Inhibitory Effects of Symbiotic Lactobacterium- and Yeast- Fermented Soy Extract on Tumor Metastasis and Proliferation

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Recently, all over the world face to serious health problem. Patients of neoplasm, allergy, and virus infection involved in immune systems are increasing continuously, since our immune systems are getting worse with daily life, food and endocrine disrupting chemicals. In the course of our studies for developing immune-response modifiers from functional foods including fermented materials and traditional medicine, we found that symbiotic Lacto bacterium- and Yeast-fermented soy extract (LYS) inhibited tumor metastasis and proliferation by the stimulation of immune systems.
Symbiotic Lacto bacterium- and Yeast-fermented Soy Extract (LYS)

Soy (No genetic recombination)

1) Water, r.t., 24 h
2) Homogenize
3) 100 º, 1h
4) Filtration

Soy Extract

Symbiotic Fermentation

1) Enzyme Reaction (Cellase, Amylase, Protease)

2) Fermentation
   *Lacto Bacterium; E. faecalis, L. helveticus, L. casei, L. sp*
   *Yeast; Saccharomyces cerevisiae*

Symbiotic Lacto bacterium- and Yeast-fermented Soy Extract (LYS)

1) 30 º, 4 d
2) 100 º, 1h
3) Freeze dry

Apply for various Assays
View
Activities of LYS

- Gastro protection (Inhibition of gastric lesions, ~1g/kg rat or mouse)
- Antiflatuents (Regulation of intestinal function)
- Anti-diabetes ( Suppressing high blood glucose level, Inhibition of aldose reductase: improvements of complication)
- Control of immune systems (anti-allergy, immune-response activator)
Effect of LYS on Macrophages Activation

Male ddY mouse (30 g)

Wash with PBS

Selection (Preculture)

Mouse peritoneal macrophage

LYS (~300 µg/ml) or LPS (10 µg/ml, positive control)

37 °C, 20 h

Measurement of Nitric oxide (NO) in the culture medium by Griess reagent

LPS; lipopolysaccharide from S. aureus

Collected from mouse peritoneal macrophage

Primary culture LYS or LPS

Measurement of NO production by Griess reagent

Activation Marker
Effect of LYS on Macrophages Activation

![Bar chart showing the effect of LYS on macrophages activation. The chart indicates that as the concentration of LYS (µg/ml) increases, the activation of macrophages also increases. There is a significant difference (p<0.01) at the highest concentration tested.

- N=6
- **p<0.01

LYS (µg/ml) vs. Macrophages Activation Response

The chart highlights the role of LYS in activating macrophages, with a notable increase in activation at higher concentrations.
Effect of LYS on Macrophages Activation

Mouse peritoneal macrophage

- LYS (~300 µg/ml)
  or
- LPS (10 µg/ml)
  37 °C, 1 h

Fluorescence Beads for phagocytic activity

37 °C, 1 h

Trypan Blue (quenching)

Measurement of fluorescence density of activated macrophage (Ex.532 nm, Em.526 nm)
Effect of LYS on Macrophages Activation

- LYS (µg/ml)
- N=6
- **p<0.01
Effect of LYS on Tumor Metastasis and Proliferation

Female C57/BL6 (4 weeks)

1 week

Injection (i.v.)
B16F10 melanoma, 10^5 cells/mouse

2 weeks

Normal diet +
Water or
1%, 2% LYS water or
1%, 2% lipoprotein water

Same condition

Counting tumor colonies in lung

Lung

Melanoma Injection

Tumor colonies

Metastasis and Proliferation

Counting tumor colonies in lung
Effect of LYS on Tumor Metastasis and Proliferation

Control

1% LYS

2% LYS

**

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Effect of LYS on Tumor Metastasis and Proliferation

N=6, Mean ± S.E. **p<0.01

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1% Lipoprotein Ext.</th>
<th>2% Lipoprotein Ext.</th>
<th>1% LYS</th>
<th>2% LYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Colonies</td>
<td>180 ± 10</td>
<td>120 ± 15</td>
<td>100 ± 20</td>
<td>80 ± 15</td>
<td>40 ± 10</td>
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Conclusion

- By in vitro treatment with LYS, macrophages were significantly activated depending on LYS concentrations. By the addition of 0.3 mg/ml LYS, production of NO and phagocytic activities of macrophages were stimulated 15-fold and 3-fold, respectively.

- By in vivo, melanoma metastasis in lung was significantly suppressed by LYS according to its dose dependence. At dose level of 2% LYS, colonies of melanoma were decreased 1/3-fold. And anti-metastasis activity of LYS was 20% stronger than positive control drug of lipoprotein.

- These data indicates that LYS inhibits tumor metastasis and proliferation by stimulating the immune systems. The LYS, symbiotic Lacto bacterium- and Yeast-fermented soy extract, is considered to be one of the immune-response modifiers.
Anti-Tumor Metastasis and Proliferation Mechanisms of LYS

LYS
Symbiotic Lacto Bacterium- Yeast-fermented Soy Extract

Immune System
( Intestinal Immune System)

Stimulation Activation

Immunocytes (Macrophage, leukocyte....) activation

Production of Cytokine, ILs, TNF

Phagocytosis

Inhibition of Tumor Metastasis and Proliferation

Prevention and Improvement of weakened Immune systems

Immunostimulator, Immunomodulator