ELECTROPHORETIC DETECTION OF PROTEIN p53 IN HUMAN LEUKOCYTES

V. D. Paponov, E. G. Shcheglova,
N. N. Yarullin, and E. G. Kupsik

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Among the many eukaryote cell proteins, much attention has been drawn in recent years to a protein with molecular weight of 53 kilodaltons (kD). The reason is that this protein (p53) is present in increased amounts in various kinds of transformed cells belonging to different species: in spontaneous teratocarcinomas and neoplastic cell lines induced by chemical agents, radiation, and viruses [5, 6, 15]. Often it is impossible to detect p53 in the normal prototypes of transformed cells. It is postulated that p53 takes part in the regulation of cell proliferation, although this has not been proved unambiguously [15].

The authors have found an acid-soluble protein with mol. wt. of about 53 kD in peripheral blood leukocytes of persons with Down's syndrome [2, 3]. It was present in different quantities in all 20 patients tested, but was virtually not discovered in 12 healthy blood donors.

The aim of this investigation was to determine the possible identity of this protein with protein p53 from mouse ascites carcinoma by comparing their electrophoretic mobilities, because the accuracy of electrophoretic determination of the molecular weight of proteins is not sufficient alone to identify them. In particular, protein p53, associated with cell transformation, has been stated in different publications to have an mol. wt. of 53 kD [5, 6], 54 kD [8, 12], and 55 kD [10]. This paper also describes experiments to detect a protein with electrophoretic mobility identical with that of a protein in the leukocytes of patients with Down's syndrome in leukocytes of patients with leukemia. To discover if protein p53 is involved in cell proliferation, the protein composition of leukocytes from healthy blood donors, cultured in the presence and absence of phytohemagglutinin (PHA), was compared.

EXPERIMENTAL METHOD

The procedures connected with taking venous blood from the donors and patients, isolating the leukocytes, and preparing specimens for electrophoretic analysis of proteins in polyacrylamide gel with sodium dodecylsulfate by Laemmli's method, were described previously [2, 3]. Densitometry of the gels after electrophoresis of proteins of the cell homogenates was carried out on "Gilford" spectrophotometer at 570 nm. Leukocytes were cultured in healthy human blood plasma with the addition of 2 volumes of Eagle's medium, and incubated at 37°C in Carrell flasks (25 ml per flask) for 72 h in the presence and absence of PHA (from Difco, USA) in a dose of 0.2 ml per 10 ml of medium (final concentration of PHA 20 μg/ml). Diploid and tetraploid strains of Ehrlich's ascites carcinoma were obtained from the Laboratory of Tumor strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The ascites suspension was injected intraperitoneally into noninbred mice in a dose of 0.5 ml per animal. Cells in ascites fluid taken from mice on the 5th day of tumor growth were washed in physiological saline, like leukocytes [2, 3], and the homogenates were used for electrophoresis.

EXPERIMENTAL RESULTS

Densitograms of gels after electrophoresis of cell homogenates of Ehrlich's ascites carcinoma (diploid and tetraploid strains) and leukocytes of a patient with Down's syndrome are shown in Fig. 1. It can be seen in Fig. 1 that a protein with mol. wt. of 53 kD, which the writers discovered previously [2, 3] in leukocytes and acid extracts of leukocytes from patients with Down's syndrome, has identical electrophoretic mobility with a protein of the same molecular weight from mouse ascites carcinoma cells known as p53.

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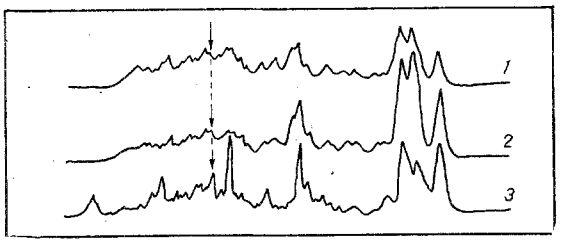

Fig. 1

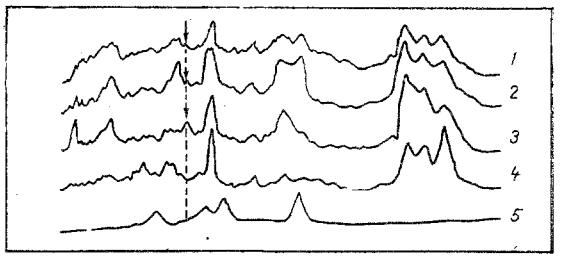

Fig. 2

Fig. 1. Densitograms of gels after electrophoresis of proteins from leukocyte homogenates of patient with Down's syndrome and Ehrlich's ascites tumor cells. 1) Proteins from cells of diploid strain of Ehrlich's ascites tumor, 2) proteins of cells of tetraploid strain of Ehrlich's ascites tumor, 3) proteins of leukocytes from patient with Down's syndrome. Here and in Figs. 2 and 3, arrow indicates protein with mol. wt. of 53 kD, detected in patients with Down's syndrome but not in healthy individuals, and proteins with corresponding electrophoretic mobility in mouse ascites cells.

Fig. 2. Densitograms of gels after electrophoresis of proteins from leukocyte homogenates of normal subjects and patients. 1) Proteins from leukocytes of patient with chronic myeloblastic leukemia, 2) proteins from leukocytes of patient with chronic myelopreblastic leukemia, 3) proteins from leukocytes of patient with Down's syndrome, 4) proteins from leukocytes of healthy blood donor, 5) marker proteins (from left to right): catalase (60 dK), ovalbumin (43 kD), aldolase (40 kD), chymotrypsinogen A (25 kD).

It cannot yet be said that proteins obtained from the different objects, and placed in class p53 on the basis of the results of electrophoretic analysis, into which the protein from leukocyte patients with Down's syndrome can be included on that basis, are completely identical. However, a high degree of similarity of amino-acid composition and of the peptide maps of p53 proteins from mouse neoplastic cell lines (methylcholanthrene-induced ascites tumor and mammary gland carcinoma) and from lymphoblastoid human cell line (Burkitt's lymphoma) has been found [6].

It is possible that there exists a nonhistone protein p53 with high evolutionary conservatism, and which, like histones, performs the same function in all cells regardless of their species and tissue of origin. This function may be associated either with cell transformation, or with intensive proliferation, or with the creation of a preliminary situation for one or both of these two processes to take place if, for example, p53 possesses protein kinase activity [6]. The discovery of protein p53 in peripheral blood leukocytes of patients with various diseases accompanied by changes in proliferative activity of cells concerned in leukocytopoiesis, is therefore interesting.

It will be clear from Fig. 2 that protein p53 is present in leukocyte homogenates from patients with chronic myeloblastic and chronic myelopreblastic leukemia. The appearance of protein p53 in the patients studied (three patients with each type of myeloid leukemia) can be ascribed to transformation of cells of the myeloid series. Another possibility, however, is that the appearance of p53 is connected with transition of a certain leukocyte population from the G_0 phase into the G_1 phase or the S phase, for the level of ^{3}H-thymidine labeling of cells of the myeloid series in the peripheral blood of patients with chronic myeloid leukemia sometimes reaches 30%, although the cells virtually never undergo mitosis, and the number of cells forming colonies in vitro (i.e., capable of proliferating) present in the blood is 50-100 times greater than normally [1].

Increased incorporation of ^{3}H-thymidine by leukocytes of patients with Down's syndrome can be explained by the presence of a population of immature leukocytes actively synthesizing DNA [4] in the peripheral blood of these patients, and this also can explain the presence of protein p53 in the leukocytes of these patients. The possibility cannot be ruled out that a disturbance leading to the appearance of p53 in the leukocytes of patients with Down's syndrome is a preliminary condition determining the tenfold increase in the frequency of transient leukemoid reactions in these patients [11]. Evidence has been obtained to suggest that the myeloproliferative reaction in patients with Down's syndrome is connected with transformation of a certain leukocyte population [7]. Since leukemoid reactions in these patients may be not only myeloproliferative in character, but also lymphoproliferative [11], the question of what type of leukocytes undergo transformation in a concrete patient requires individual analysis.

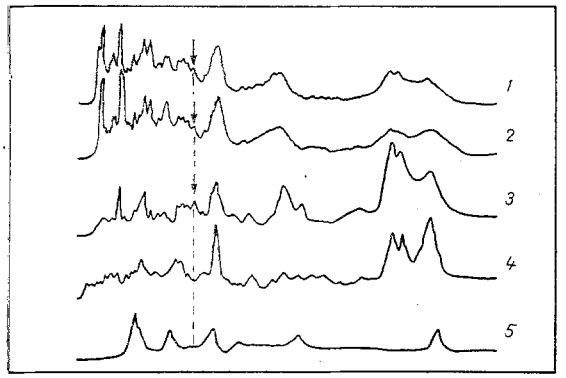

Fig. 3. Densitograms of gels after electrophoresis of proteins from leukocyte homogenates of patient with Down's syndrome and of healthy blood donor, cultured in the presence and absence of PHA. 1, 2) Leukocyte proteins from two blood donors after culture for 72 h with PHA, 3) leukocyte proteins of patient with Down's syndrome, 4) leukocyte proteins from healthy blood donors, 5) marker proteins (from left to right): albumin (67 kD), catalase, ovalbumin, aldolase, chymotrypsinogen A, cytochrome C (12.4 kD).

Figure 3 shows that protein p53 appeared in the leukocytes of two healthy donors after culture for 72 h in the presence of PHA. This can be linked with a switch to proliferation of T lymphocytes, for PHA is a specific mitogen for them [14]. Protein p53 has been found in lymphocytes from the mouse spleen cultured with concanavalin A [9], which also induces T lymphocyte proliferation [14]. After culture of lymphocytes from mouse spleen with a lipopolysaccharide that stimulates B lymphocytes, a protein with mol. wt. of 54 kD was found in their chromatin [13]. A protein with mol. wt. of 55 kD has been found in a cell culture from a 12-14-day mouse embryo [10]. It was absent in cells of a 16-day embryo, and its peptide map was similar to that of protein p53 from cells transformed by virus SV 40.

Proteins of the p53 family, appearing in tumor cells, and found in embryonic cells and after stimulation of proliferation of lymphocytes, have been discovered in human leukocytes stimulated by PHA and in peripheral blood leukocytes of patients with diseases (Down's syndrome, myeloid leukemias) accompanied by the appearance of cells capable of actively synthesizing DNA in the blood stream. Proteins of the p53 class in human leukocytes can serve as markers of pathological processes leading to the appearance of immature or transformed leukocytes, proliferating or simply synthesizing DNA in the course of replication of the genome as a whole or amplification of a restricted part of it, possibly containing the gene coding protein p53, in the blood stream. These leukocytes may consist of populations of various leukopoietic cells and may have different kinds of concomitant disturbances manifested, for example, as a much reduced life span of the leukocytes in Down's syndrome [4], but a many times increased life span of the leukocytes in chronic myeloid leukemias [1].

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