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Cancer Science & Pediatrics 2019: Yeast L-asparaginase inhibits cell growth and induces apoptosis of acute lymphoblastic leukaemia (Raji), breast cancer (MCF-7) and lung cancer (A549) cells in in-vitro system - Sahand Mazloum Ravasan - Iran University of Maragheh, Iran

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L-asparaginase is an enzyme capable of hydrolyzing the asparagine to aspartic acid and ammonia. L-asparaginase is widely used in the treatment of acute lymphoblastic leukemia (ALL) and other cancers. Here, for the first time, the effects of a novel yeast L-asparaginase from Yarrowia lipolytica were studied on human lung (A549) and breast cancer (MCF7) cell lines as the solid cancer cell lines in terms of cell growth and metastasis inhibition. Functional analysis showed the L-asparagine deprivation mediated anti-proliferation effects by apoptosis induction and changes in the expression of target genes involved in apoptosis and migration pathways. The qRT-PCR analysis showed the higher expression levels of pro-apoptosis genes, including Bax, P53, caspase 3, caspase 8, and down-regulation of Bcl-2 anti-apoptotic gene in treated cells. On the other hand, there was no increase in ROS production in the treated cells. However, L-asparaginase treatment was able to significantly induce autophagy activation in A549 cells. Besides, wound healing assay showed that L-asparaginase could considerably inhibit the migration of A549 and MCF7 cells. Taken together, our results suggested that Yarrowia lipolytica L-asparaginase might be considered for enzyme therapy against breast and lung cancers.

In previous studies Pseudomonas aeruginosa L-ASNase complete coding sequence gene, 984 bp (GenBank accession number KU161101.2) was isolated by PCR, cloned into pET28a(+) vector, expressed in E. coli DE3(BL21) pLysS, purified to apparent homogeneity and biochemically characterized. In the present work we highlight large scale production, affinity purification of the recombinant enzyme, effect of osmolytes on the stability of the L-ASNase and cytotoxicity on different cancer cell lines. Successful overexpression was achieved in E. coli as a 6-His-Tag fusion protein after 18 h of induction with lactose at a concentration of 2g/L in fermentation medium and at 37 °C.

The recombinant enzyme was purified to homogeneity using Ni^{2+} chelated Fast Flow Sepharose resin with 19758.8 specific activity and 10.28 purification fold. With respect to the effect of osmolytes on the stability of the purified enzyme, the majority of the tested osmolytes namely 5% maltose, 5% mannitol, 30% glycerol and 5% BSA were found to increase the stability of the recombinant L-ASNase as compared to the free enzyme. Triple negative breast cancer cell line, MDA-MB-231 treated with recombinant L-ASNase showed significant morphological changes and the IC50 of the purified enzyme was found to be 3.1 IU. Human leukemia cell line, THP-1 treated with L-ASNase showed apoptotic bodies and morphological changes with IC50 of the purified enzyme 1.75 IU. Moreover, the purified recombinant L-ASNase was found to induced cytotoxic effects on colorectal adenocarcinoma cell line, Caco-2 with IC50 of 68.28 IU. Results of apoptosis assay on THP-1 cells revealed that the purified L-ASNase induced early and late apoptosis at 14.16% and 7.56 respectively as compared to the control untreated cells.

Background: Cancer is one of the most important problems in the world. Today Enzymes have been intensively studied as a source of antitumor compounds. L-Asparaginase (L-ASNase) is one of the most therapeutic enzymes which used for the cancer therapy. The attendance of L-ASNase has been reported in various organisms, containing animals, plants, and microorganisms (bacteria, fungi, algae, yeast and actinomycetes) except in humans. L-ASNase enzyme which isolated from Yarrowia yeast was used for this study.

Methods: In this study, Raji, MCF7 and A549 cell lines were cultured in RPMI 1640 with 10% FBS and 5% CO2 condition. Cytotoxic effects of yeasts L-asparaginase was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Then, flow cytometry assay was exploited to measure cell death and apoptosis stage.

Results: According to our findings, yeasts L-asparaginase can inhibit cell growth in a time and dose dependent manner. Flow cytometry assay result showed that yeasts L-asparaginase was able to induce apoptosis in Raji, MCF7 and A549 cell lines. The apoptosis of raji cells is more than other cell lines and A549 is more than MCF-7.

Conclusion: Our results showed that yeasts L-asparaginase could successfully induce apoptosis in Raji, MCF7 and A549 cell lines. Therefore, it could be used as a novel and safe therapeutic candidate for cancer treatment.

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