The Anti-BRCA1 Peptide Antibody C-20 Recognizes Smooth Muscle Cells

Sir,

In 1990, a breast cancer susceptibility gene was localized in chromosome 17q (1). Mutations within this gene, designated BRCA1, are believed to account for approximately 45% of the families with a high incidence of breast cancer (2). A transfected BRCA1 gene inhibits the growth of breast and ovarian cancer cells. Therefore, BRCA1 appears to act as a tumor suppressor (3). While examining BRCA1 expression in cells of extramammary Paget's disease, we noticed that the antibody C-20, which was raised against the last 20 C-terminal amino acids of BRCA1, recognizes smooth muscle cells.

MATERIAL AND METHODS

Formalin-fixed paraffin-embedded tissue blocks (two consisting of normal skin and one of muscle) were cut into 5-micron sections, then deparaffinized, rehydrated, and incubated in hydrogen peroxide to block endogenous peroxidase activity. After blocking with normal goat serum, the sections were incubated with anti-BRCA1 antibody C-20 (Santa Cruz, CA, USA) at a concentration of 1 μg/ml. The sections were subsequently treated with secondary antibody using a Vectastain ABC-Peroxidase kit (Vector, CA, USA), followed by staining with the Vector VIP peroxidase substrate kit (Vector, CA, USA), and were then counterstained with methyl green. For immunostaining using anti-BRCA1 antibody I-20 (Santa Cruz, CA, USA) and anti-Neu antibody C-18 (Santa Cruz, CA, USA), frozen tissue sections were used. The subsequent procedures were essentially the same as for antibody C-20.

RESULTS AND DISCUSSION

Immunohistochemical staining of formalin-fixed normal skin using the anti-BRCA1 antibody C-20 showed positive staining in both the eccrine sweat gland and some dermal blood vessels. The cells that stained positively in the eccrine sweat gland were myoepithelial cells, which lay in the outer side of the secretory portion of the eccrine sweat glands (Fig. 1). The cells that stained positively in the blood vessels seemed to be vascular smooth muscle cells (Fig. 2). Endothelial cells in the blood vessels did not stain positively (data not shown). Smooth muscle cells in an angioleiomyoma also stained positively (data not shown). An arrector pili muscle stained positively (Fig. 3). However, no staining was seen in the skeletal muscles (data not shown). To summarize, positive staining was observed in several kinds of smooth muscle cells in formalin-fixed normal skin sections.

BRCA1 protein is thought to be a nuclear protein; however, it is well known that the anti-BRCA1 antibody, C-20, cross-reacts with EGFR and HER2 (4, 5). Antibody C-20 is raised against residues 1843–1862 of the BRCA1 protein, and because this region is highly conserved between BRCA1 and some members of the tyrosine kinase receptor family, such as EGFR and HER2, the antibody is thus believed to cross-react with them (4). The positive staining which we detected in several smooth muscle cells in formalin-fixed skin sections is not likely to originate from the reactions with BRCA1 itself, because another anti-BRCA1 antibody, I-20, does not stain smooth muscle cells as did C-20 (data not shown). It cannot originate from the cross-reactions with EGFR or HER2 (Neu), because the staining pattern is obviously different from

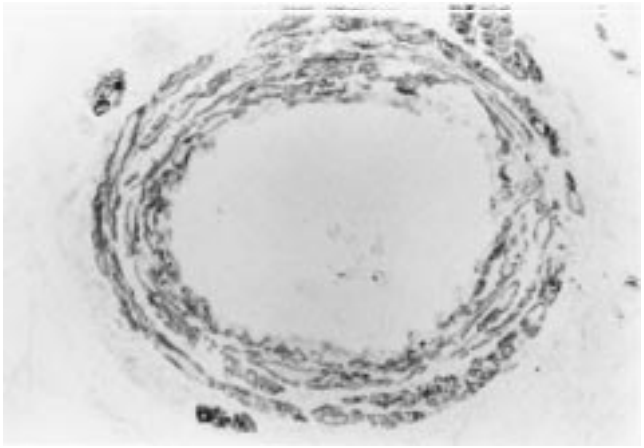

Fig. 2. Immunohistochemical staining of the dermal blood vessels with anti-BRCA1 antibody C-20. Vascular smooth muscle cells revealed positive staining (× 400).

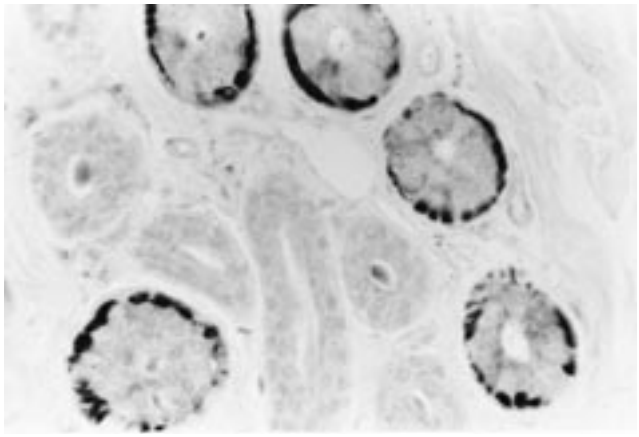

Fig. 1. Immunohistochemical staining of normal skin with anti-BRCA1 antibody C-20. Myoepithelial cells of eccrine sweat glands revealed positive staining. The ducts of the sweat glands failed to react (× 400).

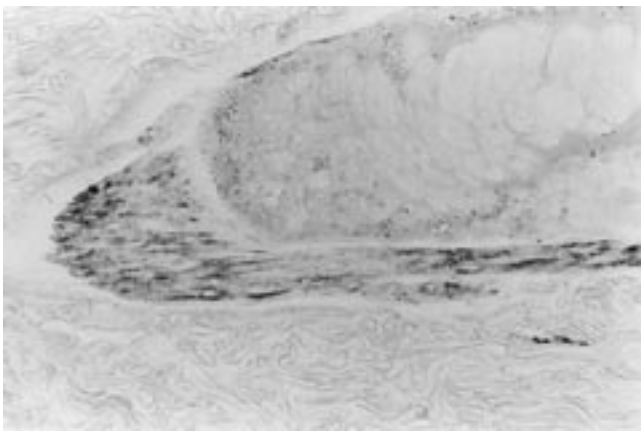

Fig. 3. Immunohistochemical staining of an arrector pili muscle with anti-BRCA1 antibody C-20. Smooth muscle cells of an arrector pili muscle revealed positive staining (× 200).

the well-known distribution of the EGFR protein in skin sections, and because the anti-Neu antibody, C-18, does not stain smooth muscle cells (data not shown).

There are known several antibodies, such as the anti-α smooth muscle actin antibody (6), the anti-smooth muscle myosin antibody (7) and the anti-desmin antibody (7), which can be used as a differentiation marker of smooth muscle cells. Although exactly which epitope antibody C-20 detects in smooth muscle cells is not clear so far, this antibody may be useful in identifying smooth muscle cells in various situations. We are now undertaking efforts to reveal to which antigen antibody C-20 reacts in smooth muscle cells.

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A Case of Pigmented Purpuric Eruption Associated with Hereditary Spherocytosis

Sir,

Pigmented purpuric eruptions are chronic conditions of unknown aetiology which comprise a group of clinical patterns of erythrocyte extravasation due to pericapillary inflammation. Progressive pigmented purpuric dermatosis (Schamberg's disease), purpura annularis telangiectodes (Majocchi's disease), pigmented purpuric lichenoid dermatosis (Gougerot and Blum's disease) and lichen aureus are the subtypes of pigmented purpuras.

Here we present a case of pigmented purpuric eruption associated with hereditary spherocytosis.

CASE REPORT

A 20-year-old man presented with the complaints of fatigue, nausea, vomiting and cutaneous eruptions. General physical examination revealed splenomegaly and icteric scleras. On dermatologic evaluation, there were red, annular and linear macules 0.5 – 3 cm across with minute telangiectases and pinhead-sized petechiaes located on the extremities and abdominal region symmetrically (Fig. 1). The lesions had started three years previously, and were characterized by waxing and waning periods. He had no drug history.

Histopathologic examination of the lesional skin biopsy specimen showed dilated capillaries surrounded by mononuclear inflammatory infiltrate and extravasation of erythrocytes in the upper dermis.

Complete blood count revealed a hemoglobin of 11.9 gm, hematocrit of 32.2% and white blood count of 6600/mm^3. Mean corpuscular hemoglobin concentration was elevated. Osmotic fragility was abnormal (0.60 – 0.45%; control: 0.45 – 0.35%), and reticulocyte count was found to be increased (8%). Total bilirubin was 2.9 mg/dl (normal: 0.2 – 1.0 mg/dl) and direct bilirubin was 0.4 mg/dl (normal: 0.1 – 0.3 mg/dl). There were spherocytes in the peripheral blood smear. Cryoglobulins and cryofibrinogens were found to be negative. Serum protein electrophoresis was within normal limits.

Hematologists advised splenectomy, but he did not consent. Because he had no complaints, we did not give any medication for the skin lesions. The patient was seen three months later. He still had skin eruption, but it was less severe. After that, he did not attend regular examinations.

DISCUSSION

Pigmented purpuric dermatoses, pigmented purpura and purpura pigmentosa chronica are the synonyms of pigmented purpuric eruptions which are preferred by some authors. Pigmented purpuric eruptions run a chronic, recurrent course in most patients. Their clinical features show considerable overlap with typical diseases (Schamberg's disease, Majocchi's disease, Gougerot and Blum's disease and lichen aureus). All are similar histologically. Extravasation of erythrocytes due to pericapillary inflammation and perivascular lymphocytic infiltration occurs.

Pigmented purpuric eruptions may be considered a purely local cutaneous inflammation characterized by lymphocytic vasculitis. Data available suggest that the vascular damage and extravasation of erythrocytes are secondary to a localized cell-mediated immunologic reaction. The fact that predominantly CD4+ T-cell infiltration occurs in lesional skin (1, 2) supports this view.

The aetiology of pigmented purpuric eruption is unknown. Some conditions, including gravity and increased venous pressure, some drugs (thiamine propyldisulfide, chlordiazepoxide)