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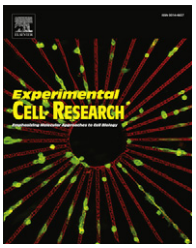

Research Article

Directional cell migration in an extracellular pH gradient: A model study with an engineered cell line and primary microvascular endothelial cells

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ABSTRACT

Extracellular pH (pH_e) gradients are characteristic of tumor and wound environments. Cell migration in these environments is critical to tumor progression and wound healing. While it has been shown previously that cell migration can be modulated in conditions of spatially invariant acidic pH_e due to acid-induced activation of cell surface integrin receptors, the effects of pH_e gradients on cell migration remain unknown. Here, we investigate cell migration in an extracellular pH_e gradient, using both model $\alpha_v\beta_3$ CHO-B2 cells and primary microvascular endothelial cells. For both cell types, we find that the mean cell position shifts toward the acidic end of the gradient over time, and that cells preferentially polarize toward the acidic end of the gradient during migration. We further demonstrate that cell membrane protrusion stability and actin–integrin adhesion complex formation are increased in acidic pH_e, which could contribute to the preferential polarization toward acidic pH_e that we observed for cells in pH_e gradients. These results provide the first demonstration of preferential cell migration toward acid in a pH_e gradient, with intriguing implications for directed cell migration in the tumor and wound healing environments.

Introduction

It is well known that the average extracellular pH (pH_e) in tumors and wounds is often more acidic than in normal tissues [1–8]. Tumors and wounds tend to be hypoxic, due to regions of damaged or irregular vasculature, resulting in anaerobic cellular metabolism and production of lactic acid [4,8,9]. Even in regions with sufficient oxygen, alterations in gene expression cause cancer cells to increase reliance on anaerobic metabolism, a phenomenon known as the Wartburg effect [8,10–12]. As glycolysis is an inefficient means of ATP production, cancer cells must increase the rate of glucose consumption and metabolism in order to maintain sufficient energy levels, which accelerates acid production [4]. However, although the average extracellular pH in these contexts is generally acidic, the pH_e environment is not spatially uniform. Rather, high resolution methods for measuring pH_e have shown significant spatial variations in pH_e profiles within tumors [3,13]. For example, pH_e gradients exist within tumors, with pH_e decreasing about 0.7 units

Abbreviations: pH_e, Extracellular pH; MVECs, Microvascular endothelial cells; AIAC, Actin–integrin adhesion complex; NHE1, Na^+/H^+ ion exchanger

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(~7.4 to ~6.7) over ~350 μm from a tumor blood vessel for human colon adenocarcinoma xenografts [13]. Furthermore, pH_e gradients have been measured at the interfaces between tumors and normal tissue, with pH_e increasing about 0.4 units (~6.9 to ~7.3) over 1 mm toward the normal tissue for human prostate tumors grown in mice [4]. Although pH_e profiles have not been measured in the wound context, the presence of oxygen gradients at wound sites implies that pH_e gradients occur in these environments as well [14–16]. Finally, in addition to gradients that span many cell lengths, pH_e gradients can also exist at the single cell level, due to localization of the Na^+/H^+ ion exchanger NHE1 to the leading edge of migrating cells [17].

It is possible that pH_e gradients could alter migration of cells that are involved in tumor growth and wound healing. Cell migration is generally mediated by binding interactions between transmembrane integrin receptors and extracellular matrix (ECM) ligands. We have previously shown that acidic pH_e promotes conversion of integrin $\alpha_v\beta_3$ to a high-affinity conformation, a process known as integrin activation, and that this can modulate cell migration in conditions of spatially uniform acidic pH_e by increasing the overall adhesiveness to the surrounding ECM [18]. However, cell migration in pH_e gradients has not yet been explored.

Studies with fibronectin surface gradients have demonstrated that cells preferentially migrate toward higher fibronectin coating concentrations, which have higher adhesiveness due to higher spatial density of adhesive ligands [19,20]. In the context of our previous work, this suggests that cells in a pH_e gradient may preferentially migrate toward lower pH_e. This could be particularly important for microvascular endothelial cells migrating within or toward tumors or wounds to initiate vascularization in hypoxic areas.

Here, we establish gradients from pH_e 6.0–7.5 and investigate the migration of model $\alpha_v\beta_3$ CHO-B2 cells and primary bovine retinal microvascular endothelial cells (MVECs) in the pH_e gradients. We examine the former engineered cell type first because this cell line exhibits a relatively well characterized integrin/fibronectin interaction, and then we validate the findings using the latter, more physiologically relevant cell type. We observe that mean cell position moves progressively toward lower pH_e over time for both cell types. We then discuss possible mechanisms underlying this shift in mean cell position with additional experiments and the aid of a computational cell migration model. We find that MVECs exhibit direction-dependent migration velocity, with cells oriented toward acid migrating faster than cells oriented away from acid. Additionally, both $\alpha_v\beta_3$ CHO-B2 cells and MVECs polarize preferentially toward lower pH_e during migration. Finally, we find that cells in acidic pH_e have longer membrane protrusion lifetimes and more actin–integrin adhesion complexes than cells in pH_e 7.4, which may contribute to regulation of directionality during cell migration in pH_e gradients.

Materials and methods

Cell culture and experimental solutions

$\alpha_v\beta_3$ CHO-B2 cells were provided by Dr. L. Griffith (Massachusetts Institute of Technology), as subcultures of a cell line developed by Dr. J. Schwarzbauer (Princeton University) and Dr. S. Corbett (University of Medicine and Dentistry of New Jersey). Cell culture media for $\alpha_v\beta_3$ CHO-B2 cells consisted of high-glucose bicarbonate-buffered Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen) containing L-glutamine and sodium pyruvate, supplemented with 10% fetal bovine serum (Hyclone), 1% antibiotics-antimyotics (Invitrogen), 1% non-essential amino acids (Invitrogen), and 500 μg/mL zeocin (Invitrogen). Bovine retinal microvascular endothelial cells were provided by Dr. I. Herman (Tufts University). Cell culture media for MVECs consisted of low-glucose bicarbonate-buffered DMEM containing L-glutamine and sodium pyruvate, supplemented with 10% bovine calf serum (Sigma) and 12.5 mM HEPES (Sigma). Both cell types were maintained in an incubator at 37 °C with 5% CO_2 and, unless otherwise noted, all cell incubations occurred under these conditions.

For cell migration, kymography, and actin–integrin adhesion complex imaging experiments, a serum and bicarbonate free version of the cell specific media described above was used. Bicarbonate-free media enabled precise control of pH during the course of experiments and has been used previously for many cell types [17, 21–25]. The pH in bicarbonate-free media was adjusted using 1 M HCl or NaOH. For a discussion of bicarbonate-free versus bicarbonate-buffered environment see the Discussion and conclusions.

Dunn chamber setup and extracellular pH gradient imaging

The Dunn chamber (Hawksley) was set up according to the manufacturer's instructions. Briefly, both wells were filled with bicarbonate-free serum-free media at pH 7.5 and covered with a coverslip, leaving a thin slit open at the outer well. The sides of the coverslip, but not the slit, were sealed to the chamber slide using a melted wax mixture consisting of 1:1:1 paraffin:beeswax:Vaseline, and the outer well was drained with a Kimwipe. The outer well was then filled with bicarbonate-free serum-free pH 6.0 media using a syringe, and the slit was sealed with the melted wax mixture. For gradient imaging, 10 μM BCECF (2′,7′-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein) (Invitrogen) was added to the bicarbonate-free serum-free $\alpha_v\beta_3$ CHO-B2 cell media. The bridge region was imaged at 4× magnification at excitation wavelengths of ~440 nm and ~495 nm, both with an emission wavelength of ~535 nm. The ratio of the 495/440 nm light intensities is linear with pH. To assess gradient stability, images were taken every hour over 8 h for chambers at 37 °C, and two independent experiments were conducted. Data points near the edges of the bridge were excluded due to artifacts in light intensity.

Cell migration measurements

Number 2 glass coverslips were coated with 30 μg/ml human plasma fibronectin (Sigma) in phosphate buffered saline (PBS) at pH 7.4 for 1 h at room temperature, then rinsed twice with PBS. Cells were plated on coverslips in serum-free media and allowed to adhere for 2–3 h before being placed in the Dunn chamber. Media loaded into the Dunn chamber wells consisted of bicarbonate-free serum-free media, and chambers were set up as described above. Cells were imaged at 4× magnification every