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Integrating Ecology and Cancer: Investigations in a Prostate Cancer Cell Model

Samantha Swartz

Comments

Also under the mentorship of Dr. Christopher Stipp

INTEGRATING ECOLOGY AND CANCER: INVESTIGATIONS IN A PROSTATE CANCER CELL MODEL

by

Samantha Swartz

A thesis submitted in partial fulfillment of the requirements
for graduation with Honors in the Biology

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Maurine Neiman
Thesis Mentor

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All requirements for graduation with Honors in the
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Lori Adams
Biology Honors Advisor

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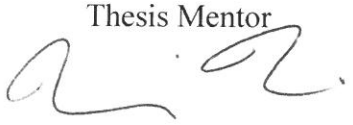

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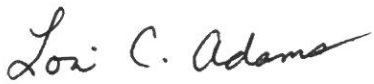

Lori Adams, PhD.

Biology Honors Advisor

Abstract

Evolution and ecology can be used to gain a better understanding of cancer. Here, my focus is on applying concepts and tools from evolution and ecology to generate new insights into the importance of the tumor microenvironment and tumor heterogeneity for cancer progression. I used a model prostate cancer cell line called PC-3, which contains both an epithelial cell type (PC-3E) and a mesenchymal cell type (TEM4-18) that are maintained at about a 75:25 PC-3E:TEM4-18 ratio in the parental population. Mesenchymal cells are particularly interesting because they have the ability to metastasize. Data of the growth rates of each cell type in isolation suggested that PC-3E cells have a higher growth rate than the TEM4-18 cells. Because of this, it unclear what mechanisms maintain the TEM4-18 cells in the parental population. To begin to understand how the dangerous mesenchymal cells are maintained, I combined the PC-3E and TEM4-18 cells at equal proportions in one flask and monitored the population for 6 weeks, noting how the proportions of each cell type changed as the experiment progressed. The experiment resulted in the mesenchymal cells depleting rapidly from the population. We next combined the mesenchymal population with the parental population in equal proportions and performed the experiment again. We found that, rather than depleting rapidly as they did with the epithelial cells, the mesenchymal cells were maintained in the population for the duration of the experiment. This result was striking because the composition of the parental cell line should be very similar to the composition of a combined population of PC-3 and TEM4-18 cells. Finally, we cultured each cell type in isolation and compared the growth rates obtained from these experiments to the growth rates found in the experiments with two combined cell types. While the mesenchymal and epithelial cell types had similar growth rates in all conditions, the parental cells had different growth rates when cultured alone vs. when cultured with another cell line. We concluded that the different proliferative success of the TEM4-18 cells in the different environments was likely due to microenvironmental differences between the parental cell line and the PC-3E + TEM4-18 combination. This research integrates the ideas of evolution and cancer to gain an understanding of metastatic cells. An evolutionary approach is especially powerful in the wake of growing knowledge of tumor heterogeneity and the tumor microenvironment, and may lead to novel methods of treating cancer.

Acknowledgements

A special thank you to Dr. Maurine Neiman and Dr. Chris Stipp, who mentored me with patience and compassion and helped me increase my scientific knowledge exponentially.

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Introduction

Cancer Microenvironments

There is a growing appreciation for the important role of the microenvironments of cancer cells in research aimed at combating cancer (Korolev, Xavier, & Gore, 2014). These microenvironments include surrounding somatic and cancer cells, accessibility of growth factors and oxygen, and predation by immune cells. (Atkispis & Nesse, 2013). In a tumor microenvironment, each cell undergoes a unique interaction with its surrounding cells and has access to distinct nutrients and resources (Marusyk & Polyak, 2010). Microenvironments can also impose natural selection on cancer cells in a manner analogous to the way the external environment imposes natural selection on an organism (Atkipis & Nesse, 2013). Although researchers are aware that the tumor microenvironment affects cancer cell proliferation, how this knowledge can be used to treat and predict tumor development remains unclear.

Tumor Heterogeneity

Cells within a single tumor have a variety of multiple genotypes, referred to as "tumor heterogeneity". A single tumor may also house hundreds of genotypes (Gillies *et al.,* 2012), and subpopulations of tumor cells with distinct genotypes or phenotypes can be isolated from a single tumor. In fact, genotypic subpopulations have been isolated from every major histological cancer type (Heppner, 1984). These subpopulations can differ in morphology, karyotype, growth rate, enzymes, metastatic ability, and sensitivity to treatment agents (Heppner, 1984). This phenotypic and genetic variation means that a tumor is likely to have at least some heritable variants with notable survival advantages. This scenario can lead to evolution by natural selection as cancer cells compete with other cell types in a cancer population, and competition with surrounding cells selects for those cells that propagate the most rapidly (Ducasse *et al.,* 2014).

Mesenchymal and Epithelial Cells

Heterogenous tumor populations can contain many cell types that are classified into the broad categories of epithelial and mesenchymal cells. Epithelial cells are characterized by tight cell-to-cell junctions and limited mobility (Larue & Bellacosa, 2005). Mesenchymal cells are motile and do not form the cell adhesions common to epithelial cells. This motility allows the cells to metastasize, invading a new tissue by crossing the tissue's endothelial barrier (Drake et al., 2009). This invasive process allows cancer to spread throughout the body.

Current cancer treatments like chemotherapy and radiation often kill subsets of cancerous cells, but these treatments also select for cells that are resistant to the treatment. These cells tend to spread throughout the body and colonize new regions, making cancer especially difficult to treat. Because only mesenchymal cells can metastasize, these cells are particularly dangerous. For this reason, it is important to understand the mechanisms underlying their maintenance of mesenchymal cells within a diverse tumor population. Finding a way to combat cells that can metastasize is essential to improving cancer treatment and survivability.

Cancer cells compete with one another within a tumor, but a tumor can still maintain several cell types. The microenvironments in which epithelial and mesenchymal cells live could affect the proliferation and, therefore, the maintenance of each cell line in a tumor. My project integrated the ideas of the tumor microenvironment, tumor heterogeneity, and metastasis by looking at the way the surrounding cells in a cancer population affect the growth rate of mesenchymal cells. I used a prostate cancer cell line called PC-3, which contains both epithelial and mesenchymal cell types.

Model Cell Line

PC-3, a prostate adenocarcinoma, provides a powerful setting in which to test how the tumor microenvironment can affect the growth of a specified cell type within a tumor. The PC-3 cell line is heterogeneous, containing at least two cell types: **1)** PC-3E cells are an epithelial cell type that can be isolated from the PC-3 parental line via detection of expression of E-cadherin, a transmembrane protein that plays an important role in cell adhesion (Drake et al., 2009) and **2)** TEM4-18 cells are mesenchymal cells that are isolated from PC-3 by their ability to cross a transendothelial barrier. These two PC-3-derived cell types can also be labeled with different fluorescent markers, allowing each cell type to be easily distinguished from one another using fluorescent microscopy.

In the parental PC-3 cell line, PC-3E cells make up about 75% of the total population. E-cadherin negative cells, including TEM4-18, make up the other 25% (Drake et al., 2009). The PC-3 cell line has been stable at this 75:25 PC-3E:TEM4-18 ratio for decades (Kaighn, M.E., 1979). This stability is especially surprising in light of unpublished data from Marion Vanneste in the lab of Prof. Michael Henry at the University of Iowa, which shows that the TEM4-18 and PC-3E cell lines have different growth rates when each cell type is grown in isolation. The TEM4-18 cells grow at 0.79 doublings per day, and the PC-3E cells grow at 1.03 doublings per day. These growth rates underpin the prediction that the PC-3E cells should outgrow the TEM4-18 within six weeks. Instead, both cell types are maintained indefinitely in the parental population.

This contradiction suggests that there may be important differences between the microenvironments of each cell type cultured on its own and the parental population. This microenvironmental difference could have implications for the way mesenchymal cells proliferate and metastasize. Guided by this idea, I specified the objective of my project:

determine the impact of the cell microenvironment has in the maintenance of the 75:25 PC-3E:TEM4-18 ratio observed in the PC-3 parental cell line.

Experimental Approach

I examined the behavior of the mesenchymal cells in different environments to better understand how they are maintained in the parental population. I combined the PC-3E and TEM4-18 cells, and used physical counts and flow cytometry to quantify their growth. Because Henry lab data on cell line growth rates suggested that PC-3E epithelial cells would outgrow the TEM4-18 cells after six weeks, we chose to run the experiment for six weeks. The PC-3E cells that I used were already labeled with green fluorescent protein (GFP), a dye that allows the cells to be visualized under fluorescence. The PC-3 cells were virally transduced to express GFP. The TEM4-18 cells used in this experiment were labelled with mCherry, a red fluorescent protein. These fluorescent markers were essential to analyzing the numbers of each cell type at each time point in my experiments.

I combined TEM4-18 cells with an equal proportion of PC-3E epithelial cells. Once each week, I harvested, counted, and preserved the combination, for a period of six weeks. I repeated the same procedure with an equal combination of PC-3 parental cells and TEM4-18 cells for another six-week period. I also cultured each cell type individually to determine a baseline value for the population doubling time of each cell type.

Cells from each time point were analyzed using flow cytometry, which gave the relative proportions of each cell type at each time point. I then used these proportions to quantify the proportion and population doublings of each cell type over time. I was able to then infer from the data the success of each cell type in each condition.

Materials and Methods

Cell Culture

I obtained PC-3E and TEM4-18 cells from a liquid nitrogen archive. These cells had been previously isolated from a parental tumor cell population using the procedures outlined in Drake *et al*., 2009. I cultured cells in growth medium consisting of DMEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) with 10% fetal bovine serum, 1% non-essential amino acids, 1% glutamine, and puromycin and G418 selection agents. In the initial experiment, 500,000 GFP-labeled PC-3E epithelial and 500,000 mCherry-labeled TEM4-18 cells were introduced into a single 75 mm^3 T-flask (T-75) and incubated for seven days at 37 ˚C in the growth medium described above. Seven days proved to be an appropriate amount of time for this culture period because the cells had replicated enough to track changes in cell proportion, but the population had not grown so large that resource depletion was likely to be a problem.

After seven days, I removed the flask from incubation and removed the media with a vacuum. I rinsed the cells with 10 mL PBS, to remove any residual growth media from the cell. Then I exposed the cells to 1 mL of the digestive enzyme trypsin. Trypsin detaches the cells from the bottom of the flask, which is required to count the individual cells. I placed the flask back into the incubator for 3-5 minutes until all cells were detached from the bottom of the flask. I added the floating cells and trypsin to 9 mL of growth medium, creating a cell suspension. Cells (total and cells/mL) were then counted in a LUNA II cell counter. I used these cell count numbers to calculate the volume of cell suspension that would contain 1,000,000 cells. I then added this volume to a new T75 flask, along with enough growth medium to equal 12 mL total. This amount of media is suggested for a T-75, easily covering the bottom of the flask where the cells reside and providing enough nutrients for cells to thrive for at least seven days. I repeated this

incubation/counting/resuspension process every seven days, for a total of 6 weeks. I used these data to quantify the number of cells at each time point and to calculate the population doublings over the course of the experiment.

I hypothesized that when the TEM4-18 and PC-3E were combined in equal proportions, the TEM4-18 cells would deplete to even less than the 25% of the population that is normal in the parental cell line. This was based on the Henry lab growth rate data showing that the intrinsic growth rate of the PC-3E cells is higher than that of the TEM4-18 cells. If the TEM4-18 cells were to deplete as predicted by the data, it would suggest that the PC-3E and TEM4-18 cells when combined both had growth rates that were similar to their growth rates in isolation.

I repeated this initial experiment (Experiment #1, Table 1) with some modifications. The purpose of these experiments was to compare growth rates of cell types when cultured alone and when cultured in combination with other cell types. The cell types and harvesting factors used in each experiment are listed in Table 1.

	Cell types used	Harvesting Agent	Labelling	Additional Modification
1.	**PC-3E + TEM4-18**	Trypsin	PC-3E - green TEM4-18 - red	
2.	**PC-3 + TEM4-18**	EDTA	PC-3 - green TEM4-18 - red	
3.	**PC-3**	EDTA	none	Media contained no puromycin
4.	**PC-3E**	Trypsin	green	
5.	**TEM4-18**	Trypsin	red	
6.	**PC-3+TEM4-18**	Trypsin	PC-3 - green TEM4-18 - red	

Table 1: Summary of the conditions that I used for all six of the experiments that I performed. "Additional modifications" are with respect to departures from the conditions that I used for Experiment #1.

Experimental Modifications

In later experiments I used the same procedures with some modifications:

Experiment 2) To explore whether the mesenchymal cells would behave differently in different microenvironments, I combined TEM4-18 cells with PC-3 parental cells instead of PC-3E epithelial cells. I could then use the relative growth rate of the mesenchymal cells in each condition to analyze whether the different cell microenvironment caused a change in proliferation rate. I expected the TEM4-18 cells to deplete slightly and then be maintained at around 25% of the population, their normal proportion in the parental cell line. This result would suggest that the parental cell line contains some protein or secreted factor that is sufficient to maintain the mesenchymal cells.

Experiment 3) I cultured the PC-3 parental population alone to generate a comparison of its growth by itself to its growth when combined with TEM4-18 cells. This would help me determine whether a microenvironment including mesenchymal cells affected the proliferation rate of the parental cells. It was not necessary to use fluorescently labelled cells in this experiment, because culturing alone meant that I did not need to distinguish amongst different cell types. For this same reason, I left puromycin (which is associated with labelling genes) out of the growth media used for this experiment. I hypothesized that the growth rate of the parental cell line would be consistent across all experiments. A consistent growth rate would suggest that combining the parental cell line with other cell types does not affect it proliferation.

Experiment 4 & 5) PC-3E and TEM4-18 cells were each cultured alone to generate a comparison of their growth alone to their growth when combined with other cell types. As with the PC-3 parental population, this comparison would help illuminate whether the presence of other cell types in the microenvironment caused changes to proliferation rate for each cell type. I hypothesized that the growth rate of the mesenchymal cells in isolation would be the same as its

growth rate in the parental population, and that the TEM4-18 cell growth rate when combined with PC-3E cells would be lower than when cultured in isolation.

Experiment 6) PC-3 and TEM4-18 cells were cultured together using trypsin as a harvesting agent. I used this experiment to provide a more direct comparison to my initial experiment than offered by Experiment #2, because Experiment #2 differed from the initial experiment in terms of harvesting agent used. I predicted that the result would be very similar to the results from Experiment #2, as these two experiments were nearly identical. This would mean the result is reproducible and harvesting agent has no effect on the results of the experiment.

Formalin Fixation

I fixed a 2 mL sample of the cells that were harvested at each time point with 5% formalin fixative to preserve them for flow cytometry at a later time. I first rinsed cells in 10 mL PBS and then spun the cells down in a centrifuge at 900 RPM for 3 minutes. Next, I resuspended the cells in 2 mL formalin fixative and left the cells in this fixative for 10 minutes. I rinsed the cells again and spun them down in 10 mL PBS at 900 RPM for 3 minutes. Finally, I resuspended the cells in 2 mL PBS and placed the resuspended cells in a 4 ˚C refrigerator, until flow cytometry analysis.

Viral Transduction

The majority of cell lines used in my experiments were fluorescently labelled with either GFP or mCherry. The purpose of this labelling was to distinguish the cell types by color during flow cytometry analysis. The PC-3E epithelial and TEM4-18 cells that I originally obtained were already labeled with fluorescent markers. The available PC-3 parental cell line, however, was unlabeled. I labelled the PC-3 cells with GFP by transducing these cells with a GFP viral vector. First, I transfected GP2-293 packaging cells with the viral vector according to the Qiagen Quick-

Start Protocol using Effectene Transfection Reagent. After 24 hours, I filtered the infected media through a 0.45 μm filter and added this media to a culture of PC-3 cells, along with 4 μg/mL polybrene, a cationic polymer used to increase efficiency of transduction. I cultured the GP2-293 packaging cells for an additional day, then filtered the infected media and then again added this filtered media to the PC-3 cells. Adding the infected media twice increased the probability that PC-3 cells would be infected and, therefore, become labelled with GFP.

Flow Cytometry

I used flow cytometry to quantify the number of each cell type at each time point and to gauge E-cadherin expression. A Benton Dickinson LSR II flow cytometer counted the number of green (GFP labelled) and red (mCherry labelled) cells in each sample and calculated these numbers as a percent of the total.

Data Analysis

I used Graphpad Prism 7 to analyze the data from flow cytometry. I converted raw data including total cell number, number of cells plated, and proportion of each cell type from each time point into growth curves for proportion of cell type vs. time and population doublings of each cell type vs. time. I compared these growth curves to model curves generated from the grow rates of the sublines growing in isolation.

Results & Discussion

When the TEM4-18 cells were combined in equal proportion with the PC-3E epithelial cells, the TEM4-18 cells depleted to less than 1% of the population after four weeks. When the TEM4-18 cells were instead combined in equal proportion with the PC-3 parental cells, the TEM4-18 cells rose to greater than 50% of the population and were maintained at roughly this level for the duration of the experiment. The PC-3 parental population should contain many of the rapidly proliferating PC-3E cells, so it is curious that the epithelial cells within the PC-3 parental population do not outgrow the mesenchymal cells and cause them to be removed from the parental population. I further investigated the differences in proliferation success of the mesenchymal cells by culturing each of the three cell lines alone and tracking the growth rate of each line over time. The PC-3E cells grew at similar rates when cultured alone vs. when cultured with the TEM4-18 cells. The TEM4-18 cells also showed a similar growth rate when cultured alone vs. when cultured with PC-3E or PC-3 cells. The PC-3 parental cell line, however, had a lower growth rate when combined with TEM4-18 cells than when cultured alone (0.29 doublings/day vs 0.43 doublings/day).

TEM4-18 + PC-3E Epithelial Cells

I combined the TEM4-18 cells and PC-3E epithelial cells in roughly equal proportions (0.562 and 0.438, starting frequencies, respectively) into a single T-75 flask on Day 0 of Experiment #1. On Day 7, I harvested the cells with trypsin, counted the cells, and transferred a sample of 1,000,000 cells from the original flask into a new flask. In addition, I fixed about 2 mL of cell suspension in 5% formalin at each time point. This process was repeated every seven days for six weeks.

The relative frequency of each cell type was monitored over time using fluorescent microscopy, After six weeks, there were very few mCherry-labelled TEM4-18 cells, as inferred by visual observation of the absence of red cells under the fluorescent microscope. For this reason, I ended the experiment at Day 42, performing the normal harvesting, counting, and fixing procedures, but not returning any cells to the incubator.

I also used flow cytometry to quantify the proportions of each cell type at each time point. I used a sample of about 20% of my total cell population for this procedure, introducing the potential for sampling error. After flow cytometry, I applied these cell type proportion estimates to the total cell number that was recorded at each time point to evaluate how the proportions of each cell type changed over the six weeks of the experiment (Figure 1). I also obtained data from Marion Vanneste in the Henry Lab that tracked PC-3E and TEM4-18 growth rates when each cell type was grown in isolation over 72 hours. From these data, I created a model that predicted the proportions of each cell type each time point given the population doubling vs. time of each cell type when it was cultured alone. This model would allow me to compare the growth rates of PC-3E and TEM4-18 cells when they were cultured alone vs. when they were cultured with one another. If the growth rates of these two cell types were different, it would suggest that the presence of another cell type caused a microenvironmental difference that affected the proliferation rates of the cell types.

In the model, the TEM4-18 population steadily declined, reaching less than 1% after 35 days. I observed strikingly similar results in my experiment, with the TEM4-18 population comprising only 1% of the population after only 28 days. The similarities between the model and my own experimental results suggest that combining the PC-3E and TEM4-18 cell types does not have an effect on the growth rate of either cell type. This result confirms my hypothesis that the TEM4-

18 cells would rapidly deplete when combined with the PC-3E epithelial cells. The result also indicates that the PC-3E cells do not help maintain the mesenchymal cells in the parental population. They may, in fact, have the opposite effect.

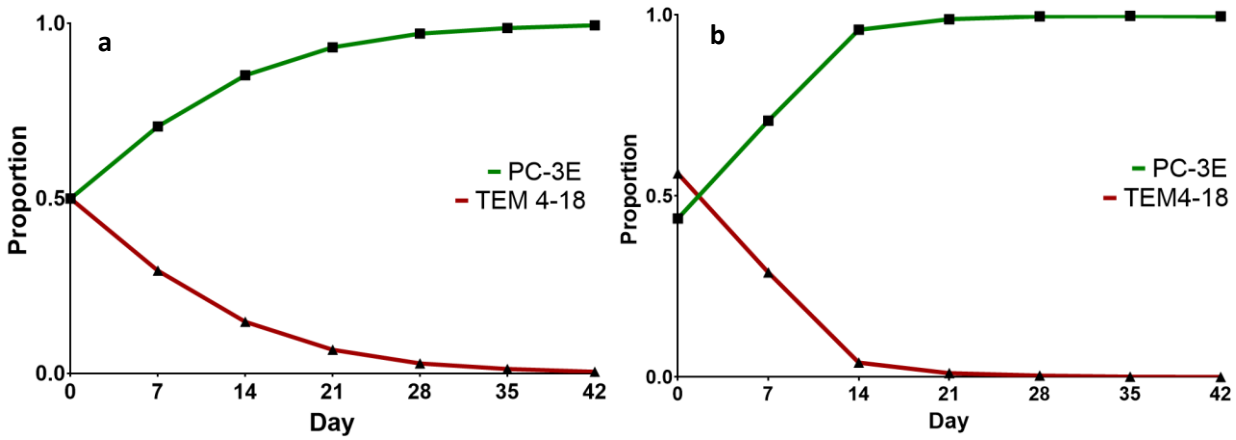

Figure 1: Results from the combination of TEM4-18 and PC-3E cells closely resembles the result predicted by a model created from previous data a) Model created from the manipulation of the population doubling vs. time data obtained from Marion X on population doubling vs. time for TEM4-18 and PC-3E cells. The experimental results b) closely follow the pattern shown in this model. b) Results from my experiment monitoring PC-3E and TEM4-18 cultured together over six weeks. The experiment was initiated with roughly equal proportions of each of the two cell types, but after just 14 days the TEM4-18 cells comprised less than 1% of the total population. After 28 days, the TEM4-18 cells were almost completely extinct in the population.

The composition of the cells in a combination of TEM4-18 and PC-3E cells should be similar to the composition of cells in the parental PC-3 population, although the parental population has a 75:25 ratio of PC-3E:TEM4-18, and my experiment used a 50:50 ratio. If the epithelial cells proliferate so quickly relative to the mesenchymal cells, how are the mesenchymal cells maintained in the PC-3 parental population, which contains both cell types? How is it possible that the PC-3E cells do not outgrow the TEM4-18 cells in the parental population?

TEM 4-18 + PC-3 Parental Cells

To further explore how the TEM4-18 cells are maintained in the parental population, I performed the first experiment again, but with PC-3 parental cells instead of PC-3E epithelial cells. It was

important to find out if the TEM4-18 cells behaved differently in the two conditions, because the result would give clues about the relevance of microenvironment to the proliferation of the TEM4-18 cells. If the mesenchymal cells were maintained when combined with PC-3 cells but not when combined with PC-3E epithelial cells, it would suggest that the parental cell line contains some cell type or secreted factor that is not present in the epithelial population and helps maintain the mesenchymal cells.

The first step in this process was transfection and transduction of unlabeled PC-3 parental cells to make the cells express GFP, which I needed both for flow cytometric cell counts and for analyzing the relative proportions of each cell type at each time point with fluorescent microscopy. Following successful transduction and transfection, I set up this experiment as for Experiment 1, with the exception of using EDTA instead of trypsin as a harvesting agent to avoid removing cell surface factors.

I cultured the cells for 42 days, during which I visualized the results week-to-week with fluorescent microscopy. I also used flow cytometry at the end of the experiment to quantify the relative frequency of each cell type at each time point. The results of this experiment contrasted sharply with results from Experiment 1, which compared TEM4-18 to PC-3E epithelial cells (Figure 2). In this second experiment, the initial proportions of TEM4-18 to PC-3 parental cells was 0.497 to 0.503, After one week, the TEM4-18 cells comprised 54% of the total population. After 21 days, TEM4-18 cells made up 77% of the total population. TEM4-18 cells remained at or above 70% for the remainder of the experiment. Prior to this experiment, I hypothesized that the TEM4-18 cells would be maintained at around 25% of the total population when combined with TEM4-18 cells. While the TEM4-18 cells were maintained, they were actually maintained at a higher proportion (~70%) than I expected. The maintenance of TEM4-18 when combined

with PC-3 but loss of TEM4-18 when combined with PC-3E epithelial cells indicates that these two different types of cell combinations differ in a fundamental way, begging the question of the nature of this difference.

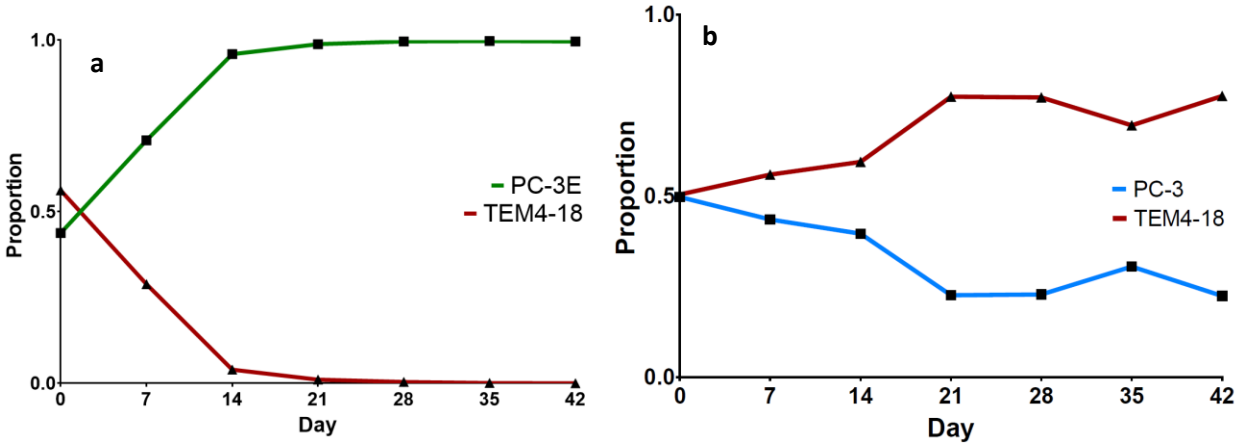

Figure 2: TEM4-18 cells behaved differently when combined with PC-3 cells than when combined with PC-3E cells. a) TEM4-18 cells were combined with PC-3 parental cells. The initial proportions of the two cell types were about 50/50, but after three weeks the TEM4-18 cells comprised 77% of the population. These cell type proportions are strikingly different from Experiment 1 (b) in which TEM4-18 cells were combined with PC-3E cells and quickly died out.

Growth Rates of Individual Cell Types

To get a better understanding of how surrounding cells affect proliferation rates, I grew PC-3 parental, PC-3E epithelial, and TEM4-18 mesenchymal cell types each in culture on their own. This experiment would allow me to quantify the growth rates of each cell type without the influence of other cell types. The experimental set-up was otherwise essentially the same as the other experiments. I used trypsin as the harvesting agent in each of these experiments, and I monitored the cells week-to-week with fluorescent microscopy. I did not use flow cytometry for these single cell line experiments because there was no need to distinguish between cell types. Instead, I counted the cells each week with a cell counter. I then used these data to calculate the

cumulative population doublings at each time point and compared these values with the population doublings vs. time for the cell lines in the first two experiments (Figure 3).

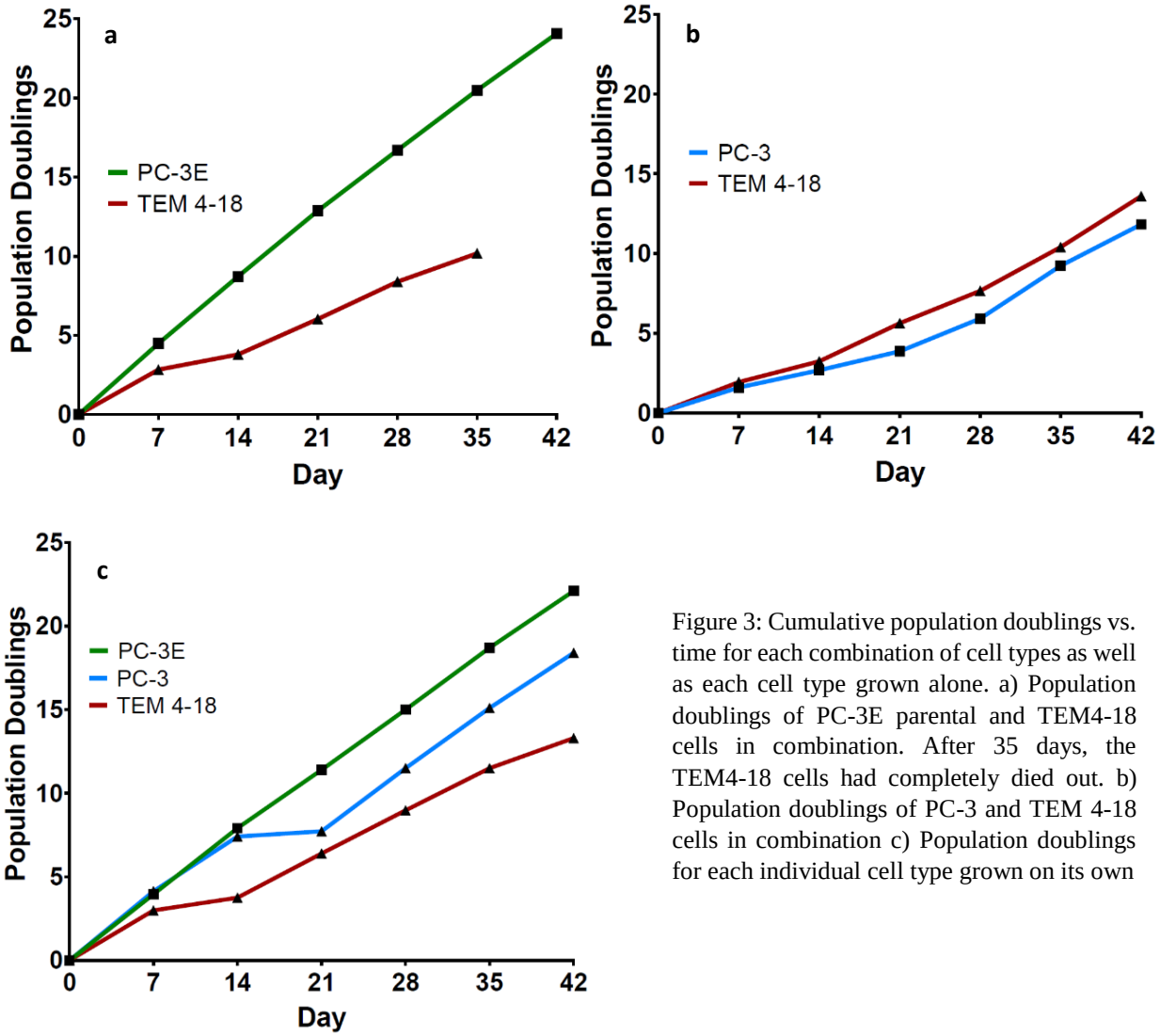

Figure 3: Cumulative population doublings vs. time for each combination of cell types as well as each cell type grown alone. a) Population doublings of PC-3E parental and TEM4-18 cells in combination. After 35 days, the TEM4-18 cells had completely died out. b) Population doublings of PC-3 and TEM 4-18 cells in combination c) Population doublings for each individual cell type grown on its own

Because the PC-3 parental cell line should contain around 75% epithelial, PC-3E type cells, I expected growth rate of the PC-3 parental cell line to be very similar to the growth rate of PC-3E epithelial cell line. Based on the model created from Marion's data, I expected the TEM4-18 cell line to consistently double fewer times than the PC-3 or PC-3E cell lines in all conditions. From

the population doubling data collected, I calculated days/doubling for each cell line in each condition (Figure 4). Replication is needed to statistically compare these outcomes.

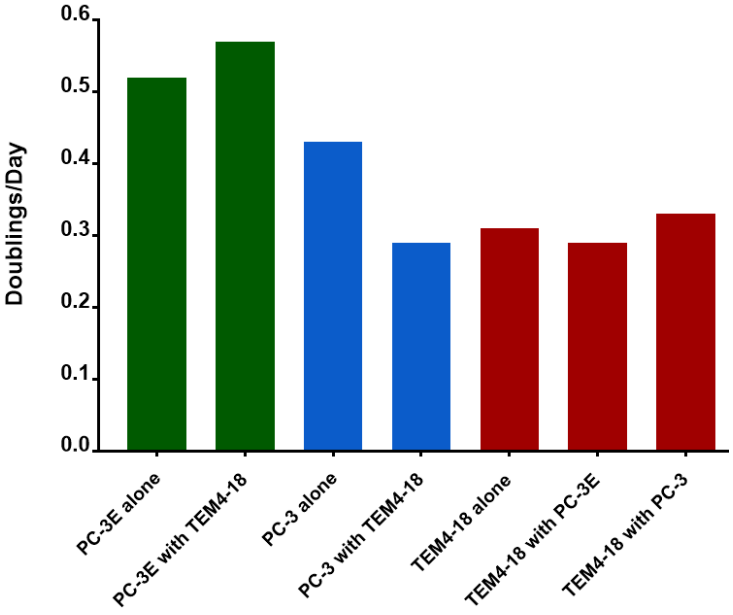

Figure 4: Doublings/day of each cell line cultured on its own and in combination with other cell lines. The PC-3E doublings/day are represented in green; PC-3 parental cell line in blue; TEM4-18 cell in red. These data made it possible to analyze whether the presence of another cell type affected the proliferation rate of the PC-3E, PC-3 or TEM4-18 cells.

PC-3E cells had the most rapid doubling time of any cell line (0.52 doublings/day when cultured alone). This growth rate advantage of the PC-3E cells was the most marked (0.57 doublings/day) when combined with the TEM4-18 cells. TEM4-18 cells showed a fairly consistent growth rate across the different experiments (0.29-0.33 doublings/day). This slower growth rate (compared to PC-3E) was consistent with the preliminary data (Vanneste, unpublished) suggesting that TEM4-18 cells do not experience population doubling as rapidly as PC-3E cells. The PC-3 cell line grew at a relatively high growth rate (0.43 doublings/day) when cultured alone, but a low rate (0.29 doublings/day) when cultured with the TEM4-18 cells.

Overall, both the PC-3E and TEM4-18 cell lines showed consistent growth rates in each experiment. The PC-3 parental cell line, however, showed a marked difference in growth rate (0.43 vs. 0.29 doublings/day) when cultured alone vs. when cultured with TEM4-18. This suggests that the addition of TEM4-18 cells changes the microenvironment of the PC-3 parental

population in a way that slows the proliferation of PC-3 cells. It is also important to note that the PC-3 cell line contains about 75% PC-3E epithelial cells. One might expect the PC-3E epithelial cells within the parental population to outcompete the TEM4-18 cells that were combined with the parental population in Experiment 2. Instead, the TEM4-18 cells thrived for the six weeks of the experiment.

There are several potential explanations for these results. One possibility is that parental PC-3 population may contain a protein or secreted factor, not present in the PC-3E epithelial cell line, that facilitates the growth of the TEM4-18 population. Conversely, the TEM4-18 population may secrete some factor that diminishes the proliferation of the PC-3 cell line. This latter explanation would explain the lower growth rate (0.29 doublings/day) of the PC-3 cell line when these cells are combined with TEM4-18 cells than when they are grown alone (0.43 doublings/day). Another possibility is that isolated PC-3E epithelial cells undergo a phenotypic switch that provides them with resistance to growth inhibition that may come from other cells when they are in the parental population. This phenomenon might explain why the PC-3E cell line doubles more quickly than the PC-3 parental cell line (0.52 vs. 0.43 doublings per day for each cell line cultured in isolation). A deeper understanding of factors secreted by each cell line and the physical effects of the mesenchymal and epithelial cells on one another are needed to determine which, if any, of these ideas provides explanatory power.

Replications

In the initial experiment, I combined PC-3E epithelial cells and TEM4-18 cells in equal proportions and cultured this combination for six weeks. The TEM4-18 cell line depleted to less than 1% of the total population within 28 days. To validate this result, I replicated the experiment with no modifications, and I obtained virtually identical results.

In Experiment #2, PC-3 parental cells and TEM4-18 cells were combined in equal proportions. The TEM4-18 cells rose to above 50% of the total population and remained at a proportion of about 70% until Day 42 when the experiment was terminated. I attempted to replicate this experiment using trypsin as a harvesting agent rather than the EDTA because the former provides a faster means of harvesting. Although all conditions were identical, the result of this replicate did not match the first replicate; in this second case, the TEM4-18 cells depleted to less than 1% of the population after 28 days.

My results from Experiment 1, where the PC-3E and TEM4-18 cells were combined, seem robust in light of my ability to successfully replicate the result. In both experiments, the TEM4-18 depleted to less than 1% of the population within six weeks of beginning the experiment. The result of the second experiment is more difficult to confirm. In both replicates, TEM4-18 cells were combined in equal proportion with PC-3 parental cells. In the first replicate, the TEM4-18 cells grew to more than 50% of the population, and comprised about 70% of the total population after six weeks. In the second replicate, the TEM4-18 cells rapidly died out, depleting to less than 1% of the population in just 4 weeks. In particular the use of EDTA vs. trypsin as harvesting agents may have been responsible for the different results from the two replicates. This experiment could be performed again, with replicates differing only in harvesting agent used, to evaluate the extent to which this explanation applies.

Conclusions

The concepts of ecology and evolution can be used to gain a better understanding of cancer. These concepts are both particularly useful when considering the way cancer cells within a tumor population compete with one another and the effects that the microenvironment of each cancer

cell can have on the cell's proliferation. My work has focused on how surrounding cells may affects the proliferation of a mesenchymal cell type within a tumor population.

TEM4-18 cells are a mesenchymal subpopulation within the larger PC-3 prostate cancer cell line. TEM4-18 cells have metastatic potential, making them a particularly dangerous cell type. In the PC-3 cell line, mesenchymal cells are maintained at about 25% of the total population. When epithelial and mesenchymal subpopulations are isolated from this PC-3 cell line and cultured separately, the PC-3E cell line has a higher growth rate (0.52 doublings/day) than the TEM4-18 cell line (0.31 doublings/day). This result would suggest that the PC-3E epithelial cells should outgrow the TEM4-18 cells in the parental population. However, both cell types are maintained in the parental population, raising the question of how the TEM4-18 cells persist.

To explore this contradiction, I cultured PC-3E epithelial, TEM4-18 mesenchymal, and PC-3 parental cells alone and in combination with one another. The TEM4-18 and PC-3E cells both have similar growth rates in each condition, but the PC-3 parental cell line grows at different rates when cultured alone vs. when cultured with the TEM4-18 cell line. These results suggest that surrounding cells can have notable effects on the proliferation of cancer cells.

Future research aimed at identifying these differences and, in particular, identifying a factor that either helps or harms the proliferation of the TEM4-18 cells could make it possible to target these cells in cancer treatment. Even if targeting the TEM4-18 cells *in vivo* proves challenging, a deeper understanding of how the tumor microenvironment effects mesenchymal cells would be beneficial in the treatment of cancer. The PC-3 parental population, as a heterogenous cell line, is a great model for studying how the various cell types in a tumor might affect the growth of one another. These ideas extend beyond this project, this cell line, or even this cancer type – knowledge of tumor microenvironments can be a powerful tool for treating cancer.

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