activation of the c-fos promoter. The c-fos proto-oncogene has been implicated as a regulator of estrogen-mediated cell proliferation.

Apoptosis and cell protection: TOX3 was demonstrated to be expressed downstream of a cytoprotective cascade together with CITED1, a transcriptional regulator that does not bind directly to DNA and enhances transcription mediated by diverse transcription factors, such as estrogen receptors, SMAD, or early growth response (EGR) 2. CITED1 expression parallels with estrogen receptors ERα and ERβ.

TOX3 over-expression protects neuronal cells from cell death caused by endoplasmic reticulum stress or BAX over-expression through the induction of repression of pro-apoptotic transcripts and anti-apoptotic transcripts, which associates with enhanced transcription implicating isolated estrogen-responsive elements (ERE) and estrogen-responsive promoters. TOX3 also interacts with native CREB and induces the CREB-responsive BCL-2 promoter. Co-expression of CITED1 inhibits the CREB-responsive BCL-2 promoter and by contrast, co-expression of CREB inhibits TOX3-mediated transcription from the estrogen-responsive complement C3 promoter. Results have suggested that TOX3 can mediate cytoprotective transcription from the BCL-2 promoter or the complement C3 promoter, based on the predominance of either phosphorylated CREB or CITED1 within the transcriptionally active complex.

Regulation of estrogen: TOX3 is also shown to regulate Trefoil Factor Family 1 (TFF1), the estrogen-regulated protein, in an estrogen-independent and tamoxifen-insensitive way. TFF1, a small cysteine-rich secreted protein, is normally expressed at highest levels in the mucosa of the gastrointestinal tract and also is extensively expressed at high levels in malignant breast epithelial cells where its expression is regulated by estrogen. Even in the absence of estrogen, TFF1 was upregulated by TOX3. Additionally, TOX3 induces enhancer RNAs that have been involved in *TFF1* gene regulation.

FOXA1 and ESR1: It is known that a SNP near its 5′end and promoter appears to be strongly associated with breast cancer susceptibility. Breast cancer–associated SNPs are also enriched for FOXA1 and ESR1 transcription factor–binding sites and H3K4me1 histone modification. This SNP alters a FOXA1, a protein required for estrogen receptor-α (ER) function, with disease susceptibility associated with enhanced FOXA1 binding, disordered enhancer function, and a reduce in *TOX3* gene expression. The data may offer a validation for prophylactic treatment of high-risk patients with FOXA1 inhibitors.

TOX3 and bone metastasis of breast cancer: Some TOX3 expressing tumors are linked with adverse outcome, and increased expression of TOX3 mRNA that has been involved in breast cancer metastatic to bone. In microarray analysis, between the genes upregulated by TOX3 was the well-investigated pro-metastatic gene *CXCR4*. It has been indicated that IGF-1 cooperates with CXCR4 signaling to advance bone marrow metastasis of breast cancer, potentially one factor in the aggressive behavior of LumB tumors. Since CXCR4 is upregulated by TOX3 and is highly expressed in LumB tumors, this could improve the migratory properties of MCF-7 cells. Therefore, IGF-1 could play a role in regulation of TOX3 expression. MCF-7 cells treated with IGF-1 indicated significant, albeit modest, upregulation of TOX3 expression, as well as the known IGF-1 target gene GAPDH.

TOX3$^+$ and therapeutic modalities for breast cancer: TOX3 expression is regarded a tumor promoter rather than a tumor suppressor in breast cancer. It has been suggested that TOX3 expression decreases in breast cancer and is correlated with the disease risk. Furthermore, TOX3 has the potential to regulate ER target gene expression in the appearance of limiting concentrations of estrogen. This potential property underlines the treatment modalities for TOX3$^+$ breast cancer. It is also suggested that TOX3 and related biomarkers, such as SCUBE2, TFF1, TFF3, AGR2, CEACAM6, TSPAN1 or CXCR4 are helpful for the diagnosis, prognosis and treatment as well as classification of different breast cancer subtypes. Knocking down of TOX3 by siRNA suggests that even low levels of TOX3 may affect estrogen activation of the *TFF1* gene.

Additionally, estrogen treatment in cells over-expressing TOX3 results in a hyper-responsive *TFF1* gene expression. Expression of TOX3 may be implicated in the resistance to endocrine therapy which may occur in some LumB cancers. In this respect, cells over-expressing TOX3 had a better capability to survive under estrogen-deprived conditions.

IGF-1 treatment in MCF-7 cells can also cause TOX3 up-regulation. This growth factor has been shown to result in breast cancer development and endocrine resistance during stabilization of FOXA1 protein in MCF-7 cells. Furthermore, FOXA1 alters the pattern of ER binding in poor outcome/metastatic ER$^+$ breast cancer leading to a situation similar to that in ER$^+$ good outcome breast cancer.

Conclusion: *TOX3* gene amplification is associated with reduced risk of the disease and metastasis. TOX3 significantly is associated with increased breast cancer cell proliferation, migration, survival, tumor development. Many studies confirm a key role for TOX3 in breast cancer pathology. However, TOX3 plays dual and conflicting roles in cancer initiation and progression which remains to be investigated.

Use of bacterial ghosts as novel drug delivery systems to improve cancer treatment

Amin Afkhami-Poustchi [a], Maryam M. Matin [a,b,c,*]
[a] *Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran* [b] *Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran* [c] *Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran*
E-mail address: matin@um.ac.ir

Extended Abstract

Introduction: The bacterial ghost (BG) system represents a novel and progressive approach in the development of bacterial mediated cancer immunotherapy. The empty inner space of BGs can be filled with drugs, proteins, DNA, enzymes and other compounds. The induced lysis process does not harm the essential structural components of the bacteria, giving rise to immunologically active particles capable of stimulating the host immune system and delivering specific antigen (Ag) to professional antigen-presenting cells (APCs) or active substances to the target cells.

Production of bacterial ghosts: BGs are produced by expression of cloned gene E from bacteriophage ϕX174 resulting to cell lysis in Gram-negative bacteria, such as, *Escherichia coli* K12 strains, *Klebsiella pneumoniae*, *Mannheimia (Pasteurella) haemolytica*, *Neisseria meningitidis, Salmonella typhimurium, Vibrio cholera, Helicobacter pylori,* and others. Expression of gene E can be placed under transcriptional control of either the thermo sensitive EpL/pR-cI857 promoter, or under chemical inducible promoter repressor systems, like lacPO or the tol expression system.

Gene E codes for 91 amino acids and exerts its lytic function by fusion of the inner and outer cell membranes, forming a specific transmembrane tunnel structure through which all the cytoplasmic content is expelled, thus leaving a bacterial envelope called a BG devoid of nucleic acids, ribosomes and other intracellular constituents. The inner membrane (IM) and outer membrane (OM) structures of BGs remain intact during the lysis process. Electron microscopy studies and enzymatic studies clearly showed a sealed periplasmic space at the border of the lysis tunnel. The efficiency of the E-mediated lysis process, and quantification of generated BGs and non lysed viable bacteria are determined by flow cytometry assays using a specific dye that is sensitive to the changes of discriminatory power of membrane potential and stains only cells that have lost membrane potential (BGs or dead bacteria).

Bacterial ghosts as advanced drug delivery systems: Many diseases including cancers require the systemic administration of highly aggressive drugs to already immunocompromised patients. Deleterious and often severe side effects result from a lack of cellular and tissue selectivity. Another major issue is the poor solubility of some drugs used in cancer treatment. Considering these limitations, the development of a safer and more efficient drug delivery system (DDS) is the priority for future advanced cancer treatments. Recently, bacterial ghosts made from the colonic commensal *Mannheimia haemolytica* were used for *in vitro* delivery of doxorubicin (DOX) to human colorectal adenocarcinoma (Caco-2) cells.

Adherence studies showed that the *M. haemolytica* ghosts targeted the Caco-2 cells and released the loaded DOX within the cells. Cytotoxicity assays showed a two folds enhancement in cytotoxic and anti-proliferative activity in cells incubated with DOX-loaded ghosts as compared with cells for which DOX was directly added to the culture media. This phenomenon might be caused by degradation of DOX-loaded BGs within the endo-lysosome of target cells allowing DOX to bypass the multi-drug resistance (MDR) efflux pumps and resulting in enhanced accumulation of DOX in the cytoplasm and then in the nuclear area of target cells. Current work with bacterial ghosts lies in the investigation of the carrier capacity of the cytoplasmic lumen. This intracellular space of BGs can be filled either with water-soluble substances or emulsions such that the drug(s) of interest can be coupled to streptavidin anchored on the inside of the cytoplasmic membrane. Moreover, bacterial ghosts can be filled and sealed for the delivery of fluid, non-anchored substances. In a recent study, *E. coli* ghosts were filled with the reporter substance calcein and were sealed by fusion with membrane vesicles to maintain inner membrane integrity. Adherence and uptake studies showed that murine macrophages and human Caco-2 cells took up the bacterial ghosts, and calcein was released within the cells.

Bacterial ghosts as immunologically active particles: Because of the unique structure of the BG's envelope with preserved pathogen-associated molecular patterns (PAMPs), BGs can be used in biomedicine alone as an adjuvant or as a delivery vehicle for drugs or genes. The inner space of BG's empty envelope can be loaded with a combination of peptides, drugs or foreign DNA which gives us an opportunity to design new types of polyvalent vaccines. BGs have excellent DNA loading capacity varying from 4000 to 6000 plasmid copies per BG depending on the concentrations of DNA solution used. BGs loaded with plasmid DNA are efficiently internalized and phagocytozed by both professional APCs and tumor cells. Cross-presentation of Ag delivered to dendritic cells (DCs) by BGs can activate both $CD4^+$ and $CD8^+$ T cells and stimulate the immune system to enhance the immune response against Ag expressed by target cells. Inner and outer membrane structures of BGs including lipopolysaccharide (LPS) and other PAMPs remain intact after protein E-mediated lysis of Gram-negative bacteria. Thus, beside possessing a high loading capacity; BGs carry highly effective molecules for the stimulation of cross presentation by DCs on their surface, specially, tumor-associated antigens (TAAs). BGs with their intact envelope structures are not only immune stimulatory to professional phagocytes but are also capable of providing stimulatory signals to tumor cells. It is known that melanoma cells have the capacity to behave as non-professional APCs and can phagocyte both apoptotic and live cells, and it was recently shown that melanoma cells actively respond to exposure to BGs by increasing their rate of phagocytosis. Using BGs for gene delivery to the immunocompetent cells, in particular DCs as well as tumor cells, could initiate or restore the immune response against the delivered TAAs as well as induce and increase the expression of target genes by APCs and tumor cells.

Conclusions: These observations indicate high capacity of BGs to target various histological types of cancers. BGs are very useful non-living carriers, as they can carry foreign antigens, nucleic acids and drugs in one or more cellular locations simultaneously. Optimization and improvement of the selected prospective model type of BGs would help to progress the development of microbial-mediated disease treatment and drug delivery systems and their application in future clinical trials.

Keywords: Bacterial ghost (BG), Drug delivery, Tumor therapy, Doxorubicin loaded BG

A brief review on protective effect of camel milk in cancer

Seyed Mousalreza Hosseini [a], Mahdi Yousefi [b], Said Zibaee [c], Ali Taghipour [d], Roshanak Salari [e], Mohammadreza Noras [f,*]

[a] *Department of Medicine Internal, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran* [b] *School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran* [c] *Razi Vaccine and Serum Research Institute of Mashhad, Iran* [d] *Epidemiology & Research Methodology Faculty of Health, Mashhad University of Medical Sciences, Iran* [e] *Department of Traditional Persian Pharmacy, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran* [f] *School of Persian and Complementary Medicine, Mashhad University Medical of Sciences, Mashhad, Iran*

E-mail address: Norasmr901@mums.ac.i

Extended Abstract

Introduction: Cancer is one of the most major and prevalent health problems around the world that shows high mortality rate. There have been many researches on the advantages of camel milk (CM) in cancers. Camel milk is very rich in nutrition and it is used in the treatment of different diseases. Traditionally, the use of camel milk in the treatment and prevention of various diseases existed in Iran, India and Arabic countries. Recent research findings prove the effectiveness of camel milk in the treatment of diabetes, milk and food allergies, autism, liver toxicity, hepatitis and cancers. Cell culture and animal studies introduce camel milk as a new option in cancer treatment.

Materials and method: Electronic databases including PubMed, Scopus, and Cochrane library were searched to access articles giving any in vitro, in vivo, and human evidences on the efficacy of CM in the treatment of cancer. Out of 80 records were found in