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Targeting of Prostate Tumor with Genetically Altered Salmonella

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Background: Recently, the use of genetically altered Salmonella for cancer therapy has gained increased attention with the remarkable finding that genetically modified *Salmonella typhimurium* preferentially replicate within solid tumors at a ratio of 1000:1 when compared to non-cancerous tissue, destroying cancer cells without causing septic shock that is typically associated with wild-type *S. typhimurium* infections. The mechanisms for the preferential infestation remain obscure although several hypotheses have been proposed including the possibility of bacteria protection from immune surveillance, increased bacteria escape through leaky blood vessels in tumor tissue, preferential attraction to specific nutrients that are accumulated in cancer tissue and perhaps a combination of several other factors. Here we propose that tumor cells provide increased cytoskeletal components that are used by Salmonella for construction of the vacuoles that house and protect the bacteria after host cell invasion. It is known that Salmonella successfully utilizes the host cell's microfilament system for engulfment and incorporation [1] and it utilizes the host cell's microtubule and kinesin sources for formation of structures to establish the vacuoles within host cells [2]. Fast proliferating cancer cells provide an abundance of tubulin and kinesin that is not available to such extents in resting interphase cells, thus providing an ideal environment to gain enriched supply of molecular components that are needed for vacuole formation and bacterial cell division. We studied the interactions of various Salmonella strains with PC-3M prostate cancer cells to investigate the possibility of using genetically modified Salmonella strains as effective prostate cancer treatment. Prostate cancer is the most common malignancy in the older male population and second only to mortality caused by lung cancer. As our life expectancy increases more men will be affected by the disease. Using bacteria for cancer cell destruction combined with the possibility for utilization as delivery systems is likely to allow for more effective treatment of different sub-populations of prostate tumor cells.

Methods: We have analyzed *S. typhimurium*-infected PC-3M human prostate cancer cells with confocal immunofluorescence microscopy [3] and transmission electron microscopy (TEM) [4] at 20 min, 4 hrs, and 8 hrs after inoculation. For fluorescence and immunofluorescence microscopy *S. typhimurium*-infected PC-3M human prostate 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. For TEM, bacteria were fixed in 2.5% glutaraldehyde in 0.1M HEPES (pH 7.0-7.4) containing 0.2% tannic acid. Ultrastructural analysis with TEM revealed bacteria inside the vacuoles within the host cells.

Results: Our results show that the bacteria attach and modify the infected PC-3M cell's microfilament system for engulfment and bacteria incorporation. At 4 hrs after inoculation, Salmonella are seen in the membrane-bounded Salmonella-containing vacuoles (SCVs) frequently associated with bundles of microtubules and intermediate filaments. The destruction of the PC-3M prostate cancer cells includes deterioration of mitochondria with loss of cristae at 4 hrs after inoculation and an accumulation of small vesicles in the cytoplasm.

Conclusions: Our data demonstrate that 1) genetically modified *S. typhimurium* interact with PC-3M prostate cancer cells like control wild type *S. typhimurium* and 2) genetically modified *S. typhimurium* accumulate within the tumor cells in membrane-bounded SCVs similarly to wild-type *S. typhimurium*, resulting in destruction of mitochondria followed by destruction of the entire prostate cancer cell. These data allow the conclusion that genetically modified Salmonella can be used for destruction of cancer cells and as drug delivery systems in future experiments.

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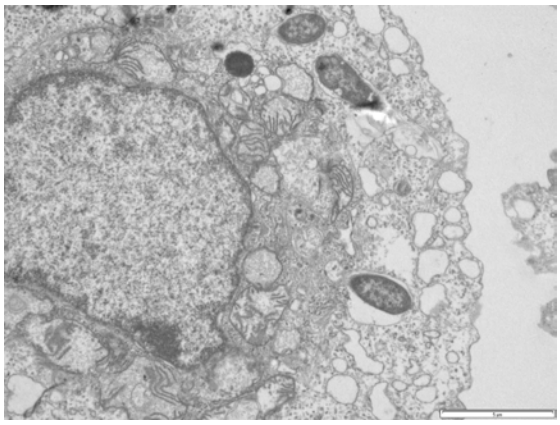

Figure 1 shows a TEM image of *S. typhimurium* within the *Salmonella* containing vacuole. Seen here also are mitochondria with deteriorated mitochondrial membranes.

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