracted by RNA isolation Kit (TAKARA, Japan). Two micrograms of total RNA was reverse-transcribed using Phusion RT-PCR Kit (NEB, Finland) as described in the manufacture's protocol. Cycle based PCR was used to semi-quantitate the JMJD1B or Rb1 gene level. GADPH were also used as an internal loading control. All the samples were determined within 3 months after collection. The primers used for the PCR were designed as follows, JMJD1B Fw : AAGCATAGAGGGGAAAGATG, JMJD1B Rv: CGTTTTCTTTGGTGTAAGTG, (expected size: 260 bp); Rb1 Fw : TGCATGGCTCTCAGATTCAC, Rb1 Rv: CAGACAGAAGGCGTTCACAA (expected size: 331 bp); GAPDH Fw: TCCCATCACCATCTTCCA, GAPDH Rv: CATCACGCCACAGTTTCC, (expected size: 376 bp). For Real-time PCR, the reactions were performed in a Real-time PCR system (Illumina, USA) using KAPA SYBR FAST Reaction Mix (Genetics, Japan). Thermo-cycling was done according to the instruction at 60 °C annealing temperature in a final volume of 10 µl including Taq DNA polymerase. To correct for differences in both RNA quality and quantity between samples, data were normalized using the ratio of the target cDNA concentration to that of GAPDH.

Western blot analysis

Equal amount of protein samples were used for western blot analysis using anti-JMJD1B (AVIVA) and anti-Erk2 (Epitomics) antibody, and quantified by densitometry. All the western blots were repeated at least three times and the representative data were shown.

RESULTS AND DISCUSSION

To investigate the possibility of using medicinal herb, extracts of many herbs and spices including Peppermint, Garlic, Garam masala, Black pepper, Red pepper, Habanero chilli, Cinnamon, Oregano, Coriander, Turmeric, Basil, Lemongrass were added into cell culture medium of Daudi or Jurkat cells and the levels of the gene expression including JMJD1B were examined. We employed RT-PCR analysis to quantify the expression level of the gene. Total RNA was isolated 24 hr after herbal extracts treatment for detection of JMJD1B and the levels of mRNA were determined by the conventional semi-quantitative RT-PCR. As shown in Figure 1, the JMJD1B gene expression level greatly increased in the treatment of Peppermint or Lemongrass extracts at the final concentration 50 µg/ml, compared with the untreated ethanol vehicle group. Expressions of retinoblastoma 1 (Rb1) gene and

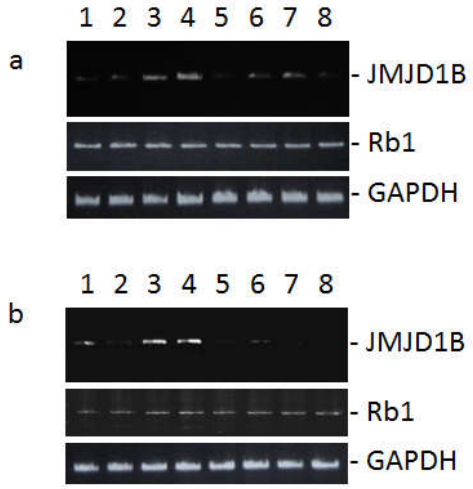

Figure 1. Semiquantitative RT-PCR was performed using primers specific to JMJD1B, Rb1, or GAPDH on 100 ng total RNA prepared from Daudi (a), or Jurkat (b) cells treated without (lane 1) or with extracts of herbs (lane 2-8: Coriander, Lemongrass, Peppermint, Habanero chilli, Oregano, Black pepper, Garam masala, respectively) at the final concentration 50 µg/ml for 24 hr. Specific expression was determined in relation to the expression of the housekeeping gene GAPDH used as an internal loading control. At least four independent experiments were done, and typical paired results were documented.

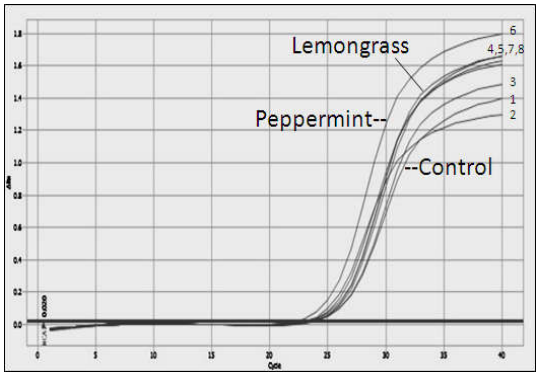

Figure 2. Fluorescence data for PCR amplification plots of the JMJD1B gene in Daudi cells stimulated by each herbs and spices (number 1-8: Control ethanol, Garlic, Cinnamon, Coriander, Turmeric, Peppermint, Lemongrass, Habanero chilli, respectively). Data were generated by the thermal-Cycler software on Illumina eco Real-Time PCR Detection System. No product was amplified in the no-template sample or when reverse transcriptions were omitted.

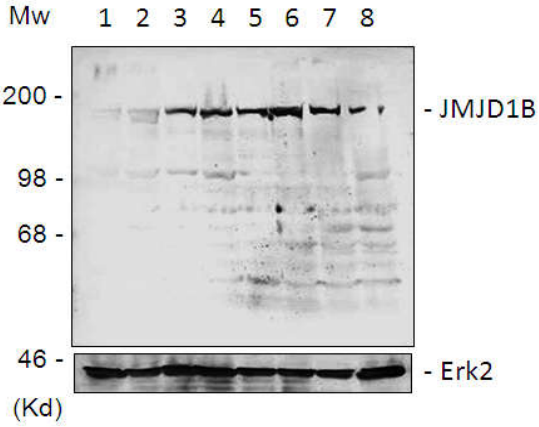

Figure 3. Several herbs extracts including Peppermint induced the expression of JMJD1B protein. Daudi cells were treated without (lane 1) or with extracts of herbs (lane 2-6: Garlic, Cinnamon, Coriander, Turmeric, Peppermint, Lemongrass, Habanero chilli, respectively) at the final concentration 50 µg/ml for 72 hr. After treatment, cell lysates were isolated, the level of JMJD1B protein was detected by Western blot analysis using anti-human JMJD1B antibody. Western blot with anti-Erk2 antibody was also shown as equal levels of protein loading. At least three independent experiments were done, and typical paired results are shown.

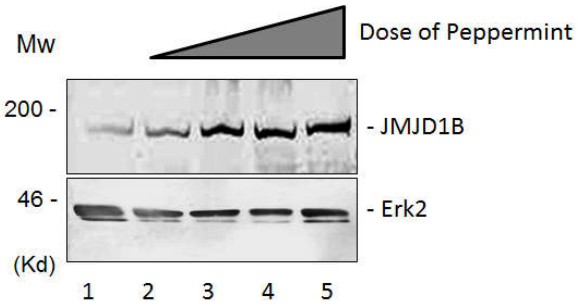

Figure 4. Dose dependent induction of JMJD1B protein expression by Peppermint. Daudi cells were treated without (lane 1) or with Peppermint extract at the final concentration 10 μg/ml (lane 2), 50 μg/ml (lane 3), 100 μg/ml (lane 4), 150 μg/ml (lane 5) for 72 hr. The levels of protein were detected by Western blot analysis using anti-human JMJD1B antibody as Figure 3. Western blots with anti-Erk2 antibody were also shown as equal levels of protein loading in each experiment.

housekeeping GAPDH gene were unaltered in the same conditions (Figure 1). There was little difference also on the results of gene expressional profile between Daudi and Jurkat cells (Figure 1). In addition, similar results were also obtained from the quantitative real-time PCR analysis (Figure2). Similar results were obtained when PCR products of K562 cells were analyzed (data not shown). To exclude the possibility of carry-over DNA contamination, reactions containing all RT-PCR reagents including primers without sample RNA were preformed as negative controls. No such RNA contamination was detected (data not shown).

To further examine the expression status of protein level, Western-blot method was performed to analyze the JMJD1B protein in the cells stimulated by those herbs and spices. As shown in Figure 3, the Peppermint or Lemongrass dramatically induced the protein expression of JMJD1B, but not Erk2 protein, in Daudi cells. The JMJD1B protein expression was also up-regulated by several herb extracts, which was approximately agreed with the result of RT-PCR shown in Figure 1. We then addressed a question whether the herb can induce CNOT3 expression at dose dependent manner. After treating the cells with a set of different concentrations of the Peppermint, Western blotting revealed that JMJD1B protein, but not Erk2, expression was increased with the increasing concentrations of the extracts. Final concentration 100μg/ml of Peppermint extract enhanced the JMJD1B expression by more than 80% in Daudi cells (Figure 4).

Little is known about the relationship between tumor suppressor gene-expression and food ingredients. Traditional chinese medicine has been used for thousands of years. Most chinese herbal formula consist of several herbal components and have been used to treat various diseases. For example, Asian ginseng is the most commonly used medicinal herb in Asian countries, and it is often prescribed for cancer patients as a complementary remedy (Fishbein *et al.,* 2009). The ethanol extract of Asian ginseng significantly suppressed tumor growth with concomitant down-regulation of cell proliferative marker, and it exhibited specific cytotoxicity to cancer cells (Wong *et al.,* 2010). The mRNA level of the wild type p53 gene significantly increased in Kanglaite experimental group compared with the control group (Bao *et al.,* 2004). However, further molecular mechanisms by the herbs largely remain to be elucidated.

In the present study, we have focused on the modification of JMJD1B expression in Daudi cells by treatment of several popular herbs, and presented here that expression level of JMJD1B significantly increased with Peppermint extract. The results indicated that Peppermint potently increased JMJD1B expression and offer support for its potential use in the prevention of hematological malignancies. To our knowledge, there are no reports on the effects of Peppermint or Lemongrass on JMJD1B expression. The active components of this material are unknown. There might be ingredient(s) that are critical for its effects. On the other hand, the whole herb could be important of the maximal effect of the agent. The JMJD1B gene might be complicatedly regulated by various transcription factors. Although the precise mechanism of the action of Peppermint in the hematopoietic cells remains unclear, here, we show that some popular food herbs are the potent transcriptional activator for certain genes. More studies including in vivo experiments need to be undertaken to elucidate the precise molecular mechanisms of this medicinal herb.

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