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ORIGINAL ARTICLE

Effects of antibiotic antitumor drugs on nucleotide levels in cultured tumor cells: an exploratory method to distinguish the mechanisms of antitumor drug action based on targeted metabolomics

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Nucleotides;
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Abstract Nucleotide pools in mammalian cells change due to the influence of antitumor drugs, which may help in evaluating the drug effect and understanding the mechanism of drug action. In this study, an ion-pair RP-HPLC method was used for a simple, sensitive and simultaneous determination of the levels of 12 nucleotides in mammalian cells treated with antibiotic antitumor drugs (daunorubicin, epirubicin and dactinomycin D). Through the use of this targeted metabolomics approach to find potential biomarkers, UTP and ATP were verified to be the most appropriate biomarkers. Moreover, a holistic statistical approach was put forward to develop a model which could distinguish 4 categories of drugs with different mechanisms of action. This model can be further validated by evaluating drugs with different mechanisms

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ANOVA, analysis of variance; ATP, adenosine triphosphate; AUC, area under the curve; CDP, cytidine diphosphate; CTP, cytidine triphosphate; dATP, deoxyadenosine triphosphate; dCDP, deoxycytidine diphosphate; dCTP, deoxycytidine triphosphate; dGMP, deoxyribonucleic monophosphate; dGTP, deoxyguanosine triphosphate; DMEM, Dulbecco's modified eagle's cell culture media; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; dUDP, deoxyuridine diphpsphate; dUTP, deoxyuridine triphosphate; EC, energy charge; EDTA, ethylene diamine tetra-acetic acid; FCS, fetal calf serum; GDP, guanosine diphosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PCA, principal component analysis; RNA, ribonucleic acid; ROC, receiver operating characteristic; RPMI-1640, Roswell Park Memorial Institute-1640; TBAHS, tetrabutylammonium hydrogen sulfate; TCA, trichloroacetic acid; UDP, uridine diphosphate; UTP, uridine triphosphate

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Antibiotic anticancer drugs;
Principal component analysis;
Ion-pair HPLC

of action. This targeted metabolomics study may provide a novel approach to predict the mechanism of action of antitumor drugs.

1. Introduction

Nucleotides are the foundation of all physiological functions. Cancer is a kind of genetic disease and genetic changes will bring about disturbances of nucleotide pools. Therefore, the fluctuation of nucleotide pools in cells can be considered as biomarkers to help reveal the occurrence and development of malignancies, and may be used to evaluate therapeutic effects in many different kind of cancers.

The relationship between nucleotide levels and malignancies has been verified by many studies. Growth stage changes in tumor cells are usually accompanied by alterations of nucleotide pools; for example, a higher rate of utilization of purine precursors has been shown during the proliferation phase than plateau phase[1]. Some studies have indicated that tumors are associated with abnormal concentrations of normal or modified nucleosides[2–4]. Moreover, the levels of nucleotide pools in tumor cells are different from normal cells. For example, the levels of GMP and GDP both were higher in colon cancer tissues than in normal colon tissues, while only GMP was increased in gastric carcinoma[5]. The activity of *de novo* synthesis of pyrimidine in MCF7 cells was 4.4 times higher than in MCF10A cells[6].

Metabolomics, representing holistic thinking derived from genomics and proteomics, is widely used in the areas of disease diagnosis, drug development, plant metabolomics, nutritional science and microbial metabolomics[7–11]. It aims at quantitative analysis of all low molecular mass metabolites and searches for the relationship between metabolites and changes in physiology and pathophysiology by combining different analytical technologies with calculation methods. The levels of nucleotides in cells will be altered significantly by perturbations provided by pharmaceuticals or environmental factors[12,13]. Clayton et al.[14] first proposed the concept of "pharmaco-metabonomics" in 2006: analyzing metabolic profiles of biological samples could be a way to predict variations of metabolic phenotypes after treatment with drugs. Over the last several decades, many scholars have investigated the dynamic changes in cell metabolism, cell physiological status, and the activity of key enzymes after drug treatment. Zhang et al.[15] did research on the effect of DNA synthetase inhibitor–aphidicolin on deoxyribonucleotide pools in human pancreatic carcinoma PANC1 cells and found that all components were increased except dUTP. Sokoloski et al.[16] found that the level of ATP decreased and UTP increased in HL60 cell lines after inhibiting the activity of glycinamide nucleotidyltransferase. Studies by Iwasaki et al.[17] showed that ribonucleotide reductase inhibitors could prevent *de novo* dCTP biosynthesis which benefited are-CTP mixing into DNA, suggesting that the antitumor activity of this category could be reinforced by combination with nucleotide reductase inhibitors. Studies on the effects of gemcitabine on ribonucleotides pools in leukemia cell lines and some solid tumors showed that the level of dNTP in cells was related to drug susceptibility, and wide differences among NTP levels in different tumor cells reflected different interactions between cells and drugs[18–19]. Monitoring these changes in nucleotide pools will help to predict the therapeutic effects of antitumor drugs and reveal the targets of drug action. Although basic analysis methods applied to cells have long been used to aid in research on effects of drugs on metabolites, the study of tumor cells for distinguishing different mechanisms of drug action has not been investigated.

In our previous research, Zhang et al.[20] developed a reliable, simple and reproducible method to determine the levels of 12 nucleotides simultaneously by using an ion-pair HPLC and analyzed the differences in nucleotide levels between normal cells and tumor cells. The method above was further used to explore three categories of chemotherapeutic drugs with different mechanisms of action by Liu et al.[21]. Three classes of antitumor drugs, including antimetabolic agents, agents directly acting on DNA and antimitotic agents, were selected to explore their effects on nucleotides in cells in order to find potential biomarkers associated with drugs. Based on the results of several analytic approaches, GMP and ATP were chosen as the best potential biomarkers for DNA-damaging drugs, while ATP, UDP and GMP were identified for two other categories of drugs. However, the method we used was not sufficient to develop a model to distinguish antitumor drugs with different mechanisms of action individually.

In this pilot study, daunorubicin (DNR), epirubicin (EPI) and dactinomycin D (ACD), with completely different mechanisms of action were evaluated and the best potential biomarkers were found. Based on the earlier study, all 12 chemotherapeutic drugs were classified into four groups by mechanism of drug action, leading to a new and discriminatory model. Moreover, different cell sensitivities to drugs were found which could affect the ability to accurately recognize mechanisms of drug action.

2. Methods

2.1. Chemicals and reagents

All standards (UTP-Na, ADP-Na_2, AMP-Na_2, ATP-Na_2, CTP-Na_2, GDP-Na_2, GMP-Na_2, UDP-Na_2, dUTP-Na_3, dATP-Na_3, dCTP-Na_3, dGTP-Na_4) were purchased from Sigma (St. Louis, USA). Tetrabutylammonium hydrogen sulfate (TBAHS) was obtained from Tianjin Kermel Chemical Reagent Co., Ltd. Methanol (HPLC grade) was obtained from Shandong Yuwang Industrial Co., Ltd. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypsin were provided by Amresco LLC (Solon, USA). All other chemical reagents used, analytical grade, were purchased from Tianjin Bodi Chemical Holding Co., Ltd. Dulbecco's modified eagle's cell culture media (DMEM), Roswell Park Memorial Institute (RPMI) 1640 media, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and fetal calf serum (FCS) were purchased from Gibco-BRL (Grand Island, USA).