Physical Models of Malignant Invasion

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Statement of Purpose: Much effort is currently being directed at elucidating the genetic, molecular, and cellular changes accompanying tumor progression through invasion and metastasis. Here, we consider the principles underlying the net effect of such changes on the tumor as a whole and connect this to tumor behavior using two tumor models, namely brain and prostate cancer. We ask whether there might be simplifying principles by which some aspects of tumor behavior can be predicted from quantifiable physical properties of the tumor as it becomes malignant. Two properties that are well-established as markers of malignancy are decreased tumor cohesion and increased affinity between tumor cells and components of the stroma into which they invade. We have developed methods aimed at quantifying these properties, and by identifying changes in their key molecular regulators and modulating them, are able to functionally revert aggressive tumors to less invasive phenotypes.

Methods: To measure tumor cohesion, we employ tissue surface tensiometry, a method predicated on the principle that tumor (and other) tissue can be modeled as resembling simple fluids. We measure aggregate cohesion (expressible as surface tension) and connect this to the spreading velocity (substrate wetting) of brain tumor cell lines. *Glioblastoma multiforme* (GBM) represents approximately 60% of all gliomas and 30% of all intracranial tumors. The hallmark of these aggressive tumors is their ability to invade the brain rapidly, with a mean survival of 12-15 months. This invasive capacity renders tumors resistant to complete surgical resection chemotherapy, and radiation therapy. Despite aggressive treatment, tumors tend to recur within 3-6 months. In order to contain these recurrent tumors it is crucial that the tumor be a defined structure that is capable of being distinguished from normal tissue. One possible strategy is to increase tumor cohesion to a point that discourages the escape of tumor cells from the mass. We explore the role of $\alpha5\beta1$ integrin-fibronectin interaction *vis a vis* fibronectin matrix assembly (FNMA) in mediating tumor cohesion and dispersal velocity. To explore the physical basis of prostate cancer invasion and metastasis, we present a model in which the interaction between tumor and stromal cells can be viewed as a liquid miscibility phenomenon, rooted in the same thermodynamic principles that govern the interactions between simple fluids. We apply an analogy of the high energy interaction between ethanol and water, or the lower energy of interaction between oil and water, to test the hypothesis that the model can differentiate between such interactions in mixtures of prostate cancer (CaP) and stromal cells. Preventing the spread of prostate cancer would significantly reduce the morbidity and mortality associated with this disease. Impeding the interaction between tumor and stromal cells is a possible strategy for therapeutic intervention. We also explore the role of FNMA in mediating miscibility between tumor and stromal cells.

Results: For brain tumors we show that three cell lines derived from pathologically-identical GBM tumors have different tissue surface tensions which correlated inversely with their dispersal velocities. Moreover, treatment with Dexamethasone (Dex), MEK inhibitor (MEKi), or Geldanamycin (GA) restores FNMA and that this results in increased surface tension and decreased dispersal velocity. The different agents regulated FNMA distinctively in different cell lines; Dex optimally up-regulating FNMA in U-87MG cells, GA optimally restoring FNMA in cells of U-118MG. For tumor-stromal interaction in prostate cancer we show that the high affinity interaction between invasive prostate cancer cells and stromal cells contrasts with the sorting of non-invasive tumor cells and stroma. We also show that the miscibility model is able to detect a shift in the affinity of interaction between tumor cells and the predominant stromal cell type as cancer progresses to a more invasive phenotype. In addition, we found that transfection of invasive cells with $\alpha5\beta1$ integrin reactivates FNMA whereas transfection with a receptor with a mismatched cytoplasmic domain of $\alpha5$ integrin fails to do so. Only cells expressing wild-type $\alpha5$ receptor demonstrated differential ability to sort from stromal cells and were effectively rendered less miscible. We show that FN matrix assembly (FNMA) can also be pharmacologically induced via treatment with the MEK inhibitor PD98059. Treated cells exhibit a greater capacity to compact in 3D culture and that this is due to increased compaction and cohesion. This leads to decreased miscibility between tumor and stromal cells.

Conclusions: The paradigms of tumor liquidity and tumor-stromal cell miscibility are novel in that they utilize simple physical principles to explain complex genetic, biochemical and cellular changes associated with acquisition of invasiveness. TST effectively reduces these complex chemical and cellular changes into quantifiable physical properties. Merging information obtained from TST with an analysis of the molecular and cellular components underlying changes in tumor physical properties, and the effects on tumor-stromal miscibility will bridge our understanding of the functional consequences of gene expression and its subsequent influence on tumor behavior. Devising strategies aimed at altering tumor cohesion, maintaining or even restoring compartmentalization between tumor and stromal cells, and reducing invasion and metastasis, are critical to successful treatment of invasive and metastatic cancers.