

RESEARCH ARTICLE

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Antibodies against specific extractable nuclear antigens (ENAs) as diagnostic and prognostic tools and inducers of a profibrotic phenotype in cultured human skin fibroblasts: are they functional?

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Abstract

Background: The importance of systemic sclerosis (SSc) autoantibodies for diagnosis has become recognized by their incorporation into the 2013 ACR/EULAR classification criteria. Clear prognostic and phenotypic associations with cutaneous subtype and internal organ involvement have been also described. However, little is known about the potential of autoantibodies to exert a direct pathogenic role in SSc. The aim of the study is to assess the pathogenic capacity of anti-DNA-topoisomerase I (anti-Topo-I) and anti-centromeric protein B (anti-Cenp-B) autoantibodies to induce pro-fibrotic markers in dermal fibroblasts.

Methods: Dermal fibroblasts were isolated from unaffected and affected skin samples of ($n = 10$) limited cutaneous SSc (LcSSc) patients, from affected skin samples of diffuse cutaneous (DcSSc) patients ($n = 10$) and from healthy subjects ($n = 20$). Fibroblasts were stimulated with anti-Topo-I, anti-Cenp-B IgGs, and control IgGs in ratios 1:100 and 1:200 for 24 h. Cells were also incubated with 10% SSc anti-Topo-I⁺ and anti-Cenp-B⁺ whole serum and with 10% control serum for 24 h. Viability was assessed by MTT test, while apoptosis was assessed by flow cytometry. Activation of pro-fibrotic genes ACTA2, COL1A1, and TAGLN was evaluated by quantitative real-time PCR (qPCR), while the respective protein levels alpha-smooth-muscle actin (α -SMA), type-I collagen (Col-I), and transgelin (SM22) were assessed by immunocytochemistry (ICC).

Results: MTT showed that anti-Cenp-B/anti-Topo-I IgGs and anti-Cenp-B⁺/anti-Topo-I⁺ sera reduced viability (in a dilution-dependent manner for IgGs) for all the fibroblast populations. Apoptosis is induced in unaffected LcSSc and control fibroblasts, while affected LcSSc/DcSSc fibroblasts showed apoptosis resistance. Basal mRNA (ACTA2, COL1A1, and TAGLN) and protein (α -SMA, Col-I, and SM22) levels were higher in affected LcSSc/DcSSc fibroblasts compared to LcSSc unaffected and to control ones. Stimulation with anti-Cenp-B/anti-Topo-I IgGs and with anti-Cenp-B⁺/anti-Topo-I⁺ sera showed a better induction in unaffected LcSSc and control fibroblasts. However, a statistically significant increase of all pro-fibrotic markers is reported also in affected LcSSc/DcSSc fibroblasts upon stimulation with both IgGs and sera.

Conclusions: This study suggests a pathogenic role of SSc-specific autoantibodies to directly induce pro-fibrotic activation in human dermal fibroblasts. Therefore, besides the diagnostic and prognostic use of those autoantibodies, these data might further justify the importance of immunosuppressive drugs in the early stages of the autoimmune disease, including SSc.

Keywords: Systemic sclerosis, Fibrosis, Autoantibodies, Fibroblasts, Centromeric protein B, Topoisomerase I

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Autoantibodies To Nuclear Antigens

Richard A. McPherson



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Autoantibodies was published and presented in November 2006 at the International Congress of Autoimmunity in Sorrento a small town in Campania Italy The Congress also celebrated the 100th anniversary of the first routine test for autoantibodies An autoantibody is a type of antibody that is produced by the immune system and that fights one or more of a person's own proteins These autoantibodies cause autoimmune diseases such as lupus erythematosus The authors and editors of this book provide a critical review of autoantibodies and their primary functions They cite a number of major developments in the field of autoantibodies including the detection of autoantibodies in which a healthy person is a carrier the discovery that autoantibodies can be both pathogenic and protective in some cases and the development of a device that will help monitor and detect a specific autoantibody using a small amount of serum and proteomic arrays Aside from the pathogenic and protective autoantibodies the book also discusses irrelevant autoantibodies as these may be relevant for future research It also addresses the importance of the autoantibodies in a person's body Clinical physicians as well as scientists interested in the significance of autoantibodies in the human body will find this book relevant It will also be of interest to those who suffer from an autoimmune disease Includes an exhaustive list of autoantibodies not covered by other publications Short reviews can easily be checked for quick reference information Both basic and clinical aspects are covered Henry's Clinical Diagnosis and Management by Laboratory Methods E-Book Richard A. McPherson,Matthew R. Pincus,2011-09-06 Recognized as the definitive book in laboratory medicine since 1908 Henry's Clinical Diagnosis and Management by Laboratory Methods edited by Richard A McPherson MD and Matthew R Pincus MD PhD is a comprehensive multidisciplinary pathology reference that gives you state of the art guidance on lab test selection and interpretation of results Revisions throughout keep you current on the latest topics in the field such as biochemical markers of bone metabolism clinical enzymology pharmacogenomics and more A user friendly full color layout puts all the latest most essential knowledge at your fingertips Update your understanding of the scientific foundation and clinical application of today's complete range of laboratory tests Get optimal test results with guidance on error detection correction and prevention as well as cost effective test selection Reference the information you need quickly and easily thanks to a full color layout many new color illustrations and visual aids and an organization by organ system Master all the latest approaches in clinical laboratory medicine with new and updated coverage of the chemical basis for analyte assays and common interferences lipids and dyslipoproteinemia markers in the blood for cardiac injury evaluation and related stroke disorders coagulation testing for antiplatelet drugs such as aspirin and clopidogrel biochemical markers of bone metabolism clinical enzymology hematology and transfusion medicine

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with antiphospholipid antibody syndrome APS A positive lupus anticoagulant LA test and or IgG aPL tests were associated with most APS related events and organ damage Lupus nephritis tobacco smoking LA positivity and the use of statins and or corticosteroids were strongly associated with damage accrual while hydroxychloroquine seemed to be protective IgA aPL was not uncommon 16% in Swedish cases of SLE and analysis of IgA aPL may add information among clinically suspected APS patients testing negative for LA and other aPL isotypes Despite modern management and tax funded health care with universal access almost two thirds of the patients accrued organ damage over time and the main causes of death were identified as malignancy infection and cardiovascular disease We could confirm well established risk factors for organ damage such as APS hypertension and or the use of corticosteroids but we also observed that other factors such as pericarditis haemolytic anaemia lymphopenia and myositis seems to be of importance in this view Paper IV We also demonstrated that levels of the extracellular matrix protein osteopontin OPN was correlated with disease activity in patients with recent onset SLE In addition OPN levels reflected global organ damage and were associated with APS and could have potential as a valuable biomarker in SLE Paper III Additional studies are warranted to further establish the clinical and mechanistic relevance of ANA seroconversion OPN as well as the importance of IgA aPL Vigilance for malignancies a restricted use of corticosteroids and prevention of cardiovascular disease and APS events are among modifiable factors to prevent organ damage and premature mortality This thesis emphasizes the importance of autoantibodies in the pathogenesis and diagnosis of SLE The autoantibody profile can be of great importance for tailored therapy in order to minimize the risk of organ damage accrual morbidity as well as mortality *Advances in Immunology* ,1982-10-11 *Advances in Immunology*

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