IPD Project Details

Project ID: IPD4220

Project Title: Proteomic analysis of Caenorhabditis elegans wound model reveals novel molecular players involved in repair

Description: Now a days, wound healing is becoming a global threat that impact economy of the country severely. Though the steps involved in wound healing are well characterised, direct therapeutics for the accelerated wound healing is comparatively less. This is solely because of the incomplete mechanism of healing and the lack of knowledge on molecular players involved in wound healing. Hence, in the present study, we have investigated the molecular players involved in wound healing process using a versatile model organism Caenorhabditis elegans through proteomic analyses. Especially, we have employed the high through put proteomic analyses tools such as 2-D GE and LCMS/MS analysis to uncover the molecular players involved in wound healing. As a result of LC-MS/MS analysis at 0 and 24 h, a total of 435 proteins were regulated in both unwounded and wounded conditions in which 13 and 10 proteins were significantly differential regulated. Bioinformatics analyses result suggested that molecular players involved in cortical actin cytoskeleton organization, protein phosphatase binding, and proton transporting ATP synthase activity were identified at 0 h; skeletal muscle myosin thick filament assembly, actin filament depolymerization were identified at 24 h.

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Sample Preparation: 100 µg of proteins from both unwounded and wounded samples at 0 and 24 h were taken for SDS-PAGE.

Peptide Separation: In-solution trypsin digestion was performed following alkylation using 20 ?l of 20 mM IAA (Iodoacetamide) at 37 °C for 30 min in dark and reduction using 10 mM DTT. After that, trypsinization was inhibited by the addition of 2 ?L of formic acid to the microfuge tubes for 20 min. Finally, the trypsin digested peptides were centrifuged for 5 min at 20,817 ×g at 4 °C and the supernatants were subjected to LC-MS/MS analysis (Mir and Balamurugan, 2019). Protein profiling analysis has been outsourced at LC-MS/MS instrumentation facility from NIPER, Kolkata.

Protein Characterization: The trypsin digested peptides were subjected to LC-MS/MS analysis by employing liquid chromatography using Agilent 6545 ESI-LC/qToF-MS/MS and operated/controlled by Mass Hunter Workstation software (version B.08.00) and the post processing of data was performed using Spectrum Mill software (version B.06.00). The taxonomy was set as C. elegans and mass tolerance was set to 20 ppm and product mass tolerance was set to 600 mDa. Initially, fractionation of peptides was performed using 1st Reversed Phase (RP) column at high pH (pH 8.0) which is the first dimension separation, subsequently subjected to 2nd RP column at a low pH (pH 2.0) which is the second dimension. In connection to the 2D separation, peptides from the nano-LC column were subjected to MS analysis. The parameters used in this study were previously described by Dharmaprakash et al., 2014. The LC-MS/MS raw data from each sample and each replicate were subjected to Spectrum Mill software (version B.06.00) for the protein identification. The C. elegans protein sequences were retrieved from NCBI which was used for protein identification and the FDR (False Discovery Rate) of proteins was set to 4 %. Meanwhile, the post processing of LC-MS/MS raw data was planned in such a way that the resulting protein must have at least one fragment ion match and one peptide match. Trypsin was selected as the primary enzyme with a specificity of at least one missed cleavage site. Mass tolerance of precursor ions was set to 10 ppm and 20 ppm for their respective fragment ions. Carbamidomethylation (cysteine residues) was selected as fixed modification and oxidation (methionine residues) was selected as variable modification.

Experiment Type: Top-down

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Wounds and injuries No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: iodoacetamide derivatized residue

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