Developing project ideas

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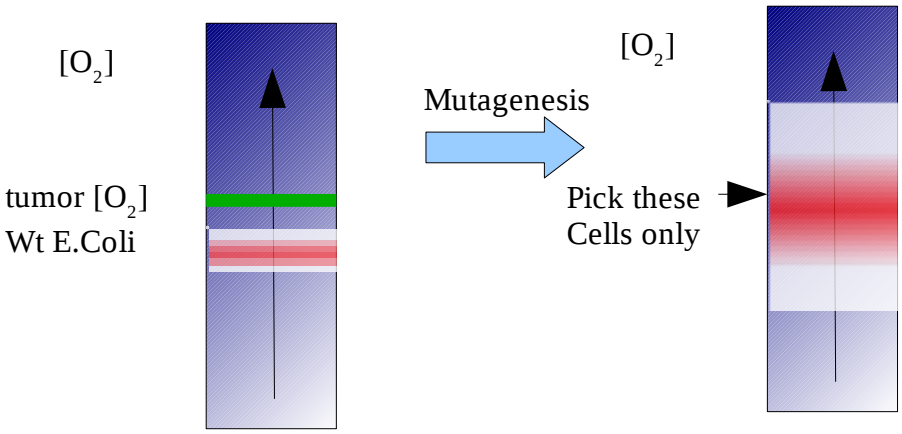
Chemotaxis - Best / most feasible approach

- Energy taxis (there are no true oxygen receptors involved in a taxis process)
- Directed evolution of E.Coli to navigate to oxygen concentrations typical for tumors



Chemotaxis – impact

????

- Maybe more interesting for locating metastasis

Invasion – Problems

- After invasions, cells need to lyse the host's membrane engulfing it, the release the cytotoxic agent
- Connecting the conditions “low oxygen” and “high bacterial density” with an AND gate

Invasion – Approaches

- **Top-Paper: Potential therapeutic applications of recombinant, invasive E. coli** (Pubmed ID 15141160)
- Besides of Yersinia *inv* E.Coli must express Listeria *hly* which lyses the phagosomal membrane
- E.Coli is lysed in the phagosome, *hly* gene product (listeriolysin O, LLO) and potential therapeutic gene/protein are released → cytoplasm
- System was efficient in delivering protein. Not so efficient in delivering genes. RNA????
- Positive side effect:Phagosomal lysis is a danger signal inducing even cancerous cells to activate the immune system: *“The concomitant presence of apoptotic/necrotic cells, bacterial products such as lipopolysaccharides and bacterial DNA could act as ‘danger signals’ for these infiltrating cells. The local abundance of these danger signals associated with he phagocytosis of dead cancer cells by activated macrophages and DCs is likely to initiate an immune response against specific cancer antigens.”*
- **AND-Gate:** An interesting strictly binary AND-Gate is presented in Pubmed ID 16408021. In short, Condition A e.g. high cell density (Lux) would lead to expression of a *inv* mRNA with a modified Kozak sequence. Condition B would lead to expression of a 16S rRNA recognizing the modified sequence (“orthogonal mRNA-Ribosome pair”). Only if (A AND B) *inv* will be

expressed.

- Might be difficult to adapt. Also, patent protected (which does not mean we cannot apply it). More straightforward approach: low oxygen leads to expression of Lux receptor, Lux promoter → *inv*

Invasion: Feasibility – Impact

- Adapting the Anderson (Pubmed 16330045) paper and with an AND Gate such as Pubmed ID 16408021 and *hly* expression (Pubmed ID 15141160) could be feasible, as it builds largely on existing work. HSV-tk (Herpes Simplex Virus Thymidine Kinase) or another pot. Cytotoxic protein could be delivered by such an approach.
- Potential high impact for gene / drug delivery
- Only related patent: *inv* nucleic acids, US 5338842 (1994) → in my opinion, making a cancer-sensing biobrick out of it is OK, as this patent is very vague about areas of application.

Intracellular Sensing – Approach

- **Top-Paper: Specific Regression of Human Cancer Cells by Ribozyme-Mediated Targeted Replacement of Tumor-Specific Transcript** (PubMed ID 16040278)
- In short, a Tetrahymena ribozyme is able to recognize a RNA message specifically, cleave it and bind it to an exon attached to its 3' end. This was used to sense hTERT (Telomerase Reverse Transcriptase) RNA and attach HSV-tk exonic RNA to it.
- The construct was shown to work well and kill cancer cells efficiently.

Intracellular Sensing – Feasibility - Impact

- RNA-sensors would generally be interesting for synthetic biology and medicine.
- Problem: US 6010904 (Patent, 2000). We could still work on it, but we could not create a truly open source BioBrick from it.
- Alternative: Sensing cancer metabolites using RNA antiswitches. (PubMed 15723047). No classic sensing can be applied if using invading bacteria as mode of delivery, as the bacteria will be lysed in the phagsosome.