Disorders in Restructuring of T-Cell Receptor γ-Chain in Malignant Skin Lymphomas

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Disorders in restructuring of T-cell receptor γ-chain in DNA from skin biopsy specimens and peripheral blood lymphocyte of patients with malignant skin lymphomas at different stages of the disease were detected by PCR. Nucleotide sequences of monoclonal T-cell receptors were determined. The study showed that DNA sequences from the skin and lymphocytes did not coincide in some cases.

Key Words: *T-cell receptor; malignant skin lymphoma*

The study of the course and mechanisms regulating malignant processes is a key problem of medical biochemistry today. Neoplastic proliferation of clones of abnormal T and B cells and/or their precursors underlies the development of malignant skin lymphomas (MSL) [2,4]. Tumor development in MSL is associated with expansion of monoclonal T cells and production of numerous copies of the same site of γ-chain gene of T-cell receptor (TCR) by daughter cells in the epidermis. Presumably, the mechanism of somatic recombination of functional genes of TCR is impaired in patients with MSL, which modulates the functions of T cells [1].

Detection of these clones by PCR is a convenient method for the early diagnosis of MSL. It is important to determine statistical regularity between the incidence of impaired restructuring in variable regions of TCR and disease severity, as well as to evaluate the identity of nucleotide sequences of clonal populations of the skin and lymphocytes. Detection of the incidence of monoclonal population with a certain V (variable) site in cases with impaired restructuring of TCR γ-chain will improve our understanding of the mechanisms of disorders accompanying tumor processes.

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We studied disorders in restructuring of TCR γ-chain in MSL in the peripheral blood lymphocytes and skin by PCR.

MATERIALS AND METHODS

Disorders in restructuring of TCR γ-chain in the peripheral blood lymphocytes and skin biopsy specimens in health and MSL were evaluated on the material from 20 normal subjects and 34 patients with different stages of T-cell lymphoma of the skin (8 patients with stage I, 9 with stage II, and 17 with stage III disease). The patients' ages varied from 25 to 69 years, the disease duration from 7 months to 20 years.

Lymphocytes were isolated from citrate blood, skin biopsy specimens were collected from MSL foci in patients with different stages of disease.

All patients were hospitalized for examination and treatment at Clinical Dermatology Department of Central Institute for Skin and Sexually Transmitted Diseases. The stage of the disease was determined on the basis of clinical and histological findings. Clinical manifestations corresponded to the classical Alibert—Bazin form.

Skin specimens from normal subjects, collected during cosmetic operations, served as the control. Biopsy specimens were collected before treatment. Informed consent was obtained from all examinees. Lymphocytes were isolated in Ficoll—verograffin density

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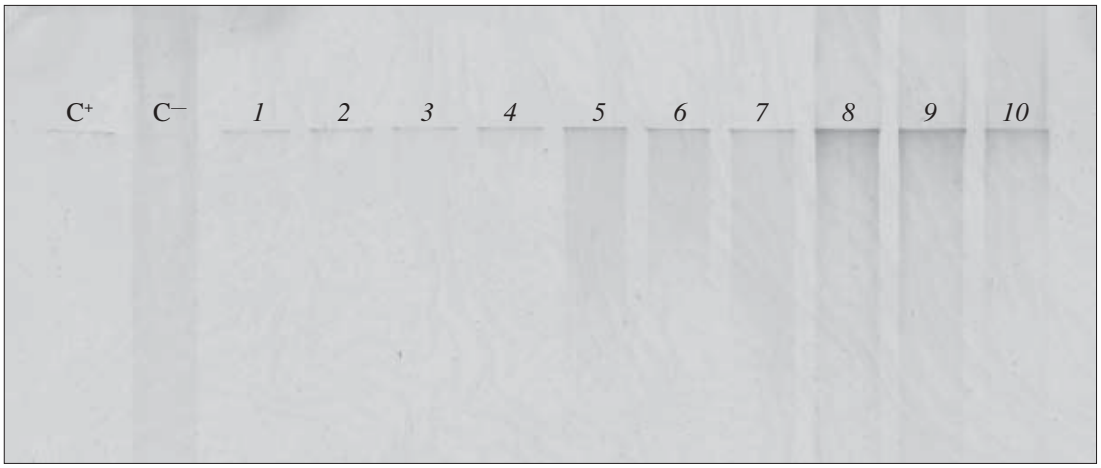

Fig. 1. Evaluation of silver nitrate staining sensitivity. C+: positive control (DNA from Jurkat cells); C–: negative control (DNA from donor peripheral blood lymphocytes); *1-10*: dilutions of Jurkat cells with donor peripheral blood lymphocytes.

gradient. Human genome DNA was isolated from lymphocytes and skin samples of donors and patients with MSL by the standard method using proteinase K. *E. coli* top F10 strain and Jurkat, SeAx and MyLa cells were used in the study.

DNA fragments were cloned in pGEM-T vector system (Promega), PCR was carried out with DNA-specific primers V1-8 (5'-TGCAGCCAGTCAGAAA TCTTAC-3'; gene sites V1-V8), V9 (5'-TGCAGGT CACCTAGAGCAACCT-3'; gene site V9), V10 (5'-AGCAGTTCCAGCTATCCATTTCC-3'; gene site V10), V11 (5'-TGCAGCCAGTCACACCTAGAG-3'; gene site V11), Jr (5'-GACAACARGAGTTGTTCC AC-3'; degenerated primer for all sites of J gene). PCR was carried out using genome DNA template from lymphocytes and skin with 10 pmol of VnJr primers. Thirty amplification cycles consisting of predenaturing at 95°C (3 min), denaturing at 95°C (1 min), annealing of primers at 60°C (1 min), and elongation of chains at 72°C (1 min) were carried out. PCR products were purified using QIAquick PCR Purification Kit (QIAGEN). The resultant fragments were separated in 2% agarose gel, stained with ethidium bromide, and visualized in UV light.

DNA of Jurkat (V1-8, V11), MyLa (V9), SeAx (V10) cells served as positive control. Cell cultures were kindly provided by Cell Culture Collection of Institute of Cytology, Russian Academy of Sciences.

DNA of donors served as a negative control. Electrophoresis was carried out in polyacrylamide-agarose gel (5% polyacrylamide, 0.25% agarose, 10% glycerol) in TBE buffer in a vertical system with cooling (Protean II xi Cell, Bio-Rad) on 200×240 mm slides at constant power of 6-8 W for 6-15 h at 4°C, with subsequent staining with silver nitrate [4]. The gel was wrapped into cellophane membrane and photographed with a Kodak DC120 digital camera. DNA nucleotide sequences were determined with an automated sequencer 373A ABI (Applied Biosystems).

RESULTS

The sensitivity of the method (0.1-5% neoplastic cells, depending on the type of somatic restructuring and studied material, Fig. 1) was determined by serial dilutions of Jurkat cells with donor lymphocytes.

No monoclonal population of TCR γ-chain was detected in normal donor skin and peripheral blood lymphocytes.

In patients with stage I MSL, TCR monoclonal population was detected in 62% skin biopsy specimens and in 12% peripheral blood lymphocyte DNA (Table

TABLE 1. Number of MSL Patients with Monoclonal Population of TCR γ-Chain in Peripheral Blood Lymphocytes and Skin Biopsy Specimens from the Foci

Material	Stage I MSL (*n*=8)		Stage II MSL (*n*=9)		Stage III MSL (*n*=17)	
	positive reaction, *n*	% of total number of patients	positive reaction, *n*	% of total number of patients	positive reaction, *n*	% of total number of patients
Skin	5	62	7	77	17	100
Lymphocytes	1	12	4	45	12	70

1). In MSL stage II the disorders in TCR γ-chain genome restructuring were observed in 77% of skin DNA and 45% lymphocyte DNA samples, and at stage III of the disease these disorders were observed in 100 and 70% cases, respectively.

Hence, genome DNA from skin biopsy specimens of patients with MSL is the most informative material for detection of the monoclonal TCR type, because the probability of appearance of malignant lymphocyte population from the skin in the peripheral blood of MSL patients is rather low at the early stage of the disease.

We failed to determine the incidence of any of the V regions in disorders of TCR restructuring. Analysis demonstrated similar incidence of all regions in restructuring. Primer pair V1-8 and Jr showed the best results in PCR analysis. No relationship between the disease stage and identity of nucleotide sequences of skin and peripheral blood lymphocyte TCR in MSL patients was detected.

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