CancerVaccine: Novel proteinase treatment of melanoma cancer cells produces a cancer vaccine with enhanced protective adaptive immune response

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BioMedicure

Developing Cures for Cancer

Objectives

- The challenge is to make an allogeneic whole cell cancer vaccine:
  1) Harmless nonviable preparation
  2) With enhanced recognition as foreign
  3) Preserving all cancer cell specific antigen information
  4) Defining optimal starting preparation made out of enriched cancer stem cells
- CancerVaccine™ produced from enriched cancer stem cell preparations equals the efficacy of that from tumor cells

Product properties from this novel procedure include the following:

1) "Giant liposomes" or whole-cell cancer vaccines, harmless derivatives of enriched cancer stem cells.
2) Force immune system to recognize cancer vaccines as foreign.
3) Cancer cell-specific antigens preserved including but limited to mutant DNA, RNA, protein, lipoprotein, phosphorylated protein, glycosylated protein and carbohydrates.
4) Cancer specific antigens are remembered in immune system.
5) Immune system prime immune responses against cancer cells specifically.

Material & Methods

A. CancerVaccine™ Safety Tests

Tumorase™proteinase treated cancer cells i.e. CancerVaccine™ maintain but do not propagate *in vitro* in flasks even with an optimal medium, the same used for the source cancer cell lines. Giant liposome shape, size differences and lack of attachment activity were observed under microscope for up to two weeks. Nude or wild type mice were injected with a CancerVaccine™ ($1x10^4$ to$1x10^6$ cells in 100 uL phosphate buffer saline per animal) subcutaneously with a 27 gauge needle. 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 sterile facilities at local vivarium CROs. All procedures comply with IACUC regulations.

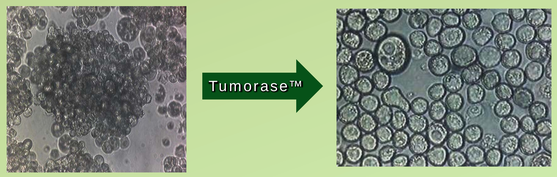
B. CancerVaccine™ Vaccination and Efficacy Tests

Cell preparations were treated with Tumorase™ to make "giant liposome" cancer vaccines preserving all antigens in forms of mutant DNA, RNA, proteins and metabolites. These vaccines were used to vaccinate wild-type mice twice biweekly or weekly for up to five weeks. 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 (W x L x H)/2, where W, L and H stands for tumor width, length and height in mm respectively.

Results

A. CancerVaccine™ preparation using the proprietary proteinase preparation Tumorase™

CancerStemCell™ → Tumorase™ → CancerVaccine™



B. CancerVaccine™ Safety Study

- **Whole cell cancer vaccine preparation no viable cells and no *in vivo* toxicity observed**

In Vitro

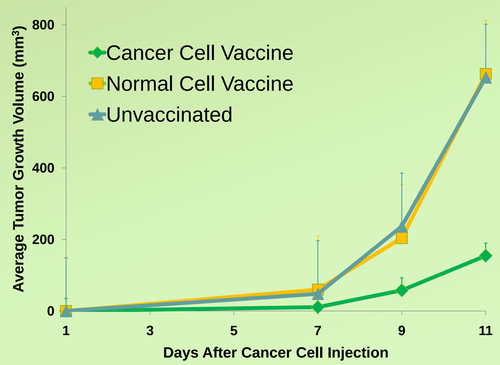
CancerVaccine™	Cancers Tested	Cancer Cell Growth
Human cancer types	13	NONE
Mouse cancer types	10	NONE
Rat cancer types	9	NONE
Chicken cancer types	2	NONE
Dog cancer types	1	NONE
Guinea pig cancer types	1	NONE

In Vivo

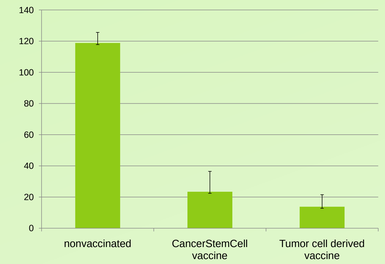
CancerVaccine™	Vaccinated Animals	Tumor Growth
Human prostate cancer	40 male nude mice	NONE
Human breast cancer	40 female nude mice	NONE
Human lung cancer	20 each male and female nude mice	NONE
Mouse melanoma	35 each male and female mice	NONE

C. CancerVaccine™ Efficacy

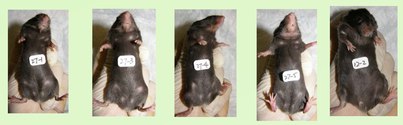
- **CancerVaccine™ from cancer cell line is active while normal cell whole cell vaccine is inactive**



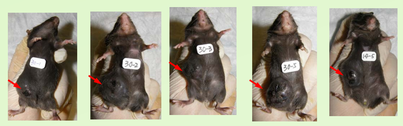
- **CancerVaccine™ from CancerStemCell™ an enriched cancer stem cell preparation: Efficacy equal to that from dissociated tumor cells**



Vaccinated group → Cancer free



Unvaccinated group → Large tumors



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

CancerVaccine™ Mechanism

CancerVaccine™ → **"Foreign cell" recognition by immune system** → Antigen profiling presentation → Cancer cell specific antigen identification → Cancer cell specific antigen memory → **Cancer cell specific antibody production** → **Immune responses to kill cancer cells specifically** → Cancer Prevention Cancer treatment

Summary

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

Discussion

Advantages

- Tumorase™ treated nonviable cancer cells now recognized as foreign enhancing antigen presentation processes
- 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 foreign, 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 processes with equal opportunity

All lead to more potent immune responses covering a wide range of cancer cell mutations or antigens. Therefore, CancerVaccine™ is expected to be an improvement over other cancer vaccines including gamma-ray irradiated whole cell cancer vaccines and single antigen DNA and peptide vaccines or monoclonal antibody.