

## **Study on anti-tumor effect of sericin**

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Since cancer is one of the major causes of deaths worldwide, increasing efforts are being made to prevent it. Chemoprevention of cancer is a means of cancer control by which this disease can be prevented completely, slowed, or reversed partially or substantially by the administration of one or more naturally occurring or synthetic chemical agents.

The silk protein, sericin, is the main constituent of silk (20-30% of the total cocoon weight), enveloping the fibroin with successive sticky layers. When the cocoon is used for silk textiles, the sericin is mostly removed from the cocoon and disposed of without any use. Recently, sericin has been found to have a strong antioxidant activity. Sericin is also known to have a skin moisturizing and antiwrinkle effect due to its high content of serine. Thus, sericin has been suggested a potential usefulness of application for cosmetics.

The purpose of this study was to investigate the influence of sericin on colon and skin tumorigenesis. In this study, I postulated that sericin suppresses colon and skin tumorigenesis by a mechanism involving reduction in oxidative stress.

### ***Effect of sericin on 1.2-dimethylhydrazine - induced colon tumorigenesis in mice***

Sericin is a dietary fiber-like protein with low digestibility (namely, "resistant protein"). Consumption of dietary fibers is well known to be associated with lower incidence of colon tumors. Oxidative stress has been considered to be associated with tumorigenesis. Therefore, consumption of sericin may inhibit colon tumorigenesis by its dietary fiber-like and antioxidant properties. To test this hypothesis, male CD-1 mice were fed a diet containing 30 g/kg sericin for 115 d, and given once per week injection of 1.2-dimethylhydrazine (DMH) (10 mg/kg body weight) for the initial 10 wk. The results showed a strong inhibitory effect of dietary sericin against the development of colon adenoma in mice. Mechanistic studies have shown that consumption of sericin significantly reduced the BrdU labeling index, a marker for cell proliferation and the protein expression of proliferation-related genes, *c-myc* and *c-fos*, suggesting that sericin suppresses colon tumorigenesis by reducing cell proliferation. Further analysis showed that dietary sericin significantly inhibited colonic oxidative stress markers including 8-hydroxydeoxyguanosine (8-OHdG) and 4-hydroxynonenal (4-HNE) and inducible nitric oxide synthase (iNOS). From these results, sericin appears to suppress colon tumorigenesis by reducing oxidative stress, cell proliferation and nitric oxide production.

Because of low digestibility of sericin together with its antioxidant property, undigested sericin in the large intestine may suppress colonic oxidative stress, leading to suppression in tumor development. This study was further conducted to examine this hypothesis. Male Wistar rats were fed a diet containing 30 g/kg sericin for 28 d, and given an injection of DMH one wk before initiation of the experiment. The results showed that the contents of the large intestine of rats fed sericin suppressed lipid peroxidation in the homogenate of colon mucosa and Fenton-type reaction

systems-induced DNA oxidation compared to those of control rats. The pattern of amino acids in the large intestine of rats fed sericin was very similar to that of amino acids in sericin. The results supported the hypothesis that undigested sericin in the large intestine suppresses colon tumorigenesis by reducing colonic oxidative stress.

#### ***Effect of sericin on chemical - and UV radiation - induced skin tumorigenesis in mice***

It has been reported that reactive oxygen species (ROS) and oxygen-derived free radicals are generated by ultraviolet (UV) radiation and various chemicals and their important roles in the pathogenesis of skin cancer are being increasingly recognized. I postulated that topical application of sericin may suppress chemical - and UV radiation - induced skin tumor promotion through its antioxidant activity.

To examine the influence of sericin on chemical-induced skin tumorigenesis, sericin was applied topically to 7,12-dimethylbenz [ $\alpha$ ] anthracene (DMBA) - initiated female ICR mouse skin at the doses of 2.5 and 5 mg twice per wk for 16 wk, 30 min prior to each promotion treatment with TPA. The protective effect of sericin was evident in terms of significant reduction in tumor incidence and tumor multiplicity at the doses of 2.5 and 5 mg per application, compared to the control group without receiving sericin. The expression of tumor necrosis factor (TNF)- $\alpha$  protein, an endogenous tumor promoter, and the level of 4-HNE in normal epidermis were significantly reduced in both sericin treatment groups. In additional experiment, sericin at the dose of 5 mg was applied topically to the dorsal mouse skin 30 min before application of a TPA, and the same doses of TPA and sericin were applied twice at an interval of 24 h. The results indicated that sericin treatment inhibited double TPA treatment - induced morphological changes reflecting inflammatory response, including leukocyte infiltration, hyperplasia and proliferating cell nuclear antigen (PCNA), a marker for cell proliferation. Furthermore, sericin treatment significantly suppressed the elevation in 4-HNE level and in the expressions of *c-fos*, *c-myc* and COX-2 in normal epidermis induced by double application of TPA. These results suggest that sericin possesses protective effect against tumor promotion in mouse skin by suppressing oxidative stress, inflammatory responses and TNF- $\alpha$ .

To examine the influence of sericin on UV - induced skin tumorigenesis, HR-1 hairless mice were treated with 180 mJ/cm<sup>2</sup> of UVB once daily for 1 and 7 d. The treatment for 7 d caused red sunburn lesions of the skin. The intensity of red color and area of these lesions were inhibited by the topical application of sericin at the dose of 5 mg after UVB treatment. The application of sericin significantly reduced UVB - induced elevations in 4-HNE, the expression of COX-2 protein and PCNA-labeling index in the UVB exposed epidermis. In additional experiment, HR-1 hairless mice were treated with 200 nmol of DMBA followed one wk later by irradiation with 180 mJ/cm<sup>2</sup> of UVB twice per wk for 22 wk. The protective effect of sericin was evident in terms of significant reduction in tumor incidence and tumor multiplicity at the dose of 5 mg. The results imply that sericin possesses photoprotective effect against UVB - induced acute damage and tumor promotion by reducing oxidative stress, COX-2 and cell proliferation in mouse skin.

Taken together, this study suggested that sericin could be a useful chemopreventive agent against skin cancer including chemical and UV - induced skin cancer.

In conclusion, this study has provided evidence that sericin has a strong anti-tumor activity in colon and skin, and further suggested that the mechanisms might be at least in part mediated by reducing oxidative stress.

**Key words:** sericin, anti-tumor effect, oxidative stress, resistant protein