ADVANCES IN TUMOUR PREVENTION, DETECTION AND CHARACTERIZATION

editor: C. Maltoni

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BIOLOGICAL CHARACTERIZATION OF HUMAN TUMOURS

editors
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Cesare Maltoni

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detection and characterization

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Biological characterization of human tumours

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Introduction

There is a very strong current of opinion in all field of cancer research and in most countries, that considers that what is called cancer research should be aimed at bringing some benefit to the cancer patient in a foreseeable future — or better still, be aimed at preventing cancer.

The coordinating committee for human tumour investigations that organized this symposium — the Sixth in a biennal series — was formed in 1961 under the inspiration of Professor Franz Bergel, FRS, with the avowed aim of encouraging research workers to come closer to the problems of human cancer. One feature of the committee's work was to foster closer contacts and therefore deeper understanding between the clinician and laboratory scientist.

The present symposium followed that pattern and in the book are an important group of papers presenting biochemical guidelines for cancer chemotherapy. Scientists from laboratories in Europe and the United States describe attempts to achieve a more selective and therefore more effective therapy based on an exploitation of the biochemical parameters that, if only marginally, distinguish the cancer cell from the normal.

The chapter devoted to classical and modern techniques for characterizing tumours takes a critical look at histological classification in the light of more modern techniques of cell kinetics and biochemistry. The concept that a tumour may be characterized by its clinical behaviour is discussed.

A great deal has already been published on the carcinogenicity of vinyl chloride, but the chapter in the present volume presents the current situation in review for those who have not been involved in the problem. There is no doubt that occupational carcinogenesis is an important aspect of cancer prevention since the hazards are identifiable and the work situation is one that is susceptible to control.

At each symposium, one type of cancer is looked at in depth and in the present volume, it is malignant melanoma that is presented. Epidemiological studies clearly related incidence of malignant melanoma to the amount of skin exposed to sunlight. Histological classification is discussed particularly in relation to prognosis, and the possibilities of immunotherapy of this tumour were considered. The problems of surgery and application of hyperthermic treatment were also discussed.

Four papers deal with the role of hormones particularly in relation to cancer of the breast, cervix and endometrium.

Ten further papers deal with recent work on various clinical and experimental aspects.

The Editors

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Chapter I

Biochemical guidelines in cancer chemotherapy

Molecular basis of malignancy*

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The objectives

The purpose of this paper is to analyze the strategy of the cancer cell as it is expressed in the biochemical phenotype. When a normal cell is transformed to a cancer cell, two major biochemical events emerge. These alterations in gene expression relate to the neoplastic transformation itself and to the degrees in the expression of the malignant state.

The alterations in the genotype in neoplasia are expressed in the various biological aspects of the transformed cells, including growth rate, invasiveness and ability to metastasize, and also in the morphological, immunological and biochemical properties. We have been able to detect these alterations in the genotype by analyzing the biochemical phenotype through the application of the molecular correlation concept as an experimental and conceptual tool.

This paper concisely analyzes the approaches and results achieved by the molecular correlation concept and presents the integrated evidence for the presence and emergence of the enzymatic and metabolic imbalance as it is linked with *(a)* the neoplastic transformation, and *(b)* the degrees in the expression of malignancy.

Difficulties in the early studies of biochemistry and chemotherapy of neoplasia

During the 1940's and the early 1950's, the understanding of the biochemistry of the cancer cell progressed relatively little because of various drawbacks in the methodics and the conceptual approaches employed. The first problem was caused by the constant shifting from tumor to tumor, from human neoplasms to viral-induced avian tumors, from murine leukemias to rat solid tumors, etc., in the hope that one peculiar metabolic or enzymatic alteration, 'lesion' or 'deletion' would be found that would characterize 'the' cancer cell. A second major difficulty lay in the measurement of metabolic pathways or enzymes where frequently the guideline appeared to be chiefly the ease and feasibility of the assay. However, since some of the enzymes were assayed incorrectly and their function in intermediary metabolism was not understood, the investigations yielded little conceptual advance. As a result of the apparent lack of meaningful metabolic systems, the lack of emphasis on comparing the biochemistry of the neoplastic tissues with their homologous counterparts, and the failure to select enzymes whose function and significance were clearly established, some workers suggested that the biochemistry of cancer yielded only a picture of 'diversity' and random alterations that provided no rhyme, no reason, no pattern. Some of these misconceptions survive in the current literature.

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Such problems in the metabolic studies on cancer had their counterpart in the apparent lack of progress in the chemotherapy of neoplastic diseases. The selective chemotherapy of tumors in man had a slow start, not because of lack of drugs or lack of well directed efforts, but because of the circumstances in which the anticancer drugs had to be tested. In those early trials, chemotherapy was mostly restricted to terminal cases. However, the condition of the patient and the enormous tumor burden provided little chance for the identification and establishment of clinically useful drugs. A detailed analysis of the successful overcoming of these and other difficulties by clinicians and scientists who achieved appropriate conditions for the testing of anticancer drugs in man has been reviewed elegantly (Frei, 1972; Zubrod, 1972). Important aspects of the breakthrough in clinical chemotherapy have been the learning of the pharmacology and toxicology of the anticancer drugs and the development of capabilities in dealing with the unavoidable bone marrow and other toxicities through biochemical rescue, the availability of sterile laminar flow rooms and various immunological techniques that have brought chemotherapeutic cures for ten neoplastic diseases in man.

Progress in understanding the molecular basis of malignancy: The molecular correlation concept

A major step towards recognition of the pattern of metabolic imbalance in neoplastic cells was achieved through a detailed analysis of a number of key enzymes and metabolic pathways in the very rapidly growing NK (Novikoff) hepatoma. The biochemical pattern was compared with suitable homologous control tissues such as liver of normal adult rats and developing and regenerating rat liver. By focussing the studies on these truly comparable systems, a definite biochemical pattern in the tumor was identified (Weber and Cantero, 1959). Subsequently, a new liver neoplasm, the 5123-D, which had a very slow growth rate and was histologically similar to normal liver, became available from H.P. Morris. In a comparative study of the slow-growing 5123-D hepatoma, the very rapidly growing NK hepatoma, and the normal liver, it was recognized (Weber, 1961) that there were a number of prerequisites which should permit elucidation of the biochemical basis of malignancy. In 1961, the essential aspects of the conceptual and experimental methods of the molecular correlation concept were introduced into the studies of the biochemistry and metabolic regulation of neoplastic cells (Weber, 1961).

The molecular correlation concept specified the need to analyze the neoplastic transformation and progression in *(a)* a meaningful biological model system by, *(b)* examining key enzymes and opposing and competing metabolic pathways and *(c)* comparing the results with those obtained in an array of appropriate control tissues (Weber, 1974*a*).

(a) Biological model system: Spectrum of hepatomas of different growth rates and malignancy

Whereas the 5123-D tumor in 1961 was very similar to normal liver, the very rapidly growing NK tumor appeared to be the example of the full-blown hepatic neoplastic transformation. In order to analyze the molecular correlates of neoplastic transformation and malignancy, there was need for a series of hepatomas that exhibited different biological malignancy, growth rates and histological degrees of differentiation. In our studies hepatomas were obtained chiefly from H.P. Morris and also from A.B. Novikoff and M.D. Reuber and others. This made it possible to set up a spectrum of liver tumors of different malignancy that provided a biological model system for the testing of the conceptual and experimental approaches of the molecular correlation concept.

(b) The concept of key enzymes

In this Laboratory biochemical work was undertaken only on enzymes and metabolic pathways the function of which was elucidated. A great deal of metabolic and regulatory studies were also

carried out to clarify the kinetics and regulatory behavior of these enzymes and pathways. As a consequence of such investigations, the concept of key enzymes was introduced in the investigations on metabolic regulation and neoplasia. Our studies revealed that metabolic regulation is exerted through control of a number of key enzymes, especially those that oppose each other in antagonistic pathways of synthesis and catabolism. By concentrating on determining the behavior of key enzymes in a spectrum of liver tumors of different growth rates, the pattern of imbalance was recognized in all metabolic pathways examined so far in this Laboratory (for reviews, see Weber, 1973*b*, 1974*a*,*b*).

(c) The need for appropriate control tissues

It was established that for the evaluation and interpretation of the biochemical pattern and its specificity to neoplasia, the biochemistry of a number of control systems had to be compared with that of liver tumors (Weber, 1974*b*). For this purpose, the liver of adult normal rats of the same strain, sex, age and weight as the tumor-bearing rats are used. It is inadvisable to use the liver of the tumor-bearing rat, the host liver, because it is subject to numerous hormonal and nutritional influences and these artifacts frequently and unpredictably invalidate a comparison between the biochemistry of the host liver and that of the tumor.

Among the valid, relevant control systems is the regenerating liver that is to be compared with the liver of the sham-operated controls. In the study of differentiation, the postnatal period from ages 5 or 6 days to adulthood is meaningful. It is an error to attempt to draw conclusions from assays on rat liver in embryo or during immediate postnatal development because the embryonic liver in the rat is a hemopoietic organ. Unless appropriate corrections are made for this fact, the results cannot be interpreted readily and the conclusions may be misleading.

Classifications achieved through application of the molecular correlation concept

Careful work carried out in this Laboratory and in other Centers has led us to recognize the following relationships in which the biochemical strategy of the cancer cell is expressed (Weber, 1974*a*).

(a) Malignancy-linked alterations: Class 1

These enzymatic and metabolic alterations are linked with the increase in tumor malignancy and growth rate in the model system. The key enzymes and metabolic pathways in this group are indicators of the degrees in the expression of the malignant properties in the different lines of cancer cells.

(b) Transformation-linked alterations: Class 2

There are certain enzymatic and metabolic alterations that occur in all hepatomas irrespective of growth rate and malignancy. Since the reprogramming of gene expression that is manifested in the emergence of these biochemical alterations appears even in the slowest growing, least malignant hepatomas, we assume that these metabolic alterations are linked with the neoplastic transformation per se.

(c) Coincidental alterations: Class 3

A number of biochemical changes occur in the hepatomas without any relationship to growth rate, malignancy or transformation. In contrast to the key enzymes that belong to Classes 1 and 2, the enzymes in Class 3 are present in an excess and they apparently do not become limiting to

the processes of intermediary metabolism. These latter enzymes are not rigorously controlled and their ups and downs represent the randomness and the diversity by which some research workers have allowed themselves to be misled to believe that there is no pattern in the biochemistry of the cancer cell.

Are the alterations in tumor cells ordered or random? With the experience the molecular correlation concept provides, it is clear that this is an erroneous question. The experimental results reveal that neoplastic cells have both ordered and random manifestations. It seems inadvisable to concentrate on the randomness and diversity because the job is to discover the underlying pattern that relates to the neoplastic transformation and the progressive expression of the degrees of the malignancy. The experimental evidence indicates that what is important for neoplasia is ordered; what is not, is the randomness and the diversity.

This conclusion draws attention to the fact that in the design of chemotherapy the drugs are to be directed against the key enzymes that show a linking with the degrees in the expression of malignancy or with the malignant transformation per se.

Analysis of the molecular basis of malignancy in cancer cells

The biochemical imbalance of the cancer cells has been examined in detail in this Laboratory for a number of metabolic areas and key enzymes. Table 1 provides a partial list of the areas investigated. In the following a brief summary of the imbalance will be given and an attempt will be made to pinpoint the selective advantages that this enzymatic and metabolic imbalance confers to the cancer cell.

Carbohydrate metabolism: Enzymatic and metabolic imbalance

Carbohydrate metabolism was the first testing ground of the molecular correlation concept and a series of original papers and reviews were devoted to this subject (Weber, 1961, 1974*a,b*). Table 2 gives the present view of the imbalance of carbohydrate metabolism in neoplastic liver.

This Table shows various aspects of the malignancy-linked metabolic imbalance that operates in the spectrum of hepatomas of different growth rates. These biochemical alterations confer selective advantages to the cancer cells.

TABLE 1 *Characterization of biochemical imbalance in the following metabolic areas*

Carbohydrate (gluconeogenesis, glycolysis)
Pentose phosphate (oxidative and non-oxidative pathways)
Pyrimidine (de novo and salvage pathways)
Purine (IMP synthesis, degradation and utilization)
Urea cycle
Ornithine utilization
Polyamine biosynthesis
Membrane cAMP synthesis and degradation
Protein and amino acid
Conclusions
An ordered pattern is revealed in
malignancy-linked (Class 1)
and transformation-linked (Class 2)
alterations of key enzymes and opposing pathways

TABLE 2 *Carbohydrate metabolism: Phenotypic evidence for reprogramming of gene expression in neoplasia*

Synthetic enzymes	Key gluconeogenic enzymes*	Decreased
Catabolic enzymes	Key glycolytic enzymes**	Increased
Metabolic imbalance	Ratios of key glycolytic/key gluconeogenic enzymes	Increased
Isozyme shift	High K_m isozymes+	Decreased
	Low K_m isozymes++	Increased
Relation to malignancy	Alterations are co-variant with growth rate	Malignancy-linked imbalance
Biological role	(*a*) Imbalance in glycolytic/gluconeogenic enzymes leads to increase in glycolysis	Confers selective advantages to cancer cells
	(*b*) Isozyme shift leads to decreased responsiveness to physiological controls	

* Glucose-6-phosphatase (G-6-Pase), fructose-1,6-diphosphatase (FDPase), phosphoenolpyruvate carboxykinase, pyruvate carboxylase. ** Hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase. + Glucokinase, liver-type pyruvate kinase. ++ HK, muscle-type pyruvate kinase.

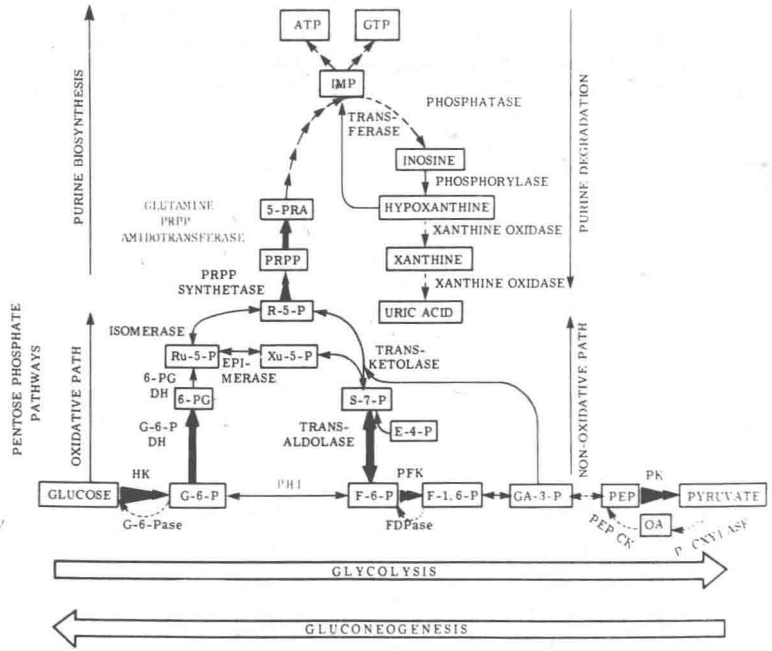

FIG. 1 *Integrated pattern of glycolysis, gluconeogenesis, pentose phosphate pathways, purine synthesis and degradation. The imbalance in hepatomas is indicated by the thick (increased activity) and dotted (decreased activity) arrows.*

Pentose phosphate metabolism: Phenotypic evidence for reprogramming of gene expression in neoplasia

The various aspects of the reprogramming of gene expression in pentose phosphate metabolism may be briefly summarized. The main alterations are the increases in all hepatomas in the activities of the key enzymes, G-6-P dehydrogenase and transaldolase, that should provide an increased potential for pentose biosynthesis (Weber et al., 1974). The enzyme that is involved in the immediate utilization of ribose-5-phosphate, PRPP (phosphoribosylpyrophosphate) synthetase, is increased in the rapidly growing hepatomas. These observations indicate that both the production and the utilization of pentose phosphate may be increased in transformed cells (Fig. 1) and this alteration in gene expression should confer selective advantages to the cancer cells.

It is important that the increase in G-6-P dehydrogenase and transaldolase occurs in all hepatomas; thus, these alterations in gene expression are linked to the neoplastic transformation per se.

The increased capacity for production of ribose-5-phosphate and its enzymatic conversion to PRPP provides an increased potential for the biosynthesis of this precursor used both by the de novo and the salvage pathway of purine biosynthesis (Fig. 1).

Purine metabolism: Phenotypic evidence for reprogramming of gene expression in neoplasia

As summarized in Table 3, there is evidence for a marked imbalance in purine biosynthesis and degradation in the hepatoma spectrum. Glutamine PRPP amidotransferase, the first enzyme committed to de novo purine biosynthesis, is increased in all the hepatomas (Katunuma and Weber, 1974; Prajda et al., 1975). Thus, the increase is a transformation-linked alteration in gene expression. There is also evidence that the 5'-nucleotidase is decreased in these hepatomas. Current studies of Prajda and Weber (to be published) indicate that the rate-limiting enzyme of purine degradation, xanthine oxidase, is decreased in all hepatomas. It was also observed that

TABLE 3 *Purine metabolism: Phenotypic evidence for reprogramming of gene expression in neoplasia*

Synthetic enzymes	Key enzymes of IMP synthesis*	Increased
Degradative enzymes	Key enzymes of IMP catabolism**	Decreased
Enzymatic imbalance	Ratios of key synthetic/catabolic enzymes***	Increased
Metabolic imbalance	Ratios of pathways of synthesis of IMP/catabolism of IMP	Increased
Relation to malignancy	Ratio of imbalance is co-variant with growth rate	Malignancy-linked imbalance
	Amidotransferase increased in all hepatomas	Transformation-linked imbalance
Biological role	(*a*) Imbalance in anabolic/catabolic enzymes of IMP metabolism leads to increased de novo IMP biosynthesis	Confer selective advantages to cancer cells
	(*b*) Imbalance provides increased synthetic potential and decreased loss of percursors for purine biosynthesis	

* PRPP synthetase, glutamine PRPP amidotransferase.
** 5'-Nucleotidase, xanthine oxidase.
*** Glutamine PRPP amidotransferase/xanthine oxidase.

TABLE 4 *DNA metabolism: Phenotypic evidence for reprogramming of gene expression in neoplasia*

Synthetic enzymes	Key enzymes of UMP*, TTP**, and DNA^{+} synthesis	Increased
Degradative enzymes	Key enzymes of UMP and thymidine (TdR) catabolism^{++}	Decreased
Enzymatic imbalance	Ratios of synthetic/catabolic enzymes	Increased
Metabolic imbalance	Ratios of TdR to DNA/TdR to CO_2 pathways	Increased
Relation to malignancy	Alterations are co-variant with growth rate	Malignancy-linked imbalance
Biological role	(*a*) Imbalance in anabolic/catabolic enzymes of UMP metabolism leads to increased de novo DNA synthesis	Confers selective advantages to cancer cells
	(*b*) Imbalance in anabolic/catabolic enzymes of TdR metabolism leads to increased salvage pathway to DNA synthesis	

* Aspartate transcarbamylase, dihydroorotase. ** Ribonucleotide reductase, dCMP deaminase, dTMP synthase, TdR kinase, dTMP kinase. $^{+}$ DNA polymerase. $^{++}$ Dihydrouracil dehydrogenase (DH), dihydrothymine DH.

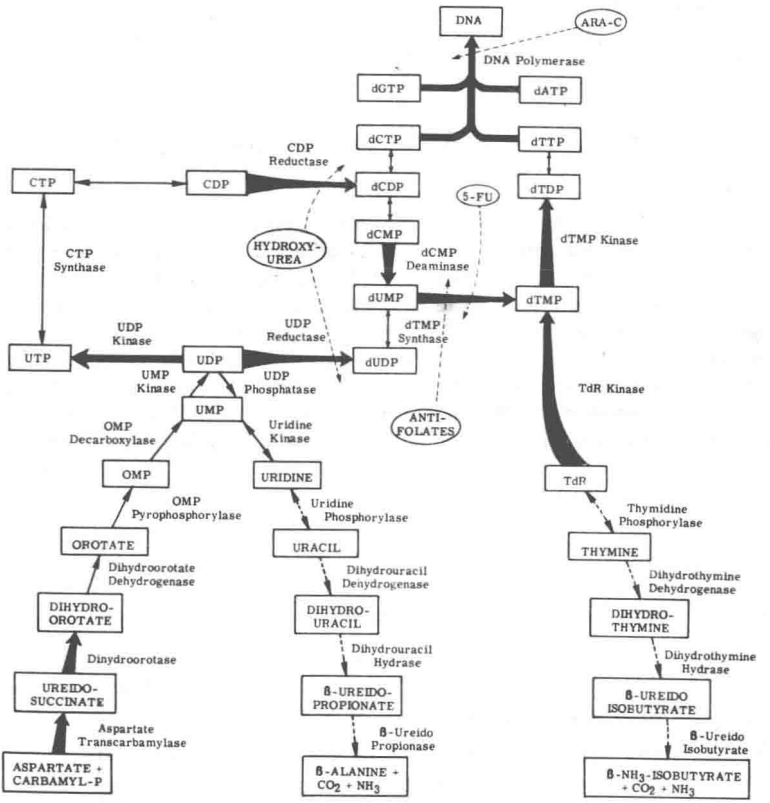

FIG. 2 *Integrated pattern of de novo and salvage pathways of pyrimidine synthesis and degradation. The imbalance in the hepatomas is indicated by the thick and dotted arrows. The attacking points of some of the chemotherapeutic drugs used in man are shown for cytosine arabinoside (ARA-C), the anti-folates and hydroxyurea.*