Biochemical Pharmacology, Vol. 58, pp. 539–555, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00035-0

COMMENTARY

Role of Antimetabolites of Purine and Pyrimidine Nucleotide Metabolism in Tumor Cell Differentiation

Sigrid Hatse, Erik De Clercq and Jan Balzarini†*

Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium

ABSTRACT. Transformed cells are characterized by imbalances in metabolic routes. In particular, different key enzymes of nucleotide metabolism and DNA biosynthesis, such as CTP synthetase, thymidylate synthase, dihydrofolate reductase, IMP dehydrogenase, ribonucleotide reductase, DNA polymerase, and DNA methyltransferase, are markedly up-regulated in certain tumor cells. Together with the concomitant down-modulation of the purine and pyrimidine degradation enzymes, the increased anabolic propensity supports the excessive proliferation of transformed cells. However, many types of cancer cells have maintained the ability to differentiate terminally into mature, non-proliferating cells not only in response to physiological receptor ligands, such as retinoic acid, vitamin D metabolites, and cytokines, but also following exposure to a wide variety of non-physiological agents such as antimetabolites. Interestingly, induction of tumor cell differentiation is often associated with reversal of the transformation-related enzyme deregulations. An important class of differentiating compounds comprises the antimetabolites of purine and pyrimidine nucleotide metabolism and nucleic acid synthesis, the majority being structural analogs of natural nucleosides. The CTP synthetase inhibitors cyclopentenylcytosine and 3-deazauridine, the thymidylate synthase inhibitor 5-fluoro-2′-deoxyuridine, the dihydrofolate reductase inhibitor methotrexate, the IMP dehydrogenase inhibitors tiazofurin, ribavirin, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) and mycophenolic acid, the ribonucleotide reductase inhibitors hydroxyurea and deferoxamine, and the DNA polymerase inhibitors ara-C, 9-(2-phosphonylmethoxyethyl)adenine (PMEA), and aphidicolin, as well as several nucleoside analogs perturbing the DNA methylation pattern, have been found to induce tumor cell differentiation through impairment of DNA synthesis and/or function. Thus, by selectively targeting those anabolic enzymes that contribute to the neoplastic behavior of cancer cells, the normal cellular differentiation program may be reactivated and the malignant phenotype suppressed. BIOCHEM PHARMACOL **58**;4:539–555, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. purine and pyrimidine nucleotide metabolism; transformation-related metabolic imbalance; tumor cell differentiation; antimetabolites; nucleoside analogs; nucleic acid synthesis

The origin and progression of malignancy is the result of an accumulation of mutations, allowing cells to uncouple the balance between cellular proliferation and differentiation. In certain cases, the defective growth regulation of cancer cells may be bypassed by the action of substances inducing a reactivation of the normal differentiation program. The purpose of this overview article is to delineate the three-way relationship existing between purine/pyrimidine nucleotide metabolism, malignant transformation, and tumor cell differentiation. The pivotal role of distinct key enzymes of nucleotide metabolism, which may serve as targets for differentiation therapy of neoplastic diseases, will be highlighted.

KEY ENZYMES OF PURINE AND PYRIMIDINE BIOSYNTHESIS

Pyrimidine Nucleotide Metabolism (Fig. 1)

The pyrimidine nucleotide *de novo* synthetic pathway assembles UMP from glutamine, aspartic acid and PRPP‡. CTP is synthesized from UTP by the rate-limiting enzyme CTP-S. The formation of the deoxyribonucleotides dUDP and dCDP from the corresponding ribonucleotides UDP

* Research Assistant of the "Fonds voor Wetenschappelijk Onderzoek - Vlaanderen (F.W.O.)".

† Corresponding author: Dr. Jan Balzarini, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium. Tel. 32-16-337352; FAX 32-16-337340; E-mail: jan.balzarini@rega.kuleuven.ac.be

‡*Abbreviations*: APRT, adenine phosphoribosyltransferase; ara-C, 1-β-D-arabinofuranosylcytosine; c^3Urd, 3-deazauridine; CPEC, cyclopentenylcytosine; CTP-S, CTP synthetase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dNTP, 2′-deoxynucleoside 5′-triphosphate; EICAR, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide; FdUrd, 5-fluoro-2′-deoxyuridine; 5-FU, 5-fluorouracil; HGPRT, hypoxanthine guanine phosphoribosyltransferase; HMBA, hexamethylene-bisacetamide; HU, hydroxyurea; IMPDH, IMP dehydrogenase; MPA, mycophenolic acid; MTX, methotrexate; PMEA, 9-(2-phosphonylmethoxyethyl)adenine; PRPP, 5-phosphoribosyl-1-pyrophosphate; RR, ribonucleotide reductase; SCID, severe combined immunodeficiency; SHMT, serine-hydroxymethyl transferase; THF, tetrahydrofolate; TR, tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide); and TS, thymidylate synthase.

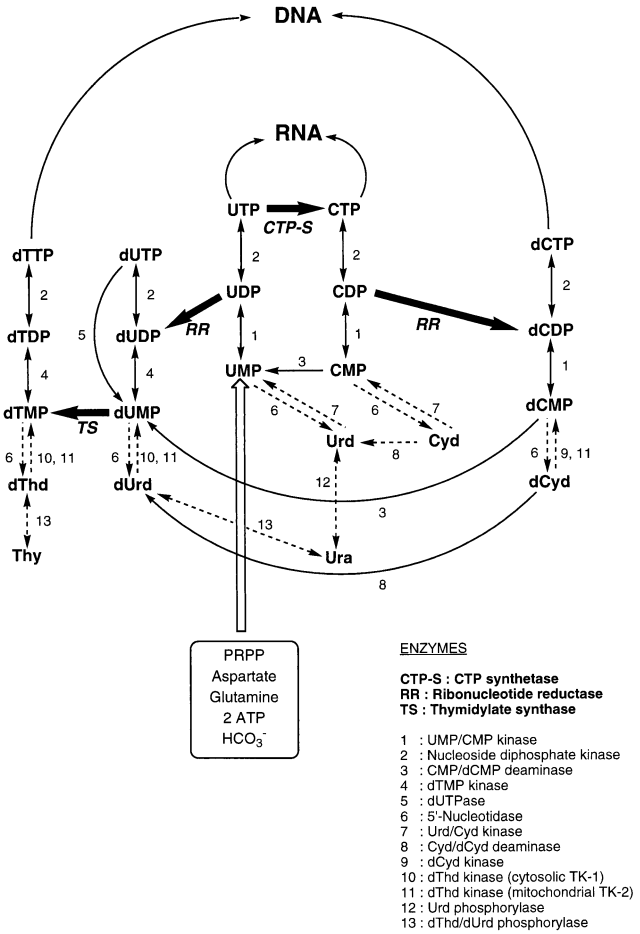

FIG. 1. ***De novo*** **and** ***salvage*** **biosynthesis of pyrimidine ribo- and 2′-deoxyribonucleotides.**

and CDP is controlled by RR. TS, another key enzyme of the pyrimidine nucleotide pathway, catalyzes the conversion of dUMP into dTMP. The deoxynucleoside triphosphates dTTP and dCTP eventually provide the pyrimidine nucleotide substrates for DNA polymerization. RR, which shows an enzymatic activity that is orders of magnitude lower than that of any other enzyme involved in purine and pyrimidine metabolism, is the rate-limiting enzyme of the entire process of DNA synthesis [1]. The salvage enzymes uridine/cytidine kinase, deoxycytidine kinase, and thymidine kinase provide significant alternative routes for the supply of pyrimidine nucleoside monophosphates.

Purine Nucleotide Metabolism (Fig. 2)

PRPP, the precursor for purine nucleotide synthesis, is produced from ribose-5-phosphate and ATP by PRPP synthetase. The central purine nucleotide IMP is formed from PRPP via a series of ten reactions. IMP can be further converted to ITP, but a highly active ITPase hydrolyzes ITP back to IMP. The adenine nucleotides AMP, ADP, and ATP are synthesized from the branch-point nucleotide IMP via the intermediate succinyl-AMP. The other branch of the purine nucleotide pathway leads to guanylate formation via XMP, which is synthesized from IMP through the action of IMPDH. XMP is further converted to GMP, and subsequently to GDP and GTP. Among the enzymes involved in purine nucleotide biosynthesis, IMPDH has the lowest enzymatic activity [1]. RR converts ADP and GDP to dADP and dGDP, which provide the purine substrates for DNA synthesis after further conversion to dATP and dGTP. The purine bases adenine, hypoxanthine, and guanine are salvaged to their respective nucleotides AMP, IMP, and GMP by the highly active, PRPP-consuming salvage enzymes APRT (in the case of adenine) and HGPRT (in the case of hypoxanthine and guanine).

GENETIC ALTERATIONS THAT CONFER SELECTIVE GROWTH ADVANTAGES ON CELLS DURING TRANSFORMATION

Role of Oncogenes in Neoplastic Transformation

Particularly relevant with regard to the development of neoplasia are genetic events that turn proto-oncogenes, encoding proteins that act as agonists of cell growth, into oncogenes, or hyperactive alleles of the normal cell growth-promoting genes. This may happen through gene rearrange-

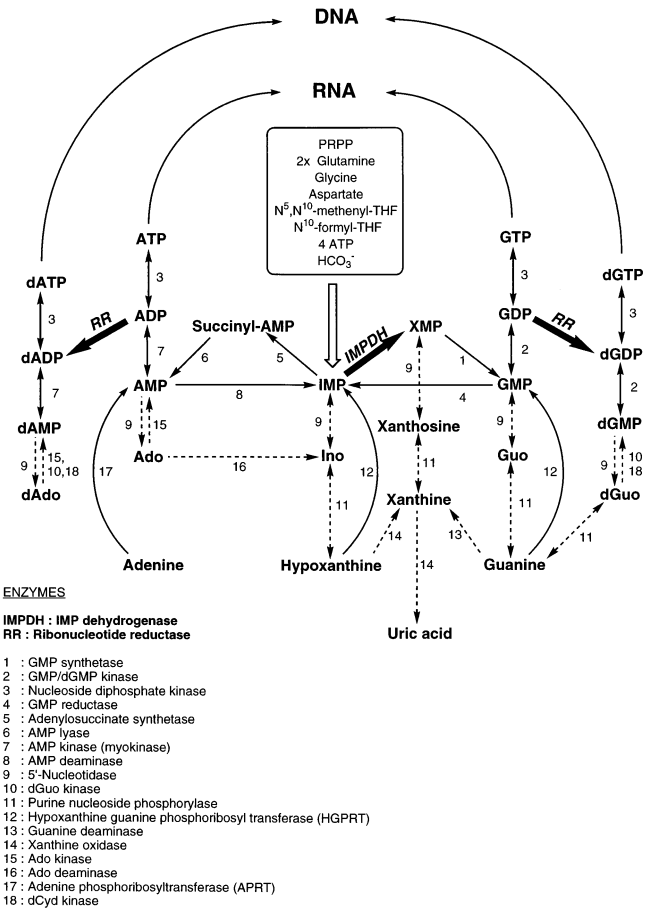

FIG. 2. ***De novo*** **and** ***salvage*** **biosynthesis of purine ribo- and 2′-deoxyribonucleotides.**

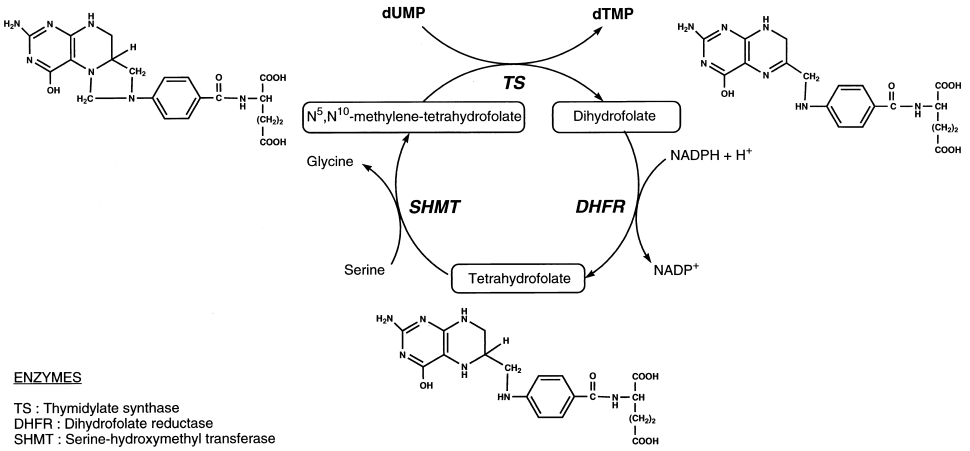

FIG. 3. Thymidylate synthase complex.

ments or amplification, or through mutations leading to uncontrolled or constitutive gene expression. For example, the c-*myc* proto-oncogene was found to be amplified or overexpressed in many tumors [2]. The Myc protein is a transcription factor that modulates a set of growth-promoting genes, including several cyclin genes [3].

Alternatively, point mutations may occur that create a modified protein with altered functional activity, for instance oncogenic forms of Ras protein. However, the genetic background of a normal cell does not allow progression toward malignancy by one single oncogenic mutation. Additional events, such as the inactivation of tumor suppressor genes controlling critical cell cycle checkpoints and DNA repair mechanisms, and alterations in the biochemical program are required before the malignant phenotype emerges.

Transformation-Associated Reprogramming of Purine and Pyrimidine Nucleotide Metabolism

The maximal proliferative capacity of a cell is limited by the activities and abundances of the different rate-limiting enzymes of the essential purine and pyrimidine nucleotide and carbohydrate anabolic pathways. The biochemical schedule of transformed cells has been reprogrammed to support continued proliferation through a metabolic and enzymatic imbalance, which becomes more pronounced with progression to a more malignant phenotype [1]. Such imbalance implies a shift in the anabolic direction, as manifested by increased activities of rate-limiting enzymes of the synthetic (anabolic) pathways, concomitant with decreased activities of the opposing enzymes of the degradation (catabolic) pathways. Consistent with this concept, an altered nucleotide metabolism has been observed in many cancers. Identification of the biochemical differences between a certain tumor cell line and the normal cells from which the neoplasm originates may offer possibilities for the design of a selective, enzyme-targeted chemotherapy of this particular type of malignancy.

CTP FORMATION. Compared with the other nucleotide pools, the intracellular level of cytosine nucleotides is relatively small in normal cells. It has been reported that the amount of cytosine nucleotides is elevated in several types of tumor cells, compared with their non-proliferating counterparts [4, 5]. Moreover, a rough correlation could be established in different normal and neoplastic tissues between the activity of CTP-S, the rate-limiting enzyme of CTP biosynthesis, and the cell proliferation rate [6]. Also, Kizaki *et al.* [7] have found that the specific activity of CTP-S is closely linked to the transformational state of the cell and is increased up to 11-fold in rapidly growing hepatomas, compared with normal liver tissue.

THYMIDYLATE FORMATION. *De novo* dTMP formation from dUMP is controlled by the TS complex, consisting of the enzymes TS, DHFR, which catalyzes the reduction of DHF to THF, and SHMT, which mediates the methylene transfer from serine to THF, leading to N^5,N^{10}-methylene-THF (Fig. 3) [8].

Certain types of tumor cells exhibit increased activity of DHFR [9–11] and TS [11–13]. In addition, Hengstschläger *et al.* [14] found that high expression levels of DHFR and the salvage enzyme thymidine kinase are sustained throughout all cell cycle phases in transformed cells, whereas both DHFR and thymidine kinase are highly S phase-specific in normal cells.

GUANYLATE FORMATION. The tumor-specific elevation of IMPDH activity is an intensively studied and well-documented manifestation of the metabolic imbalance characteristic of transformed cells [1, 15–18]. Interestingly, it has been found that there exist at least two different isozymes of IMPDH, of which only the type II isoform has been linked to the transformed state of the cells [19]. Hence, the use of drugs that preferentially inhibit the type II IMPDH isozyme may represent an attractive strategy for selective anticancer chemotherapy. Since GTP, besides its role as a substrate for

RNA polymerization, also participates in the regulation of diverse other cellular functions, such as protein synthesis, GDP-mannose-requiring lipid synthesis, 5′-"cap" formation in mRNA, signal transduction via GTP-binding proteins, activation of CTP synthetase [7], and energy donor in the conversion of IMP to succinyl-AMP [20], a relatively small variation in the intracellular GTP pool level may have a large impact on the global regulation of cell growth and division.

DEOXYRIBONUCLEOTIDE METABOLISM. RR and DNA polymerase, the two rate-limiting enzymes in DNA biosynthesis, also were found to be implicated in the neoplastic program of cancer cells [21–24]. RR is composed of two nonidentical subunits. The R1 subunit, which binds the nucleotide diphosphate substrates and allosteric regulators, is expressed constitutively in cycling cells. In contrast, the expression of the R2 subunit, which contains the iron-stabilized free radical essential for catalytic activity, is strictly S-phase-correlated [25].

Elford *et al.* [26] have reported a 200-fold difference in the enzymatic activity of RR between slow- and very fast-growing rat hepatomas. This represents the most striking elevation of any enzymatic activity in these tumors and presumably results from an increased rate of enzyme synthesis. Also, a mutation of the R1 subunit, conferring resistance to the allosteric inhibitor dATP and resulting in significantly increased dATP and dGTP pools, has been found in murine lymphosarcoma cells [27]. Moreover, the R2 subunit appeared to be overexpressed in premalignant breast lesions [28]. Fan *et al.* [29] recently found that altered expression of the R2 component of RR, in cooperation with the *ras* oncogene, may be critically involved in malignant progression.

EFFECTS OF (DEOXY)RIBONUCLEOTIDE POOL IMBALANCES ON TRANSFORMATION AND MUTATION RATE. It is obvious that the diverse alterations of purine and pyrimidine nucleotide metabolism in cancer cells are reflected in deviations of the intracellular nucleotide pools. Examples of transformation-specific pool size deviations include the imbalance between the pyrimidine nucleotide pools in rat rhabdomyosarcoma R1 cells [30], and the decreased ratios of purine:pyrimidine and uracil:cytosine nucleotides in myeloid and lymphoblastic leukemia cells [31, 32]. Similarly, a decreased uracil:cytosine ribonucleotide ratio was demonstrated by Slingerland *et al.* [33] in rat pheochromocytoma PC-12 cells in comparison with adrenal medulla tissue.

Perturbation of (deoxy)ribonucleotide pools may have an important impact on the progression of malignancy. It is generally accepted that the unbalanced supply of dNTP constituents (particularly dCTP/dTTP) may alter mutation rates during DNA replication. A deoxyribonucleotide present in excess increases the probability of misincorporation and may inhibit excision repair mechanisms [27]. In this respect, neoplastic transformation may be considered as a self-stimulating process: metabolic adaptations to unlimited proliferation can cause dNTP pool fluctuations, which, in turn, may increase the incidence of additional mutations.

ALTERED DNA METHYLATION PATTERNS IN TRANSFORMED CELLS. Cytosine residues in specific CpG sequences of newly synthesized DNA are enzymatically methylated in the 5-position, according to the methylation pattern present in the parental strand [34]. These specific DNA methylation patterns have been suggested to play a role in the ordered switching-on of genes during embryonic development, giving rise to different cell types containing identical genetic information [35]. The DNA methyltransferase gene was shown to be up-regulated by cellular oncogenic pathways [34], and DNA methylation changes are among the most common molecular abnormalities in human malignancies [36]. In addition to regional hypermethylation, which may lead to the silencing of tumor suppressor and growth-regulatory genes, cancer cells also exhibit a widespread hypomethylation [34].

Interrelations between Nucleotide Metabolism and Oncogene Function

The Ras protein is a GTP-binding protein that participates in the transmission of growth-stimulatory signals from membrane receptors to the nucleus [37]. Exchange of bound GDP for GTP switches the Ras protein from an inactive to an active form. Thus, in the case of Ras protein function, there exists a direct link between oncoprotein activity and nucleotide metabolism.

Chu and coworkers [38] have characterized a ribonucleoprotein complex in cultured human colon cancer cells, consisting of TS protein complexed by a 275-nucleotide sequence located in the C-terminal coding region of c-myc mRNA. It is noteworthy that, unlike most enzymes involved in nucleotide metabolism, TS is not subject to allosteric or feedback regulation by nucleotides. However, TS protein controls the translation of its own mRNA in an autoregulatory manner. Hence, the sequestration of TS protein by c-myc mRNA may disrupt the translational autoinhibition of the enzyme, leading to the synthesis of new TS protein. Thus, the relative stoichiometry of c-myc mRNA and TS mRNA may represent an important determinant of *de novo* thymidylate synthesis. In addition, photoaffinity labelling studies have revealed that the Myc protein specifically binds dTTP and, to a lesser extent, other nucleoside di- and triphosphates [39]. Thus, it appears that nucleotides not only act as precursors of DNA and RNA synthesis, but may also participate in the coordinate regulation of several critical aspects of cellular functioning.

INDUCTION OF TUMOR CELL DIFFERENTIATION: AN ATTRACTIVE STRATEGY FOR CANCER THERAPY

Neoplastic transformation favors cellular proliferation by causing a block in the maturation program [40]. However,

TABLE 1. Overview of tumor cell differentiation models in cell culture

Cell line	Origin	Differentiation lineage	Differentiation markers	Ref.
LEUKEMIA				
HL-60	Human acute promyelocytic leukemia	Granulocytic	Morphology (nuclear constriction) Nitroblue tetrazolium reduction Chloroacetate esterase CD67 expression	53, 54
		Monocytic	Morphology Nitroblue tetrazolium reduction Non-specific esterase CD14, CD11b expression	
K562	Human chronic myelogenous leukemia	Erythroid	Hemoglobin synthesis Glycophorin A expression Acetylcholine esterase	55
FEL	Murine Friend virus-induced leukemia	Erythroid	Hemoglobin synthesis	56
SOLID TUMORS				
BeWo	Human choriocarcinoma	Trophoblastic	Giant cell formation Alkaline phosphatase Placental hormones	57
RCHO	Rat choriocarcinoma	Trophoblastic	Giant cell formation Alkaline phosphatase Placental hormones Loss of cytotrophoblast-specific antigen	58
C6	Rat astrocytoma	Glial	Morphological changes Glial fibrillary acidic protein	45
SK-N-SH	Human neuroblastoma	Neuronal	Neurite extension Neurotransmitter biosynthesis	59
SK-MEL-131	Human melanoma	Melanocytic	Cell morphology (melanosomes) Tyrosinase activity Melanin production Melanocyte-specific antigens	60
F9	Mouse teratocarcinoma	Endodermal	Serine protease plasminogen activator Loss of F9 surface antigen	61
MCF-7	Human breast carcinoma	Milk-secretory breast cells	Morphology Alkaline phosphatase Lipid accumulation and casein	62
NON-MALIGNANT				
Human keratinocytes	Epidermis	Epidermal	Stratification Cornified envelope Keratins, involucrin Increased integrin expression	63

various agents, physiological as well as non-physiological, have been found to be able to force cancer cells to rescue their differentiation program. The physiological differentiation inducers represent agents that are involved in the control of normal differentiation and development, such as retinoids [41, 42], vitamin D metabolites [42, 43], the different cytokines that regulate hemopoietic stem cell differentiation along particular lineages [44], and phenylacetate, a natural metabolite of phenylalanine [45]. The majority of these natural substances trigger differentiation after binding to specific membrane or nuclear receptors. The non-physiological differentiating agents include 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) [46, 47], sodium butyrate [48], tyrosine kinase inhibitors such as the isoflavone genistein [49], anthracyclines [50], the polar-apolar compounds DMSO and HMBA [46, 51], and those compounds that interfere with nucleotide metabolism (see below).

Models for Studying Differentiation Induction

The therapeutic potential of differentiation inducers has been most widely studied in leukemias [52] and neural crest-derived tumors such as neuroblastoma, pheochromocytoma, and melanoma. To evaluate the differentiation-inducing potential of (newly discovered) drugs, preclinical models are required in which specific phenotypic characteristics of the mature cell type can be measured easily. Morphological changes, appearance or disappearance of cell surface antigens, enzymes, and secretory products are typical differentiation markers. In Table 1, a number of commonly used *in vitro* tumor cell differentiation models are summarized. The non-malignant keratinocyte model is useful to gain insight into the precise regulation of continuous proliferation and differentiation in the normal epidermis. This is of high importance in view of the possible application of differentiation inducers for the treatment of

malignant as well as benign proliferative skin disorders (e.g. squamous cell carcinoma and psoriasis).

The absence of a biological microenvironment makes the interpretation of results obtained from *in vitro* studies with differentiating agents speculative and incomplete. Unfortunately, reliable *in vivo* differentiation models are rather scarce, and they all encounter the difficulty of how to provide unequivocal evidence that the antitumor activity of the tested drug is based on the induction of tumor cell differentiation and not any other mechanism(s). Indeed, *in vivo* antitumor activity may be achieved through direct cytotoxic and/or differentiation-inducing effects of the agent at the cellular level, through indirect drug action via immunomodulatory effects or via stimulation of the production of normal proliferation/differentiation mediators, or through a combination of both direct and indirect mechanisms [64].

In vivo differentiation of human leukemia cell lines (e.g. HL-60, K562, U937, and ML1) can be studied by subcutaneous (s.c.) or intraperitoneal (i.p.) injection of tumor cells into immunocompromised mice (SCID mice or athymic nude mice) [65, 66]. However, since several observations suggest that functional T-cells and macrophages may be involved in the modulation of leukemic cell differentiation, these models do not offer an ideal context to study the *in vivo* antileukemic effect of differentiation-inducing agents [64]. *In vivo* retinoic acid-induced differentiation of malignant embryonal carcinoma, a solid tumor that is well-known for its ability to differentiate into benign tissue, has been demonstrated in mice [67]. Also, Anderson and Crowle [68] have reported regression and differentiation of neuroblastoma tumors in mice upon treatment with differentiation-inducing drugs. Tumor growth in nude mice inoculated with human colon carcinoma HCT-15 cells was shown to be inhibited by treatment with *N,N*-dimethylformamide, which induces HCT-15 cell differentiation *in vitro* [69]. Furthermore, phenylacetate was shown to prolong survival of cerebral gliosarcoma-bearing rats through induction of tumor cell differentiation [70]. Another interesting *in vivo* model was established in our laboratory by inoculation of rat choriocarcinoma RCHO cells [71] under the kidney capsule of syngeneic WKA/H rats. This model was used successfully to demonstrate the *in vivo* antitumor efficacy of acyclic nucleoside phosphonate derivatives [72]. Due to the lack of immunogenicity of trophoblastic tissue, this *in vivo* rat choriocarcinoma model offers the advantage that the tumor can be easily studied in its natural background.

Antimetabolites of Purine and Pyrimidine Nucleotide Metabolism as Differentiation-Inducing Agents

Among the purine and pyrimidine nucleoside class of differentiation inducers, three subgroups can be distinguished, i.e. agents that interfere with the *de novo* synthesis of DNA and RNA precursors (antifolates and inhibitors of the rate-limiting enzymes CTP-S, TS, RR, and IMPDH), inhibitors of DNA synthesis (e.g. aphidicolin, ara-C, the acyclic nucleoside phosphonate PMEA), and compounds that perturb DNA methylation patterns (DNA methyltransferase inhibitors and *S*-adenosylhomocysteine hydrolase inhibitors).

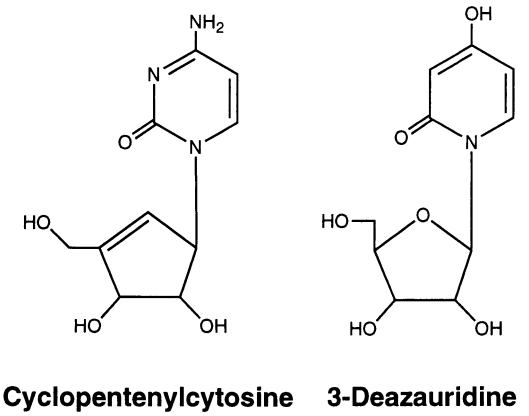

FIG. 4. Chemical structures of the CTP synthetase inhibitors cyclopentenylcytosine and 3-deazauridine.

CTP SYNTHETASE INHIBITORS (FIG. 4). CPEC, a carbocyclic analogue of cytidine, and c^3Urd are potent and specific inhibitors of CTP-S [73, 74]. CPEC and c^3Urd are phosphorylated intracellularly to their respective triphosphate forms, which competitively inhibit CTP-S. The triphosphate of CPEC is approximately 3-fold more inhibitory against CTP-S than is that of c^3Urd [74]. c^3Urd and CPEC have been reported to induce differentiation of HL-60 cells and to down-regulate the c-*myc* oncogene [75, 76]. Moreover, Moyer *et al.* [77] have demonstrated antitumor activity of CPEC in leukemia- and melanoma-bearing mice. As single agents, c^3Urd and CPEC have not gained much clinical attention. However, the drugs have generated substantial interest as biological response modifiers because of their pronounced synergism with ara-C and 5-aza-2′-deoxycytidine through CTP and dCTP depletion, activation of deoxycytidine kinase, and inhibition of (deoxy)cytidine deaminase [78, 79].

TS INHIBITORS (FIG. 5). Inhibition of *de novo* dTMP formation by the metabolite FdUMP represents one of the potential actions of the fluoropyrimidine derivative 5-FU. 5-FU can be converted to FdUrd by the action of dThd/dUrd phosphorylase, and the subsequent formation of FdUMP from FdUrd is catalyzed by dThd kinase. Alternatively, 5-FU may be converted to FUMP, either directly through the action of uracil or orotate phosphoribosyl transferase(s) or by a two-step reaction via FUrd. FUDP is recognized as a substrate by RR, resulting in FdUDP and, eventually, FdUMP formation. FdUMP strongly inhibits TS through formation of a ternary complex (TS/FdUMP/N^5,N^{10}-methylene-THF) analogous to the ternary intermediate that is normally formed with dUMP [80]. Despite the high activity of the enzymes dUTPase, which converts

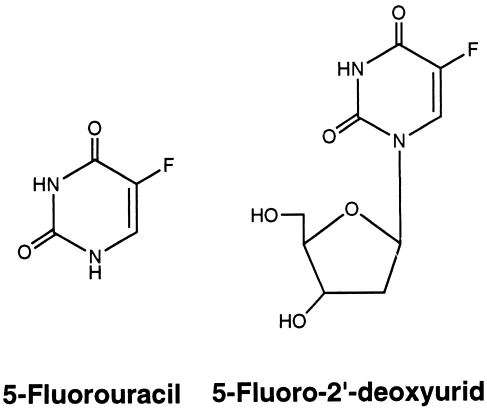

FIG. 5. Chemical structures of the thymidylate synthase inhibitors 5-fluorouracil and 5-fluoro-2′-deoxyuridine.

dUTP to dUMP (Fig. 1), and uracil-DNA glycosidase, which removes uracil residues from DNA, the excessive accumulation of dUMP, resulting from TS inhibition, may lead to misincorporation of dUTP into DNA [81]. In addition, FdUTP, formed by further phosphorylation of FdUMP, may be incorporated into DNA as well, causing genetic miscoding and DNA damage [80, 82]. Incorporation of FUTP into RNA represents another important mechanism of antitumor action of 5-FU, which causes inhibition of rRNA maturation [83]. Although 5-FU represents a major constituent of chemotherapy of cancers of the gastrointestinal tract, breast, and head and neck, its impact on tumor cell differentiation is poorly documented. However, a pronounced differentiation-inducing effect of 5-FU was observed in human erythroleukemia K562 cells [84] and in human keratinocytes [85]. Also, Kafka *et al.* [86] have found a synergistic differentiation-inducing effect of 5-FU and interferon-γ in HL-60 cells at concentrations ineffective by themselves. Furthermore, exposure to FdUrd, the deoxyribosyl derivative of 5-FU, enhances neurite development in fetal rat brain neurons [87] and triggers differentiation in murine erythroleukemia Friend [88] and teratocarcinoma F9 cells [61].

ANTIFOLATES (FIG. 6). THF is an essential cofactor in enzymatic reactions that require the delivery of C_1 (methyl, formyl, methenyl) entities. Hence, THF plays an important role in the *de novo* biosynthesis of IMP (Fig. 1), as well as in the formation of thymidylate from dUMP by TS (see above and Fig. 3). Inhibition of DHFR causes dTMP depletion through interruption of the TS cycle [8] (Fig. 3) and results in the rapid accumulation of DHF, a strong inhibitor of the *de novo* purine nucleotide biosynthesis enzyme AICAR transformylase [80]. Alternatively, certain folate analogs can directly inhibit folate-dependent enzymes, such as TS, through competition at the cofactor binding site.

MTX, a well-known structural analogue of folic acid, is a potent inhibitor of DHFR and, consequently, of *de novo* thymidylate and purine nucleotide synthesis. MTX was

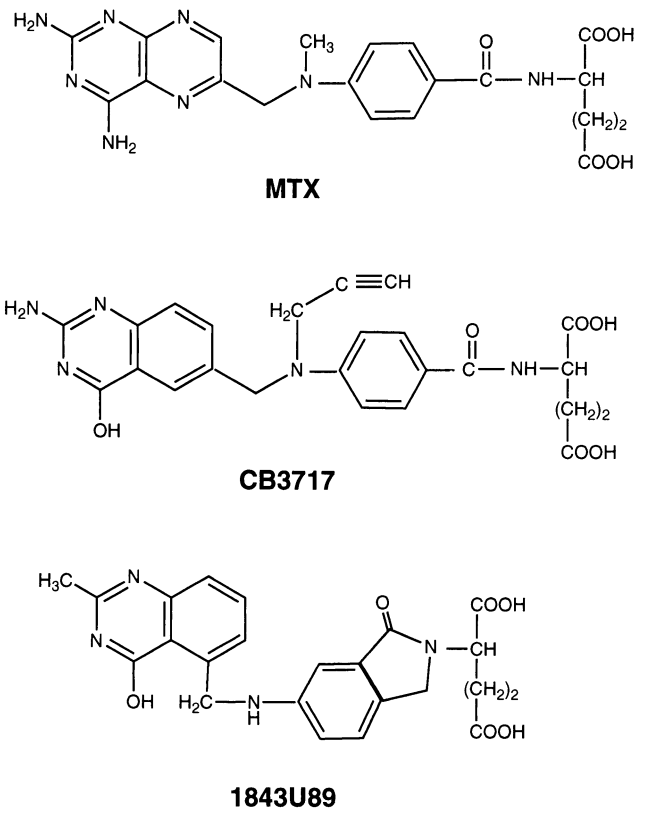

FIG. 6. Chemical structures of the antifolates MTX, CB3717, and 1843U89.

found to be a potent differentiation inducer in human and rat choriocarcinoma cells [57, 58], as well as in human keratinocytes [89], human promyelocytic HL-60 cells [90], and human neuroblastoma LA-N-1 cells [91]. Moreover, the fact that MTX-induced differentiation is prevented by thymidine suggests that the cytodifferentiating effects of MTX mainly result from thymine nucleotide depletion and concomitant inhibition of DNA synthesis [89, 92, 93].

The antifolate 5,10-dideazatetrahydrofolic acid (DDATHF) selectively inhibits glycinamide ribonucleotide transformylase, a folate-dependent enzyme that catalyzes the first of the two one-carbon transfer reactions in the *de novo* purine nucleotide biosynthetic pathway. DDATHF strongly induces differentiation of HL-60 cells, presumably through depletion of intracellular GTP pools [94].

N^{10}-Propargyl-5,8-dideazafolic acid (CB3717) [95] is a folate analogue that directly inhibits TS [8]. Many other antifolates that selectively target TS, such as the benzoquinazoline derivative 1843U89 [96] and AG#85 [97], have been designed and synthesized recently. The latter compound strongly induces keratinocyte differentiation [85].

IMPDH INHIBITORS (FIG. 7). The metabolic effects of IMPDH inhibition are GTP (and dGTP) depletion and inhibition of RNA-primed DNA synthesis [98]. Depletion of guanine nucleotides has been suggested as the primary trigger for differentiation induced by IMPDH inhibitors [99].

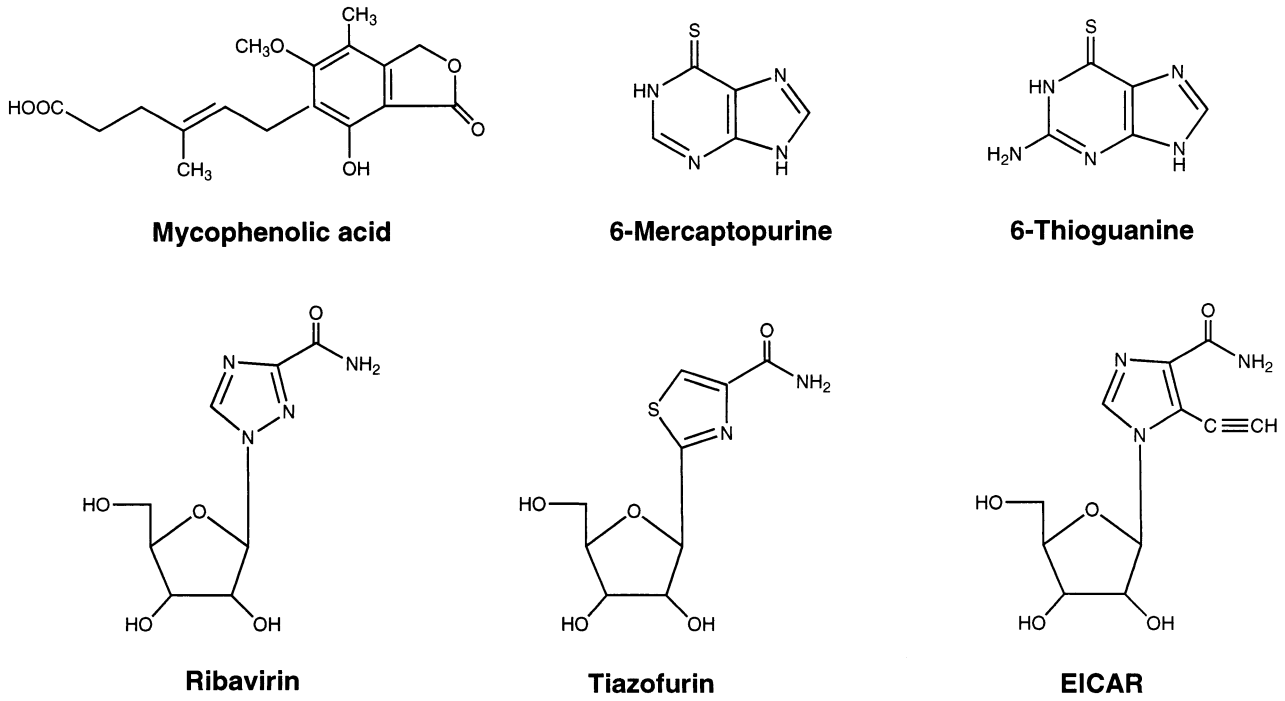

FIG. 7. Chemical structures of the IMPDH inhibitors mycophenolic acid, 6-mercaptopurine, 6-thioguanine, ribavirin, tiazofurin, and EICAR.

MPA, a natural compound produced by the fungus *Penicillium stoloniferum*, inhibits IMPDH activity by simultaneously mimicking the nicotinamide portion of the NAD^+ cofactor and a catalytic water molecule [100]. The monophosphate form of the antiviral agent ribavirin [101, 102] was shown to act as a competitive inhibitor of IMPDH [80]. TR is phosphorylated intracellularly to its monophosphate form, which then is converted to the active metabolite thiazole-4-carboxamide adenine dinucleotide or TAD. This NAD^+ analogue, containing TR instead of the nicotinamide riboside moiety, inhibits IMPDH activity through competition for the NAD^+ cofactor-binding site of the enzyme [103]. A similar dinucleotide metabolite (i.e. EAD) [104] is also formed from the monophosphorylated form of EICAR, a recently described IMPDH inhibitor [20, 105]. The 6-thio analogs of the natural purine bases hypoxanthine and guanine, namely 6-mercaptopurine and 6-thioguanine, are converted by HGPRT into thio-IMP and thio-GMP, respectively, which both inhibit IMPDH [102, 106].

MPA was found to induce maturation of MCF-7 breast cancer cells, which could be reversed by repletion of the intracellular GTP pools [62]. Kiguchi *et al.* [107, 108] have reported the differentiation-inducing properties of MPA and TR in human T-lymphoblastoid leukemia cells and in human melanoma SK-MEL-131 and HO cells. Also, TR displays a marked differentiation-inducing effect in human neuroblastoma cells [109]. Furthermore, MPA, TR, EICAR, and ribavirin strongly induce erythroid differentiation of human K562 leukemia cells [110–112]. TR proved to be able to induce complete hematologic remission, presumably through induction of leukemic cell differentiation, in several leukemic patients with poor prognosis [113]. Also, when administered as its ester prodrug form, mycophenolate mofetil, MPA showed a marked *in vivo* antitumor activity in athymic nude mice bearing diverse human and mouse tumors [114]. Although much less intensively studied, the abilities of 6-mercaptopurine and 6-thioguanine to induce differentiation have been demonstrated in neuroblastoma cells [115] and in murine erythroleukemia cells [116]. The finding that an IMPDH antisense oligomer is able to induce HL-60 and K562 cell maturation [117] further substantiates the important role of this enzyme in the process of differentiation.

Several reports point to decreased GTP binding of signal transduction proteins, such as the Ras oncoprotein, as an important factor in differentiation induction by IMPDH inhibitors [108, 118–121]. However, this hypothesis is contradicted by a recent study of Pilz *et al.* [122], who found no change in the activation state of Ras when HL-60 cells underwent differentiation in response to DMSO or inhibitors of guanylate synthesis.

RIBONUCLEOTIDE REDUCTASE INHIBITORS (FIG. 8). HU, a well-known inhibitor of RR, destabilizes the iron center of the enzyme, thereby destroying the tyrosine free radical essential for enzyme activity [123]. Deferoxamine also strongly inhibits RR through chelation of ferric ion. Both inhibitors block DNA synthesis and induce monocytic/macrophage maturation of HL-60 cells [124]. Increased expression of trophoblastic differentiation markers was observed in rat RCHO and human JEG-3 and BeWo

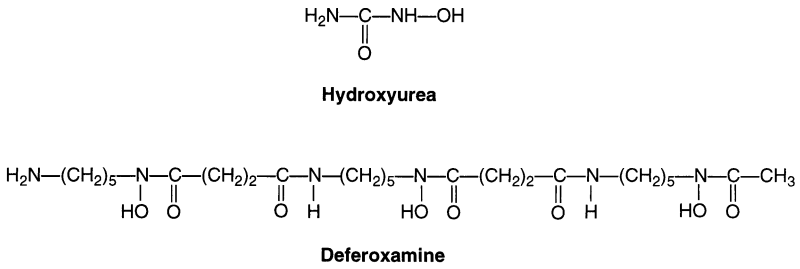

FIG. 8. Chemical structures of the ribonucleotide reductase inhibitors hydroxyurea and deferoxamine.

choriocarcinoma cells following exposure to HU ([125, 126]; Hatse *et al.*, unpublished data). LoPresti *et al.* [127] have reported the enhancement by HU of nerve growth factor-induced neuronal differentiation of neuroblastoma SHSY5Y cells. Also, like several other agents interfering with DNA synthesis, HU was found to be a potent inducer of murine F9 teratocarcinoma cell differentiation [61]. In the erythroleukemia K562 cell line, HU-induced differentiation (as demonstrated by hemoglobin production) was associated with up-regulation of both c-*jun* and c-*fos* transcription factors [128]. Moreover, HU has been found to reduce the severity of the clinical symptoms associated with sickle-cell anemia through *in vivo* induction of fetal hemoglobin synthesis [129].

Studies of Creasey and Wright [130] on HU-resistant rat myoblast cell lines suggested that RR, either directly or indirectly (i.e. by causing fluctuations in the DNA precursor pools), can influence cellular differentiation. In six independently selected HU-resistant mutants, elevated reductase activity was accompanied by a largely decreased capacity for morphological and biochemical differentiation. The dNTP pool imbalance in the HU-resistant cells, resulting from a non-coordinate increase of the four dNTP levels, may possibly modulate components of the differentiation program. Interestingly, a variant of the human K562 erythroleukemia cell line, resistant to the DNA synthesis inhibitor PMEA, exhibited a similar decreased sensitivity to differentiation induction by a variety of structurally and functionally unrelated substances [110]. In contrast, Barbat *et al.* [131] found that the ability of human colon carcinoma cells to develop resistance to the antimetabolites MTX and 5-FU was restricted to cells that were committed to differentiation.

DNA POLYMERASE INHIBITORS (FIG. 9). The triphosphorylated metabolite of the deoxycytidine analogue ara-C, ara-CTP, is a potent inhibitor of mammalian DNA polymerases α and β, through competition with the natural substrate dCTP [132]. Upon incorporation into the nascent DNA chain, ara-CMP residues strongly obstruct further DNA elongation, resulting in the accumulation of short strands of DNA [133, 134]. The differentiation-inducing properties of ara-C have been most profoundly studied in the human erythroleukemia K562 cell line [135]. Furthermore, ara-C induces maturation of human neuroblastoma [136], melanoma [137], breast carcinoma MCF-7 [138], and myeloid leukemia HL-60 [139] cells, as well as in mouse teratocarcinoma F9 cells [140]. The observation that inhibition of DNA synthesis precedes the appearance of differentiation markers in ara-C-exposed human myeloblastic ML-1 cells [141] suggests that ara-C induces differentiation by inhibiting cell proliferation.

Aphidicolin selectively inhibits the activity of DNA polymerase α in competition with pyrimidine deoxyribonucleoside triphosphates [142]. In the presence of aphidicolin, monocyte-specific phenotypic changes occur in HL-60 cells [139]. Also, erythroid differentiation is triggered by aphidicolin in human K562 cells [143], and myogenic differentiation is induced in the human rhabdomyosarcoma cell line KFR [144]. Furthermore, aphidicolin proved able to induce keratinocyte differentiation [85], and

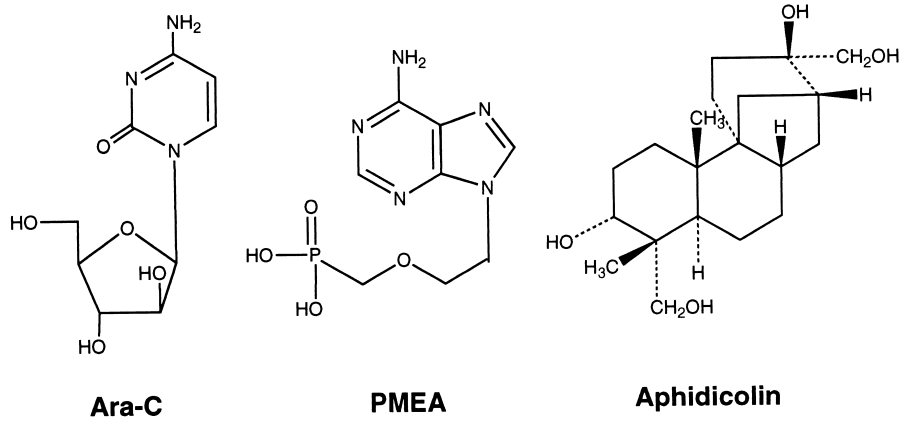

FIG. 9. Chemical structures of the DNA polymerase inhibitors ara-C, PMEA, and aphidicolin.

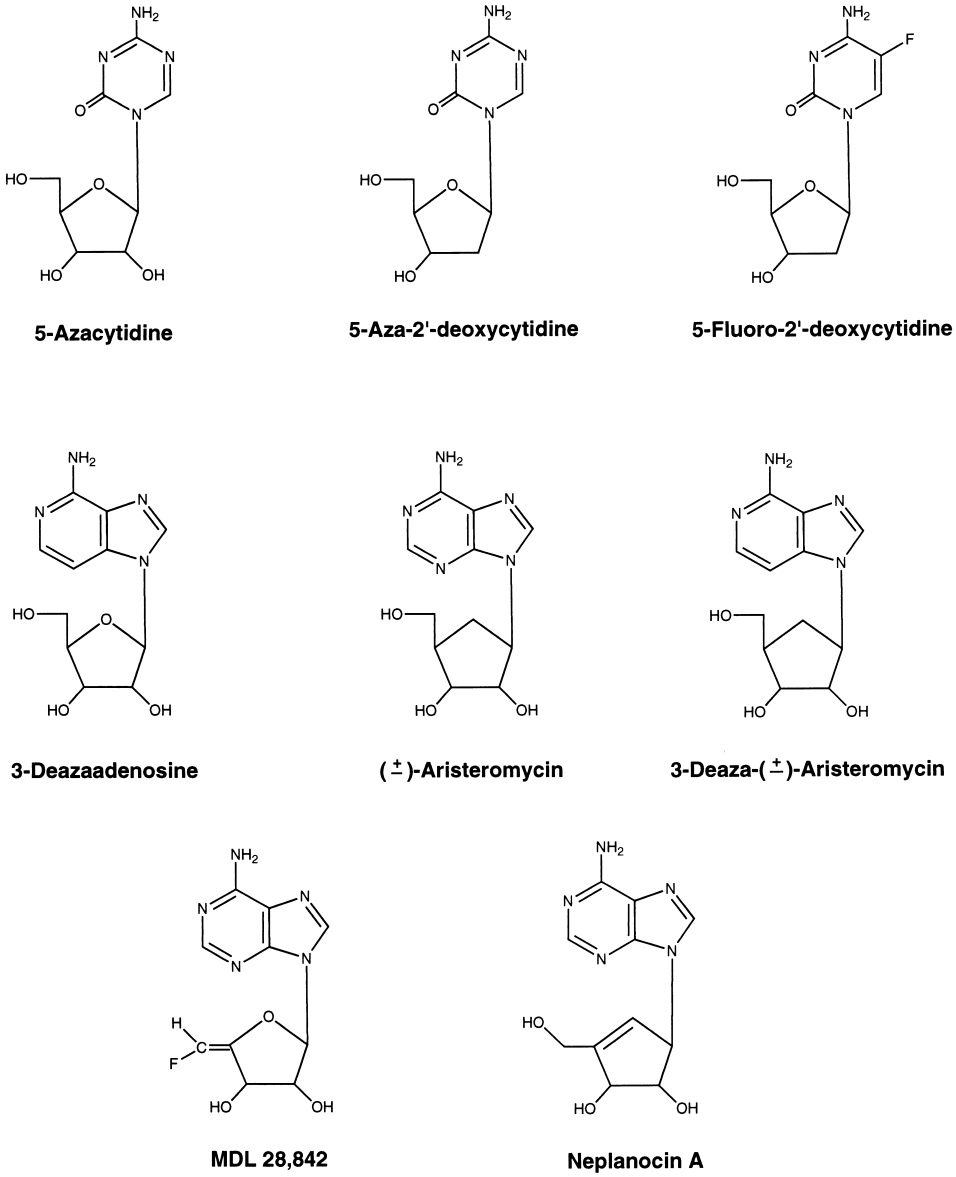

FIG. 10. Chemical structures of the DNA methylation inhibitors 5-azacytidine, 5-aza-2′-deoxycytidine, and 5-fluoro-2′-deoxycytidine and the *S*-adenosylhomocysteine hydrolase inhibitors 3-deazaadenosine, aristeromycin, 3-deazaaristeromycin, MDL 28,842, and neplanocin A.

acts synergistically with nerve growth factor in inducing differentiation of SH-SY5Y neuroblastoma cells [127].

The acyclic nucleoside phosphonate PMEA, a potent broad-spectrum antiviral drug [145, 146], is an analogue of (d)AMP that is converted intracellularly to its mono- and diphosphorylated forms, PMEAp and PMEApp [147]. The active metabolite PMEApp competitively inhibits DNA polymerases [148]. When incorporated into DNA, PMEA causes DNA chain termination due to the lack of the hydroxyl group required for further polymerization. Consequently, S phase progression is strongly impeded in PMEA-exposed cells, resulting in a marked accumulation of the four dNTPs (Hatse *et al.*, unpublished data). This is in agreement with the finding that *de novo* synthesis of deoxyribonucleotides continues when DNA synthesis is blocked [149]. PMEA was found recently to be a potent inducer of differentiation in human erythroleukemia K562 and myeloid HL-60 cells [150] and rat choriocarcinoma RCHO cells [58]. Moreover, PMEA is endowed with potent antitumor activity *in vivo* in choriocarcinoma-bearing rats [72].

INHIBITORS OF DNA METHYLATION (FIG. 10). Local DNA hypermethylation may decrease the accessibility of gene regulatory sequences for transcription and transactivation factors and, hence, may down-modulate gene expression. Temporally regulated DNA demethylation has been suggested to play a critical role in orchestrating gene expression profiles during differentiation [34]. For example, Kao *et al.* [151] recently demonstrated that the strict lineage- and

stage-specific expression of myeloid nuclear differentiation antigen (MNDA) in maturing normal granulocytes and monocytes is controlled by specific DNA demethylation of the promoter region of the *MNDA* gene. Thus, DNA methyltransferase inhibitors possibly could restore the function of growth-regulatory genes that have become silenced in transformed cells and reactivate the original cellular program of tumor suppression and differentiation [152].

Analogs of cytosine, modified in the 5-position, are expected to markedly reduce the proportion of methylated cytosine residues in DNA, due to their intrinsic resistance to methylation after incorporation into DNA. As a result, these compounds may activate differentiation-related gene expression leading to the mature phenotype of the cell. 5-Azacytidine, 5-aza-2′-deoxycytidine (decitabine), and 5-fluoro-2′-deoxycytidine indeed were found to induce normal differentiation of mouse embryo cells into muscle cells, adipocytes, and chondrocytes [35]. Also, 5-azacytidine triggers differentiation in human promyelocytic HL-60 cells [153] and Friend erythroleukemia cells [154], and 5-aza-2′-deoxycytidine induces maturation of human lung cancer A549 cells [155] and melanoma M21 cells [137].

An alternative strategy to perturb DNA methylation is based on *S*-adenosylhomocysteine hydrolase as the target enzyme. *S*-Adenosylhomocysteine is a competitive inhibitor of *S*-adenosylmethionine-dependent methyltransferases, including DNA methyltransferase [156]. Several nucleoside analogs, including neplanocin A [157], 3-deazaadenosine, aristeromycin [158], 3-deaza-(±)-aristeromycin [159], and MDL 28,842 [160], have been reported to inhibit *S*-adenosylhomocysteine hydrolase and to induce differentiation in different cell lines. Although DNA methylation is reduced markedly in HL-60 cells exposed to 3-deaza-(±)-aristeromycin [159] and neplanocin A [157], biological transmethylation pathways other than DNA methylation, such as mRNA 5′-"cap" formation, may be involved as well. For instance, the decline in c-myc mRNA levels, observed in HL-60 cells following exposure to neplanocin A, may result from decreased mRNA stabilization, due to inhibition of RNA methylation by the drug [157].

Differentiation-Related Modulation of Purine and Pyrimidine Nucleotide Metabolism

The activities of several enzymes of purine and pyrimidine nucleotide metabolism change during induced differentiation of tumor cells. For example, the activities of the anabolic enzymes thymidine kinase and TS, which are both overexpressed in human histiocytic U-937 and promyelocytic HL-60 cells as compared with normal leukocytes, markedly decline upon phorbol ester-induced differentiation, whereas the enzymes of the catabolic pathways (e.g. thymidine phosphorylase) are inversely regulated [161–163]. Moreover, Horie *et al.* [164] have identified differentiation-specific nuclear factors involved in the regulation of TS gene expression during HL-60 cell maturation. Also, Schwartz *et al.* [165] have demonstrated that differentiating keratinocytes lose the capacity to salvage extracellular thymidine due to a rapid decline in thymidine kinase activity.

Knight *et al.* [16] reported that the marked decrease in IMPDH activity, occurring within 3–6 hr after HL-60 cells had been exposed to retinoic acid or dimethylformamide, clearly preceded the appearance of differentiation markers. Moreover, HL-60 cell maturation in response to diverse differentiation-inducing agents was shown to be associated with a marked decrease in intracellular guanylate levels [166] and was prevented in the presence of exogenous guanine or guanosine [167]. Together with the fact that IMPDH inhibitors are potent inducers of differentiation, these observations point to a possible role of intracellular guanine nucleotide concentrations in the regulation of cell differentiation. However, in other studies, using T-lymphoid cells, no correlation between differentiation induction and IMPDH expression could be established [107], indicating that the relationship between nucleotide metabolism and cellular proliferation and differentiation may vary among different cell types.

In addition to the above-mentioned alterations in thymidylate and guanylate metabolism, differentiation induction in HL-60 cells also was shown to be accompanied by a marked decrease in the expression of the ribonucleotide reductase R1 subunit [168]. Furthermore, active demethylation of DNA was shown to be a very early event in the process of HMBA-induced differentiation of mouse erythroleukemia cells [169], and a marked decrease in *S*-adenosylhomocysteine hydrolase activity was observed prior to differentiation-related phenotypic changes in HL-60 cells exposed to DMSO [170].

Differentiation-Related Modulation of Oncogene Expression

Numerous reports have been published on the effects of differentiation inducers on the expression and/or function of several oncogenes. In particular, chemically induced differentiation has been associated with a substantial decrease of c-myc mRNA [126, 171, 172]. Also, Shimizu *et al.* [173] have demonstrated extensive losses of amplified c-*myc* genes in spontaneously differentiated HL-60 cells. However, several studies have revealed that c-*myc* down-regulation is not obligatory for differentiation and strongly depends on the nature of the inducing agent ([84, 174–176]; Hatse *et al.*, unpublished results). It has also been shown that down-regulation of c-*myc* gene expression alone is insufficient to trigger tumor cell maturation [125]. The IMPDH inhibitor tiazofurin was shown to down-regulate the expression of both the c-*myc* and c-*Ki-ras* oncogenes *in vitro* as well as *in vivo* [111, 120]. Interestingly, increased expression of GTPase-activating proteins, which inactivate Ras by stimulating the dephosphorylation of Ras-bound GTP to GDP, has been observed upon differentiation induction in neuroblastoma cells [177].

CONCLUDING REMARKS AND PERSPECTIVES

Clinical and experimental evidence suggests an inverse relationship between differentiation and malignancy. On the one hand, antimetabolites of purine and pyrimidine nucleotide metabolism that specifically target those enzymes that have become hyperactive during neoplastic transformation appear to be potent differentiation inducers in diverse model systems. On the other hand, the deregulation of particular oncogenes and metabolic enzymes in cancer cells is often reversed during tumor cell differentiation. Since the transformation-associated metabolic imbalances may differ among diverse tumors, optimal differentiation in various tissues may be achieved by different drugs at different doses. Importantly, the use of drugs that specifically inhibit the hyperactive proliferation-promoting enzymes of cancer cells may be expected to confer a certain degree of selectivity to the anticancer therapy.

Except for the DNA methylation-perturbing agents, inhibition of DNA replication is common to all the antimetabolites discussed. Presumably, inhibition of the proliferation program seems to represent the principal factor in the onset of antimetabolite-induced differentiation. In addition, it is likely that the (deoxy)ribonucleotide pool imbalances, elicited by the antimetabolites, play a crucial role in activating the differentiation program. Nucleotide antimetabolites can thus clearly be distinguished from physiological differentiation inducers such as retinoic acid, vitamin D metabolites, and cytokines, which act through direct stimulation of the differentiation pathways by specific receptor-mediated signals. For these natural differentiation-inducing substances, the eventual loss of the self-renewal capacity of the cells is a consequence rather than a cause of their differentiation-inducing effect.

Since proliferation arrest due to growth factor withdrawal, nutrient starvation, or high cell density does not stimulate spontaneous differentiation, reduction of the growth rate as such is not sufficient to trigger cellular differentiation. The reversible growth arrest (G_0 stage) resulting from unfavorable external conditions clearly differs from the aberrant situation created by nucleic acid-perturbing agents that directly interfere with the DNA replication machinery. Apparently, duplication of the cellular genome in the S phase of the cell cycle is a critical event, during which the cells are highly susceptible to differentiation induction by agents that disturb the tight regulation of DNA precursor synthesis and utilization. Moreover, unlike exponentially growing cells exhibiting extensive DNA biosynthesis, stationary phase murine erythroleukemia cells with diminished DNA replication activity were found to be unable to undergo DMSO-induced differentiation. Together with the fact that retinoic acid-induced HL-60 cell differentiation also was shown to be initiated in the S phase, these findings support the general hypothesis that early events in the onset of cell differentiation may depend on S phase-specific processes. Consistent with this concept is the fact that differentiation can be induced in K562 cells by compounds that affect nucleotide metabolism, but not by vinblastine, an inhibitor of mitosis, or by agents that interfere with RNA or protein synthesis.

Until now, very few differentiation-inducing agents have entered the clinic for the treatment of human cancers. Although childhood neuroblastoma shows the highest rate of spontaneous regression through maturation into benign ganglioneuroma, the clinical success of differentiation-inducing drugs (13-*cis* and all-*trans* retinoic acid and interferon-γ) for the treatment of pediatric patients with neuroblastoma has been disappointing. In contrast, all-*trans* retinoic acid proved remarkably effective for differentiation therapy of acute promyelocytic leukemia, which is characterized by a chromosomal translocation affecting the retinoic acid receptor RARα. Another example of successful differentiation therapy is the treatment of highly aggressive human choriocarcinoma tumors with MTX, a potent differentiation inducer of human BeWo choriocarcinoma cells and rat RCHO choriocarcinoma cells. However, it remains difficult to demonstrate unequivocally that the remarkable therapeutic efficacy of MTX *in vivo* results from tumor cell differentiation. For ara-C and tiazofurin, *in vivo* tumor cell differentiation has clearly been demonstrated in certain cases. Yet the contribution of tumor cell differentiation to the eventual antitumor activity of these drugs is not fully understood.

Experimental data indicate that the combination of antimetabolites of nucleotide metabolism with differentiation inducers such as retinoic acid could greatly enhance the efficacy of differentiation therapy of malignancies. Therefore, the synergistic differentiation-inducing effects afforded by combinations of agents from both classes of differentiation-inducing compounds should be further explored. Also, the contribution of tumor cell differentiation to the eventual therapeutic success of currently applied chemotherapeutic drugs such as ara-C, 5-FU, MTX, and others awaits further investigation.

The authors are indebted to the A.S.L.K. Cancer Fund and the Belgian Geconcertcerde Onderzoeksacties (Grant N° 95/5) for support of their research.

References

1. Weber G, Biochemical strategy of cancer cells and the design of chemotherapy. *Cancer Res* **43:** 3466–3492, 1983.
2. Garte SJ, The *c-myc* oncogene in tumor progression. *Crit Rev Oncog* **4:** 435–449, 1993.
3. Henriksson M and Lüscher B, Proteins of the Myc network: Essential regulators of cell growth and differentiation. *Adv Cancer Res* **68:** 109–182, 1996.
4. Mandel P, Wintzerith M, Klein-Pete N and Mandel L, Comparative investigation of the free nucleotides of an ascitic hepatoma and of normal or regenerating liver. *Nature* **198:** 1000–1001, 1963.
5. Van den Berg AA, van Lenthe H, Busch S, de Korte D, Roos D, van Kuilenburg ABP and van Gennip AH, Evidence for transformation-related increase in CTP synthetase activity

in situ in human lymphoblastic leukemia. *Eur J Biochem* **216:** 161–167, 1993.
6. Genchev DD, Activity of cytidine triphosphate synthetase in normal and neoplastic tissues. *Experientia* **29:** 789–790, 1973.
7. Kizaki H, Williams JC, Morris HP and Weber G, Increased cytidine 5′-triphosphate synthetase activity in rat and human tumors. *Cancer Res* **40:** 3921–3927, 1980.
8. Costi MP, Thymidylate synthase inhibition: A structure-based rationale for drug design. *Med Res Rev* **18:** 21–42, 1998.
9. Nano R, Gerzeli G, Invernizzi R and Supino R, A qualitative and quantitative cytochemical assay of dihydrofolate reductase in erythroid cells. *Acta Histochem* **85:** 51–58, 1989.
10. Taylor C, Jalava A and Mai S, c-Myc dependent initiation of genomic instability during neoplastic transformation. *Curr Top Microbiol Immunol* **224:** 201–207, 1997.
11. Volm M, Sauerbrey A and Zintl F, Dihydrofolate-reductase and thymidylate-synthase in childhood acute lymphoblastic leukemia. *Anticancer Res* **14:** 1377–1382, 1994.
12. Hashimoto Y, Shiotani T, Eble JN, Glover JL and Weber G, Increased thymidylate synthase (EC 2.1.1.45) activity in normal and neoplastic proliferation. *Cancer Biochem Biophys* **10:** 1–10, 1988.
13. Vlaykova T, Jekunen AP, Kesomaa M, Kairemo KJ, Pyrhonen S and Wasenius VM, Increased thymidylate synthase gene expression in metastatic melanoma. *Oncology* **54:** 146–152, 1997.
14. Hengstschläger M, Mudrak I, Wintersberger E and Wawra E, A common regulation of genes encoding enzymes of the deoxynucleotide metabolism is lost after neoplastic transformation. *Cell Growth Differ* **5:** 1389–1394, 1994.
15. Jackson RC, Weber G and Morris HP, IMP dehydrogenase, an enzyme linked with proliferation and malignancy. *Nature* **256:** 331–333, 1975.
16. Knight RD, Mangum J, Lucas DL, Cooney DA, Khan EC and Wright DG, Inosine monophosphate dehydrogenase and myeloid cell maturation. *Blood* **69:** 634–639, 1987.
17. Collart FR, Chubb CB, Mirkin BL and Huberman E, Increased inosine-5′-phosphate dehydrogenase gene expression in solid tumor tissues and tumor cell lines. *Cancer Res* **52:** 5826–5828, 1992.
18. Huberman E, Glesne D and Collart F, Regulation and role of inosine-5′-monophosphate dehydrogenase in cell replication, malignant transformation and differentiation. *Adv Exp Med Biol* **370:** 741–746, 1994.
19. Nagai M, Natsumeda Y, Konno Y, Hoffman R, Irino S and Weber G, Selective up-regulation of type II inosine 5′-monophosphate dehydrogenase messenger RNA expression in human leukemias. *Cancer Res* **51:** 3886–3890, 1991.
20. Balzarini J, Karlsson A, Wang L, Bohman C, Horská K, Votruba I, Fridland A, Van Aerschot A, Herdewijn P and De Clercq E, Eicar (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide): A novel potent inhibitor of inosinate dehydrogenase activity and guanylate biosynthesis. *J Biol Chem* **268:** 24591–24598, 1993.
21. Baril EF, Jenkins MD, Brown OE, Laszlo J and Morris HP, DNA polymerases I and II in regenerating rat liver and Morris hepatomas. *Cancer Res* **33:** 1187–1193, 1973.
22. Ove P, Laszlo J, Jenkins MD and Morris HP, Increased DNA polymerase activity in a series of rat hepatomas. *Cancer Res* **29:** 1557–1561, 1969.
23. Staub M, Szpaszokukockaja T, Bencsáth M, Antoni F and Lapis K, DNA polymerase and thymidine kinase activities in MC-29 virus-induced transplantable hepatoma and the effect of cytostatic treatment on these activities. *Chem Biol Interact* **41:** 181–192, 1982.
24. Takeda E and Weber G, Role of ribonucleotide reductase in expression of the neoplastic program. *Life Sci* **28:** 1007–1014, 1981.
25. Björklund S, Skog S, Tribukait B and Thelander L, S-Phase specific expression of mammalian ribonucleotide reductase R1 and R2 subunit mRNAs. *Biochemistry* **29:** 5452–5458, 1990.
26. Elford HL, Freese M, Passamani E and Morris HP, Ribonucleotide reductase and cell proliferation. I. Variations of ribonucleotide reductase activity with tumor growth rate in a series of rat hepatomas. *J Biol Chem* **245:** 5228–5233, 1970.
27. Meuth M, The molecular basis of mutations induced by deoxyribonucleoside triphosphate pool imbalances in mammalian cells. *Exp Cell Res* **181:** 305–316, 1989.
28. Jensen RA, Page DL and Holt JT, Identification of genes expressed in premalignant breast disease by microscopy-directed cloning. *Proc Natl Acad Sci USA* **91:** 9257–9261, 1994.
29. Fan H, Villegas C and Wright JA, Ribonucleotide reductase R2 component is a novel malignancy determinant that cooperates with activated oncogenes to determine transformation and malignant potential. *Proc Natl Acad Sci USA* **93:** 14036–14040, 1996.
30. Slingerland RJ, van Kuilenburg AB, Bodlaender J, Van Lenthe H, Kreuk E, Voute PA and van Gennip AH, Imbalance between the pyrimidine ribonucleotide pools in rat rhabdomyosarcoma R1 cells. *Adv Exp Med Biol* **370:** 279–282, 1994.
31. de Korte D, Haverkort WA, de Boer M, van Gennip AH and Roos D, Imbalance in the nucleotide pools of myeloid leukemia cells and HL-60 cells: Correlation with cell-cycle phase, proliferation, differentiation, and transformation. *Cancer Res* **47:** 1841–1847, 1987.
32. de Korte D, Haverkort WA, Roos D, Behrendt H and van Gennip AH, Imbalance in the ribonucleotide pools of lymphoid cells from acute lymphoblastic leukemia patients. *Leuk Res* **10:** 389–396, 1986.
33. Slingerland RJ, Bodlaender JM, Van Lenthe H, van Kuilenburg ABP and van Gennip AH, Imbalance between the pyrimidine ribonucleotide pools of rat pheochromocytoma PC-12 cells. *Clin Chem Enzymol Commun* **5:** 315–319, 1993.
34. Szyf M, DNA methylation properties: Consequences for pharmacology. *Trends Pharmacol Sci* **15:** 233–238, 1994.
35. Jones PA and Taylor SM, Cellular differentiation, cytidine analogs and DNA methylation. *Cell* **20:** 85–93, 1980.
36. Issa JP, Baylin SB and Herman JG, DNA methylation changes in hematologic malignancies: Biologic and clinical implications. *Leukemia* **11** (Suppl 1)**:** S7–S11, 1997.
37. Hall A, A biochemical function for ras–at last. *Science* **264:** 1413–1414, 1994.
38. Chu E, Takechi T, Jones KL, Voeller DM, Copur SM, Maley GF, Maley F, Segal S and Allegra CJ, Thymidylate synthase binds to c-*myc* RNA in human colon cancer cells and *in vitro*. *Mol Cell Biol* **15:** 179–185, 1995.
39. Satav JG, Modak MJ and Studzinski GP, Photoaffinity labeling of human c-*myc* protein with deoxythymidine triphosphate. *Lab Invest* **63:** 551–556, 1990.
40. Marks PA and Rifkind RA, Differentiating factors. In: *Biological Therapy of Cancer* (Eds. De Vita VT Jr, Hellman S and Rosenberg SA), pp. 754–762. Lippincott, Philadelphia, 1991.
41. Breitman TR, Selonick SE and Collins SJ, Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* **77:** 2936–2940, 1980.
42. James SY, Williams MA, Kelsey SM, Newland AC and Colston KW, The role of vitamin D derivatives and retin-

oids in the differentiation of human leukaemia cells. *Biochem Pharmacol* **54:** 625–634, 1997.
43. Binderup L, Vitamin D analogs: New regulators of cancer cell growth and differentiation. *Bioorg Med Chem Lett* **3:** 1891–1896, 1993.
44. Metcalf D, The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. *Nature* **339:** 27–30, 1989.
45. Stockhammer G, Manley GT, Johnson R, Rosenblum MK, Samid D and Lieberman FS, Inhibition of proliferation and induction of differentiation in medulloblastoma- and astrocytoma-derived cell lines with phenylacetate. *J Neurosurg* **83:** 672–681, 1995.
46. Huberman E, Heckman C and Langenbach R, Stimulation of differentiated functions in human melanoma cells by tumor-promoting agents and DMSO. *Cancer Res* **39:** 2618–2624, 1979.
47. Tötterman TH, Nilsson K and Sundström C, Phorbol ester-induced differentiation of chronic lymphocytic leukemia cells. *Nature* **288:** 176–178, 1980.
48. Leder A and Leder P, Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. *Cell* **5:** 319–322, 1975.
49. Constantinou A and Huberman E, Genistein as an inducer of tumor cell differentiation: Possible mechanisms of action. *Proc Soc Exp Biol Med* **208:** 109–115, 1995.
50. Casazza AM, Anthracyclines as inducers of tumor cell differentiation. In: *Bioactive Molecules* (Ed. Lown JW), Vol. 6, pp. 715–734. Elsevier Science, Amsterdam, 1988.
51. Arcangeli A, Carlá M, Del Bene MR, Becchetti A, Wanke E and Olivotto M, Polar/apolar compounds induce leukemia cell differentiation by modulating cell-surface potential. *Proc Natl Acad Sci USA* **90:** 5858–5862, 1993.
52. Hozumi M, Fundamentals of chemo-differentiation therapy of myeloid leukemia. *Anticancer Res* **14:** 1177–1192, 1994.
53. Birnie GD, The HL60 cell line: A model system for studying human myeloid cell differentiation. *Br J Cancer* **58** (Suppl IX): 41–45, 1988.
54. Koeffler HP, Induction of differentiation of human acute myelogenous leukemia cells: Therapeutic implications. *Blood* **62:** 709–721, 1983.
55. Andersson LC, Jokinen M and Gahmberg CG, Induction of erythroid differentiation in the human leukaemia cell line K562. *Nature* **278:** 364–365, 1979.
56. Friend C, Scher W, Holland JG and Sato T, Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro*: Stimulation of erythroid differentiation by DMSO. *Proc Natl Acad Sci USA* **68:** 378–382, 1971.
57. Friedman SJ and Skehan P, Morphological differentiation of human choriocarcinoma cells induced by methotrexate. *Cancer Res* **39:** 1960–1967, 1979.
58. Hatse S, Naesens L, De Clercq E and Balzarini J, Potent differentiation-inducing properties of the antiretroviral agent 9-(2-phosphonylmethoxyethyl)adenine (PMEA) in the rat choriocarcinoma RCHO tumor cell model. *Biochem Pharmacol* **56**: 851–859, 1998.
59. Abemayor E and Sidell N, Human neuroblastoma cell lines as models for the *in vitro* study of neoplastic and neuronal cell differentiation. *Environ Health Perspect* **80:** 3–15, 1989.
60. Thomson TM, Real FX, Murakami S, Cordon-Cardo C, Old LJ and Houghton AN, Differentiation antigens of melanocytes and melanoma: Analysis of melanosome and cell surface markers of human pigmented cells with monoclonal antibodies. *J Invest Dermatol* **90:** 459–466, 1988.
61. Nishimune Y, Kume A, Ogiso Y and Matsushiro A, Induction of teratocarcinoma cell differentiation. Effect of the inhibitors of DNA synthesis. *Exp Cell Res* **146:** 439–444, 1983.
62. Sidi Y, Panet C, Wasserman L, Cyjon A, Novogrodsky A and Nordenberg J, Growth inhibition and induction of phenotypic alterations in MCF-7 breast cancer cells by an IMP dehydrogenase inhibitor. *Br J Cancer* **58:** 61–63, 1988.
63. Font J, Braut-Boucher F, Pichon J, Noel-Hudson MS, Muriel MP, Bonnet M, Wepierre J and Aubery M, A new three-dimensional culture of human keratinocytes: Optimization of differentiation. *Cell Biol Toxicol* **10:** 353–359, 1994.
64. Reiss M, Gamba-Vitalo C and Sartorelli AC, Induction of tumor cell differentiation as a therapeutic approach: Preclinical models for hematopoietic and solid neoplasms. *Cancer Treat Rep* **70:** 201–218, 1986.
65. Machado EA, Gerard DA, Lozzio CB, Lozzio BB, Michell JR and Golde DW, Proliferation and differentiation of human myeloid leukemic cells in immunodeficient mice: Electron microscopy and cytochemistry. *Blood* **63:** 1015–1022, 1984.
66. Zhang SY, Zhu J, Chen GQ, Du XX, Lu LJ, Zhang Z, Zhong HJ, Chen HR, Wang ZY, Berger R, Lanotte M, Waxman S, Chen Z and Chen SJ, Establishment of a human acute promyelocytic leukemia-ascites model in SCID mice. *Blood* **87:** 3404–3409, 1996.
67. Speers WC, Conversion of malignant murine embryonal carcinomas to benign teratomas by chemical induction of differentiation *in vivo*. *Cancer Res* **42:** 1843–1849, 1982.
68. Anderson DW and Crowle AF, Regression and differentiation of neuroblastoma tumors in mice treated with differentiating agents—prostaglandin E_1 and a phosphodiesterase inhibitor RO 20–1724. *Cancer Lett* **16:** 287–295, 1982.
69. Dexter DL, Spremulli EN, Matook GM, Diamond I and Calabresi P, Inhibition of the growth of human colon cancer xenografts by polar solvents. *Cancer Res* **42:** 5018–5022, 1982.
70. Ram Z, Samid D, Walbridge S, Oshiro EM, Viola JJ, Tao-Cheng JH, Shack S, Thibault A, Myers CE and Oldfield EH, Growth inhibition, tumor maturation, and extended survival in experimental brain tumors in rats treated with phenylacetate. *Cancer Res* **54:** 2923–2927, 1994.
71. Verstuyf A, Sobis H, Goebels J, Fonteyn E, Cassiman JJ and Vandeputte M, Establishment and characterization of a continuous *in vitro* line from a rat choriocarcinoma. *Int J Cancer* **45:** 752–756, 1990.
72. Hatse S, Naesens L, Degrève B, Segers C, Vandeputte M, Waer M, De Clercq E and Balzarini J, Potent antitumor activity of the acyclic nucleoside phosphonate 9-(2-phosphonylmethoxyethyl)adenine in choriocarcinoma-bearing rats. *Int J Cancer* **76:** 595–600, 1998.
73. McPartland RP, Wang MC, Bloch A and Weinfeld H, Cytidine 5′-triphosphate synthetase as a target for inhibition by the antitumor agent 3-deazauridine. *Cancer Res* **34:** 3107–3111, 1974.
74. Kang GJ, Cooney DA, Moyer JD, Kelley JA, Kim H-Y, Marquez VE and Johns DG, Cyclopentenylcytosine triphosphate: Formation and inhibition of CTP synthetase. *J Biol Chem* **264:** 713–718, 1989.
75. van Kuilenburg ABP, Van den Berg AA, Meinsma JR, Slingerland RJ and van Gennip AH, Inhibition of CTP synthetase induces differentiation of HL-60 cells and down-regulation of the c-*myc* oncogene. *Adv Exp Med Biol* **370:** 761–764, 1995.
76. Glazer RI, Cohen MB, Hartman KD, Knode MC, Lim M-I and Marquez VE, Induction of differentiation in the human promyelocytic leukemia cell line HL-60 by the cyclopentenyl analogue of cytidine. *Biochem Pharmacol* **35:** 1841–1848, 1986.
77. Moyer JD, Malinowski NM, Treanor SP and Marquez VE, Antitumor activity and biochemical effects of cyclopentenyl cytosine in mice. *Cancer Res* **46:** 3325–3329, 1986.
78. Moriconi WJ, Slavik M and Taylor S, 3-Deazauridine (NSC

126849): An interesting modulator of biochemical response. *Invest New Drugs* **4:** 67–84, 1986.
79. Bouffard DY, Momparler LF and Momparler RL, Enhancement of the antileukemic activity of 5-aza-2′-deoxycytidine by cyclopentenyl cytosine in HL-60 leukemic cells. *Anticancer Drugs* **5:** 223–228, 1994.
80. Christopherson RI and Lyons SD, Potent inhibitors of *de novo* pyrimidine and purine biosynthesis as chemotherapeutic agents. *Med Res Rev* **10:** 505–548, 1990.
81. Canman CE, Radany EH, Parsels LA, Davis MA, Lawrence TS and Maybaum J, Induction of resistance to fluorodeoxyuridine cytotoxicity and DNA damage in human tumor cells by expression of *Escherichia coli* deoxyuridine triphosphatase. *Cancer Res* **54:** 2296–2298, 1994.
82. Danenberg PV, Heidelberger C, Mulkins MA and Peterson AR, The incorporation of 5-fluoro-2′-deoxyuridine into DNA of mammalian tumor cells. *Biochem Biophys Res Commun* **102:** 654–658, 1981.
83. Heidelberger C, Danenberg PV and Moran RG, Fluorinated pyrimidines and their nucleosides. *Adv Enzymol Relat Areas Mol Biol* **54:** 58–119, 1983.
84. Yang Y-W and Chang Y-H, Induction of erythroid differentiation by 5-fluorouracil in K562 leukemia cells. *Jpn J Cancer Res* **86:** 948–955, 1995.
85. Schwartz PM, Barnett SK and Milstone LM, Keratinocytes differentiate in response to inhibitors of deoxyribonucleotide synthesis. *J Dermatol Sci* **9:** 129–135, 1995.
86. Kafka M, Dvilansky A and Nathan I, Mechanism of interaction between interferon-γ and antineoplastic agent on the differentiation of HL-60 promyelocytic cells. *Exp Hematol* **18:** 153–158, 1990.
87. Oorschot DE, Effect of fluorodeoxyuridine on neurons and non-neuronal cells in cerebral explants. *Exp Brain Res* **78:** 132–138, 1989.
88. Flickinger RA and Richman R, The effect of induction of hemoglobin synthesis in cultured Friend cells on the number of initiation sites for replication and transcription. *Cell Differ* **14:** 59–71, 1984.
89. Schwartz PM, Barnett SK, Atillasoy ES and Milstone LM, Methotrexate induces differentiation of human keratinocytes. *Proc Natl Acad Sci USA* **89:** 594–598, 1992.
90. Bodner AJ, Ting RC and Gallo RC, Induction of differentiation of human promyelocytic leukemia cells (HL-60) by nucleosides and methotrexate. *J Natl Cancer Inst* **67:** 1025–1030, 1981.
91. Ross SA, Jones CS and DeLuca LM, Retinoic acid and methotrexate specifically increase PHA-E-lectin binding to a 67-kDa glycoprotein in LA-N-1 neuroblastoma cells. *Int J Cancer* **62:** 303–308, 1995.
92. Burres NS and Cass CE, Inhibition of methotrexate-induced differentiation of cultured human choriocarcinoma (BeWo) cells by thymidine. *Cancer Res* **47:** 5059–5064, 1987.
93. Sokoloski JA, Beardsley GP and Sartorelli AC, Mechanism of the induction of the differentiation of HL-60 leukemia cells by antifolates. *Cancer Commun* **1:** 199–207, 1989.
94. Sokoloski JA, Pizzorno G, Beardsley GP and Sartorelli AC, Evidence for a relationship between intracellular GTP levels and the induction of HL-60 leukemia cell differentiation by 5,10-dideazatetrahydrofolic acid (DDATHF). *Oncol Res* **5:** 293–299, 1993.
95. Jones TR, Calvert AH, Jackman AL, Brown SJ, Jones M and Harrap KR, A potent antitumor quinazoline inhibitor of thymidylate synthetase: Synthesis, biological properties and therapeutic results in mice. *Eur J Cancer* **17:** 11–19, 1981.
96. Duch DS, Banks S, Dev IK, Dickerson SH, Ferone R, Heath LS, Humphreys J, Knick V, Pendergast W, Singer S, Smith GK, Waters K and Wilson R, Biochemical and cellular pharmacology of 1843U89, a novel benzoquinazoline inhibitor of thymidylate synthase. *Cancer Res* **53:** 810–818, 1993.
97. Appelt K, Bacquet RJ, Bartlett CA, Booth CLJ, Freer ST, Fuhry MAM, Gehring MR, Herrmann SM, Howland EF, Janson CA, Jones TR, Kan C-C, Kathardekar V, Lewis KK, Marzoni GP, Matthews DA, Mohr C, Moomaw EW, Morse CA, Oatley SJ, Ogden RC, Reddy MR, Reich SH, Schoettlin WS, Smith WW, Varney MD, Villafranca JE, Ward RW, Webber S, Webber SE, Welsh KM and White J, Design of enzyme inhibitors using iterative protein crystallographic analysis. *J Med Chem* **34:** 1925–1934, 1991.
98. Catapano CV, Dayton JS, Mitchell BS and Fernandes DJ, GTP depletion induced by IMP dehydrogenase inhibitors blocks RNA-primed DNA synthesis. *Mol Pharmacol* **47:** 948–955, 1995.
99. Zoref-Shani E, Lavie R, Bromberg Y, Beery E, Sidi Y, Sperling O and Nordenberg J, Effects of differentiation-inducing agents on purine nucleotide metabolism in an ovarian cancer cell line. *J Cancer Res Clin Oncol* **120:** 717–722, 1994.
100. Sintchak MD, Fleming MA, Futer O, Raybuck SA, Chambers SP, Caron PR, Murcko MA and Wilson KP, Structure and mechanism of inosine monophosphate dehydrogenase in complex with the immunosuppressant mycophenolic acid. *Cell* **85:** 921–930, 1996.
101. Streeter DG, Witkowski JT, Khare GP, Sidwell RW, Bauer RJ, Robins RK and Simon LN, Mechanism of action of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad-spectrum antiviral agent. *Proc Natl Acad Sci USA* **70:** 1174–1178, 1973.
102. Périgaud C, Gosselin G and Imbach J-L, Nucleoside analogs as chemotherapeutic agents: A review. *Nucleosides Nucleotides* **11:** 903–945, 1992.
103. Lui MS, Faderan MA, Liepnieks JJ, Natsumeda Y, Olah E, Jayaram HN and Weber G, Modulation of IMP dehydrogenase activity and guanylate metabolism by tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide). *J Biol Chem* **259:** 5078–5082, 1984.
104. Balzarini J, Stet L, Matsuda A, Wiebe L, Knauss E and De Clercq E, Metabolism of EICAR (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide), a potent inhibitor of inosinate dehydrogenase. *Adv Exp Med Biol* **431**: 723–728, 1998.
105. De Clercq E, Cools M, Balzarini J, Snoeck R, Andrei G, Hosoya M, Shigeta S, Ueda T, Minakawa N and Matsuda A, Antiviral activities of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide and related compounds. *Antimicrob Agents Chemother* **35:** 679–684, 1991.
106. Elion GB, The purine path to chemotherapy. *Science* **244:** 41–47, 1989.
107. Kiguchi K, Collart FR, Henning-Chubb C and Huberman E, Cell differentiation and altered IMP dehydrogenase expression induced in human T-lymphoblastoid leukemia cells by mycophenolic acid and tiazofurin. *Exp Cell Res* **187:** 47–53, 1990.
108. Kiguchi K, Collart FR, Henning-Chubb C and Huberman E, Induction of cell differentiation in melanoma cells by inhibitors of IMP dehydrogenase: Altered patterns of IMP dehydrogenase expression and activity. *Cell Growth Differ* **1:** 259–270, 1990.
109. Pillwein K, Schuchter K, Ressmann G, Gharehbaghi K, Knoflach A, Cermak B, Jayaram HN, Szalay SM, Szekeres T and Chiba P, Cytotoxicity, differentiating activity and metabolism of tiazofurin in human neuroblastoma cells. *Int J Cancer* **55:** 92–95, 1993.
110. Hatse S, De Clercq E and Balzarini J, Evidence for distinction of the differentiation-inducing activities and cytostatic properties of 9-(2-phosphonylmethoxyethyl)adenine and a

variety of differentiation-inducing agents in human erythroleukemia K562 cells. *Mol Pharmacol* **50:** 1231–1242, 1996.
111. Olah E, Natsumeda Y, Ikegami T, Kote Z, Horanyi M, Szelenyi J, Paulik E, Kremmer T, Hollan SR, Sugar J and Weber G, Induction of erythroid differentiation and modulation of gene expression by tiazofurin in K-562 leukemia cells. *Proc Natl Acad Sci USA* **85:** 6533–6537, 1988.
112. Yu J, Lemas V, Page T, Connor JD and Yu AL, Induction of erythroid differentiation in K562 cells by inhibitors of inosine monophosphate dehydrogenase. *Cancer Res* **49:** 5555–5560, 1989.
113. Tricot GJ, Jayaram HN, Lapis E, Natsumeda Y, Nichols CR, Kneebone P, Heerema N, Weber G and Hoffman R, Biochemically directed therapy of leukemia with tiazofurin, a selective blocker of inosine 5′-phosphate dehydrogenase activity. *Cancer Res* **49:** 3696–3701, 1989.
114. Tressler RJ, Garvin LJ and Slate DL, Anti-tumor activity of mycophenolate mofetil against human and mouse tumors *in vivo*. *Int J Cancer* **57:** 568–573, 1994.
115. Prasad KN, Differentiation of neuroblastoma cells induced in culture by 6-thioguanine. *Int J Cancer* **12:** 631–636, 1973.
116. Gusella JF and Housman D, Induction of erythroid differentiation *in vitro* by purines and purine analogs. *Cell* **8:** 263–269, 1976.
117. Tsutani H, Inai K, Imamura S, Ueda T and Nakamura T, Induction of cell differentiation by IMPDH antisense oligomer in HL-60 and K562 human leukemia cell lines. *Adv Exp Med Biol* **370:** 757–760, 1994.
118. Kharbanda SM, Sherman ML and Kufe DW, Effects of tiazofurin on guanine nucleotide binding regulatory proteins in HL-60 cells. *Blood* **75:** 583–588, 1990.
119. Hata Y, Natsumeda Y and Weber G, Tiazofurin decreases Ras-GTP complex in K562 cells, *Oncol Res* **5:** 161–164, 1993.
120. Weber G, Nagai M, Natsumeda Y, Eble JN, Jayaram HN, Paulik E, Zhen W, Hoffman R and Tricot G, Tiazofurin down-regulates expression of c-Ki-*ras* oncogene in a leukemic patient. *Cancer Commun* **3:** 61–66, 1991.
121. Weber G, Hata Y and Prajda N, Role of differentiation induction in action of purine antimetabolites. *Pharm World Sci* **16:** 77–83, 1994.
122. Pilz RB, Huvar I, Scheele JS, Van den Berghe G and Boss GR, A decrease in the intracellular guanosine 5′-triphosphate concentration is necessary for granulocytic differentiation of HL-60 cells, but growth cessation and differentiation are not associated with a change in the activation state of Ras, the transforming principle of HL-60 cells. *Cell Growth Differ* **8:** 53–59, 1997.
123. Krakoff IH, Brown NC and Reichard P, Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. *Cancer Res* **28:** 1559–1565, 1968.
124. Kaplinsky C, Estrov Z, Freedman MH, Gelfand EW and Cohen A, Effect of deferoxamine on DNA synthesis,DNA repair, cell proliferation and differentiation of HL-60 cells. *Leukemia* **1:** 437–441, 1987.
125. Arbiser JL, Arbiser ZK and Majzoub JA, Differential regulation of choriocarcinoma gene expression by DNA synthesis inhibitors. *Endocr J* **40:** 263–268, 1993.
126. Arbiser JL, Arbiser ZK and Majzoub JA, Effects of hydroxyurea and cyclic adenosine monophosphate/protein kinase A inhibitors on the expression of the human chorionic gonadotropin alpha subunit and c-*myc* genes in choriocarcinoma. *J Endocrinol Invest* **16:** 849–856, 1993.
127. LoPresti P, Poluha W, Poluha DK, Drinkwater E and Ross AH, Neuronal differentiation triggered by blocking cell proliferation. *Cell Growth Differ* **3:** 627–635, 1992.
128. Adunyah SE, Chander R, Barner VK and Copper RS, Regulation of c-jun mRNA expression by hydroxyurea in human K562 cells during erythroid differentiation. *Biochim Biophys Acta* **1263:** 123–132, 1995.
129. Charache S, Dover GJ, Moyer MA and Moore JW, Hydroxyurea-induced augmentation of fetal hemoglobin production in patients with sickle cell anemia. *Blood* **69:** 109–116, 1987.
130. Creasey DC and Wright JA, Involvement of ribonucleotide reductase in cellular differentiation. *Biosci Rep* **4:** 299–309, 1984.
131. Barbat A, Pandrea I, Cambier D, Zweibaum A, and Lesuffleur T, Resistance of the human colon carcinoma cell line HCT-8 to methotrexate results in selection of cells with features of enterocytic differentiation. *Int J Cancer* **75:** 731–737, 1998.
132. Yoshida S, Yamada M and Masaki S, Inhibition of DNA polymerase-α and -β of calf thymus by 1-β-D-arabinofuranosylcytosine 5′ triphosphate. *Biochim Biophys Acta* **477:** 144–150, 1977.
133. Ross DD, Chen SRS and Cuddy DP, Effects of 1-β-D-arabinofuranosylcytosine on DNA replication intermediates monitored by pH-step alkaline elution. *Cancer Res* **50:** 2658–2666, 1990.
134. Dijkwel PA and Wanka F, Enhanced release of nascent single strands from DNA synthesized in the presence of arabinosylcytosine. *Biochim Biophys Acta* **520:** 461–471, 1978.
135. Luisi-DeLuca C, Mitchell T, Spriggs D and Kufe DW, Induction of terminal differentiation in human K562 erythroleukemia cells by arabinofuranosylcytosine. *J Clin Invest* **74:** 821–827, 1984.
136. Ponzoni M, Lanciotti M, Montaldo PG and Cornaglia-Ferraris P, γ-Interferon, retinoic acid, and cytosine arabinoside induce neuroblastoma differentiation by different mechanisms. *Cell Mol Neurobiol* **11:** 397–413, 1991.
137. Steigerwald SD and Pfeifer GP, Variable DNA methylation changes during differentiation of human melanoma cells. *Exp Cell Res* **178:** 41–50, 1988.
138. Abe M and Kufe D, Effects of maturational agents on expression and secretion of two partially characterized high molecular weight milk-related glycoproteins in MCF-7 breast carcinoma cells. *J Cell Physiol* **126:** 126–132, 1986.
139. Griffin J, Munroe D, Major P and Kufe D, Induction of differentiation of human myeloid leukemia cells by inhibitors of DNA synthesis. *Exp Hematol* **10:** 774–781, 1982.
140. Nishimune Y, Kosaka M, Nishina Y, Sumi T, Sakuda M, Takeda M and Matsumoto K, Inhibition of DNA synthesis causes stem cell differentiation: Induction of teratocarcinoma F9 cell differentiation with nucleoside analogs of DNA-synthesis inhibitors and their inducing abilities counterbalanced specifically by normal nucleosides. *Biochem Biophys Res Commun* **163:** 1290–1297, 1989.
141. Craig RW, Frankfurt OS, Sakagami H, Takeda K and Bloch A, Macromolecular and cell cycle effects of different classes of agents inducing the maturation of human myeloblastic leukemia (ML-1) cells. *Cancer Res* **44:** 2421–2429, 1984.
142. Ikegami S, Taguchi T and Ohashi M, Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase-α. *Nature* **275:** 458–460, 1978.
143. Murate T, Kagami Y, Hotta T, Yoshida T, Saito H and Yoshida S, Terminal differentiation of human erythroleukemia cell line K562 induced by aphidicolin. *Exp Cell Res* **191:** 45–50, 1990.
144. Cinatl J Jr, Cinatl J, Driever PH, Rabenau H, Novak M, Stefanik M, Kornhuber B and Doerr HW, Aphidicolin induces myogenic differentiation in the human rhabdomyosarcoma cell line KFR. *Cell Biol Int* **18:** 271–278, 1994.
145. De Clercq E, Holý A, Rosenberg I, Sakuma T, Balzarini J

and Maudgal PC, A novel selective broad-spectrum anti-DNA virus agent. *Nature* **323:** 464–467, 1986.
146. De Clercq E, Sakuma T, Baba M, Pauwels R, Balzarini J, Rosenberg I and Holý A, Antiviral activity of phosphonylmethoxyalkyl derivatives of purines and pyrimidines. *Antiviral Res* **8:** 261–272, 1987.
147. Balzarini J, Hao Z, Herdewijn P, Johns DG and De Clercq E, Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. *Proc Natl Acad Sci USA* **88:** 1499–1503, 1991.
148. Cherrington JM, Allen SJW, Bischofberger N and Chen MS, Kinetic interaction of the diphosphates of 9-(2-phosphonylmethoxyethyl)adenine and other anti-HIV active purine congeners with HIV reverse transcriptase and human DNA polymerases α, β and γ. *Antiviral Chem Chemother* **6:** 217–221, 1995.
149. Nicander B and Reichard P, Relations between synthesis of deoxyribonucleotides and DNA replication in 3T6 fibroblasts. *J Biol Chem* **260:** 5376–5381, 1985.
150. Balzarini J, Verstuyf A, Hatse S, Goebels J, Sobis H, Vandeputte M and De Clercq E, The human immunodeficiency virus (HIV) inhibitor 9-(2-phosphonylmethoxyethyl)adenine (PMEA) is a strong inducer of differentiation of several tumor cell lines. *Int J Cancer* **61:** 130–137, 1995.
151. Kao WY, Briggs JA, Kinney MC, Jensen RA and Briggs RC, Structure and function analysis of the human myeloid cell nuclear differentiation antigen promoter: Evidence for the role of Sp1 and not of c-Myb or PU. in myelomonocytic lineage-specific expression. *J Cell Biochem* **65:** 231–244, 1997.
152. Bender CM, Pao MM and Jones PA, Inhibition of DNA methylation by 5-aza-2′-deoxycytidine suppresses the growth of human tumor cell lines. *Cancer Res* **58:** 95–101, 1998.
153. Christman JK, Mendelsohn N, Herzog D and Schneiderman N, Effect of 5-azacytidine on differentiation and DNA methylation in human promyelocytic leukemia cells (HL-60). *Cancer Res* **43:** 763–769, 1983.
154. Creusot F, Acs G and Christman JK, Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2′-deoxycytidine. *J Biol Chem* **257:** 2041–2048, 1982.
155. Saitoh F, Hiraishi K, Adachi M and Hozumi M, Induction by 5-aza-2′-deoxycytidine, an inhibitor of DNA methylation, of Le^y antigen, apoptosis and differentiation in human lung cancer cells. *Anticancer Res* **15:** 2137–2144, 1995.
156. Palmer JL and Abeles RH, The mechanism of action of *S*-adenosylhomocysteinase. *J Biol Chem* **254:** 1217–1226, 1976.
157. Linevsky J, Cohen MB, Hartman KD, Knode MC and Glazer RI, Effect of neplanocin A on differentiation, nucleic acid methylation, and c-myc mRNA expression in human promyelocytic leukemia cells. *Mol Pharmacol* **28:** 45–50, 1985.
158. Mizutani Y, Masuoka S, Imoto M, Kawada M and Umezawa K, Induction of erythroid differentiation in leukaemic K562 cells by an S-adenosylhomocysteine hydrolase inhibitor, aristeromycin. *Biochem Biophys Res Commun* **207:** 69–74, 1995.
159. Aarbakke J, Miura GA, Prytz PS, Bessesen A, Slørdal L, Gordon RK and Chiang PK, Induction of HL-60 cell differentiation by 3-deaza-(±)-aristeromycin, an inhibitor of *S*-adenosylhomocysteine hydrolase. *Cancer Res* **46:** 5469–5472, 1986.
160. Paller AS, Arnsmeier SL, Clark SH and Mirkin BL, Z-4′,5′-Didehydro-5′-deoxy-5′-fluoroadenosine (MDL 28,842), an irreversible inhibitor of *S*-adenosylhomocysteine hydrolase, suppresses proliferation of cultured keratinocytes and squamous carcinoma cell lines. *Cancer Res* **53:** 6058–6060, 1993.
161. Shiotani T, Hashimoto Y, Fujita J, Yamauchi N, Yamaji Y, Futami H, Bungo M, Nakamura H, Tanaka T and Irino S, Reversal of enzymic phenotype of thymidine metabolism in induced differentiation of U-937 cells. *Cancer Res* **49:** 6758–6763, 1989.
162. Hashimoto Y, Shiotani T, Fujita J, Yamaji Y, Futami H, Yamauchi N, Bungo M, Nakamura H, Tanaka T and Irino S, Reversal of enzymic phenotype of thymidine metabolism in induced differentiation of HL-60 cells. *Leuk Res* **13:** 1123–1129, 1989.
163. Mullan PB, McKenna PG and McKelvey-Martin VJ, Activities of potential tumour marker enzymes during induced differentiation in HL-60 and U-937 cells. *Br J Biomed Sci* **54:** 91–99, 1997.
164. Horie N, Nozawa R and Takeishi K, Identification of cellular differentiation-dependent nuclear factors that bind to a human gene for thymidylate synthase. *Biochem Biophys Res Commun* **185:** 127–133, 1992.
165. Schwartz PM, Barnett SK and Reuveni H, Thymidine salvage changes with differentiation in human keratinocytes *in vitro*. *J Invest Dermatol* **97:** 1057–1060, 1991.
166. Lucas DL, Webster HK and Wright DG, Purine metabolism in myeloid precursor cells during maturation. Studies with the HL-60 cell line. *J Clin Invest* **72:** 1889–1900, 1983.
167. Wright DG, A role for guanine ribonucleotides in the regulation of myeloid cell maturation. *Blood* **69:** 334–337, 1987.
168. Mann GJ, Musgrove EA, Fox RM and Thelander L, Ribonucleotide reductase M1 subunit in cellular proliferation, quiescence and differentiation. *Cancer Res* **48:** 5151–5156, 1988.
169. Razin A, Levine A, Kafri T, Agostini S, Gomi T and Cantoni GL, Relationship between transient DNA hypomethylation and erythroid differentiation of murine erythroleukemia cells. *Proc Natl Acad Sci USA* **85:** 9003–9006, 1988.
170. Chiba P, Plas A, Wessels JM and De Bruyn CH, *S*-Adenosylhomocysteine hydrolase activity during differentiation of HL-60 cells. *Biosci Rep* **4:** 687–694, 1984.
171. Bianchi Scarrà GL, Romani M, Coviello DA, Garrè C, Ravazzolo R, Vidali G and Ajmar F, Terminal erythroid differentiation in the K-562 cell line by 1-β-D-arabinofuranosylcytosine: Accompaniment by c-*myc* messenger RNA decrease. *Cancer Res* **46:** 6327–6332, 1986.
172. Lachman H and Skoultchi AI, Expression of c-myc changes during differentiation of mouse erythroleukaemia cells. *Nature* **310:** 592–594, 1984.
173. Shimizu N, Nakamura H, Kadota T, Kitajima K, Oda T, Hirano T and Utiyama H, Loss of amplified c-*myc* genes in the spontaneously differentiated HL-60 cells. *Cancer Res* **54:** 3561–3567, 1994.
174. Dotto GP, Gilman MZ, Maruyama M and Weinberg RA, c-*myc* and c-*fos* expression in differentiating mouse primary keratinocytes. *EMBO J* **5:** 2853–2857, 1986.
175. Gómez-Casares MT, Delgado MD, Lerga A, Crespo P, Quincoces AF, Richard C and Léon J, Down-regulation of c-myc gene is not obligatory for growth inhibition and differentiation of human myeloid leukemia cells. *Leukemia* **7:** 1824–1833, 1993.
176. Toffoli G, Viel A, Bevilacqua C, Maestro R, Tumiotto L and Boiocchi M, In K562 leukemia cells treated with doxorubicin and hemin, a decrease in c-*myc* mRNA expression correlates with loss of self-renewal capability but not with erythroid differentiation. *Leuk Res* **13:** 279–287, 1989.
177. Burchill SA, Berry PA and Lewis IJ, Changing expression of GTPase activating proteins with differentiation in neuroblastoma. *J Neurol Sci* **126:** 126–132, 1994.