Gene Expression Patterns 10 (2010) 53–59

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Expression of the AMACO (VWA2 protein) ortholog in zebrafish

Jan M. Gebauer [a], Kristina Røkenes Karlsen [b], Wolfram F. Neiss [b], Mats Paulsson [a,c,d], Raimund Wagener [a,*]

[a] Center for Biochemistry, University of Cologne, D-50931 Cologne, Germany
[b] Department of Anatomy I, Medical Faculty, University of Cologne, D-50931 Cologne, Germany
[c] Center for Molecular Medicine Cologne (CMMC), University of Cologne, D-50931 Cologne, Germany
[d] Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, D-50931 Cologne, Germany

ARTICLE INFO

Article history:
Received 4 March 2009
Received in revised form 20 October 2009
Accepted 21 October 2009
Available online 25 October 2009

Keywords:
AMACO
VWA2
VWA domain
EGF-like domain
Basement membrane
Myoseptum
Pronephros
Zebrafish
Danio rerio

ABSTRACT

AMACO is a basement membrane associated protein that belongs to the VWA domain-containing protein superfamily. In addition to three VWA domains it contains two EGF-like domains, a cysteine-rich domain and a unique domain. Mouse AMACO has been partially characterized, but its function remains unknown. The zebrafish genome contains a single AMACO ortholog gene on chromosome 12. The domain structure is completely conserved between zebrafish and mouse and the first EGF-like domain, carrying a rare *O*-glucosylation and *O*-fucosylation consensus sequence, has the highest identity at the protein level. RT-PCR shows strongest AMACO expression during development, starting at the 5 somite stage. An antibody specific for zebrafish AMACO detected expression mainly in myosepta but also in skin, pronephros, pituitary gland, otic capsule and gills. *In situ* hybridization revealed that the muscle precursor cells of the somites express the protein that is laid down in the myosepta.

1. Results and discussion

AMACO (VWA2 protein) is a member of the von Willebrand factor A (VWA) domain-containing protein superfamily (Whittaker and Hynes, 2002). The protein consists of an N-terminal VWA domain that is followed by a cysteine-rich domain, an epidermal growth factor (EGF)-like domain and two more VWA domains. At the C-terminus another EGF-like domain and a unique domain are present (Sengle et al., 2003). Recently, an *O*-glucosylation and *O*-fucosylation was detected on the first EGF-like domain (Gebauer et al., 2008) and it was shown that both fully elongated glycan chains are attached in close proximity on the same EGF-like domain. The function of this rare glycosylation remains unclear and, in contrast to in thrombospondin 1 (Wang et al., 2007), secretion efficiency is not affected by lack of glycosylation (Gebauer et al., 2008). Mouse AMACO has a very restricted expression pattern. It is present in mouse kidney, lung, choroid plexus and skin and is associated with certain basement membranes that underlie epithelial cells (Sengle et al., 2003; Gebauer et al., 2009). Mouse AMACO supports integrin-mediated cell attachment, but as the RGD motif responsible for this activity is not conserved in humans this is probably not a major role of AMACO (Gebauer et al., 2009). Human AMACO was alternatively named CCSP-2 (colon cancer secreted protein-2), as it was shown to be highly up-regulated in colon cancer. In addition, epitope tagged AMACO was detected in blood of mice bearing transfected human cancer xenografts, indicating a potential use of AMACO as a serum marker for colon cancer (Xin et al., 2005). Further, polymorphisms in the AMACO gene were shown to be associated with dominant protection against type 1A diabetes (Eller et al., 2004). The zebrafish (*Danio rerio*) is a powerful model organism for the study of vertebrate development. The embryos develop rapidly, with all organs having been formed by 72 h post fertilization. The externally developing embryos are produced in large numbers, are optically clear and can be monitored by simple microscopic observation. Here, we describe the expression of the AMACO ortholog gene in zebrafish by RT-PCR, *in situ* hybridization and immunohistochemistry. The comprehensive study of zebrafish AMACO expression forms the basis for future studies of AMACO function in the zebrafish system.

1.1. Cloning and bioinformatical analysis of zebrafish AMACO

In a BLAST search with the murine AMACO sequence as query the zebrafish AMACO gene was identified. The corresponding cDNA was partially cloned by RACE and RT-PCR and remaining sequences

* Corresponding author. Address: Institute for Biochemistry II, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, D-50931 Cologne, Germany. Tel.: +49 221 478 6990; fax: +49 221 478 6977.
E-mail address: raimund.wagener@uni-koeln.de (R. Wagener).

1567-133X/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2009.10.005

deduced from the genomic sequence. The cDNA has an open reading frame of 2385 bp, encoding a protein consisting of 795 amino acid residues (Fig. 1). The prediction of the signal peptide using neural networks (NN) and hidden Markov models (HMM) trained

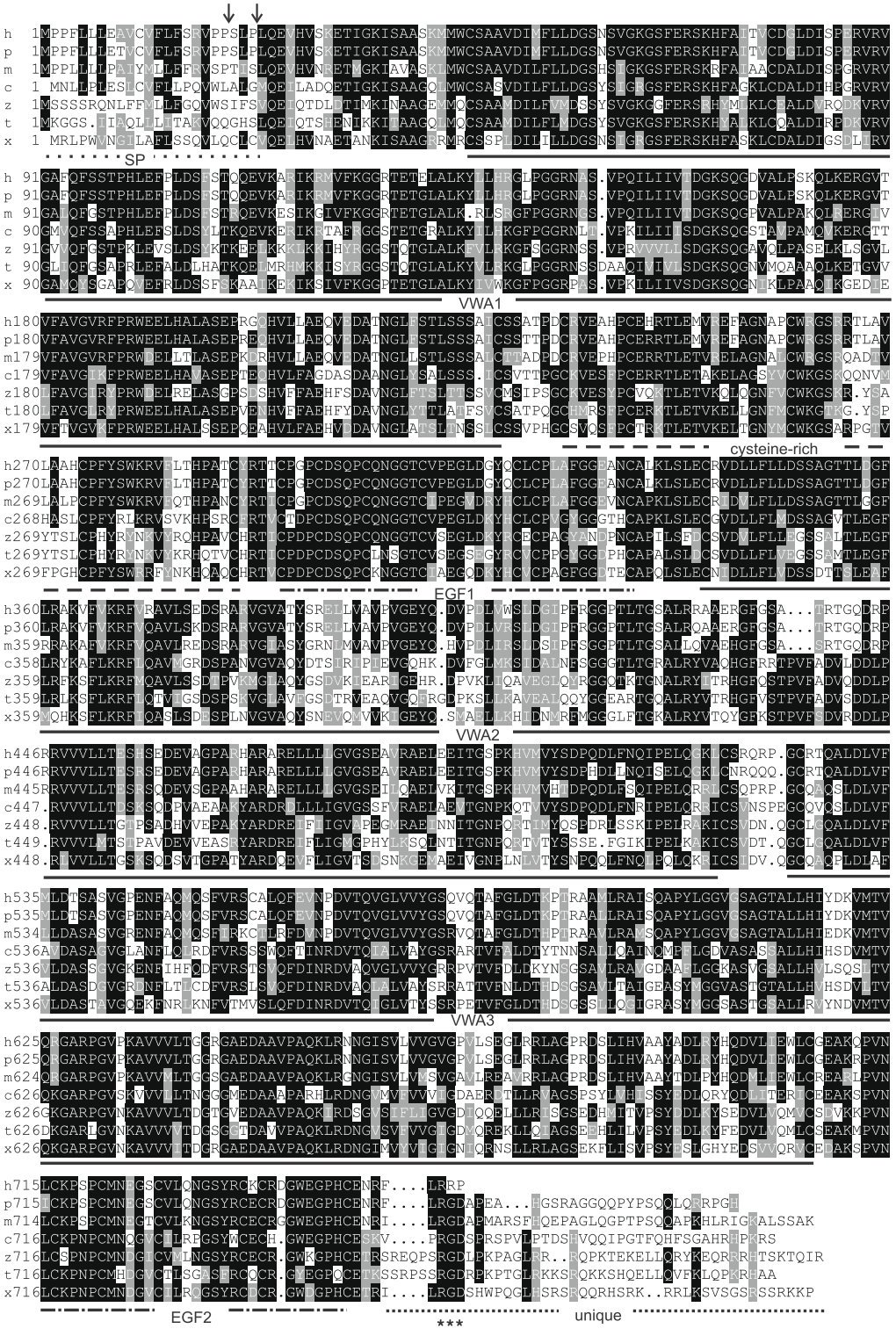

Fig. 1. Amino acid sequence alignment of zebrafish with those of other vertebrates. The amino acid sequences of human (h), chimpanzee (p), mouse (m), chicken (c) zebrafish (z), *Tetraodon nigroviridis* (t) and *Xenopus tropicalis* (x) are shown. The zebrafish amino acid sequences were deduced from cDNA sequences deposited in the database under Accession No. FJ797566 and the zebrafish genome project, respectively. The other sequences were obtained from the NCBI database http://www.ncbi.nlm.nih.gov/: *Tetraodon nigroviridis*, CAG12610, completed by the signal peptide sequence deduced from the genomic sequence; human, CAD60276; chimpanzee, Map Viewer Gnomon model: hmm55852, amino acid residues 520–523 were corrected using genomic sequence information; chicken, XP_421769.2 corrected using sequence information from EST BU271772.1; *Xenopus laevis*, Q6DCQ6. Solid bars mark the position of the VWA domains; dashed bars indicate the EGF-like domains and the cysteine-rich domain and dotted bars indicate the signal peptide and the unique domain. Arrows mark the predicted signal peptide cleavage sites in zebrafish. Note that the second site is predicted in all other species. The conserved RGD-motif is indicated by asterisks.