CHINESE HAMSTER LUNG CELLS TRANSFORMED WITH THE HUMAN HA-RAS-1 ONCOGENE: 5-AZACYTIDINE MEDIATED INDUCTION TO ADIPOGENIC CONVERSION.

Roberto Gambari[1] and Demetrios Spandidos[2].

(1) Centro di Studi Biochimici sul Morbo di Cooley, Ferrara; (2) Beatson Institute for Cancer Research, Glasgow, Scotland.

FHO6N1-1 and FHO6T1-1 are Chinese hamster lung cells transformed with the human normal Ha-ras-1 proto-oncogene or the activated T24-Ha-ras-1 oncogene, respectively (1). In this study we analysed the effects of the demethylating agent 5-azacytidine (5-azaCR) on the capability of these two cell lines to express differentiated functions. Table I shows that when FHO6N1-1 cells are treated with 12 μM-50 μM 5-azaCR a proportion of the colonies analysed show adipogenic conversion.

TABLE I. Effects of 5-azaCR on adipogenic conversion of FHO6N1-1 and FHO6T1-1 cells.

	5-azaCR (μM)			
	-	12	50	100
FHO6N1-1 cells.				
Total colonies scored	476	26	28	22
Adipogenic colonies	3	1	8	2
% of differentiation	0.1	4	31	9.8
FHO6T1-1 cells.				
Total colonies scored	511	312	72	89
Adipogenic colonies	0	0	0	0
% of differentiation	0	0	0	0

Cells were cultured as indicated for 20 days. After 7 and 14 days the cells were seeded into secondary cultures.

The morphology of the adipocyte-like FHO6N1-1 cells is shown in Fig.1. All the inclusions showed in Fig.1C are stainable with Oil Red O stain (2)(Fig.1E) but not with May-Grunwald/Giemsa stain (Fig.1D).

Adipogenic colonies from 5-azaCR treated FHO6N1-1 cells might be subcloned -FHO6N1-1(Adipo) cells- and the majority of them was found to maintain the capability to differentiate into adipocytes even when cultured in the absence of 5-azaCR. Since this differentiation is associated with a sharp decrease in the rate of cell proliferation(data not shown),a long term culture of FHO6N1-1(Adipo) cells in the absence of 5-azaCR leads to a back selection of undifferentiated cells (Table II). In sharp contrast with these findings, no adipogenic conversion was observed in 5-azaCR treated FHO6T1-1 cells,which contain the T24Ha-ras oncogene integrated and expressed at high levels (1). It is of interest to point out that FHO6N1-1 cells are immortalized but not tumorigenic, while FHO6T1-1 cells do induce tumors when injected into mice (1).

TABLE II. Stability of the differentiated phenotype of FHO6N1-1(Adipo) cells cultured in the absence of 5-azacytidine.

Days	Total colonies scored	Adipogenic colonies	%
10	17	16	91
30	66	61	92
44	25	22	88
60	71	54	76
240	68	3	3.2

In this experiment FHO6N1-1(Adipo) cells were seeded into secondary cultures weekly.

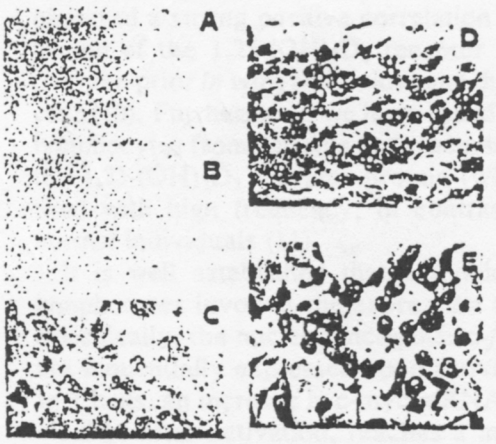

Fig.1: Morphology of FHO6T1-1(A), FHO6N1-1 (B) and FHO6N1-1(Adipo) cells(C). D,E: FHO6N1-1(Adipo) cells stained with May-Grunwald/Giemsa stain (D) or with May-Grunwald/Giemsa stain plus Oil Red O stain (E).

Taken together these results suggest that immortalized Chinese hamster FHO6N1-1 cells can undergo adipogenic differentiation. Since no differentiation was on the contrary observed in FHO6T1-1 cells, these two cell lines might be considered as a useful experimental model system to study the relationship between the tumor phenotype associated with the activation of the Ha-ras-1 oncogene and the capability to express differentiated functions.

Acknowledgments: Work supported by CNR grants n.83022014.04 and n.84.00728.04.

References: (1) Spandidos DA and Wilkie NM, Nature 310:469, 1984; (2) Walker C and Shay JW, Differentiation 25:259, 1984.

0309-1651/86/030173-01/$03.00/0