Molecular cloning and the allergenic characterization of tropomyosin from forcipomyia taiwana

江啟峰、江主惠、李美芳

E-mail: 322199@mail.dyu.edu.tw

ABSTRACT

Background：Forcipomyia taiwana is a tiny blood-sucking midge widely distributed in Taiwan. Like mosquito bites, midge bites can cause allergic reactions. Invertebrate tropomyosins are the panallergens responsible for cross-reactions between crustacea, insects, mites, nematodes, and different classes of mollusks. There are two purposes in this study. Firstly, we aimed to produce recombinant F. taiwana tropomyosin and investigate its allergenicity. Secondly, we aimed to investigate the inflammatory reaction of recombinant F. taiwana tropomyosin in human skin cell line, Hs68. Materials and Methods：We isolated total RNA of F. taiwana and synthesized cDNA by RT-PCR using degenerate primers designed according to tropomyosin sequences of silverfish (Lepisma saccharina). The PCR product was ligated into TA cloning vector and sequenced to confirm the identity of the insert, and then subcloned into pET30a expression system. Recombinant protein was over-expressed in E.coli BL21 and purified using the His-Tag affinity column chromatography. The IgE-binding reactivity of purified recombinant protein was evaluated by immunoblot and ELISA. In addition, the skin fibroblast Hs68 cells were stimulated with rFor t 4 to evaluate the immunogenicity. Results：The cloned F. taiwana tropomyosin, named For t 4, comprised an 855-bp open reading frame and encoded a 32 kDa protein. The deduced amino acid sequence shared 58~67% identity with previously known allergenic tropomyosins. ELISA analysis revealed that rFor t 4 reacted with 24% (6/25) of F. taiwana-sensitized subjects. In addition, rFor t 4 showed 20~50% inhibition of IgE binding to F. taiwana crude extract by inhibition ELISA. It also stimulates IL-8 proteins secretion and up-regulates mRNA expression of IL-8, MCP-1, eotaxin and GM-CSF from human skin fibroblasts. Conclusions：Tropomyosin represents a minor allergen in F. taiwana extract. It is hoped that rFor t 4 will be useful for developing specific in vitro and in vivo diagnostics tools and immunotherapy of F. taiwana allergy.

Keywords : Forcipomyia taiwana、allergy、For t 4、tropomyosin、Hs68 cells

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