Characterization of cryptic antinuclear autoantibody target antigens

Zitao Zeng

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Abstract

Objective: To identify and characterize target autoantigens of sera that produce antinuclear autoantibody (ANA) patterns in indirect immunofluorescence assay (IFA) but do not contain autoantibodies against a multiplicity of established nuclear autoantigens.

Methods: Thirty-six serum samples with pre-determined ANA against unknown nuclear autoantigens (UNA) as determined by IFA and immunoblot were collected in the Clinical Immunological Laboratory Prof. Dr. med Winfried Stöcker. UNA sera and controls were incubated with different substrates, including HEp-2 cells and monkey liver cryosections fixed on cover glass, HEp-2 cell homogenate, cell-free supernatant lysate and nuclei to form immune complexes. Different buffer systems were tested for their subsequent extraction and RIPA lysis buffer (1x PBS pH 7.4, 1% (v/v) NP40, 1% w/v) deoxycholate, 0.1% (w/v) SDS including 1 mM Protease inhibitor cocktail) was chosen for immunoprecipitation (IP). The immunoprecipitate was subjected to SDS-PAGE and Western blot with the autologous index serum as the immunoprobe. Immunoreactive proteins were then subjected to mass spectrometry to identify the candidate autoantigens. The candidate autoantigen PSME3 was recombinantly expressed in *E.coli*. The recombinant protein was incubated with the index serum in Western blot to verify the reaction with the patient's antibodies. Moreover, it was tested for its ability to neutralize the ANA reactivity in IFA.

Result: IP using fixed HEp-2 cells or cell fractions was able to isolate autoantigens that were detected by the autologous sera in Western blot. Among them, IP using fixed cells resulted in lowest number of irrelevant proteins in the precipitate. IP with nuclear extract and monkey liver did generally not lead to conclusive identifications. Five target autoantigens were identified by mass spectrometry: DNA-directed RNA polymerase II subunit RPB1 (POLR2A) with three UNA sera and tight junction protein zonula occludens-1 (TJP1), ATP-dependent RNA helicase A (DHX9), proteasome activator complex subunit 3 (PSME3) and structural maintenance of chromosomes flexible hinge domain-containing protein 1 (SMCHD1) with individual other UNA sera. PSME3 could be prepared from recombinant *E.coli* in sufficient amounts for subsequent immunoassays. The recombinant protein reacted with the index serum in Western blot whereas 82 control sera were negative. Moreover, recombinant PSME3 was able to abolish the ANA reaction of the index serum in IFA.

Conclusion: Five candidate nuclear autoantigens were identified, among them two candidates that have not been described before. PSME3 was successfully verified by demonstrating specific reactivity of its recombinant homologue with the index serum. The next step will be the recombinant expression, preparation and verification of the other candidates. The prevalences of ANAs against these antigens can then be determined in cohorts of patients with systemic autoimmune diseases in large scale as part of my dissertation.