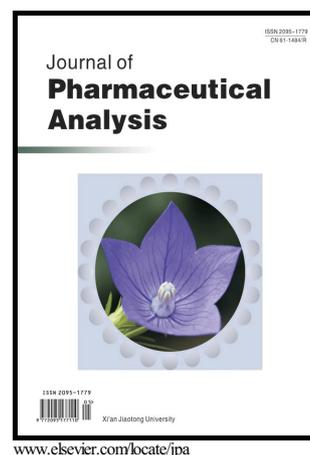


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Preparation of monoclonal antibody against human KIAA0100 protein and northern blot analysis of human KIAA0100 gene

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Abstract

Monoclonal antibodies (MAbs) are important tools for the study of proteins' function and structure. But there has been no report as to the preparation of MAbs against human KIAA0100 protein up to date. Here, firstly, we generated the mouse MAb against human KIAA0100 protein using purified recombinant 6xHistidine (6xHis)-tagged human KIAA0100 protein segment (1557-2234) as an antigen; secondly, the mRNA expression of human KIAA0100 gene was detected in U937 cells using Northern blot analysis. Our results showed that the mouse MAb against human KIAA0100 protein could sensitively recognize the human KIAA0100 protein using western blot analysis and immunocytochemistry analysis. Besides, western blot analysis revealed that human KIAA0100 gene possibly encoded two different protein products (254kDa and <250kDa) in U937 cells. Moreover, Northern blot analysis confirmed human KIAA0100 gene possibly produced two different mRNA products (6000bp-10000bp and 5000bp-6000bp) in U937 cells. Collectively, these results provide a basis for large-scale production of the MAb against human KIAA0100 protein, which will be useful for the study of human KIAA0100 protein's function/structure and MAb-targeted drugs in future.

Keywords

KIAA0100; Monoclonal antibody; Northern blot; Western blot; Immunocytochemistry

1. Introduction

Since 1975, when Köhler and Milstein proposed the method of hybridoma technology and prepared mouse MAbs, many antibodies specific to various antigens have been obtained [1, 2]. The MAbs have many advantages in comparison with polyclonal antibodies, such as high specificity, easy preparation, and good reproducibility. Nowadays, they are applied to the diagnosis and therapy of an array of human disorders, including cancer and infectious diseases [3]. Besides, MAbs are also important tools for the study of proteins' function and structure.

Human KIAA0100 gene was identified from a cDNA library of human immature myeloid cell line KG-1 in 1995 [4]. It belongs to the HUGE database, which contains more than 2,400 members identified in the Kazusa cDNA sequencing project [5-8]. Northern blot analysis showed its mRNA was

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