Tumor Targeting Gene Therapy System
BACKGROUND
CANCER

First cause of death

No effective therapies
extreme hypoxia

poor vascularization and moderate hypoxia

necrosis
Cancer

First cause of death

No effective therapies

Searching for better one
Initiation of Bacteria Therapy

Streptococcus vs Cancer cells

1800s
- First discovered the phenomenon

1860s
- First applied in clinical case

See you, I do. Smash you, I will.
1990s

Yale University

Attenuated *Salmonella Typhimurium*
ADVANTAGES

- Highly colonize hypoxic areas
- Distinct genetic background
Without msbB & purl

Be more talented without the two genes!

But…
Little effect on tumor regression.
OPTIMIZATIONS

Part I

Auxotrophic mutants

Part II

Anaerobic promoters & Drug genes

Gene Therapy System
OPTIMIZATIONS

Part I
Auxotrophic mutants

Part II
Anaerobic promoters & Drug genes

Gene Therapy System
PART I

—- Mutants selecting and screening
Gene A to be knocked out in VNP
PKD4 provides Kanamycin cassette for recombination
PKD46 takes a recombination tool called the Red system.
Electroporate PCR products into bacteria
Gene A is knocked out successfully

Lost Gene A and PKD46
The optimized bacteria has a better ability to target tumors and may be safer.
The optimized bacteria accumulates more in tumor than in normal tissues.
The ratio of optimized bacteria is 50 times higher than that of VNP.
As a result, the optimized bacteria indeed is better at targeting tumors, possibly also has a higher tumor expression and safety.
OPTIMIZATIONS

Part I
 Auxotrophic mutants

Part II
 Anaerobic promoters & Drug genes

Gene Therapy System
PART II

-----hypoxia-induced gene expression
Literature-based promoter searching

|--------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|

Reference:
1. Use of the Nirb promoter to direct the stable expression of heterologous antigens in S. almonella oral vaccine strains – development of a single-dose oral tetanus vaccine. Biotechnology. 10, 888-892(1992)
# Library-based promoter searching

<table>
<thead>
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PLASMID CONSTRUCTION

Cut with E & S

Mix & Ligate

Cut with E & X
*E. Coli* NirB is a hypoxia specific promoter in VNP

Pic 1. Fluorescent microscopic analysis under aerobic and anaerobic condition in VNP.

Pic 2. Quantification of expression after aerobic and anaerobic incubation
The highly tumor-targeting specificity in vivo

**Pic 3.** The expression of GFP in tumor tissue and liver

\[ \text{pLacZ: a constitutive promoter.} \]

\[ \text{N: necrotic and hypoxia area in tumor tissue.} \]

\[ \text{V: vital tumor cells} \]
As a result, VNP with the anaerobic promoters can have more advantageous expression in hypoxia and necrotic tumor microenvironment.
On-going experiments
More screened strains are coming
More anaerobic are under examination.

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Igem

Literature
Drugs combined are on the way.

TRAIL was effectively expressed in VNP20009 under hypoxia.
BACTERIAL GENE THERAPY SYSTEM

Screened strains

Anaerobic promoters

Drugs combined

TUMOR-TARGETING DELIVERY & TUMOR-SPECIFIC EXPRESSION
A tenable choice for tumor therapy.
Human Practice
—— by 静云
What did we do?

1 Experience a real iGEM
2 Collaboration
3 Explore a better iGEM
1 Experience a real iGEM Academic salon

In Nanjing University
1 Experience a real iGEM

Introduction of synthetic biology and biosafety

In Jinling High School
2 Collaboration

Communication with SEU
### Biobrick curse

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3 Explore a better iGEM
Online survey

http://www.surveymonkey.com/s/3C6HVDM
3 Explore a better iGEM
Online survey questions

1. Have you ever experienced the following problems when you enter the iGEM official website? (Multiple choice)
2. Have you ever felt helpless when you met problems listed above (in Q1) while there are no exchange platform to get assistance?
3. If we could set up an exchange platform, which form do you think is the best?
4. Which of the following information you think we should include in the exchange platform? (Multiple choice)
5. Do you think we need to set up a committee to manage the platform?
6. In your opinion, who should be in charge of the platform if we set one?
7. Have you ever felt it difficult to contact with other teams’ members? Do you prefer the iGEM committee to provide the contact information of all the participants (E-mail address for example) under their permission?
8. As an iGEM team (especially when it’s your first time to attend this competition), do you think you know little about the criteria for the Special Prizes? And do you prefer the iGEM committee to provide more detailed explanations for these awards?
9. When using the BioBricks provided by iGEM committee, have you found some BioBricks fail to function?
10. Thank you for taking time to fill in this questionnaire. It will be nice of you to write down your team name (required), name (optional) and e-mail address (optional) in the box below so as to make it possible for us to mention your help in our wiki. You can also comment about your views on the iGEM competition.
3 Explore a better iGEM
Online survey results

Have you ever felt helpless when you met problems listed above (in Q1) while there are no exchange platform to get assistance?

Chart 1

Agree: 83.40%
Disagree:

Chart 1
Online survey results

Chart 2

Preference for the form of exchange platform

- Forum
- iGEMers' social network
- E-mail
- Other
Chart 3

Have you found some BioBrikcs fail to function?
3 Explore a better iGEM

We hope

- I: Set up an exchange platform
- II: Check the validity of the parts
Our members
Our members
Abstract

Salmonella selection

Salmonella typhimurium VNP20009 (VNP) has unique characteristics of high tumor targeting and low toxicity. It can highly colonize central parts of tumor tissues. Our project aims to decrease the influence of the bacteria on non-tumor tissues.
Acknowledgement

Instructors:
Pr. Zi-Chun Hua, suggest the whole program design.
Pr. Yi-xin Shi, Assistant Professor, instruct gene konck-out technology.

Advisers:
Dr. Xia-Wei Cheng, guide anaerobic culture.
Dr. Xiao-Xin Zhang, construct animal models.

Sponsors:
NJU Affair Office ,
the School of Life Science, Nanjing Univ. ,
the State Key Laboratory of Pharmaceutical Biotechnology.

The cooporation of all team members and suggestions from other iGem teams,especially USTC & SEU.
Thank you

Thanks for your attention !