ONCOLOGY

CD24 and CD70 as Differential Markers of Human Hepatocellular Carcinoma Cells in Culture

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We developed a new method for evaluation of the purity of cell population in primary cultures of hepatocellular carcinoma. Expression of potential surface molecular markers on primary cultures of renal tumors was assayed by the method of flow cytofluorometry. CD24 and CD70 were identified as differential markers, which allowed us to distinguish cancer cells from tumor stromal cells *in vitro*. A negative marker CD90 was expressed on fibroblasts of the tumor stroma, but not on cancer cells. Combined use of these three markers allows evaluation of the severity of tumor culture contamination with stromal cells.

Key Words: *hepatocellular carcinoma; cell culture; surface markers; flow cytofluorometry*

Hepatocellular carcinomas (HCC) are characterized by poor prognosis. Five year survival is typical of only 40% patients with renal tumors. Modern therapeutic methods for HCC are low effective (except for the surgical method) [1]. The use of primary cultures from tumor cells is a promising approach to the development of new methods for the diagnostics and therapy of renal cancer. Adaptation of the primary culture to *in vitro* growth allows us to isolate and increase the yield of pure population of tumor cells for molecular, genetic, and immunological studies, testing of antitumor drugs, and other researches [4].

It is difficult to isolate pure cultures of cancer cells from tumor tissue because of possible contamination of these cultures with tumor stromal cells with high proliferative activity [7]. Tumor and non-tumor cells should be separated at the initial stage of isolation of primary cultures.

The method of flow cytofluorometry in combination with specific antibodies against surface antigens was shown to be effective in comparative study for the expression of surface molecules. This method can be used for identification of markers specific for tumor and stromal cells. These markers serve to confirm the tumor nature of cell cultures and to separate the cells for isolation of a homogeneous population.

This work was designed to identify surface markers of human HCC, which allowed us to distinguish cancer cells from tumor stromal cells in the culture.

MATERIALS AND METHODS

Primary cultures of renal cancer were obtained from tumor tissue samples of HCC patients. Comparative study involved cultures of human embryonic fibroblasts (HEF) and human embryonic kidney (HEK).

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Tumor tissues were isolated after surgical treatment and excision of renal carcinoma. Tumor tissue was minced and placed in DMEM/F-12 nutrient medium (Gibco) containing 10% fetal bovine serum (FBS, HyClone), 0.1 mg/ml collagenase, 0.01% hyaluronidase, and 0.01% dispase. Tumor fragments were incubated overnight at 37°C with constant agitation. The cell suspension was passed through a filter (70 μ pore diameter, Becton Dickinson). The cells were cultured in collagen-coated culture flasks (Greiner) with DMEM/F-12 medium (Gibco) containing 10% FBS (HyClone) and insulin transferrin selenite (PanEko) under standard conditions (5% CO_2, 37°C).

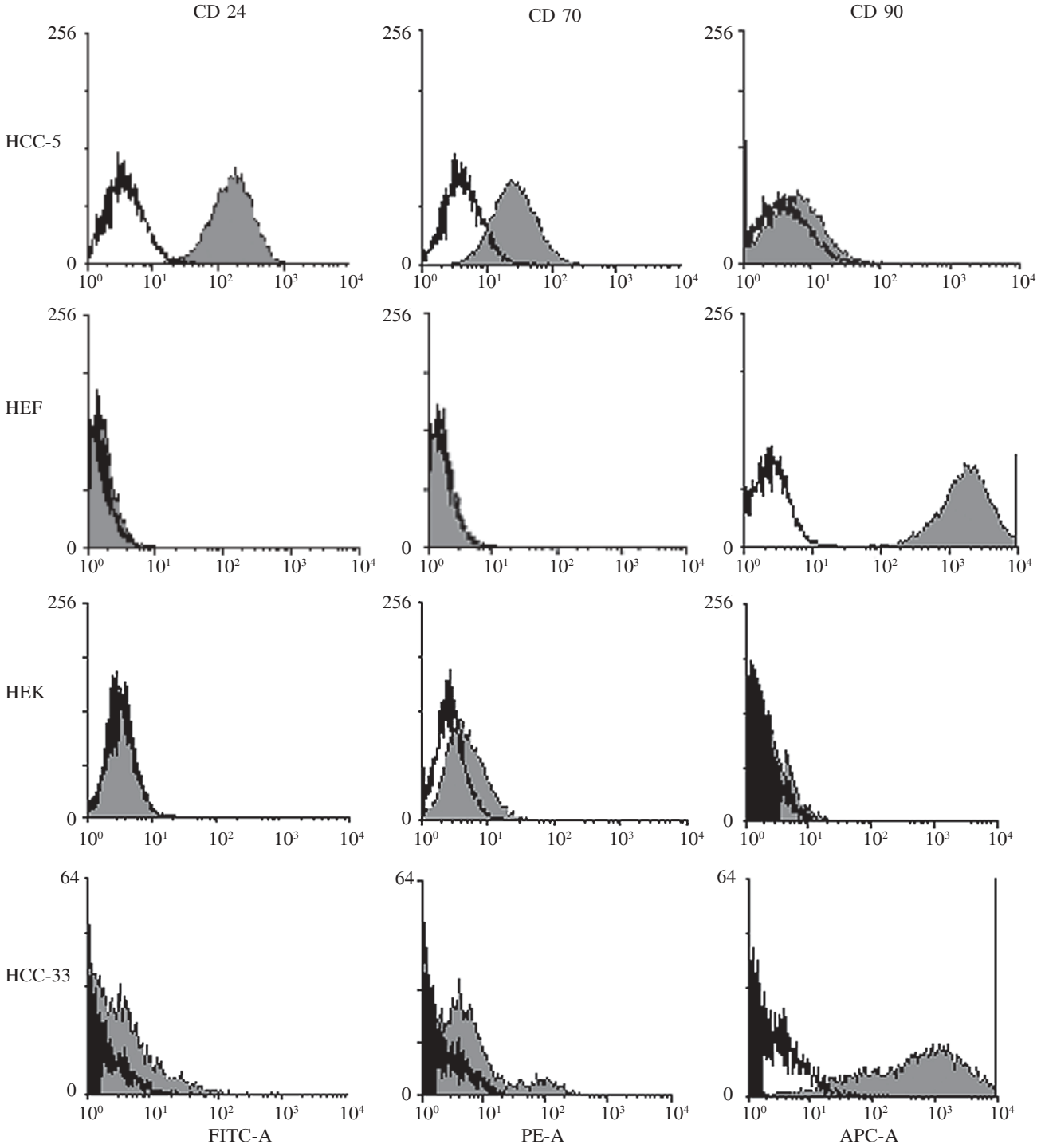

Fig. 1. Expression of CD24, CD70, and CD90 in cell cultures. Abscissa, fluorescence intensity; ordinate, number of recorded events (cell count). Thick line, isotopic control.

Immunocytochemical staining involved monoclonal antibodies against surface antigens of human CD10, CD24, CD34, CD70, CD71, and CD90 cells conjugated with one of the following fluorochromes: allophycocyanin (APC), FITC, and phycoerythrin (PE, Becton Dickinson). Isotypic antibodies labeled with the corresponding fluorescent dyes of a similar manufacturer served as the control. Fluorescence was measured on a FACSAria cytofluorometer/sorter (Becton Dickinson).

RESULTS

Expression of markers was evaluated from the histogram for fluorescence of a dye conjugated with specific antibodies. The CD34 marker expressed on endothelial cells [8] was identified in none of the cell cultures, which reflects the absence of contamination with endothelial cells from the tumor environment. The test cultures did not express CD10 and CD71 surface epithelial markers.

Figure 1 illustrates the expression of CD24 and CD70 surface markers in the HCC-5 culture. Morphological characteristics of cells in this culture were typical of epithelial cells over several passages (Fig. 2, *a*). Fluorescence of CD24 and CD70 was 156.29±23.00 and 26.86±6.37 units, respectively.

The CD24 molecule of nephron epithelial differentiation is a small glycosylated protein acting as P selectin ligand (adhesion molecule on the surface of activated platelets) [6]. Another marker CD70 serves as ligand for CD27 receptors only. These molecules belong to a superfamily of tumor necrosis factor receptors [5]. CD70 can trigger apoptosis in T lymphocytes, which allows malignant cells to avoid the immune defense system [3]. High expression of CD24 and CD70 on the cell surface illustrates tumor nature of this culture.

As differentiated from the tumor culture of HCC-5, the culture of HEF was characterized by high expression of CD90 (1569.0±204.7, Fig. 1). This molecule belongs to the superfamily of immunoglobulin-like receptors previously identified on human skin fibroblasts [2]. Expression of these markers was low in the culture of HEK (Fig. 1).

During culturing of another HCC (HCC-3), morphologically epithelial cells were progressively replaced with long spindle cells, whose morphological signs were similar to those of fibroblasts (Fig. 2, *b*). Cytofluorometry showed that the mixed culture contains 2 cell populations with different expression of CD24, CD70, and CD90 (Fig. 1). CD24 expression was low in 89% cells, which corresponded to 2.35±2.14 fluorescence. However, fluorescence of 11% cells was 20.63±3.43 (Fig. 3, *a*). The distribution of fluorescence was similar for CD70 (2.47±2.03, 89% cells; 56.53±11.03, 11% cells; Fig. 3, *b*). The majority of cells in the HCC-3 culture (90%) exhibited significant expression of the CD90 molecule, which was manifested in high fluorescence (432.45±131.25). However, the remaining cells (10%) did not express this marker. This conclusion was derived from low fluorescence (13.17±3.69 units, Fig. 3, *c*). Hence, simultaneous expression of CD24 and CD70 markers for cancer cells and no expression of the CD90 marker for fibroblasts allowed us to reveal tumor cells in the culture. The majority of cells were identified as fibroblasts due to high expression of the CD90 marker.

Contamination of tumor cells with stromal fibroblasts with higher proliferative activity could occur at the initial stage of isolation of the primary culture from renal cancer (HCC-3). It was followed by a progressive replacement of cancer cells from the culture. Cytofluorometric study showed that the number of cancer and stromal cells in the HCC-3 culture is 10 and 90%, respectively. However, none of the remaining five cultures of HCC contained stromal cells.

Our results indicate that CD24 and CD70 are differential markers for renal cancer in the culture. Immunocytochemical staining with antibodies against

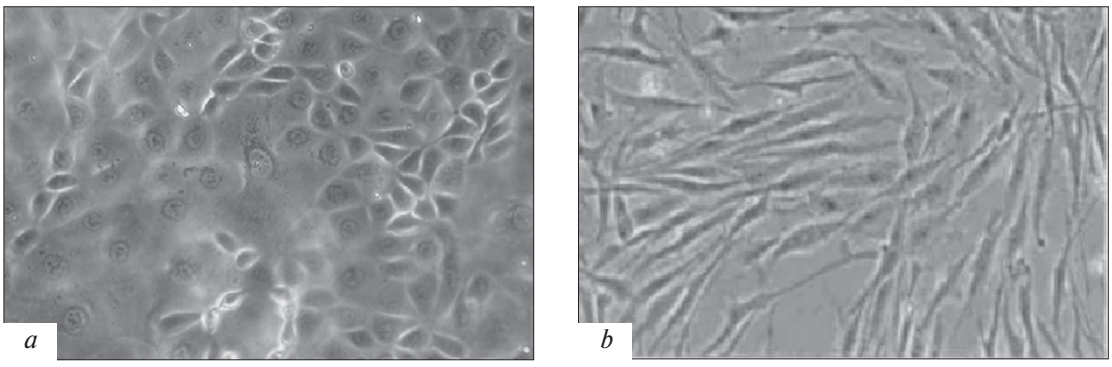

Fig. 2. Morphology of HCC cultures (phase contrasting, ×20). HCC-5 culture (*a*) and HCC-3 culture (*b*).

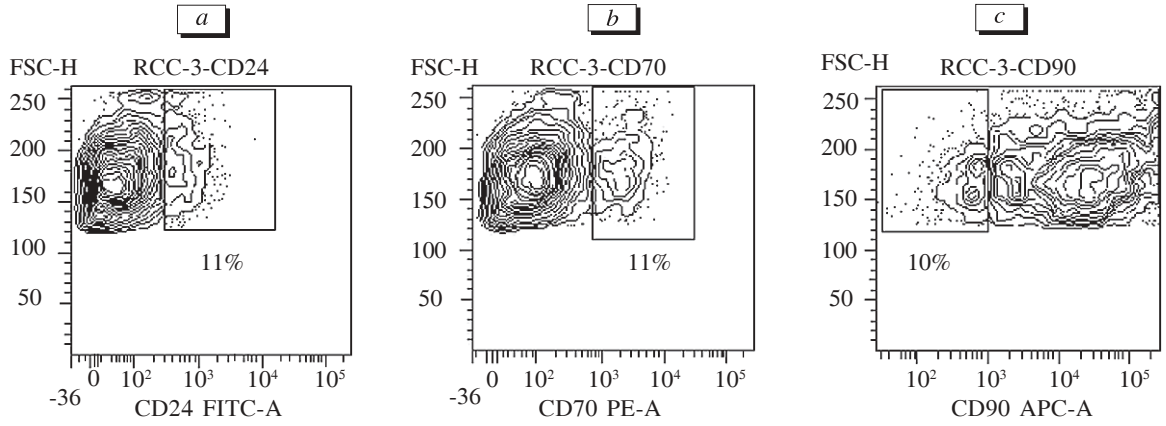

Fig. 3. Ratio between cancer and stromal cells in the HCC-3 culture. CD24 (*a*), CD70 (*b*), and CD90 (*c*). Abscissa, fluorescence intensity (expression of molecules); ordinate, direct light scattering. Number of events (cell count) in the rectangular gate is expressed in percent.

CD24 and CD70 at various stages of culturing can be used to confirm the tumor nature of cell cultures and to separate the cells for isolation of homogeneous populations.

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