Generation and Characterization of a High-affinity Monoclonal Antibody for MUC1 Measurement in Breast Cancer

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Breast mucin is secreted by breast tumor cells and serves as a marker for breast cancer. Thus, antibodies against breast mucin will be valuable in the development of immunotherapy and laboratory diagnostic tests. Monoclonal antibodies (MAbs) against breast cancer–associated antigen were generated and characterized. Balb/c mice were immunized with breast cancer–associated antigen CA15-3, and subsequently splenocytes from immunized mice were fused with myeloma cells. After fusion, culture supernatants from hybridomas surviving HAT medium were screened by enzyme-linked immunosorbent assay (ELISA). A total of eight hybridomas producing MAbs against breast cancer showed significant levels of antibody activity against CA15-3. Two selected stable hybridomas were adapted into CELLine CL 350 bioreactors, and the MAbs produced were characterized for their subclass, specificity, and affinity. The MAbs were of high specificity and affinity as shown by ELISA. The MAbs produced may represent a powerful tool and are considered promising reagents for use in diagnosis and detection of early stage of the disease.

Introduction

Breast cancer is one of the most common cancers in women worldwide, with 3738 new cases reported in Malaysia in 2003. Therefore, specific and sensitive methods for early diagnosis and therapy are necessary to detect, target, and treat breast cancer.

MUC1, a highly glycosylated transmembrane protein, also known as polymorphic epithelial mucin, episialin, DF3 antigen, epithelial membrane antigen, or CA15-3, is a member of the mucin family. It is normally present on the apical surface of most glandular epithelial-type cells, including cells in breasts, pancreas, salivary glands, and lungs. In contrast to healthy tissue, many adenocarcinomas, especially malignant breast tumors, overexpress MUC1 in the form of the underglycosylated molecule. It can be found on the entire cell surface and substantial amounts are shed into the circulation. This underglycosylated form of MUC1 makes MUC1 a unique candidate for therapy and for use as a tumor marker.

A number of assays based on competitive or sandwich-type immunoassay have been employed to measure circulating levels of MUC1 protein. Being a product of MUC1, breast-associated antigen CA15-3 plays an important role as a serum tumor marker in the management of breast cancer patients. Although multiple serum-based tumor markers have been described for breast cancer, not much work has been done in Malaysia to develop locally produced MAbs for use in development of diagnostic assays for cancer management and therapy. In this study, we report the generation of murine MAbs against breast cancer antigen with the aim of producing MAbs for local diagnosis and therapy. We describe the generation, purification, and characterization of stable hybridomas secreting specific MAbs against CA15-3 and the scale-up production of these MAbs to laboratory level via an in vitro culture method. Due to their characteristics, these MAbs are promising reagents for use in the diagnosis and therapy of breast cancer.

Materials and Methods

Hybridoma cells secreting specific MAb against CA15-3 protein were generated by fusion of myeloma cells with splenocytes from mice immunized with CA15-3. The use of animals and immunization protocol were approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia (FIS/06/04/2009/CSH[R]).

Immunization

Immunization was carried out based on standard protocols. CA15-3 (Sigma-Aldrich, St Louis, MO) was dissolved in normal saline and emulsified with an equal volume of

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