



Quantitative proteomic analysis of GnRH agonist treated GBM cell line LN229 revealed regulatory proteins inhibiting cancer cell proliferation

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Abstract

Background: Gonadotropin-releasing hormone (GnRH) receptor, a rhodopsin-like G-protein coupled receptor (GPCR) family member involved in GnRH signaling, is reported to be expressed in several tumors including glioblastoma multiforme (GBM), one of the most malignant and aggressive forms of primary brain tumors. However, the molecular targets associated with GnRH receptor are not well studied in GBM or in other cancers. The present study aims at investigating the effect of GnRH agonist (Gosarelin acetate) on cell proliferation and associated signaling pathways in GBM cell line, LN229.

Methods: LN229 cells were treated with different concentrations of GnRH agonist (10^{-10} M to 10^{-5} M) and the effect on cell proliferation was analyzed by cell count method. Further, total protein was extracted from control and GnRH agonist treated cells (with maximum reduction in cell proliferation) followed by trypsin digestion, labeling with iTRAQ reagents and LC-MS/MS analysis to identify differentially expressed proteins. Bioinformatic analysis was performed for annotation of proteins for the associated molecular function, altered pathways and network analysis using STRING database.

Results: The treatment with different concentrations of GnRH agonist showed a reduction in cell proliferation with a maximum reduction of 48.2% observed at 10^{-6} M. Quantitative proteomic analysis after GnRH agonist treatment (10^{-6} M) led to the identification of a total of 29 differentially expressed proteins with 1.3-fold change (23 upregulated, such as, kininogen-1 (KNG1), alpha-2-HS-glycoprotein (AHSG), alpha-fetoprotein (AFP), and 6 downregulated, such as integrator complex subunit 11 (CPSF3L), protein FRG1 (FRG1). Some of them are known [KNG1, AHSG, AFP] while others such as inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2), ITIH4, and LIM domain-containing protein 1 (LIMD1) are novel to GnRH signaling pathway. Protein-protein interaction analysis showed a direct interaction of KNG1, a hub molecule, with GnRH, GnRH receptor, EGFR and other interactors including ITIH2, ITIH4 and AHSG. Overexpression of KNG1 after GnRH agonist treatment was validated using Western blot analysis, while a significant inhibition of EGFR was observed after GnRH agonist treatment.

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