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SHORT COMMUNICATION

THE EFFECT OF EXOGENOUS HUMAN *RAS* AND *MYC* ONCOGENES IN MORPHOLOGICAL DIFFERENTIATION OF THE RAT PHEOCHROMOCYTOMA PC12 CELLS

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Abstract—An electroporation technique was employed to study the effect of oncogenes H-*ras* and c-*myc* after their short-term expression in the rat pheochromocytoma PC12 cells. It was found that within 6 days after electroporation the mutant T24 H-*ras*1 gene induced differentiation of PC12 cells whereas the c-*myc* blocked NGF-induced differentiation. The induction of differentiation by the T24 H-*ras* gene may suggest a physiological role of the *ras* gene in cell differentiation as well as in cell proliferation.

Key words: Oncogenes, Pheochromocytoma, Differentiation.

The main action of oncogenes is the induction of cell proliferation,[9,16] although in some cases their action has been found to inhibit cell proliferation.[7,13] Oncogenes also play a role in differentiation[10] in some cases blocking or in others inducing differentiation.[18] Such roles have now clearly emerged for members of the *ras* oncogene family where these genes can either block[2,4,12] or induce[1,3,6,11] differentiation. The *myc* oncogene has also been shown to block differentiation in some systems.[8]

In the present study the effect of *ras* and *myc* oncogenes in rat pheochromocytoma PC12 cells was examined[5] after introducing these genes by electroporation. The results suggest a differential response of these cells to the two oncogenes. Whereas the mutant T24 H-*ras*1 induces, the c-*myc* blocks NGF-induced differentiation.

EXPERIMENTAL PROCEDURES

Cell culture

A subclone (PC12-A) of the rat pheochromocytoma cells was used in these studies. The cells were grown in Ham's SF12 medium containing 15% fetal calf serum (FCS).

Plasmids and electroporation

The construction of plasmids pH06T1 and pH06N1 carrying the human mutant T24 or normal H-*ras*1 gene respectively in the vector Homer 6[14] and pMCGM1, carrying the human c-*myc* gene[15] have been previously described. Plasmids, pH06T1 and pH06N1 also carry the SV40 and Moloney LTR enhancer sequences whereas plasmid pMCGM1 carries only the Moloney LTR enhancer sequences. Electroporation of plasmid DNA into recipient PC12-A cells was carried out as previously described[17] using 10 μg plasmid DNA per 5×10^6 cells at 2 kV/cm. NGF was purchased from Sigma.

RESULTS

An electroporation technique[17] was used to transfer and express plasmid DNA into a subclone (PC12-A) of the rat pheochromocytoma cell line PC12 (our unpublished results). Six days after electroporation with the plasmid pH06T1 which carries the human T24 H-*ras*1 gene in the plasmid

vector Homer 6, transfectants had undergone neuronal differentiation (Fig. 1B) similar to that induced by NGF (Fig. 1C). Neither the Homer 6 vector alone (Fig. 1A) nor the normal H-*ras*1 gene, plasmid pH06N1 (data not shown), elicited this effect.

When plasmid pMCGM1 which carries the human c-*myc* gene was introduced into PC12-A cells (Fig. 2A) NGF-induced differentiation was blocked (Fig. 2B).

DISCUSSION

In the present study a short-term electroporation technique was employed to study the effects of oncogenes *ras* and *myc* on the differentiation properties of the rat pheochromocytoma PC12 cell line. There was a differential response between the two oncogenes in that the human T24-*ras*1 gene induces differentiation and the c-*myc* gene blocks the NGF-induced process of differentiation.

The results on the effects of *ras* and *myc* genes on PC12 rat pheochromocytoma differentiation are similar to those obtained with the mutant HT1080 human N-*ras*[6] and the human c-*myc*[7] genes. The PC12 cells were also found to differentiate on infection with retroviruses carrying the H-*ras* or K-*ras* genes[11] or on microinjection of oncogenic H-*ras* p21 protein but not the normal p21.[1,3]

Block of differentiation by activated *ras* genes has also been observed, i.e. the human T24 H-*ras*1 gene, when introduced by transfection into F4-12 B2TK$^-$ mouse erythroleukemic cells interferes with differentiation as shown by the failure of about 80% of stable transfectants to differentiate when induced by hexamethylene bis-acetamide (HMBA).[18] The T24 H-*ras*1 gene also inhibited adipocytic cell differentiation.[4] In addition the T24 H-*ras*1 gene prevents mouse muscle myoblast differentiation[12] and inhibits myogenesis of the muscle cell line BC3H1.[2] Thus, depending on the cell system the same *ras* gene can either induce or block differentiation.

The implication of the above studies is that oncogenes might play a physiological role in differentiation as well as in proliferation.

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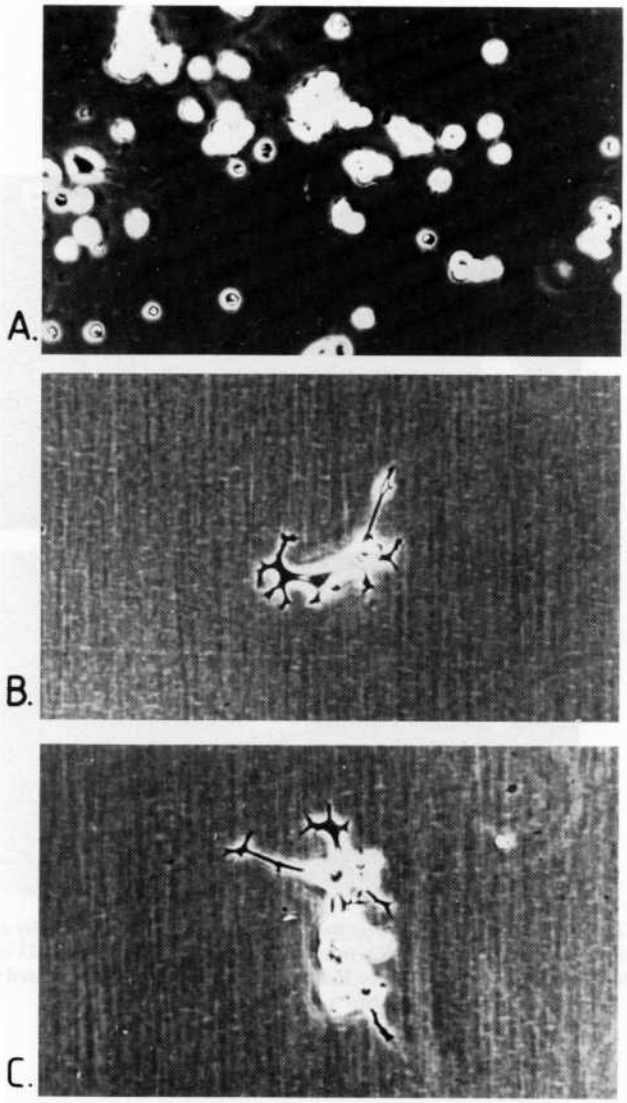

Fig. 1. T24 H-*ras*1- and NGF-induced differentiation in PC12 cells. (A) Cells transfected with Homer 6. (B) Cells transfected with pH06T1. (C) Cells exposed to 20 ng/ml of NGF. Cells were photographed at 6 days post-treatment.

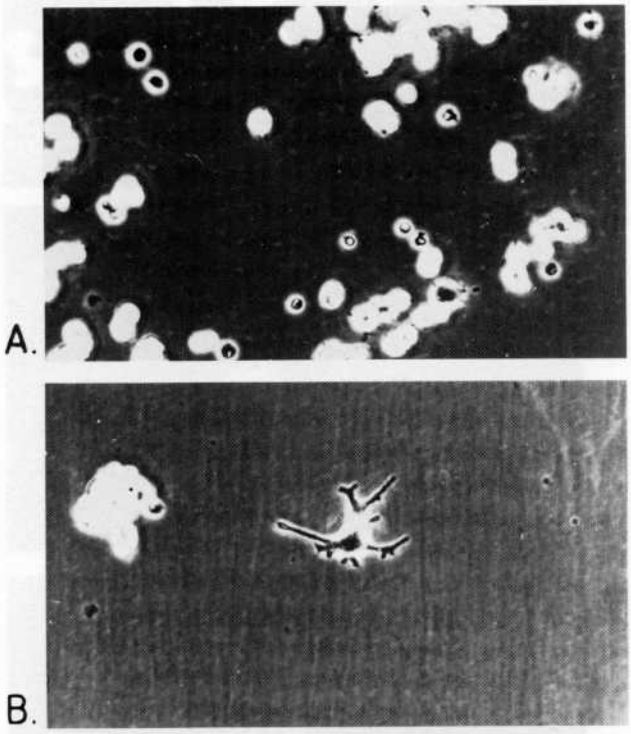

Fig. 2. Block of NGF-induced differentiation by the human *myc* gene transfected by electroporation into PC12 rat pheochromocytoma cells. (A) Cells transfected with plasmid pMCGM1 carrying the human *myc* gene. (B) Cells transfected with the vector Homer 6. Both cultures were treated with 20 ng/ml NGF and photographed at 6 days post-treatment.