Continuing iGem 2008: Perspectives for fighting tumors in live mice

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3 Approaches to let microbes specifically kill tumor cells

- Bacteria are more concentrated in tumors due to immunosuppression by at least 3 orders of magnitude[1]

I. **Chemotaxis** can be used to amplify this pre-existing gradient. Possible tumor sensors are:
   - Low oxygen → Aerotaxis is a system widespread in Nature and should thus be easy to adapt for iGem
   - High bacterial cell density → The project started in 2008 can be continued, and combined with Aerotaxis. Possibly, a two-stage process could be applied:
     1. Mice are infected with an AI-2 producing strain of pathogenic bacteria ("Bait"). After some time, these bacteria naturally assemble in the tumors. Possibly, bait can be engineered to be less likely to survive outside tumors.
     2. Mice are then injected with the killer strain. Killer strain would naturally concentrate in tumors by roughly three orders of Magnitude, but due to the presence of the Bait and possibly Aerotaxis, this gradient is steepened.
   - Other ways to sense tumors by Chemotaxis might be difficult to implement in the frame of an iGem project, as no natural system can be easily adapted

II. Previous work[1] omitted the step of bacteria cell density amplification by chemotaxis. All bacteria residing in tumors (as sensed by low oxygen [fdhF-Promoter] and high bacterial cell density [LuxQ]) were to express the *inv* gene of *Y. pseudotuberculosis*, and thus to invade the tumor cells: **Environmentally controlled invasion of tumor cells by bacteria.** This approach might work alone, but can be improved by combining it with Chemotaxis as outlined in (I).
   - Delivery of cell poison is more efficient if released from inside the cancerous cell (application of inv)

III. An additional control step *might* be added: **Checking whether the cell is cancerous *after invasion***. From the inside, it might be easier to check whether the invaded cell is cancerous or not as a cancerous cell is very different from a healthy cell. Even if not combined with (I) and (II), bacteria that invade cells unspecifically and kill only cancerous cells would by specific.
   - Some mRNAs are upregulated in cancer cells, so a way to check whether an invaded cell is cancerous or not I can think of is to
     1. Induce Lysis of the bacterium
     2. Lysis releases Killer-RNA made by the bacterium: Killer-RNA forms a stable secondary structure loop prohibting translation, unless regulatory tail hybridizes with cancer-specific RNA (which must be more stable than forming a loop). Killer RNA encodes highly efficient eukaryotic cell poison.

1 JC Anderson et al.: Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria in: J. Mol. Biol. 355 (2006) pp. 619-627

5 Project Columns

Implementing these ideas, or parts of them, would involve forming up to 5 project columns

I. Getting iGem 2008 to work, also in Vivo (Killer finds tumor by seeking microbial or bait density)
II. Implementing an Aerotaxis system
III. Adapting the work by Anderson et al[1] (for approach II)
IV. Killer-RNA Development or something else related to approach III
V. Directed Evolution (see below)

Applying Directed Evolution to Chemotaxis engineering

When I thought about how to get the LuxQ-Tar-Fusion receptor to work, it occurred to me that engineering signal cascades is difficult. It might be an interesting project column to develop high-throughput techniques for directed evolution of signal cascades. Just an idea:

- Fuse Receptor (LuxQ-Tar) to N-terminal half of GFP
- Fuse Kinase (CheA) to C-terminal half of GFP
- Error-prone PCR or something related
- Sort cells by FACS → Fluorescent cells do show Tar-CheA interaction and can be studied by swarm plates etc.