Heterogeneity of isozyme expression in tumor cells does not correlate with metastatic potential

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The major purpose of these studies was to determine whether the expression of isozymes by tumor cells was heterogeneous among tumor cell subpopulations within a neoplasm and whether expression of one or another isozyme correlated with metastatic potential of tumor cells.

The expression levels of 40 isozymes were determined in 56 cell lines, many of them clonal, from nine different murine and human tumors. The enzymes chosen for study are involved in nucleotide, carbohydrate and pentose phosphate metabolism, and as such are indicators of the general metabolic and differentiaional status of the cell. The tumors studied included two murine and two human malignant melanomas, four murine fibrosarcomas, and one human prostatic adenocarcinoma. The lines isolated from these tumors consisted of cells that are tumorigenic non-metastatic, tumorigenic low metastatic and tumorigenic highly metastatic. Clonally derived cell lines from a given tumor differed in their expression of a number of different isozymes, including adenosine deaminase, creatine phosphokinase-B and lactate dehydrogenase. Different patterns of isozyme expression were observed among different tumor types as well as between tumors of the same type; however, there were no differences in isozyme expression for any enzyme tested that correlated with metastatic ability of tumor cells.

Introduction

Over the past decade, many studies have demonstrated that human and experimental animal neoplasms consist of subpopulations of cells with distinctly different biological properties. This heterogeneity has been shown for a number of biological properties including growth rate, immunogenicity, karyotype, drug sensitivity and antigenicity [7, 11, 23, 24]. Additionally, in recent years, it has become evident that cells populating tumors are also heterogeneous with respect to their metastatic abilities [6, 17, 21, 22]. The ability of a tumor cell to produce metastases is governed by more than one cellular characteristic. This process is selective, in that only some cells in the primary tumor are endowed with all the required characteristics necessary for successful metastasis [7, 30, 31]. Since the major cause of death from neoplasia is attributable to metastatic disease, it is imperative to identify underlying biological, biochemical and ultimately, genetic differences between those neoplastic cells able to metastasize and those that cannot.

Tumor cells are associated with a large number of chromosomal and biochemical alterations that distinguish them from normal cells and tissue. These alterations include differences in enzyme activities and in isozyme expression, and many are characteristic of specific types of tumors (reviewed in [1, 12, 13, 26, 27]). Moreover,
differences between non-metastatic and metastatic cells regarding lytic or degradative enzymes have been reported [3, 19, 33]. It stands to reason, therefore that biological differences among tumor cell subpopulations might also be reflected in the expression of enzyme loci. In the present study, we investigated the expression of numerous enzymes in various cell lines and clonal isolates of several human and murine tumor systems with different metastatic potential. The enzymes studied were chosen so as to be indicative of the general metabolic status of the cell lines. We were particular to include enzymes that are coded for by multiple genetic loci, the products of which (isoenzymes) are electrophoretically separable. Since such isoenzymes have tissue specific patterns of expression, their relative activities have proven useful in observing the pattern of gene expression and assessing the differentiaional status in tumor cells [25]. In addition, the activity of several of these enzymes have been shown by Weber et al. to correlate with tumor progression [34, 35]. Thirty enzymes, encoded by 40 different genetic isozyme loci, were screened. This investigation was performed to determine if isozyme expression is heterogeneous between cell subpopulations in tumors and if differences in expression of particular isozymes correlated with metastatic potential of tumor cells.

Materials and methods

Mice

Specific-pathogen-free inbred male C3H/HeN mice were obtained from the Animal Production Area, Frederick Cancer Research Facility (Frederick, MD). Within a single experiment, all mice were age matched and were 8–10 weeks old.

Cell lines

Cell lines derived from nine different tumors were used in this study (table 1). These tumors consisted of two murine (K1735, B16) and two human (A375, DX3) malignant melanomas, five murine fibrosarcomas, (UV2237M, UV2237M-ADM, UV2240, UV1591, UV1316) and one human prostate adenocarcinoma (PC3). The origin of each of these lines except the clonal isolates from UV2237M have been detailed elsewhere [4–6, 8, 9, 15, 16, 18]. UV2237M was cloned by plating 0·5 cells/well in 96-well plates. Wells with single cells were identified 18 h later and were then expanded in culture.

In vitro cell culture

Cell cultures were maintained on tissue culture plastic in Eagle’s minimal essential medium supplemented with 10 per cent fetal bovine serum (MA Bioproducts, Walkersville, MD), sodium pyruvate, non-essential amino acids, L-glutamine and vitamins (Gibco, Grand Island, NY) at 37°C in a humidified incubator containing 5 per cent CO₂. The PC3 and PC3M cell lines were grown in RPMI-1640 medium supplemented with L-glutamine and 10 per cent fetal bovine serum.

Each cell line was prepared for isozyme analysis by harvesting monolayers from two T175 flasks (Falcon Plastics, Cockeysville, MD) with 0·25 per cent trypsin and 0·02 per cent sodium EDTA. The flasks were tapped sharply after 1 min to dislodge the monolayers, and the cells were washed in an excess medium with 10 per cent fetal bovine serum. Cell suspensions were centrifuged and washed once in Hanks’