

ABSTRACT

Man Ching Leung

Title: Identification of Human Hair Follicle Antigens Targeted in the Presumptive Autoimmune Hair Follicle Disorder *Alopecia Areata* and their Potential Functional Relevance *in vitro*

Keywords: hair follicle; alopecia areata; trichohyalin; autoantigens; proteomics; tissue culture; immunoprecipitation; 2D gel electrophoresis; LC-MALDI-TOF/TOF Mass Spectrometry; immunohistochemistry

Alopecia areata (AA) is a putative autoimmune hair loss disorder. It mainly affects the scalp hair but can also involve body hair, and can also affect the nail and the eye. While there are may be several lines of evidence to support the autoimmune basis of AA, there is still very little information on the hair follicle autoantigen(s) involved in its pathogenesis.

In this project, serum antibodies (AA=10, control=10) were used to immunoprecipitate AA-relevant target antigens from normal human scalp hair follicle extracts. These immunoprecipitates were analysed by LC-MALDI-TOF/TOF mass spectrometry for target protein identification. This part of the project involved substantial methods development.

Trichohyalin was immunoprecipitated by all AA sera, but by only 5 normal sera. Importantly, the mean Mascot scores of the AA group was significantly higher than the normal group ($p=0.005$). Keratin 16 was also identified from immunoprecipitates as another potential AA-relevant target antigen.

Functional studies by *ex vivo* whole hair follicle organ culture using commercial antibodies to trichohyalin and keratin 16 significantly inhibited hair fibre elongation compared to controls.

Indirect immunofluorescence studies revealed that AA sera contained higher immunoreactivity against normal human scalp anagen hair follicles compared to normal sera. Immunoreactivities were mainly in the outer root sheath and inner root sheath, and less so to the medulla and hair bulb matrix. Double immunofluorescence studies of AA and normal serum with anti-trichohyalin antibody (AE15) revealed co-localisation of 9 of the AA sera antibodies with trichohyalin in the inner root sheath (mostly in Henle's, less in Huxley's/inner root sheath cuticle), but only weakly in 3 normal sera.

This study supports the involvement of an antibody response to anagen-specific hair follicles antigens in AA. Moreover, there may be some evidence that these antibodies may have a pathogenic role.