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Anti-tumor and anti-metastasis activities of honey bee larvae powder by suppressing the expression of EZH2

Masakatsu Kageyama[a,c,1], Kejuan Li[b,1], Shuang Sun[a], Guoqing Xing[d], Ran Gao[e], Zhongfang Lei[a], Zhenya Zhang[a,*]

[a] Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8572, Japan
[b] School of Clinical Medicine, Chengdu University of Traditional Chinese Medicine, 37 Twelve Bridge Road, Jinniu District, Chengdu, 610075, PR China
[c] DHC Corporation, Laboratories, Division 2, 2-42 Hamada, Mihama-ku, Chiba, 261-0025, Japan
[d] Nature Industry of Tsukuba Co., Ltd., 4-31-8 Kenkyugakuen, Tsukuba, Ibaraki, 305-0817, Japan
[e] Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, Beijing, 100021, PR China

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ABSTRACT

Honey bee larvae products have been widely used as traditional daily supplements and complementary medicine for health promotion. However, there is little scientific evidence about their bioactivities. This study was designed to examine the anti-tumor and anti-metastasis effects of honey bee larvae powder (HLP) and explore the underlying mechanism. A subcutaneous transplantation model (murine breast cancer cell 4T1-LUC) and lung metastasis model (murine melanoma cell B16-F10) were established to evaluate the anti-tumor and anti-metastasis effects of HLP. Honey bee larvae powder extract (HLE) was obtained by 70% ethanol extraction, and its chemical composition was determined according to physiochemical methods. Cell Counting Kit-8 assay was performed to test the cytotoxicity of HLE, and qRT-PCR assays were conducted to examine the mRNA levels of tumor marker EZH2 in HLE-treated tumor cells. In vivo xenograft tumor assays in BALB/c mice revealed dose-dependent suppression of tumor growth and lung metastasis showing an inhibition rate of 37.5% and 70.4% at 6 g/kg HLP-administered group with no toxicity to the animals. In vitro studies indicated that HLE showed no cytotoxicity to cancer cells at doses up to 1000 μg/mL, however, it significantly decreased EZH2 mRNA levels in HLE (1000 μg/mL)-treated B10-F10 cells (28.49%) and 4T1-LUC cells (26.75%). Further studies to elucidate the mechanisms involved and to isolate the active components of honey bee larva may provide more valuable information for its development and application in cancer treatment.

1. Introduction

Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis [1]. The clinical development of a cancer chemopreventive agent necessitates mechanism-based efficacy with minimal side effects. Broadly, most chemotherapeutic drugs work by impairing mitosis, effectively targeting rapidly dividing cells [2,3]. Since these drugs cause damage to cells, they are termed cytotoxic. In order to develop new ways to treat cancer and improve patient's quality of life, extremely expensive drug screening and designing have been conducted worldwide. However, the therapeutic power of natural compounds remains underexplored, despite the fact that many natural compounds are affordable and have a long history of human use.

Honey bee-derived products have been widely used as traditionally daily supplements and complementary medicine for health promotion [4]. The main active ingredients of honey bee products have been considered to be composed of phytosterols, phytoestrogens (lignins and flavonoids), amino acids, oligopeptides, and enzymes [5,6]. Modern pharmaceutical research shows that honey bee products, like royal jelly, has been discovered diverse nutritional and pharmacological functions in human/mouse model such as vasodilative and hypotensive activities, anti-hypercholesterolemic activity, estrogenic activity, anti-inflammatory activity, and antitumor activity [7–10]. Although the honey bee larvae also contain considerable proteins, lipids, sugars,

Abbreviations: HLP, honey bee larvae powder; HLE, honey bee larvae powder extract; TNF-α, tumor necrosis factor alpha; CCK-8, cell counting Kit-8; H&E, hematoxylin and eosin; TPC, total phenolic content; TFC, total flavonoids content; EZH2, Enhancer of Zeste Homolog 2; qRT-PCR, quantitative real-time polymerase chain reaction

* Corresponding author.
E-mail addresses: mkageyam@dhc.co.jp (M. Kageyama), likejuan2005@sina.com (K. Li), sunshuang2013@gmail.com (S. Sun), kei@nakahara2001.co.jp (G. Xing), gaoran@cnilas.org (R. Gao), lei.zhongfang.gu@u.tsukuba.ac.jp (Z. Lei), zhang.zhenya.fu@u.tsukuba.ac.jp (Z. Zhang).
[1] These authors contributed equally to this work and therefore share the first authorship.

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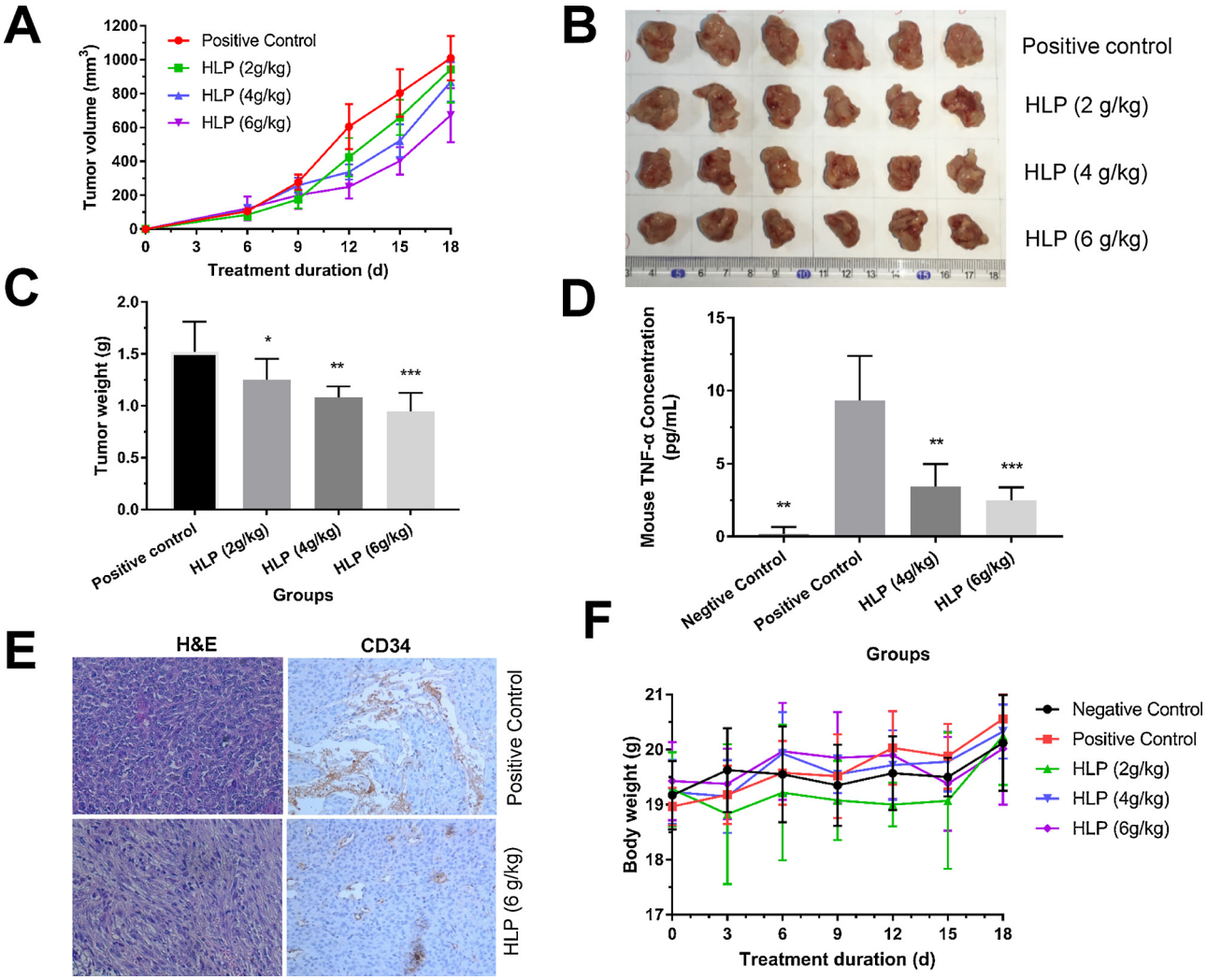

Fig. 1. Tumor growth suppression effect of honey bee larva powder in BALB/c mice tumor xenograft assay. (A) Growth curve of the tumor volume during the tumor growth suppression experiment. (B) Image of dissected tumors in the control and honeybee larva powder treated mice. (C) Average tumor weight in the control and honeybee larva powder treated mice. (D) Mouse TNF-α concentration in the serum of control and honeybee larva powder treated mice. (E) Anti-angiogenic effects of honeybee larva powder in 4T1-LUC xenografts tumors for immunohistochemical CD 34 staining and hematoxylin and eosin staining. (F) Body weight changes during tumor growth suppression experiment. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ denote the statistically significant differences between the treated and control groups.

vitamins, amino acids and numerous minerals, most of them have not been fully utilized and are usually discarded by the beekeepers, which is obviously a great waste for beekeeping.

In fact, in the “Shen nong ben cao jing”, the earliest extant classic Traditional Chinese Medicine pharmacology, honey bee larvae are classified into the top grade, suggesting their great health benefits and therapeutic potentials without toxicity [11]. A previous study revealed that lyophilized powder of honey bee larvae alleviated depression associated with tinnitus by regulating the activity of hypothalamic-pituitary-adrenal axis [6]. Most recently, we found that oral administration of freeze-dried powder of honey bee larvae inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice [12]. To the best of our knowledge, there is no research on the anti-tumor effect of honey bee larvae powder.

Enhancer of Zeste Homolog 2 (EZH2), a key component of the polycomb repressive complex 2 (PRC2) complex, has been found to regulate several cellular processes, including cell fate determination, cell cycle regulation, senescence, cell differentiation and carcinogenesis [13]. Several studies have shown that EZH2 is aberrantly overexpressed in various malignant tumors, such as prostate cancer [14], breast cancer [15], hepatocellular carcinoma [16], and others. Additionally, it was reported that EZH2 acts a critical factor in promoting tumor growth and metastasis in many malignant tumor models [17,18]. Therefore, as a new biomarker, EZH2 can be considered a novel target for the treatment of malignant tumors. In the present study, for the first time, we examined the anti-tumor effect of honey bee larvae powder (HLP) on the development of tumors in BALB/c mice both in the subcutaneous and the tail vein xenograft model. Then, the honey bee larvae powder extract (HLE) was obtained, and its chemical composition, cytotoxicity and the mRNA levels of EZH2 in HLE-treated cells were evaluated.

2. Materials and methods

2.1. Preparation of honey bee larvae powder and honey bee larvae extract

The freeze-dried powder of honey bee larvae (approximately 21 days old) in all experiments performed in this study was obtained from Nakahara Co., Ltd. (Saitama, Japan).

To obtain the honey bee larvae extract (HLE), 60 g of HLP was extracted twice with 70% ethanol (600 mL) at room temperature for 24 h, concentrated via vacuum evaporation and freeze-dried. The obtained HLE was stored at −20 °C until further use.