CoCa coli

PROBIOTIC IMMUNOTHERAPY AGAINST COLON CANCER

BIND. DETECT. SECRETE.

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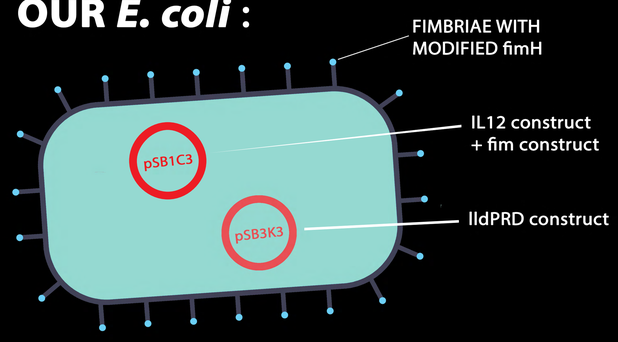
COLON CANCER

It is the 3rd most common cancer worldwide in terms of its incidence and 2nd in mortality. It has claimed over 1.8 million lives in 2018 alone. The disease can happen at any age and the developing countries are worst hit by the disease due lack of effective treatment measures.

Currently available therapies are costly and often associated with systemic toxicity. Our project offers an off-beat treatment method against colon cancer and we envision that it can be developed as a targeted therapy for any type of cancer in future.

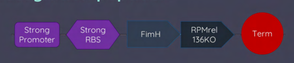
Our project aims to engineer a bacterium "Coca coli" which will be able to -
1. Synthesize and secrete IL12, which suppresses tumour growth.
2. Specifically target cancer cells to reduce off-target release of IL12

OUR *E. coli* :



TUMOUR RECOGNITION MODULE

In order to limit the interaction of our bacteria with colon cancer cells, . we tried modifying the type I fimbriae by addition of RPMrel, a tumor recognition peptide.

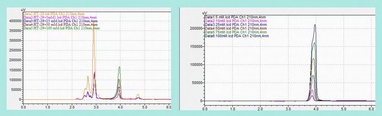


The bacteria expressing the modified fimbriae was not able to be tested due to technical difficulties. Thus we analyzed the efficiency of our recognition module using mathematical modelling.

WETLAB

LACTATE LEVELS IN CANCER

Tumor cells reprogram their metabolism making their microenvironment rich in lactic acid by a phenomenon called Warburg effect. Here we have quantified the amount of lactate produced by colon cancer cells (HT-29) using HPLC.

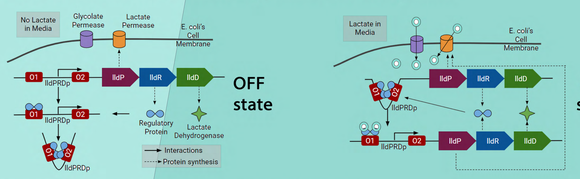


The amount of lactate produced by HT29 cells after 24hrs was found to be :

16±2 mM

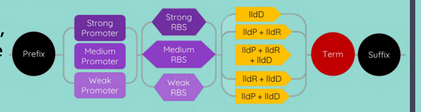
TUMOUR RESPONSE AND CLEARANCE MODULE

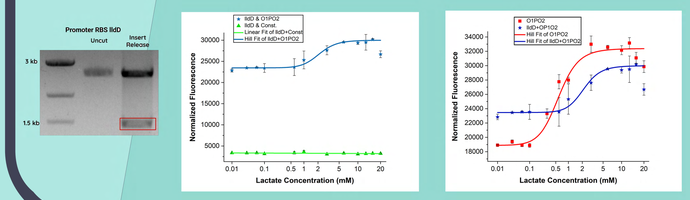
Coca-coli is engineered in such a way that it is able to sense lactate levels in the microenvironment and will be triggered only when lactate level crosses the threshold characteristic of colon cancer microenvironment. This is achieved using the lactate inducible regulatory sequence, O1-J23117-O2 developed by ETH-Zurich and the structural genes of lldPRD operon.

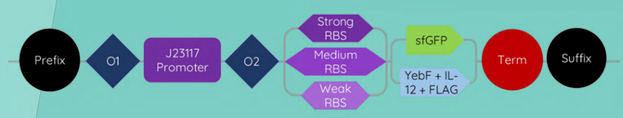


MODULATION OF LACTATE :

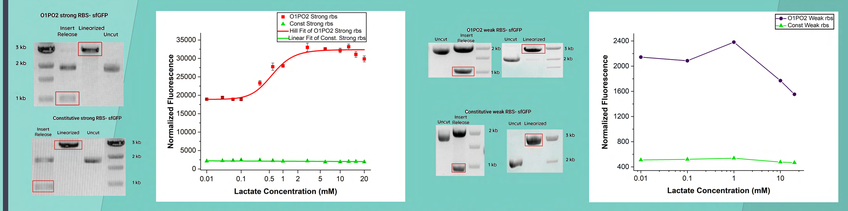
We see an increase of threshold, suggesting that using lldD as the modulator will have an intended effect on the system.







RESPONSE TO LACTATE BY sfGFP REPORTER :



The construct with strong RBS gives a clear sigmoidal pattern for detection of lactate with a threshold of around **0.45 mM**.

TUMOUR CLEARANCE BY RELEASE OF IL12 :

To check for protein production, we cultured BL21 (DE3) having a 1 mM lactate induction.

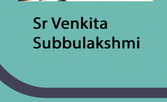
The gel images below show no visible band difference between pellet and supernatant fractions. This indicates that the protein is not produced.

OUTREACH & INTEGRATED HUMAN PRACTICES

For outreach, we focused on spreading awareness about synthetic biology and its potential prospects in our country amongst students.

Prof G Ambika

Dr Ambrish Saxena



Sr Venkita Subbulakshmi

MODELLING

Module 1: THE LACTATE OPERON

Through this module we hoped to understand the IL-12 expression profile by each of our constructs. But, no previous model existed for this - so we began from scratch, with a background of the well-studied lactate operon.

Step 1: Equations for change in concentrations of RBS responsible for the translation of lldP, lldR and lldD mRNAs.

$$\frac{dM_x}{dt} = Dk_M P_{\tau_x o}(O_x) - (\gamma_m + \mu)M_x$$

Step 2: Equations for the translation of mRNA

$$\frac{dx}{dt} = k_x e^{-\mu\tau_x^o} M_{P\tau_x^o}(t) - (\gamma_x + \mu)E_x$$

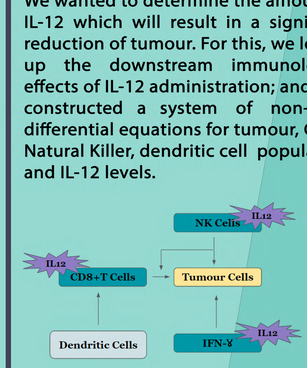
Step 3: Accounting for the intracellular lactate concentration

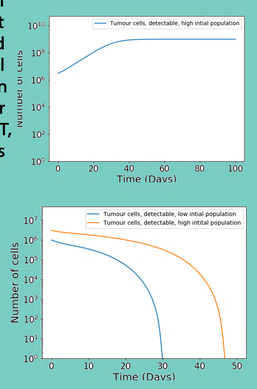
$$\frac{dL}{dt} = k_L B_L(L_c)Q - k_l B_l(L)Q - \phi_M M(L)D - (\gamma_L + \mu)L - \kappa LB$$

Our ultimate aim: to vary the extracellular lactate levels (L_c) and predict the changes observed in IL-12/sfGFP production.

Module 2: COCA-COLI vs. CANCER

We wanted to determine the amount of IL-12 which will result in a significant reduction of tumour. For this, we looked up the downstream immunological effects of IL-12 administration; and then constructed a system of non-linear differential equations for tumour, CD8+T, Natural Killer, dendritic cell populations and IL-12 levels.

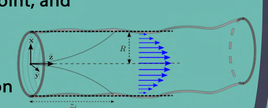




Module 3: COCA-COLI in the COLON

Aim: To study the flow of bacteria from its point of release in the cecum until the tumour-site by:
1. Understanding the flow and distribution profile of bacteria until this point, and
2. Its attachment kinetics

Our Results and Propositions:
1. A capsule, that releases the bacteria in the cecum, right before the colon



2. Diffusion-Advection model to ensure the transit of this bacteria to the walls of the colon, as described by the equation -

$$\frac{\partial c}{\partial t} = D_t \nabla^2 c - v_z \frac{\partial c}{\partial z}$$

3. A minimal length (Z_l) from the opening of the colon before which the tumour cannot be targeted, which was about 10cm.

4. An expression for the number of bacteria that will be present at a particular point on the colon :

$$c(R, t_m) = \frac{e^{-1} c_0}{\pi R^2}$$

5. Attachment-Detachment kinetics of the bacteria: We calculated the time constant of detachment (τ_R) by taking into account the shear forces. We found a favourable situation arises when we administer CoCa coli when peristalsis is not occuring.

ACHIEVEMENTS

- Integrated inputs from the industry experts in the development of our project
- Assembled *E. coli* which recognize specific lactate concentrations
- Formulated a model for movement of our bacteria through the colon and for determining the amount of IL12 required to reduce tumours

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