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Up-regulation of fatty acid oxidation in the ligament as a contributing factor of ankylosing spondylitis: A comparative proteomic study

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ABSTRACT

Objectives: The present study first utilized a standardized shotgun proteomic analysis method to determine differences in protein expression of fibroblasts in the ligament between AS patients and healthy controls.

Methods: Proteins extracted from primarily cultured FLLs from 35 AS patients and 10 normal subjects were analyzed by automated 2D-Nano-LC-ESI-MS/MS. Differentially expressed proteins were screened by 2-sample *t*-test and fold change. Bioinformatics analysis of differentially expressed proteins was based on the IPA. Fatty acid β-oxidation-related proteins and INSR pathway-related proteins in the ligament were confirmed by real-time PCR and Western blot.

Results: A total of 556 differential proteins were screened in AS. Of them, 322 proteins were up-regulated and the remaining 234 proteins were down-regulated. GO and pathway analyses showed that six fatty acid β-oxidation-related proteins (HADHB, ECHS1, ACSL4, ACADM, ACSL1 and HADH) were up-regulated in FLL cells, which was consistent with the results obtained from real-time PCR, Western blot and MS, while INSR pathway-related proteins (INSR, IRS1, PI3K and PKC) was low in the ligament of AS as compared with that in healthy controls.

Conclusion: The lower body fat level in AS maybe due to up-regulation of fatty acid β-oxidation-related enzymes regulated by INSR/PI3K/PKC pathway.

Abbreviations: FLL cells, Fibroblast-like ligament cells; AS, Ankylosing spondylitis; HC, Healthy controls; IPA, Ingenuity pathway analysis; HADHB, Trifunctional enzyme subunit beta; ECHS1, Enoyl-CoA hydratase; ACSL4, Long-chain-fatty-acid-CoA ligase 4; ACADM, Medium-chain specific acyl-CoA dehydrogenase; ACSL1, Long-chain-fatty-acid-CoA ligase 1; HADH, Hydroxyacyl-coenzyme A dehydrogenase; INSR, Insulin receptor; IRS1, Insulin receptor substrate 1; PI3K, Phosphoinositide-3-kinase; PKC, Protein kinase C.

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Biological significance
Ankylosing spondylitis (AS), a common spondyloarthropathy, is an inflammatory rheumatic disease with a predilection for the axial skeleton. Clinical hallmarks of AS include sacroiliitis, uveitis, enthesitis and persistent spinal inflammation. The pathogenic mechanism of disease causation and perpetuation remains poorly understood. In this study, we primarily cultured fibroblast cells from ligament biopsies, knowing that fibroblast cells are dominant cells in the diseased ligament. One of the characteristic pathologic changes in AS is inflammation of the attachment points, including the muscle, ligament and bone or joint capsule. Inflammation of the tendon attachment point is usually non-bacterial and can lead to pain and swelling of the tendon ligament. To obtain more information, we used Shotgun proteomic analysis based on multidimensional liquid chromatography tandem mass spectrometry (LC-MS/MS). we firstly mixed the lysates of FLL cells derived from the ligaments of 35 AS patients and 10 normal subjects, identified proteins by automated 2D-Nano-LC-ESI-MS/MS method, GO and pathway analyses showed that six fatty acid β-oxidation-related proteins (HADHB, ECHS1, ACSL4, ACADM, ACSL1 and HADH) were up-regulated in the ligament, which was consistent with the results obtained from real-time PCR, Western blot and MS, while INSR pathway-related proteins (INSR, IRS1, PI3K and PKC) was low in the ligament of AS as compared with that in healthy controls. We also find that AS subjects had significantly lower body mass index (BMI) and BMI Z-scores compared with that in healthy controls. The results remind us that up-regulation of fatty acid β-oxidation-related proteins lower the body fat content, which is a new discovery contributing to the progression of AS.
This is the first report on fatty acid oxidation in AS. It was found that the body fat level was low in AS due to high fatty acid oxidation, suggesting that insulin signaling may play an important role in the metabolic switch from predominant to fatty acid metabolism that characterizes the ligament of AS. One mechanism for this transition is increased expression of genes that regulate the rate of fatty acid oxidation. This effect may be mediated by PI3K, a downstream mediator of many receptor tyrosine kinases, including the INSR. This is a newly discovered factor contributing to the progression of AS.

1. Introduction

Ankylosing spondylitis (AS), a common spondyloarthropathy, is an inflammatory rheumatic disease with a predilection for the axial skeleton. Clinical hallmarks of AS include sacroiliitis, uveitis, enthesitis and persistent spinal inflammation. The diagnosis of AS is usually difficult because the pathogenic mechanism of disease causation and perpetuation remains poorly understood [1,2], although it is thought to be immune-mediated and has a strong genetic association with the class I human Leukocyte antigen allotype HLA-B27 [3]. Additional genes, including endopeptidase ERAP1 and IL23R receptor (IL23R), are also believed to been associated with AS [4–6]. Recent studies on the structure and function of ERAP1 have demonstrated altered N-terminal peptide trimming by the AS-associated ERAP1 variant K528R, supporting a key role for major histocompatibility complex I-associated antigen processing in AS pathogenesis [7,8].

Recent proteomic studies have demonstrated that protein expression is altered in autoimmune diseases. A primary aim of clinical proteomics is to identify biomarkers for the diagnosis and therapeutic intervention of disease by comparing proteomic profiles in normal and diseased conditions and between different physiological states so as to provide new opportunities to examine the physiology and pathophysiology of biological samples of AS [9]. Fischer et al. [10] first integrated proteomic and metabolomic techniques to find new biomarker candidates for the diagnosis of AS using nano-liquid chromatography mass spectrometry analysis. They detected and quantitated proteins and small compounds including endogenous peptides and metabolites in sera from 18 AS patients and 9 healthy individuals and finally identified a total of 316 proteins, of which combined levels of serum amyloid P component and inter-α-trypsin inhibitor heavy chain 1 were found to have a high value for the diagnosis of AS. It was found that one molecular feature identified as a Vitamin D3 metabolite was down-regulated in AS. The ratio of this vitamin D metabolite *versus* vitamin D binding protein serum levels was also altered in AS compared with controls. These changes may contribute to pathological skeletal changes in AS. Li et al. [11] reported that serum amyloid A, apolipoprotein A (ApoA)-IV, ApoA-IV precursor, haptoglobin 2, ceruloplasmin (Cp) and immunoglobulin superfamily 22 were over-expressed by more than 3 fold in the sera of AS patients compared with healthy volunteers (HVs). Using proteomic techniques coupled with systems biology analysis, Wright et al. [12,13] found that monocytes in AS and rheumatoid arthritis (RA) patients expressed high levels of proteins that involved a variety of inflammatory pathways compared with those in HVs. Monocytes from AS patients showed statistically significant differential protein expression within the ubiquitin proteasome pathway (UPP) as compared with those from HVs. In addition, proteosome activator (PA) 28, a member of the UPP, could increase the generation of HLAB27-restricted epitopes, Ingenuity Pathway Analysis (IPA) showed that AS autoantigens from both arrays demonstrated a bias towards proteins expressed in connective and skeletal tissues [12].