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BRCA1 Tumor Suppressor Gene Product Shares Immunoreactive Epitopes with a Protein Present in Seminal Plasma

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Objectives: To develop immunofluorometric procedures for measuring BRCA1 protein in various biological fluids and tissue extracts.
Design and Methods: Five commercially available monoclonal and polyclonal antibodies against BRCA1 were evaluated for developing competitive and non-competitive immunofluorometric procedures for BRCA1. Biotinylated and nonbiotinylated peptides were used to assess the specificity of the antibodies for blocking experiments and for the competitive immunoassay. Extensive studies to exclude cross-reactivity and non-specific effects in the non-competitive immunoassay were undertaken. Seminal plasmas as well as breast tumor extracts, amniotic fluids and cerebrospinal fluids were analyzed.
Results: We designed novel methods for measuring BRCA1 immunoreactivity. One configuration based on the "sandwich-type" immunoassay principle was used for further studies. We discovered that seminal plasma contains an immunoreactive protein which appears to possess the D-20 (aminoterminal) and C-20 (carboxy-terminal) epitopes of BRCA1. Molecular weight identification using gel filtration chromatography has shown that the immunoreactive species has a molecular weight between 660 and 160 KDa.
Conclusions: We identified for the first time a protein in seminal plasma that shares immunoreactive epitopes with the BRCA1 tumor suppressor protein. We are currently purifying this protein in order to examine if it is homologous or identical to BRCA1.

KEY WORDS: BRCA1; familial breast cancer; ovarian cancer; seminal plasma components; prostate cancer; cancer susceptibility genes.

Introduction

Breast cancer is the most common cancer among North American women. By the year 2000, it is estimated that worldwide, breast cancer will account for 500,000 deaths annually (1). About 10% of breast cancers are due to inherited genes, two of which, BRCA1 and BRCA2, have now been cloned (2–4). BRCA1, the breast and ovarian cancer susceptibility gene, is localized to human chromosome 17q21. Mutations in this gene account for approximately 95% of families with increased incidence of early onset breast and ovarian cancer and 45% of families with increased incidence of breast cancer alone (5,6). BRCA1 is believed to encode a tumor suppressor protein since transfected BRCA1 gene inhibits the growth of breast and ovarian cancer cells but not of other malignant cells. Most inherited BRCA1 mutations produce truncated proteins that vary in length from 5% to 99% of full-length protein. However, missense mutations that alter cysteines in a motif called the RING finger are also found. The C-terminal end of the BRCA1 protein appears to be essential for normal BRCA1 function in breast epithelial cells because patients inheriting codon 1853 stop mutations, develop very early onset breast cancer. The BRCA1 gene encodes a 1863 aminoacid protein and it is expressed in several tissues including the breast and ovary.

The amino-terminal domain of BRCA1 possesses high sequence homology with a zinc finger topology (3). This topology, C_3HC_4, is referred to as the "zinc-ring domain," "RING finger," or A-box and is found in several viral proteins, proto-oncoproteins, and regulatory and transcription factors (7,8).

The BRCA1 zinc-ring domain and its structure and function are of particular interest because it is the location of some of the most frequently occurring mutations linked to breast and ovarian cancer. The 185delAG mutation (deletion of two nucleotide base pairs in exon 2) in the zinc-ring domain has been shown to occur in 1 in 100 Ashkenazi Jewish individuals. This is in comparison with the estimated 1 in 833 frequency of all BRCA1 mutations in the general population (9,10).

The mechanism by which the loss of function of

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