Cancer Update

Current topics in research

Cancer Update: Current topics in research

Based on an international educational seminar held in Tokyo on July 15–16, 1989

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Preface

With changes in society, culture, and lifestyle, diseases are becoming more and more complicated and diverse. Cancer is no exception. We have to study not only the disease itself, but also increasingly wider fields including the effects of surgery, radiotherapy, and chemotherapy, as well as the quality of life.

It was an honor and a pleasure to have been able to hold such a symposium as "Cancer Update," with eminent physicians in the field of cancer therapy from the USA reviewing the most up-to-date knowledge and achievements.

I have always been interested in and had great respect for the planning and programs of the Educational Symposia of the American Society of Clinical Oncology (ASCO). The programs are more thorough each year and I am always impressed by their timeliness. I had been thinking that it would be useful if more physicians from Japan could attend such seminars. That was why I suggested a seminar like the ASCO Educational Symposia when Taiho Pharmaceutical Co., Ltd., was considering what type of event to hold in commemoration of the 5th anniversary of the launch of tegafur. They agreed with my idea and asked me to organize the symposium.

Dr Setsuo Fujii, who developed tegafur, joined me as co-organizer of the meeting. We also asked Drs Tamaki Kajitani and Kiyoji Kimura to act as advisors and Drs Kazuo Ota, Hisashi Furue, Hisanobu Niitani, Shigeru Tsukagoshi, and Hisashi Majima to become program committee members. Although ASCO's seminars are excellent, we could not directly reproduce them in Japan and we had many discussions before we eventually completed the final program. Looking back, I am quite satisfied that the meeting sustained the spirit of ASCO's Educational Symposia. I am also very pleased that, in addition to the seminar, we also had a panel discussion with all the presenting physicians as panelists and Drs Tsukagoshi and Majima as chairpersons.

The seminar, held on July 15–16, 1989, was a great success, with 600 delegates attending, thanks to the 8 physicians from the USA and all those who contributed to its organization. I would like to express my sincere thanks to all those concerned and also to Mr Kobayashi, President of Taiho Pharmaceutical Co., Ltd.

Lastly, I would also like to express my deepest regret at the loss of Dr Setsuo Fujii, who died on August 4, 1989. We will cherish his memory always.

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Preface

Surgery and radiotherapy will cure 30–40% of patients who develop cancer. Most of the rest die because of systemic tumor, ie, metastases. The successful treatment of micro- or macrometastatic disease must be systemic.

In 1947, Farber introduced the first successful treatment of leukemia and Rhodes introduced the nitrogen mustards in 1945 and 1946. These early temporary successes prompted a major investment in cancer chemotherapy. New agents were discovered and by the mid- and late 1960s, the principles of therapeutic research in cancer were established. This included such clinical variables as dose, schedule, and combination and sequential therapy. The proper application of these principles led to the curative treatment first of acute lymphocytic leukemia, then of Hodgkin disease and non-Hodgkin lymphoma, for testis cancer, and for some of the childhood solid tumors.

In 1970 the curative intent focus shifted to adjuvant chemotherapy. This occurred primarily because of the recognition that microscopic tumor deposits are much more readily eradicated by chemotherapy than macroscopic disease. Initially in breast cancer and osteogenic sarcoma, it was demonstrated that adjuvant chemotherapy was substantially effective, decreasing mortality by 25% in premenopausal patients with breast cancer and some 60–70% in patients with osteogenic sarcoma. More recently, the use of adjuvant chemotherapy in the form of fluorouracil plus levamisole has decreased mortality in patients with the appropriate stage of colorectal cancer.

Finally, neoadjuvant chemotherapy was developed in an effort to reduce and render more operable selected solid tumors. In addition, neoadjuvant chemotherapy provided systemic treatment up front, which is ideal, based on our knowledge of clonal evolution to drug resistance. Neoadjuvant chemotherapy has proven effective in head and neck cancer, stage III breast cancer, invasive bladder cancer, and other selected diseases. While tumor regression has been achieved in a major way in these programs, survival improvement has not yet been demonstrated.

Perhaps the most compelling reality of modern cancer therapeutic research is related to the impact of the rapid advances in molecular biology and immunology. Increasingly, such findings are being applied to clinical material. Oncogenes and oncogene products, such as growth factors, can be secreted by tumor cells, often interacting with adjacent normal cells to maintain the malignant phenotype. Indeed, there are circumstances where a tumor cell produces a growth factor and also a surface receptor for the growth factor, allowing for autocrine maintenance of the neoplastic state. Molecular biological products such as tumor necrosis factor, the interferons, and hematopoietins will have a significant effect on therapy today and increasingly in the immediate future. The control of transcription, such as with antisense compounds, may also control the neoplastic phenotype. The ability to identify such an oncogene product that, for example, has enzymatic activity, allows

for the application of crystallography, X ray diffraction, and computer graphing of the 3-dimensional enzyme, followed by selected site synthesis of analogues.

It has been estimated that information and concepts relating to basic tumor biology are doubling every 7 years. Such advances cannot help but have a profound positive impact in the clinic in the immediate and long-range future. This cancer update thus serves the very important role of bringing to the physicians the expanding and changing body of knowledge that is cancer.

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Special lecture I

Biochemical strategy of cancer cells and enzyme-pattern-targeted chemotherapy

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Introduction

Cancer is the second most common cause of death in the USA and it is a major clinical and biological problem. An understanding of the biochemical differences between normal and cancer cells should provide paradigms for problem solving in cancer research and yield approaches in the rational design of chemotherapy. Studies carried out in my laboratory with guidance of the molecular correlation concept identified important aspects of the biochemical strategy of cancer cells [1,2]. The approach adopted by our laboratory is indicated in Table 1.

Table 1 Biochemical basis of cancer chemotherapy.

1) Transformation	Commitment to continued replication Biochemical program of quantitative imbalance
2) Progression	Escalation in expression of neoplasia; growth and spread Amplification of quantitative and qualitative biochemical imbalance
3) Drug treatment	Should interfere with the biochemical program of the commitment to replication and cause selective cell death

Biochemical basis of cancer chemotherapy

In the biochemistry of cancer cells there are transformation- and progression-linked alterations. In the progression of the cancer cells there is an escalation in the expression of neoplastic properties and, correspondingly, an amplification of the quantitative and qualitative biochemical differences which distinguish cancer cells from normal ones [2]. Drug treatment should interfere only with the biochemical program of the commitment to replication and it should cause selective

death of the cancer cells. The host cells should adapt and recover from toxicity. The biochemical studies have revealed operation of gene logic in cancer cells. The following will illuminate the operation of gene logic in pyrimidine and purine metabolism in cancer cells which forms the basis for antimetabolite chemotherapy. It will also underline the international nature of cancer research carried out in my laboratory.

Pyrimidine metabolic imbalance in cancer cells

An outstanding aspect of gene logic is the reciprocal regulation of the activities of key enzymes and metabolic pathways [1,2]. The operation of reciprocal regu-

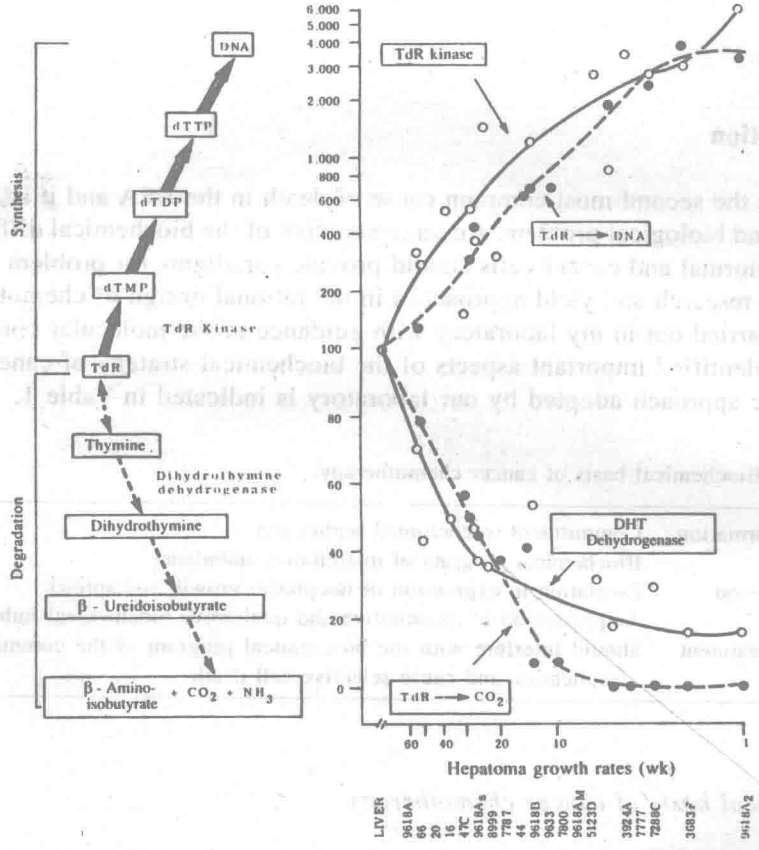

Fig 1 Reciprocal behavior of activities of opposing key enzymes, thymidine (TdR) kinase and dihydrothymine (DHT) dehydrogenase (assayed in cytoplasmic extracts), and of synthetic and catabolic pathways of thymidine (measured in tissue slices by the incorporation of thymidine into DNA and by the degradation of thymidine to CO_2). Activities were expressed as percentages of values of liver of control normal rats. Heavy lines indicate the enzymic activities that were increased and broken lines the activities that were decreased.

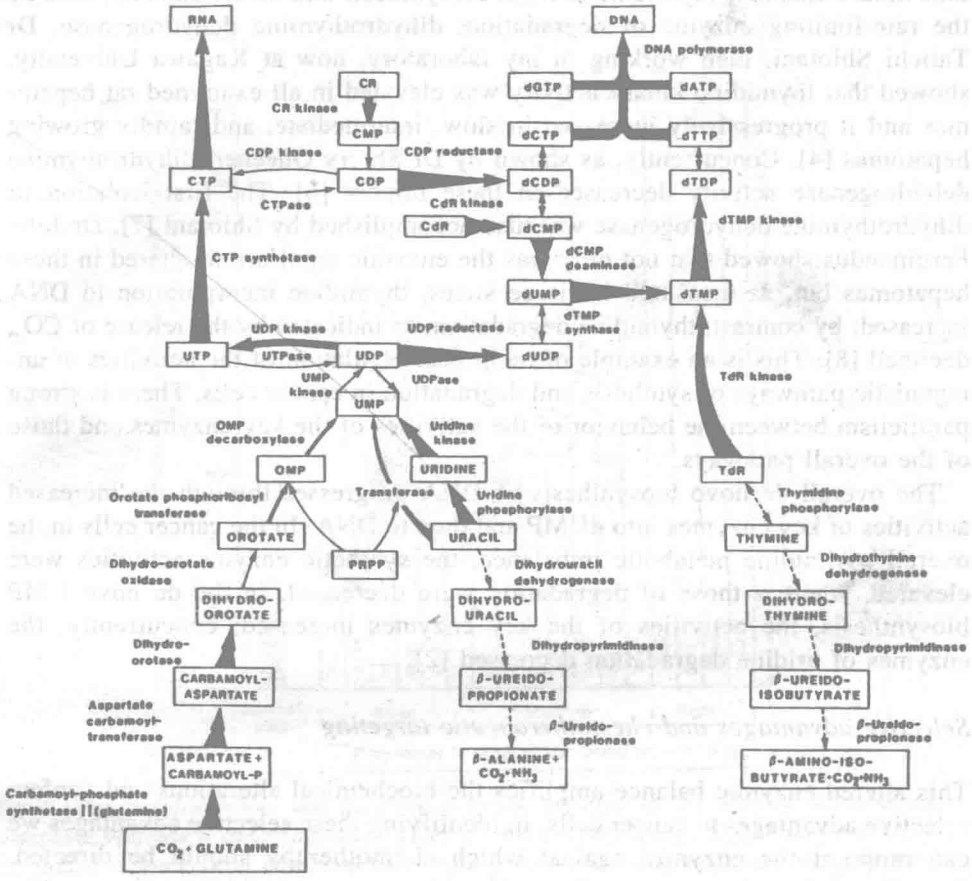

Fig 2 Biochemical strategy of pyrimidine metabolism of cancer cells as revealed in the integrated reprogramming of gene expression manifested in the imbalance of activities of key enzymes of de novo and salvage biosynthetic and degradative pathways. Tapered, thick arrows, enzymic activities that are increased with tumor proliferative rate (progression-linked alterations); broken arrows, enzymic activities that are decreased; straight thick arrows (eg, UDP kinase, orotidine-5'-monophosphate decarboxylase), enzymic activities that are increased in all the tumors (transformation-linked); dotted straight arrows, (eg, thymidine phosphorylase), enzymic activities that are decreased in all the neoplasms. Arrows with normal thinness, no relation found with transformation and progression (eg, dihydroorotate oxidase) or the behavior has not yet been determined (eg, CDP kinase). Heavy lines indicate the enzymic activities that were increased and broken lines the activities that were decreased.

lation was first shown in carbohydrate metabolism in our laboratory in 1968 [3]. Reciprocal regulation in thymidine metabolism was demonstrated by Japanese and American colleagues (Figs 1 and 2) [1,2,4,5]. As shown in Fig 1, thymidine metabolism involves its utilization to DNA and its degradation to CO_2. Thymi-

dine kinase channels thymidine to DNA biosynthesis and its action is opposed by the rate-limiting enzyme of degradation, dihydrothymine dehydrogenase. Dr Taiichi Shiotani, then working in my laboratory, now at Kagawa University, showed that thymidine kinase activity was elevated in all examined rat hepatomas and it progressively increased in slow, intermediate, and rapidly growing hepatomas [4]. Concurrently, as shown by Dr Sherry Queener, dihydrothymine dehydrogenase activity decreased in these tumors [6]. The first isolation of dihydrothymine dehydrogenase was also accomplished by Shiotani [7]. Dr John Ferdinandus showed that not only was the enzymic equilibrium altered in these hepatomas but, as measured in tissue slices, thymidine incorporation to DNA increased; by contrast, thymidine degradation, as indicated by the release of CO_2, declined [8]. This is an example of reciprocal regulation of the activities of antagonistic pathways of synthesis and degradation in cancer cells. There is strong parallelism between the behavior of the activities of the key enzymes and those of the overall pathways.

The overall de novo biosynthesis of DNA progresses through the increased activities of key enzymes into dUMP and then to DNA. In the cancer cells in the overall pyrimidine metabolic imbalance, the synthetic enzyme activities were elevated, whereas those of degradation were decreased. In the de novo UMP biosynthesis, the activities of the key enzymes increased; concurrently, the enzymes of uridine degradation decreased [2].

Selective advantages and chemotherapeutic targeting

This altered enzymic balance amplifies the biochemical alterations and confers selective advantages to cancer cells. In identifying these selective advantages we can pinpoint the enzymes against which chemotherapy should be directed. Therefore, it is an important matter to identify such potential target enzymes [2].

The first and rate-limiting enzyme of UMP biosynthesis is carbamoyl phosphate synthase II. Dr Takashi Aoki of Juntendo University, working in my laboratory, showed that synthase II activity increased in all tumors (transformation-linked) and gradually increased in the intermediate and rapidly growing hepatomas (progression-linked alteration) (Fig 3) [9]. There are a number of such transformation- and progression-linked increased enzymic activities in de novo DNA biosynthesis and they have been used as clinical targets of cancer drug treatment.

Relationship of de novo and salvage activities: clinical implications

The specific activities of the key enzymes of de novo pyrimidine biosynthesis are low, whereas those of the salvage enzymes are high (Fig 4) [2]. This relationship of de novo and salvage activities has an important implication for clinical chemotherapy. The following example should illuminate the significance of these relationships (Figs 4 and 5).

In dTMP biosynthesis the rate-limiting enzyme is ribonucleotide reductase.

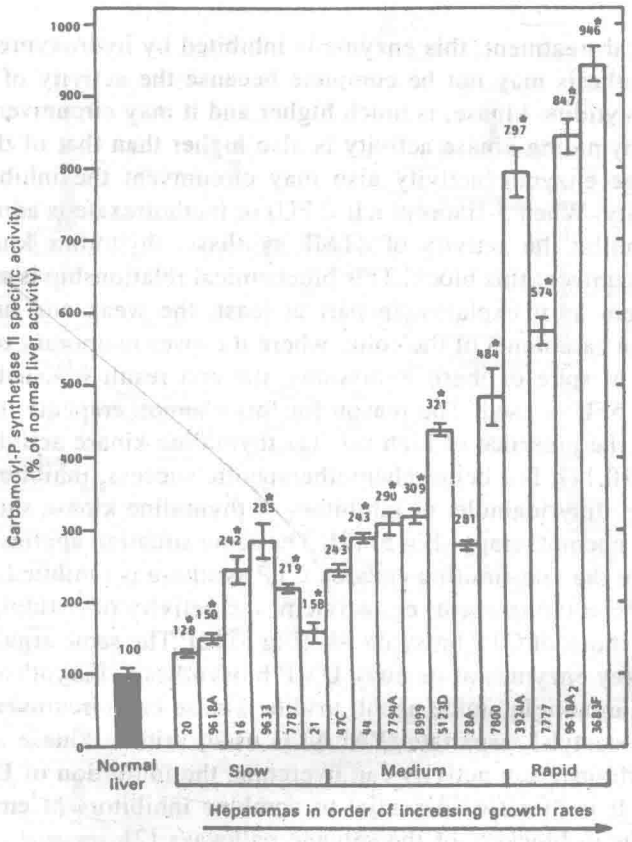

Fig 3 The transformation- and progression-linked behavior of the activity of carbamoyl-phosphate synthase II in hepatomas of different growth rates. Mean specific activities (± SE) are plotted as percentages of normal liver value. Asterisks indicate values significantly different from that of the normal liver ($p<0.05$).

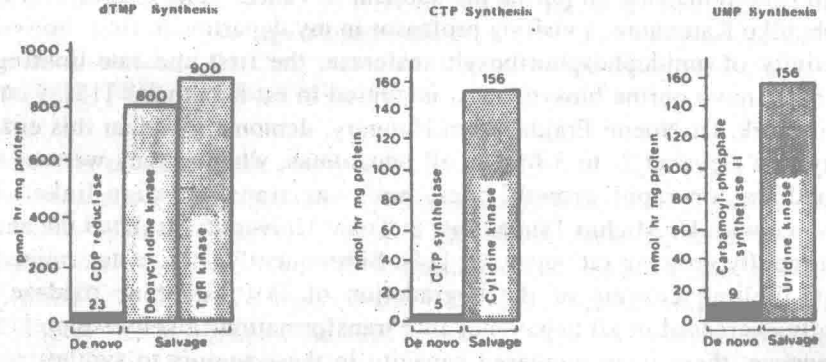

Fig 4 Comparison of the specific activities of the rate-limiting and salvage pyrimidine enzymes involved in the synthesis of dTMP (left), CTP (middle), and UMP (right) in normal rat liver. TdR, thymidine.

When, in clinical treatment, this enzyme is inhibited by hydroxyurea, the block of DNA biosynthesis may not be complete because the activity of the salvage enzyme, deoxycytidine kinase, is much higher and it may circumvent the block. Furthermore, thymidine kinase activity is also higher than that of the reductase and this salvage enzymic activity also may circumvent the inhibition of the reductase activity. When 5-fluorouracil (5FU) or methotrexate is administered in the clinic to inhibit the activity of dTMP synthase, thymidine kinase salvage activity can circumvent this block. This biochemical relationship is significant in clinical treatment as it explains, in part at least, the weak and undependable action of 5FU in carcinoma of the colon where it causes temporary remissions in 20% of cases. In spite of these remissions, the end result is usually the same whether or not 5FU is used. The reason for this chemotherapeutic failure is the overlooking of the presence of high salvage thymidine kinase activity in human colon tumors [10,11]. For better chemotherapeutic success, inhibitors of thymidine uptake, eg, dipyridamole, or inhibitors of thymidine kinase should be used in combination chemotherapy (Fig 5) [2]. The same situation applies to CTP biosynthesis. When the rate-limiting enzyme CTP synthase is inhibited in the tumor cells by an antiglutamine agent, eg, acivicin, the activity of cytidine kinase can circumvent the block of CTP biosynthesis (Fig 5) [2]. The same argument applies to blockers of key enzymes of de novo UMP biosynthesis. If synthase II activity is inhibited by an antiglutamine agent, uridine kinase can circumvent the block. If *N*-phosphonoacetyl-L-aspartate (PALA) is used, uridine kinase and/or uracil phosphoribosyltransferase activity can overcome the inhibition of UMP biosynthesis (Fig 5). It is therefore essential to combine inhibitors of enzymes of de novo pathway with blockers of the salvage pathways [2].

Purine metabolic imbalance in cancer cells

The enzymic imbalance in purine metabolism of cancer cells is shown in Fig 6. Dr Nobuhiko Katunuma, a visiting professor in my department, first showed that the activity of amidophosphoribosyltransferase, the first and rate-limiting enzyme of de novo purine biosynthesis, increased in rat hepatomas [12]. Continuing this work, Dr Noemi Prajda, from Hungary, demonstrated that this enzymic activity was elevated 2- to 3-fold in all hepatomas, whether they were of slow, intermediate, or rapid growth rates, and was transformation-linked [13]. Katunuma, with Dr Michio Tsuda, now at Tokai University, isolated the enzyme from a rapidly growing rat hepatoma [14]. Subsequently, Prajda determined that the rate-limiting enzyme of the degradation of IMP, xanthine oxidase, was markedly decreased in all hepatomas in a transformation-linked fashion [15]. In consequence, there is an increased capacity in these tumors to synthesize IMP and a marked decline in the capacity to degrade it. This is the operation of reciprocal regulation in purine metabolism. In consequence, there is an overwhelming capacity in cancer cells to make IMP that can be channeled to the