CD44 Determination by Western Blot with the Sapphire Biomolecular Imager

In collaboration with the Lompardía Lab, University of Buenos Aires, Argentina

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Introduction

CD44 is the main receptor for hyaluronic acid and is expressed in several kinds of cells. Its overexpression has been associated with a poor prognosis in various types of cancer, including glioblastoma.[1,2] Glioblastoma represents the main primary malignant neoplasm of the central nervous system. This pathology exhibits high mortality rates and few therapeutic alternatives, making it important to identify novel therapeutic targets.[3] Therefore, it is interesting to be able to determine the expression of CD44 in human glioblastoma cells. Western blotting is a highly useful assay for this purpose. Additionally, studying cells that do not express the receptor can provide insight into its role in cancer cell biology. Western blotting is an important step to confirm that expression of a protein is disrupted in a knockout cell line. In this application note, we describe CD44 expression in protein extract from LN229 glioblastoma cells using the Azure Biosystems Sapphire™ Biomolecular Imager.

Methods

LN229 CD44 -/- and LN229 wild type (WT) cells were grown for 24 hours at 37°C in a 5% CO_2 atmosphere. Cells were then lysed with hypotonic buffer. After centrifugation (13000 rpm for 30 minutes), equal amounts of protein were resolved by SDS-polyacrylamide gel electrophoresis and transferred onto a PVDF membrane (Osmonics Inc., Gloucester, MA). The membrane was blocked and incubated with anti-CD44 IM7 monoclonal antibody overnight at 4°C, followed by incubation with a horseradish peroxidase-labeled secondary antibody for 2 hours at 37°C. The reaction was developed using a chemiluminescent detection system. Images were obtained with Azure Biosystems Sapphire™, with an exposure time of 20 seconds and 3 x 3 binning settings.

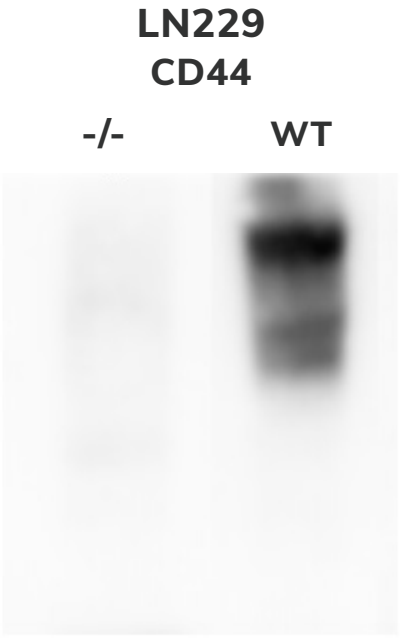

Figure 1. CD44 detection by Western blot assay using Azure Biosystems Sapphire™.

Results and Discussion

Figure 1 shows that wild type LN229 cells exhibit high CD44 expression, while silenced cells do not express the receptor. The Azure Biosystems Sapphire™ Biomolecular Imager was useful for checking the proper silencing of CD44 and produced images of excellent quality. It is worth noting that the Sapphire™ Biomolecular Imager's ability to acquire images at different time points and accumulate them, and to capture images at a variety of exposure times, is highly useful for chemiluminescent assays. In addition to chemiluminescence, the Sapphire™ Biomolecular Imager also detects near-infrared and visible light fluorescence, enabling imaging of single- and multicolor fluorescent Westerns. The Sapphire is a highly versatile and flexible imaging system for a variety of Western blotting applications.

References

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3. Weller M, Reifenberger G. Beyond the World Health Organization classification of central nervous system tumors 2016: what are the new developments for gliomas from a clinician's perspective? *Curr Opin Neurol* 2020; **33**: 701–706

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