

Author's response to reviews

Title: Bacteria under SOS evolve anticancer phenotypes

Authors:

Shatha F Dallo (Shatha.Dallo@utsa.edu)

Tao Weitao (tao.wei@utsa.edu)

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Author's response to reviews: see over

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Dear Editor,

I appreciate your considering revision of the current manuscript for publication. I have addressed all the reviewers' comments with my best effort. Accordingly, I have made significant revision as colored red: new references in background, new evidence for the hypothesis, sequence of figures. I believe that through answering the critiques, the quality this work is further improved.

Sincerely yours,
Tao

Comments from Jiyuan Sun:

The revised manuscript and the additional letter answer my questions well regarding the anti-drug proteins, biofilm, etc, I support the hypothesis to be published, although there are still some pitfalls mentioned by other reviewer. I suggest the authors to add more parts to address the pitfalls addressed by other reviewers.

Comments from ANANDA M CHAKRABARTY

The authors' response and revision are unconvincing and this manuscript requires major compulsory revisions. The ability of facultative anaerobic bacteria such as Salmonella or Clostridia, with or without additional cloned genes, to target tumors and grow in the hypoxic core of the tumors, has been known for many years. The authors, however, propose to use an aerobic bacterium *P. aeruginosa* that is also a known opportunistic pathogen. As far as I know, nothing is known about this bacterium's ability to directly target tumors in preference to normal tissues. Thus the authors need to perform at least one critical experiment to base their hypothesis on. They should use several human cancer cell lines (breast, melanoma, lung, ovarian, colon, etc), AND THEIR NORMAL COUNTERPARTS, in presence and absence of anticancer drugs. They should then use green fluorescent protein (GFP)-tagged *P. aeruginosa* cells, with or without attenuation of key virulence factors, and use such cells in an infection assay with the cancer and normal cells, in presence and absence of anticancer drugs, looking for green fluorescence in gently washed cancer and normal cells after various periods of infection. If they can show that there are many more GFP-tagged *P. aeruginosa* cells bound to cancer cells (any cancer cell line will do) than the corresponding normal cells, then they will have some basis to study preferential adherence/invasion of cancer cells by *P. aeruginosa*. Additionally, if they can show that in presence of certain drugs, *P. aeruginosa* becomes intracellular in cancer cells, BUT NOT IN NORMAL CELLS, then they will have an excellent basis to put forward the present hypothesis. In absence of such data, the hypothesis has no ground to stand on.

I agree that the first revised manuscript needs further revision. The reviewer also suggested an interesting experiment to test natural tumor propensity of *P. aeruginosa*, but the expected data may not be relevant to the hypothesis because this hypothesis focuses on evolution of new phenotypes rather than direct adhesion to cancer. I apologize for missing some important citations and being unable to make this point clear. Below is the elaboration, and I hope it is convincing this time.

As reviewed by Chakrabarty [1], *S. enterica* serovar Typhimurium, a facultative anaerobe, shows propensity to tumors, and the antitumor effects appear to be encoded by *Salmonella* pathogenicity island 2. Besides, *Clostridium novyi*, an anaerobe, is thought to be able to grow in anaerobic core of the tumors and to deprive the tumors of oxygen and essential nutrients. As mentioned by this reviewer, these antitumor activities are encoded by the bacterial genomes and are played under hypoxic conditions. But such conditions limit application of

bacterial antitumor approaches. To challenge the limitation, our hypothesis predicts that bacteria can evolve the cancer adhesion-invasion phenotypes. That is, these phenotypes may not be naturally expressed in the bacterial nature habitat but can be induced intentionally. So our hypothesis is not based on the bacterial natural antitumor propensity but on the SOS-induced molecular evolution of new phenotypes. Apparently, *P. aeruginosa*, an aerobe, does not tend to migrate to the anaerobic zone of tumor core. Its ecological niche may not be necessarily tumor though *P. aeruginosa* can attach to and penetrated human lung epithelial cells derived from a human bronchus alveolar carcinoma [2]. Thus, while the experiments the reviewer suggested may provide insights about *P. aeruginosa* natural propensity to tumor, such results are not reasonably the empirical basis of this hypothesis about evolution of new phenotypes.

Although tumor does not seem to be the natural habitat of opportunistic pathogens, such as *P. aeruginosa*, this bacterium does have antitumor potential; in fact, it has genes encoding antitumor proteins. Azurin is a periplasmic antitumor protein in *P. aeruginosa* (reviewed by Mahfouz et al [3]). Release of Azurin depends on contact with cancer cells, and it targets preferentially cancer cells but marginally normal cells[4]. Additionally, Laz and Pa-CARD displays cytotoxic activity against leukemia cells [5]. Our hypothesis suggests that such proteins and new candidates would emerge when bacteria interact with cancer cells in the presence of anticancer drugs and the induced SOS. Because of significance of the review and research papers discussed above, they are quoted in this article, and revision is made accordingly.

1. Chakrabarty AM: **Microorganisms and Cancer: Quest for a Therapy.** *J Bacteriol* 2003, **185**(9):2683-2686.
2. Carterson AJ, Honer zu Bentrup K, Ott CM, Clarke MS, Pierson DL, Vanderburg CR, Buchanan KL, Nickerson CA, Schurr MJ: **A549 Lung Epithelial Cells Grown as Three-Dimensional Aggregates: Alternative Tissue Culture Model for Pseudomonas aeruginosa Pathogenesis.** *Infect Immun* 2005, **73**(2):1129-1140.
3. Mahfouz M, Hashimoto, W., Das Gupta. T.K., Chakrabarty, AM.: **Bacterial proteins and CpG-rich extrachromosomal DNA in potential cancer therapy.** *Plasmid* 2007, **57**(1):4-17.
4. Yamada T, Fialho AM, Punj V, Bratescu L, Gupta TKD, Chakrabarty AM: **Internalization of bacterial redox protein azurin in mammalian cells: entry domain and specificity.** *Cellular Microbiology* 2005, **7**(10):1418-1431.
5. Kwan JM, Fialho, A.M., Kundu. M., Thomas. J., Hong. C.S., Das Gupta. T.K, Chakrabarty. A.M.: **Bacterial proteins as potential drugs in the treatment of leukemia.** *Leuk Res* 2009, **33**(10):1392-1399.