COMMUNICATIONS IN PROLACTIN RESEARCH

DATA REPORT

Sequencing Report of a Monoclonal Vasoinhibin Antibody

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Abstract

Background: Vasoinhibin is an antiangiogenic fragment of the pituitary hormone prolactin which is of interest in diseases of the eye, the heart, and pregnancy complications. This is the first report of the sequence of a monoclonal antibody against vasoinhibin.
Methods: Whole transcriptome shotgun sequencing of a hybridoma cell culture producing a monoclonal anti-vasoinhibin antibody was performed and the protein sequences of the complementarity determining regions were determined by Kabat, IMGT and Chotia definitions.
Results: A mouse IgM-kappa antibody was identified, and the complete protein sequences were determined. The complementarity determining regions were identified according to Kabat, IMGT, and Chotia definitions.
Conclusions: The sequences of the antibody can be used for its recombinant production and antibody engineering.

Keywords
16 K PRL, 16-kDa PRL, prolactin, vasoinhibin, monoclonal antibody

Abbreviations
vi-mab, monoclonal anti-vasoinhibin antibody; CDRs, complementarity determining regions; HC, heavy chain; LC, light chain; VH, variable heavy; VL, variable light

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Introduction

Vasoinhibin, an antiangiogenic fragment of the pituitary hormone prolactin (PRL), is of interest in the context of proliferative retinopathies (1, 2), retinopathy of prematurity (3), peripartum cardiomyopathy (4, 5) and preeclampsia (6, 7). Vasoinhibin has also been referred to as 16 K PRL or 16-kDa PRL (8). Monoclonal antibodies against vasoinhibin without cross-reactivity to PRL have been generated recently and have been shown to be useful for immunoneutralization and employment in a sandwich ELISA (7, 9). The present study reports the full protein sequence of a monoclonal anti-vasoinhibin antibody which can be used for the recombinant production of the antibody (subject to licensing) and antibody engineering procedures. The vi-mab (monoclonal anti-vasoinhibin antibody), produced by the hybridoma technique, is commercially available at Davids Biotechnologie GmbH (Regensburg, Germany).

Materials and Methods

Sequencing of hybridoma cells expressing a vi-mab, generated as reported earlier (9), was performed by whole transcriptome shotgun sequencing (RNA-Seq). This is a proprietary approach to antibody sequencing developed exclusively at Absolute Antibody (Oxford, UK). Total RNA was extracted from cells and a barcoded cDNA library generated through RT-PCR using a random hexamer. Next Generation Sequencing was performed on an Illumina HiSeq sequencer. Contigs were assembled using a proprietary approach and data were mined for antibody sequences identifying all viable antibody sequences (i.e. those not containing stop codons). Variable heavy and variable light domains were identified separately and relative abundance of each identified gene was reported in transcripts per million (TPM). The species and isotype of the identified antibody genes were confirmed. Sequences were compared with known aberrant (i.e. non-functional) antibody genes that are present in many hybridomas and these genes were removed from analysis. For each chain the variable domain is reported along with the constant domain. The heavy chain and light chain type was identified based on the sequence of the constant domain. For each heavy and light chain two measurements of abundance are reported. TPM (transcripts per million) is an abundance measurement from next generation sequencing which scores the relative abundance of each gene in the whole transciptome pool. The percent column

converts the TPM into a percentage antibody gene abundance. This enables simple identification of high and low abundance antibody genes. The data is automatically filtered to only report antibody genes with an abundance of greater than 1%. The complementarity determining regions (CDRs) have been automatically identified on a 'CDR identification' worksheet using Excel formulas working to the Kabat, IMGT and Chotia definition for CDRs. CDR identification was only performed for the primary VH and VL sequences.

Results

The complete sequencing of the primary heavy and light chain demonstrated the protein sequence of a HC and LC of a mouse IgM-kappa antibody (Table 1). The CDRs were determined according to Kabat, IMGT and Chotia and constitute the sequences presented in Table 2.

Discussion

The vi-mab, the sequence of which is shown here, is the first vasoinhibin-specific monoclonal antibody reported. The antibody originates from a mouse, which was injected with an immunizing peptide comprising amino acids 40–58 of mature human PRL (FDKRYTHGRGFITKAINSC) and subsequent monoclonal antibody production by the hybridoma technique. The sequences of the antibody can be used for its recombinant production and antibody engineering, such as species switch, isotype switch, subtype switch, reformatting to scFv, Fab, engineered Fc, or bispecificity.

Table 1 Protein sequences of HC and LC variable and constant regions.

Protein sequence	TPM[1]	%[2]
Primary HC sequence, Mouse IgM, variable: EVKLVESGGGLVQPGGSLRLSCATSGFTFTDYYMSWVRQPPGKALEWLGFIRNKANGY TTEYSASVKGRFTISRDNSQSILYLQMNTLRAEDSATYYCARDRGYYDYWGQGTTLTVSS	16294.85	6.42
Primary HC sequence, Mouse IgM, constant: ESQSFPNVFPLVSCESPLSDKNLVAMGCLARDFLPSTISFTWNYQNNTEVIQGIR TFPTLRTGGKYLATSQVLLSPKSILEGSDEYLVCKIHYGGKNRDLHVPIPAVAE MNPNVNVFVPPRDGFSGPAPRKSKLICEATNFTPKPITVSWLKDGKLVESGFTT DPVTIENKGSTPQTYKVISTLTISEIDWLNLNVYTCRVDHRGLTFLKNVSSTCAA SPSTDILTFTIPPSFADIFLSKSANLTCLVSNLATYETLNISWASQSGEPLETKIKIM ESHPNGTFSAKGVASVCVEDWNNRKEFVCTVTHRDLPSPQKKFISKPNEVHKH PPAVYLLPPAREQLNLRESATVTCLVKGFSPADISVQWLQR GQLLPQEKYVTSAPMPEPGAPGFYFTHSILTV TEEEWNSGETYTCVVGHEALPHLVTERTVDKSTGKPTLYNVSLIMSDTGGTCY		
Primary LC sequence, Mouse kappa, variable: DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYK VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLEIK	237588.64	93.58
Primary LC sequence, Mouse kappa, constant: RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLN SWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC		

[1]transcripts per million; [2]percentage antibody gene abundance.

Table 2 CDRs of the vi-mab according to Kabat, IMGT and Chotia.

	Kabat	IMGT	Chothia
CDR-H1	DYYMS	GFTFTDYY	GFTFTDY
CDR-H2	FIRNKANGYTTEYSASVKG	IRNKANGYTT	RNKANGYT
CDR-H3	DRGYYDY	ARDRGYYDY	DRGYYDY
CDR-L1	RSSQSLVHSNGNTYLH	QSLVHSNGNTY	RSSQSLVHSNGNTYLH
CDR-L2	KVSNRFS	KVS	KVSNRFS
CDR-L3	SQSTHVPWT	SQSTHVPWT	SQSTHVPWT

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The antibody has been tested in a sandwich ELISA to determine vasoinhibin concentrations in aqueous solutions and human serum (7, 10). A recommended concentration for coating a microplate is 4 μg/ml. The antibody is suitable for neutralizing the antiangiogenic activity of vasoinhibin (7); this feature is expected as the immunizing peptide includes the HGR-motif, which is the antiangiogenic determinant of vasoinhibin (11).

Review Board Statement

The animal studies have been registered, reviewed and approved in 2019 by the government of Lower Franconia in Würzburg, Germany.

Conflict of Interest Statement

The vi-mab and the sequence information are subjects of an international patent application filed by J.T. (international application no.: PCT/EP2024/073698) at the European Patent Office. The commercial production and distribution of the antibody has been licensed to Davids Biotechnologie GmbH, Regensburg, Germany (website: https://davids-science.de/).

References

1. Triebel J, Bertsch T, Clapp C. Prolactin and vasoinhibin are endogenous players in diabetic retinopathy revisited. Frontiers in Endocrinology. 2022;13:994898. DOI: 10.3389/fendo.2022.994898
2. Triebel J, Robles-Osorio ML, Garcia-Franco R, Martinez de la Escalera G, Clapp C, Bertsch T. From bench to bedside: Translating the prolactin/vasoinhibin axis. Frontiers in Endocrinology. 2017;8:342. DOI: 10.3389/fendo.2017.00342
3. Zepeda-Romero LC, Vazquez-Membrillo M, Adan-Castro E, Gomez-Aguayo F, Gutierrez-Padilla JA, Angulo-Castellanos E, et al. Higher prolactin and vasoinhibin serum levels associated with incidence and progression of retinopathy of prematurity. Pediatric Research. 2017;81(3):473–9. DOI: 10.1038/pr.2016.241
4. Hoes MF, Arany Z, Bauersachs J, Hilfiker-Kleiner D, Petrie MC, Sliwa K, et al. Pathophysiology and risk factors of peripartum cardiomyopathy. Nature Reviews Cardiology. 2022;19(8):555–65. DOI: 10.1038/s41569-021-00664-8
5. Triebel J, Clapp C, Martinez de la Escalera G, Bertsch T. Remarks on the prolactin hypothesis of peripartum cardiomyopathy. Frontiers in Endocrinology. 2017;8:77. DOI: 10.3389/fendo.2017.00077
6. Lenke L, Martinez de la Escalera G, Clapp C, Bertsch T, Triebel J. A dysregulation of the prolactin/vasoinhibin axis appears to contribute to preeclampsia. Frontiers in Endocrinology. 2019;10:893. DOI: 10.3389/fendo.2019.00893
7. Zamora M, Harris D, Davies N, Ebnet J, Radermacher P, Brucker C, et al. Immunometric and functional measurement of endogenous vasoinhibin in human sera. Frontiers in Endocrinology. 2024;15:1345996. DOI: 10.3389/fendo.2024.1345996
8. Triebel J, Robles JP, Zamora M, Martinez de la Escalera G, Bertsch T, Clapp C. Regulator of angiogenesis and vascular function: A 2019 update of the vasoinhibin nomenclature. Frontiers in Endocrinology. 2019;10:214. DOI: 10.3389/fendo.2019.00214
9. Muller N, Robles JP, Zamora M, Ebnet J, Markl-Hahn H, Martinez de la Escalera G, et al. Development of vasoinhibin-specific monoclonal antibodies. Frontiers in Endocrinology. 2021;12:645085. DOI: 10.3389/fendo.2021.645085
10. Triebel J, Harris D, Davies N, Ebnet J, Neugebauer L, Friedrich C, et al. Mass spectrometric and immunologic detection of prolactin-derived vasoinhibin in human serum. Clinical Laboratory. 2024;70(11). DOI: 10.7754/Clin.Lab.2023.240635
11. Robles JP, Zamora M, Siqueiros-Marquez L, Adan-Castro E, Ramirez-Hernandez G, Nunez FF, et al. The HGR motif is the antiangiogenic determinant of vasoinhibin: Implications for a therapeutic orally active oligopeptide. Angiogenesis. 2022;25(1):57–70. DOI: 10.1007/s10456-021-09800-x