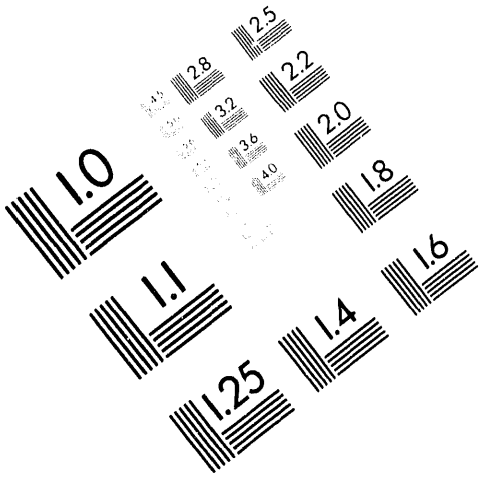

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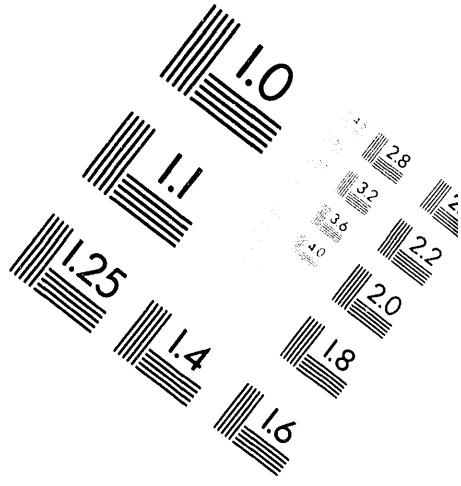

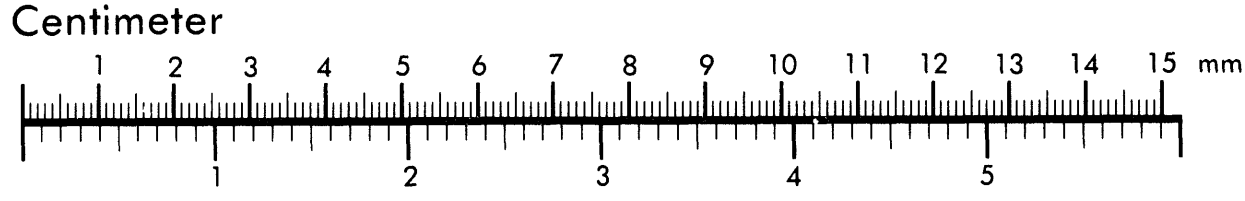

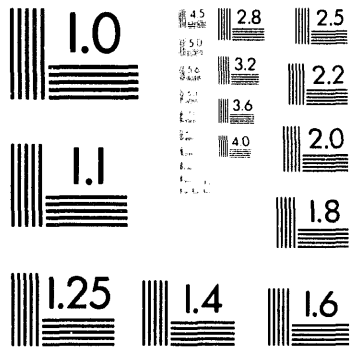

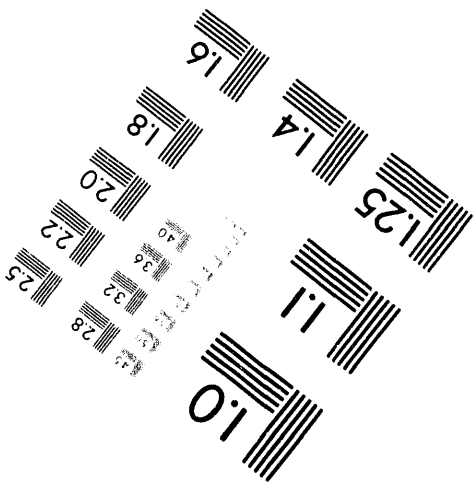

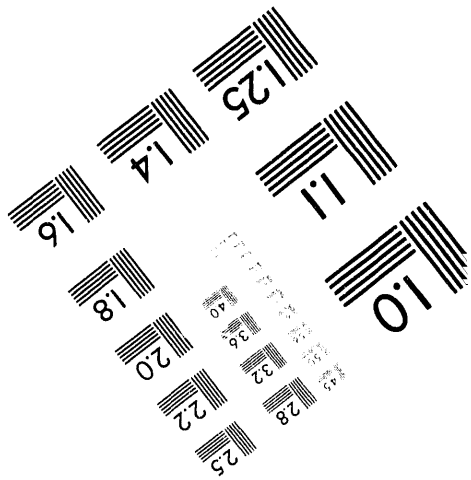

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For submission to Cancer Research; Advances in Brief Category

Increased IMP Dehydrogenase Gene Expression in Solid Tumor Tissues and Tumor Cell Lines

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Abbreviations: IMPDH: IMP dehydrogenase

ABSTRACT

IMP dehydrogenase, a regulatory enzyme of guanine nucleotide biosynthesis, may play a role in cell proliferation and malignancy. To assess this possibility, we examined IMP dehydrogenase expression in a series of human solid tumor tissues and tumor cell lines in comparison with their normal counterparts. Increased IMP dehydrogenase gene expression was observed in brain tumors relative to normal brain tissue and in sarcoma cells relative to normal fibroblasts. Similarly, in several B- and T-lymphoid leukemia cell lines, elevated levels of IMP dehydrogenase mRNA and cellular enzyme were observed in comparison with the levels in peripheral blood lymphocytes. These results are consistent with an association between increased IMP dehydrogenase expression and either enhanced cell proliferation or malignant transformation.

INTRODUCTION

IMP dehydrogenase (EC 1.1.1.205, IMPDH) is the enzyme that catalyzes the formation of XMP from IMP and is the rate-limiting enzyme in the *de novo* synthesis of guanine nucleotides (1). IMPDH is coded by two distinct genes termed Type I and II (2, 3) and has an essential role in providing the necessary precursors for DNA and RNA biosynthesis, as evidenced by the abrupt cessation of DNA synthesis when cells are treated with IMPDH inhibitors (4). The essential nature of IMPDH activity is manifested in an association between IMPDH activity and cellular proliferation. In general, normal tissues that exhibit increased cell proliferation also exhibit increased IMPDH activity (5). A similar relationship between increased proliferation and elevated enzyme activity has been found among a panel of rat hepatomas having varied growth rates (1). Moreover, the IMPDH activities in these hepatomas were disproportionately higher than those of normal tissue (1). In addition, it was found that IMPDH inhibitors induce differentiation of a variety of human tumor cells, including HL-60 promyelocytic (6, 7) and CEM T-lymphoid leukemia cells (8), MCF-7 breast cancer cells (9), and several melanoma cell lines (10). This differentiation is a consequence of the reduction in guanine nucleotides attributable to inhibition of the activity of IMPDH. These studies implicate IMPDH activity and the level of guanine nucleotides in the regulation of cell proliferation and differentiation, and malignancy. To assess its involvement in the control of cell proliferation and malignant transformation, we analyzed IMPDH gene expression in a series of human solid tumor tissues and tumor cell lines in comparison with their normal counterparts.

MATERIALS AND METHODS

Materials. The human fetal skin and muscle fibroblasts were obtained from Flow Laboratories, Inc. (McLean, VA). Other cell lines were obtained from the American Type Culture Collection (Rockville, MD). Tumor tissue samples were obtained after surgical procedures and stored in tissue culture medium until RNA isolation. Normal brain tissue used in this study was removed at autopsy, stored overnight at 4

°C, and then frozen on dry ice for transport to the laboratory. Normal tissue used for the extraction of RNA included cerebral and cortical brain samples.

Cell culture. Cells were grown in RPMI 1640 medium supplemented with 15% fetal calf serum, penicillin (100 U/ml), streptomycin (100 ug/ml), and glutamine (2 mM) in a humidified incubator supplied with a constant amount of 8% CO_2 in air. Growth-rate experiments were performed by seeding 5×10^4 cells in 5 ml of medium in 60-mm Petri dishes and determining cell number per plate in successive 24-h intervals. The generation time was derived from the slope of the linear reciprocal plots defined by regression analysis of the data.

Human peripheral blood lymphocytes were isolated from freshly drawn peripheral blood by Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) gradient centrifugation as described (11). The mononuclear fraction contained predominantly lymphocytes (85-90%), with monocytes comprising the remaining percentage. Immediately after purification, the lymphocyte fraction was resuspended in the appropriate buffer for either immunoblot blot analysis or for isolation of total cellular RNA.

Measurement of IMPDH protein and activity. For analysis of cellular IMPDH activity, cells were washed in 20 mM Tris-HCl, pH 8.3, 50 mM NaCl, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride and were homogenized to disrupt the cells. After the mixture was centrifuged for 5 min at 4 °C in a microfuge, the supernatant was removed for determination of enzyme activity as previously described (2). One unit of enzyme activity is the amount forming 1 μmol of NADH/min at 37 °C under the prescribed assay conditions. Protein concentration was determined by the Bradford method (12).

RNA isolation and analysis. Electrophoretic separation of the cellular proteins and immunoblot analyses were carried out as previously described (13). The anti-IMPDH antibody was prepared against the purified Chinese hamster protein (13) and shown

by immunoblot analysis to react with IMPDH from Chinese hamster and human cells (13).

RNA was purified by centrifugation through a CsCl cushion as described by Chirgwin *et al.* (14). Northern blot analyses were performed as previously described (13). Ethidium bromide staining intensity and hybridization with a 18S ribosomal RNA probe derived by *Eco*RI digestion of plasmid pB (Dr. James Sylvester, Hahnemann University, Philadelphia, PA) were used for the assessment of RNA quantities in each lane. For the hybridization analysis, we used a 1.3-kilobase pair IMPDH probe derived by *Eco*RI digestion of the human cDNA clone (2).

RESULTS

To assess the involvement of IMPDH gene expression in the control of cell proliferation and malignant transformation, we examined the steady-state level of IMPDH mRNA in tissue samples from neuronal tumors and normal brain. The tumors used in this study included neuroblastomas, astrocytomas, and a Ewing's sarcoma. Northern blot analysis of total RNA indicated increased steady-state levels of a 2.2-kilobase transcript corresponding to the Type II IMPDH message in all the tumor samples (Fig. 1) and in tumor sample CM-6 in particular in comparison to the normal brain tissue samples. None of the tissue samples contained detectable levels of a 3.7-kilobase transcript corresponding to the Type I IMPDH message. Southern blot analysis of genomic DNA isolated from the CM-6 tumor tissue indicated that there were the same number of copies of the IMPDH gene in DNA prepared from the CM-6 tumor and several normal tissue samples, eliminating the possibility that the increased IMPDH mRNA is a consequence of IMPDH gene amplification (data not shown).

To determine whether increased IMPDH gene expression is the cause of the elevated levels of IMPDH activity in tumor cells, we examined the steady-state level of IMPDH mRNA in several lymphocytic leukemia cell lines and in normal peripheral blood lymphocytes. Relative to peripheral blood lymphocytes, an elevated steady-state level of Type II IMPDH mRNA was consistently observed in RNA

isolated from the leukemic cell lines (Fig. 2A). A similar pattern was observed for the amounts of cellular IMPDH detected with the specific IMPDH antibody using the Western blotting technique (Fig. 2B) and for measurements of the IMPDH activity in these cells (data not shown).

The marked differences in expression, amount, and activity of IMPDH between the normal and tumor cells may be associated with the low level of cell proliferation in the normal cells versus the active proliferation of the tumor cells. To affirm this possible relationship, we examined IMPDH expression in sarcoma cells and proliferating cultured normal human fibroblasts (Fig 3). The differences in the levels of IMPDH mRNA and cellular enzyme between the normal and tumor cells were not as prominent as those observed between the brain tumors and normal brain or between leukemic cells and the normal peripheral blood cells. However, all of the sarcoma cells had higher levels of Type II IMPDH mRNA, and larger amounts of enzyme, and greater IMPDH activity than the normal fibroblasts (Fig. 3, Table 1). Yet, none of the sarcoma or fibroblast samples contained detectable levels of the Type I IMPDH message. These differences may be attributable in part to a difference in the proliferation rate of the various cell types, because the 37-h doubling time of the normal fibroblasts is greater than that of the sarcoma cells (Table 1). However, factors in addition to cell proliferation appear to influence the activity of the enzyme, because an absolute correlation between IMPDH activity and cellular proliferation rate was not always observed for the tumor cell lines.

DISCUSSION

Our results show increased expression of Type II IMPDH in several solid tumor tissue samples in comparison to samples of normal brain tissue. Although considerable heterogeneity in the level of IMPDH mRNA was observed in the solid tumor samples, all tumor samples contained higher IMPDH mRNA levels than the normal brain samples. The variation in the steady-state level of IMPDH mRNA in the solid tumor samples may, in part, reflect the different contributions of normal and tumor tissue in the samples. However, the relatively low level of IMPDH

mRNA observed in normal brain tissue indicates the nature of the tumor sample is the major factor that contributes to the heterogeneity in IMPDH expression in the different samples. In the case of the CM-6 tumor sample, the elevated level of IMPDH expression was not a consequence of gene amplification. Thus, the enhanced expression of the IMPDH gene in these tumor sample is most likely due to other regulatory events.

Our analysis also demonstrates an increased IMPDH expression in sarcoma cells relative to normal fibroblasts and in leukemia cells relative to peripheral blood lymphocytes. This increased expression is manifested by higher levels of IMPDH mRNA, protein and activity in the tumor cells. In all these samples, there was a correspondence between the steady-state level of IMPDH mRNA, the amount of cellular enzyme, and to a large degree, the activity of the enzyme. The wide variation in the steady-state level of IMPDH mRNA indicates there is a range over which the cell can modulate the enzyme quantity. In nonproliferating cells such as peripheral blood lymphocytes, low levels of IMPDH message and protein are present which is consistent with measurements of IMPDH activity in these cells (15-16). Although higher levels of IMPDH expression were observed in proliferating normal human fibroblast cells, all of the sarcoma cells had increased IMPDH mRNA levels relative to the fibroblasts. On the basis of the differing proliferation rates of normal fibroblasts and the sarcoma cells and also the brain tumors and normal brain tissue, it is likely that part of the increase in mRNA IMPDH in the tumor cells is attributable to their increased proliferation rate. These results are consistent with an association between IMPDH gene expression and cell proliferation and perhaps indicate an obligatory decrease of IMPDH expression with the cessation of cell proliferation. Our studies do not discern whether the increased expression of IMPDH, which is associated with enhanced cell proliferation, is a prerequisite or a consequence of the acquired malignant phenotype.

One consistent pattern observed in this study is a general correlation between the level of IMPDH mRNA, the amount of cellular enzyme, and to a large degree, the activity of IMPDH. Such a correlation was also observed in a series of Chinese

hamster variant cell lines with different levels of IMPDH gene amplification (13). In these cells, the level of gene amplification paralleled the levels of IMPDH mRNA, protein, and activity. Similar results were obtained by an analysis of IMPDH mRNA and enzyme activity in several human leukemias (16). Although we do not exclude other regulatory controls, our data suggest that regulation of the steady-state level of IMPDH mRNA is a primary means by which to modulate cellular IMPDH activity and is the basis for the increased IMPDH activity which is observed in tumors.

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Table 1. Proliferation rate and IMPDH activity in normal fibroblasts and sarcoma cells

Cells	Doubling Time (h)	Specific Activity (mU/mg of protein)
Normal Fibroblasts	37	0.8
Fibrosarcoma Hs913	27	1.9
Fibrosarcoma HT 1080	24	1.2
Osteosarcoma U2-OS	23	3.7

FIGURE LEGENDS

Fig. 1. Northern blot analysis of IMPDH steady-state mRNA and protein levels in tumor and normal brain tissue. The same filter was used for hybridization to both the IMPDH and 18S ribosomal RNA probes.

Fig. 2. Northern (A) and immunoblot (B) analysis of IMPDH steady-state mRNA and protein levels in normal and leukemic blood cells. The T-lymphoid leukemias are I, MOLT-4 and II, CEM cells; the B lymphoid leukemias are I, RAJI and II, CCL-155 cells.

Fig. 3. Northern (A) and immunoblot (B) analysis of IMPDH steady-state mRNA and protein levels in normal fibroblasts and sarcoma cells. The normal fibroblasts are of human fetal skin and muscle origin. The sarcoma cells are I, osteosarcoma U2-OS; II, fibrosarcoma HT1080; and III, fibrosarcoma Hs913t.

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