Role of Acid-Sensing Ion Channels in Human Adipose-Derived Stem Cell Response to Acute vs Gradual Acid Stress

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INTRODUCTION

Intervertebral disc degeneration (IVDD) is a potentially debilitating condition with an estimated economic burden of one hundred billion dollars a year (Katz, J. *The Journal of Bone and Joint Surgery,* 2006). Current IVDD treatments only address symptoms and do not restore disc functionality (Xin, J. Et al. *Orthopaedic Surgery*, 2022), so there is a need for treatments to address IVDD's underlying etiology. Stem cell therapies are a promising potential treatment (Clouet, J. Et al. *Advanced Drug Delivery Reviews,* 2019), but the low pH of the intervertebral disc (IVD) (7.1 in healthy, 6.5 in degenerated) (Liu, J. Et al. *Stem Cells and Development,* 2017), can impede the survival, differentiation and function of implanted stem cells (Li, H. Et al. *Exp Biol Med*, 2012). While still controversial, it has been suggested that Acid-Sensing Ion Channels (ASICs) play a role in the IVD cell response to low pH (Gao Y. Et al. *Channels,* 2019). Some studies suggest that the activation of ASIC in IVD cells by low pH inhibits anabolic activity and cell proliferation, contributing to IVDD (Gilbert H. Et al. *Sci Rep,* 2016). Others suggest that ASIC is essential for IVD cell viability in low-pH environments (Uchiyama Y. Et al. *Journal of Bone and Mineral Research,* 2007). The goal of the present study is to improve understanding of the role that ASIC plays in stem cells' response to low pH. We examined the cell counts, cell morphology, and mRNA expression for ASIC and differentiation markers in human adipose-derived mesenchymal stem cells (hADMSCs) following exposure to various pH conditions.

METHODS

hADMSCs (Lonza) were seeded into 6-well plates at a density of 2.5 x 10^5 cells per well and cultured under DEMEM with 10% FBS and 1% ABAM 7.4 pH for 24 hours. Media of varying pH (7.4, 7.1, 6.8, 6.5) were prepared by adjusting the concentration of sodium bicarbonate based on a preprepared pH curve. The cells were then exposed to gradual (pH 7.1, 6.8, and 6.5 over three days) or sudden (pH 6.5 for three days) changes in pH, then cultured at 6.5 pH for three additional days. Cells maintained at pH 7.4 served as a control. Media changes occurred on days 1, 2, and 3. In half the experiments, cells were pre-treated with an ASIC inhibitor, Amiloride (100 μM). At the end of the prescribed periods, the cells were fixed with 10% formalin and stained with Coomassie blue and DAPI to evaluate cell morphology and cell count. In addition, total RNA was isolated, and RT-qPCR was performed using custom primers and commercial kits. The ΔΔCt method was used to quantify the expression of ASIC1, ASIC3, SOX9, PAX1, and FOXF1. Experiments were conducted in duplicate and independently at least three times. Numerical data were analyzed statistically using ANOVA tests on R.

RESULTS

hADMSCs maintained under control (7.4 pH) conditions exhibited a spindle shape and uniform distribution across the substrate. In contrast, cells exposed to a sudden change in pH (to 6.5) and cultured at the low pH for six days exhibited a stellate shape with noticeable clustering. The cells exposed to a gradual change in pH from 7.4 to 6.5 over six days exhibited a stellate shape and mild clustering. Cells pre-treated with Amiloride exhibited similar morphologies to their untreated counterparts in all groups. hADMSCs cultured at 6.5 pH had a significantly ($P<0.05$, n=3) lower count than cells cultured at 7.4 pH after six days. In contrast, hADMSCs exposed to gradual changes in pH from 7.4 to 6.5 had a similar cell count to cells cultured at 7.4 pH after six days. However, hADMSCs gradually exposed to low pH in the presence of Amiloride had a significantly ($P<0.01$, n=3) lower count than the gradual group without Amiloride. Compared to the control, the cells exposed to both sudden and gradual changes in pH expressed significantly ($P<0.01$, n=3) lower ASIC1, SOX9, PAX1, and FOXF1 mRNA. ASIC3 expression was similar in all three groups. Moreover, a pair-wise comparison revealed that SOX9 expression was significantly ($P<0.05$, n=3) lower in the cells exposed to a sudden pH change than in cells exposed to a gradual change. The results of a time-course experiment provided evidence that ASIC1 and SOX9 expressions dropped after switching from pH 7.4 to 7.1 and continued decreasing as the pH decreased from 7.1 to 6.8 and to 6.5. There was no significant difference in gene expression for any of the low pH groups between groups with and without amiloride.

DISCUSSION

The observed change in cell morphology under low pH was not altered by the presence of Amiloride, which implies that this event did not result from ASIC activation. In contrast, the variation in cell counts under gradual changes in pH with and without amiloride may suggest that ASICs play a role in increasing hADMSC survival in low pH environments. The time-course results provided evidence that ASIC1 and SOX9 expressions dropped after switching from pH 7.4 to 7.1. Since ASICs are sensitive to the extracellular pH, a reduction in ASIC1 expression may indicate a compensation mechanism for hADMSCs to cope with acidic conditions. The lower expressions of SOX9, PAX1, and FOXF1 in both sudden and gradual exposure compared to control suggest that IVD-like differentiation marker expression can be negatively affected by even a small change in pH (from 7.4 to 7.1). In addition, the results of the present study demonstrated that inhibiting ASIC is not sufficient to prevent the negative effects of low pH on IVD-like differentiation. For successful treatment of IVDD, implanted cell survival in the IVD is the necessary first step. In this regard, our findings suggest that gradual acclimation of cells to low pH in vitro and retaining ASIC3 expression may help improve hADMSCs' survival in vivo. While the present study provides insight into hADMSCs' response to one factor of the IVDs microenvironment, there are still other environmental conditions (e.g., high pressure, low oxygen, and low glucose) that will also need to be considered when developing cell therapies for IVDD.

SIGNIFICANCE/CLINICAL RELEVANCE

Stem cell therapies present a promising treatment option for IVDD, but the low pH environment of the IVD remains a barrier for the successful implementation of these treatments. This study provides insights into hADMSCs' response to low pH and the role ASIC plays in this response for use in future cell therapies for IVDD.

IMAGES AND TABLES