Section 4
Cancer and Infectious Diseases

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Recent Advances in Antimetabolite Cancer Chemotherapies

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1. INTRODUCTION

An antimetabolite interferes with the formation or utilization of a normal cellular metabolite. Most antimetabolites interfere with the enzymes involved in the synthesis of new DNA, are incorporated into the newly formed DNA, or in some cases both processes are important to an agent's efficacy. As a result, many antimetabolites are derivatives of the building blocks of DNA itself, such as the nucleoside based inhibitors, or analogs of critical cofactors such as the antifolates. A variety of key cellular pathways have been disrupted with antimetabolite therapy, including inhibition of the thymidine and purine nucleotide biosynthesis pathway, and the inhibition of ribonucleoside reductase. Given their mechanism of action, it is not surprising that the observed benefits of antimetabolites are often accompanied by significant toxicity, due to the fact that the affected cellular metabolites are critical to both normal and cancer cells. Single antimetabolite agents can act on a single pathway, or on multiple pathways at once, but in either instance, they are often used in combination with other therapies in the clinic.

2. MECHANISMS OF ANTIMETABOLITE CANCER CHEMOTHERAPY

2.1. Thymidine biosynthesis

Critical to the cell's process of replication is its ability to synthesize thymidine. This process involves several key enzymes including thymidylate synthase (TS),

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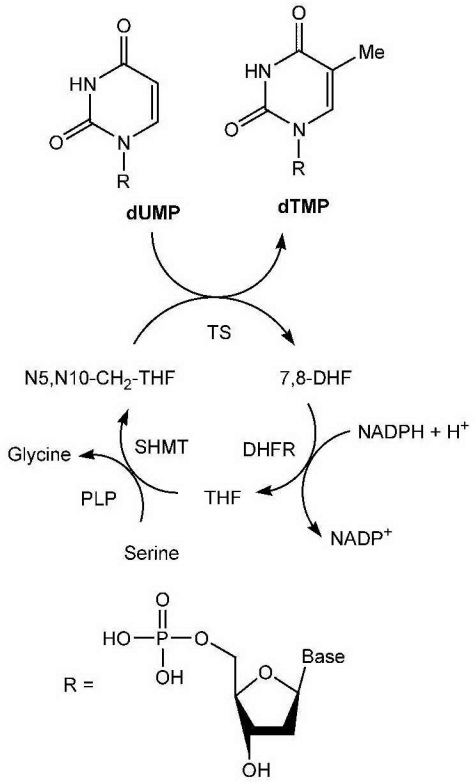

Fig. 1. Key steps in thymidine biosynthesis.

dihydrofolate reductase (DHFR), and serine hydroxymethyl transferase (SHMT) (Fig. 1).

The methylation of deoxyuridine 5′ monophosphate (dUMP) to produce deoxythymidine 5′ monophosphate (dTMP) is mediated by TS [1]. The methyl group for dTMP is provided by N5,N10-methylene tetrahydrofolate (N5,N10-CH_2-THF) through its conversion to 7,8-dihydrofolate (7,8-DHF). The 7,8-DHF must then be converted to tetrahydrofolate (THF) by DHFR [2], followed by further transformation back to N5,N10-CH_2-THF through the action of SHMT [3]. Therefore, inhibition of TS, DHFR, or SHMT with an appropriate agent would interrupt the process of thymidine biosynthesis. Low thymidine levels cause defects in DNA which in turn activates stress response elements, such as the Fas ligand/Fas death pathway leading to apoptosis [4]. It has also been proposed that defects in this Fas-dependent apoptotic signaling pathway are one cause of cellular resistance to drugs.

2.2. Purine nucleotide synthesis

The cell's ability to provide the needed purine nucleotides for DNA and RNA synthesis is also critical to its survival. The *de novo* biosynthesis of purine nucleotides involves 10

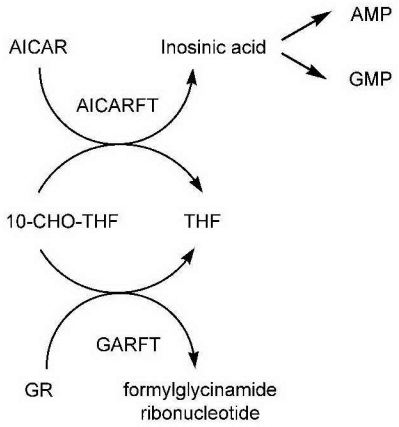

Fig. 2. Key step in purine biosynthesis.

separate enzyme catalyzed reactions starting with 5-phosphoribosyl-1-pyrophosphate and leading to inosinic acid [5]. Both adenosine monophosphate (AMP) and guanine monophosphate (GMP) are then derived from inosinic acid (Fig. 2). The third step in this process is the biosynthesis of formylglycinamide ribonucleotide from glycinamide ribonucleotide (GR) via glycinamide ribonucleotide formyltransferase (GARFT). The last two steps in the synthesis of inosinic acid occur via a bifunctional enzyme having both aminoimidazolecarboxamide ribonucleotide formyltransferase (AICARFT) and inosine monophosphate cyclohydrolase (IMPCH) activity. This enzyme has been shown to be made up of a 39 kDa carboxy-terminal AICARFT active fragment along with a 25 kDa amino-terminal IMPCH active fragment [6]. Both GARFT and AICARFT catalyze the transfer of a formyl group from 10-CHO-tetrahydrofolate (10-CHO-THF) to GR or aminoimidazolecarboxamide ribonucleotide (AICAR) respectively, returning THF as the second product of the reaction.

2.3. Ribonucleoside reductase

The synthesis of new DNA within a cell requires the production of deoxynucleotides. The four required deoxynucleotides (adenosine, guanosine, cytidine, and thymidine) are produced as by reduction of the appropriate ribonucleotide substrate with ribonucleoside reductase, also referred to a nucleoside diphosphate reductase (NDPR) [7]. The resulting oxidized form of NDPR can then be reduced back to NDPR by the action of glutaredoxin, which is in turn oxidized to thioredoxin [8]. NDPR is a dimer, with each monomer made up of two subunits: a larger (M1) and a smaller (M2) subunit. The M1 subunit contains two allosteric sites involved in regulation of the overall activity of the enzyme and the enzyme's substrate specificity. The deoxynucleoside triphosphates bind to this allosteric site, and regulate their own synthesis. The M2 subunit is responsible for the key reduction reaction, carrying a tightly bound iron atom that stabilizes the tyrosyl free radical critical to reduction. Deoxynucleotide pools in proliferating cells are

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