Pharmacoproteomic Assessment of Three Sulfated Molecules on Human Osteocyte Receptors and Extracellular Proteomes

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Abstract

The CS employed in scientifc studies is generally derived from animal sources by extraction and purifcation

and in clinical trials Although benefcial efects of orally administrated CS in OA patients have been reported caution should be exercised in the study or use of diferent CS formulations, because the species or tissue of origin could result in great diferences in CS structural organization or disaccharide composition. Probably for this reason, recent meta-analysis and large scale clinical trials have demonstrated variable efects on OA symptoms, yielding conficting result.

The diferentially labeled co-resolved proteins within each gel were imaged at 100 dots/inch resolution using a

the glass plates, and the 16-bit image fle format images were exported for data analysis. After imaging for CyDyes,

Introduction

Semi-automated image analysis was performed with Progenesis SameSpots V3.2 so ware (Nonlinear Dynamics). Image quality control was rst performed to identify saturated spots. Multiplexed analysis was selected for DIGE experiments, and a representative gel image was chosen as reference. Spots were detected, and their normalized volumes were ranked on the basis of analysis of variance *p* values, fold changes, and statistical power (which re ect our con dence in the ability of the experimental data to nd the di erences that do actually exist).

Subjective heading

e gel spots of interest were manually excised and transferred to microcentrifuge tubes. Samples selected for analysis were in-gel reduced, alkylated, and digested with trypsin according to the method of Sechi and Chait). e samples were analyzed using the MALDI-TOF/TOF mass spectrometer 4800 Proteomics Analyzer (ABSCIEX, Framingham, MA) and 4000 Series Explorer™ so ware (ABSCIEX). Data Explorer version 4.2 (ABSCIEX) was used for spectra analyses and generating peak picking lists. All of the mass spectra were internally calibrated using autoproteolytic trypsin fragments and externally calibrated using a standard peptide mixture (Sigma-Aldrich). TOF/TOF fragmentation spectra were acquired by selecting the 10 most abundant ions of each MALDI-TOF peptide mass map (excluding trypsin autolytic peptides and other known background ions.

Syphilis is an infection caused by Treponema pallidum. Usually, *T.* pallidum is transmitted through sexual intercourse. In addition, syphilis greatly increases the risk of infection and transmission of acquired immune de ciency syndrome . In recent years, the global incidence of syphilis has increased because of the ability of *T.* pallidum to evade host immune defenses and spread from the initial site of infection to other organs and tissues. Hence, it is also termed a “stealth pathogen. How *T.* pallidum overcomes the immune response and damages tissue is incompletely understood. Explaining the pathogenesis and immune mechanism of action of *T.* pallidum has become a key link to controlling syphilis.

Discussion

e monoisotopic peptide mass ngerprinting data obtained by MS and the amino acid sequence tag obtained from each peptide fragmentation in MS/MS analyses were used to search for protein candidates using Mascot version 2.2 from Matrix Science. Peak intensity was used to select up to 50 peaks/spot for peptide mass ngerprinting and 50 peaks/precursor for MS/MS identi cation. Tryptic autolytic fragments, keratin, and matrix-derived peaks were removed from the data set used for the database search. e searches for peptide mass ngerprints and tandem MS spectra were performed in the UniProt knowledgebase (2010_09 release version, August 10, 2010), by searching in the UniProtKB/Swiss-Prot database, containing 519,348 entries. Fixed and variable modi cations were considered (Cys as *S*-carbamidomethyl derivate and Met as oxidized methionine, respectively), allowing one trypsin missed cleavage site and a mass tolerance of 50 ppm. For MS/MS identi cations, a precursor tolerance of 50 ppm and MS/MS fragment tolerance of 0.3 Da were used. Identi cations were accepted as positive when at least ve peptides

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For SILAC experiments, freshly isolated chondrocytes were recovered and plated at low density in SILAC Dulbecco's modi ed Eagle's medium lacking arginine and lysine and supplemented with

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