Modeling the Effect of Melanoma Tumor Cells When in the Presence of Natural Killer Cells

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Introduction

Cancer is the 2nd leading cause of death in the United States. Current treatments primarily consist of chemotherapy and radiation, which have significant side effects, such as tissue damage, bleeding, and secondary carcinogenesis. There is an increasing need to provide treatments, which are more specific and less traumatic. The aim of this project was to model a relatively new adoptive immunotherapy to investigate the immune response against cancer. To achieve this goal, experimental and mathematical modeling were used to predict how natural killer (NK) cells interact with and kill melanoma cells.

Research objectives

Experimentation using NK cells and melanoma cells has been outlined to create the following objectives:

- Predict interaction between NK cells and tumor cell populations in combined in vitro cultures
- Create a mathematical model that can be used to develop and improve immunotherapies for cancer patients
  - Use MATLAB, to develop continuous partial differential equations based on data from published literature
  - Experiments are expected to validate the model and allow for further modifications
- Perform experiments to validate model
  - Experiments performed at the University of Tennessee involved a migration assay that allows the cells to be injected individually and observed over time
  - Parameters will be validated through examining cell directional movement and velocity.

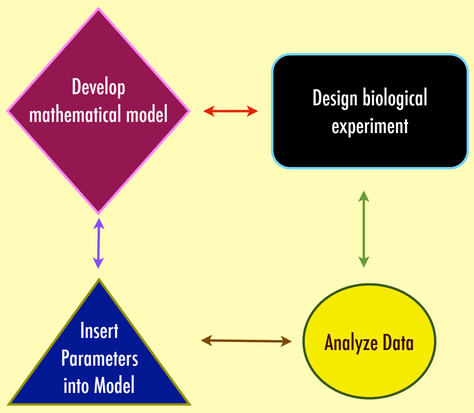

Figure 1: Diagram showing steps to integrate biological and mathematical modeling

Variables

State Variables	Definitions
NK	natural killer cells
NKR	natural killer cells with NKG2D receptors
TMICA	Tumor bound with MICA
sMICA	soluble MICA
MMP	matrix metalloproteinases
Tumor	tumor NOT bound to MICA
NKR-sMICA	natural killer cells with NKG2D receptors bound to soluble
NKR-TMICA	natural killer cells with NKG2D receptors bound to TMICA
TumorD	dead tumor
MMP-TMICA	matrix metalloproteinases bound to TMICA
CT	cytokines secreted by tumor
ECM	extracellular matrix

Parameters	Definitions
k	binding/dissociation rate
D	diffusion rate
λ	chemotaxis rate
p	production rate
G	growth rate
δ	rate at which ECM is broken down by MMP

Mathematical Model

Partial differential equations

$$\frac{\partial[MMP]}{\partial t} = -k_3[MMP][TMICA] + k_{-3}[MMP - TMICA] + p_R NKR + p_B NK + D_{mmp}\nabla^2 MMP$$

$$\frac{\partial[Tumor]}{\partial t} = k_6[MMP - TMICA] + D_T(ECM)\nabla^2 Tumor + G_T Tumor$$

$$\frac{\partial[NKR]}{\partial t} = -k_1[NKR][SMICA] + k_{-1}[NKR - SMICA] - k_2[NKR][TMICA] + k_{-2}[NKR - TMICA] + k_5[NKR - TMICA] + D_{NKR}(ECM)\nabla^2 NKR + \lambda_{NKR}(ECM)\nabla\cdot(NKR\nabla C_T)$$

$$\frac{\partial[SMICA]}{\partial t} = -k_1[NKR][SMICA] + k_{-1}[NKR - SMICA] + k_6[MMP - TMICA] + D_{sMICA}\nabla^2 sMICA$$

$$\frac{\partial[NK]}{\partial t} = k_4[NKR - SMICA] + D_{NK}(ECM)\nabla^2 NK + \lambda_{NK}(ECM)\nabla\cdot(NK\nabla C_T)$$

$$\frac{\partial[TMICA]}{\partial t} = -k_2[NKR][TMICA] + k_{-2}[NKR - TMICA] - k_3[MMP][TMICA] + k_{-3}[MMP - TMICA] + D_{TMICA}(ECM)\nabla^2 TMICA + G_{TMICA} TMICA$$

$$\frac{\partial[Tumor^D]}{\partial t} = k_5[NKR - TMICA]$$

$$\frac{\partial[C_T]}{\partial t} = p_{TB} Tumor + p_{TMICA} TMICA + D_{C_T}\nabla^2 C_T$$

$$\frac{\partial[ECM]}{\partial t} = -\delta * MMP * ECM$$

Simulation Results

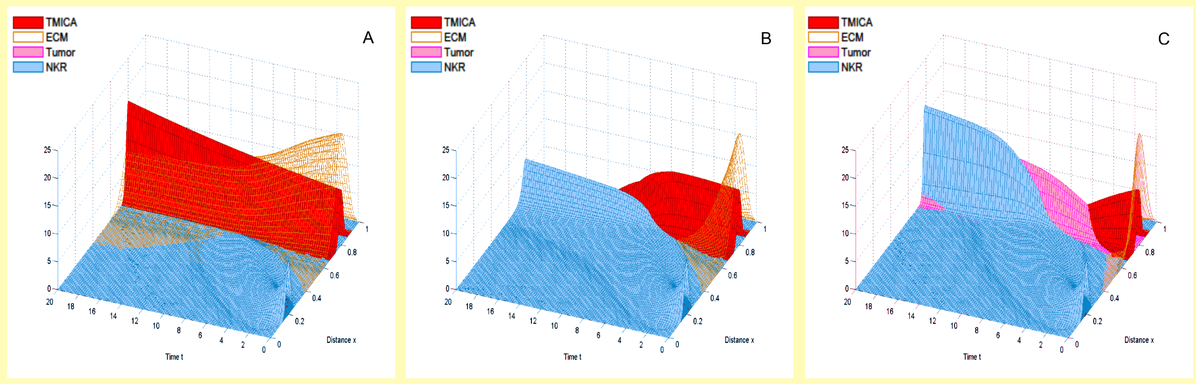

Figure 2. Graphs depicting the effects of MMPs released by NK cells on Tumor growth when in the presence of an extracellular matrix A.) Low MMP levels. B.) Normal MMP levels. C.) High MMP levels.

Biological model

- Blood separation performed to remove mononuclear cell layer
- Mononuclear cell layer separated into specific immune cells through a Percoll™ gradient
- Immune cell fractions tested using flow cytometry to analyze for purity of NK cells
- NK and A375 cells were placed in two separate wells in Delta T dish
- Images of Delta T dish were taken using an automated stage microscope
- Quantitative analysis performed using image software
- Information received from quantitative analysis utilized to validate mathematical model

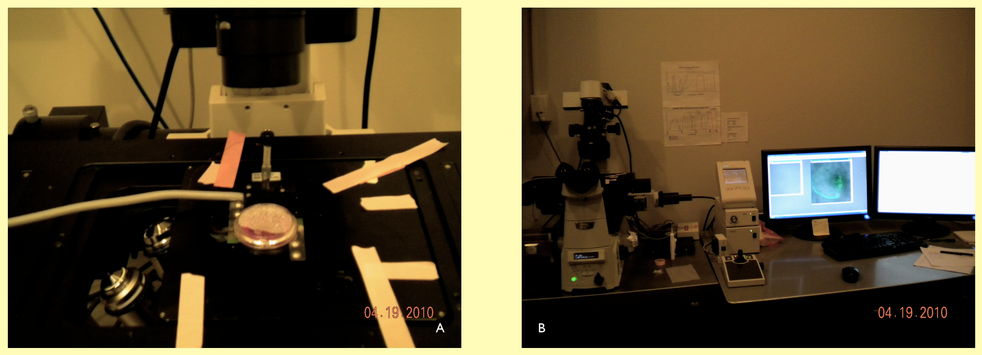

Figure 3: A.) Image of delta T dish on heated stage B.) Image of system set-up

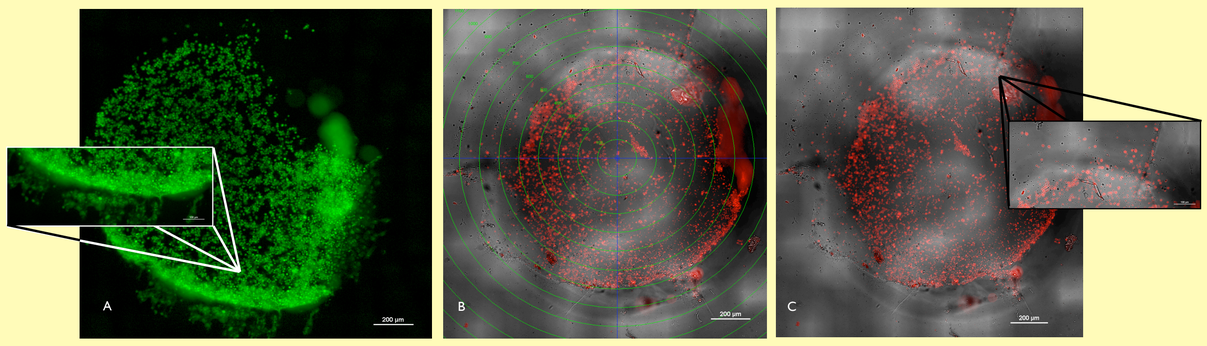

Figure 4: A.) Magnification of A375 cells migrating out of well -FITC B.) Large scan image of Mononuclear cells with concentric circle overlay-bright field and TRITC C.) Magnification of Mononuclear cells migrating out of well-bright field and TRITC

Conclusions

- Developed assay to provide data for mathematical model
- Biological models can be developed to provide quantitative data for a mathematical model
- Multiple biological experiments are needed to provide data for all parameters in biological model

Future work

- Simulate mathematical model that takes receptor density into account
- Perform time-lapse imaging with biological experiments
- Begin validating mathematical model with experimental data

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