Formulation and Evaluation of therapeutic vaccine microparticles for Breast Cancer

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ABSTRACT SUMMARY
This research focusses on developing therapeutic microparticulate vaccine for treatment of breast cancer. Delivering protein via oral route is a challenge and thus the aim was to formulate and determine the efficacy of orally delivered breast cancer vaccine microparticles.

INTRODUCTION
Breast cancer is one of the most fatal diseases amongst women all over the world. Currently, radiation therapy or chemotherapy is the choice of treatment for breast cancer. Immunotherapy involves use of immune system modifying drugs in combination with immunostimulant proteins (vaccine). Whole cell lysate obtained from 4TO7 cancer cells was used as the vaccine and along with a blend of polymer was spray dried into microparticles.

Microparticles were intended for delivery via the oral route. M-cells in the intestine provide a great site for uptake of microparticles. Mucosal immune system has a great amount of dendritic and other antigen presenting cells which can phagocytose and process the antigen microparticles and present them to appropriate T cell for further generation of adaptive immune response. T–regulatory cells (T-regs) play an important role in suppressing the immune system acting against its own cells. Hence it is important to suppress these cells in order to generate an immune response against cancerous cells which are also body’s own cells. Cyclophosphamide was used as a T-reg inhibitor.

Tumor was first induced into the animals and treated using immunotherapy which included T-regulatory cell suppressor cyclophosphamide (i.p) and whole cell lysate vaccine microparticles administered orally. We hypothesized that this immunotherapy will inhibit tumor growth and build memory immune cells for fighting against future attacks.

EXPERIMENTAL METHODS
Whole cell lysate was prepared using murine breast cancer 4TO7 cells. Briefly, cells were lysed using hypotonic buffer and freeze thaw cycles. Cellular membrane fragment proteins were collected using centrifugation and characterized for protein content. Cellular lysate was then spray dried using enteric coating polymers. Release of protein was studied in gastric pH and intestinal pH. 10% w/v suspension of particles was prepared in appropriate buffer and samples were taken after 30 min to detect protein released in gastric fluid. Release study was carried out to ensure protection from harsh pH conditions of stomach. These microparticles were characterized for size, zeta and protein loading studies.

Post characterization studies, tumor was induced into mice using 10⁶ 4TO7 murine breast cancer cells. Following tumor induction, 10mg of vaccine loaded microparticles were administered orally after 3 days. Antigen loading was 2% w/w. Cyclophosphamide was administered 2 days before administering vaccine microparticles. Animals were used as per protocol approved by Institutional Animal Care and Use Committee and Mercer University. Various control groups were maintained. After 3 doses the animals were monitored for tumor growth. After sacrificing animals, immune organs were removed and processed for flow cytometer analysis. Immune organs were studied for the expression of adaptive immune response like CD8 cell, T memory cell and T-regulatory cells.

RESULTS AND DISCUSSION
Whole cell lysate protein content was 2.00 ± 0.5 mg/ml. The particle size range was 900-4000 nm. Zeta potential was -9.2 ± 2.1 mV.
Particle size of around 1-2 µ is found to be optimum for cellular uptake. Half-life of protein release was 3 hrs. Less than 30% of protein released in gastric pH conditions (Figure 1). 70% of protein in particles was available for uptake by M-cells in intestine. Vaccinated animals receiving Cyclophosphamide showed highest inhibition in tumor growth compared to unvaccinated mice (p<0.05) (Figure: 2). CD8 levels were comparatively higher in vaccinated animals (p<0.05). Higher CD8 levels show the development of adaptive immune response (Figure 3).

![Release study](image1)

Fig 1: Release of lysate protein from microparticles at stomach pH (pH=3) conditions (0.5 hrs) and intestine pH (pH=5) conditions

![Tumor volume](image2)

Fig 2: Reduction in tumor volume in animals which received vaccine and adjuvants.

![CD8 levels](image3)

Figure 3. Higher CD8 levels in group receiving Oral Vaccine and Cyclophosphamide (p<0.05)

CONCLUSION
Oral vaccination was able to induce an adaptive immune response against tumor growth. Protection of protein from harsh pH conditions of stomach was achieved using enteric coating cellulosic polymers. Reduction in tumor growth can be attributed to the increased CD8 levels.

REFERENCES

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