

A 'GELLAB' COMPUTER ASSISTED 2-D GEL ANALYSIS OF STATES OF DIFFERENTIATION IN HEMATOPOIETIC CELLS

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1 INTRODUCTION

We have used a relatively global approach to the analysis of gene expression at the level of protein synthesis in an attempt to seek more meaningful classifications of human leukemias and a better understanding of their biology. The technique of two-dimensional polyacrylamide gel electrophoresis (2-D gels) allows us to examine the relative synthetic rates of up to 1000 of the major cellular proteins. The pattern of proteins displayed reflects both the underlying differentiation and the metabolic status of the leukemic cell population. From this pattern we are able to be able to subclassify human leukemias, both in terms of their differentiation and their state of growth, and to find sets of proteins whose genetic expression is regulated in common. Analysis of such data may permit a precise localization of blocks in the differentiation of leukemic cells.

2 METHODS

For these studies purified populations of cells have been biosynthetically pulse-labeled with [^3H]-leucine and cellular proteins have been subjected to 2-D gel electrophoresis and autoradiography (1, 2, 3). Each spot in the resulting pattern reflects the relative synthetic rate of a particular polypeptide during the labeling period. To quantitate and compare such images, we have developed the GELLAB computer system which is capable of accurately and automatically locating and quantifying over 90% of the spots in each image, comparing similar images, building a unified data base, and performing a variety of statistical analyses (4, 5, 6, 7).

3 RESULTS

Our initial studies characterized the sets of proteins whose relative synthetic rates are altered in normal lymphocytes during growth activation by lectins (1, 2). Subsequent work showed similar results in long-term human lymphoblastoid cell lines whose growth rates were modulated by varying culture durations. The synthetic rates of these proteins may serve as measures of the growth (cell cycle) status of leukemic cells. Additional work established that a GELLAB computerized analysis of 2-D gels could distinguish states of differentiation when a pair of autologous lymphoblastoid cell lines (T vs. B cell) were compared (3). Furthermore, differing pathways of

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myeloid differentiation (granulocytic vs. monocyctic) during the induction of differentiation in the human promyelocytic cell line HL-60 may be defined using a GELLAB analysis of protein synthesis patterns (8). The identification of sets of proteins characterizing such differentiation is useful in the analysis of human leukemias (9).

Our most recent clinical data base contains 58 2-D gel images from leukemic cells from 26 patients (7 AML, 2 ALL, 5 CLL, and 12 HCL). Initial analysis suggests that approximately 10% of the proteins detected show significant qualitative or quantitative differences between these major classes of leukemia. The use of a correlation coefficient of the spot densities in each possible pair of gel images (a 'density/density' plot) provides a summary statistic expressing the overall relatedness of the various leukemic samples (9). Table 1 shows the average correlation coefficients obtained for the 3 major classes of samples.

Table 1. Average Correlation Coefficients of 2-D gel images of leukemic samples.

	AML	HCL	CLL
AML	.52	.42	.23
HCL	-	.68	.51
CLL	-	-	.87

The identification of individual proteins of particular interest in the 2-D gel studies permits the generation of monoclonal antibodies reactive with them (8). Such antibodies may then be used to explore the function of such proteins. Proteins showing increased relative synthetic rates in growth-activated normal cells show higher rates in most acute than in most chronic leukemias. The considerable variation in the 2-D gel patterns within each major category suggests that new and more meaningful subclassifications will be possible. Ultimately, correlation of genomic composition at the level of DNA (chromosomes) with genetic expression at the level of protein synthesis will be possible.

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