CD43 Expression on Jurkat Cells and U937 Myeloid Cells Cultured in Hypoxic Conditions

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Introduction

Hypoxia describes an environment with a limitation of oxygen. Hypoxic conditions have been shown to affect leukocytes in a manner that aids the development of diseases (Kong *et al.* 2004). CD43, also known as sialophorin, leukosialin, or gpL 115, is a highly conserved 95-135 kDa transmembrane sialoglycoprotein on the surface of most hematopoietic cells. CD43 extends about 45 nm from the cell membrane resulting in potential steric hindrance with other cells and molecules (Cyster *et al.* 1991). Negative sialic acid residues are abundant within the extracellular domain causing lymphocyte repulsion due to the like negative charge on each cell surface. As a result of these structural elements, CD43 functions primarily as an anti-adhesive molecule prohibiting interactions with other cells (Ardman *et al*. 1992). Ironically, CD43 has also been found to function *in vitro* as an adhesive molecule (Rosenstein *et al.* 1991). The effects of physiological conditions and stimuli on the expression of CD43 are not entirely understood. And, expression of CD43 is defective in a medical condition known as Wiskott-Aldrich syndrome (Shelley *et al*. 1989). Therefore, a greater understanding of this cell surface molecule is important. In this study, our primary objective was to investigate the effects of hypoxic growth conditions on CD43 expression when compared with normal oxygen levels. Flow cytometry and Western blot analysis were utilized to examine the hypoxic effects on CD43 expression.

Materials and Methods

Jurkat cells and U937 myeloid cells were cultured in normal and hypoxic conditions by Dr. Simon Shelley. Incubation occurred at 37°C and 21% oxygen for the control cultures and 8% oxygen for the hypoxic cultures. Hypoxic cultures were placed in the hypoxic chamber 48 hours prior to lysate preparation and flow cytometry analysis. Cells were lysed in 1 mL Nonidet P-40 (NP-40) lysis buffer, 10 µl EDTA, and 10 µl protease inhibitors for 30 minutes on ice.

Western blot protein samples were electrophoresed in 8% tris-glycine gels in nonreducing conditions and then transferred onto nitrocellulose membrane. The membrane was blocked for one hour with 7% BSA/PBS, washed with wash buffer (1X PBS 0.05% Tween 20), and exposed to primary Ab L10 at 1:100 for one hour (Ab provided by Dr. Simon Shelley).

The membrane was washed, incubated in secondary Ab for 30 minutes, and detected using a Pierce Chemiluminescent Detection Module.

Flow cytometry preparation on ice: cells were blocked, washed with PBS, and incubated in primary Ab L10 for 10-15 minutes. Cells were washed, incubated in FITC-conjugated secondary Ab for 10-15 minutes, washed again, and then analyzed using a Becton Dickinson FACScan flow cytometer.

Results

Western blot analysis detected a strong signal revealing no quantitative or qualitative changes in the expression of CD43. FACS analysis also indicated that expression of CD43 on the surface of Jurkat cells and U937 myeloid cells was not affected by hypoxia.

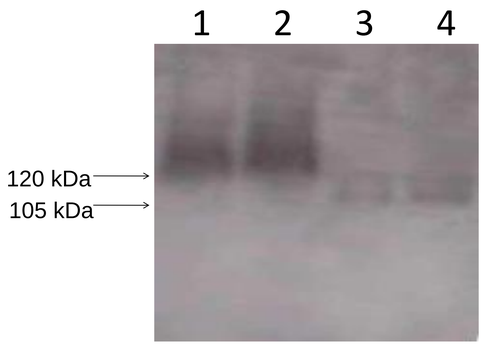

Figure 1. Comparison of CD43 expression on Jurkat and U937 cells. Lane 1, Jurkat cells normal oxygen levels; lane 2, Jurkat cells hypoxic conditions; lane 3, U937 cells normal oxygen levels; lane 4, U937 cells hypoxic conditions.

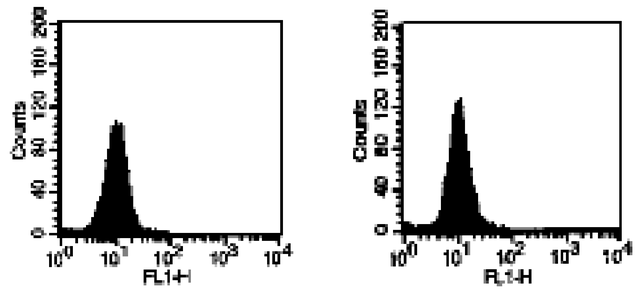

Figure 2. Representative FACS of U937 myeloid cells grown under normal (*left*) and hypoxic conditions *(right*). Cells were incubated with primary antibody L10 followed by incubation with a FITC-conjugated secondary antibody.

Discussion

In this study we examined CD43 expression in hypoxic conditions on the surface of Jurkat Cells and U937 myeloid cells. Our western blot showed differences in molecular weight of CD43 between the two cell types, but no effects resulting from the hypoxic conditions. Likewise, flow cytometry analysis showed no significant differences in CD43 expression on the surface of either cell type between normal oxygen levels and hypoxic conditions. Therefore, CD43 is not down regulated on the surface of Jurkat cells or U937 myeloid cells under hypoxic conditions.

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Resources

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