Project Log

Team Name: oncoCURES
Class: Intro to Biological Engineering Design (20.020)
Project: Dynamic Metastasis Imaging

Anonymous students MK and NM, with mentor anonymous student RA

-Start of Entries-

Wednesday, February 25

- discussed the prompts for the team contract
- put together a contacts list
- came up with 5 ideas for our presentation
    - signaling, environment, genetic, chemotherapy, and virus

Wednesday, March 4

- figured out 3 ideas to go forward with
- eliminated chemotherapy and redefined environment
- chose signaling and genetics, virus and bacteria, and competition and decoys
- divided up the ideas amongst the team members
    - MK: Competition and Decoys
    - NM: Signaling and Genetics
    - Anonymous student AJ: Virus and Bacteria
    - planned meeting for the weekend

Sunday, March 8

- worked on presentation slides
- brainstormed the pros and cons of each
- discussion on safety and security of the proposed technology
- could not decide which one we are leaning towards

Tuesday, March 10

- modified draft of the PowerPoint presentation
- decided to redo third idea
    - problem: decoys have been done better, competition is difficult
    - problem: could trigger more evolution of cancer, make it worse
    - solution: markers and targeting, focus on metastasis

Wednesday, March 11

- gave 3 Ideas Presentation
- got reviews and questions
- student feedback, voting by category

Category:	Signaling and Genetics	Viruses and Suppression	Markers and Targeting
Most Important	10	2	7
Greatest Impact	7	7	6
Most Competitive	7	4	5
Greatest Certainty	5	0	11

- decided to go with Markers and Targeting

- thought there was the greatest potential for research, could take in parts

Thursday, March 12

- reviewed the ideas and the mentor feedback
- signaling and genetics - Glivec, important example to study in great depth, mimic it, small molecule inhibitors not proteins need to pick specific pathway to target what would it do
- viruses and bacteria - tried for a long time, genetic therapy, no viral therapy approved by FDA study, existing possibilities some dangers
- markers and targeting - interesting findings may not be able to achieve ambition
    - reassured decision to go with the markers/targeting idea

Tuesday, March 17

- passed on our project idea selection to Natalie
- tried to plan on system schematic
- decided to start with detectiont through marker first
    - would consider targetting/killing later if time
- drew a diagram of marker cells and killing cells

Wednesday, March 18

- AJ has left the class, sadly
- will be working closely with our advisor, RA
- learned about system levels, mapped to our own project
    - system - marker on metastatic cells
    - device - light producer, signal receptors
    - parts - lots of regulatory genes from registry of parts, promoters, terminators, we'd need eukaryotic ones
    - DNA - from registry, don't need exact for all of devices
- drew a timing diagram on the board for our project

Thursday, March 19

- problem: working with false triggering, specificity
    - solution: need two enzymes that on most basic level function as signals
    - solution: use "AND" gate for receptors only present in breast cancer
- problem: is this safe/secure?
    - solution: build so that it is harmless
    - solution: most likely will die on its own, due to human response
    - solution: don't let others know how to program these, science behind it

Tuesday, March 31

- debate on cost/buildable and development
    - problem: is this possible?
    - solution: use current research ideas, experimentation
- read up on luciferase
- different types, from underwater animals to fireflies
- reaction involves oxygen and luciferin
    - problem: hypoxia in tumor cells?
    - solution: metastasis means angiogenesis, blood vessels bring oxygen
- researching enzymes involved in breast cancer metastasis
- debating pros and cons of each one
- some occur naturally and need to be judged cancerous with increased activity

Wednesday, April 1

- have decided on COX-2 and MMP-1 enzymes
- used the products of each reaction

        - COX-2 makes prostanoids
        - MMP-1 makes collagen debris
    - need to evolve receptors
    - pull out DNA from experiment, place in chassis
    - picked out some general regulatory parts
        - decided that we should use viral promoters
            - strongest and necessary for marker to appear bright
        - used normal yeast terminators
            - will need to be humanized, account for evolutionary differences
    - redrew diagram with our selected enzymes, products, and proposed sensors

Thursday, April 2

- found exact sequence for MMP degradation
    - polypeptide strand
- strand can bind to antibodies
- developed through clonal explansion after introduced in animal
- in T-cells binding can lead to MAC or attack complex
    - problem: how to prevent full-attack on cancer cell, without detection
    - solution: disable some fo the C1+ components through inhibitors, chemicals
    - solution: use the components for our own reaction, competition
- prostanoids trigger receptor involved in G-protein cascade
- binds and phosphorylates
- black box in middle
- regulates production of light
    - could make luciferin
    - could trigger luciferase through ATP

Sunday, April 5

- wrote up Tech Spec Review
- debated the benefits/impact of our current project
    - used mainly for research
    - understanding of metastasis and how it works
    - current tests are very vague, not accurate
    - no real knowledge on how and why it spreads

Tuesday, April 7

- ran through slides and made some corrections
- finalized testing/debugging methods
    - problem: nothing happens
    - solution: in vitro, make sure receptors localize with freeze-fracture
    - solution: add collagen debris and prostanoids to see if reacting
    - solution: use GFP for products, receptors, luciferase

Wednesday, April 8

- gave Tech Spec Review presentation
- got feedback from guest panelists
    - Kuldell gave written feedback
        - advice: how will the black box work and integrate two signals
        - advice: better research on low-light imaging, CCD cameras
        - advice: consider other types of chassis, how will these move in vivo
    - Barry/Austin also spoke, asked questions, criticism
        - advice: look at magnetic sensing or skip straight to toxin release
        - advice: consider the value of understanding metastasis

        - advice: how will the sensors work in combination, look for better combo
- overall decision to reconsider enzyme activation
- look at luciferase alternatives
- decision: still going forward with project

Thursday, April 9
- considered what chassis to use
- researched cells of the immune system
    - hard to understand differences in all T-cell types, B-cells
- reading through papers on immune system

Tuesday, April 14
- talked about thresholds, how to add them to our system
    - problem: how to biologically replicate thresholds for our system
    - solution: decided on light gradients to detect, most concentrated light
    - solution: also decided that we could have molecular competition
        - needs a lot of collagen/prostanoids to activate it
- discussion of how to deal with false positives

Wednesday, April 15
- looked at the registry of parts
- talked about which promoters/terminators we should use
- do we want to keep our current ones?
- which part will we contribute
    - picking between sensors, light producer

Thursday, April 16
- discussion on what will tax the cell, too much production
    - problem: will we have luciferin in the cell?
    - solution: need to store some in the cell
    - solution: make up for not eating it, acquiring from environment
    - solution: only need limited store

Wednesday, April 22
- talked to Drew Endy about project design
- suggested changing from cascading enzyme reactions to one simple reaction
- ribozyme that needs two signals
- avoid phosphorylation, amplification or branching reactions
- can rely on prostanoids to diffuse since they are hydrophobic
    - problem: need to get collagen debris into the cell
    - solution: build transporter enzyme of sorts

Thursday, April 23
- discussed the idea of a collagen transporter
- tried to find literature on channel in nature, not much success
- looked into how this would regulate luciferase activity
    - could cut gene to activate it
    - could stop inhibiting transcription of DNA

Tuesday, April 28
- wrote an email to Roger Kamm who knows collagen structure models
    - was referred to other papers on subject
    - some useful, others a bit confusing
- wrote email to Drew Endy and Christina Smolke on ribozymes

Wednesday, April 29

- called Chris Anderson who made the tumor killing bacteria
- explained how TKB worked, what issues it had
- realized some of the problems/research is unrelated
- heard our project summary
- emailed Agi Stachowiak, chemistry expert and knowledgeable with collagen
    - arranged meeting for next week
    - received several research papers to read on collagen/MMP

Thursday, April 30

- heard back from Christina Smolke
    - need to read her paper
    - most of this is still at work in lab, not proven yet
- heard a presentation on how to give a good talk
- took some notes for future reference
    - kinds of audience, how to design slides, etc.
- for collagen transporter: read up on TRAPP enzyme and integrins from papers
    - decided that both enzymes were not what we wanted, involved mostly collagen production
    - no known channel, procollagen spat out using exocytosis from ER/Golgi apparatus
        - try to reverse this procedure?
- debated a chimeric receptor
- fuse together antibody specificity with endocystosis receptors
- decided that ribozyme would inhibit luciferase mRNA from translation
    - use our signals to in turn inhibit it

Monday, May 4

- met with Agi Stachowiak, talked about collagen structure
    - got link to website that explains collagen degradation
    - advice: look for upregulation of collagenases
    - advice: piggyback on endocytosis of collagen for breakdown
- decided that endocytosis was most likely to work
- works better than channel

Tuesday, May 5

- found possible collagen receptor
- paper mentions that it leads to endocytosis
- problem: generally also fuses with lysosome and leads to degradation
    - solution: can be delayed by chemicals
    - solution: could possibly modify liposomes to break after endocytosis
- decided to go with uPARAP/Endo 180 enzyme

Wednesday, May 6

- first round of Final Presentations
- took notes on various groups ideas
- worked on rough Powerpoint presentation template

Thursday, May 7

- looked at ribozyme paper again, tried to decipher figures
- read up on various aptamers
    - most of the research is a work in progress
    - some peptide, hormone, lipid sensors in consideration

Sunday, May 10

- compiled project log entries, made a google doc for final tweaking
- made rough draft of technical documents
- started to put together Powerpoint presentation for Wednesday

Tuesday, May 11
- finished up slides, practiced presentation
- entered Endo180 part into the registry
    - collected DNA sequence from paper

Wednesday, May 12
- polished and completed technical documents
- finalized project log

-End of Entries-

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