Toxicity and mechanism studies of Selenium nanoparticles to human Vγ9Vδ2 T cells

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Abstract

γδT cells are an important subset of T lymphocytes γδT cells can be further divided into two major subpopulations, Vδ1 and Vδ2, depending on the TCR chain Vδ2 cell subsets have the characteristics of inhibiting bacterial infection and anti-tumor, and exist only in humans and primates Studies have shown that Vδ2 T cell subsets have a very important inhibitory effect on the occurrence and development of tumors and can significantly inhibit the growth of tumor cells Selenium is one of the essential trace elements in the human body It has many important physiological effects such as anti-tumor, anti-oxidation and antagonistic heavy metals At present, there are reports in the literature that Selenium nanoparticles(SeNPs) has excellent anti-tumor effect and has great potential for development as an anti-tumor drug carrier or adjuvant However, the influence of Selenium nanoparticles on immune cell function in vitro and in vivo has not been reported yet. The purpose of this work is to study the role of Selenium nanoparticles in regulating the function of human γδ T cells (herein referred to as Vδ2 T cell subsets), and to provide new methods for cell expansion and culture of anti-tumor immune cells based on γδT cells We used flow cytometry, microplate reader, confocal microscopy and cell energy metabolism to observe the γδT cells before and after the addition of Selenium nanoparticles As a result, after adding Selenium nanoparticles, the indexes of T cells decreased significantly, and Selenium nanoparticles Shows great toxicity to T cellsThis study shows that Selenium nanoparticles, which has a wide range of applications in the field of nanomaterials, has potentially great toxicity to theimmune system, which will significantly affect the application prospect of Selenium nanoparticles materials and put forward higher requirements for the further development of Selenium nanoparticles materials.

Keywords

Nanomaterials, selenium, T cells .

1. Introduction

At present, it has been proved that γδT cells can be used as a adoptive immunotherapy cell Compared with other immune cells, γδT cells have three main advantages: 1 γδT cells have a broad-spectrum anti-tumor effect, not only through direct contact The way to kill tumor cells can also secrete a variety of cytokines such as TNF-α, which can play a non-contact anti-tumor effect[1]; 2, γδT cells do not rely on antigen-presenting cells for the recognition of tumor cells[2], nor rely on MCH can recognize and kill multiple autologous tumor cells without any effect on autologous normal cells[3]; 3 γδT cells can be massively amplified in vitro at low cost Currently used zoledronic acid combined with IL-2 culture The base formula can efficiently in vitro amplify γδT cells, and the amplification can reach 90% or more on the tenth day only, and the amplified γδT cells also have obvious killing effect on tumor cells.

Unlike "regular" αβ T cells, Vγ9Vδ2T cells can recognize phosphoantigens (PAgs), such as isopentenyl pyrophosphate (IPP), and this antigen is abundantly synthesized in tumor cells, becoming an important way for γδ cells to recognize tumor cells Zoledronate is an aminodiphosphonate that blocks a large amount of IPP synthesized in tumor cells by blocking the mevalonate pathway and

does not degrade[4], causing IPP to accumulate in tumor cells and thus activating Vgamma9Vdelta2T cells.

The position of selenium in the periodic table is the fourth cycle VI A family, which is a non-metallic element Originally discovered and named in 1817. At the beginning of the discovery, due to the high toxicity of inorganic selenium itself, people mistakenly thought that this is a highly harmful "harmful element" Therefore, since the discovery of selenium for more than 100 years, the research on selenium and selenium compounds has been stagnant until one A hundred years later in 1957, scientists demonstrated that selenium is actually a trace element necessary for human metabolism After further research, people gradually realized the important role of selenium in human function[5].

Nano is a small unit of length of one billionth of a meter long, symbolized in nm, and 1 nanometer is only equivalent to 4 times the atomic size Nanotechnology is an interdisciplinary subject that was born and emerged in the late 1980s Its main research object is 01 to 100 nanometers Through the arrangement and operation of molecules and atoms, the material and structure of materials are processed And a new technology for processing and manufacturing products with new special features Selenium nanoparticles (SeNPs) is a very important material research field For Selenium nanoparticles, properties such as particle size, shape, charge and surface modification will significantly affect its function.

2. Materials and methods.

2.1 Immune cell isolation and depletion.

Anonymized human leucocyte cones were obtained from the UK Blood Transfusion Service, and peripheral blood mononuclear cells (PBMCs) were isolated by densityadjusted centrifugation using Histopaque-1077 (Sigma- Aldrich, Dorset, UK) Contaminating red blood cells and platelets were removed using ammonium chloride solution and slow speed centrifugation, respectively PBMCs were washed three times in PBS (Sigma Aldrich) and resuspended in 45% RPMI-1640, 45% fetal bovine serum (FBS) and 10% DMSO (all from Sigma Aldrich) before being frozen and stored in liquid nitrogen Monocytes and/or cd T cells were isolated and/or depleted from PBMCs using magnetic bead separation PBMCs were labelled with either CD14 or anti-TCR-cd-conjugated microbeads (Miltenyi Biotec, Surrey, UK) according to the manufacturer's instructions and passed through magnetic columns Purity was then assessed by flow cytometric analysis of CD14 and TCR-cd expression .

2.2 Cell culture.

The PBMCs were cultured in RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 lg/ml streptomycin (all from Sigma- Aldrich) at 37° with 5% CO2 PBMCs were seeded at a density of10^6 cells/ml, and 200 ll of cell suspension was added per well of 96-well round-bottomed tissue culture plates (Corning, Corning, NY) Duration of cell culture for individual experiments is detailed in the figure legends To measure interferon-c accumulation, 1 lg/ml brefeldin A (Sigma-Aldrich) was added to the cells for the last 4 hr of culture To measure degranulation, CD107a and CD107b antibodies along with 1 lg/ ml monensin (Sigma-Aldrich) were added to the cells for the last 4 hr of culture.

2.3 Flow cytometry.

Cells were washed in flow cytometry buffer (PBS supplemented with 1% weight/volume BSA and 01% weight/volume sodium azide; all from Sigma-Aldrich), and then stained with fluorochrome-conjugated antibodies according to the manufacturer's instructions For intracellular staining, cells were simultaneously fixed and permeabilized using 4% paraformaldehyde and 01% saponin solution (Cytofix/Cytoperm kit; Becton Dickinson, Oxford, UK) before staining with fluorochrome-conjugated antibodies according to the manufacturer's instructions The antibodies used throughout this study are as follows: CD3(SK7), CD54(HCD14), CD107a(H4A3),. IFN-c(B27) (Biolegend); Vd2(B6). perforin(dG9)； granzyme B(GB12) (Caltag, Buckingham, UK).

2.4 Statistical analyses.

Gaussian distributions were assumed for all data sets Student's paired t-tests or analyses of variance followed by Bonferroni's multiple comparison tests were used to compare data sets All statistical analyses were carried out using GRAPHPAD PRISM 7 Statistical significance was reached when P < 005. and. are used throughout the figures to indicate P values of < 005, < 001 and < 0001, respectively.

3. Result.

3.1 Selenium nanoparticles causes a large number of Vgamma9Vdelta2 T cell morphological changes and increased mortality.

In order to initially explore the effect of Selenium nanoparticles on Vgamma9Vdelta2 T cells, we directly added Selenium nanoparticles to Vgamma9Vdelta2 T cell culture medium According to the experience of processing tumor cells in the literature, we chose 10uM concentration and 6-hour treatment time Then, the morphological changes, particle size changes, and PI positive rate of Vgamma9Vdelta2 T cells were observed by light microscopy.

Forward-angle scattering, FSC, is positively correlated (dimension) with cell diameter bisection, so we usually use FSC as a threshold to exclude debris and other particles to avoid interference.

Side-scattering, or SSC, refers to a scattering signal that is orthogonal to the laser beam in the direction of 90 degrees It is more sensitive to the refractive index of the cell membrane, cytoplasm, and nuclear membrane and can provide information on intracellular structure and particle properties.

PI (propidium iodide) is a nuclear staining reagent that stains DNA and is commonly used for apoptosis detection The English name is Propidium Iodide It is an analog of ethidium bromide that releases red fluorescence after the insertion of double-stranded DNA Although PI cannot pass through the living cell membrane, it can penetrate the damaged cell membrane and stain the nuclei.

Our research shows Under light microscopy, Vgamma9Vdelta2 cells exhibit an irregular shape, and their typical appearance resembles a comma. After adding Selenium nanoparticles, the cells lose this form, the particle size and size decrease, and the PI positive rate increases.

3.2 Selenium nanoparticles reduces expression of functional molecules on the surface of Vgamma9Vdelta2 T cells.

To explore the effects of Selenium nanoparticles on the function of Vgamma9Vdelta2 T cells, we screened several functional molecules on the surface of Vgamma9Vdelta2 T cells and examined the expression changes of these molecules before and after the addition of nanoselenium by flow cytometry We found a significant decrease in the expression of CD16, TCR-VD2, and CD54.

CD16, also known as FcγRIII, is a cluster of differentiation molecule found on the surface of natural killer cells, neutrophil polymorphonuclear leukocytes, monocytes and macrophages CD16 has been identified as Fc receptors FcγRIIIa (CD16a) and FcγRIIIb (CD16b), which participate in signal transduction The most well-researched membrane receptor implicated in triggering lysis by NK cells, CD16 is a molecule of the immunoglobulin superfamily (IgSF) involved in antibody-dependent cellular cytotoxicity (ADCC).

The T-cell receptor, or TCR, is a molecule found on the surface of T cells, or T lymphocytes, that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules The binding between TCR and antigen peptides is of relatively low affinity and is degenerate: that is, many TCRs recognize the same antigen peptide and many antigen peptides are recognized by the same TCR[6].

ICAM-1 (Intercellular Adhesion Molecule 1) also known as CD54 (Cluster of Differentiation 54) is a protein that in humans is encoded by the ICAM1 gene This gene encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system It binds to integrins of type CD11a. CD18, or CD11b. CD18 and is also exploited by rhinovirus as a receptor.

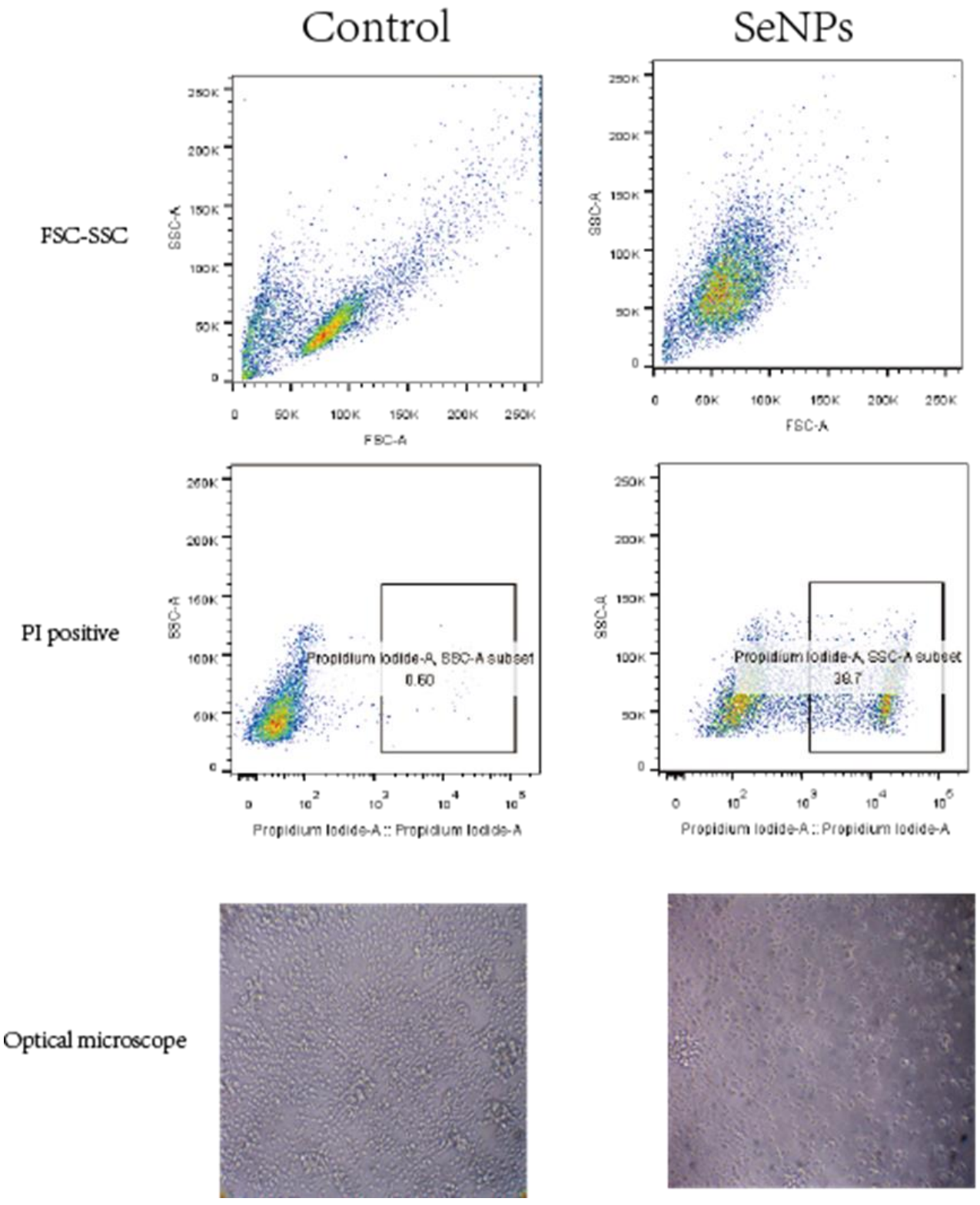

Fig 1 Fig 1 shows the most intuitive toxicity results Under the treatment of SeNPs, FSA-A in Vγ9Vδ2 Vgamma9Vdelta2 T cells became smaller and a PI-positive population appeared The statistical results showed that PI-positive Vγ9Vδ2 Vgamma9Vdelta2 T cells accounted for more than 50% of the total cell number after sodium selenite was added Light microscopy results showed that compared to the control group, Vγ9Vδ2 cells in the experimental group all lost the typical comma morphology, turned round, and produced a large number of debris.

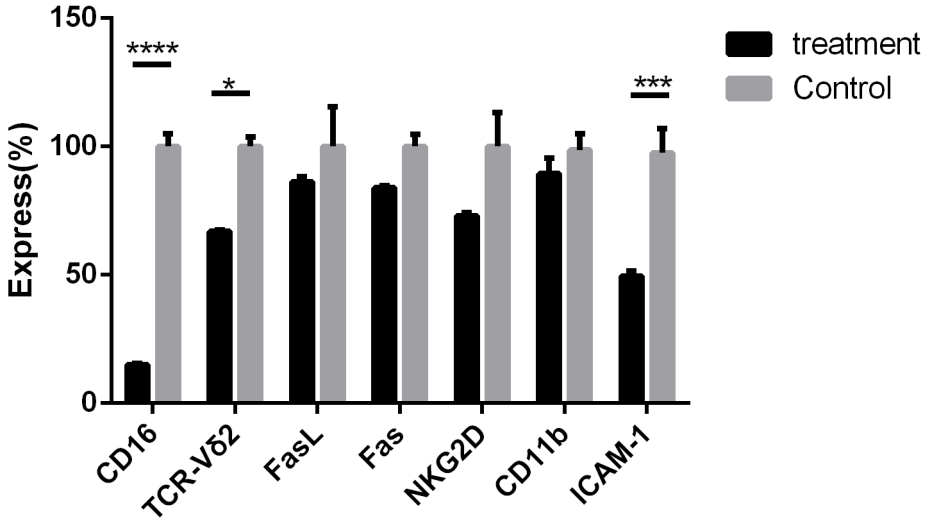

Fig.2 Fig.2 shows the changes in the expression levels of functional molecules on the surface of Vgamma9Vdelta2 T cells before and after the addition of nanoselenium We used the control group's relative fluorescence intensity (MFI) as 100%, the experiment was repeated 3 times and significant analysis was performed.

3.3 Selenium nanoparticles inhibits Vgamma9Vdelta2 T cell metabolism.

To further understand the effect of Selenium nanoparticles on the metabolism of Vgamma9Vdelta2 T cells, we verified the ability of Vgamma9Vdelta2 T cells to produce lactic acid and ATP before and after the addition of nanoselenium We used sigma kits and the results were measured using a microplate reader The production of lactic acid can reflect the strength of anaerobic respiration of cells, and the production of ATP directly reflects the level of energy metabolism of cells The experimental results showed that the metabolism of Vgamma9Vdelta2 T cells was inhibited by the addition of Selenium nanoparticles, resulting in reduced energy production.

Lactic acid (IUPAC scientific name: 2-hydroxypropionic acid) is a compound that plays a role in various biochemical processes It is a carboxylic acid and its molecular formula is C3H6O3 It is a hydroxy-containing carboxylic acid and is therefore an alpha-hydroxy acid (AHA) Its carboxyl group releases a proton in aqueous solution, producing the lactate ion CH3CHOHCOO− Lactate dehydrogenase converts pyruvate to L-lactic acid during fermentation In the general metabolism and movement, lactic acid is continuously produced, but its concentration does not generally increase.

Adenosine triphosphate (ATP) is a complex organic chemical that participates in many processes Found in all forms of life, ATP is often referred to as the "molecular unit of currency" of intracellular energy transfer When consumed in metabolic processes, it converts to either the di- or monophosphates, respectively ADP and AMP Other processes regenerate ATP such that the human body recycles its own body weight equivalent in ATP each day It is also a precursor to DNA and RNA.

3.4 Selenium nanoparticles affects Vgamma9Vdelta2 T cell functional gene expression.

To validate the effect of nanoselenium on the gene level of Vgamma9Vdelta2 T cells, we used a commercial company's transcriptome sequencing service Samples treated with nanoselenium for 6 hours were used as experimental groups and PBS was used as a control.

Transcriptome sequencing demonstrated that the expression levels of several genes changed significantly before and after Selenium nanoparticles was added After Selenium nanoparticles was added, the expression of multiple upstream apoptotic genes was up-regulated, and cell adhesion-related pathways were significantly inhibited The decrease in ICAM-1 adhesion factor

expression was consistent with the previous stage Flow cytometry experiments also demonstrated that the addition of Selenium nanoparticles resulted in early apoptosis in Vgamma9Vdelta2 T cells In order to find out which signal pathway has changed in the end, we analyzed the enrichment of these genes The results of KEGG showed that the expression of multiple genes such as DR5, PUMA, and PAG608 directed to the apoptotic pathway changed significantly.

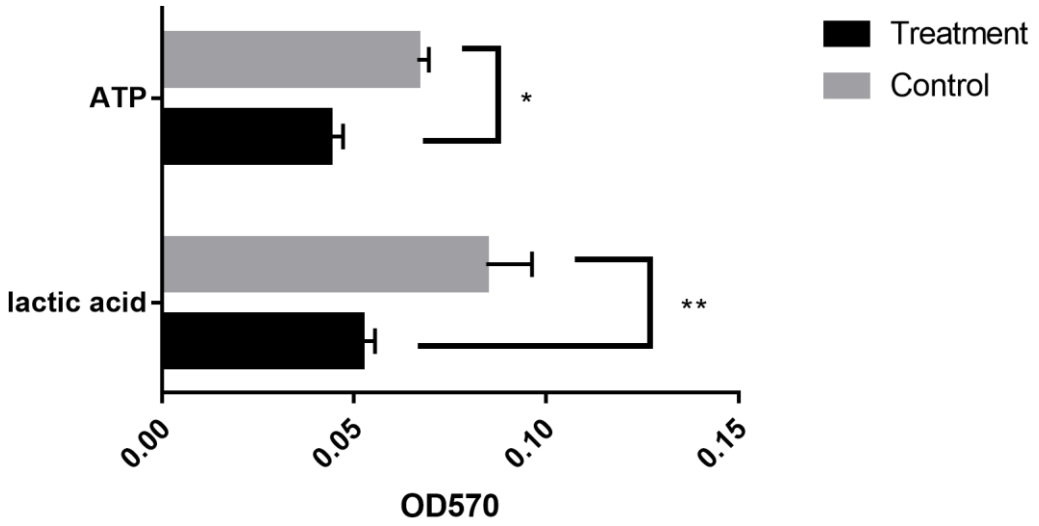

Fig 3 Fig 3 shows the changes in ATP-producing ability and lactic acid-producing ability of Vgamma9Vdelta2 T cells before and after Selenium nanoparticles treatment The change in OD570 was used to show the change in the content of the corresponding substance, and the experiment was repeated 3 times and subjected to significant analysis.

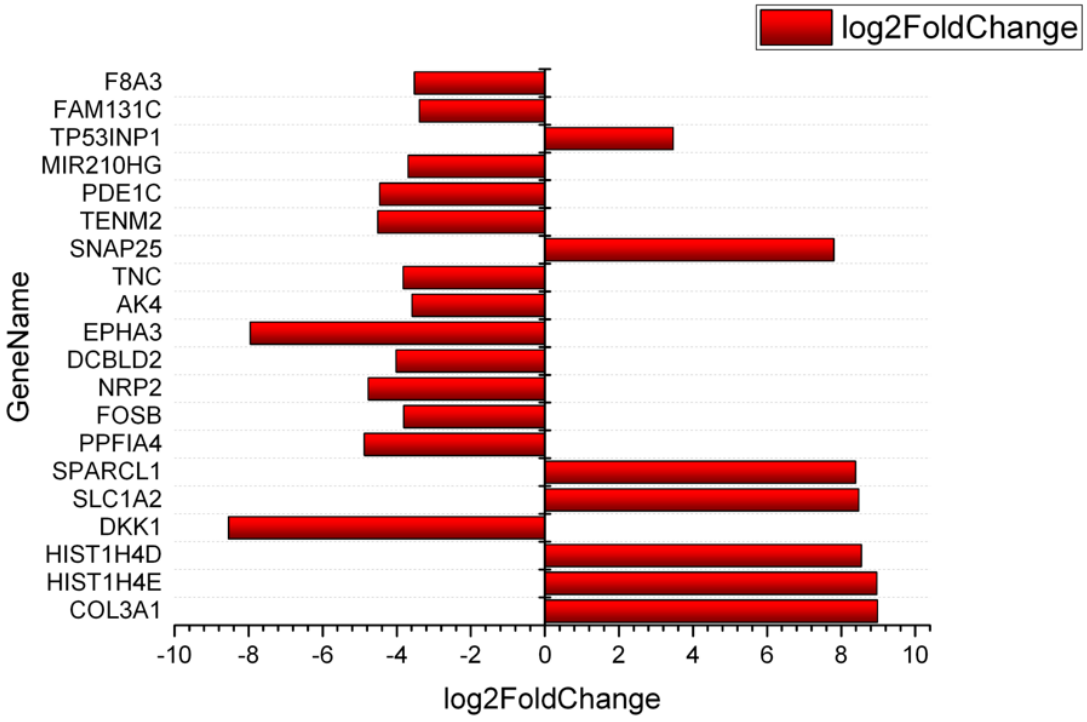

Fig 4 shows the results of our transcriptome sequencing The results show that the expression levels of a considerable number of genes have undergone significant changes after nanoselenium treatment.

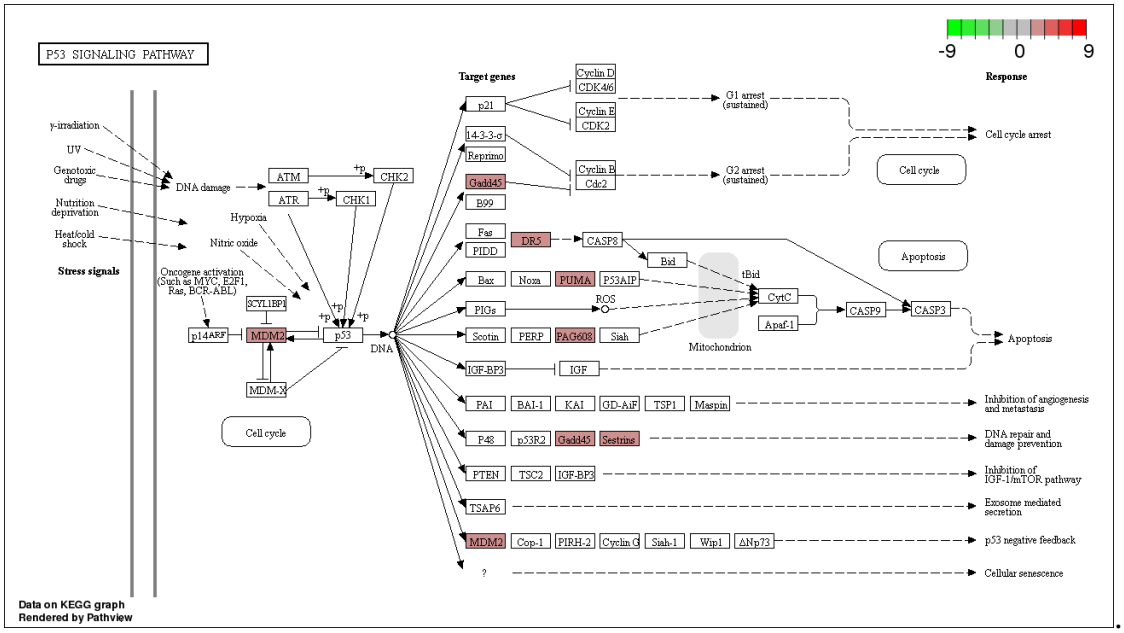

Fig 5 Activation inhibition of the P53 signaling pathway.

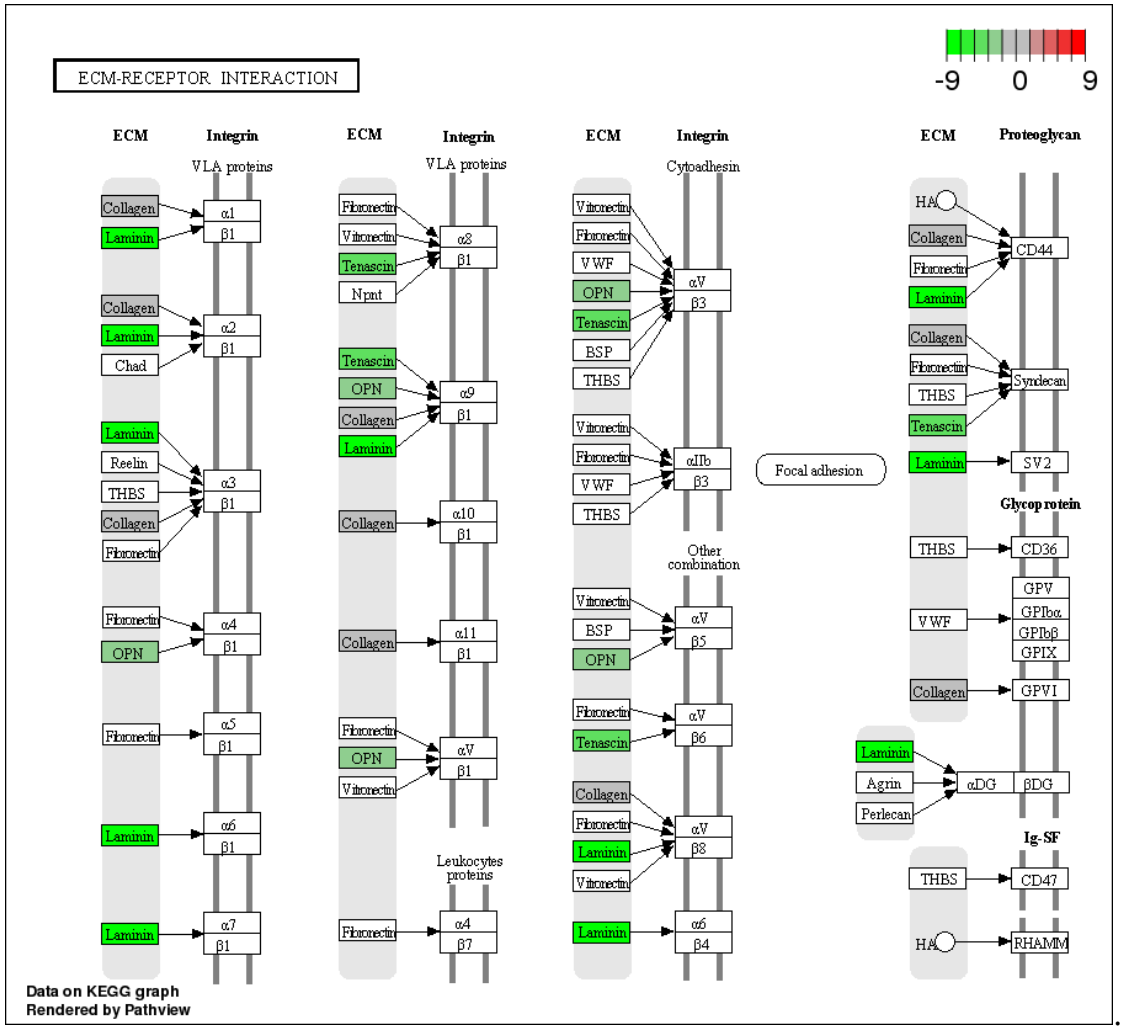

Fig 6 The expression of multiple genes is significantly down-regulated in the ECM-receptor interaction signaling pathway.

4. Conclusion.

In recent years, Selenium nanoparticles has attracted attention from many well-known research groups as a very popular field in nanomaterials, but the effects of Selenium nanoparticles on the immune system are still relatively unknown People are addicted to the powerful and highly selective killing effect of Selenium nanoparticles on tumor cells, ignoring the impact of this material on the immune system Our experiments found that the presence of Selenium nanoparticles had a very large negative effect on human Vgamma9Vdelta2 T cells Although the proportion of Vgamma9Vdelta2 T cells in the peripheral blood is very small, it plays a very important role in anti-tumor and anti-inflammatory.

Our study found that Selenium nanoparticles exerts inhibitory effects on Vgamma9Vdelta2 T cells through the following three aspects.

1, Selenium nanoparticles is cytotoxic and can easily kill Vgamma9Vdelta2 T cells or mediate cell apoptosis In terms of appearance, Vgamma9Vdelta2 T cells treated with Selenium nanoparticles become smaller, rounder, lose their inherent shape and generate a large amount of debris Flow cytometry tests revealed even more serious consequences, including massive cell death and reduced particle size.

2 Selenium nanoparticles can act on functional molecules on the cell surface, down-regulate its expression levels, including several related to Vgamma9Vdelta2 T cell recognition capabilities such as TCR-VD2 and NKG2D, and cell adhesion-associated CD54, and cell killing CD16 and so on.

3, Selenium nanoparticles can reduce the metabolism of Vgamma9Vdelta2 T cells, making it produce less lactic acid and ATP Lower metabolic levels mean less energy supply, which will lead to a decline in the physiological function of Vgamma9Vdelta2 T cells.

In summary, we conducted experiments to determine the impact of Selenium nanoparticles, a popular nanomaterial, on the human immune system This result will pose new safety challenges for the further application of Nano selenium.

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