Immunohistochemical characterization of novel murine monoclonal antibodies against human placenta-specific 1

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Abstract

Human *PLAC1* (placenta-specific 1) is a new member of cancer–testis antigens with 212 amino acids, and its expression is restricted to placenta and at much lower levels to testis. Recently, ectopic expression of the PLAC1 transcript has been demonstrated in a wide range of human tumors and cancer cell lines with a proposed function in tumor cell growth. No monoclonal anti-PLAC1 antibody applicable to immunohistochemical staining is available so far. To better understand the PLAC1 expression and localization, we aimed to produce monoclonal antibodies (mAbs) against the extracellular region of PLAC1. Mice were immunized with a synthetic peptide corresponding to the C-terminal 11 amino acids of PLAC1 conjugated with a carrier protein. Hybridomas were produced by standard protocol and screened for positive reactivity by enzyme-linked immunosorbent assay. Reactivity of final two clones was then assessed by Western blotting (WB), immunohistochemistry (IHC), and immunocytochemistry (ICC). Both clones showed a specific immunostaining pattern in human term placenta as the positive control. Reactivity was mostly localized to the cytoplasm of syncytiotrophoblasts. One of the clones showed an excellent staining signal in breast, ovary, and prostate cancer cell lines. Importantly, no reactivity was observed with human lymph node cells or prostate. None of the mAbs were able to detect PLAC1 in Western blot. Based on the present results, these mAbs can be used for detection of PLAC1 in IHC and ICC techniques. Volume 61, Number 3, Pages 363–369, 2014

Keywords: *Anti-PLAC1, immunohistochemistry, monoclonal antibody, placenta, PLAC1*

Abbreviations: *PLAC1, placenta-specific 1; WB, Western blotting; IHC, immunohistochemistry; ICC, immunocytochemistry; BSA, bovine serum albumin; PBS, phosphate-buffered saline; FBS, fetal bovine serum; TBS, Tris-buffered saline; mAbs, monoclonal antibodies.*

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1. Introduction

PLAC1 (placenta-specific 1) is a novel X-linked gene [1] and a new member of cancer–testis antigens [2, 3]. Based on an open reading frame, human PLAC1 consists of 212 amino acids, whereas mouse *Plac1* gene encodes a 173-amino-acid product. Human and murine PLAC1 proteins have 60% identity and 77% homology [1]. *In silico* analysis predicted that PLAC1 has a transmembrane region spanning from amino acids 23 to 40 of the N-terminus, suggesting that PLAC1 is localized to a membranous compartment [3, 4]. In mouse placenta, the expression

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