**Sericin Obtained from Silkworm**

**As Supplement into Culture Medium for Mammalian Cells.**

Satoshi Terada1*\**, Kyohei Kuriyama1, Ryo Yahagi1, Kohei Kurebayashi1,

Jun Takahashi 2 and Masahiro Sasaki 2

*1 Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, University of Fukui; 3-9-1, Bunkyo, Fukui, 910-8507, Japan;*

*2 R&D Center, SEIREN Co. Ltd.;* *2-3-1 Techno Port, Mikuni-cho, Sakai, 913-0038 Japan*

*\*Corresponding author: terada@u-fukui.ac.jp*

**Highlights**

* Sericin improves the proliferation of the cells in serum-free culture.
* Sericin prevents the cell from apoptosis induced by heating.
* Sericin induced the proliferation of the cells via not only EGF-signaling pathway.
* Sericin induced the proliferation via but also pathway different from EGF-signaling.

**1. Introduction**

Mammalian cells are extensively cultured for using in tissue engineering and for producing biotherapeutics production. Although mammal-derived factors are supplemented into the culture media, these mammal-derived factors have the risk of zoonotic disease and so must be replaced by non-mammal factors.

Previously, we reported that sericin hydrolysate obtained from cocoon of silkworms successfully induced the proliferation of various cells and significantly improved the cellular survival under stressful conditions.

In this study, we aimed to elucidate the mechanism how sericin promotes the proliferation of mammalian cells. For the purpose, we focused on a human keratinocyte cell line, highly depend on sericin, as well as on EGF. Using this cell line, the proliferation signal pathway from sericin was compared with that of EGF.

**2. Methods**

Human keratinocyte PHK16-0b cells were sub-cultured in Keratinocyte-SFM medium supplemented with EGF and BPE. One day later, the medium was replaced into that without both EGF and BPE in order to reset the cells. After additional 24 hours for reset, the medium was replaced to fresh one containing EGF or sericin and/or any of signal inhibitors. Then the cells were cultured for three days and the cell number was counted.

The activation or phosphorylation of signal factors including EGF-Receptor and ERK is confirmed by immunoblotting against lysates from the cells treated with sericin or EGF using anti-phosphorylated tyrosine antibodies.

**3. Results and discussion**

Both EGF-Receptor inhibitor and EGF-Receptor-neutralizing antibody inhibited the proliferation of the cells treated with sericin and EGF, implying that sericin promotes the proliferation through EGF-Receptor as well as EGF.

MAPK pathway inhibitor and JAK/STAT pathway inhibitor inhibited the proliferation of the cells treated with both factors, whereas PI3K pathway inhibitor inhibited the proliferation of the cells treated with sericin only, and failed to inhibited those with EGF; implying that sericin promotes the proliferation of the cells partly through other pathways different from EGF.

Immunoblot analysis found that phosphorylation/activation of EGF-Receptor and ERK in the cells treated with EGF and sericin were suppressed by EGF-Receptor inhibitor and EGF-Receptor-neutralizing antibody.

**4. Conclusions**

We found that sericin promotes the cell proliferation through EGFR-MAPK and EGFR-JAK/STAT signaling, as well as PI3K signaling independent of EGF.