Human tumor and normal tissue reactivity of the anti-(breast cancer) monoclonal antibody BA-Br-3 and its similarity to the anti-(epithelial membrane antigen) monoclonal antibody E29

Shuen-Kuei Liao1, Robert E. Flahart1, Bobby Kimbro1, Linda Horton1, Robert K. Oldham1, Jo Hilgers2, and Reindert van der Gaag2

1 Biotherapeutics, 357 Riverside Drive, Franklin, TN 37064, USA
2 Bioprobe BV, 1182 GM Amsterdam, The Netherlands

Summary. A mouse monoclonal antibody (BA-Br-3) raised against the breast carcinoma cell line CAMA-1 was previously shown to react with a ≈ 300-kDa globule-like glycoprotein from human milk fat also expressed in the cytoplasm and on the surface of human carcinoma cells of different histological types. In this report the reactivity of this mAb with a large number of normal and malignant human tissues was analyzed using immunoperoxidase techniques. When tested on sections of both fresh-frozen tissues and formalin-fixed, paraffin-embedded tissues, BA-Br-3 reacted with a formalin-resistant antigenic determinant expressed by normal and malignant epithelial cells. Preferential reactivity was observed at the apical portion of ductal epithelial cells in normal breast and in glandular epithelia distributed in several other organs. Reactivity with mucin-like secretions in the lumina of ducts was also found. BA-Br-3 reacted mostly in heterogeneous staining patterns with 88% of 49 breast carcinoma specimens tested, regardless of their histological type or whether they were primary or secondary neoplasms. Testing of epithelial malignant tumors other than breast carcinomas with this antibody showed that 127 of 151 (84%) were also reactive. mAb BA-Br-3 and E29 (a commercially available anti-epithelial membrane antigen) shared very similar staining patterns and distributions of reactivity with breast and other epithelial tumors. However, BA-Br-3 showed a significantly higher percentage of reactivity with melanoma (33% versus 6%, P = 0.003) and a trend toward a higher percentage of reactivity with sarcoma (55% versus 27%, P > 0.05). This antibody, therefore, defines a molecule that is a member of the mucin-like epithelial membrane antigen family. Further studies are warranted to determine its usefulness in antibody-directed cancer diagnosis, prognosis, and immunotherapy.

Introduction

The availability of monoclonal antibodies (mAb) to antigens associated with human breast tumor has created great interest in diagnostic, prognostic and therapeutic approaches to breast cancer [4, 8, 11–13, 17, 23, 24, 30, 35, 40, 44, 45]. Associated with the development of these mAb is an increasing appreciation of the importance of immunohistochemistry in facilitating fundamental research on the biology of human mammary glands and neoplasms [3, 19, 20, 22, 23, 29, 43, 47, 48]. Because of the extreme sensitivity of most mAb-recognized antigenic structures to various histological fixatives and embedding procedures, a majority of the currently available mAb to human cancers, including breast carcinomas, can only be applied to fresh or cryopreserved tissues but not to formalin-fixed, paraffin-embedded tissues [37, 39]. A search for mAb that are able to react with tumor-associated antigens in both formalin-fixed tissues and frozen tissues with comparable specificity and sensitivity is, therefore, of practical importance.

We have recently generated a murine mAb, BA-Br-3, raised against cell lysates prepared from the breast carcinoma cell line, CAMA-1 [27, 30]. Using mixed hemadsorption assays, we have initially shown that this antibody reacts with carcinoma cell lines of diverse origin but not with neuroectodermal tumor cell lines. Immunoprecipitation studies revealed that BA-Br-3 detected a glycoprotein with an apparent molecular mass of ≥ 300 kDa from reactive carcinoma cells. In this report we have conducted an extensive immunohistochemical study with BA-Br-3. We showed that this antibody selectively reacted with fresh...
Materials and methods

Monoclonal antibodies

Monoclonal antibody (mAb) BA-Br-3 (IgG1, κ) was developed by the fusion of NS-1 myeloma cells with spleen cells of a BALB/c mouse immunized with cells of the breast cancer line CAMA-1. The CAMA-1 cell line was established by Fogh et al. [15] from pleural effusion cells of a 51-year-old woman with breast adenocarcinoma. The characteristics of this breast cancer cell line have been described [25]. The procedures for mouse immunization, spleen cell preparation, and hybridoma screening, selection, and cloning have been described [29]. The BA-Br-3 hybridoma was grown in ascites and the antibody was purified using the caprylic acid method [34, 41]. Antibody purity was determined by gel permeation high-performance liquid chromatography using two Waters Protein-Pak 300 SW columns in series and by sodium dodecyl sulfate/polyacrylamide gel electrophoresis [35]. The purified material contained more than 95% mouse IgG plus a small amount of other protein contaminants. The anti-(epithelial membrane antigen) (EMA) mAb E29 (IgG1, κ) [18, 37] was obtained from DAKO corporation, Santa Barbara, Calif. For immunohistochemical analysis both mAb BA-Br-3 and E29 were used at a concentration of 5 μg/ml, previously determined to be saturating without increasing nonspecific binding to breast carcinoma tissues.

Tumor and normal tissues

Fresh-frozen tumor tissues and part of the formalin-fixed, paraffin-embedded tumor tissues were obtained from biopsy materials of cancer patients who entered the monoclonal antibody/immunoconjugate program at Biotherapeutics. Tumor tissues were examined by paraffin-embedded tumor tissues were obtained from biopsy materials of certain normal organs.

Frozen, acetone-fixed sections and formalin-fixed, paraffin-embedded sections of breast carcinoma as well as a wide variety of other carcinomas. Specifically, this antibody reacted with 80% of epithelial tumors, 33% of melanomas and 55% of sarcomas tested, as well as normal breast ductal epithelia and other epithelial cell types in certain normal organs.