

## HISTONE MODIFICATIONS AND TRADITIONAL CHINESE MEDICINALS

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**Introduction.** Histones are evolutionarily conserved proteins that abound in the cells of eukaryotes including plants and animals [1]. They form protein families and two copies of each of the structurally similar histones H2A, H2B, H3 and H4 assemble into histone octamers. DNA sequence wraps around the octamers to form nucleosomes, which constitute the subunits of chromatin. Nucleosome-nucleosome and histone-DNA interactions take place to tighten or loosen the chromatin structure, prohibiting or permitting access of the transcriptional machinery, such as RNA polymerase II and regulatory factors, to the DNA sequence. Gene activities and thus genomic functions can change independent of the DNA sequence. Chromatin structure is altered by covalent modifications to the amino acid residues in the unstructured tails of histones. For example, acetylation of the lysines in H3 and H4 N-termini neutralizes the otherwise positively charged histones, weakening the coupling between histones and negatively charged DNA sugar-phosphate backbone. The relaxing chromatin is associated with active gene transcription [2], so is cytosine hypomethylation, a covalent modification to the DNA that is found in association with histone acetylation [3]. An equally important property of histone modifications and DNA methylation is that modification patterns, once established, propagate through cell divisions. Different combinations of covalent modifications over the chromatin give rise to different cellular phenotypes. A histone code, supplementary to the DNA sequence, for cellular functions was therefore recently proposed [4].

Traditional Chinese medicine (TCM) has developed a system of theories and practices since at least 2,000 years ago and remains popular in some Far East Asian areas. In contrast to the reductionist approach of modern western medicine, TCM diagnoses a patient via inspection (e.g. tongue colour), listening/smelling, questioning and palpation (e.g. pulse-reading) [5]. Emotional, mental and environmental factors are usually also taken into account. Outcomes of the diagnostics are summarized as TCM syndromes (called *Zheng* in TCM) which are usually classified under the eight outlines: yin or yang, internal or external, cold or hot, deficiency or excess [5]. Yin and yang in TCM refer, respectively, to the materialistic and functional qualities of the body (parts). External and internal indicate the origin or direction of syndrome development. Cold and hot are manifestations of the syndrome through metabolism and body heat. Deficiency means lack of activities, such as immunodeficiency, of the body organ(s). Two examples of TCM syndromes are Lung-Stomach-yin deficiency with excessive heat and concurrent yin-yang deficiency, both being commonly diagnosed by TCM in type II diabetic patients [6]. A major feat of TCM is that Chinese herbal formulas that counteract the TCM syndromes have been developed so that once the patient's TCM syndrome is identified, the Chinese herbal formula specific to the syndrome is readily prescribed [7–9]. Due to its

diagnostic system, TCM is considered a holistic, personalized yet less specific therapy compared to modern western medicine.

As histone modifications and cytosine methylation play a role in the activity of genes, aberration in the pattern of modifications to histones and DNA, called epigenome, can lead to disease. Indeed, increasing evidence for dysregulated epigenomes in developmental, autoimmunological, metabolic and neurodegenerative disorders has been reported [10–13]. In particular, region-specific hypermethylation over a hypomethylated genome is characteristic of cancer cells [14]. Drugs that target the altered epigenome for cancer treatment have been under investigation. For example two compounds (vorinostat and romidepsin) that inhibit histone deacetylases have been FDA-approved for cutaneous T-cell lymphoma. As histone modifications and DNA methylation are evolutionarily conserved regulatory mechanisms for cell functions, chemicals, e.g. secondary metabolites, in plants and animals that modulate the human epigenome may be found. It is therefore not surprising that a recent study, of bioinformatic nature yet at the pharmacopeia scale, found 30% of ~3,000 traditional Chinese medicinals, the majority of which are herbs, were human epigenome-interacting [15]. Human epigenome-interacting herbs were extensively utilized in TCM formulas so that 99% of the studied formulas were epigenome-interacting. Furthermore, the epigenome-interacting herbs were found to serve in the formulas as the major herbs, called *Monarch* in TCM herbalism, that target patient's main syndrome [15].

**The aim of the study.** Studying the effect of herbs on phenotypes through histone modifications.

**Materials and methods.** Two public traditional Chinese medicine (TCM) databases were accessed to retrieve the chemical constituents and TCM natures of 3,294 TCM medicinals. NCBI taxonomy database was accessed to build the phylogenetic tree of the TCM medicinals. Statistical test was used to test if TCM natures of the medicinals cluster in the phylogenetic tree. A public chemical-protein interaction database was accessed to identify TCM medicinals whose constituent chemicals interact with human histone-modifying enzymes. For each histone modification, a correlation coefficient was calculated between the medicinals' TCM natures and modification modulabilities. Information of the ingredient medicinals of 200 classical TCM formulas was accessed from a public database.

**Results.** Among the basic TCM syndromes, coldness or hotness most easily discern themselves. For example, a healthy individual may be able to determine the coldness or hotness of her constitute by answering a standardized questionnaire. Yin-yang is less straightforward. However TCM asserts that sour, bitter and salty medicinals, believed to be, respectively, physiologically astringing, purging and moistening, are yin medicinals and that sweet medicinals and pungent medicinals, being nourishing and dispersing respectively, are yang medicinals [7, 16]. If one prefers sweet foods, then her constitute is likely more yin (or yang-deficient). Furthermore, cold medicinals are yin and hot medicinals are yang. In Methods, we quantified the cold-hot annotation of each TCM medicinal by assigning a seven-level cold-hot score to it. Local clustering of the colours indicates that similar TCM

properties are shared among evolutionarily related medicinals. Since evolutionary relatedness indicates similarity in the organisms' biology including their metabolomes, local clustering of the TCM properties attributable to the metabolites in the medicinals is what one would expect. We employed Moran's  $I$  [17], a statistical test for autocorrelation of adjacent phenomenon, and found a significant local clustering of the TCM cold-hotness on the phylogenetic tree ( $p = 6.4 \times 10^{-7}$ ).

It was found that 1,170 or 36% of the 3,294 TCM medicinals interact with human histone-modifying enzymes. Among the histone-modifying medicinals, 56% of them promote chromatin condensation. The cold-hot natures of TCM medicinals were found to be phylogenetically correlated. Furthermore, cold (hot) TCM medicinals were found to be associated with heterochromatinization (euchromatinization) through mainly H3K9 methylation and H3K4 demethylation. The associations were weak yet statistically significant. On the other hand, analysis of TCM formulas, the major form of TCM prescriptions in clinical practice, found that 99% of 200 government approved TCM formulas are histone-modifying. Furthermore, in formula formation, heterochromatic medicinals were found to team up with other heterochromatic medicinals to enhance the heterochromatinization of the formula. The synergy was mainly through concurrent DNMT and HDAC inhibition, co-inhibition of histone acetylation and H3S10 phosphorylation, or co-inhibition of H3K4 demethylation and H3K36 demethylation.

TCM natures (i.e. cold, cool, warm and hot) and flavours (i.e. pungent, sweet, sour, bitter and salty) have been essential properties of Chinese herbs since the early days of TCM in that the properties are closely connected to the classes of TCM syndromes they are supposed to treat. For example, yin-deficient physical constitutes and TCM syndromes are treated with yin-nourishing herbs. Similarly, cold-inclined individuals will be prescribed with warm herbs where cold can be considered warm-deficient. It is therefore of paramount importance that the TCM properties live up to the expectations of modern biology. Evolutionarily closely related species, such as Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*), have similar genomes and may be expected to deliver similar pharmacological effects to the human body compared with herbs of dissimilar genomes. Since the nucleic acid or amino acid sequences are of a modern discovery, results of the phylogenetic analysis argue that TCM cold-hot and yin-yang are qualified properties for scientific investigation. Indeed, an *in vitro* study reported bitter, sour (yin) herbs to be associated with higher anti-oxidant activity than pungent, sweet (yang) herbs [18].

Herbs and formulas differ from conventional drugs in their multi-compound composition. The effects of many of the compounds on cells are, however, unclear, let alone studies of their interactions. By focusing on histone modifications and chromatin conformations the herbs impart, we showed that herbs in a formula cooperate to condense or unpack the chromatin and that a chromatin condensing/unpacking formula is composed of herbs that are chromatin condensing/unpacking. The finding is in agreement with a recent study in which warm (cold) herbs were found to connect with other warm (cold) herbs in the herb network constructed from TCM formulas [19]. The findings may shed light on herb

combination rules for the development of complementary and new herbal prescriptions for such complex and emerging disorders as cancer and SARS.

**Conclusions.** With the data of 3,294 TCM medicinals from public resources and with the help of a chemical-protein interaction database, we found that 36% of the medicinals interact with human histone-modifying enzymes. Among the histone-modifying medicinals, 56% of them condense chromatin. Further exploration of the connection between histone modifications and TCM medicinals demonstrated that the cold-hot nature of TCM medicinals, one of the central properties of TCM, is phylogenetically correlated. Cold or yin (hot or yang) medicinals were then found to be associated with heterochromatinization through mainly H3K9 methylation and H3K4 demethylation. Studies of TCM formulas found that 99% of 200 government approved TCM formulas are histone-modifying. Furthermore, in formula formation, medicinals are combined in a way that heterochromatic medicinals team up with other heterochromatic medicinals to enhance the heterochromatinization of the formula. TCM prescriptions' modulation of the human epigenome helps elucidation of TCM pharmacology and discovery of epigenetic drugs.

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## ДОСЛІДЖЕННЯ НЕЙРОТРОПНИХ ЕФЕКТІВ КОМБІНОВАНОГО ЗАСОБУ «СЕЛЕРИН»

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**Вступ.** Порушення діяльності центральної нервової системи, пов'язані з підвищеною тривожністю та/або надмірною збудливістю, широко розповсюджені, часто мають хронічний перебіг та погіршують якість життя. Вони часто потребують фармакотерапії, для якої застосовуються переважно седативні та анксиолітичні засоби, асортимент яких є обмеженим. Результати численних досліджень вказують на те, що фітопрепарати можуть мати більш сприятливий профіль безпеки у порівнянні з синтетичними засобами. У цьому аспекті привертають увагу рослини родини селерових (*Apiaceae*), а саме яглиця звичайна (*Aegopodium podagraria* L.) та селера звичайна (*Apium graveolens* L.). Вони застосовуються як харчові, а отже препарати на їх основі мають бути безпечними навіть при тривалому застосуванні.

**Мета дослідження.** Вивчення нейротропних властивостей фітопрепарату «Селерин», який являє собою рідкий екстракт (екстрагент – 70 % спирт етиловий) суміші листя яглиці звичайної та листя селери звичайної у співвідношенні 1:1.

**Матеріали і методи.** Дослідження проводили на мишах масою 20–22 г.