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Technical report

Characterisation of the CD5 cDNA sequence from the tammar wallaby (*Macropus eugenii*)

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ABSTRACT

CD5 has previously been identified in marsupial tissues using anti-human CD5. However, despite the known cross-reactivity of the antibody in marsupial tissues, the cDNA sequence has not previously been characterised in any marsupial. This study has identified the CD5 gene in the opossum genome database and has characterised the CD5 cDNA sequence from the tammar wallaby. Both marsupial CD5 sequences have a high level of sequence identity to known eutherian CD5 sequences, are cysteine-rich and have identical structural motifs to their eutherian homologs. CD5 transcripts were strongly expressed in adult tammar wallaby spleen, mammary node and blood, and expressed at a lower level in liver, kidney and heart tissues. Characterisation of CD5 in marsupials allowed a comparison to the epitope sequence of anti-human CD5 and showed a high level of sequence identity.

Marsupials offer a unique mammalian model (Selwood and Coulson, 2006) to study the development of the immune system as marsupials are born with tissues in an immunologically immature state compared to their eutherian counterparts. To date, human anti-CD5 is one of the most successful and widely used cross-species reactive antibodies used by marsupial immunologists and pathologists (Jones et al., 1993; Hemsley et al., 1995; Old and Deane, 2001; Young and Deane, 2003). In eutherians, CD5 plays an important role in thymocyte selection through the T-cell receptor-ligand affinity (Tarakhovsky et al., 1995) and to regulate the immune response (Biancone et al., 1996) however little is known about the role it plays in marsupials. This study aimed to elucidate the cDNA sequence of a metatherian CD5 to assess its similarity to the known eutherian CD5 sequences. It is hoped that by identifying the marsupial CD5 cDNA sequence, and the epitope sequence in marsupials, it will increase the successful development of future antibodies for marsupial immune studies.

Adult tammar wallaby tissues were collected opportunistically from animals housed at Macquarie University Fauna Park (North Ryde, NSW, Australia) that had been euthanased as part of other experimental work. Total RNA was isolated from spleen tissue using TRI reagent (Molecular Research Centre, OH, USA) according to the manufacturer's protocol. cDNA for RT-PCR was synthesized using SuperScript II reverse transcription system (Invitrogen, CA, USA). RT-PCR was performed using degenerate consensus primers designed from an alignment of known eutherian CD5 sequences and the Ensembl opossum CD5 sequence. The primer sequences were 5′-AGAARAAGCARCGYCARTGGAT-3′ and 5′-CTGTCRGAGGARTTRTC-3′ (R = A/G, Y = C/T). The PCR reaction was performed using Taq polymerase (Promega, WI, USA) under the following conditions: 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s and then a final elongation at 72 °C for 10 min.

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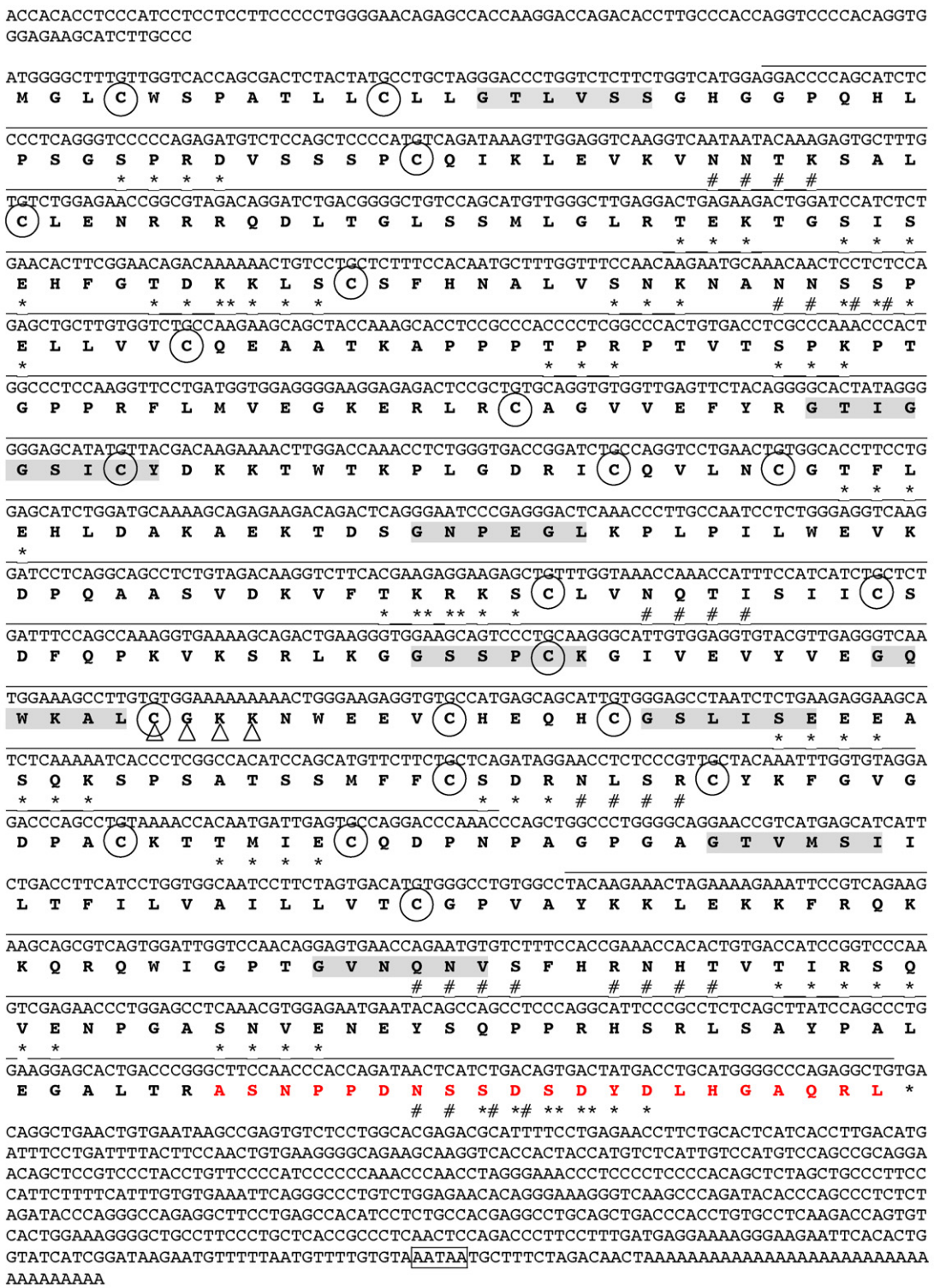

Fig. 1. Tammar wallaby CD5 cDNA and deduced amino acid sequence. The amino acid sequence is printed in bold. The poly A tail signal is boxed and cysteines are circled. The putative leader sequence is 23 amino acids in length. The region consisting of 328 amino acids after the putative leader sequence and indicated by the line above the cDNA sequence is the extracellular domain. The third region of 30 amino acids is the transmembrane domain and the last portion of the sequence of 94 amino acids is the cytoplasmic domain (indicated with the second line above the cDNA sequence). Phosphorylation sites are indicated with stars(*) and N-glycosylation sites are denoted by hashes(#). N-myristoylation sites are shaded. Potential amidation sites are marked with arrows. The red amino acid sequence corresponds to the portion of the human CD5 epitope sequence. Genbank accesssion: EU586111. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)