Jul 18, 2024

Profiling the Surfaceome of Meningioma for Immunotherapeutic Target Identification

DOI
dx.doi.org/10.17504/protocols.io.5qpvokqm9l4o/v1

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DOI: dx.doi.org/10.17504/protocols.io.5qpvokqm9l4o/v1

Protocol Citation: ABDULLAH BIN ZUBAIR, Muhammad Awais Khan, Fatima Hamid, Renee M. Hirte, mlabib, dgandhi 2024. Profiling the Surfaceome of Meningioma for Immunotherapeutic Target Identification. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.5qpvokqm9l4o/v1

Protocol status: In development
We are still developing and optimizing this protocol

Created: July 17, 2024

Last Modified: July 18, 2024

Protocol Integer ID: 103616

Disclaimer

Our research project is currently in the application phase, awaiting the acquisition of necessary data to commence a pilot study. Despite the limited availability of initial data, our investigation holds significant promise to redefine the paradigm of cancer biology. Specifically, our hypothesis posits that the molecular characteristics of cancers are intricately linked to the unique genetic makeup of individuals. By scrutinizing the DNA profiles of cancer patients, we aim to unveil correlations between genetic variations and cancer phenotypes. This pioneering approach not only has the potential to elucidate novel biomarkers for early detection and personalized treatment strategies but also to fundamentally alter our understanding of cancer etiology and progression.
The protocol described in this publication is experimental and investigational. It involves innovative methodologies that require further rigorous scientific evaluation to establish their effectiveness and safety. Researchers and healthcare professionals should exercise caution in considering the application of this protocol outside of controlled research settings. Potential risks and benefits associated with its experimental nature should be carefully weighed, and regulatory approvals sought prior to any clinical implementation. Continued research efforts are essential to validate and refine this protocol for potential future clinical use.

Abstract

This protocol aims to profile the surfaceome of meningioma tumors and matched healthy tissue to identify differentially expressed tumor cell-surface proteins and tumor-specific antigens. By using N-Glycocapture Mass Spectrometry, we will enrich and analyze the cell surface glycoproteins, allowing for the identification of potential immunotherapeutic targets. The protocol includes sample collection, tissue processing, N-Glycocapture enrichment, Mass Spectrometry analysis, and data interpretation. We anticipate that this protocol will provide valuable insights into the surfaceome of meningioma cells, enhancing our understanding of tumor biology and facilitating the development of precise, targeted immunotherapies.

Subject Terms:

1 Meningioma, Immunotherapy, Surfaceome profiling, Target identification

Keywords:

2 Meningioma, Immunotherapy, Surfaceome, Proteomics, Mass Spectrometry

Introduction

3 Meningioma is the most common primary brain tumor in adults, characterized by significant treatment challenges and variable recurrence rates. Immunotherapy has demonstrated promise in other cancer types, but the lack of specific cell surface targets unique to meningioma hampers its application in this context. This protocol aims to overcome this challenge by profiling the surfaceome of meningioma cells, identifying differentially expressed tumor-specific antigens, and validating their functional significance as potential immunotherapeutic targets.

Reagents and Equipment:

4 - N-Glycocapture Kit (Manufacturer: Glycopath. Inc , Catalog Number: Basic N-Glycan Imaging Kit)
- Mass Spectrometer (Manufacturer: Bruker, Model: TimsTOF Pro 1, Mass Analyzer: IonMob.-Q-Time-Of-Flight (IM-Qq-TOF), m/z Range: 50-40'000, LC-Detector: MS only, Chromatographic Separation: e.g. nano C18, Sample types: Proteomics, Peptides, Proteins, Digest (Sequence identification), Operation Modes: MS, MS/MS (CID, PASEF).

Guidelines and Warnings

5 This protocol needs prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s). Before proceeding with this protocol, ensure that you have obtained the necessary ethical approval. By seeking ethical approval, you demonstrate your commitment to conducting research responsibly and ensuring the protection of participants' rights and well-being. This protocol is still pending process.

Procedure

6 Tissue collection and processing

6.1 Obtain meningioma tumor tissue and matched healthy tissue during surgical resection.

6.2 Immediately transfer the collected tissue samples to a sterile container filled with ice-cold phosphate-buffered saline (PBS) to maintain tissue integrity.

6.3 Label each container with the patient's unique identifier and the type of tissue (tumor or healthy) using a waterproof marker.

6.4 Transport the tissue samples to the laboratory on ice within 30 minutes of collection.

7 Tissue preparation and homogenization:

7.1 In a sterile laminar flow hood, transfer each tissue sample to a pre-chilled petri dish.

7.2 Using a sterile scalpel, remove any visible connective tissue or blood vessels from the tissue samples.

7.3 Cut the tissue into small pieces (~1-2 mm) using sterile scissors and forceps.

7.4 Transfer the tissue pieces to pre-labeled microcentrifuge tubes containing ice-cold lysis buffer provided in the N-Glycocapture Kit.

7.5 Ensure that the tissue-to-lysis buffer ratio is appropriate (recommended ratio: 1:10) and adjust as necessary.

7.6 Homogenize the tissue samples using a tissue homogenizer or a motorized pestle until a homogeneous suspension is achieved.

7.7 Centrifuge the homogenized samples at 10,000 x g for 10 minutes at 4°C to pellet cellular debris and collect the supernatant containing the solubilized proteins.

8 N-Glycocapture enrichment

8.1 Add the appropriate volume of N-Glycocapture beads to the collected supernatant based on the manufacturer's instructions.

8.2 Incubate the sample-bead mixture on a rotator or shaker at 4°C for 2 hours to allow for the specific binding of glycoproteins to the beads.

8.3 Place the sample-bead mixture on a magnet or use a magnetic separator to separate the beads from the supernatant.

8.4 Carefully remove the supernatant without disturbing the beads and discard it.

8.5 Wash the beads three times with ice-cold wash buffer provided in the N-Glycocapture Kit, following the manufacturer's instructions.

8.6 After the final wash, resuspend the beads in the elution buffer provided in the N-Glycocapture Kit to release the captured glycoproteins.

8.7 Incubate the resuspended beads on a rotator or shaker at room temperature for 10 minutes to elute the glycoproteins.

8.8 Separate the beads from the eluted glycoproteins using a magnet or magnetic separator and collect the glycoprotein-containing supernatant.

9 Mass Spectrometry analysis

9.1 Transfer the collected glycoprotein-containing supernatant to a fresh microcentrifuge tube.

9.2 Prepare the sample for Mass Spectrometry analysis following the instrument manufacturer's guidelines, including protein digestion and peptide purification steps.

9.3 Load the purified peptides onto a Mass Spectrometer for data acquisition.

9.4 Analyze the acquired data using appropriate software to identify and quantify differentially expressed tumor cell-surface proteins and tumor-specific antigens.

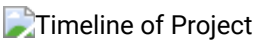
Troubleshooting

10

STEP	PROBLEM	POSSIBLE REASON	SOLUTION
6.1	Insufficient tissue samples.	Inadequate samples obtained during surgery.	Ensure proper communication between surgical team and research staff.
6.2	Samples can't be snap frozen straight away.	Inadequate area for freezing samples.	Cryofreeze/ Use dry Ice to transport samples to Lab.
6.3	Samples being mislabeled.	Human Error	Double confirmation with the surgical team before surgery regarding MRN of patient and label container before going to collect the samples.
9.1	No Results.	Expired Reagents and Kit.	Inventory overview by lab manger every 3 weeks.
9.4	Variable Results from the same patient.	Human Error, Systematic Error in Steps 6, 7, 8, and 9.	Create a back up storage of tissue samples from each patient in biobank.

Time Taken

11



Timeline of Project

Anticipated Results

12 We anticipate identifying differentially expressed tumor cell-surface proteins and tumor-specific antigens in meningioma samples compared to healthy tissue. These findings will

provide valuable insights into potential immunotherapeutic targets for precise, targeted treatments.

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Acknowledgments

14 We acknowledge the **Colon Rectal Surgery Department** at **Mayo Clinic Rochester** where the idea was in its budding phases and the process of individualized immunotherapy was considered a viable option for the future of cancer treatment plans. **Dr. David W. Larson M.D., M.B.A.,(PI)** and **Dr. Kellie L. Mathis, M.D. (PD CRS MAYO CLINIC-RST)** were the supporting group that provided vast opportunities to their researchers to make better contributions to the field of medicine.

ETH ZURICH the MS service at LOC is open to all research groups within ETH Zurich, provided the Individual, customer-specific development of fully automated processing scripts for large series of experiments (screenings), and provided knowledge and expertise to our customers as integral consulting around analytical questions with focus on mass spectrometry.