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

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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 RT-qPCR 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 LIF-mediated 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.

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