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Review

Homeostatic control of uridine and the role of uridine phosphorylase: a biological and clinical update

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Abstract

Uridine, a pyrimidine nucleoside essential for the synthesis of RNA and bio-membranes, is a crucial element in the regulation of normal physiological processes as well as pathological states. The biological effects of uridine have been associated with the regulation of the cardio-circulatory system, at the reproduction level, with both peripheral and central nervous system modulation and with the functionality of the respiratory system. Furthermore, uridine plays a role at the clinical level in modulating the cytotoxic effects of fluoropyrimidines in both normal and neoplastic tissues. The concentration of uridine in plasma and tissues is tightly regulated by cellular transport mechanisms and by the activity of uridine phosphorylase (UPase), responsible for the reversible phosphorolysis of uridine to uracil. We have recently completed several studies designed to define the mechanisms regulating UPase expression and better characterize the multiple biological effects of uridine. Immunohistochemical analysis and co-purification studies have revealed the association of UPase with the cytoskeleton and the cellular membrane. The characterization of the promoter region of UPase has indicated a direct regulation of its expression by the tumor suppressor gene p53. The evaluation of human surgical specimens has shown elevated UPase activity in tumor tissue compared to paired normal tissue.

Keywords: Uridine; Uridine phosphorylase; Pyrimidine; 5-Fluorouracil; p53; Cytoskeleton

1. Introduction: physiological and biological role of uridine

Pyrimidines are synthesized de novo in mammalian cells through a multistep process starting from glutamine and carbon dioxide to form, the pyrimidine ring in the second to last intermediate, orotic acid, which is then converted to its nucleotide form in the presence of PRPP. From the degradation of the nucleic acids and nucleotides a large portion of the pyrimidines are salvaged. The relative contribution of de novo synthesis and salvage pathway to the maintenance of the nucleotide pools varies in different cells and tissues [1]. A crucial difference between purine and pyrimidine metabolism is that purines are recycled from their bases while pyrimidines are salvaged from their nucleosides, particularly uridine. In fact, in patients with deficient pyrimidine biosynthesis, only uridine is able to overcome this pathological manifestation but uracil is not [2].

The concentration of circulating plasma uridine of approximately 3–5 μM is tightly regulated throughout different species and individuals [3–5]. The liver appears to have this homeostatic control on uridine degradation and formation [6]. Uridine is essentially cleared in a single pass through the liver and it is replaced in a highly regulated manner by "new uridine" formed by de novo synthesis [6]. We have previously reported the cellular basis for the catabolic component of this apparent paradox by the dissociation of the liver into two cell fractions, hepatocytes and a nonparenchymal cell population. Suspensions of the nonparenchymal cells were shown to rapidly cleave uridine to uracil, while in hepatocytes, this activity was barely detectable. Conversely, hepatocytes caused extensive degradation of uracil to β-alanine. These differences correlated with the

Abbreviations: PRPP, 5-Phosphorylribose 1-pyrophosphate; BAU, Benzylacyclouridine; UDPG, Uridine 5′-diphosphoglucose; UPase, Uridine phosphorylase; TNF-α, Tumor necrosis factor-α; IL-1α, Interleukin-1α; IFN α and γ, Interferon α and γ; TPase, Thymidine phosphorylase; 5-FU, 5-Fluorouracil; PALA, *N*-(phosphonacetyl)-L-aspartate; MTD, Maximum tolerated dose; TAU, 2′,3′,5′-tri-*O*-acetyluridine; NBMPR, nitrobenzylthioinosine; PD-ECGF, platelet-derived endothelial cell growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NDPK, nucleoside diphosphate kinase; NTP, nucleotide triphosphates

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uridine phosphorylase (UPase) and dihydrouracil dehydrogenase activity present in each cell type [7].

Besides the critical role of uridine in the synthesis of RNA and bio-membranes, through the formation of pyrimidine-lipid and pyrimidine-sugar conjugates, experimental and clinical evidence suggests a role for uridine in regulating a series of biological functions [8].

Uridine and its nucleosides have shown a complex effect in the regulation of vascular resistance, producing opposing effects in some tissues, either by acting directly on the smooth muscle cells or by stimulating the surrounding endothelial cells [9].

Uridine is present in the seminal fluid at millimolar concentrations, a level that is approximately three orders of magnitude higher compared to other body fluids or tissues [10]. The presence of such a high uridine concentration and its correlation with sperm motility suggests a role of uridine in spermatogenesis [11]. Furthermore, a low level of uridine in prostatic secretions of patients with prostatitis also indicates a possible role of this nucleoside in the etiology of this disease [11].

In the area of the peripheral nervous system, uridine appears to have a modulatory role. It has been shown to hyperpolarize amphibian ganglia and rat superior cervical ganglia at submillimolar concentrations, possibly resulting in an inhibitory activity [12]. Several clinical observations indicate the crucial function of pyrimidine nucleoside salvage in the maintenance of normal CNS activity [13,14]. In the treatment of a form of autism with seizures, oral uridine administration has led to improvement in speech, behavior and decreased frequency in seizures [15]. Deficiencies in the catabolism of pyrimidines due to impaired activity of one of the steps in the catabolic pathway, such as dihydropyrimidine dehydrogenase or β-ureidopropionase, have resulted in autism, convulsions, mental retardation and decreased motor coordination [14].

In animal models, uridine has been shown to potentiate dopaminergic transmission and reduce anxiety [16]. Uridine has been documented as a sleep-inducing factor in rats and shown to increase the activity of barbiturates [17,18]. In mice and rats, high doses of uridine cause a dramatic reduction in body temperature [19]; whereas uridine induces fever in human and rabbits [20,21]. The co-administration of an inhibitor of UPase, benzylacyclouridine (BAU), almost completely prevented the effect on thermoregulation, suggesting that a pyrimidine catabolite, possibly β-alanine, could alter the control of the body temperature [21].

Aside from the 'physiological' effects that we have just briefly outlined, uridine appears to have remarkable functions in tissues under stress or pathological situations and in the clinical setting. In hearts subjected to ischemia, perfusion with uridine rapidly restored myocardial ATP levels, glycogen and UDPG [22]. Similarly, uridine perfusion resulted in the maintenance of brain metabolism during ischemia or severe hypoglycemia [23,24]. Furthermore, uridine induced recovery from neuronal degeneration produced by diabetic neuropathy [25]. Uridine has been used as a 'rescue' agent in cancer therapy to decrease bone marrow and gastrointestinal toxicity, following 5-fluorouracil-based drug regimens [4,26–31]. In combination with BAU, uridine has shown some activity in reducing neurotoxicity and the effects on the bone marrow of AZT during treatment for HIV infections [32].

1.1. Role of UPase on the pharmacological activity of uridine

Plasma and intracellular concentrations of uridine are regulated by the catabolic activity of UPase and by two transport mechanisms, facilitated diffusion and Na^+-dependent active transport. UPase catalyzes the reversible phosphorolysis of uridine and to a lesser degree of thymidine. It also cleaves pyrimidine 2′- and 5′-deoxyribosides at a much lower rate [33–37]. UPase is present in most tissues and in tumors, where its activity is generally elevated [33,35,38]. The mammalian enzyme appears to be a tetrameric protein with subunits of approximately 33,000 molecular weight. Initial velocity and product inhibition studies suggest an ordered bi–bi mechanism where Pi binds first before uridine and ribose-1-phosphate is released after uracil [39]. UPase plays an important role in the homeostatic regulation of uridine concentration in plasma and tissues [22–25] as well as affects activation and catabolism of fluoropyrimidines influencing their therapeutic capacity [40–43]. The expression of UPase has been shown to be induced in different tumor cell lines, such as Colon 26 and HCT-116, when in the presence of cytokines: TNF-α, IL-1α and IFN-α and γ, and vitamin D_3 [44–46]. A similar response to cytokines has been observed for thymidine phosphorylase (TPase) [47]. Induction of UPase expression has also been reported in c-H-ras transformed NIH 3T3 cells [48]. In a murine model, hepatic UPase has been found to follow a circadian rhythm which was the inverse of that for plasma uridine concentration, re-emphasizing its role in the regulation of blood uridine level and suggesting its possible involvement in the humoral control of sleep [49].

A misconception surrounds the role of UPase and TPase. Some literature reports UPase as the pyrimidine nucleoside phosphorylase in murine tissues and TPase the main phosphorolytic enzyme in human tissues [50]. UPase, however, has been shown to be present in all human tissues and tumors, whereas TPase activity has been found reduced or absent in many human tumors [4,17,26,27,33,35,40,51–54].

1.2. "Rescue" of 5-fluorouracil toxicity

As previously mentioned, UPase has a critical role in regulating the concentration of uridine in plasma and tissues. A number of clinical studies have demonstrated the ability of uridine to reduce 5-FU toxicity, without affecting its antitumor activity, if properly administered 18–24 h following the cytotoxic agent [26–29].

The combination 'rescue regimens' of 5-FU plus uridine were initially proposed to evaluate the hypothesis that the antitumor effect of 5-FU is primarily due to the inhibition of thymidylate synthase and the host toxicity caused by the incorporation of the fluoropyrimidine into RNA [55]. In vivo studies in a murine model [56] and in vitro data [57] have clearly indicated that the incorporation of 5-FU into RNA appears to be the major cause of gastrointestinal toxicity. Results show that uridine inhibited the incorporation and avoided the cytotoxic effect, whereas thymidine did not prevent 5-FU toxicity. Furthermore, a recent study has indicated that the p53-dependent apoptosis induced by 5-FU in intestinal cells was reduced by uridine administration but not by thymidine [58].

Because of its low oral bioavailability and a rapid half-life, large doses of uridine are necessary to achieve clinically relevant concentrations in plasma causing moderate to severe toxicity including severe diarrhea as dose-limiting toxicity [4,20]. On the other hand, infusion of uridine has resulted in fevers, phlebitis, cellulitis, superior vena cava syndrome, torpor and confusion, and precluded an extensive clinical use [20,59]. Nevertheless, clinical studies of 5-FU in combination with methotrexate and PALA, have shown that patients tolerated combination therapy with delayed uridine (infused over a 72-h period starting 2 h after 5-FU administration, 3 h on and 3 h off) up to a weekly dose of 750 mg/m^2 of 5-FU, with 25% experiencing moderate mucositis (grade II). In a previous clinical trial without uridine, four out of six patients could not tolerate a 600 mg/m^2 dose of 5-FU because of mucositis, diarrhea and a decrease in performance status. In another study, 5-FU treatment could be continued with delayed administration of uridine at a weekly dose of 5-FU, which alone caused dose-limiting myelosuppression. In most of the patients who had previously developed leukopenia, the WBC increased markedly despite continued 5-FU administration [59]. In a more recent study of high-dose 5-FU with doxorubicin, high-dose methotrexate and leucovorin, oral uridine administration allowed for dose intensification of 5-FU with a 33% increase in the MTD of 5-FU in the presence of doxorubicin and a 45% increase in 5-FU MTD without doxorubicin [60]. No responses were obtained in patients with gastric cancer (0/11) when uridine was administered 2 h after 5-FU, however 2/3 patients responded with the 24-h uridine delay. This last regimen, with a 24-h interval between the administration of 5-FU and uridine, ensured rescue from 5-FU-induced hematologic toxicity without adverse impact on tumor response [60]. As indicated in pre-clinical studies, properly delayed uridine rescue results in a faster clearance of 5-FU from RNA of bone marrow and tumors and enhancement of the rate of recovery of DNA synthesis only in the bone marrow [37].

Our laboratory has shown that the problem of a rapid disposition of uridine and the administration of large doses of the nucleoside could be overcome by utilizing inhibitors of UPase, such as BAU, to conserve endogenous uridine with consequent elevation of its concentration in plasma and tissues. This approach has resulted in the reduction in animal models of host toxicity, while maintaining the antineoplastic effect of 5-FU [34]. A phase I clinical trial of oral BAU administered as a single agent has shown the ability of this inhibitor to elevate 2–3-fold the plasma uridine concentration with no significant host toxicity in patients [3].

1.3. Tri-O-acetyluridine (TAU)

A new agent 2′,3′,5′-tri-*O*-acetyluridine (TAU; PN 401) has been tested recently in a clinical trial to rescue 5-FU toxicity. TAU is a uridine pro-drug, the presence of the acetyl groups increases the hydrophobicity, therefore enhancing the gastrointestinal transport and bioavailability, and protecting this agent from the catabolism by UPase. Uridine is then progressively released by plasma esterases resulting in sustained delivery over time without most of the side effects of uridine administration. The sustained elevated level of uridine, up to 50 μM in plasma, has allowed a dose escalation of 5-FU, administered as a rapid intravenous bolus weekly for 6 weeks, from 600 to 1000 mg/m^2. Still, an oral dose of 6 g of TAU at 6-h intervals was necessary to produce significant uridine levels [61].

2. Regulation of uridine homeostasis: transport mechanisms

Besides catabolism by nucleoside phosphorylase activity, the intracellular concentration of uridine is regulated by its transport through the cell membranes. A facilitated-diffusion mechanism, which equilibrates intracellular and extracellular uridine, has been considered for many years to be responsible for intracellular uridine concentration. This non-energy dependent process displays broad substrate specificity toward synthetic and naturally occurring pyrimidine nucleosides [62–65]. In addition to competition between functional substrates, the facilitated diffusion of nucleosides present in most cell lines can be reversibly inhibited by compounds such as dipyridamole and nitrobenzylthioinosine [66]. Studies by Belt [67,68] have revealed that the facilitated diffusion mechanism in cell lines can be distinguished by the sensitivity to inhibition by NBMPR or dipyridamole. Recently, both transporters, NBMPR-sensitive (es) and NBMPR-insensitive (ei) have been cloned and characterized [69,70].

In addition to the non-concentrative facilitated diffusion mechanism, renal and gut epithelial cells were shown to possess a Na^+-dependent transporter for nucleosides [71–77]. Our laboratory revealed the potential physiological significance of this Na^+-dependent transport system by the finding that concentrations of uridine in a variety of freeze-clamped normal murine tissues far exceeded the concentration of uridine in the plasma. We were able to

demonstrate that BAU generated concentrations of uridine in selected tissues that were as much as 50–100 times that in control plasma [34]. The concentrative system in liver, kidney and gut appears to have different substrate specificity than in lymphoid cells (spleen or thymus). In addition, we have demonstrated the generality of the expression of a concentrative, active transport mechanism for uridine in a variety of normal murine tissues [53]. These observations changed the perception of the Na^+-dependent uridine transport process from a rate phenomenon observed in isolated cells to a major physiological effect that afforded therapeutic opportunities considering that the intracellular concentrations of uridine in a wide variety of neoplastic cell lines did not exceed those in the media. It has been shown that some lines express the concentrative mechanism to a very limited degree [78] but that it is overwhelmed by the equilibration of nucleoside achieved by an active facilitated diffusion mechanism. At this moment, five major Na^+-dependent active transport systems have been isolated: (1) the purine selective N1 system, or cif, shown to be present in rat intestinal epithelium, mouse enterocytes and lately in human kidney [79–82]; (2) the pyrimidine selective N2 system or cit described by Jarvis and Griffith [83], present in rabbit small intestine with selectivity for pyrimidine nucleosides and adenosine; (3) the N3 transport system (cib) isolated in rat jejunum sensitive to inhibition by both purine and pyrimidines [84]; (4) the N4 transport system, expressed in human kidneys, which is identical to the N2 system in substrate specificity [85]; and the N5, a cs transport system, found in freshly isolated human leukemia cells [86].

3. UPase: clinical, biological and regulation update

Over the past few years, we have focused on the role of UPase in regulating uridine metabolism and its intracellular levels in normal and neoplastic tissues and on the mechanisms controlling the expression and localization of UPase. We have determined that the activity of UPase is elevated in tumors as compared to their normal tissue counterpart and discovered variant uridine phosphorolytic activity in selected human tumors [54]. We have also defined the genomic structure of the UPase gene, characterized its promoter region, the p53-dependent control of its expression [87,88] and established its intracellular localization and association with cytoskeletal elements [89].

3.1. Expression and detection of UPase and identification of variant phosphorolytic activity in human tumors

TPase catalyzes the reversible phosphorolysis of thymidine to thymine and with less efficiency also contributes to the initial degradation of uridine. Since TPase has been shown to be identical to platelet-derived endothelial cell growth factor (PD-ECGF) and its angiogenic activity demonstrated [90,91], several studies have investigated the role of this protein in tumor progression and clinical outcome. Increased expression has been found in some solid tumors compared to the adjacent normal tissues [92] and TPase has been shown to be a negative prognostic indicator in bladder [93], colo-rectal [94,95], ovarian [92], pancreatic [96] and renal [97] cancers but its role is still controversial in breast carcinoma [98,99]. PD-ECGF/TPase has been shown to promote angiogenesis only when enzymatically active and in the presence of thymidine [100].

The intense investigation of the role of TPase on cancer invasiveness and malignancy has not translated to a similar interest for a potential involvement of UPase, despite its complementary phosphorolytic activity.

We have evaluated the activity of UPase in fresh tumor specimens and adjacent normal tissues of patients undergoing surgical resection of their malignancy. The enzymatic activity was variable among the different tissue specimens, but overall it was 2–3-fold higher in tumors compared with the paired normal tissue (Fig. 1). In the tissues that we were able to collect the most clinical specimens, breast ($n=28$) and colon ($n=9$) carcinomas, the difference in activity between tumor and normal tissues was statistically significant with P values of 0.012 and 0.021, respectively [54]. These results have been confirmed in a recent study in 35 human colo-rectal carcinomas also indicating higher UPase mRNA gene expression in tumor compared to paired normal tissue [101]. The same study concluded that higher UPase gene expression was a negative prognostic factor for the patient [101]. In all normal tissues and most tumor specimens evaluated in our investigation [54], UPase activity was completely inhibited by the UPase inhibitor BAU at 10 μM concentration. However, breast, head-neck, and ovarian tumors showed partial sensitivity to the inhibitor with ~ 40% of residual phosphorolytic activity still present after the addition of 100 μM BAU. Using the TPase inhibitor 5-bromo-6-aminouracil [102], we were able to establish that TPase does not significantly contribute to the BAU insensitive phosphorolytic activity present in breast tumor tissue.

The evaluation of the clinical specimens has clearly indicated that normal tissues like gastrointestinal tract and bone marrow, that are the most sensitive to fluoropyrimidine toxicity, possess UPase activity completely inhibitable by BAU. However, human breast tumors possess distinct phosphorolytic activity that is partially insensitive to the classical UPase inhibitors, therefore resulting in a more rapid degradation of uridine. This differential catabolism of the pyrimidine nucleoside could be exploited to create, in the presence of BAU, a selective rescue effect for normal tissues without affecting the antineoplastic activity in breast neoplastic tissues therefore enhancing the therapeutic index of the fluoropyrimidine.

Our study, on human clinical specimens revealing the presence of higher UPase enzymatic activity in tumor tissues as compared to paired normal tissue is in contrast with a previous report from Maehara et al. [48] indicating no difference in UPase activity between tumor and normal

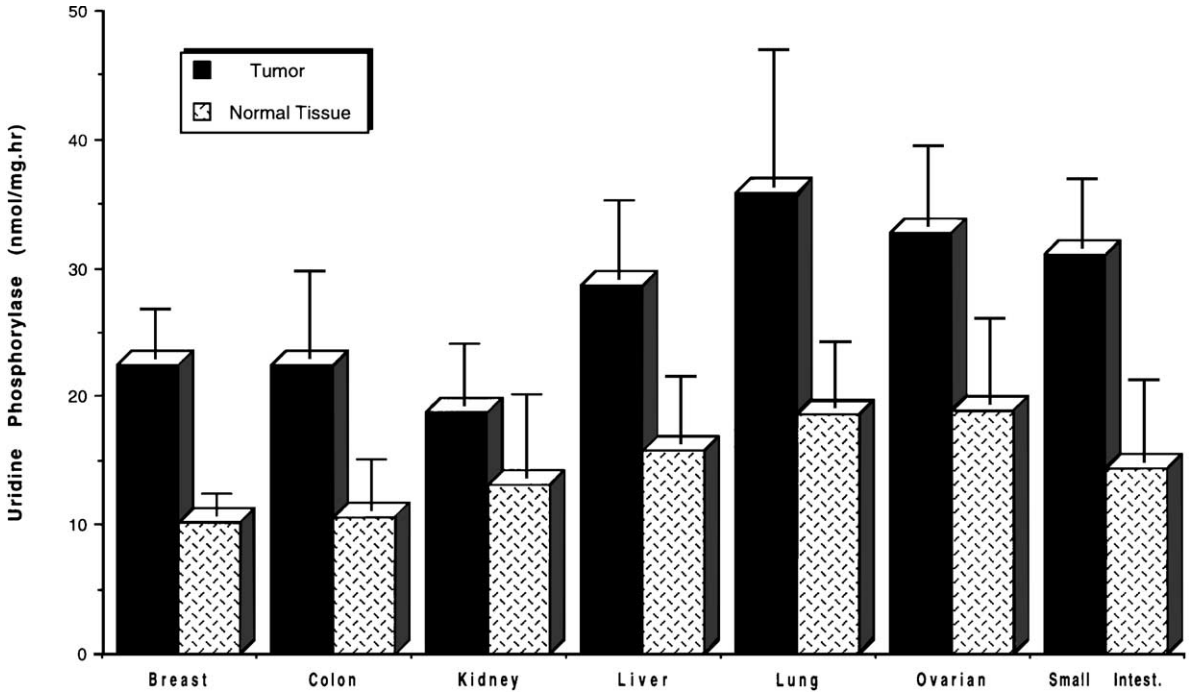

Fig. 1. UPase activity in matched pairs of human breast tumor and normal tissues following surgical excision of the malignancy.

tissues. We also disagree with another finding from the same group indicating that the main pyrimidine nucleoside phosphorylase in human is TPase with activity 20-fold higher than UPase [48]. In our evaluation, we have observed a degree of variation in the ratio of TPase to UPase activity in human tissues with an overall ratio of 2. However, in many tissues including breast tumors, the ratio was actually in favor of UPase. Our investigation has also indicated that both bone marrow and gut mucosa specimens possess low phosphorolytic activity compared to tumor tissues, suggesting that uridine rescue could specifically benefit these tissues representing the primary targets of 5-fluorouracil toxicity, as reported by Pritchard et al. [58] in a study on its effect on the intestinal mucosa.

A recent article from Kanzaki et al. [103] has examined the mRNA expression of UPase and TPase in surgical specimens of 43 patients with breast carcinoma and examined the correlation with clinical pathological factors. The investigators have found a large variation in the expression level of both genes with the highest level measured as more than 1000-fold higher than that in samples expressing the lowest level. There was a significant correlation between TPase expression and microvessel density but no correlation with UPase expression suggesting that UPase does not have any angiogenic activity in human breast carcinoma. In addition, no correlation was found between UPase gene expression and TPase expression level in those breast tumor samples. However, UPase gene expression was higher in patients who relapsed than in patients that did not and patients with high UPase mRNA levels had a significantly poorer overall survival than patients with lower levels. TPase gene expression did not correlate with either relapse or overall survival in these breast cancer patients [103]. This critical study confirms a previous study from the same group indicating a lack of correlation between clinical outcome and TPase mRNA expression in breast cancer [98]. However, it suggests that UPase could be an independent prognostic factor in breast cancer patients [103].

3.2. UPase genomic structure, characterization of its promoter region and p53-dependent control of its expression

We have recently isolated from a murine BAC library a genomic DNA fragment which included the entire murine UPase gene, whose full length approximates 18.0 kb. The UPase gene has been mapped by FISH to the murine chromosome 11A1–2. A series of oligonucleotide primers based on the cDNA sequence of murine UPase have been utilized to elucidate the intron–exon boundaries [87]. Our results indicate that the murine UPase gene consists of nine exons, ranging in length from 66 to 210 bp, and eight introns varying in size from 240 to 6.0 kb, with typical donor and acceptor sites (GT-AG rule). Exon 1, 2 and the 5′ end part of exon 3 do not encode amino acids, the first in-frame ATG codon is located in exon 3. Exon 8 encodes the C-terminus of murine UPase protein and contains a translation stop codon TGA. It also contains the first 70 bp of the 3′-untranslated region. A polyadenylation signal, AATAAA, is present at 45 bp downstream of the TGA codon [87]. We have now also concluded the characterization of the human UPase gene that presents the same basic structure with nine

exons and eight introns. Chromosomal mapping of human UPase identified its location at 7p12, a position where frequent LOH has been found in human breast cancers [104].

The sequence of the 3′-untranslated flanking region of the murine UPase gene shows a GT-rich region present 22 bp downstream of the AATAAA polyadenylation signal. A TGGGG tandem repeat, $TGGGGG(TGGGG)_4$, is present at 154 bp downstream of AATAAA polyadenylation signal, which represents a putative recombination consensus sequence found in the immunoglobulin switch region (S region), in the α-globin gene cluster, in the putative arrest sites for polymerase α, and in the deletion hot spot (exon 8) of the survival motor-neuron (SMN) gene [105–107].

The 5′ flanking region of the murine UPase gene, the immediate full-length sequence (1703 bp) that has shown promoter activity in our studies, doesn't contain canonical CAAT box although a TATA-like sequence, CAATAAAA, is present from −41 to −49 bp upstream of the transcription start point at +1 bp. The lack of both canonical TATA and CAAT consensus sequences is a feature present in a group of genes, many of which have a housekeeping function, such as *N*-ras and transforming growth factor α [108]. At the 5′ end of UPase promoter (from −1619 to −1110) we identified a series of microsatellite and minisatellite repeat bases. In addition, an abundance of promoter regulatory elements are seen in the murine UPase promoter region including the presence of the consensus motifs for GATA-1 and two transcription factors. These factors mainly function as regulatory elements in the control of cellular differentiation of hematopoietic cells [109,110]. An IRF-1-like consensus element present just upstream (from −21 to −33) of the putative transcription start site of the UPase gene represents an important transcription factor in the regulation of the interferon response system for infection, cell growth and apoptosis [111,112]. Finally, two potential proto-oncogene binding sites for C-Myb and V-Myb [113–115], and a tumor suppressor gene, p53 putative regulatory element [116–118], located in the sequence −303 bp to −294 bp, have been found in the UPase promoter region.

To explore the possible effect of p53 on UPase expression, we have analyzed the effects of p53 on the murine UPase promoter activity [88]. We found that the deletion from −1619 to −445 of the UPase promoter had no effect on the ability of p53 to inhibit gene expression, however, the inhibitory activity was altered when the promoter region between −445 and −274 bp was deleted. Using transient-expression assays in EMT6 and NIH 3T3 cells, co-transfection with the wild-type p53 construct resulted in significantly less luciferase activity in the constructs from −1619 to −445 bp, whereas down to −274 bp and more, the promoter activity was not affected. These data indicate that the region between −445 and −274 bp is susceptible to regulation by p53 in the UPase promoter. This phenomenon was further confirmed in p53 nullified cells [88]. Sequencing analysis of this region found a putative p53-binding motif AGcCTTGTCC located at −303 to −294. This binding motif differs in one base (small case base) from the consensus binding element of p53 [119]. The gel mobility shift assay and DNase I footprinting have indicated that this putative regulatory motif exhibited specific binding with the p53 protein [88].

p53 has been shown, by Linke et al. [120], to be activated by ribonucleotide depletion caused by antimetabolite drugs such as PALA even in the absence of DNA damage. As previously mentioned, the phosphorolytic activity of UPase regulating intracellular uridine levels reveals the critical role of this enzyme in modulating the pyrimidine salvage pathway. The suppressive regulation of p53 on UPase gene indicates the presence of a negative control of the pyrimidine salvage pathway by p53 through UPase, probably as a cellular self-protection mechanism in case of ribonucleotide depletion. p53 has previously been shown to: (a) activate genes that initiate apoptosis to eliminate damaged cells and protect an organism from more severe damage and (b) cause cell-cycle arrest following DNA damage to prevent the replication of altered DNA. However, so far, any indication of the contribution of p53 to damage repair is quite limited. A recent report by Tanaka et al. [121] has described a p53-induced gene, p53R2 that encodes for a protein similar to one of the two subunits of ribonucleotide reductase, the rate-limiting step in the conversion of ribonucleotides to deoxyribonucleotides. The p53 regulated R2 subunit is found in the nucleus and its expression is induced by cellular damage (γ-radiation and doxorubicin treatment) suggesting that when repair is needed, the nuclear precursors have to be concentrated near the site of damage.

Somehow, the p53-regulated suppression of UPase expression exerts similar functions to the control that p53 has on p53R2. A cellular damage causing loss or imbalance in the ribonucleotide pools could cause activation of p53 leading to suppression of UPase expression and activation of the pyrimidine salvage pathway to replenish the affected pyrimidine nucleotide pools (Fig. 2). These two p53-regu-

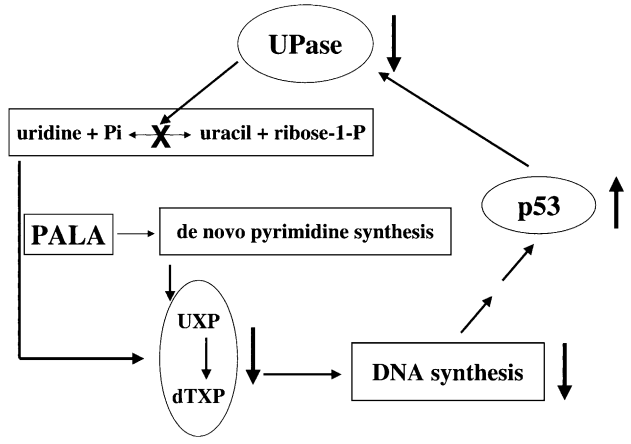

Fig. 2. p53-dependent control of UPase expression and regulation of pyrimidine salvage pathway following PALA inhibition of de novo pyrimidine biosynthesis.

lated mechanisms provide a new level of control on ribo- and deoxy-ribonucleotide pools. Under normal replication conditions, the regulating mechanisms that control the appropriate balance of nucleotides are mostly based on the direct feedback regulation of the biosynthetic enzymes by some of the precursors or final products. However, the p53R2 study and our data on UPase possibly indicate that in case of cellular damage with depletion of nucleotide pools a more sophisticated level of regulation is triggered to more rapidly provide precursors for nuclear repair [88,121].

The elucidation of the negative control regulation of p53 on the UPase gene promoter and UPase expression could also have considerable implication at the clinical level since the human UPase DNA promoter presents, as we have shown for the murine gene, a p53 regulatory element [88]. It is conceivable that mutations and loss of functionality of the p53 gene product, which is a common event in many forms of cancer [122], could alter the suppressive regulatory control on UPase resulting in higher UPase mRNA expression and elevated UPase activity seen in many tumors as compared to paired normal tissues [54].

3.3. Intracellular distribution, localization and association with vimentin

We have established that UPase is associated with the intermediate filament vimentin in NIH 3T3 fibroblasts and Colon 26 cells through co-purification studies using a 5′-amino benzylacyclouridine affinity matrix. The separation of cytosolic proteins using gel filtration chromatography yields a high molecular weight complex containing UPase and vimentin in a 1:1 stoichiometry. Immunofluorescent techniques have confirmed that UPase is associated with vimentin and that the depolymerization of the microtubule system using nocodazole results in UPase remaining associated with the collapsed intermediate filament, vimentin [89].

UPase is associated with both the soluble pool of vimentin and also with its insoluble pool, with approximately 50–70% of the total UPase present in the cytosol as a soluble protein. However, sequential cell extraction liberates an additional 15–25% UPase activity associated with a Triton-X-100 soluble fraction and a remaining 10–20% UPase activity associated with a Triton-X-100 insoluble pool [89].

The role of UPase in the salvage pathway of pyrimidine nucleoside biosynthesis does not readily translate into a role for this enzyme in association with the cytoskeleton and more specifically with the intermediate filament vimentin. While a number of theories have been proposed for the function of this network, the data are not yet conclusive. Cellular processes as diverse as differentiation, motility, signal transduction, cell division, cytoskeletal stability and vesicular trafficking have been associated with alterations in the dynamics of the intermediate filaments [123–127].

A number of proteins have been shown to be associated with the vimentin intermediate filament scaffold including p53 [128], protein kinase C [129], Yes and cGMP kinase [130,131], glycolytic enzymes creatine phosphokinase and GAPDH [132–134] and nucleoside diphosphate kinase [132,135] as well as the cross-linking proteins plectin, IFAP-300 and filamin that link intermediate filaments to other cytoskeletal elements and membranes [136–139]. It is particularly interesting to note the number of proteins involved in signal transduction and energy metabolism that have been associated with vimentin. The proposed role for NDPK in nucleotide channeling [135], its co-purification with vimentin and enzymes involved in ATP formation/regeneration [132] together with our observation of UPase co-localization with this same cellular machinery, is making it more likely that such observations are biologically relevant. UPase, a nucleoside phosphorylase and NDPK, an enzyme that provides the majority of cellular non-ATP nucleoside triphosphates have both been co-localized to the intermediate filament vimentin. Since a number of biological responses have been associated with UTP and UDP [140] through the activation of pyrimidine receptors, it is possible that vimentin may play a role in the coordination of these signaling events.

In vitro enzymatic analyses of the detergent-resistant pool of UPase demonstrated that this source of enzyme retains enzymatic activity. The UPase found in association with the polymeric vimentin network may represent a mobilizable pool of enzyme that is only active when liberated from its three-dimensional network. It is also possible that UPase, in association with the insoluble vimentin network, represents a way of localizing enzymatic activity to a particular area within the cell. Vimentin has been proposed as a network that might target mRNA to areas of active protein synthesis [124]. This function for vimentin might explain the necessity of having machinery for pyrimidine synthesis/degradation in close proximity to areas of mRNA translation.

The interdependence of the dynein and kinesin motor proteins, microtubule and intermediate filament systems and the need to furnish this cellular machinery with high quantities of nucleotide triphosphates provides a basis for investigating the mechanisms responsible for the local delivery of high quantities of NTPs to the areas of active energy utilization. The question of what role UPase may play in close proximity to such machinery is at this moment cause for speculation.

4. Conclusions

Many factors have contributed to the limited attention uridine and UPase have received despite their physiologic and pharmacological role in comparison to the interest reserved over the years to adenosine and TPase.

Adenosine has been shown to have a general inhibitory effect on neuronal activity including regulation of sleep, neuroprotection and seizure control [141]. Furthermore, this

purine nucleoside appears to have cardioprotective and immunomodulatory functions [142]. These regulatory and modulatory activities of adenosine are mediated by four subtypes of G-protein-coupled receptors [143]. As we have indicated in our introduction, uridine exerts very similar modulatory and regulatory functions to adenosine, however, no clear mechanism has been identified modulating these physiological activities. Receptors for uridine nucleotides have been mentioned and also the possible existence of a specific receptor for uridine itself has been postulated. A very recent report suggests the presence of a new receptor identified as "uridine receptor" regulating the hypnotic activity of uridine derivatives in rat brain [144]. More studies are needed to confirm this finding, to elucidate the biological and structural characteristic of the receptor, and the interactions with other receptors and substrates. It is critical to extend these studies to other organs to determine the presence of "uridine receptor(s)" not only in the central nervous system but also in the cardio-circulatory system and at the reproduction level. These studies will lead to the discovery of a new interacting molecules and the development of new class of therapeutic agents for various human diseases.

Similarly, the attention dedicated to TPase is associated to its angiogenic properties [144] and more recently, to its link to a human genetic disease, MNGIE [145], and it could be matched by UPase only after we have better defined its physiological function and its possible role in human diseases. Our discoveries of an elevated expression of UPase in human tumors [54], its altered pattern of inhibition in breast cancers [54] and the intracellular association with the cytoskeleton [89] only represent starting points to gain new insight in its biological functions and define its clinical–pathological role.

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References

[1] J.D. Moyer, J.T. Oliver, R.E. Handschumacher, Salvage of circulating pyrimidine nucleosides in the rat, Cancer Res. 41 (1981) 3010–3017.
[2] D.M. Becroft, L.I. Phillips, A. Simmonds, Hereditary orotic aciduria: long-term therapy with uridine and a trial of uracil, J. Pediatr. 75 (1969) 885–891.
[3] G. Pizzorno, L. Yee, B.A. Burtness, J.C. Marsh, J.W. Darnowski, M.Y. Chu, S.H. Chu, J.J. Leffert, R.E. Handschumacher, P. Calabresi, Clinical and pharmacological studies of Benzylacyclouridine, a uridine phosphorylase inhibitor, Clin. Cancer Res. 4 (1998) 1165–1175.
[4] C.J. Van Groeningen, A. Leyva, I. Kraal, G.J. Peters, H.M. Pinedo, Clinical and pharmacokinetic studies of prolonged administration of high-dose uridine intended for rescue from 5-FU toxicity, Cancer Treat. Rep. 70 (1986) 745–750.
[5] T.W. Traut, Physiological concentrations of purines and pyrimidines, Mol. Cell. Biochem. 140 (1994) 1–22.
[6] T. Gasser, J.D. Moyer, R.E. Handschumacher, Novel single pass exchange of circulating uridine in rat liver, Science 213 (1981) 777–778.
[7] M.P. Liu, E. Levy, R.E. Handschumacher, G. Pizzorno, Discrete roles of hepatocytes and nonparenchymal cells in uridine catabolism as a component of its homeostasis, Am. J. Physiol. 274 (1998) G1018–G1023.
[8] G.P. Connolly, J.A. Duley, Uridine and its nucleotides: biological actions, therapeutic potentials, Trends Pharmacol. Sci. 20 (1999) 218–225.
[9] R. Seifert, G. Schultz, Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides, Trends Pharmacol. Sci. 10 (1989) 365–369.
[10] G. Ronquist, F. Niklasson, Uridine, xanthine, and urate contents in human seminal plasma, Arch. Androl. 13 (1984) 63–70.
[11] G. Ronquist, B. Stegmayr, F. Niklasson, Sperm motility and interactions among seminal uridine, xanthine, urate, and ATPase in fertile and infertile men, Arch. Androl. 15 (1985) 21–27.
[12] G. Siggins, D. Gruol, A. Padjen, D. Forman, in: R.W. Ryall, J.S. Kelly (Eds.), Iontophoresis and Transmitter Mechanisms Mammalian Central Nervous System, Elsevier, New York, 1978, pp. 453–455.
[13] F.J. Gonzalez, P. Fernandez-Salguero, Diagnostic analysis, clinical importance and molecular basis of dihydropyrimidine dehydrogenase deficiency, Trends Pharmacol. Sci. 16 (1995) 325–327.
[14] G.P. Connolly, H.A. Simmonds, J.A. Duley, Pyrimidines and CNS regulation, Trends Pharmacol. Sci. 17 (1996) 106–107.
[15] T. Page, A. Yu, J. Fontanesi, W.L. Nyhan, Developmental disorder associated with increased cellular nucleotidase activity, Proc. Natl. Acad. Sci. 94 (1997) 11601–11606.
[16] N.N. Karkishchenko, I.S. Makliakov, B.V. Stradomskii, Pyrimidine derivatives: their psychotropic properties and the molecular mechanisms of their central action, Farmakol. Toksikol. 53 (1990) 67–72.
[17] A. Leyva, C.J. Van Groeningen, I. Kraal, G.J. Peters, J. Lankelma, H.M. Pinedo, Phase I and pharmacokinetic studies of high-dose uridine intended for rescue from 5-fluorouracil toxicity, Cancer Res. 44 (1984) 5928–5933.
[18] T. Kimura, J. Kuze, K. Watanabe, S. Kondo, I.K. Ho, I. Yamamoto, N3-phenacyluridine, a novel hypnotic compound, interacts with the benzodiazepine receptor, Eur. J. Pharmacol. 311 (1996) 265–269.
[19] G.J. Peters, C.J. Van Groeningen, E.J. Laurensse, J. Lankelma, A. Leyva, H.M. Pinedo, Uridine-induced hypothermia in mice and rats in relation to plasma and tissue levels of uridine and its metabolites, Cancer Chem. Pharm. 20 (1987) 101–108.
[20] C.J. Van Groeningen, A. Leyva, I. Kraal, G.J. Peters, H.M. Pinedo, Clinical and pharmacokinetic studies of prolonged administration of high-dose uridine intended for rescue from 5-FU toxicity, Cancer Treat. Rep. 70 (1986) 745–750.
[21] G.J. Peters, C.J. Van Groeningen, E. Laurensse, I. Kraal, A. Leyva, J. Lankelma, H.M. Pinedo, Effect of pyrimidine nucleosides on body temperatures of man and rabbit in relation to pharmacokinetic data, Pharm. Res. 4 (1987) 113–119.
[22] J. Aussedat, Effect of uridine supply on glycogen resynthesis after ischaemia in the isolated perfused rat heart, Cardiovasc. Res. 17 (1983) 145–151.
[23] A. Geiger, S. Yamasaki, Cytidine and uridine requirement of the brain, J. Neurochem. 1 (1956) 93–100.
[24] G. Benzi, R.F. Villa, M. Dossena, L. Vercesi, A. Gorini, O. Pastoris, Cerebral endogenous substrate utilization during the recovery period after profound hypoglycemia, J. Neurosci. Res. 11 (1984) 437–450.
[25] V. Gallai, G. Mazzotta, S. Montesi, P. Sarchielli, F. Del Gatto, Effects of uridine in the treatment of diabetic neuropathy: an electrophysiological study, Acta Neurol. Scand. 86 (1992) 3–7.
[26] T.A. Krenisky, M. Barclay, J.A. Jacquez, Specificity of mouse uridine phosphorylase. Chromatography, purification and properties, J. Biol. Chem. 239 (1964) 805–812.
[27] T.A. Krenisky, J.W. Mellors, R.K. Barclay, Pyrimidine nucleosidases,

their classification and relationship to uric acid ribonucleoside phosphorylase, J. Biol. Chem. 240 (1965) 1281–1286.
[28] J.G. Niedzwicki, M.H. El Kouni, S.H. Chu, S. Cha, Pyrimidine acyclonucleosides inhibitors of uridine phosphorylase, Biochem. Pharmacol. 30 (1981) 2097–2101.
[29] J.G. Niedzwicki, S.H. Chu, M.H. El Kouni, E.C. Rowe, S. Cha, 5-Benzylacyclouridine and 5-benzyloxybenzylacycloruridine, potent inhibitors of uridine posphorylase, Biochem. Pharmacol. 31 (1982) 1857–1861.
[30] H. Pontis, G. Degerstedt, P. Reichard, Uridine and deoxyuridine phosphorylase from Ehrlich ascites tumor, Biochim. Biophys. Acta 51 (1961) 138–147.
[31] M.P. Liu, S. Srimatkandada, R.E. Handschumacher, G. Pizzorno, Variant uridine phosphorolytic activity in human breast and head-neck tumors: a potential target for improving 5-FU therapy, Proc. Am. Assoc. Cancer Res. 37 (1996) 2786.
[32] P. Calabresi, A. Falcone, M.H. St Clair, M.C. Wiemann, S.H. Chu, J.W. Darnowski, Benzylacyclouridine reverses azidothymidine-induced marrow suppression without impairment of anti-human immunodeficiency virus activity, Blood 76 (1990) 2210–2215.
[33] R. Bose, E.W. Yamada, Uridine phosphorylase molecular properties and mechanism of catalysis, Biochemistry 13 (1974) 2051–2056.
[34] J.W. Darnowski, R.E. Handschumacher, Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine, Cancer Res. 45 (1985) 5364–5368.
[35] S.H. Chu, Z.Y. Weng, Z.H. Chen, E.C. Rowe, E. Chu, F.N.M. Naguib, M.H. El Kouni, S. Cha, M.Y. Chu, Synthesis of 5-benzyl and 5-benzyloxybenzyl 2,2′-anhydrouridines and related nucleoside analogs as inhibitors of uridine phosphorylase, Nucleosides Nucleotides 7 (1988) 91–102.
[36] A. Monks, O. Ayers, R.L. Cysyk, Effect of 5-benzylacyclouridine, a potent inhibitor of uridine phosphorylase, on the metabolism of circulating uridine by the isolated rat liver, Biochem. Pharmacol. 32 (1983) 2003–2009.
[37] D.S. Martin, R.L. Stolfi, R.C. Sawyer, Use of oral uridine as a substitute for parenteral uridine rescue of 5-fluorouracil therapy, with and without the uridine phosphorylase inhibitor 5-benzylacyclouridine, Cancer Chemother. Pharmacol. 24 (1989) 9–14.
[38] G.D. Birnie, H. Kroeger, C. Heidelberger, Studies of fluorinated pyrimidines: XVIII. The degradation of 5-fluoro-2′-deoxyuridine and related compounds by nucleoside phosphorylase, Biochemistry 2 (1963) 566–572.
[39] P.W. Woodman, A.M. Sarrif, C. Heidelberger, Specificity of pyrimidine nucleoside phosphorylases and the phosphorolysis of 5-fluoro-2′-deoxyuridine, Cancer Res. 40 (1980) 507–511.
[40] H. Ishitsuka, M. Miwa, K. Takemoto, K. Fukuoka, A. Itoga, H.B. Maruyama, Role of uridine phosphorylase for antitumor activity in 5′-deoxy-5-fluorouridine, Gann 71 (1980) 112–123.
[41] M.Y. Chu, F.N.M. Naguib, M.H. Itzsch, M.H. El Kouni, S.H. Chu, S. Cha, P. Calabresi, Potentiation of 5-fluoro-2′ -deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and benzyloxybenzylacyclouridine, Cancer Res. 44 (1984) 1852–1856.
[42] H. Eda, K. Fujimoto, S. Watanabe, T. Ishikawa, T. Ohiwa, K. Tatsuno, Y. Tanaka, H. Ishitsuka, Cytokines induce uridine phosphorylase in mouse colon 26 carcinoma cells and make the cells more susceptible to 5-deoxy-5-fluoruridine, Jpn. J. Cancer Res. 84 (1993) 341–347.
[43] S. Watanabe, A. Hino, K. Wada, J.F. Eliason, T. Uchida, Purification, cloning, and expression of murine uridine phosphorylase, J. Biol. Chem. 270 (1995) 12191–12196.
[44] S. Watanabe, T. Uchida, Cloning and expression of human uridine phosphorylase, Biochem. Biophys. Res. Commun. 216 (1995) 265–272.
[45] E.L. Schwartz, E. Wan, F. Wang, N. Baptiste, Regulation of expression of thymidine phosphorylase/platelet-derived endothelial cell growth factor in human colon carcinoma cells, Cancer Res. 58 (1998) 1551–1557.
[46] Y. Geng, E. Gheuens, E.A. De Bruijn, Activation and cytotoxicity of 5′-deoxy-5-fluorouridine in c-H-ras transformed NIH 3T3 cells, Biochem. Pharmacol. 41 (1991) 301–303.
[47] M.H. El Kouni, F.N.M. Naguib, K.S. Park, S. Cha, J.W. Darnowski, S.J. Soong, Circadian rhythm of hepatic uridine phosphorylase activity and plasma concentration of uridine in mice, Biochem. Pharmacol. 40 (1990) 2479–2485.
[48] Y. Maehara, Y. Sakaguchi, T. Kusumoto, H. Kusumoto, K. Sugimachi, Species differences in substrate specificity of pyrimidine nucleoside phosphorylase, J. Surg. Oncol. 42 (1989) 184–186.
[49] F.N.M. Naguib, J.G. Niedzwicki, M.H. Iltzsh, M.C. Wiemann, M.H. El Kouni, S. Cha, Effects of N,*N*-dimethylformamide and sodium butyrate on enzymes of pyrimidine metabolism in cultures human tumor cells, Leuk. Res. 11 (1987) 855–861.
[50] D.S. Martin, R.L. Stolfi, R.C. Sawyer, S. Spiegelman, C.W. Young, High dose 5-fluorouracil with delayed uridine "rescue" in mice, Cancer Res. 42 (1982) 3964–3970.
[51] J.G. Niedzwicki, M.H. El Kouni, S.H. Chu, S. Cha, Structure–activity relationship of ligands of the pyrimidine nucleoside phosphorylases, Biochem. Pharmacol. 32 (1983) 399–415.
[52] M.Y. Chu, F.N.M. Naguib, M.H. Itzsch, M.H. El Kouni, S.H. Chu, P. Calabresi, Potentiation of 5-fluoro-2′-deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and benzyloxybenzylacyclouridine, Cancer Res. 44 (1984) 1852–1856.
[53] J.W. Darnowski, R.E. Handschumacher, Tissue uridine pools evidence in vivo of a concentrative mechanism for uridine uptake, Cancer Res. 46 (1986) 3490–3494.
[54] M.P. Liu, D.L. Cao, R.L. Russell, R.E. Handschumacher, G. Pizzorno, Expression, characterization and detection of human uridine phosphorylase and identification of variant uridine phosphorolytic activity in selected human tumors, Cancer Res. 58 (1998) 5418–5424.
[55] H.M. Pinedo, G.J. Peters, Fluorouracil: biochemistry and pharmacology, J. Clin. Oncol. 6 (1988) 1653–1654.
[56] J.A. Houghton, P.J. Houghton, R.S. Wooten, Mechanism of induction of gastrointestinal toxicity in the mouse by 5-fluorouracil, 5-fluorouridine, and 5-fluoro-2′-deoxyuridine, Cancer Res. 39 (1979) 2406–2413.
[57] F.J. Geoffroy, C.J. Allegra, B. Sinha, J.L. Grem, Enhanced cytotoxicity with interleukin-1 alpha and 5-fluorouracil in HCT116 colon cancer cells, Oncol. Res. 6 (1994) 581–591.
[58] M.D. Pritchard, A.J.M. Watson, C.S. Potten, A.L. Jackman, J.A. Hickman, Inhibition by uridine but not thymidine of p53-dependent intestinal apoptosis initiated by 5-fluorouracil: evidence for the involvement of RNA perturbation, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1795–1799.
[59] K. Seiter, N. Kemeny, D. Martin, A. Schneider, L. Williams, J. Colofiore, R. Sawyer, Uridine allows dose escalation of 5-fluorouracil when given with *N*-phosphonacetyl-L-aspartate, methotrexate, and leucovorin, Cancer 71 (1993) 1875–1881.
[60] G.K. Schwartz, K. Christman, L. Saltz, E. Casper, V. Ouan, J. Bertino, D.S. Martin, J. Colofiore, D. Kelsen, A phase I trial of a modified, dose intensive FAMTX regimen (high dose 5-fluorouracil + doxorubicin + high dose methotrexate + leucovorin) with oral uridine rescue, Cancer 78 (1996) 1988–1995.
[61] D.P. Kelsen, D.S. Martin, J. O'Neil, G. Schwartz, L. Saltz, M.T. Sung, R. von Borstel, J.R. Bertino, Phase I clinical trial of PN401, an oral prodrug of uridine, to prevent toxicity from fluorouracil in patients with advanced cancer, J. Clin. Oncol. 15 (1997) 1511–1517.
[62] C.E. Cass, A.R.P. Paterson, Mediated transport of nucleosides in human erythrocytes. Accelerative exchange diffusion of uridine and thymidine and specificity toward pyrimidine nucleosides as permeants, J. Biol. Chem. 247 (1972) 3314–3320.
[63] C.E. Cass, A.R.P. Paterson, Mediated transport of nucleosides by human erythrocytes. Specificity toward purine nucleosides as permeants, Biochim. Biophys. Acta 291 (1973) 734–746.

[64] S.M. Jarvis, J.D. Young, Nucleoside translocation in sheep reticulocytes and fetal erythrocytes: a proposed model for the nucleoside transporter, J. Physiol. 324 (1982) 47–66.
[65] R.M. Wohlhueter, R. Marz, P.G.W. Plagemann, Thymidine transport in cultured mammalian cells. Kinetic analysis, temperature dependence and specificity of the transport system, Biochim. Biophys. Acta 553 (1979) 262–283.
[66] A.R.P. Paterson, E.S. Jakobs, E.R. Harley, C.E. Cass, M.J. Robins, Inhibitors of nucleoside transport as probes and drugs, in: Y.C. Cheng (Ed.), Development of Target Oriented Anticancer Drugs, Raven Press, New York, 1983, pp. 41–55.
[67] J.A. Belt, Nitrobenzylthioinosine-insensitive uridine transport in human lymphoblastoid and murine leukemia cells, Biochem. Biophys. Res. Commun. 110 (1983) 417–423.
[68] J.A. Belt, Heterogeneity of nucleoside transport in mammalian cells. Two types of transport activity in L1210 and other cultured neoplastic cells, Mol. Pharmacol. 24 (1983) 479–484.
[69] S.Y. Yao, A.M. Ng, W.R. Muzyka, M. Griffiths, C.E. Cass, S.A. Baldwin, J.D. Young, Molecular cloning and functional characterization of nitrobenzylthioinosine (NBMPR)-sensitive (es) and NBMPR-insensitive (ei) equilibrative nucleoside transporter proteins (rent1 and rent2) from rat tissues, J. Biol. Chem. 272 (1997) 28423–28430.
[70] C.R. Crawford, D.H. Patel, C. Naeve, J.A. Belt, Cloning of the human equilibrative, nitrobenzylmercaptopurine riboside (NBMPR)-insensitive nucleoside transporter ei by functional expression in a transport-deficient cell line, J. Biol. Chem. 273 (1998) 5288–5293.
[71] J.F. Kuttesch, J.A. Nelson, Renal handling of 2′-deoxyadenosine and adenosine in humans and mice, Chemother. Pharmacol. 8 (1982) 221–229.
[72] J.F. Kuttesch, M.J. Robins, J.A. Nelson, Renal transport of 2′-deoxytubercidin in mice, Biochem. Pharmacol. 31 (1982) 3387–3394.
[73] M. LeHir, U.C. Dubach, Uphill transport of pyrimidine nucleosides in renal brush border vesicles, Pfluegers Arch. 404 (1985) 238–243.
[74] C.W. Lee, C.I. Cheeseman, S.M. Jarvis, Na^+- and K^+-dependent uridine transport in rat renal brush border membrane vesicles, Biochim. Biophys. Acta 942 (1988) 139–149.
[75] M.E. Trimble, R. Coulson, Adenosine transport in perfused rat kidney and renal cortical membrane vesicles, Am. J. Physiol. 246 (1984) F794–F803.
[76] S.M. Jarvis, T.C. Williams, C.W. Lee, C.I. Cheeseman, Active transport of nucleosides and nucleoside drugs, Biochem. Soc. Trans. 17 (1989) 448–449.
[77] M. Schwenk, E. Hegazy, V. Lopez del Pino, Uridine uptake by isolated intestinal epithelial cells of guinea pig, Biochim. Biophys. Acta 805 (1984) 370–374.
[78] L. Dagnino, L.L. Bennett, A.R.P. Paterson, Sodium-dependent nucleoside transport in mouse leukemia L1210 cells, J. Biol. Chem. 266 (1991) 6308–6311.
[79] M. Roden, A.R.P. Paterson, K. Turnhein, Sodium-dependent nucleoside transport in rabbit intestinal epithelium, Gastroenterology 100 (1991) 1553–1562.
[80] S.L. Betcher, J.N. Forrest, R.G. Knickelbein, J.W. Dobbins, Sodium-dependent nucleoside co-transport in brush-border membranes from rabbit ileum, Am. J. Physiol. 259 (1990) 504–510.
[81] D. Vijayalakshmi, J.A. Belt, Sodium-dependent nucleoside transport in mouse epithelial cells. Two transport systems with differing substrate specificities, J. Biol. Chem. 263 (1988) 19419–19423.
[82] M.W.L. Ritzel, S.Y.M. Yao, M.Y. Huang, J.F. Elliott, C.E. Cass, J.D. Young, Molecular cloning and functional expression of cDNAs encoding a human Na^+-nucleoside co-transporter (hCNT1), Am. J. Physiol. 341 (1997) C707–C714.
[83] S.M. Jarvis, D.A. Griffith, Expression of the rabbit intestinal N2 Na^+-nucleoside transporter in *Xenopus laevis* oocytes, Biochem. J. 278 (1991) 605–607.
[84] Q.Q. Huang, C.M. Harvey, A.R.P. Paterson, C.E. Cass, J.D. Young, Functional expression of Na^+-dependent nucleoside transport systems of rat intestine in isolated oocytes of *Xenopus laevis*. Demonstration that rat jejunum expresses the purine-selective system N1 (cif) and a second, novel system N3 having broad specificity for purine and pyrimidine nucleosides, J. Biol. Chem. 268 (1993) 20613–20619.
[85] M.M. Gutierrez, C.M. Brett, R.J. Ott, A.C. Hui, K.M. Giacomini, Nucleoside transport in brush border membrane vesicles from human kidney, Biochim. Biophys. Acta 1105 (1992) 1–9.
[86] A.R.P. Patterson, W.P. Gati, D. Vijajalakshmi, C.E. Cass, M.J. Mant, J.D. Young, A.R. Belch, Inhibitor-sensitive, sodium-linked transport of nucleoside analogs in leukemia cells from patients, Proc. Am. Assoc. Cancer Res. 34 (1993) 14.
[87] D. Cao, M.A. Nimmakayalu, F. Wang, R.E. Handschumacher, P. Bray-Ward, G. Pizzorno, Genomic structure, chromosomal mapping and promoter region sequence of murine Uridine Phosphorylase Gene, Cancer Res. 59 (1999) 4997–5001.
[88] D. Zhang, D. Cao, R.L. Russell, G. Pizzorno, p53-dependent suppression of uridine phosphorylase gene expression through direct promoter interaction, Cancer Res. 61 (2001) 6899–6905.
[89] R.L. Russell, D. Cao, D. Zhang, R.E. Handschumacher, G. Pizzorno, Uridine phosphorylase association with vimentin: intracellular distribution and localization, J. Biol. Chem. 276 (2001) 13302–13307.
[90] T. Furukawa, A. Yoshimura, T. Sumizawa, M. Haraguchi, S.I. Akiyama, K. Fukui, M. Ishizawa, Y. Yamada, Angiogenic factor, Nature 356 (1992) 668.
[91] A. Moghaddam, H.T. Zhang, T.P.D. Fan, D.E. Hu, V.C. Lees, H. Turley, S.B. Fox, K.C. Gatter, A.L. Harris, R. Bicknell, Thymidine phosphorylase is angiogenic and promotes tumor growth, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 998–1002.
[92] K. Reynolds, F. Farnaneh, W.P. Collins, S. Campbell, T.H. Bourne, F. Lawton, A. Moghaddam, A.L. Harris, R. Bicknell, Association of ovarian malignancy with expression of platelet-derived endothelial cell growth factor, J. Natl. Cancer Inst. 86 (1994) 1234–1237.
[93] T.S. O'Brien, S.B. Fox, A.J. Dickinson, H. Turley, M. Westwood, A. Moghaddam, K.C. Gatter, R. Bicknell, A.L. Harris, Expression of the angiogenic factor thymidine phosphorylase/platelet-derived endothelial cell growth factor in primary bladder cancers, Cancer Res. 56 (1996) 4799–4802.
[94] C. Luccioni, J. Beaumartin, V. Bardot, D. Lefrancois, Pyrimidine nucleotide metabolism in human colon carcinomas: comparison of normal tissues, primary tumors and xenografts, Int. J. Cancer 58 (1994) 571–575.
[95] Y. Takebayashi, S. Akiyama, S. Akiba, K. Yamada, K. Miyadera, T. Sumizawa, Y. Yamada, F. Murata, T. Aikou, Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma, J. Natl. Cancer Inst. 88 (1996) 1110–1114.
[96] K. Fujimoto, R. Hosotani, M. Wada, J.U. Lee, T. Koshiba, Y. Miyamoto, S. Tsuji, S. Nakajima, R. Doi, M. Imamura, Expression of two angiogenic factors, vascular endothelial growth factor and platelet-derived endothelial cell growth factor in human pancreatic cancer, and its relationship to angiogenesis, Eur. J. Cancer 34 (1998) 1439–1443.
[97] Y. Imazono, Y. Takebayashi, K. Nishiyama, S. Akiba, K. Miyadera, Y. Yamada, S. Akiyama, Y. Ohi, Correlation between thymidine phosphorylase expression and prognosis in human renal cell carcinoma, J. Clin. Oncol. 15 (1997) 2570–2573.
[98] M. Toi, S. Hoshina, T. Taniguci, Y. Yamamoto, H. Ishitsuka, T. Tominaga, Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer, Int. J. Cancer 64 (1995) 79–82.
[99] S.B. Fox, M. Westwood, A. Moghaddam, M. Comley, H. Turley, R.M. Whitehouse, R. Bicknell, K.C. Gatter, A.L. Harris, The angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase is up regulated in breast cancer epithelium and endothelium, Br. J. Cancer 73 (1996) 275–278.
[100] M. Haraguchi, K. Miyadera, K. Uemura, T. Sumizawa, T. Furukawa,

K. Yamada, S.I. Akiyama, Y. Yamada, Angiogenic activity of enzymes, Nature 368 (1998) 198.
[101] H. Uetake, W. Ichikawa, M. Kirihara, M. Tajima, Z. Nihei, K. Sugihara, Relationship between gene expression level of phosphorylating enzymes for 5-FU and chemosensitivity by drug response assay in human colorectal carcinoma, Proc. Am. Assoc. Cancer Res. 42 (2001) 3327.
[102] P. Langen, G. Etzold, B. Barwolff, B. Preussel, Inhibition of thymidine phosphorylase by 6-amino-thymine and derivatives of 6-aminouracil, Biochem. Pharmacol. 16 (1987) 1833–1837.
[103] A. Kanzaki, Y. Takebayashi, H. Bando, J.F. Eliason, S. Watanabe, H. Miyashita, M. Fukumoto, M. Toi, T. Uchida, Expression of uridine and thymidine phosphorylase genes in human breast carcinoma, Int. J. Cancer 97 (2002) 631–635.
[104] R.J. Osborne, M.G. Hamshere, A genome-wide map showing common regions of loss of heterozygosity/allelic imbalance in breast cancer, Cancer Res. 60 (2000) 3706–3712.
[105] C.A. Gritzmacher, Molecular aspects of heavy-chain class switching, Crit. Rev. Immunol. 9 (1989) 173–200.
[106] R.D. Nicholls, N. Fischel-Ghodsian, D.R. Higgs, Recombination at the human alpha-globulin gene cluster: sequence features and topological constraints, Cell 49 (1987) 369–378.
[107] E. Hahnen, J. Schonling, S. Rudnik-Schoneborn, K. Zerres, B. Wirth, Hybrid survival motor neuron genes in patients with autosomal recessive spinal muscular atrophy: new insights into molecular mechanisms responsible for the disease, Am. J. Hum. Genet. 59 (1996) 1057–1065.
[108] K. Hagiwara, G. Stenman, H. Honda, P. Sahlin, A. Andersson, K. Miyazono, C.H. Heldin, F. Ishikawa, F. Takaku, Organization and chromosomal localization of the human platelet-derived endothelial cell growth factor gene, Mol. Cell. Biol. 11 (1991) 2125–2132.
[109] S.H. Orkin, GATA-binding transcription factors in hematopoietic cells, Blood 80 (1992) 575–581.
[110] M. Maeda, K. Kubo, T. Nishi, M. Futai, Roles of gastric GATA DNA-binding proteins, J. Exp. Biol. 199 (1996) 513–520.
[111] C.R. Escalante, J. Yie, D. Thanos, A.K. Aggarwal, Structure of IRF-1 with bound DNA reveals determinants of interferon regulation, Nature 391 (1998) 103–106.
[112] Y.C. Henderson, M. Chou, A.B. Deisseroth, Interferon regulatory factor-1 induces the expression of the interferon-stimulated genes, Br. J. Haematol. 96 (1997) 566–575.
[113] C. Grandori, R.N. Eisenman, Myc target genes, Trends Biochem. 22 (1997) 177–181.
[114] S. Ishida, S. Takada, K. Koike, Isolation and analysis of cellular DNA fragments directly binding to c-Myc protein, Leukemia 3 (1997) 399–401.
[115] C. Rudolph, J.P. Halle, G. Adam, Accelerated proliferative senescence of rat embryo fibroblasts after stable transfection of multiple copies of the c-Myc DNA-binding sequence, Exp. Cell. Res. 239 (1998) 361–369.
[116] M.E. Anderson, B. Woelker, M. Reed, P. Wang, P. Tegtmeyer, Reciprocal interference between the sequence-specific core and nonspecific C-terminal DNA binding domains of p53: implications for regulation, Mol. Cell. Biol. 17 (1997) 6255–6264.
[117] B.F. Muller-Tiemann, T.D. Halazonetis, J.J. Elting, Identification of an additional negative regulatory region for p53 sequence-specific DNA binding, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6079–6084.
[118] G.W. Verhaegh, M.O. Parat, M.J. Richard, P. Hainaut, Modulation of p53 protein conformation and DNA-binding activity by intracellular chelation of zinc, Mol. Carcinog. 21 (1998) 205–214.
[119] W.S. el-Deiry, S.E.- Kern, J.A. Pietenpol, K.W. Kinzler, B. Vogelstein, Definition of a consensus binding site for p53, Nat. Genet. 1 (1992) 45–49.
[120] S.P. Linke, K.C. Clarkin, A. Di Leonardo, A. Tsou, G.M. Wahl, A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage, Genes Dev. 10 (1996) 934–947.
[121] H. Tanaka, H. Arakawa, T. Yamaguchi, K. Shiraishi, S. Fukuda, K. Matsui, Y. Takei, Y. Nakamura, A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage, Nature 404 (2000) 42–49.
[122] D.E. Fisher, The p53 tumor suppressor: critical regulator of life and death in cancer, Apoptosis 6 (2001) 7–15.
[123] Y.H. Chou, O. Skalli, R.D. Goldman, Intermediate filaments and cytoplasmic networking: new connections and more functions, Curr. Opin. Cell Biol. 9 (1997) 49–53.
[124] O. Skalli, R.D. Goldman, Recent insights into the assembly, dynamics, and function of intermediate filament networks, Cell Motil. Cytoskeleton 19 (1991) 67–79.
[125] J.E. Eriksson, P. Opal, R.D. Goldman, Intermediate filament dynamics, Curr. Opin. Cell Biol. 4 (1992) 99–104.
[126] R.M. Evans, Vimentin: the conundrum of the intermediate filament gene family, BioEssays 20 (1998) 79–86.
[127] R.D. Goldman, S. Khoun, Y.H. Chou, P. Opal, P.M. Steinert, The function of intermediate filaments in cell shape and cytoskeletal integrity, J. Cell Biol. 134 (1996) 971–983.
[128] O. Klotzsche, D. Etzrodt, H. Hohenberg, W. Bohn, W. Deppert, Cytoplasmic retention of mutant tsp53 is dependent on an intermediate filament protein (vimentin) scaffold, Oncogene 16 (1998) 3423–3431.
[129] A. Spudich, T. Meyer, L. Stryer, Association of the beta isoform of protein kinase C with vimentin filaments, Cell Motil. Cytoskeleton 22 (1992) 250–256.
[130] K.B. Pryzwansky, T.A. Wyatt, T.M. Lincoln, Cyclic guanosine monophosphate-dependent protein kinase is targeted to intermediate filaments and phosphorylates vimentin in A23187-stimulated human neutrophils, Blood 85 (1995) 222–230.
[131] J. Ciesielski-Treska, G. Ulrich, S. Chasserot-Golaz, D. Aunis, Immunocytochemical localization of protein kinases Yes and Src in amoeboid microglia in culture: association of Yes kinase with vimentin intermediate filaments, Eur. J. Cell Biol. 68 (1995) 369–376.
[132] A. de S. Otero, Copurification of Vimentin, energy metabolism enzymes, and a MER5 homolog with nucleoside diphosphate kinase, J. Biol. Chem. 272 (1997) 14690–14694.
[133] F.J. Doherty, J.A. Wassell, R.J. Mayer, A putative protein-sequestration site involving intermediate filaments for protein degradation by autophagy. Studies with microinjected purified glycolytic enzymes in 3T3-L1 cells, Biochem. J. 241 (1987) 793–800.
[134] B.S. Eckert, S.J. Koons, A.W. Schantz, C.R. Zobel, Association of creatine phosphokinase with the cytoskeleton of cultured mammalian cells, J. Cell Biol. 86 (1980) 1–5.
[135] V.P. Pinon, G. Millot, A. Munier, J. Vassy, G. Linares-Cruz, J. Capeau, F. Calvo, M.L. Lacombe, Cytoskeletal association of the A and B nucleoside diphosphate kinases of interphasic but not mitotic human carcinoma cell lines: specific nuclear localization of the B subunit, Exp. Cell Res. 246 (1999) 355–367.
[136] T.M. Svitkina, A.B. Verkhovsky, G.B. Borisy, Plectin sidearms mediate interaction of intermediate filaments with microtubules and other components of the cytoskeleton, J. Cell Biol. 135 (1996) 991–1007.
[137] R. Foisner, W. Bohn, K. Mannweiler, G. Wiche, Distribution and ultrastructure of plectin arrays in subclones of rat glioma C6 cells differing in intermediate filament protein (vimentin) expression, J. Struct. Biol. 115 (1995) 304–317.
[138] H.Y. Yang, N. Lieska, A.E. Goldman, R.D. Goldman, A 300,000-mol-wt intermediate filament-associated protein in baby hamster kidney (BHK-21) cells, J. Cell Biol. 100 (1985) 620–631.
[139] K.D. Brown, L.I. Binder, Identification of the intermediate filament-associated protein gyronemin as filamin. Implications for a novel mechanism of cytoskeletal interaction, J. Cell Sci. 102 (1992) 19–30.
[140] C.M. Anderson, F.E. Parkinson, Potential signalling roles for UTP and UDP: sources, regulation and release of uracil nucleotides, Trends Pharmacol. Sci. 18 (1997) 387–392.

[141] T.V. Dunwiddie, S.A. Masino, The role and regulation of adenosine in the central nervous system, Annu. Rev. Neurosci. 24 (2001) 31–55.
[142] H.T. Sommerschild, K.A. Kirkeboen, Adenosine and cardioprotection during ischaemia and reperfusion: an overview, Acta Anaesthesiol. Scand. 44 (2000) 1038–1055.
[143] K.N. Klotz, Adenosine receptors and their ligands, Naunyn-Schmiedeberg's Arch. Pharmacol. 362 (2000) 382–391.
[144] T. Kimura, I.K. Ho, I. Yamamoto, Uridine receptor: discovery and its involvement in sleep mechanism, Sleep, (2001) 251–260.
[145] I. Nishino, A. Spinazzola, M. Hirano, Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder, Science 283 (1999) 689–692.