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Letter to the Editor

INTEGRINS: UTILITY AS CELL TYPE- AND STAGE-SPECIFIC MARKERS FOR HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA

Dear Editor:

Integrin molecules, which consist of α and β subunits, are major receptors for extracellular matrix (ECM), and it is generally accepted that proliferation and differentiation signals are produced through interactions between integrins and ECM (2). The constituent ECM protein which binds to integrin is determined by the specific subunits that compose the integrin (2). These findings suggest that the expression pattern of various integrins may provide useful information in discriminating cell type and/or differentiation stage. Volpes et al. demonstrated the utility of integrins as cell lineage markers of hepatic differentiation by immunohistochemical staining for tissue specimens (13,14).

We have established a human sarcomatoid CC cell line, ETK-1, from a patient with cholangiocarcinoma (CC) and demonstrated that ETK-1 cells possess stem-cell-like properties and that they can differentiate along both hepatocytic and biliary epithelial lineages (4). The ETK-1 cells showed transformation at a high rate by differentiation-inducing 5-azacytidine treatment and the converted cell line, MEK, whose phenotype corresponds to that of hepatocellular carcinoma (HCC), was successfully established (4). When the ETK-1 cells were transplanted to nude mice, the xenografts were histopathologically diagnosed as well-differentiated CC (4). We could obtain the NEC cell line from the xenograft. Moreover, sarcomatoid CC cell line, SSP-25, and well-differentiated CC cell line, RBE, were established from another patient (3). SSP-25 also showed the tendency to differentiate along a biliary epithelial lineage (3). The expression of known marker proteins by these cell lines is shown in Table 1. Next, we compared the expression pattern of integrins in these cell lines and in other previously described cell lines, HCC cell lines, (HepG2 and HuH7) and a well-differentiated CC cell line (H1) (7), and then revaluated *in vitro* the role of integrins as marker proteins.

As we previously reported (3,4), ETK-1 and SSP-25 are in an immature stage, and NEC and RBE are in a mature stage along a biliary epithelial lineage. MEK is committed to a hepatocytic lineage. Normal hepatocytes show a low constitutive level of Fas antigen expression on their cell membranes (5). We found that Fas antigen was expressed by the HCC cells tested. It has been reported that thrombopoetin (TPO) is also produced in the liver (1), and we demonstrated that TPO is expressed by HCC cells, but not by CC cells, independent of differentiation stage.

We investigated the expression of integrin molecules in the described cell lines by flow cytometry (Table 2, Fig. 1). We quantified integrin expression by fluorescence intensity. Interestingly, the distribution of integrin subunits differed in the ETK-1, MEK, and NEC cell lines, which have the same origin. As compared to ETK-1 cells, integrin α_6 was markedly upregulated in NEC cells. In addition, the amount of integrin α_3 and β_4 expressed by NEC cells was also increased. In contrast to ETK-1 and NEC, integrin α_1 was positive and integrins α_3 and β_4 were negative in MEK cells. Similarly, the tendency that maturer CC cells express more integrins α_3, α_6, and β_4 was shown between SSP-25 and RBE.

After considering the results, we arrived at the following conclusions: 1) Integrins α_3 and β_4 are expressed in all CC cell lines but not in HCC cell lines, and integrin α_1 expression is demonstrated only in HCC cell lines. Therefore, integrins α_1, α_3, and β_4 are useful in distinguishing cells with a hepatocytic or biliary epithelial lineage. 2) Higher amounts of integrins α_3 and β_4 are expressed in more differentiated CC. 3) Integrin α_4 is mostly not expressed by HCC and CC cell lines. 4) Integrin β_1, which is a subunit of the very late antigen (VLA) family, is expressed by all HCC and CC cell lines. 5) Integrin α_2 is expressed by almost all CC and HCC cell lines. 6)

TABLE 1

THE EXPRESSION OF MARKER PROTEINS IN HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA CELL LINES

	HuH7	HepG2	MEK	ETK-1	SSP-25	NEC	RBE	H1
AFP[1]	+	+	+	−	−	−	−	−
Albumin[1]	+	+	+	−	−	−	−	−
TPO[2]	+	+	+	−	−	−	−	−
Fas[3]	+	+	+	−	−	−	−	−
CK19[4]	+	+	+	+	+	+	+	+
Vimentin[4]	−	−	−	+	+	−	+	−
CEA[4]	−	−	−	−	−	+	+	+
CA19-9[4]	−	−	−	−	−	+	+	+
Mucin[5]	−	−	−	−	−	+	+	+

Antigen expression was examinaed by [1]Northern blot, [2]RT-PCR, [3]flow cytometry, [4]immuno cytochemistry, or [5]PAS staining.

AFP: α-fetoprotein, TPO: thrombopoietin, CK19: cytokeratin 19, CEA: carcinoembryonic antigen, CA19-9; carbohydrate antigen 19-9.

TABLE 2

DISTRIBUTION OF INTEGRIN MOLECULES IN HEPATOCELLULAR CARCINOMA AND CHOLANGIOCARCINOMA CELL LINES

	HuH7	HepG2	MEK	ETK-1	SSP-25	NEC	RBE	H1
Integrin α_1	−	+	+	−	−	−	−	−
Integrin α_2	+	+	+	+	+/−	+	+	+
Integrin α_3	−	−	−	+	+	+	+	+
Integrin α_4	−	−	−	−	+/−	−	−	−
Integrin α_5	+	−	−	−	+	−	+	+
Integrin α_6	+	+	+	−	+/−	+	+	+
Integrin β_1	+	+	+	+	+	+	+	+
Integrin β_4	−	−	−	+	+/−	+	+	+

% of positive calls; + > 60% > +/− > 20% > −

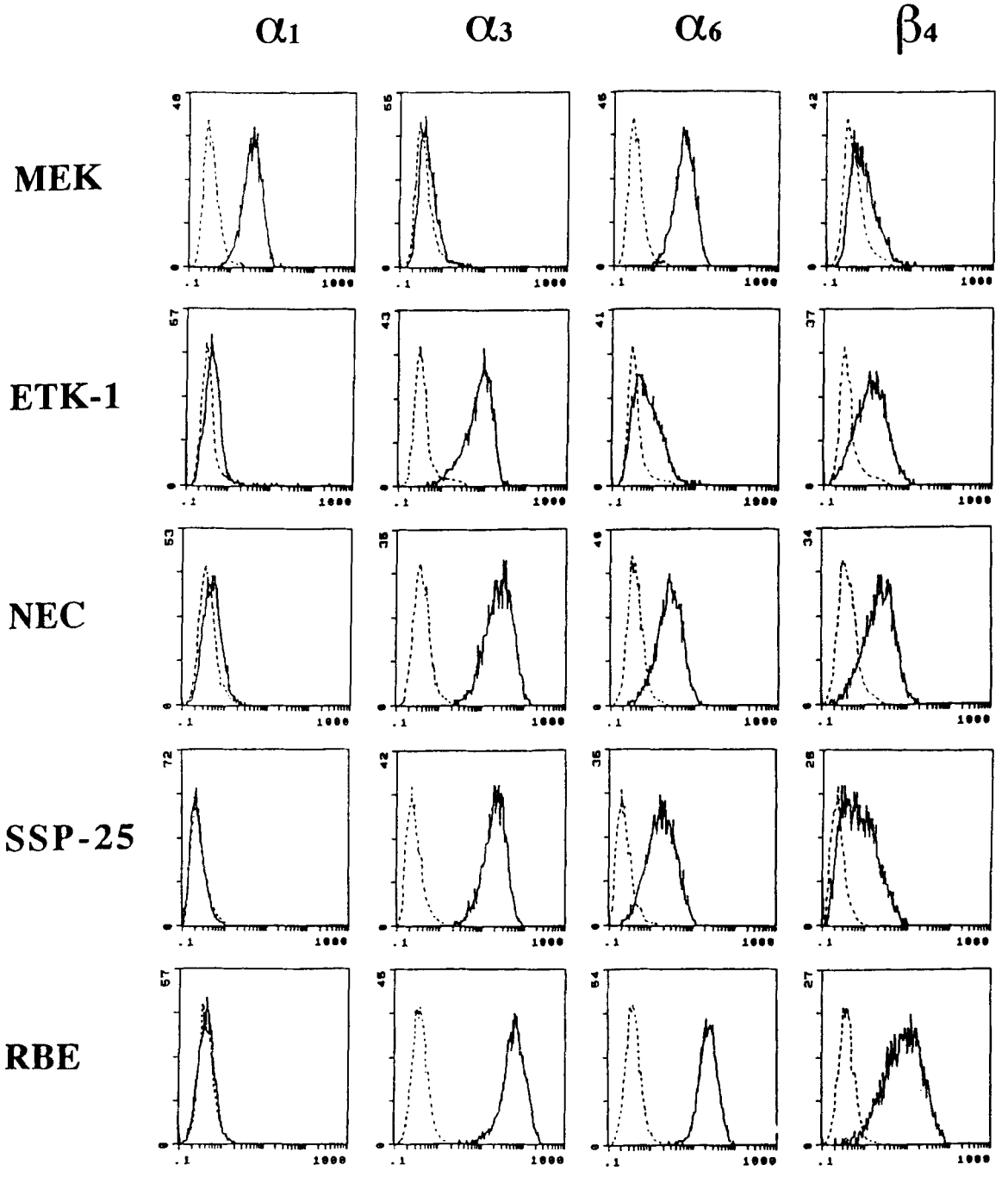

FIG. 1. Flow cytometry analysis detecting the expression of integrins α_1, α_3, α_6, and β_3 in ETK-1, MEK, NEC, SSP-25, and RBE cells. Data are presented as histograms of cell number (*y* axis) versus fluorescence intensity (log scale, *x* axis). *Dotted curves* represent negative controls.

Integrin α_5 is not uniformly expressed by HCC and CC cell lines. 7) Integrin α_6 is generally positive in CC and HCC cell lines. However, the expression is decreased in immature CC cells.

Some protein markers for HCC and CC, like albumin, α-fetoprotein, γ-glutamyl transpeptidase, cytokeratin 19, carcinoembryonic antigen, and carbohydrate antigen 19-9 are well known and utilized. Clinicopathologically, dedifferentiated HCC and CC cells, as well as hepatic stem-cells reported as "oval cells" (11), have been demonstrated in patients (8,12). It is often difficult to distinguish if these undifferentiated cells have a biliary epithelial or a hepatocytic lineage based on expression of these marker proteins because biliary epithelial cells and hepatocytes are derived from common precursor cells which retain characteristics of both biliary epithelial cells and hepatocytes (6,9,10). Therefore, we need to identify other markers

which can help to distinguish between the biliary epithelial and hepatocytic lineages, especially in immature HCC and CC. We investigated the expression pattern of various integrin subunits in ETK-1, MEK, NEC, SSP-25, RBE, and other HCC and CC cell lines, and clearly showed that integrins α_1, α_3, and β_4 are useful in discriminating between HCC and CC, even in immature stages.

When the mechanisms of differentiation or conversion of any cell lineage are studied, it is very important to correctly identify the cell type of immature cells. For this purpose, it is useful to ascertain the expression pattern of integrin molecules of cells within the biliary epithelial or hepatocytic lineage.

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