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Research Findings of IT-sparassis crispa Project: A joint R&D project with academia and public-sector organization

Standardization of sparassis crispa and discovery of pharmacological activity

Sparassis crispa can act as estrogen substitute

In October 2014, Intertrade Co., Ltd. embarked on a joint R&D project with academia and public-sector organization to determine the active ingredients of sparassis crispa (IT-sparassis crispa Project) with Tokyo Women’s Medical University (TWMU) and National Institute of Advanced Industrial Science and Technology (AIST).

The research team established a standard strain of sparassis crispa and discovered active ingredients of the fungus as outlined below. The team has filed a patent application related to the research findings. It will continue studies of efficacy and other properties of the fungus in greater detail.

**Points**

- **Fungus genome used in search for active ingredients and quality control**
  
  Genome projects\(^1\) have been completed for 10,000 species, but only for 25 or so species of fungus (including mold). Intertrade collaborated with TWMU and AIST to determine the whole genome sequence of sparassis crispa. This has enabled the Company to establish the sparassis crispa that it supplies as a standard strain for use in the search for active ingredients and for quality control purposes.

- **New cell activity discovered in sparassis crispa extract**
  
  The research team discovered new cell activity in extract from a standard strain of sparassis crispa. The newly discovered cell activity is known as silent estrogen, which retains the advantages of normal estrogen\(^2\) activity without the adverse effect of stimulating cancer cell multiplication. The active ingredient is a candidate for use in estrogen preparations\(^3\), antiestrogen and anticancer agents, anti-arteriosclerotic agents, and health foods.

1. **Background**

   Various physiological activity in sparassis crispa has been reported, such as antitumor effects, lowering blood sugar levels, and immune activation effects. In the majority of studies, beta-glucans (macromolecular polysaccharides) were reported as the active ingredient and few other active ingredients were identified. Although beta-glucans are not digested or absorbed by the human body, they are known to be responsible for various physiological activity such as modulating immunological activity of the intestinal tract.

   Research findings including those from a clinical study with human subjects carried out by Intertrade in 2013 demonstrated that some phenomena cannot be explained solely by the effect of beta-glucans. The Company therefore continued the search for other ingredients that might be involved. Collaboration with TWMU and AIST in a joint project with academia and public sector organization made it possible to investigate the fungus at cellular...
and molecular levels to identify active ingredients.

A sudden decrease in levels of estrogen (the female sex hormone) is known to be the cause of menopausal disorder*4 and various diseases. Estrogen secretion peaks for women in their 20s and 30s and gradually declines, and is sharply reduced before menopause. Estrogen deficiency can lead to hormone imbalances, which are believed to be responsible for the autonomic dysfunction and psychological symptoms of menopausal disorder. It is also known that LDL cholesterol levels rise and arteriosclerosis progresses after menopause, both of which are linked to estrogen deficiency. Hormone supplementation therapy is a treatment for estrogen deficiency that reduces these symptoms by direct administration of estrogen. However, an adverse effect associated with estrogen’s cell multiplication activity is the increased risk of breast cancer and endometrial cancer. For this reason, estrogen therapy*5 using plant-derived estrogen-like compounds with no cell multiplication activity or limited adverse effects is considered a promising alternative. Compounds with estrogen activity that do not induce cell multiplication are also known as silent estrogen.

2. Research findings

1) Established standard strain of sparassis crispa by full genome analysis

   Genome projects have been completed for 10,000 species, but only for 25 or so species of fungus (including mold). The research team’s study was the first full genome analysis of sparassis crispa. As a result, the team was able to determine the whole genome sequence of sparassis crispa, with an estimated genome size of around 34 megabases (34Mb), and discovered that the genomic information contained approximately 10,000 genes. The full genome analysis data has been registered with the DNA Data Bank of Japan (DDBJ).

   It is difficult to distinguish among different taxonomic groups of sparassis crispa, often resulting in confusion between two or more species. Establishing a standard strain by determination of the whole genome sequence will facilitate stable supply of the fungus and quality control regarding the efficacy and quantity of active ingredients. It will also allow future use of information on useful genes contained in the full genome analysis data.

2) Estrogen effect of sparassis crispa

   AIST had established a new measurement method for hormonal activity using DNA chips*6 to evaluate the active ingredients of chemical substances and natural products. This method, which involves the profiling of the effect of chemical substances on the expression of specific genes, was used in this study to obtain more accurate measurements than conventional cell-based assays. Cell-based assays do not detect estrogen-like compounds that do not show estrogen’s cell multiplication activity, because they assume cell multiplication is evidence of hormonal activity. By using this new method, AIST has succeeded in demonstrating that certain Kampo (Japanese traditional herbal) medicines and health foods contain estrogen-like compounds with anti-arteriosclerotic and other effects and isolating some of these compounds.

   The research team tested sparassis crispa extract using the new measurement method based on DNA chips, discovering estrogen-like effects on genes subsequently confirmed at the protein level. The effect is a new mechanism of action unlike known estrogen effects, because no estrogen-like cell multiplication activity was observed.
These research findings hold implications for the use of *sparassis crispa* in a new estrogen preparation for estrogen therapy, drug discovery, and the development of new hormone therapies with no cancer risk using the newly discovered compound.

3) Lipid metabolism improvement effect of *sparassis crispa*

Dried cultured *sparassis crispa* was orally administered to mice once daily over 10 weeks, after which their lipid metabolism was evaluated by blood biochemistry tests. Both total cholesterol and free fatty acid values were low after the study period. The results of this study suggest that *sparassis crispa* has a lipid metabolism improvement effect.

4) Evaluating the safety of *sparassis crispa*

The acute toxicity of *sparassis crispa* was evaluated by single administration to mice of dried cultured *sparassis crispa* and hot water extract of *sparassis crispa* (SCE). No changes attributed to dried cultured *sparassis crispa* and SCE were noted in general condition and bodyweight after administration, or in necropsy observations.

3. Outlook

These research findings have revealed potential for the use of *sparassis crispa* in estrogen preparations that are safer than those currently on the market. The IT-*sparassis crispa* Project will continue studies to identify and find uses of active ingredients in addition to planned human clinical studies to study efficacy and safety in humans in more detail.

The research team plans to progress development of products that harness the estrogen-like properties of *sparassis crispa* and explore ways to use *sparassis crispa* extract and ingredients in health foods, drug discovery, and diagnostics, which is one of the objectives of the project.

【Glossary】

*1 Genome Project*

Genome refers to all genetic information contained in the DNA of an organism. Genome projects are scientific studies that aim to determine the sequence of nucleotide base pairs that make up the DNA of an organism and identify and map all of the genes. Genome projects so far have identified and mapped all the genes of organisms such as viruses, bacteria, and animals.

*2 Estrogen*

Estrogen (also known as one of the follicular hormones or female sex hormones) is a steroid hormone made from cholesterol in the granulosa cells mainly of the ovaries. Physiological functions of estrogen include stimulation of mammary cell multiplication, control of the release of eggs from the ovaries, development of a distinctly female brain (female gender awareness) and reduction of arteriosclerosis, but not all is known about its effects. Estrogen activity can be examined by various biochemical, molecular biological, cytobiological, and biological tests such as ligand binding assay, reporter gene assay, gene expression profiling, enzyme activity assay, enzyme-linked
immunosorbent assay (ELISA) antibody test, cell multiplication test, and bacteria, plant, and animal tests. These tests can also measure estrogen-like compounds, which react in the same way as estrogen. Estrogen-like compounds such as endogenous estrogens, phytoestrogens, and synthetic and environmental estrogens are chemical substances that mimic some or all of the properties of estrogen, including physiological action and genetic effects.

*3 Estrogen preparations

Estrogen preparations are a large group of chemical compounds that mimic or inhibit the action of estrogens such as 17-β estradiol and narrowly defined as agents used in estrogen therapy. Estrogen preparations are either estrogen receptor agonists (with the same action as estrogen) or estrogen receptor antagonists, which either block the binding of estrogen-like compounds with estrogen receptors or inhibit the action of estrogen that has bound to estrogen receptors. Tamoxifen and raloxifene are examples of estrogen preparations. They were developed for the prevention and treatment of osteoporosis, cardiovascular disease, and breast cancer. However, both have adverse effects such as hot flashes and tamoxifen is known to increase the risk of developing endometrial cancer. Thus the development of new estrogen preparations is a priority for pharmaceutical companies.

*4 Menopausal disorder

Menopausal disorder is a syndrome caused by hormone imbalances associated with estrogen deficiency due to reduced ovarian function. Symptoms include those associated with autonomic dysfunction such as increased heart rate, palpitations, and unstable blood pressure; hot flashes (flushed face and sudden feeling of warmth); hyperhidrosis; headaches; dizziness; back pain; numbness; hyperesthesia; joint pain; and psychological symptoms such as anxiety and irritability. The number of menopausal women in Japan is estimated at 20 million, of whom many experience physical and psychological distress due to estrogen depletion. Approximately 20–30% of these women have been medically diagnosed with menopausal disorder.

*5 Estrogen therapy

Estrogen therapy uses estrogen or medication that stimulates or inhibits the secretion or effects of estrogen to alleviate symptoms of menopausal disorder arising from a sudden decline in estrogen levels during menopause and prevent osteoporosis linked with hormone reduction. It is also used for the treatment of gender dysphoria. Patients may receive estrogen supplementation or estrogen replacement therapy (administration of a chemical substance with estrogen activity). Estrogen therapy is used for the treatment of diseases and symptoms caused or induced by estrogen depletion, including ovarian dysfunction or menopause, autonomic dysfunction, sleep cycle disturbance, cognitive disorders, impaired motor function, mood disorders, eating disorders, and cardiovascular disease. Estrogen therapy can increase the risk of a range of severe adverse events including ischemic stroke, myocardial infarction, thromboembolism, cerebrovascular disease, and endometrial cancer. Many studies have been undertaken to discover or synthesize new chemical substances that do not have these adverse effects, such as nonsteroidal estrogens that resemble natural estrogen and antiestrogens (estrogen receptor antagonists).
*6 DNA chip

DNA chip or DNA microarray is an analysis tool in which a high concentration of nucleic acid sequences are bound to a silicon or glass slide substrate divided into tens and hundreds of thousands of sections. The technology makes it possible to study the expression of tens and hundreds of thousands of genes at once. Total gene expression data obtained by this method is called a gene profile.

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