Mapping tumor cell sensitivity and resilience to targeted therapy from 2D to 3D

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We explore how the external environment impacts the growth of a non-small cell lung cancer line PC9 in the presence and absence of targeted therapy. Prior studies in 2D cell culture conditions determined that the PC9 cell line contains a heterogeneous distribution of cell response under targeted therapy. In this work, we determine a mapping from 2D experiments measuring proliferation, quiescence, and apoptosis to the growth of cell clusters suspended in an extracellular matrix (ECM). We find that cell suspension in an ECM shifts the balance of cell fates towards apoptosis, increasing the sensitivity of cells to targeted therapy.

I. Purpose

Inhibitors of the epidermal growth factor receptor such as erlotinib, are effective in the initial treatment of non-small cell lung cancer. With time, tumors that initially shrink under therapy frequently reemerge and grow. Of general interest is the impact of *de novo* resistance, due to tumor heterogeneity present prior to drug therapy, on the rebound time of the tumor. Previously, we isolated 89 discrete subline clones from the commonly studied PC9 cell line by monitoring the asymptotic drug induced proliferation (DIP) rate under erlotinib therapy. We model the DIP rate as being composed of three cell fates: proliferation, quiescence, and apoptosis. The rates associated with these three cell fates can be constrained *via* live cell imaging [1].

In this work we examine the role of 3D suspension in extracellular matrix in determining proliferation and the distribution of cell fates. Mapping experimental results from 2D *in vitro*, 3D *in vitro*, to 3D *in vivo* has often proved difficult, as the cell environment leads to changes in protein expression altering the balance in signaling pathways such that different final cell states are favored. Specifically, we ask: is there a simple mathematical transformation from 2D cell culture conditions to 3D cell culture conditions and how does suspension in an ECM alter the distribution of possible cell fates?

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2D Experiments: We examine four PC9 sublines having DIP rates under erlotinib therapy from the most negative (i.e., drug sensitive) to the most positive (i.e., drug resilient) in 2D cell culture conditions. Cells containing nuclei labeled with histone-RFP and expressing the cell cycle marker FUCCI are seeded at low density in 96 well plates. Each well is scanned every 1 hour for 5 days, with drug treatment beginning on the 2nd day. The subsequent time series are tracked and the following quantities extracted: the number of cells as a function of time, the distribution of intermitotic times, and the number of cell death events over the total time course. This data is used to constrain the fractional proliferation model yielding rates for 2D proliferation, entry into quiescence, and apoptosis [1].

3D Experiments: Multiple samples of each of the four PC9 sublines are seeded at low densities in 3D suspended cell culture in each of three extra cellular matricies: collagen 1, Matrigel, and Hydronic Acid hydrogels. The colonies grow for three days before drug treatment and for 14 days under therapy. Each day, a sample from each of the experimental conditions is stained with sytox blue, an apoptosis marker, the matrix digested, and the cells extracted. The total cell number is determined as well as the number of cells currently proliferating and the apoptotic cell fraction. The 3D DIP rates are extracted from asymptotic cell counts and compared to the 2D cell data. To better constrain the mapping from 2D to 3D, we acquire confocal stacks of cell clusters each day. We determine the cluster size, morphology, and spatial distribution of proliferating and dying cells in the presence and absence of targeted therapy.

II. Results:

We find that cells suspended in 3D matrices show increased sensitivity to erlotinb with the rate of cell death depending on the extracellular matrix composition. For example, PC9 subclones that experienced less than 10% of cell death under treatment in 2D culture, experience up to 40% cell death in Matrigel after ten days of treatment with 1 μM erlotinb. After 14 days of therapy, the resilient cell clones continue to show cell proliferation. We interpret the results in the context of EGFR signaling pathways and a shift in balance from cell proliferation to apoptosis.

References

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