Journal of Glycomics and Lipidomics

Extended Abstract

Label free quantitative proteomics approach unravels the pleiotropy of buffalo leukemia inhibitory factor (BuLIF) in COS-1 cells

Sudarshan Kumar

Abstract

Leukemia inhibitory factor (LIF) is a pleotropic molecule which performs diverse functions in a context dependent manner. Bovine LIF (BuLIF) is an essential media component in in-vitro stem cell culture and also considered essential in the early stages of pregnancy. However, the exact molecular mechanism behind the diverse actions of this molecule is unknown except the stat3 mediated canonical pathways in stem cell pluripotency. We produced a stably transfected COS-1_BuLIF cell line which expressed high amount of LIF in media. The integration of BuLIF into genome was confirmed by PCR followed by sequencing. We found that LIF induces dome like structure formation which is indicative of BuLIF action via stat3 pathway. Further, pure rBuLIF was purified from this cell line which was found to be 58.99 kDa and 48.9 kDa protein with and without glycosylation respectively which was confirmed by western blot and nLC-MS/MS. The time lapse and concentration-dependent assay of purified LIF showed maximum inhibition at 72 hours and half-maximal effective concentration (EC50) to be 0.0555 ng/mL, corresponding to a specific activity of >1.6�?107 units/mg and identified IC50 value for migrating cells to be 77.8 ng/ml. The biological activity of pure rBuLIF was tested using multiple assays such as BrdU, MTT, migration, caspase 3/7, western and RTqPCR which indicated that it is growth inhibitory in nature and it doesn�??t activate apoptosis. To further, elucidate the molecular mechanism behind its growth inhibitory action we used high-resolution LC-MS/MS-based label-free quantification (LFQ) approach to identify the DEPs (differentially expressed proteins) and deep bioinformatics analysis on Cytoscape platform for determination of non-canonical pathways. The MS/MS data recognized 2083 proteins which consequently, illustrated the LIFmediated cascade for the activation of MEK/ERK, Ras, mTOR, Hippo and RAP1 pathways in addition to three well known PIP3, STAT3 and MAPK pathways. Thus, we conclude that rBuLIF is growth inhibitory in nature in fibroblast cells (COS-1) and this action is mediated via the regulation of multiple signalling pathways in addition to three canonical pathways in a highly context-dependent manner.

Leukemia Inhibitory Factor (LIF) may be a pleotropic molecule which performs diverse functions during a context dependent manner. Bovine LIF (BuLIF) is an important media component in in-vitro somatic cell culture and also considered essential within the early stages of pregnancy. However, the exact molecular mechanism behind the diverse actions of this molecule is unknown except the stat3 mediated canonical pathways in stem cell pluripotency. One of the "cells that are self-replicating, are derived from human embryos or human fetal tissue, and are known to become cells and tissues of the three primary germ layers. Although human pluripotent stem cells could also be derived from embryos or fetal tissue, such stem cells aren't themselves embryos." (From the National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells.) "Self-replicating" means the cell can divide and to make cells indistinguishable from it. The three primary germ layers (called the ectoderm, mesoderm, and endoderm) are the first layers of cells within the embryo from which all tissues and organs develop. Human pluripotent stem cells also are referred to as human embryonic stem cells. We amplified BuLIF from cumulus oophorus cells of cow ocytes by RT-PCR. The nucleotide sequence was determined by bidirectional sequencing and submitted to NCBI database as GenBank (accession number HQ616665). A multiple sequence alignment from different species revealed its high similarity with sheep (98.77%) and cattle (96.62%) in comparison to pig (86.77%), dog (88.15%), and human (87.38%). The signal peptide was of 22 amino acids which was highly conserved in all organisms, except for goat where at the third position valine was replaced with aspartic acid (V–D). Further amino acid changes in position 124–132 were seen, and it was clearly remarkable that the C-terminal end of the sequences is fully conserved among the species.We produced a stably transfected COS-1_BuLIF cell line which expressed high amount of LIF in media. The integration of BuLIF into genome was confirmed by PCR followed by sequencing. We found that LIF induces dome like structure formation which is indicative of BuLIF action via stat3 pathway (https://doi.org/10.1159/000465507). Further, pure rBuLIF was purified from this cell line which was found to be 58.99 kDa and 48.9 kDa protein with and without glycosylation respectively which was confirmed by western blot and nLC-MS/MS. The time lapse and concentration-dependent assay of purified LIF showed maximum inhibition at 72 hours and half-maximal effective concentration (EC50) to be 0.0555 ng/mL, corresponding to a specific activity of >1.6×107 units/mg and identified IC50 value for migrating cells to be 77.8ng/ml. The biological activity of pure rBuLIF was tested using multiple assays like BrdU, MTT, migration, Caspase 3/7, western and RT-qPCR which indicated that it's growth inhibitory in nature and it doesn't activate apoptosis. To further, elucidate the molecular mechanism behind its growth

inhibitory action we used high-resolution LC-MS/MS-based LFQ approach to identify the DEPs (Differentially Expressed Proteins) and deep bioinformatics analysis on Cytoscape platform for determination of non-canonical pathways.

The MS/MS data recognized 2083 proteins which consequently, illustrated the LIF-mediated cascade for the activation of MEK/ERK, Ras, mTOR, Hippo, and RAP1 pathways in addition to three well know PIP3, STAT3, and MAPK pathways.

This work is partly presented at 2th International Conference and Expo on Proteomics and Molecular Medicine

Sudarshan Kumar
National Dairy Research Institute, India E-mail: kumarsudershan@gmail.com