Data Article

Topology and adenocarcinoma cell localization dataset on the labyrinthin biomarker

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Keywords

Pan-tumor target, biomarker, neoantigen, adenocarcinoma, tumor associated antigen, tumor specific antigen, labyrinthin, ASPH

Abstract

The discovery and characterization of tumor associated antigens is increasingly important to advance the field of immuno-oncology. Data on the 1) topology, 2) sequence analyses, 3) and cell surface localization are presented as evidence in support of labyrinthin as a pan-adenocarcinoma marker. Multiple bioinformatics tools were used to collectively predict calcium binding domain(s), N-myristoylation sites, kinase II phosphorylation sites, and transmembrane domain(s) with predicted N- and C-termini orientations. Amino acid (a.a.) sequence homology comparisons were found and alignments made for labyrinthin (255 a.a.) vs. the intracellular aspartyl/asparaginyl beta-hydroxylase (ASPH; 758 a.a.) and the ASPH-gene related protein junctate (299 a.a.), which are both type II proteins. The epitopes for commonly used MCA 44-3A6 anti-labyrinthin and FB-50 anti-ASPH/junctate antibodies were present in all the proteins. The selective expression of labyrinthin was therefore investigated by third party fluorescent activated cell sorter (FACS) analyses of MCA 44-3A6 binding to non-permeabilized adenocarcinoma and normal cell line models. Labyrinthin was detected on non-permeabilized A549 human lung adenocarcinoma cells, but not on normal WI-38 human lung fibroblasts nor primary cultures of normal human glandular-related cells. Microscopic images of immunofluorescent labelled MCA 44-3A6 binding to A549 cells at random cell cycle stages complement the FACS results and further showed labyrinthin persisted on the cell surfaces along with some cell internalization within 20 minutes. The dataset presented herein is related to research article: "Labyrinthin: A distinct pan-adenocarcinoma diagnostic and immunotherapeutic tumor specific antigen" by Babich *et. al* 2022, *Heliyon* [1].

Specifications table

Subject	Biological Sciences
Specific subject area	Cancer Research

Type of data	Supplementary Table, Figures, Images, Graph
How the data were acquired	Retrospective chart and data review from multiple computer analyses of protein topology performed at academic medical centre. Computer generated graph derived from a protein-cell membrane orientation analysis program. Amino acid sequence comparison of homologies for labyrinthin via National Center for Biotechnology Information database. FACS analysis of antigen on surface of non-permeabilized human adenocarcinoma cell model vs. multiple primary human normal cells. Immunofluorescent antibody labelling of cell surface antigen (derived via previously reported FACS study [2] and microscopic image of localization.
Data format	Raw and analysed
Description of data collection	Labyrinthin amino acid sequence data entered and topology analysed using seven different online tools. Amino acid sequence of proteins (labyrinthin, ASPH, junctate) obtained via search tool on the NCBI website. Alignments and sequence identity performed manually. FACS analysis: Cells were commercially obtained and grown according to the instructions from the American Type Culture Collection (A549 human lung adenocarcinoma and WI-38 normal human lung fibroblast) and Cambria/Lonza (normal primary cells cultures).
Data source location	LabyRx Immuno-Oncology Limited Sacramento, California USA Stanford Research Institute International Menlo Park, California USA University of Illinois, Chicago Chicago, Illinois USA Raw data source (amino acid sequence comparisons): National Center for Biotechnology Information USA
Data accessibility	With the article
Related research article	M Babich, A Sharma, T Li, JA Radosevich. Labyrinthin: A distinct pan-adenocarcinoma diagnostic and immunotherapeutic tumor specific antigen. Heliyon, e08988, 2022.

Value of the data

- Identification and characterization of neoantigens [3, 4] such as labyrinthin [5] are rare yet increasingly important for cancer treatment. Insight on labyrinthin as a pan-adenocarcinoma

target labyrinthin for clinicians and cancer researchers is provided and consistent with 1) the convenient location of labyrinthin on the cell surface of adenocarcinomas; 2) appearance throughout the A549 human lung adenocarcinoma cell cycle; and 3) not modulated from the cell surface.

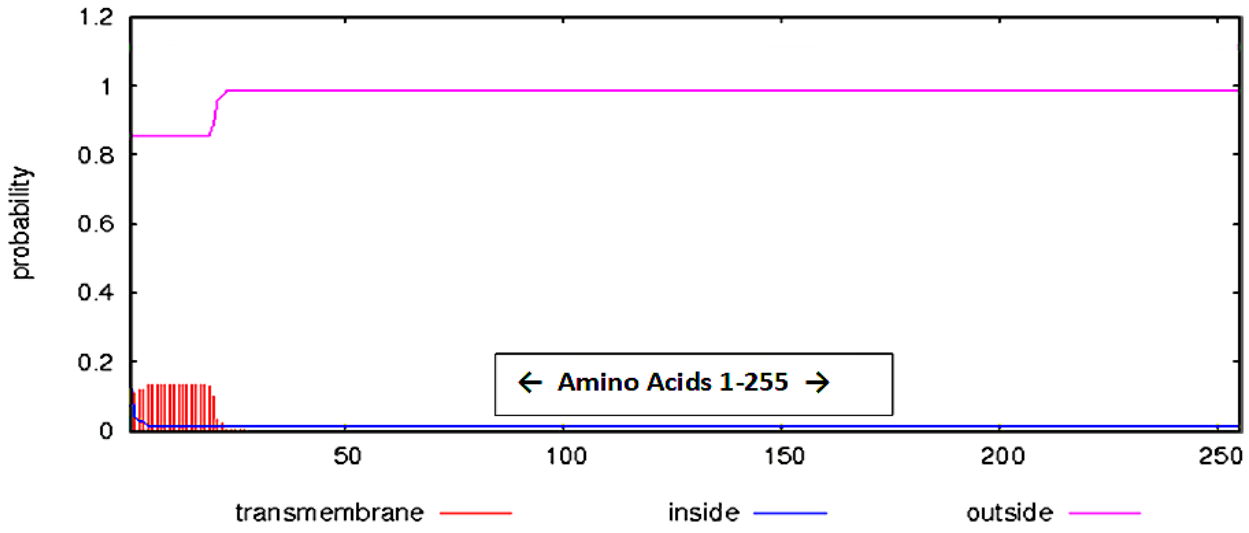
- Various program algorithms predicted a transmembrane domain and/or signal peptide for labyrinthin that coincides with N-myristoylation to support N-terminus membrane localization as a type II protein [6, 7] and a calcium receptor. Labyrinthin may thus be key to explaining the long-known phenomena of elevated calcium and irregular signaling in cancer cells [8, 9]; the data provide a basis to elucidate the mechanism of labyrinthin action in adenocarcinomas.
- FACS analysis of intact cells supports the notion of labyrinthin as a pan-adenocarcinoma target and showed: 1) A549 and WI-38 cells as labyrinthin positive and negative controls, respectively; and that 2) normal human primary cultures do not have labyrinthin.
- Though no extracellular protein similar to adenocarcinoma-specific labyrinthin has been identified by protein Basic Local Alignment Search Tool (U.S. National Library of Medicine; Request I.D. UUSZ7ZY4013, Job Title:gb AFL00846.1), preliminary findings with SCC40 tongue squamous cell carcinoma and MCF7 mammary adenocarcinoma cells may either represent anomalies or reflect true rare exceptions of labyrinthin expression that may occur in nature.
- The data raise a concern that many of the research approaches that led to the conclusion about ASPH as a cancer target [10, 11] may unwittingly have been studying labyrinthin due to shared epitopes and nucleotide sequences (for over/underexpression studies) that likely affected labyrinthin. This is particularly timely because ASPH and labyrinthin-based treatments have already been FDA approved for clinical trials.
- The data provide a basis to explore distinctions between ASPH and labyrinthin such as localization (which affects diagnostic and therapy strategies) with developed tools (e.g., antibodies, sense/antisense sequences) for each of the respective proteins.

1. Data description

A

TOPOLOGY	PREDICTED AMINO ACIDS & COMMENTS	PROGRAM
Calcium binding: EF-hand domain Calcium receptor	 a.a. 32-55 a.a. 33-44*	ScanProsite
N-myristoylation sites	a.a. 7-12, 30-35, 215-220	ScanProsite
Casein kinase II phosphorylation sites	a.a. 55 - 58, 70 - 73, 132-135, 146-149, 173, 176	ScanProsite
	TM a.a. 1-17; strongly preferred model: N-terminus inside/C-terminus outside	TMPred
	TM indeterminate; signal peptides identified between a.a. 2-18	Quick2D

Transmembrane domain	TM predicted but sequence indeterminate; labyrinthin mostly outside with signal peptides in a.a. 1-17 identified	Protter
	TM indeterminate	PROTEUS2
	TM 2-16; C-Terminus cytoplasmic	MemBrain
	TM 2-20; C-terminus outside (high probability)	TMHMM (see plot below)



B **LABYRINTHIN - JUNCTATE HOMOLOGY**

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MAEDKETKHGGHKNGRKGGLSGTSFFTWFMVIALLGVWTSVAVVWFDLVDYEEVLAKAKDFRYNLSEVLQ  70
GKLGIYDADGDGDFDVDDAKVLLGLKERSTSEPAVPPEEAEPHTEPEEQVPVEAEPQNIEDEAKEQIQSL 140
LHEMVHAEHVEGEDLQQEDGPTGEPQQEDDEFLMATDVDDRFETLEPEVSHEETEHSYHVEETVSQDCNQ 210
DMEEMMSEQENPDSSEPVVEDERLHHDTDDVTYQVYEEQAVYEPLENEGIEITEVTAPPEDNPVEDSQVI 280
VEEVSIFPVEEQQEVPPDT                                                    299
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C **LABYRINTHIN - ASPH HOMOLOGY**

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MAQRKNAKSSGNSSSSGSGSGSTSAGSSSPGARRETKHGGHKNGRKGGLSGTSFFTWFMVIALLGVWTSV 70
AVVWFDLVDYEEVLGKLGIYDADGDGDFDVDDAKVLLGLKERSTSEPAVPPEEAEPHTEPEEQVPVEAEP 140
QNIEDEAKEQIQSLLHEMVHAEHVEGEDLQQEDGPTGEPQQEDDEFLMATDVDDRFETLEPEVSHEETEH 210
SYHVEETVSQDCNQDMEEMMSEQENPDSSEPVVEDERLHHDTDDVTYQVYEEQAVYEPLENEGIEITEVT 280
APPEDNPVEDSQVIVEEVSIFPVEEQQEVPPETNRKTDDPEQKAKVKKKKPKLLNKFDKTIKAELDAAEK 350
LRKRGKIEEAVNAFKELVRKYPQSPRARYGKAQCEDDLAEKRRSNEVLRGAIETYQEVASLPDVPADLLK 420
LSLKRRSDRQQFLGHMRGSLLTLQRLVQLFPNDTSLKNDLGVGYLLIGDNDNAKKVYEEVLSVTPNDGFA 490
KVHYGFILKAQNKIAESIPYLKEGIESGDPGTDDGRFYFHLGDAMQRVGNKEAYKWYELGHKRGHFASVW 560
QRSLYNVNGLKAQPWWTPKETGYTELVKSLERNWKLIRDEGLAVMDKAKGLFLPEDENLREKGDWSQFTL 630
WQQGRRNENACKGAPKTCTLLEKFPETTGCRRGQIKYSIMHPGTHVWPHTGPTNCRLRMHLGLVIPKEGC 700
KIRCANETKTWEEGKVLIFDDSFEHEVWQDASSFRLIFIVDVWHPELTPQQRRSLPAI             759
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Fig 1. Labyrinthin topology and amino acid sequence comparisons with junctate and ASPH.

(A) Labyrinthin topology as analyzed by several computer programs with different areas of focus, each having their own distinct advantages. The TMHMM plot is shown because it most closely depicts a composite of the results.

(B) Junctate, a member of the ASPH gene family, is compared with labyrinthin. All 255 labyrinthin amino acids (bold lettering) are contained within junctate.

(C) Other than a substitution at ASPH position 312 (glutamic acid instead of aspartic acid), there are no sequences in labyrinthin (bold lettering) that are not contained within ASPH (a.k.a., AAH, HAAH).

(B) & (C) Epitopes for mouse monoclonal anti-labyrinthin antibody MCA 44-3A6 [12] and the widely used FB-50 [10, 11] are shown in red and blue, respectively. RK/KR and KKXX motifs essential for ER targeting of proteins, and RR motifs that maintain type II proteins in the ER [13, 14, 15] are underlined (bars).

*Partial sequence identified

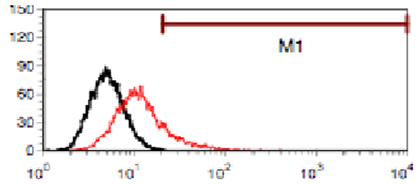
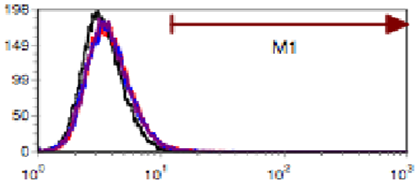
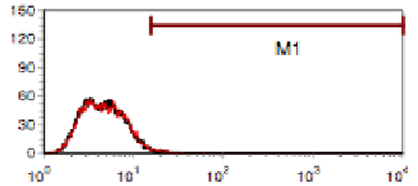
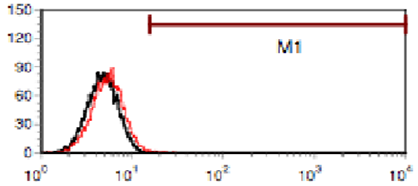
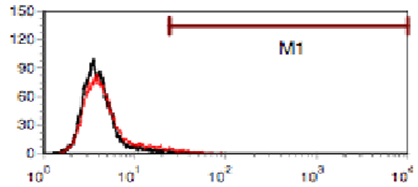
	Extracellular
Positive control A549 *Source ATCC*	
Negative control WI-38 *Source ATCC*	
Normal primary cell culture Human astrocytes *Source Cambrex/Lonza*	
Normal primary cell culture Renal proximal tubule epithelial cells *Source Cambrex/Lonza*	
Normal primary cell culture Small airway epithelial cells *Source Cambrex/Lonza*	

Fig. 2. Selectivity of cell surface adenocarcinoma marker labyrinthin by FACS analysis.

The presence or absence of labyrinthin on non-permeabilized human A549 lung adenocarcinoma and normal cells is shown. Because adenocarcinomas are neoplasia of epithelial tissue that have glandular origin, normal primary cultures (Cambrex/Lonza Bioscience) of human astrocytes, renal proximal tubule epithelial cells, and small airway epithelial cells were selected as counter models of study. Experiments were performed by a third-party (Stanford Research Institute International, Menlo Park, CA) for independent confirmation. Tracings represent results from at least duplicate preparations for each of the cell cultures.

It should be noted that preliminary studies for the above included non-adenocarcinoma cancer cell lines. Unexpectedly, SCC40 tongue squamous cell carcinoma had 8.8% labyrinthin-positive cells and MCF7 mammary adenocarcinoma was negative. However, consistent with the idea that labyrinthin is selectively located on adenocarcinoma cells, all other non-adenocarcinoma cells were negative (BT 20 mammary gland carcinoma, T47D mammary gland ductal carcinoma, and SC15 squamous cell lung carcinoma; data not shown).

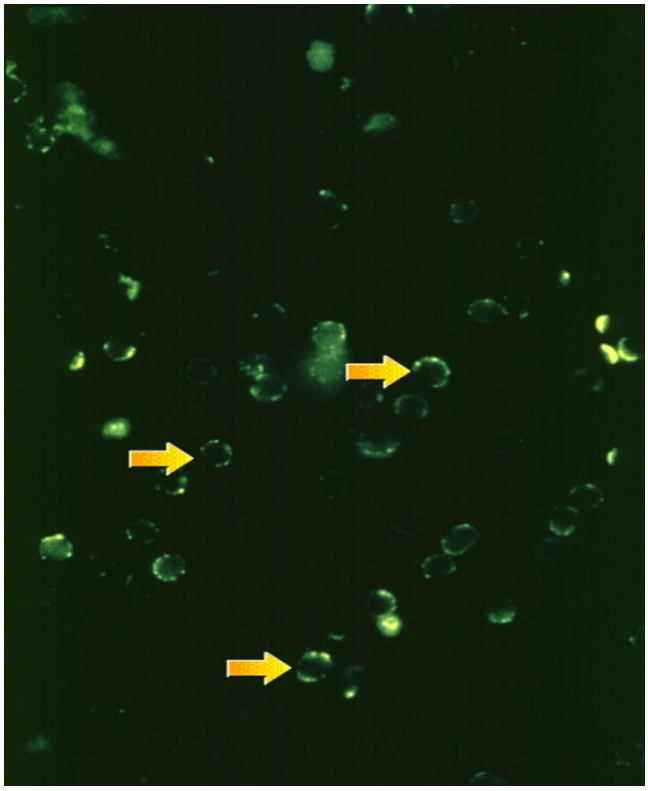


Fig. 3. Immunofluorescence of MCA 44-3A6 Binding to A549 Cells: Cell cycle and Surface localization. A549 cells at random stages of their cell cycle were incubated with immunofluorescent labelled MCA 44-3A6 antibody and examined by fluorescence microscopy (100x shown). The cells were not permeabilized in order to visualize labyrinthin location via antibody-targeted cell surface epitopes. Intense staining is noted on cell surfaces (arrows) with some internalization of antibody and/or antibody-labyrinthin. Magnification 20x.

2. Experimental design, materials and methods

2.1. Topology analysis

Sequences for labyrinthin, junctate and ASPH were obtained from the National Center for Biotechnology Information. The following online tools were used to analyse various topology characteristics: ScanProsite, TMPred, Quick2D, Protter, PROTEUS2, MemBrain, TMHMM v2.0.

2.*2. Fluorescent activated cell sorter analysis*

FACS was performed independently (Stanford Research Institute International; California, USA)

2.2.1. Reagents and cell lines

Mouse monoclonal anti-labyrinthin antibody MCA 44-3A6 was provided by the current lab [2] as grown from standard hybridoma technology and purified by 50% saturated ammonium sulphate precipitation and ion exchange chromatography or by a commercial kit (Pierce™ Antibody Clean-up Kit).

Cytofix/Cytoperm solution, Perm/Wash buffer, biotinylated goat anti-mouse antibody, FITC-conjugated goat anti-mouse antibody, FITC-conjugated streptavidin, and propidium iodide were obtained from Becton Dickinson. BSA, trypan blue, and sodium azide were obtained from Sigma, and PBS was purchased from Media Tech. Cell lines were obtained and grown as recommended by the American Type Culture Collection or provided by Cabrex/Lonza. In addition to the respective labyrinthin-positive and negative A549 human lung adenocarcinoma and WI-38 normal human lung fibroblast cells, BT 20 mammary gland carcinoma, T47D mammary gland ductal carcinoma, SSC15 squamous cell lung no-adenocarcinoma, MCF7 mammary adenocarcinoma, and SCC40 tongue squamous cell carcinoma cells were used in preliminary studies.

2.2.2. Cell Staining and Flow Cytometry

Cells were counted by hemacytometer using trypan blue to visualize dead cells and cells were stained at 500,000 cells/tube. For extracellular staining, cells were incubated with indicated concentrations/dilutions of 44-A36 antibody for 30 min on wet ice and then washed twice with PBS containing 1% BSA and 0.05% sodium azide (FACS buffer). Cells were then incubated with indicated concentrations of biotinylated goat anti-mouse antibody for 30 min on wet ice (in some experiments, biotinylated goat antibody was diluted in normal goat serum). After washing twice with FACS buffer, cells were incubated with indicated concentrations of FITC-conjugated streptavidin for 30 min on wet ice, followed by washing twice with FACS buffer. Propidium iodide was added to cells immediately before FACS analysis. Unstained controls were not incubated with 44-A36 antibody, but were incubated with all subsequent antibodies.

2.2.3. FACS cell labeling confirmation and detection of labyrinthin at various cell cycle stages.

Cells used in previous FACS studies [2] were examined to 1) confirm sufficient labeling and 2) observe labyrinthin localization at random stages of the cell cycle. A sample of A549 cells grown to 50-80 confluence and prepared for FACS analysis were viewed under a glass coverslip by fluorescence microscopy. Images were obtained 20-30 minutes following antibody incubation and cell washing.

Ethical Statement

Not applicable.

CRediT author statement

Michael Babich: Conceptualization, Methodology, Software-based analysis, Writing the final draft. **Ankit Sharma**: Data curation, Writing initial draft, Reviewing and editing, Reference work. **Tianhong Li**: Investigation, Research direction. **James A. Radosevich**: Supervision, Experiments, Reviewing.

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Declaration of interests

Michael Babich and James Radosevich are principals, and Ankit Sharma is a research fellow, at LabyRx Immuno-Oncology Limited.

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