Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 96-102

MESSENGER RNA POPULATIONS AND THEIR NUCLEAR PRECURSORS IN CULTURED HUMAN GLIOMA AND FETAL BRAIN CELLS

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Received November 24, 1982

SUMMARY: Using labelled single copy DNA to cytoplasmic messenger RNA from a glioma cell line, it is shown by the excess RNA hybridization technique that a human glioma and a human fetal brain cell line both contain the mid and low abundancy classes of cytoplasmic messenger RNA. However, the high abundancy class present in the glioma cells is absent from the hybridization profile of the fetal cell line. Most of the nuclear RNA species complementary to this single copy DNA were present in the low abundancy class of both cell types; the mid-abundancy class was present in much lower concentration than in glioma cytoplasmic RNA and the high abundancy class was essentially absent. The extent of formation of S_1-nuclease resistant hybrids indicated that some of the messengers which are present in the high abundancy class in the cytoplasm of glioma cells are present in the lower abundancy classes of fetal brain cells. Thus the glioma cells appear to exhibit a higher degree of specialization potential than the embryonic cells.

A human glioblastoma multiforme cell line, a clone from this line and a human fetal brain cell line have been characterized previously (1-4). The effect of potential growth modifiers on the rate of proliferation and the cell cycle of these cells was subsequently studied (5-7). These culture systems offer the potential to compare the genetic expression and the regulation of cellular

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0006-291X/83/010096-07$01.50/0

proliferation in a human brain tumor cell and its embryonic counterpart. Such comparisons are of importance since the theory that oncogeny represents blocked ontogeny (8) implies that upon neoplastic transformation cells retain, or obtain in varying degrees, phenotypic characteristics of the embryonic cell.

The genetic expression in different cellular phenotypes may be compared by molecular hybridization. Many earlier studies have involved comparisons of messenger RNA populations in the cells during phenotypic changes induced by, for example, differentiation (9). The messenger RNA sequences in the tumor cell has also been compared to the differentiated cell of origin (10). In such comparisons it was found that related cell types often differ within the limits of the techniques in the relative messenger abundance, rather than in the presence, or absence, of specific messengers, or groups of messengers.

In the present investigation the cytoplasmic messengers and the nuclear pre-messengers of the glioma and fetal brain cell lines were compared by RNA excess hybridization with single copy DNA (cDNA) prepared to the cytoplasmic messenger RNA of the glioma cells. The results suggest that these tumor cells represent a later stage in ontogeny than do the 16 week fetal cells.

MATERIALS AND METHODS

Cell Culture: The isolation, growth and cloning of the cell lines has been described in detail previously (5). Briefly, the glioma line was originally derived from a glioblastoma multiforme biopsy obtained from the temporo-parietal region of a 72 year old woman. The fetal cell line was originally derived from the cerebral hemispheres of a 16 week old therapeutically aborted fetus. The cells were cloned by the glass-chip technique (11), then grown in roller bottles in Eagles Minimal Essential Medium (MEM) plus 5% NCTC-135 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (Flow Labs, Rockville, MD) and 10 ml/l of antibiotic antimycotic mixture (Gibco).

Cell Fractionation: Confluent monolayer cells were harvested, rinsed twice with ice-cold phosphate, then treated for 7 min with buffer containing 1% NP40 (12) before gently homogenizing. Nuclei were pelleted from the homogenate at 10,000 rpm x 10 min at 3° C, resuspended in the lysis buffer and repelleted again to eliminate cytoplasmic contamination associated with the perinuclear membrane. The lack of gross cross-contamination of the nuclear and cytoplasmic fractions was confirmed by light microscopy.

RNA Purification: The cytoplasmic fraction was adjusted to 0.1 MTris -0.5% sodium dodecyl sulfate, 100 mM EDTA, pH 9.0, prior to deproteinization by phenol at pH 9.0 and 4°C(13). The nuclear RNA was similarly deproteinized at 65°C with redistilled phenol, after dissolving the nuclear pellet in 10 vols. of buffer containing 140 mM NaCl, 50 mM sodium acetate, pH 5.0, 10 mM EDTA and 0.5% sodium dodecyl sulfate (14). The RNA was precipitated twice at -20°C from 0.15 M sodium acetate, pH 5.1, then lyophilized and stored in liquid nitrogen.

Preparation of cDNA: DNA complementary to cytoplasmic messenger RNA of cloned glioma cells, was synthesized by incubating 30µg of cytoplasmic RNA with 5 units of "avian myeloblastosis virus" reverse transcriptase (obtained from Dr. J. Beard, Life Sciences, Inc., St. Petersberg, FL), 0.5 µg of oligo(dT) primer (P-L-Biochemicals, Inc., Milwaukee, WI), 1.0 mM each of dATP, dGTP, dTTP and 250 µCi of [^{3}H]-dCTP (54.9 Ci/m mol, New England Nuclear Corp., Boston, Mass.) in 100 µl of 50 mM Tris-HCl, pH 8.2 buffer containing 10 mM dithiothreitol, 5.0 mM KCl, 5 mM Mg acetate and 12.5 µg/m of actimomycin D for 60 min. at 37°C (15). After hydrolyzing the RNA with alkali, the DNA was recovered by chromatography on a Sephadex G-50-Chelex 100 with 50 µg. of liver tRNA as carrier, and sized by 5% slab polyacrylamide gel electrophoresis. The gel loading buffer contained 80% formamide, 10mM NaOH, 0.1% (w/v) xylene cyanol and bromophenol blue and the DNA was heated to 95°C for 5 min in this buffer before electrophoresis. The running buffer was Tris (90mM, pH 8.3), boric acid (90mM), EDTA (10mM) and urea (8.3 M). Fluorography was according to Laskey and Mills (16).

Hybridization studies: The cDNA:RNA hybridization was performed in double siliconized glass capillaries containing in a reaction volume of 4.0 µl, 0.03 µg/ml of cDNA (5000 cpm) and from 0.8 µg/ml to 9.0 mg/ml of RNA in 240 mM Na_2HPO_4/NaH_2PO_4, pH 7.0 and 2.0 mM EDTA. The hybridization was carried out with total cytoplasmic or nuclear RNA since 30-40% of the messengers may exist in a poly A deficient fraction which does not bind to oligo (dT)-cellulose (17-19).

The reaction mixtures in the heat-sealed capillaries were boiled 4 min, then incubated at 70°C to achieve the appropriate Rot value. Hybrid formation was assayed using S_1 nuclease treatment to destroy single stranded nucleic acid (20). The data were analyzed with the aid of a computer. By subjecting 3 consecutive points at a time on the data plot (Fig. 1) to least square analysis, an estimate was obtained of the slope within each overlapping triplet, together with a confidence interval around each slope. It was assumed that the data consisted of non-overlapping straight lines and that all error terms were normal with mean zero and common variance.

RESULTS AND DISCUSSION

Upon acrylamide gel electrophoresis most of the cDNA migrated as a broad band within the size range of 600 to 1600 nucleotides, with a single band at 180 nucleotides. The glioma [^{3}H] cDNA was hybridized to excess glioma, or fetal brain cytoplasmic RNA with the Rot values extending over 7 orders of magnitude. The results are shown in Fig. 1. In the homologous hybridization (i.e. glioma cDNA vs. glioma

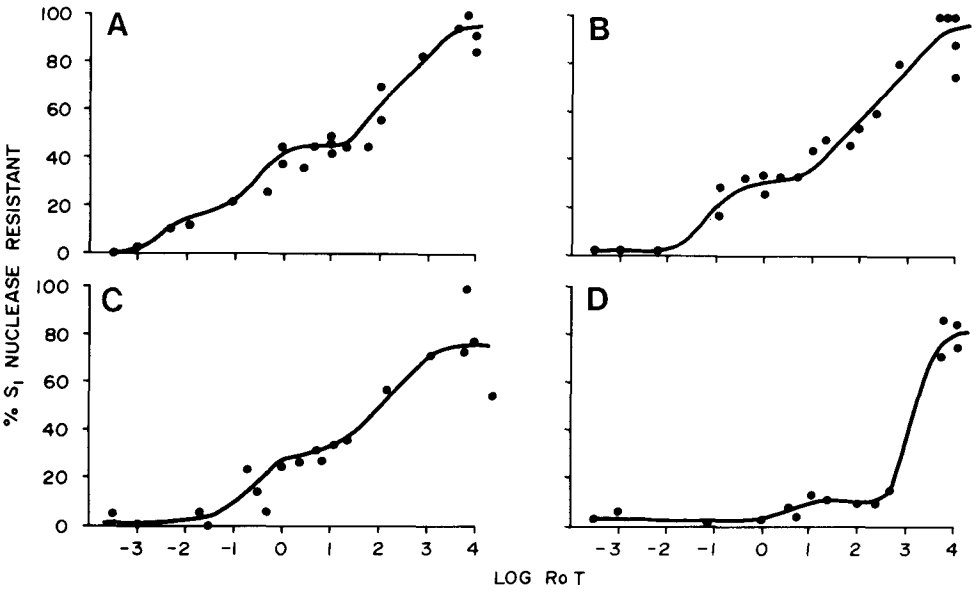

Fig. 1. Hybridization of single copy DNA to cytoplasmic messenger RNA of human glioma cells with (A) the homologous cytoplasmic messenger RNA from the glioma cells, (B) the cytoplasmic messenger RNA from fetal brain cells (C) the nuclear (messenger precursor) RNA from glioma cells and (D) the nuclear RNA from fetal brain cells.

cytoplasmic messenger RNA) three RNA abundancy classes were observed (Fig. 1A). The cumulative total of cDNA hybridizing in the high, intermediate and low abundancy classes was 14%, 45% and 94% at Rot values of 10^{-2}, 10 and 10^4, respectively. In the heterologous hybridizations (i.e. glioma cDNA vs. fetal brain RNA) only the intermediate and low abundancy classes were observed (Fig. 1B) with 30% and 94% of the cDNA hybridizing at Rot values of 1.0 and 10^4, respectively. Most of the cDNA formed S_1 resistant hybrids in both cases. This suggests that essentially all of the messenger species present in the glioma cells are also present in the fetal brain cells, but those represented in the high abundancy fraction of the glioma cells are present in much lower concentration in the fetal cell line.

Upon hybridization of the cDNA to cytoplasmic messenger with glioma cell nuclear RNA, only the intermediate and low abundancy classes were detected, with 32% and 75% of the cDNA hybridizing at Rot values of 10 and 10^4, respectively (Fig. 1C). Therefore, 75% of the

cDNA nuclear RNA hybrids were S_1 nuclease resistant at saturating concentrations of nuclear RNA, as compared to 94% in the homologous hybridizations with glioma cytoplasmic RNA (see above).

In the heterologous reaction between fetal brain cell nuclear RNA and the glioma cDNA probe (Fig. 1D), again only the mid and low abundancy classes were present. Approximately 10% and 80% of the cDNA was in S_1-nuclease resistant hybrids at Rot values of approximately 300 and 10^4. Again these data confirm that most of the messenger sequences in the nuclear RNA are present at a lower abundancy than their counterparts in the cytoplasm, there being an absence of the high abundance species and a markedly lower concentration of the midabundant species. Since essentially all of the sequences of fetal cell nuclear RNA which hybridizes with the single copy DNA is located in the low abundant class, there is a significant shift of the Rot 1/2 value i.e. from approximately 2.0 with the glioma cell nuclear RNA, to approximately 3.0 with the fetal cell nuclear RNA, reflecting the increased complexity. Differences in other $Rot_{1/2}$ values shown in Fig. 1 are marginal. The presence of the abundant class of messenger RNA's in the cytoplasm, but not the nucleus of the human glial cells, reflects the operation of post-transcriptional controls (21).

In related studies (C. Icard-Liepkalns, unpublished observations) it was shown that the template activity in the reticulocyte lysate translation system of the messenger RNA isolated from glioma and fetal brain cell lines, was comparable to that of pure globin messenger RNA. Upon analysis of the [^{35}S]-methionine labeled translation products by two dimensional acrylamide gel electrophoresis and fluorography, a number of proteins were observed which represented products of the high and mid-abundant messenger RNA's. Predictably some were common to both cell types, while the concentration of others depended on the cell type. None of these proteins can at present be identified with known proteins.

In general it is accepted that abundant messenger RNA's are characteristic of the differentiated state (10). They are also characteristic of cells where the corresponding gene(s) has been amplified, as for example, under the selective pressure of chemotherapy (22). The fact that the glioma nuclei do not contain abundant species while the cytoplasm does, is easily explained by differential post-transcriptional selection of the individual messenger species (21).

Tumor cells appear to be blocked in development, somewhere between fetal (embryonic) cells and the fully differentiated cells (8). Because of a lack of appropriate normal embryonic cells for comparison it is common to compare the biochemical and morphological characteristics of the tumor with the corresponding differentiated cell (e.g. liver with hepatoma) in order to attempt to identify the lesion leading to loss of growth control. Since tumor cells also have many characteristics similar to embryonic cells, with the exception of loss of growth regulation and other neoplastic attributes, it is equally important to also carry out studies on the other side of the spectrum. Since the results of the present study indicate that the glioma cells are biochemically more highly differentiated than the embryonic cells, the two human cell lines should be very useful in future investigations related to growth regulation in neoplasia.

ACKNOWLEDGEMENT

This investigation was supported by a grant from the American Cancer Society (V.A.L.) and grant GM/CA2922 from the National Institutes of Health (T.E.W.). We thank Mr. M.G. SRIRAM of the Division of Computing Services (O.S.U) for expert assistance on the statistical analysis of these data.

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