2nd International
Stem Cells & Regenerative Medicine Congress
19-21 April, 2017 Mashhad-Iran

Prodrug converting enzyme gene delivery by E. coli for colon cancer therapy

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Abstract

Metastasis is the main cause of death in patients with malignancies. Increasing evidence supports the cancer stem cell (CSC) hypothesis, which postulates that a subpopulation of malignant cells are resistant to conventional therapies resulting to tumor relapse and metastasis. Glycyrrhizic acid (GL), a glucuronide compound extracted from licorice roots, has been shown as a potent anti-cancer agent with possible effects on cancer stem cells through various routs, for example, inhibition of adhesion molecules expression and suppression of inflammatory pathways such as NF-κB, HMGB-1 and self-renewal pathways such as Wnt signaling and also COX-2 production in tumor microenvironment. GL and glycyrrhetinic acid (GA), its aglycon and active metabolite, are potent inhibitors of 11-β-hydroxysteroid dehydrogenase type 2 (11βHSD2). Inhibition of 11βHSD2 blocks COX-2 production and suppresses colon carcinogenesis and metastasis. E. coli bacteria expressing β-glucuronidase can selectively colonize in solid tumors and convert glucuronide compounds to cytotoxic agents specifically at the tumor site. DH5α competent cells were transformed with pRESETB-lux/βG and plasmid extraction was done by miniprep method. Restriction digestion by EcoRI and luciferase activity were carried out to validate the plasmid integrity and correct transformation. In next step, we examined anti-cancer effects of this system on CT26 colon carcinoma cells. For in vitro cytotoxicity analysis, CT26 cells were seeded overnight in 96 well plates and then treated with E. coli DH5α-lux/βG and graded concentrations of GL. The cytotoxic effects on CT26 cells were then verified by MTT assay. IC50 value after 24 h incubation, 3 times PBS washing and additional 24 h in fresh medium, was determined as 36.62 µM (30.13 µgml-1). The following step, will be performed in vivo in BALB/c mice. Heterogeneous subpopulation of cancer cells could be treated with conventional therapies which eliminate cancer cells but not CSCs and result to tumor relapse. In CSC targeted therapies, the immune system could be stimulated or immune checkpoints inhibited, and then CSCs are killed and tumor loses its ability to generate new cancer cells. GL and GA have been shown as potent anti-cancer agents with possible effects on cancer stem cells and could be introduced in cancer therapy as herbal products.

Keywords: Glycyrrhizic acid, E. coli, Anti-inflammatory agents, Tumor Therapy, Colon Cancer, Metastasis

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