

VB1-008: Identification of a fully-human antibody specific for CD44E and CD44-isoform-3

Francina Chahal^a, Jeannick Cizeau^a, , Nick Glover^b, and Glen C. MacDonald^a.

^aViventia Biotech Inc. 147 Hamelin St., Winnipeg, MB, Canada, R3T 3Z1; ^b5060 Spectrum Way, Suite 405, Mississauga, ON, L4W 5N5

ABSTRACT

During the course of their disease, cancer patients often produce antibody responses to their tumors. Humoral tumor immunity has been correlated, in some cases, with a better prognosis and longer survival. Viventia's product development platforms, Hybridomics™, ImmunoMine™, and UnLock™, have been designed to identify patient tumor-specific antibodies and their cognate tumor-associated antigen. One hybridoma-derived antibody, VB1-008, an IgG MAb, generated from the PBLs of a breast cancer patient, demonstrates preferential binding to the breast cancer tissues/cell lines as tested by immunohistochemistry and flow cytometry with limited normal tissue reactivity. Immunoprecipitation with tumor cell membrane fractions from VB1-008-reactive cell lines showed VB1-008 to react with a ~110 kDa protein under mild reducing conditions that further resolved over time into one ~50 kDa band following 1D and two spots varying in pI upon 2D-PAGE analysis. LC-MS/MS analysis of the 1D and 2D spots identified CD44-isoform-3/CD44E and a truncated version of alpha-fetoprotein (AFP). More specifically, with the use of overlapping peptides, VB1-008 reactivity was restricted to the peptide sequence-TNMDSSH, from the unique region constituting the junction of Exon 5 and the variable region, v8 of CD44-isoform-3 and CD44E. CD44E and CD44-isoform-3 differ from each other by only 2 amino acid changes at positions 221 and 230; however, conserving the epitope, TNMDSSH, in both. A synthetic peptide representing the sequence, TNMDSSH, conserved in both isoforms, competes 96% of the original binding by VB1-008. A similar peptide of the same length generated according to the sequence of variable regions v7-v8 of a parent isoform, CD44-isoform 2, MDMDSSH, showed no binding or competition to VB1-008. Extensive analysis by flow cytometry demonstrated that AFP was not present on the cell surface suggesting that this truncated version of AFP was associated with CD44 at the cytosol/membrane interface. The known over-expression of CD44E and CD44-isoform-3 with certain cancers validates the "mining" of the human immune response for the identification of fully-human antibodies specific for drugable cell surface tumor-associated target antigens.