What’s next for ligand membrane disrupting peptides?

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Potential Novel Treatments

- Alpha melanocyte-stimulating hormone MDP to treat melanomas
- Gene therapy to treat cancer
- Use of ligand membrane disrupting peptides (MDPs) to treat a non-neoplastic reproductive disease
Use of truncated alpha-melanocyte stimulating hormone MDPs for treatment of melanomas
Melanoma

- A malignant skin tumor that originates in the melanocytes.

- Not the most common type of skin cancer but causes the most deaths.

- 2007 estimates—
  60,000 new cases and 8,000 deaths.
Synthesis of melanin by melanocytes is regulated mainly by alpha melanocortin hormone (αMSH) and ACTH.

Both hormones bind the melanocortin receptor (MC1R) which is overexpressed on the surface of melanoma cells.
Experimental Peptide and Design

Truncated αMSH (9mer) linked to phor21

Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro--phor21

1.) Implantation of $1 \times 10^6$ B16 melanoma cells.

2.) 2.0 mg/kg IP of appropriate peptide administered once/day for 3 days on days 8, 9 and 10 post tumor implantation.

3.) Necropsy on day 11.
CPE results from female animals with tumors

CPE: Significance of MSHphor21 v baseline  P≤0.001  
MSHphor21 v saline  P≤0.002

<table>
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<tr>
<th>treatment</th>
<th>number in group</th>
<th>CPE</th>
<th>group avg CPE</th>
<th>SD</th>
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<tr>
<td>Baseline (pretreatment)</td>
<td>5</td>
<td>4,3,4,4,3</td>
<td>3.6</td>
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<td>Saline</td>
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<td>MSHphor21</td>
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<td>GnRHphor21</td>
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</table>
Melanoma tumor CPE in C57/BL6 mice

MSHphor21 treatment v controls

- baseline
- saline
- phor21
- MSHphor21
- GnRHphor21
- MSH

CPE
$\alpha$MSH-Phor 21

No evidence of toxicity noted in histological examination or in clinical chemistries.
In vitro melanoma study

- Identification of another melanoma “ligand” (any small protein binding to a cell surface receptor)

- Use of a phage display peptide library

- Target cell is the SK-MEL-28—ATCC #HTB-72
General Methods

6 rounds of positive and negative selections were employed

Positive selections used tumor cells as targets.

Negative selections used a variety of normal human cells.
100 plaques were selected.

Sequence analysis demonstrated that 31 of the first 50 clones had identical sequences coding for TSSMRNLGHPIP.
This peptide (SK-MEL) and SK-MEL phor21 are being synthesized and will be evaluated *in vitro* and *in vivo* in nude mice.
Gene Therapy
During the early 1990’s Dr. Richard Cooper developed a transposon-based vector with which we were able to efficiently insert foreign DNA into a variety of hosts including fish, mice, pigs, dogs, cats, quail and chickens.
Disease Resistance

Hyalaphora cecropia

Cecropin B

ATS  IS10L  APR  Cecropin B  polyA  IS10R

732 bp  1035 bp  406 bp

stop codon

732 bp  1035 bp  406 bp
Sterilization and Anti-Cancer Constructs
Gene constructs: $0.14/mg

Peptides: $14.00/mg
Our gene constructs expressing either ligand MDPs or MDPs alone have been shown to be safe and effective in mice, cats, dogs and cows.
Dog Sterilization
Untreated and treated uteri at 5X
Average weight of female reproductive tracts in each treatment group

Different letters are significantly different from one another.

*  P≤0.25 (not significant)
** P≤0.0011
*** P≤0.0122
**** P≤0.003

Reproductive tract includes midcervix, uterine body and horns and both ovaries.
Use of GnRH MDP and/or MDP β-hCG to treat a non-neoplastic reproductive disease
Endometriosis

Endometriosis is a disease caused by the implantation and growth of endometrial tissue outside the uterus.

In the US, it affects over 10 million women. Estimated to be present:
- in 15-20% of all reproductive-age women
- in 40% of all infertile women
- in 80% of those with deep chronic pelvic pain
Endometriosis - current treatment

Long-acting gonadotropin-releasing hormone agonists of GnRH (leuprolide) have been used to cause so-called chemical sterilization.

- These long-acting agonists cause pituitary receptor down-regulation and ovarian suppression.
Endometriosis – setting the stage

Over the last 16 years, it has been reported that ectopic endometrial cells recovered from diagnostic and surgical procedures on women with clinical endometriosis demonstrate functional receptors for both hCG and GnRH and GnRH agonists.
Endometriosis – the idea

**Hypothesis**

- Ligand MDPs targeted to these ectopic cells will result in their death

- Selective destruction of these cells offers obvious advantages over current treatments which only suppress the proliferation of these abnormally localized cells
Endometriosis – novel treatment

What is needed to test this idea?

An animal model of endometriosis that utilizes non-human primates

Currently, this does not exist
Collaborators

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