

Effects of HIV on metabolic and biological pathways of CD4⁺ T lymphocytes

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Received July 28, 2017; Accepted December 12, 2017

DOI: 10.3892/etm.2018.5749

Abstract. The effects of human immunodeficiency virus (HIV) on the metabolic and biological pathways of cluster of differentiation (CD)4⁺ T lymphocytes were investigated. A total of 150 patients with acquired immune deficiency syndrome (AIDS) and 50 healthy individuals who were admitted to hospital for physical examination during the period of June 2016 to January 2017, were selected as subjects in the present study. According to the virus load, 150 AIDS patients were divided into three groups: i) Viral load >10⁶ copies/ml (group A, n=39), ii) 10⁴ copies/ml < viral load <10⁵ copies/ml (group B, n=76), and iii) viral load <10⁴ copies/ml (group C, n=35). The relationship between viral loads in the three groups and CD4⁺ T lymphocyte counts was assessed. Active lymphocytes were isolated from T lymphocytes in the subjects, and the ratio of Th1 to Th2 was measured by flow cytometry. Effects of HIV on human T-lymphocyte differentiation were observed. Differences in T-lymphocyte metabolites were detected by proton nuclear magnetic resonance and their biological pathways analyzed. The results showed that CD4⁺ T-cell counts were decreased with the increase of the viral loads of patients. The viral loads of AIDS patients differentiated T lymphocytes. In other words, high viral loads accelerated the differentiation of T lymphocytes into Th1 cells. In the high HIV viral load group, the levels of glycerol phosphodiesterase, 7-dehydrocholesterol, p-hydroxyphenylacetic acid, cholesterol and deoxyuridine were increased, but the levels of 3-methoxytyramine, cytidine deaminase, deoxycorticosterone and 3-hydroxybutyric acid were decreased. The viral loads of AIDS patients are associated with CD4⁺ T-cell counts and the ratio of CD4⁺ T to CD8⁺ T cells. At the same time, HIV viral loads can affect the lipid

biosynthesis of T-lymphocyte membranes, thus affecting the differentiation and proliferation of T lymphocytes and finally intervening its mediated immune responses.

Introduction

Acquired immune deficiency syndrome (AIDS) is a type of acquired immune deficiency syndrome caused by human immunodeficiency virus (HIV) infection. The number of AIDS patients has been on the increase annually (1). The main transmission modes of the disease include sexual, blood and mother-to-fetus transmission.

At present, the main diagnosis of AIDS is based on laboratory tests. The number of peripheral blood cluster of differentiation (CD)4⁺ T lymphocytes and viral load can be used as important test indicators to assess the severity of AIDS patients, determine the progression of the disease and evaluate the prognosis of AIDS patients following antiviral therapy (2). The CD4⁺ T lymphocyte is one of the central cells involved in immune responses *in vivo*. HIV mainly infects CD4⁺ T lymphocytes. T lymphocytes in healthy individuals contain approximately 65% of CD4⁺ T lymphocytes and 35% of CD8⁺ T lymphocytes (3,4). Once HIV infection occurs, the body's immune system is damaged resulting in decreased CD4⁺ T lymphocyte and dysfunction as well as increased CD8⁺ T lymphocyte levels, eventually leading to CD4⁺/CD8⁺ imbalance. Therefore, the main assessment indicator is immune system damage condition in AIDS patients. In other words, the CD4⁺ T lymphocyte expression level is used to determine whether the immune cell function is disordered (5-7). The detection of viral load in the peripheral blood of patients is the most important factor affecting the progress of AIDS (8).

Therefore, in the present study, we analyzed the relationship of viral loads with CD4⁺ T-cell counts, T lymphocyte differentiation and metabolites in AIDS subjects. The aim of the present study was to provide a theoretical basis for conducting antiviral therapies as early as possible to reduce the incidence and death rate of AIDS.

Materials and methods

General materials. A total of 150 AIDS patients admitted to Qingdao Sixth People's Hospital (Shandong, China) from June

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Key words: viral load, acquired immune deficiency syndrome, human immunodeficiency virus, CD4⁺ T lymphocytes

2016 to January 2017 and 50 healthy individuals admitted to hospital for physical examination during the same period were selected as subjects. There were no significant differences in sex and age between the two groups ($P > 0.05$). Therefore, after the effects of the above factors on the test results were excluded, the data were comparable.

Inclusion criteria for the study were: i) Patients aged 18-65 years; ii) either males or females were included; iii) all the subjects were diagnosed as anti-HIV-1-positive patients; iv) patients had not received antiviral therapy and had complete baseline data records; and v) subjects and their family members signed the informed consent to participate in the study.

Exclusion criteria for the study were: i) Patients infected with hepatitis viruses; ii) patients with autoimmune diseases; iii) patients with malignant tumors; iv) patients with severe cardiovascular or cerebrovascular diseases; and v) mental patients.

Grouping of subjects. According to the viral load, 150 patients with AIDS were divided into three groups: i) Viral load $> 10^6$ copies/ml (group A, $n=39$), ii) 10^4 copies/ml $<$ viral load $< 10^5$ copies/ml (group B, $n=76$), and iii) viral load $< 10^4$ copies/ml (group C, $n=35$).

Detection of HIV viral loads. Ethylenediaminetetraacetic acid dipotassium salt dihydrate (EDTA-K2) anticoagulants were taken and centrifuged for 10 min at $1,300 \times g$ to separate the plasma. The separated plasma was stored in the refrigerator at -70°C for standby application. Plasma ($1,100 \mu\text{l}$) was taken from each sample and the HIV RNA content in the plasma samples was detected by reverse transcriptase-polymerase chain reaction in an automatic virus loader (Roche Diagnostics, Basel, Switzerland). During the operation, an internal standard for quantification, a strong positive, a weak positive and a negative control were set, respectively.

Detection of the number of CD4⁺ T lymphocytes. The levels of CD4⁺ T lymphocytes were detected in accordance with the Guidelines for Quality Assurance of CD4⁺ T Lymphocytes Detection and the National AIDS Test Specification as follows: The test specimens and Immuno-Trol™ controlled blood were operated simultaneously; the CD4 fluorescent-marked antibodies ($20 \mu\text{l}$) and mixed anticoagulants ($50 \mu\text{l}$) were successively added to the tubes for detection using flow cytometry (FCM), and then incubated for 15 min at room temperature. The CaliBRITE™ beads and FACSCOMP™ software were used to calibrate the FCM before the operation. Hemolysin was added to the specimens and incubated in the dark at room temperature for 15 min, and the levels of CD4⁺ T lymphocytes were detected using the aforementioned software. The results were retrieved and automatically analyzed by MultiSET™ software.

Detection of T-lymphocyte differentiation. Active lymphocytes were isolated from T lymphocytes in subjects, and T lymphocytes and CD3⁺CD8⁺IFN- γ +(Th1)/CD3⁺CD8⁺IL-4+(Th2) cells were isolated and analyzed by FCM. At the same time, the ratio of Th1 to Th2 was measured by FCM, and the effects of HIV on human T-lymphocyte differentiation were observed.

Table I. Results of HIV viral load detection.

HIV viral load (copies/ml)	Percentage (n, %)
$> 10^6$	39/150 (26.00)
10^4 - 10^5	76/150 (50.67)
$< 10^4$	35/150 (23.33)

Detection of metabolites of T lymphocytes cultured in vitro and analysis of their biological pathways. The metabolites of T lymphocyte lysates were analyzed using proton nuclear magnetic resonance. The differences in T-lymphocyte metabolites among groups were analyzed, and the biological pathways were then analyzed.

Statistical analysis. The data in this study were all analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) software. The measurement data were analyzed by analysis of variance. The data results were expressed as mean \pm standard deviation, and verified using a t-test and Chi-square test. The Mann-Whitney U non-parametric test was performed for variables. The grouping variable was 'group' and test variable was 'chemical shift value (shift)'. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Detection of HIV viral load. Results of the detection on the HIV viral load of AIDS patients showed that the highest HIV viral load was 3.8×10^7 copies/ml and the lowest was 6.2×10^2 copies/ml in 150 patients. Of these, 39 subjects had $> 10^6$ copies/ml HIV viral load, 76 patients had 10^3 - 10^5 copies/ml HIV viral load and 35 subjects had $< 10^4$ copies/ml HIV viral load, accounting for 26.00, 50.67 and 23.33%, respectively (Table I).

Association between the HIV viral load of AIDS patients and CD4⁺ T lymphocyte counts. CD4⁺ T lymphocyte counts of subjects in group A were < 50 cells/ μl , accounting for 71.79% of the total number of group A; CD4⁺ T lymphocyte counts of subjects in group B were mainly ≤ 400 cells/ μl . In group C, 23 subjects had 200-399 cells/ μl CD4⁺ T lymphocyte counts, accounting for 65.71% of the total subjects in group C (Table II).

Association between HIV viral load and T lymphocyte differentiation. HIV viral loads of AIDS patients differentiated T lymphocytes. In other words, high HIV viral loads accelerated the differentiation of T lymphocytes to Th1 cells (Table III).

Detection of metabolites of human T lymphocytes cultured in vitro. The Mann-Whitney U non-parametric test was performed for the variables. The test results showed that different HIV viral loads exerted significant effects on the metabolites of T lymphocytes. There were a total of 11 chemical shift values representing significant changes in metabolites (Table IV).

We searched the Human Metabolome Database and selected endogenous metabolites with high matching degree

Table II. Association between the HIV viral load of AIDS patients and CD4⁺T lymphocyte counts.

Groups	CD4 ⁺ T lymphocyte counts (cells/ μ l)				χ^2 value	P-value
	<50	50-199	200-399	\geq 400		
A (n, %)	28/39 (71.79)	4/39 (10.26)	5/39 (12.82)	2/39 (5.13)	10.764	0.001
B (n, %)	20/76 (26.32)	28/76 (36.84)	20/76 (26.32)	8/76 (10.53)	6.979	0.004
C (n, %)	0/35 (0.00)	12/35 (34.29)	23/35 (65.71)	0/35 (0.00)	12.582	<0.01
χ^2 value	11.524	8.221	9.275	13.124	-	-
P-value	<0.01	0.001	0.007	0.001	-	-

in the database. After checking the displacement variables, we found that most metabolites were intermediates metabolized by lipids, end proteins, which were closely related to organic acid, amino acid, purine pyrimidine, lipid metabolism and other metabolic pathways. The detection results showed that glycerol phosphocholine diesterase, 7-dehydrocholesterol, p-hydroxyphenylacetic acid, cholesterol and deoxyuridine levels were increased in the high HIV viral load group, whereas 3-methoxytyramine, cytidine deaminase, deoxycorticosterone and 3-hydroxybutyric acid were reduced (Table V).

Discussion

HIV-1 AIDS is a kind of cellular immunodeficiency disease. Its main feature is that after T lymphocytes are damaged and HIV-1 enters the body, the disease does not immediately develop into AIDS, but goes through an asymptomatic period (9,10). The current international assessment determines the progression of AIDS mainly based on two indicators, that is, HIV-1 viral loads in the peripheral blood and CD4⁺ T lymphocyte counts (11). Foreign scholars have found that the expression levels of CD4⁺ T lymphocytes in AIDS patients are closely related to their HIV-1 virus loads. HIV-1 can directly attack CD4⁺ T lymphocytes, causing the body's immune dysfunction and different degrees of damage to other immune cells. Another study has suggested that, CD4⁺ T lymphocyte count levels can accurately reflect the immune status of the body, and the exacerbation of HIV-1 AIDS patients can be reflected by largely reduced expression levels of CD4⁺ T lymphocytes in the short term (12,13).

In the present study, the detection results of the viral load of AIDS patients showed that the highest viral load in the 150 patients was 3.8×10^7 copies/ml while the lowest was 6.2×10^2 copies/ml, in which 39 subjects had more than 10^6 copies/ml HIV viral load, 76 patients had 10^3 - 10^5 copies/ml HIV viral load and 35 subjects had less than 10^4 copies/ml HIV viral load, accounting for 26.00, 50.67 and 23.33%, respectively. Results of the analysis of the relationship between viral loads and CD4⁺ T lymphocyte counts in AIDS patients showed that CD4⁺ T lymphocyte counts of subjects in group A were less than 50 cells/ μ l, accounting for 71.79% of the total number of group A; while CD4⁺ T lymphocyte counts of subjects in group B were mainly no more than 400 cells/ μ l. In group C, 23 subjects had 200-399 cells/ μ l CD4⁺ T lymphocyte counts, accounting for 65.71% of the total subjects in group C. It could be inferred that the higher the viral load was, the lower was the CD4⁺ T

Table III. Association between HIV viral load and T lymphocyte differentiation (mean \pm SD).

Groups	T-lymphocyte differentiation (Th1/Th2)
A (n=39)	35.28 \pm 4.37
B (n=76)	26.45 \pm 2.91
C (n=35)	18.75 \pm 2.37
F-value	15.97
P-value	0.003

lymphocyte count. The occurrence mechanism may include the following six aspects: i) HIV infection may directly kill CD4⁺ T lymphocytes; ii) HIV DNAs not integrated in the cytoplasm could infect CD4⁺ T lymphocytes; iii) intracellularly replicated HIV may cause interference with the synthesis of proteins within the cells; iv) CD4⁺ T lymphocytes were damaged by specific cytotoxicity of cytotoxic T lymphocytes; v) Gp41 and gp120-mediated cell fusion cause accelerated infection of CD4⁺ T lymphocytes and cause death; and vi) immune dysfunction existed in patients prior to a sharp decrease in the expression levels of CD4⁺ T lymphocytes (14-16). The HIV viral load reflects the number of replicated HIVs within the body, and the number of CD4⁺ T lymphocytes represents the number of HIV-infected target cells, thus both can be used independently for the prediction of disease development and prognosis of HIV-1 AIDS patients (17,18). CD4⁺ T lymphocyte counts indicate different immune levels of the human body. A low or reduced CD4⁺ T lymphocyte count level has a parallel relationship with disease progression, which is a useful indicator for following disease development and is closely related to opportunistic infection and patient survival time. Thus, the more the number of replicated HIVs is, the more the number of CD4⁺ T lymphocytes are and the less the number of residual CD4⁺ T lymphocytes.

Changes in metabolic quantities reflect metabolic trends in major biochemicals, suggesting metabolic status and metabolic cell regulation, including energy, nucleotide and amino acid metabolism (19,20). From the present study, it was found that the differences between the different HIV viral load groups were significant, including differences in cholesterol, adenosine and deoxyinosine (dI). Adenosine and dI are the compounds of adenine metabolism. dI is the metabolite of deoxyadenosine (dA) after it has been hydrolyzed by adenosine deaminase for

Table IV. Displacement of metabolites of T lymphocyte cell lysates.

Shift (ppm)	z	P-value	Correlation coefficient	Coefficient
0.03	-2.265	0.009	-	-2.87214
0.67	-2.887	0.002	+	8.10393
1.35	-2.887	0.002	-	-29.7471
1.39	2.727	0.004	-	-2.02653
3.39	2.887	0.002	-	-3.03678
3.63 ^a	-2.727	0.004	+	18.4743
3.67	-2.727	0.004	+	15.6276
3.72	-2.082	0.041	+	8.52371
3.35	-2.882	0.002	-	-1.08921
3.43	-2.722	0.004	+	0.434467
3.47	-2.882	0.002	+	0.50965
3.51	-2.882	0.002	+	0.476585
3.59	-2.882	0.002	+	1.40816
4.12	-2.882	0.002	-	-1.493351
4.16	-2.882	0.002	-	-1.35287

^a '+', upregulation; '-', downregulation.

Table V. Metabolites of T lymphocyte cell lysates and relevant metabolic pathways.

Shift (ppm)	Endogenous	Possible endogenous metabolites	Pathway
0.03	Adenine	Adenine	Purine metabolic pathway
0.67	Cholesterol	Cholesterol	Bile acid metabolic pathway
1.35/1.39	Deoxycorticosterone	Deoxycorticosterone	Steroid metabolic pathway
3.39	L-carnitine	L-carnitine	Long-chain fatty acid β-oxidation metabolic pathway
3.63/3.67	7-Dehydrocholesterol	7-Dehydrocholesterol	Steroid metabolic pathway
3.72	Deoxyuridine	Deoxyuridine	Pyrimidine metabolic pathway
3.35	3-Methoxytyramine	3-Methoxytyramine	Tyrosine metabolic pathway
3.43/3.47/3.51	P-hydroxyphenylacetic acid	P-hydroxyphenylacetic acid	Tyrosine metabolic pathway
3.59	Glycerophosphocholine	Glycerophosphocholine	Glycerophosphoric acid metabolic pathway
4.12	Cytidine	Cytidine	Pyrimidine metabolic pathway
4.16	(R)-3-hydroxybutyric acid	(R)-3-hydroxybutyric acid	Ketone body metabolic pathway

deamination (21,22). Adenosine belongs to the intracellular endogenous nucleoside, which can participate in the synthesis of adenylyate, adenine and adenosine triphosphate, which are involved in cell energy metabolism. Adenosine and dA can lead to the accumulation of dA triphosphate (dATP), thus inhibiting the nucleotide reductase and eventually leading to lymphopenia and causing immune deficiency. The over-accumulation of dATP can inactivate adenosine homocysteine hydrolase, which affects involvement of the enzyme in the decomposition of adenosine homocysteine, and ultimately inhibits the physiological action of adenosylmethionine-dependent trans methylation and interferes with methylation-dependent processes. dA can inhibit adenosine homocysteine hydrolase, which induces the accumulation of adenosine homocysteine

hydrolase in lymphoblasts (23,24). dA is closely associated with lymphocyte toxicity and dATP accumulation as well as adenosine homocysteine hydrolase inactivation. Adenosine deaminase has a detoxification effect on adenosine and dA. Adenosine and dA can stimulate the adenylyate cyclase system, upregulate adenylyate deaminase activity, and ultimately accelerate the metabolism of T-lymphocyte nucleotides (25,26). The present study indicated that HIV viral load affected adenosine deaminase to catalyze the deamination of adenosine and dA, reduced their toxicity to lymphocytes, and then affected T lymphocyte-mediated immune responses (25,26). Cholesterol is an important substance for cell metabolism, which is involved in the formation of cell membranes, vitamin D and steroid hormones. It is also involved in maintaining the

physiological function of T lymphocytes (27,28). The results suggested that HIV viral loads may regulate the lipid biosynthesis of T lymphocyte membranes and affect the proliferation of T lymphocytes, thus interfering with its immune processes.

In summary, the viral loads of AIDS patients are associated with CD4⁺ T lymphocyte count and the ratio of CD4⁺T/CD4⁺8T. At the same time, HIV viral loads can affect the lipid biosynthesis of T lymphocyte membranes, thus affecting the differentiation and proliferation of T lymphocytes and finally interfering its mediated immune responses. Therefore, the levels of CD4⁺ T lymphocytes and viral loads of AIDS patients should be regularly measured in order to begin antiviral therapies as early as possible, thus reducing the incidence and death rates of AIDS.

Competing interests

The authors declare that they have no competing interests.

References

- Berhan Y and Berhan A: A meta-analysis of risky sexual behaviour among male youth in developing countries. *Aids Res Treat* 2015: 580961, 2015.
- Yang D, Zhao H, Gao G, Wei K, Zhang L, Han N, Xiao J, Li X, Wang F, Liang H, *et al*: Relationship between CD4(+) T lymphocyte cell count and the prognosis (including the healing of the incision wound) of HIV/AIDS patients who had undergone surgical operation. *Zhonghua Liu Xing Bing Xue Za Zhi* 35: 1333-1336, 2014 (In Chinese).
- Zhang ZN, Bai LX, Fu YJ, Jiang YJ and Shang H: CD4(+)IL-21(+) T cells are correlated with regulatory T cells and IL-21 promotes regulatory T cells survival during HIV infection. *Cytokine* 91: 110-117, 2017.
- He H, Xue J, Wang W, Liu L, Ye C, Cong Z, Kimata JT, Qin C and Zhou P: Efficient transduction of human and rhesus macaque primary T cells by a modified human immunodeficiency virus Type 1-based lentiviral vector. *Hum Gene Ther* 28: 271-285, 2017.
- Bhattar S, Mehra B, Bhalla P and Rawat D: Evaluation of baseline CD4⁺ T cell counts and ART requirement in newly diagnosed HIV seropositive individuals in a tertiary care hospital of Northern India. *J Clin Diagn Res* 10: 18-21, 2016.
- Majekodunmi AO, Thorne C, Malyuta R, Volokha A, Callard RE, Klein NJ and Lewis J: European Paediatric HIV/HCV Co-infection study group in the European Pregnancy and Paediatric HIV Cohort Collaboration and the Ukraine Paediatric HIV Cohort Study in EuroCoord: Modelling CD4 T cell recovery in Hepatitis C and HIV co-infected children receiving antiretroviral therapy. *Pediatr Infect Dis J* 36: 123-129, 2017.
- Abate D, Fison M, Saldan A, Cofano S, Mengoli C, Sgarabotto D, d'Agostino C, Barzon L, Cusinato R, Toscano G, *et al*: Human cytomegalovirus-specific T-cell immune reconstitution in preemptively treated heart transplant recipients identifies subjects at critical risk for infection. *J Clin Microbiol* 50: 1974-1980, 2012.
- Deeks SG, Lewin SR and Havlir DV: The end of AIDS: HIV infection as a chronic disease. *Lancet* 382: 1525-1533, 2013.
- Parkin NT: Measurement of HIV-1 viral load for drug resistance surveillance using dried blood spots: Literature review and modeling of contribution of DNA and RNA. *AIDS Rev* 16: 160-171, 2014.
- Touzard Romo F, Gillani FS, Ackerman P, Rana A, Kojic EM and Beckwith CG: Monitored viral load: A measure of HIV treatment outcomes in an outpatient setting in Rhode Island. *R I Med J* (2013) 98: 26-30, 2014.
- Yuan J, Wang J, Crain K, Fearn C, Kim KA, Hua KL, Gregory PD, Holmes MC and Torbett BE: Zinc-finger nuclease editing of human *cxcr4* promotes HIV-1 CD4⁺ T cell resistance and enrichment. *Mol Ther* 20: 849-859, 2012.
- Shu ST, Emert-Sedlak LA and Smithgall TE: Cell-based fluorescence complementation reveals a role for HIV-1 nef protein dimerization in AP-2 adaptor recruitment and CD4 co-receptor down-regulation. *J Biol Chem* 292: 2670-2678, 2017.
- Bakhshayesh-Karam M, Tabarsi P, Mirsaiedi SM, Amiri MV, Zahirifard S, Mansoori SD, Masjedi MR and Velayati AA: Radiographic manifestations of tuberculosis in HIV positive patients: Correlation with CD4⁺ T cell count. *Int J Mycobacteriol* 5: S244-S245, 2016.
- De Crignis E and Mahmoudi T: HIV eradication: Combinatorial approaches to activate latent viruses. *Viruses* 6: 4581-4608, 2014.
- Gorry PR, Francella N, Lewin SR and Collman RG: HIV-1 envelope-receptor interactions required for macrophage infection and implications for current HIV-1 cure strategies. *J Leukoc Biol* 95: 71-81, 2014.
- Yi G, Choi JG, Bharaj P, Abraham S, Dang Y, Kafri T, Alozie O, Manjunath MN and Shankar P: CCR5 gene editing of resting CD4(+) T cells by transient ZFN expression From HIV envelope pseudotyped nonintegrating lentivirus confers HIV-1 resistance in humanized mice. *Mol Ther Nucleic Acids* 3: 198, 2014.
- Schweitzer F, Mikkola V, Timmen-Wego M, Jensen B, Fätkenheuer G and Kaiser R: Longitudinal comparison of HIV-1 plasma viral load and cellular proviral load. *J Int AIDS Soc* 17: 19669, 2014.
- Hakre S, Chavez L, Shirakawa K and Verdin E: HIV latency: Experimental systems and molecular models. *FEMS Microbiol Rev* 36: 706-716, 2012.
- Sookoian S, Castaño GO, Scian R, Fernández Gianotti T, Dopazo H, Rohr C, Gaj G, San Martino J, Sevic I, Flichman D, *et al*: Serum aminotransferases in nonalcoholic fatty liver disease are a signature of liver metabolic perturbations at the amino acid and Krebs cycle level. *Am J Clin Nutr* 103: 422-434, 2016.
- Dudley J, DelBello MP, Weber WA, Adler CM, Strakowski SM and Lee JH: Tissue-dependent cerebral energy metabolism in adolescents with bipolar disorder. *J Affect Disord* 191: 248-255, 2016.
- Yoneshima Y, Abolhassani N, Iyama T, Sakumi K, Shiomi N, Mori M, Shiomi T, Noda T, Tsuchimoto D and Nakabeppu Y: Deoxyinosine triphosphate induces MLH1/PMS2- and p53-dependent cell growth arrest and DNA instability in mammalian cells. *Sci Rep* 6: 32849, 2016.
- Jeong YC, Zhang F, Geter DR, Wood AJ, Schisler MR, Gollapudi B and Bartels MJ: Measurement of deoxyinosine adduct: Can it be a reliable tool to assess oxidative or nitrosative DNA damage? *Toxicol Lett* 214: 226-233, 2012.
- Mishra V and Ronning DR: Crystal structures of the *Helicobacter pylori* MTAN enzyme reveal specific interactions between S-adenosylhomocysteine and the 5'-alkylthio binding subsite. *Biochemistry* 51: 9763-9772, 2012.
- McLaughlin M, Kong J, Belyk KM, Chen B, Gibson AW, Keen SP, Lieberman DR, Mileczek EM, Moore JC, Murray D, *et al*: Enantioselective synthesis of 4'-ethynyl-2'-fluoro-2'-deoxyadenosine (EFdA) via enzymatic desymmetrization. *Org Lett* 19: 926-929, 2017.
- Lonhi MS, Moss A, Bai A, Wu Y, Huang H, Cheifetz A, Quintana FJ and Robson SC: Characterization of human CD39⁺ Th17 cells with suppressor activity and modulation in inflammatory bowel disease. *PLoS One* 9: e87956, 2014.
- Tapbergenov SO, Sovetov BS and Tapbergenov AT: Features of influence adenosine, AMP and hyperadrenalinemiya on the immune status, metabolic enzymes of purine nucleotides and the antioxidant defense system. *Biomed Khim* 62: 645-649, 2016 (In Russian).
- Nus M, Sage AP, Lu Y, Masters L, Lam BYH, Newland S, Weller S, Tsiantoulas D, Raffort J, Marcus D, *et al*: Marginal zone B cells control the response of follicular helper T cells to a high-cholesterol diet. *Nat Med* 23: 601-610, 2017.
- Galimzyanov TR, Lyushnyak AS, Aleksandrova VV, Shilova LA, Mikhalyov II, Molotkovskaya IM, Akimov SA and Batishchev OV: Line activity of ganglioside GM1 regulates the raft size distribution in a cholesterol-dependent manner. *Langmuir* 33: 3517-3524, 2017.



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