Anti Metabolite Theory

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ABSTRACT

Inhibition of the key element controlling the metabolic process would decrease or completely inhibit the synthesis of the intracellular key molecules. The broad field called antimetabolites originated from this magnificent simple rationale. While Wood's works on antibacterial antimetabolites were going on, Hitchings found that cells such as bacterial, tumoral or protozoal cells that grew and proliferated rapidly required large amount of nucleic acids. In particular, the prevention of synthesis by changing the substrates of key enzymes of nucleotide or nucleic acidmetabolism, and the information obtained by the introduction of synthetic nucleotide analogs into DNA and RNA synthesis has improved our knowledge in this field. The expansion of our knowledge regarding the metabolism of key enzymes controlling the nucleotide metabolism can provide us with new perspectives in treatment of diseases with cancer and viral diseases in the first place. After all, enzymes, which are complex protein structures control all the chemical reactions including those related to nucleic acid. While studies carried out starting from the beginning of the century on enzymes targeting the nucleotide metabolism had found a valuable place thanks to the antimetabolite theory, the number of groups working on the structural characteristics and kinetics of enzymes in particular have started to decrease gradually. The major challenges in this area are that enzyme kinetics studies are largely dependent on instrument infrastructure, are labor intensive, and the results of the study are not satisfactory when applied to technology. In addition to the lack of popularity of such studies, long working periods, difficulties in publishing and citing studies limit the field. Enzyme kinetics is currently the most challenging multidisciplinary field in biochemistry. The limitations mentioned above discouraged the scientists working in this field and the field started to shrink. Despite all the difficulties, we must continue the work necessary to understand the structural features and behavior of this "working class" that controls the metabolism of nucleotides and nucleic acids. It can be predicted that the antimetabolite theory will come to the fore again in the treatment of viral diseases that emerge as an important health problem and in the treatment of many important diseases, especially cancer. The purpose of this article is to shed light on the field again.

Key Words: Antimetabolite Theory, Purine and Pyrimidine Metabolism Enzymes, Nucleic acid Metabolism Enzymes, Enzyme Kinetics

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ÖZET

Metabolik süreci kontrol eden anahtar enzimlerin inhibisyonu, hücre içi anahtar moleküllerin sentezini azaltır veya tamamen inhibe eder. Antimetabolit teori olarak tanımlanan bu geniş alan muhteşem basit bir mantıktan kaynaklanmaktadır. Wood'un antibakteriyel antimetabolitler üzerindeki çalışmaları devam ederken, Hitchings hızla büyüyen ve çoğalan bakteriyel, tümöral veya protozoal hücreler gibi hücrelerin büyük miktarda nükleik asit ihtiyacı olduğunu keşfetti.Özellikle nükleotid veya nükleik asit metabolizmasının anahtar enzimlerinin substratlarını değiştirerek sentezin önlenmesi ve sentetik nükleotid analoglarının DNA ve RNA sentezine dahil edilmesiyle elde edilen sonuçlar bu alandaki bilgimizi geliştirmiştir.Nükleotid veya nükleik asit metabolizmasını kontrol eden anahtar enzimlerin metabolizmasına ilişkin bilgilerimizin genişlemesi, bize ilk etapta kanser ve viral hastalıkların tedavisinde yeni perspektifler sağlamıştır. Sonuçta, karmaşık protein yapıları olan enzimler, nükleik asitle ilgili olanlar dahil tüm kimyasal reaksiyonları kontrol eder. Yüzyılın başından itibaren nükleotid metabolizmasını hedefleyen enzimler üzerine yapılan çalışmalar antimetabolit teorisi sayesinde değerli bir yer bulurken, son dönemde özellikle enzimlerin yapısal özellikleri ve kinetiği üzerinde çalışan grupların sayısı giderek azalmaya başlamıştır. Bu alandaki en büyük zorluklar; enzim kinetiği çalışmalarının büyük ölçüde cihaz altyapısına bağlı olması, emek yoğun olması ve çalışmanın sonuçlarını teknolojide uygularken tatmin edici sonuçlar elde edilmemesidir. Bu tür çalışmaların popüler olmamasının yanı sıra uzun çalışma süreleri, çalışmaların yayınlanması ve kaynak gösterilmesindeki zorluklar alanı sınırlamaktadır. Enzim kinetiği biyokimyadaki en zorlu multidisipliner alanlardan bir tanesidir. Yukarıda belirtilen sınırlamalar, bu alanda çalışan bilim adamlarını caydırdı ve alan küçülmeye başladı. Tüm zorluklara rağmen, nükleotidlerin ve nükleik asitlerin metabolizmasını kontrol eden bu "işçi sınıfının" yapısal özelliklerini ve davranışını anlamak için gerekli çalışmalara devam etmeliyiz. Önemli bir sağlık sorunu olarak ortaya çıkan viral hastalıkların tedavisinde ve başta kanser olmak üzere birçok önemli hastalığın tedavisinde antimetabolit teorisinin yeniden ön plana çıkacağı tahmin edilebilir. Bu yazının amacı sahaya yeniden ışık tutmaktır

Anahtar Sözcükler: Antimetabolit Teori, Purin ve Pirimidin Metabolizması Enzimleri, Nükleik Asit Metabolizması Enzimleri, Enzim Kinetiği

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INTRODUCTION

Inhibition of key enzymes that control metabolic processes is the basis of the antimetabolite theory (1,2). Changing the natural substrate of the enzymes involved in nucleotide and nucleic acid metabolism causes inhibition of metabolic processes. The development of this logic has increased the importance of the antimetabolite theory (3,4). Studies of Elion carried out in 1951 on 6-mercaptopurin (6-MP), which is a purine analog, are pioneering studies in use of antimetabolites in cancer treatment (5). Study of Burchenal and colleagues conducted on children with acute leukemia started the clinical process of the use of purine analogs in treatments of humans (6). Greenberg played a pioneering role in this area with the definition of hypoxanthine ribonucleotide (inosinic acid, IMP) biosynthesis pathway (7). Inhibition of inosinate dehydrogenase by the nucleotide forms of 6-MP and entry of 6-MP in DNA structure in the form of thioguanine is the basis of inhibition mechanism (8). 6-MP was approved by American Food and Drug Administration (FDA) in 1953, and thioguanine was approved by the same agency in 1966. A rationale similar to the one used by Woods on folic acid inhibitors has led the way to the use of antimetabolites in cancer treatment. The idea of inhibiting the synthesis of active folic acid, which is carbon source in the synthesis pathway for both purine and pyrimidine has resulted in the discovery of methotrexate. Methotrexate is an inhibitor of Dihydrofolate reductase (DHFR), which occupies a central position in the metabolic pathway. DHFR is responsible for the conversion of dihydrofolate to tetrahydrofolate and ultimately to 10-formyl tetrahydrofolate. The last compound provides the formyl group for glycinamide ribonucleotide formyltransferase (GARFT) and aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFT). Thus, the inhibition of DHFR results in depletion of intracellular pools of reduced folates and ultimately in reduced synthesis of purines and pyrimidines (9). The same rationale has been applied to the use of folic acid metabolism inhibitors in cancer treatment in later periods. Pemetrexed disodium heptahydrate is a novel multitargeted antifolate that inhibits 3 enzymes involved in folate metabolism and purine and pyrimidine synthesis. These enzymes are thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase (10). Pemetrexed acts like methotrexate. It hinders multiple enzymes needed for de novo production of the thymidine and purine nucleotides. Normal DNA and RNA production is prevented (11). Pemetrexed was approved by FDA in 2004. Synthesis of the pyrimidine analog, 5-flurouracil (5-FU) by Robert Duschinsky marked a new era in cancer treatment (12).

Antimetabolite theory lies under the studies of Robert Duschinsky on the pyrimidine analog,5 Fluorouracil. Structural similarity of 5-flourouracil with the thymine base made it possible to take a place in the treatment of various cancer types. Flouro is similar in structure to uracil and is converted to two active metabolites (FdUMP and FUTP) that inhibit the activity of the enzyme thymidylate synthetase. The enzyme normally converts uracil to thymidine by adding a methyl group at the fifth carbon of the pyrimidine ring. 5-FU mimics the natural base and functions to inhibit DNA synthesis. dUTP and FdUTP are incorporated into DNA so that it cannot function normally. In addition, FUTP is incorporated into RNA leading to faulty translation of the RNA. Thus, the synthesis of multiple forms of RNA (messenger, ribosomal, transfer and small nuclear RNAs) is blocked. These combined actions on DNA and RNA are cytotoxic to the rapidly dividing cancer cells. 5-FU was approved by FDA in 1962. Again, another fluorinated uracil compound, Capecitabine, was approved by FDA in 2005. Capecitabine, an orally administered triple prodrug of 5-FU. Fluorouracil it then metabolized both normal and tumor cells to 5-fluoro-2'-deoxyuridine 5'-monophosphate FdUMP and 5- fluorouridineriphosphate (FUTP). FdUMP inhibits DNA synthesis and cell division by reducing normal thymidine production, while FUTP inhibits RNA and protein synthesis by competing with uridine triphosphate for incorporation into the RNA strand (13). Discovery of Pentostatin, a potent inhibitor of Adenosine deaminase (ADA), which is one of the key enzymes of the catabolic pathway for purine and controls the Adenosine metabolism displays the significance of inhibitors of catabolism. Inhibition of this enzyme results in the accumulation of pentostatin (20-deoxycoformycin; dCF) deoxyadenosine (dAdo) and adenosine (Ado). dAdo is phosphorylated by deoxycytidine kinase in lymphocytes to deoxyadenosine monophosphate (dAMP), which is subsequently converted to deoxyadenosine triphosphate (dATP). Both dATP and CdATP cause an initial accumulation of DNA strand breaks in lymphocytes and this results in the activation of p53, the release of cytochrome c from mitochondria, and apoptosis.

CdA has several unique mechanisms of action over dAdo and these include the incorportation of CdATP into DNA, the inhibition of DNA polymerase b, and the phosphorylation of CdA to CdATP by deoxyguanosine kinase in mitochondria. These additional modes of action produce further DNA breaks in CdA-treated cells and explain the more potent activity of CdA compared to dCF and the greater myelosuppression with this agent. The cells die by apoptosis, but the DNA strand breaks also cause the activation of poly (ADPribose) polymerase (PARP), with resultant cellular depletion of nicotinamide adenine dinucleotide (NAD) and ATP. The induction of necrosis by PARP activation may explain the activity of these analogs in some patients with p53 mutations. (14). Accumulation of these two substrates in the medium leads to several events that trigger one another in the organism. Pentostatin was approved by FDA in 1991. Triggering of such significant reactions independent from each other through ADA inhibition is important since it shows importance of adenosine metabolism. Together with the 5' nucleotidase (5'NT), adenosine deaminase has a very important place as the key enzyme of the immune system as well as the cancer metabolism. Azacytidine is a pyrimidine analog that inhibits DNA methyltransferase (DNMT) to interrupt DNA synthesis. Azacytosine-guanine dinucleotides are recognized by the DNA methyltransferases as natural substrate and the enzymes will initiate the methylation reaction by a nucleophilic attack. This results in the establishment of a covalent bond between the carbon-6 atom of the cytosine ring and the enzyme. The bond is normally resolved by beta elimination through the carbon-5 atom, but the reaction is blocked with azacytosine, where carbon-5 is substituted by nitrogen. Thus, the enzyme remains covalently bound to DNA and its DNA methyltransferase function is blocked. In addition, the covalent protein adduction also compromises the functionality of DNA and triggers DNA damage signaling, resulting in the degradation of trapped DNA methyltransferases. As a consequence, methylation marks become lost during DNA replication (15). Azacytidine was approved by FDA in 2004. Cytarabine (AraC) is phosphorylated into a triphosphate form (Ara-CTP) involving deoxycytidine kinase (dCK), which competes with dCTP for incorporation into DNA, and then blocks DNA synthesis by inhibiting the function of DNA and RNA polymerases (16,17).

Cytarabine was approved by FDA in 1969. Hydroxyurea is a monohydroxyl-substituted urea (hydroxycarbamate) antimetabolite. Hydroxyurea selectively inhibits ribonucleoside diphosphate reductase, an enzyme required to convert ribonucleoside diphosphates into deoxyribonucleoside diphosphates, thereby preventing cells from leaving the G1/S phase of the cell cycle. Hydroxyurea is well absorbed after oral administration, converted to a free radical nitroxide in vivo, and transported by diffusion into cells where it quenches the tyrosyl free radical at the active site of the M2 protein subunit of ribonucleotide reductase, inactivating the enzyme (18) It was originally approved in 1967 as an antineoplastic drug for use in multiple Cancers. Although approved as a chemotherapeutic agent, its diverse set of mechanisms eventually led to FDA approval for the treatment of sickle cell disease (SCD) in 1998. Gemcitabine is phosphorylated in the cytoplasm by deoxycytidine kinase (dCK) to the monophosphate (dFdCMP) and then phosphorylated again by pyrimidine nucleoside monophosphate kinase (UMP-CMP kinase) to give gemcitabine diphosphate (dFdCDP) (19). The first phosphorylation by dCK is considered the rate-limiting step for dFdCDP and dFdCTP production. Gemcitabine triphosphate (dFdCTP) uses during DNA synthesis. Afterwards, another nucleotide triphosphate (dNTP) is incorporated, making the polymerase unable to proceed and then chain elongation is stopped (20). Gemcitabine was approved by FDA in 1996. George H. Hitchings opened another vision in the antimetabolite theory by describing the immune suppressive effects of Azathioprine, a pyrimidine analog of the early period (21). Azathioprine that was approved for the first time in 1968 was later re-approved for the treatment of several non-cancer diseases including rheumatoid arthritis and multiple sclerosis. Mycophenolate mofetil (MMF, CellCept(R)) is a prodrug of mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase (IMPDH). This is the rate-limiting enzyme in de novo synthesis of guanosine nucleotides. T- and B-lymphocytes are more dependent on this pathway than other cell types are. Moreover, MPA is a fivefold more potent inhibitor of the type II isoform of IMPDH, which is expressed in activated lymphocytes, than of the type I isoform of IMPDH, which is expressed in most cell types. MPA has therefore a more potent cytostatic effect on lymphocytes than on other cell types. This is the principal mechanism by which MPA exerts immunosuppressive effects. Three other mechanisms may also contribute to the efficacy of MPA in preventing allograft rejection and other applications.

First, MPA can induce apoptosis of activated T-lymphocytes, which may eliminate clones of cells responding to antigenic stimulation. Second, by depleting guanosine nucleotides, MPA suppresses glycosylation and the expression of some adhesion molecules, thereby decreasing the recruitment of lymphocytes and monocytes into sites of inflammation and graft rejection. Third, by depleting guanosine nucleotides MPA also depletes tetrahydrobiopterin, a co-factor for the inducible form of nitric oxide synthase (iNOS). MPA therefore suppresses the production by iNOS of NO, and consequent tissue damage mediated by peroxynitrite. CellCept(R) suppresses T-lymphocytic responses to allogeneic cells and other antigens. The drug also suppresses primary, but not secondary, antibody responses. The efficacy of regimes including CellCept(R) in preventing allograft rejection, and in the treatment of rejection, is now firmly established. CellCept(R) is also efficacious in several experimental animal models of chronic rejection (22). Mycophenolate (Mofetil) was approved as an immunosuppressive drug by FDA in 2000. During the same period, the use of a purine analog of Xanthine Oxidase, a key enzyme in the catabolism of purines, created a new area in the use of antimetabolites (23,24,25). Currently, allopurinol is commonly used in the treatment of hyperuricemia and gout.

Rationale of the antimetabolite theory has also worked in the treatment of viral infections. Acyclovir, an acyclic purine nucleoside analog, is a highly potent inhibitor of herpes simplex virus (HSV), types 1 and 2, and varicella zoster virus, and has extremely low toxicity for the normal host cells. This selectivity is due to the ability of these viruses to code for a viral thymidine kinase capable of phosphorylating acyclovir to a monophosphate, this capability is essentially absent in uninfected cells. The acyclovir monophosphate (acyclo-GMP) is subsequently converted to acyclovir triphosphate (acyclo-GTP) by cellular enzymes. The amounts of acyclo-GTP formed in HSV-infected cells are 40 to 100 times greater than in uninfected cells. Acyclo-GTP acts as a more potent inhibitor of the viral DNA polymerases than of the cellular polymerases. The DNA polymerases of HSV-1 and HSV-2 also use acyclo-GTP as a substrate and incorporate acycto-GMP into the DNA primer-template to a much greater extent than do the cellular enzymes, the viral DNA polymerase binds strongly to the acyclo-GMP-terminated template, and is thereby inactivated (26,27). Azidothymidine (AZT, 3 -azido-3 –deoxythymidine) is a frontline antiviral agent for the treatment of human immunodeficiency virus-1 (HIV-1) and prevention of transmission from mother to offspring. Like many antiviral drugs approved for HIV-AIDS therapy, AZT acts as an inhibitor of HIV reverse transcriptase (RT). AZT is believed to act as a DNA chain terminator because the 3 -hydroxyl group of thymidine is replaced with an azide group, rendering it impossible for the following nucleotide to be incorporated by DNA polymerases. Its therapeutic potential results from the fact that it is readily incorporated by HIV-RT but less well by cellular DNA polymerases (28). The newest and most important work on the antimetabolite theory is on remdesivir (RDV). RDV is a phosphoramidate prodrug of a 1-cyano-substituted nucleotide analogue (29). Its triphosphate form (RDV-TP) resembles ATP and is used as a substrate of several viral RNAdependent RNA polymerase (RdRp) enzymes or complexes. Remdesivir, being a prodrug, is metabolized into its active form competes with ATP for incorporation into RNA and inhibits the action of viral RNA-dependent RNA polymerase. This results in the termination of RNA transcription and decreases viral RNA production (30,31,32). Remdesivir is authorized by FDA for the treatment of hospitalized patients with severe COVID-19 disease on 1 May 2020.

Nowadays, some alternative treatments have started to take their place in the cancer treatment in addition to the treatment with antimetabolites. Panitumumab (INN), formerly ABX-EGF, is a fully human monoclonal antibody specific to the epidermal growth factor receptor (also known as EGF receptor, EGFR, ErbB-1 and HER1 in humans).EGFR is a transmembrane protein. Panitumumab works by binding to the extracellular domain of the EGFR preventing its activation. This results in halting of the cascade of intracellular signals dependent on this receptor (33). Panitumumab was approved by FDA in 2006. Rational therapies that target the RAS pathways might inhibit tumour growth, survival and spread. Several of these new therapeutic agents are showing promise in the clinic and many more are being developed. Targeting RAS signalling pathways in cancer therapy (34). Metabolic alterations in cancer can be driven by changes in signalling pathways involving kinases such as PI3K and mTOR, and transcription factors, including hypoxia inducible factor and MYC. These are important targets for cancer therapy in general and cancer metabolism in particular. Cancer cells increase their rate of glucose and glutamine metabolism for bioenergetic and anabolic purposes. These important external carbon sources are diverted to generate DNA, proteins and lipids that are required for cancer cell growth. Cancer-specific isoforms of enzymes involved in energy metabolism, anabolism and adaptation to low oxygen may be new druggable targets for cancer therapy with potentially improved therapeutic indices compared with current therapy (35). Emerging studies have begun to demonstrate that mitochondrial metabolism is potentially a fruitful arena for cancer therapy. Mitochondria have a well-recognized role in the production of ATP and the intermediates needed for macromolecule biosynthesis, such as nucleotides. Mitochondria also participate in the activation of signaling pathways. Overall, accumulating evidence now suggests that mitochondrial bioenergetics, biosynthesis and signaling are required for tumorigenesis. In this Perspective, we highlight recent developments in targeting mitochondrial enzyme metabolism for the treatment of cancer (36). Many proteins regulated by ubiquitylation control cellular processes relevant to tumorigenesis, such as cell-cycle progression, apoptosis, receptor downregulation and gene transcription. In recent years, substantial progress has been made in understanding the molecular basis of ubiquitin action in cancer-relevant processes. The ubiquitin system is a network of proteins dedicated to the ubiquitylation of cellular targets and the subsequent control of numerous cellular functions (37).

The Role of Key Enzymes of Purine and Pyrimidine Metabolism in Antimetabolite Theory

Extensive knowledge is available in the literature about the relations of the pyrimidine and pürine metabolism with numerous diseases including cancer, immune diseases, viral diseases, ischemia-reperfusion, atherosclerosis and tuberculosis. Use of the nucleotide forms of this metabolic pathway in the synthesis of DNA and RNA has rendered this metabolic pathway a key element. Nucleic acids defined as polynucleotides are not primary molecules, and are synthesized by specific enzymes using nucleotides. Inhibition of the enzymes of the pyrimidine and purine pathway through irreversible and competitive inhibition mechanisms limits or inhibits the replication and transcription processes. Another issue that adds to the value of the antimetabolite theory is the use of some synthetic analogs in place of the natural substrates of enzymes and entry of the analog molecules into the DNA and RNA synthesis in the intracellular medium because of their similarity with the natural substrates, and inhibit both of these key molecules. We have a lot to discover about PRPP synthetase, hypoxanthine guanine transferase (HGPRT), ribonucleotide reductase (RR), and thymidine kinase (TK) . In addition, adenosine deaminase (ADA), 5 'nucleotidase (5'NT) and citidine deaminase (CD), which have an important role in purine and pyrimidine degradation pathways, may be important targets in this field. In general, the de-novo pathway, salvage enzymes and catabolic pathway enzymes are increased in cancer cells and other cells with high rates of proliferation. As a result, the general expectation for cancer patients is the increased uric acid levels. The reason for the activation of all the three pathways in the cancer cell is related to the need for nucleotides (38,39,40,41,42,43,44). In the catabolism of purine with xanthine oxidase, generation of oxygen radicals and hydrogen peroxide in both steps during the use of hypoxanthine and xanthine as substrates to synthesize uric acid makes this metabolic pathway a key in another sense. Xanthine oxidase (XO) plays the most important role in the enzymatic production of oxygen derivative radicals. It is possible that xanthine oxidase plays a significant role particularly in cancer metabolism in regard to the unification of nucleotides and radical metabolisms.In this respect, Xanthine oxidase is an important target as regards the use of new generation antimetabolites in both cancer treatment and prevention of intracellular damage arising from damages related to radicals (45,46,47). Hypoxanthine is the common substrate of both XO and HGPRT, and this places it in the position of an intersection. It is the key enzyme for the HPRT purine salvage pathway that uses hypoxanthine and guanine as substrates. The enzyme catalyzes the conversion of guanine and hypoxanthine to the respective nucleoside monophosphates, by using PRPP as donor of the phosphoribosyl moiety. The enzyme transforms guanine and hypoxanthine produced in the catabolic pathway to IMP and GMP. IMP and GMP synthesis are important with respect to the shortening of the metabolic pathway and the energy balance. Conversion of IMP and GMP into ATP and GTP, respectively, is important for cells if the proliferation and growth rates are high.

The problematic issues for this enzyme are that it has two substrates, its kinetics is more complex as compared to the other single substrate enzymes; and limitation of methods based on enzyme activity measurements or use of radioactive substrates limit the studies on this enzyme (48,49). Blockage of this pathway particularly in cancer cells can significantly reduce the DNA synthesis and proliferation rate of the cells that proliferate rapidly. Studies on (HPRT) are generally about the Lesch–Nyhan disease. This condition that presents with neurologic defects and hyperuricemia fully displays the significance of the purine metabolism for the brain. HPRT is found to have an important role in the regulation of the pluripotent human stem cells purinergic signaling system (50,51). Phosphoribosyl diphosphate (PRPP), which is the third substrate of HGPRT is an important intermediate in cellular metabolism. PRPP is used in both purine de nova pathway and purine salvage pathway, and as a ribose and phosphate source in pyrimidine synthesis, and it is the key substrate in direct nucleotide synthesis from the base. HGPRT can play a key role in cancer treatment as the key enzyme to prevent PRPP synthesis targeted according to the antimetabolite theory. HGPRT still carries important secrets in terms of enzyme science. PRPP is synthesized by PRPP synthetase. PRPP is ubiquitously found in living organisms and is used in substitution reactions with the formation of glycosidic bonds. PRPP synthetase enzyme is the first and key step in nucleotide synthesis. Purine is subject to negative allosteric adjustments of the enzyme end-products of its metabolism.Increase of PRPP in tumor cells is seen with poor prognosis. Together with this, down regulation of this enzyme inhibits the cell proliferation and tumor growth. With the increase of our knowledge on the diversity of enzymatic isoforms of PRPP synthetase and better understanding of its kinetic properties, its specific inhibitors can be the target of antimetabolites (52). Phosphorylase activity being shown for the 5’ nucleosidase, one of the key enzymes of the purine pathway, in addition to its phosphatase activity contrary to what is believed, diversity of substrates and particularly use of synthetic pyrimidines as substrate provide investigators with important possibilities. 5 'NT has kinase and phosphatase activity in the organism and this feature makes the enzyme special.This shows us the importance of studies on enzymes and enzyme kinetics. 5’ NT enzyme that transfers phosphate to various intracellular nucleotides can have an important place in the antimetabolite theory like the other kinases (53,54,55). Thymidine kinase has an effect similar to that of the kinase activity of 5’NT, and has substrate specificity. TK1 converts the deoxythymidine (dT) nucleoside to deoxythymidine monophosphate (dTMP). It takes the phosphate group from ATP enzymatically (56). Thymidine kinase enzyme can be used for diagnosis and prognosis in cancer patients.his enzyme, of which the molecular structure has been clarified, can be a key enzyme in the discovery of new active metabolites. It can be used in key enzyme of salvage pathway. The most basic property of kinases is related to the definition of their substrate affinities.In vitro description of the kinetics related to the use of synthetic nucleoside analogs by kinases and investigation of their effects on proliferation of tumor cells will widen the usage areas as antimetabolites of substrates of kinases including thymidine kinase. Discovery of new substrates outside the natural substrates of catabolic enzymes in purine and pyrimidine metabolism such as phosphatase and deaminase can open new horizons in the nucleoside metabolism. Cytidine deaminase deaminate cytidine to uridine play an important role in a variety of pathways from bacteria to man. Ancestral members of this family were able to deaminate cytidine only in a mononucleotide or nucleoside context. This enzyme plays a key role particularly in the catabolic pathway of purines. Enzymes that Recently, a family of enzymes has been discovered with the ability to deaminate cytidines on RNA or DNA. The first member of this new family is an RNA-binding cytidine deaminase (APOBEC1) which deaminates apolipoprotein B messenger RNA to generate a premature stop codon. APOBEC1 has the conserved active site motif found in Escherichia coli cytidine deaminase. In addition, APOBEC1 has a unique motif containing 2 phenylalanine residues and an insert of 4 amino acid residues across the active site motif. This motif is present in APOBEC family members including activation-induced cytidine deaminase (AID), APOBEC2, and APOBEC3A through APOBEC3G. AID is essential for initiating class-switch recombination, somatic hypermutation, and gene conversion. The APOBEC3 family is unique to primates. APOBEC3G is able to protect cells from human immunodeficiency virus and other viral infections. This function is not unique to APOBEC3G; other APOBEC3 family members also have this ability. Overexpression of enzymes in this family can cause cancer, suggesting that the genes for the APOBEC family of proteins are proto-oncogenes.

These expanding evidences suggest that the APOBEC family of cytidine deaminases plays an important role in antiviral innate immunity and might be a novel target for an antiviral therapy (57,58,59,60,61).

CONCLUSION

Antimetabolite theory is based on a very simple rationale. While the simplicity of the rationale has remained valid starting from the introduction of the theory, limitations of this field direct the investigators to more popular areas with different reasons. The rate of development of the knowledge on nucleic acids and studies on the application of this knowledge to technology have overshadowed especially this important field. Key enzymes that carry basic information in nucleic acid and purine-pyrimidine metabolism are at the center of the antimetabolite theory. Increasing our knowledge of enzymes will not only pave the way for the use of new antimetabolites in this field, but also improve our knowledge of other important metabolic functions, particularly nucleic acid and nucleotide metabolism. Generation of synthetic proteins by bioengineering, discovery of antimetabolites that target membrane receptors of cancer cells, synthesis of key enzyme inhibitors through protein-protein interactions could lead to new developments. If we improve our knowledge of the kinetics of key enzymes of nucleotide and nucleic acid metabolism, We will be able to make significant advances in this area. Although there is a long road ahead, antimetabolite theory will guide us in this area.

Conflict of interest

No conflict of interest was declared by the author.

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