University of Westminster

Targeting cancer stem cells using a molecular toolkit

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The Problem

Research has shown that cancer stem cells exhibit elevated levels of aldehyde dehydrogenase (ALDH)-activity. This activity has been linked to the ability of these cells to be resistant to many of the current chemotherapy treatments. As such, cancer stem cells are being increasingly investigated as a source of cancer recurrence which is seen in many patients. ALDH has been identified as a biomarker in a number of primary cancers and cancer cell lines. However, 19 isoforms of ALDH exist and more work is required to determine the role which these different isoforms play in cancer stem cells.

How would iGEM work?

The key of our constructs are the promoters. It is estimated that the expression level of our chosen ALDH isoforms is 10 times greater in cancer stem cells than it is in regular cancer cells. The experiments will be done using MCF7 cell line.

Below we illustrate how the iGEM constructs would act in cancer tissue. (1) Identify. (2) Isolate and (3) Eliminate.

(1) Upregulation of expression of the first construct, results in red fluorescence. An ALDH inhibitor will be added so that only those cells which exhibit high expression levels will be detected. This allows us to locate and quantify cancer stem cells providing a useful tool for further study.

(2) Transiently transfected cells will be treated with hygromycin for selection of cells which have taken up plasmid. Then cells will be transfected to neomycin-containing media for selection of those cells with high resistance to neomycin.

(3) Doxycycline induction would lead to expression of the cre-recombinase enzyme. This enzyme cleaves the sequence between the two lox sites, excising the terminator and killing the end. This allows expression of herpes simplex thymidine kinase which confers susceptibility to the glucocorticoid, effectively killing those cells with high expression of ALDH.

Human Practice

The interview

Interviewing Dr. Miriam Dawe, Lead Researcher at the Agilent Breast Cancer Unit and co-editor of Oncology news, made us more aware of the difficult nature of cancer diagnosis, and the potential issues we may encounter during the development of this project.

The survey

We engaged with the general public regarding their interest and depth of knowledge in biology and to gauge their perception of the applications of gene therapies. These include the following questions:

Public Engagement at University

We have pioneered iGEM at the University of Westminster. We have generated a buzz about synthetic biology and the opportunities iGEM can offer both students and staff at the university. We have engaged with students from a number of non-science departments including computing, art and design and architecture. At Freshers’ Fair we signed up 46 undergraduate students keen to be involved in iGEM 2013. On the 2nd of October, we held a presentation evening for staff and students to present iGEM and our project.

References


Modifying Plug-n-Play To meet iGEM Standards

Plug-n-play is a modular assembly strategy introduced to iGEM by the DTU-Denmark 2011 iGEM team. This assembly method is particularly relevant for designing and constructing mammalian parts and offers a number of advantages:

- It is a ligation free assembly method
- Multiple parts can be ligated together in a single ligation reaction
- Allows for parts holding illegal sites to be tested
- Allows for developing interchangeable molecular tools.

We added the iGEM trisacid prefix and suffix to each construct, making them RFP compatible.

Linera Primer Design

The Phusion® PCR system is used to generate the linker primers. A uracil base is added at the end of the linker. Treatment of PCR products with USER enzyme mix generates homogenous overhangs allowing for seamless ligation. Allows for in-frame expression of proteins.

The Team

We are a mix of undergraduate and Masters students in the Life Sciences Department at the University of Westminster, based in central London.