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Nathaniel Sangster
Lincoln University, nathaniel.sangster@lincoln.edu

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Development of DNA Vaccination Approach for Tumor Immunotargeting

By: Nathaniel Sangster
Background

- Conventional treatments for various forms of cancer include surgery, radiation, and chemotherapy.

- In recent years immunotherapy emerged as a prominent modality to treat cancer.

- Immunotherapy is aimed at the activation of the host immune system against malignancy.
Their have been recent advances in the development of suitable immunotherapy treatments that may prove effective in the fight against cancers.

### Vaccination
- Peptide vaccines
- Dendritic cell vaccines
- Virus based vaccines
- Micro-organisms
- DNA vaccines

### “Supplementation”
- Tumor-specific T cell (CTL)
- Generation of tumor-reactive T cells expressing engineered T cell or chimeric receptors.
- Tumor-specific antibodies

### “Modification”
- Inhibition of regulatory T cell
- Addition of Adjuvants
- Alteration of tumor-derived Ag
- Systemic activation of T cells via blockade of CTLA-4
Advantages and Disadvantages of DNA Vaccines

- As opposed to viral vaccines, DNA vaccines are relatively inexpensive and are often easily produced.

- DNA vaccines are relatively easy to design.

- Thus far, no significant safety concerns were raised regarding DNA vaccines.

- Immune response consists of not only antibodies, but also T-cells, which have been proven effective for killing cancerous cells. (DNA vaccination was recently approved by the USDA for melanoma therapy in dogs)

Nevertheless:

- DNA vaccines were not effective in inducing cancer-targeting immune response in therapeutic settings (at least in humans)
How They Work

The mechanism for DNA vaccination includes:

- Construction and production of a plasmid DNA encoding an antigen (cancer-specific gene) and its delivery into the host cells in vivo.

- Expression of the antigen.

- Activation of the immune response to the antigen (acquired as a protein by the Antigen Presenting Cells (APC) of the host) (indirect presentation).

- Antigen can be expressed in the APC leading to the direct presentation of the antigen-derived peptide(s) by the APC via MHC class I and activation of the CD8+ cytotoxic T cells (direct presentation).
Development of Vaccine

- The first DNA vaccine to be tested was against mutant mouse GNAQ.
- GNAQ is a signaling molecule that is expressed in all cells.
- Point mutation in the GNAQ (and highly homologous GNA11)-encoding genes (Q209L) was shown to be responsible for the development of more than 70% of uveal melanomas in humans.
- At present, there is no uveal-melanoma-specific immunotherapy. In fact, there is no mutation-specific therapy for melanoma in general.
- Analysis of protein sequences of the wild type and mutant GNAQ using SYFPEITHI epitope prediction database (http://www.syfpeithi.de) showed that GNAQ mutant peptide \texttt{FRMVDVGGL} has a high probability of being loaded and presented by mouse and human MHC class I.
Development of Vaccine

- We hypothesize that DNA vaccination may result in the expression of the mtGNAQ in the APC, presentation of the mutant peptide, and induction of the mutation specific immune response.

We also proposed that vaccination efficacy could be enhanced by inclusion of:

- VP22 and PADRE immune-activating epitopes into vaccine composition

- pBOOST2–mIRF7/3 vector encoding interferon response factors 7 and 3, which has been proven to increase immune response after DNA vaccination

- Chemokines (CCL20 and CCL21), which are chemotactic for immature APC and T cells, respectively.
We suggest that administering pEF1–VP22–mutGNAQ–PADRE DNA vaccine along with other enhancement approaches will lead to the induction of the mutant GNAQ–specific immune response in vivo.

The DNA vaccine used in this experiment contained the backbone plasmid pEF1–VP22–PADRE (VPG) (designed in Dr. Alexeev laboratory) that contained human Elongation Factor 1 promoter, herpes simplex virus VP22 cDNA, multicloning site, and pan T cell activating PADRE epitope.
Development of Vaccine

Expression vectors used for vaccination

pEF1-mCCL20
6402 bp
6xHis
Amp(R)
Bsd(R)
V5 epitope
mCCL20 cDNA
SV40 pA
BGH pA
BGH reverse primer
EF-1alpha forward primer
T7 primer
T7 promoter
3'-T overhang

pCUB-CCL21
5170 bp
Amp(R)
CCL21 cDNA
CMV enhancer
SV40 polyA
pUbC
pCUB
P(BLA)
ORI
CCL21 3' UTR

pEF1-VP22-mtGNAQ-PADRE (VPG)
7884 bp
Amp(R)
Bsd(R)
VP22
PADRE
mtGNAQ-PADRE
SV40 pA
BGH pA
EF-1alpha forward primer
T7 primer
3'-T overhang

pBOOST2-samIRF73
1008 bp
samIRF73
SV40 pA

pCUB-CCL21
5170 bp
Amp(R)
CCL21 cDNA
CCL21 3' UTR

VPG 1001
Full Kozak
EF-1alpha forward primer
VP22
T7 promoter
3'-T overhang

VPG 1002
NcoI (959)

VPG 1004
NcoI (2077)

F1 origin
SV40 early promoter

PvuII (309)
PvuII (3115)

BamHI (1949)
BamHI (2683)

P(BLA)
CMV enhancer

NotI (915)
NotI (2077)

XbaI (1756)
XbaI (2089)

BglII (503)
BglII (4777)
BglII (5684)
Analysis of antigen expression in vivo

Intradermal electroporation of the Green Fluorescent Protein (GFP) encoding plasmid

This analysis confirmed efficient in vivo electroporation-based expression of the antigen in the skin.

RT-PCR – based analysis of the antigen expression in mouse skin 48 h after electroporation of the pEF1–VP22–mtGNAQ–PADRE plasmid.

Lanes 1 and 3 – treated skin
Lanes 2 and 4 – untreated skin

This analysis confirmed expression of the Ag in the skin after electroporation.
Experimental Design

- In these experiments, **three groups** of C57BL6 mice were used. Each group consisted of three mice.

- Each group was intradermally electroporated with vaccines four separate times, with 4–5 days in–between each vaccination.

- Electroporation was used to allow the target cells to receive the DNA vaccine.
Treatment groups

- Group 1 mice received an initial dose of chemokine CCL20 alone to recruit APC. This was followed by the full vaccine four days later.

- Group 2 mice received doses of pEF1-VP22-mutGNAQ-PADRE along with pBOOST2-IRF7/3.

- Group 3 mice received doses of the full vaccine: pEF1-VP22-mutGNAQ-PADRE along with pBOOST2-IRF7/3 and cytokine CCL21.
Timetable of Vaccinations

G1 received: CCL20
G2&G3 received: vaccine

G1,G2&G3 received: vaccine

G1 received: CCL20
G2&G3 received: vaccine

G1,G2&G3 received: vaccine

Spleens were harvested

7/05/12  7/09/12  7/13/12  7/18/12  7/26/12
Upon completion of their respective vaccination series (2 weeks after last immunization), two mice from each group were euthanized. Their blood was drawn and their spleens were harvested.

Splenocytes were isolated from each of the harvested spleens.

- Isolate the spleen from the mouse and put it in a petridish.
- Squash spleen using syringe plunger.
- Disrupt the cells with a 18G needle fitted onto syringe.
- Transfer into a tube and centrifuge.
- Lysis of red blood cells.

Collection of the Splenocytes
For further experimentation.
Induction of immune response to wild type and mutant GNAQ was assessed by IFNγ ELISpot assay using the isolated splenocytes.

**ELISPOT assay**

- IFNγ Capture Abs are bound to the wells

Target (GNAQ wt or mutant) cells and Effector (splenocytes) are added to the wells and incubated for 48 h

- T cell-produced IFNγ is captured

- IFNγ is detected by another Ab

- Colorimetric reaction results in the development of spots
Results

Average Number of Spots per Group

Number of spots correspond to a number of antigen-reactive T cells
To examine the presence of the GNAQ-specific antibodies, we conducted a sandwich ELISA.

**Diagram 1: Illustration of Sandwich ELISA method.**
Results

ELISA assay / serum antibodies tested against wild type GNAQ
Results

ELISA assay / serum antibodies tested against mutant GNAQ
Cellular and animal models for testing mutant GNAQ-specific immune response

Cell model:
Spontaneously immortal mouse melanocytes (Melan-a) were transduced with mtGNAQ cDNA to generate cells expressing both wild type and mutant GNAQ. Cells resembled parental melanocytes, however with darker pigmentation and increased proliferation in vitro.

Light microscopy and indirect immunofluorescent detection of GNAQ in mtGNAQ-expressing melanocytes
Animal Model:
On contrary to parental melanocytes, mtGNAQ expressing cells produce lesions resembling human blue nevus after intradermal injection of $1 \times 10^6$ cells.

These models will be used in future studies for the intra-lesional vaccination and for the inoculation of the lesions into control and vaccinated mice in prophylactic setting.
Conclusions

1. Electroporation, although not yet optimized, provide an effective means to express antigen in the skin.
2. DNA vaccine encoding mutant GNAQ induces *mutation-specific immune response* as defined by the IFNγ ELISpot and ELISA assays conducted against cells expressing wild type and mutant protein.
3. Pre-treatment of the skin with CCL20 prior to vaccination does not enhance cytotoxic immune response but induces strong antibody response. This suggests indirect route of vaccine presentation (Group 1).
4. Vaccination with pEF1-VP22-mtGNAQ-PADRE and pBOOST leads to the preferential induction of the cytotoxic response via direct route of antigen presentation (Group 2).
5. Based on ELISpot assay, inclusion of CCL21 cDNA into the vaccine composition enhances immunogenicity of the vaccine and induction of both cytotoxic and antibody responses.
6. Non-tumorigenic mouse melanocytes expressing mutant GNAQ is a suitable mouse model to test immune responses.
Future Directions

- Assess mutant GNAQ-specific immune response in vaccinated mice by inoculation of the lesions.

- Test the intra-lesional vaccination strategy using mouse “blue nevus” model.
Generation of other DNA vaccines

We initiated construction of two new DNA vaccines encoding model antigen – chicken ovalbumin (Ova) and Gastro-Intestinal (GI) tract cancer associated antigen – Guanylyl Cyclase C (GCC).

Cloning Strategy:

Amplify GCC-S1 and Ova by PCR adding NotI an XbaI sites

mGCC-Not 5’-CGGCGGGGTATGACGTCACCTGGGGCTTGGCTGTG-3’
mGCC-Xba 5’-TCTAGATCGAACCACCTTTGTACAAAGAAAGCTGGG-3’

Ova-Not 5’-CGGCGGGCTATGGGCTCCATCGGCCGCAG-3’
Ova-Xba 5’-CCTCTAGATTAAGGGAACACATCTGCACAAAGAAAGACGC-3’
Future Directions

- Finish cloning of the GCC and Ova into pEF1–VP22 vector.

- Use new DNA vaccines encoding Ova and GCC for vaccination.
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