Harmless Derivatives of Cancer Cells Induce Adaptive Immune Responses Against Cancer Cells

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Developing Cures for Cancer

Abstract

Cancer vaccines are harmless derivatives or variants of cancer cells that induce specific adaptive or acquired immune responses. BioMedicure is developing cancer vaccine products useful both for prevention and treatment. Results for CancerVaccine™ will be presented using a B16 melanoma c57 wild-type mice model. Major breakthroughs are 1) the immune system recognizes CancerVaccine™ as foreign, 2) CancerVaccine™ antigens are presented on surfaces of professional antigen presenters without interference of self-recognition molecular patterns, 3) Differences between CancerVaccine™ and normal cells are observed, 4) CancerVaccine™-specific antigens are remembered by B-cells and antibodies specific to CancerVaccine™-specific antigens are produced, 5) Challenged cancer cells are labeled by CancerVaccine™-specific antibodies, and 6) Cytotoxic T-cells kill antibody-labeled cancer cells. CancerVaccine™ may be used for cancer treatment or prevention in a cancer type specific manner.

Objectives

The challenge is to make the whole cancer cell vaccine harmless, with enhanced recognition as foreign and preserve all cancer specific mutations or antigen information.

The cancer vaccines described here elicit polyclonal antibodies to multiple mutations or antigens in cancer cells.

Properties elicited from this novel procedure include the following:

1) Whole-cell cancer vaccines harmless forms of cancer cells.
2) Enhanced immune system recognition of cancer vaccine whole cells as foreigners.
3) Cancer cell-specific antigens preserved including but limited to DNA, RNA, protein, lipoprotein, phosphorylated protein, glycosylated protein and carbohydrates mutations.
4) Stimulated immune responses with enhanced killing of cancer tissue.
5) Not introduced are new non-cancer-specific-antigens.

Further Reading

1. Qian, Y. 2008. Proteinases destroy cancer tumor's solid structure and kill cancer cells locally. United States of America, Patent and TradeMark Office publication No: 20080014190
2. Qian, Y. 2009. Proteinase-engineered cancer vaccine induces immune responses to prevent cancer and to systemically kill cancer cells. United States of America, Patent and TradeMark Office publication No: 20090162405
3. Li, J. et al, 2009. Whole tumor cell vaccine with irradiated S180 cells as adjuvant. Vaccine 27: 558–564

Materials & Methods

A. CancerVaccine™ Production

Cancer cell lines, including human prostate tumor line CRL-2505 or 22Rv1, human breast adenocarcinoma tumor line HTB-26 or MDA-MB-231, human lung carcinoma tumor line HTB-177 or NCI-H460, mouse melanoma tumor cell line CRL-6475 or B16-F10 and a normal mouse epidermis cell line CRL-2007 were purchased from American Type Culture Collection (ATCC, Manassas, VA). Cells were grown in RPMI-1640 Medium (ATCC, Manassas, VA), Eagle's Minimum Essential Medium (30-2003, ATCC, Manassas, VA) or Leibovitz's L-15 medium (ATCC, Manassas, VA) with 5% fetal bovine serum USDA Premium (9871-5200, USA Scientific, Ocala, FL) in tissue culture flasks under conditions described previously(1). Cells are harvested when confluent or cover more than 90% area of the tissue culture flask, as seen under an inverted microscope (PhotoZoom, Cambridge Instruments, Cambridge, MA). Cancer vaccines are made from these cell lines by treating with Tumorase™ as previously described(2).

B. CancerVaccine™ Safety Tests

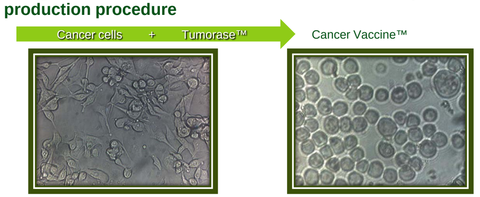
Tumorase™ treated cancer cells i.e. CancerVaccine™ maintain but do not propoagate *in vitro* in flasks even with an optimal medium, the same used for the source cancer cell lines. Cell shape differences and lack of attachment activity were observed under microscope for two weeks. Forty male nude mice were injected with a human prostate tumor line derived CancerVaccine™ ($1x10^6$ cells in 100 uL phosphate buffer saline or PBS per animal) subcutaneously with a syringe and a 27 gauge needle. In the same manner, 40 female nude mice were injected with a human breast adenocarcinoma tumor line derived CancerVaccine™ ($1x10^6$ cells in 100 uL PBS per animal) and 20 males and 20 females with a human lung carcinoma tumor line derived CancerVaccine™ ($1x10^6$ cells in 100 uL PBS per animal). Athymic nude mice (3-4 weeks old, NCR nu/nu) and wild-type mice (C57BL/6, 23 days old) were purchased from Simonsen Labs (Gilroy, CA) or Charles River (Hollister, CA), and directly delivered to a sterile facilities at either Molecular Diagnostics Services (San Diego, CA) or Bio-Quant, Inc (San Diego, CA). Procedures comply with IACUC regulations.

C. CancerVaccine™ Vaccination and Efficacy Tests

Mouse B16 melanoma tumor cell cultures or normal cell lines were treated with Tumorase™ to make vaccines and used to vaccinate wild-type mice 3-5 times weekly(2) or twice biweekly. Male and female mice of various age were sub-Q vaccinated (~100,000 to 2 million treated cancer cells or treated normal cells in 100 uL PBS). Two weeks after the last vaccination 100,000 to 1 million living cancer melanoma cells were injected in order to challenge the immune system. Tumor growth was measured, recorded and calculated by multiplying WxLxH 3-dimensions, where W, L and H stands for tumor width, length and height in mm respectively. Vaccines made from normal cells had no effect (data not shown).

Results

A. Using Tumorase™ to make CancerVaccine™: A simple multi-functional production procedure



B. CancerVaccine™ Safety Study

In Vitro

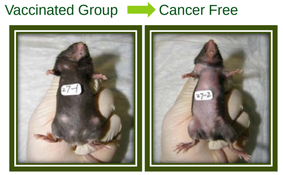
CancerVaccine™	Cancer Cell Lines Tested	Cancer Cell Growth
Human prostate cancer	2	NONE
Human breast cancer	4	NONE
Human lung cancer	4	NONE
Human melanoma	2	NONE
Human colon cancer	1	NONE
Mouse melanoma	1	NONE

In Vivo

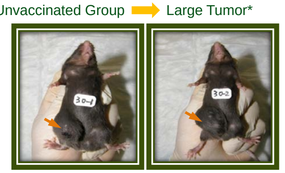
CancerVaccine™	Vaccinated Animals	Tumor Growth
Human prostate cancer	40 male nude mice	NONE
Human breast cancer	40 female nude mice	NONE
Human lung cancer	20 each male and female nude mice	NONE
Mouse melanoma	35 each male and female mice	NONE

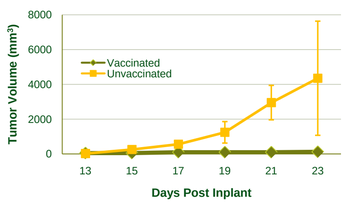
C. CancerVaccine™ Vaccinated vs. Unvaccinated

Vaccinated Group ➡ Cancer Free



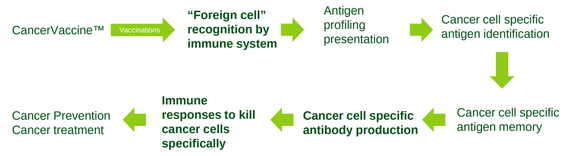
Unvaccinated Group ➡ Large Tumor*





*B16 melanoma in c57 wild type mice

CancerVaccine™ Mechanism



Summary

A. CancerVaccine™ is safe
B. Tumorase™ treated cancer cells are dead *in vitro* and harmless *in vivo*
C. CancerVaccine™ characteristics
   - Cell proliferation or clump formation not observed
   - Cell round shapes due possibly to cytoskeleton collapse
   - Whole cell without cell surface proteins, including molecular self-recognition patterns in major histocompability complex (MHC) I & II
   - Antigens preserved including those for intracellular DNA, RNA, proteins, lipoproteins, phosphorylated proteins, carboxylated proteins and carbohydrates mutations
D. CancerVaccine™ induces strong immune responses against cancer cells thanks to polyclonal antibodies against the multiple antigens specific to cancer cells.

Discussion

Advantages

- Tumorase™ treated non viable cancer cells now recognized as foreign enhancing the antigen presentation process
- Preserved are full complement of intracellular antigens that are general to heterogeneous cancer cells as well as specific to the cancer type
- Does not introduce new antigens that may confuse the immune system
- Simple production with potential improved quality consistency for antigens

CancerVaccine™ strong efficacy may result from

- Enhanced recognition of CancerVaccine™ as foreigners, thus more CancerVaccine™ cells are engulfed by macrophage and dendritic cells for antigen presentation process
- Larger amount of any specific antigen for antigen presentation process
- More antigen species being forced to go through the antigen presentation process

All of these advantages lead to a more potent immune response covering a wide range of cancer cell mutations. Therefore, CancerVaccine™ is expected to be an improvement over other cancer vaccines including gamma-ray irradiated whole cell cancer vaccines, DNA and peptide vaccines, monoclonal antibody against one oncogene products, dendritic cells, T-cells, and others including lipoproteins, carboxylated proteins, cytokines and interleukins.