Simple and sensitive assays to detect autoantibodies against alpha 2 macroglobulin-like 1 in paraneoplastic pemphigus sera

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Introduction

Autoantibodies against alpha-2-macroglobulin-like-1 (A2ML1) and desmocollin 3 (DSC3) have been found in 60% of paraneoplastic pemphigus (PNP) patients and it has been suggested that these autoantibodies might be pathogenic in a subset of PNP patients. However the function(s) of A2ML1 in the epidermis and the role of anti-A2ML1 and anti-DSC3 autoantibodies in PNP patients are yet to be defined.

Aim of the project

To ultimate aim of the project is to develop sensitive and quantitative assays to detect the Abs against A2ML1 and extend them for the diagnosis of more common autoimmune skin diseases by expressing relevant EGFP-tagged antigens. The availability of novel diagnostic tools with high specificity and sensitivity is important to ensure the correct classification of affected patients, proper disease management and for their prognosis.

Results

1) Expression and characterization of EGFP-A2ML1 and DSC3-EGFP

Figure 1. In gel fluorescence detection of EGFP-tagged proteins. The culture medium of transfected human embryonic kidney (HEK) 293T cells expressing the indicated proteins was size-fractionated on 10% SDS-PAGE, which was scanned for fluorescence.

Figure 2. EGFP-A2ML1 inhibitory activity towards chymotrypsin. A fluorometric protease activity assay kit with casein-TAMRA conjugated as substrate, was used to test the inhibitory activity of EGFP-A2ML1 towards chymotrypsin. Red fluorescence released by casein cleavage was measured with a fluorometer. RFU, relative fluorescence unit. SC substrate control; PC positive control; VC vehicle control (PBS).

2) EGFP-A2ML1 and DSC3-EGFP allow an easy and rapid detection of positive human PNP sera.

Figure 3. Immunoprecipitation (IP) of sEGFP-A2ML1 with PNP human sera. IPs were performed with the culture medium of transfected HEK 293T cells expressing sEGFP-A2ML1 or sEGFP. Fluorescence associated with the protein A-Sepharose beads was measured with a fluorometer. N, normal serum. RFU, relative fluorescence unit.

Figure 4. Immunoprecipitation (IP) of DSC3-ECD-EGFP with PNP human sera. IPs were performed with the culture medium of transfected HEK 293T cells expressing DSC3-ECD-EGFP. Fluorescence associated with the beads was measured with a fluorometer. N, normal serum; RFU, relative fluorescence unit.

Figure 5. A2ML1-ELISA analysis of PNP sera. The culture medium of HEK 293T cell expressing sEGFP-A2ML1 or sEGFP was immobilized on ELISA plates that were incubated with PNP sera, diluted 1:10. A donkey anti human-IgG-horse radish peroxidase with tetramethylbenzidin as substrate was used for detection. The sEGFP background was subtracted from the sEGFP-A2ML1 values. With this method and the presently set cutoff value, 42 % of the tested PNP sera (n=36) were positive. Among them, 25 % were strongly positive, while none of the sera of pemphigus vulgaris (PV, n=20) and bullous pemphigoid patients (BP, n=20) were positive.

Conclusions

We developed **easy methods** to **test** and **quantify** the **reactivity of human sera against A2ML1 or DSC3** and similar assays may be applied for the detection of autoantibodies directed against other target antigens.

References

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