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Breast Tumor Targeting with Genetically Altered Salmonella.

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Background: Breast cancer is one of the most common life-threatening cancers and second in mortality only to lung cancer. One out of eight women will develop breast cancer in her lifetime and breast cancer also affects a small percent of the male population. Although breast cancer therapy, traditionally involving surgery, radiation, chemotherapy, and hormone treatment has improved cancer survival, resistance to anti-cancer drugs is still the primary reason for breast cancer mortality. There is a need for new approaches to treat breast cancer, especially in women with advanced tumor progression. Recently, it has been shown that genetically modified *Salmonella typhimurium* preferentially replicate within solid tumors (1000:1 compared to non-cancerous tissue) destroying cancer cells without causing septic shock that is typically associated with wild-type *S. typhimurium* infections. Furthermore, these bacteria can be utilized as delivery systems to effectively target different sub-populations of tumor cells. We have started to explore the mechanisms by which *S. typhimurium* interact with breast cancer cells with the goal to increase therapeutic efficiency. We have currently available to us several thousand *S. typhimurium* strains that differ from each other genetically and will allow us to engineer strains that are optimal in appropriate lipopolysaccharide surface for attachment and penetration into tumor cells, antibiotic sensitivity, metabolic poverty of entire pathways as well as individual nutrients.

Methods: We have analyzed *S. typhimurium*-infected MCF-7 human breast cancer cells with confocal immunofluorescence microscopy, with scanning electron microscopy (SEM) and with transmission electron microscopy (TEM). For fluorescence and immunofluorescence microscopy *S. typhimurium*-infected MCF-7 human breast cancer cells were fixed with 3.7% paraformaldehyde and processed using rhodamine-phalloidin to stain microfilaments, FITC-conjugated anti-tubulin antibody to stain microtubules and DAPI to stain DNA. Double and triple immunofluorescence staining was performed to determine the effects of *Salmonella* on the tumor cell's cytoskeleton and on DNA. Cells were prepared for SEM and TEM as described before [1,2].

Results: Our results show that the bacteria attach tightly to the MCF-7 cell surface while interfering with the actin cytoskeleton, modifying the infected cell's microfilament system for engulfment and bacteria incorporation. The microtubule cytoskeleton did not appear modified by the bacteria at 20 and 30

minutes after infection as analyzed with immunofluorescence and electron microscopy.

Conclusions: Our data demonstrate that 1. *S. typhimurium* interacts with MCF-7 breast cancer cells and 2. genetically modified *S. typhimurium* has the potential to deliver compounds such as anticancer therapeutic proteins or pro-drug converting enzymes to destroy various subpopulations of fast proliferating cancer cells that would be more specific and effective than less targeted administration with commonly used drugs.

[1] Schatten, H., Ripple, M., Balczon, R., Weindruch, R., and Taylor, M. J. Cellular Biochem. 76:463-477, 2000.
[2] Schatten, H., Wiedemeier, A., Taylor, M., Lubahn, D., Greenberg, M.N., Besch-Willifird, C., Rosenfeld, C., Day, K., and Ripple, M. Biol. of the Cell 92:331-340, 2000.
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