Journal of Functional Foods 89 (2022) 104914

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Effects of dietary selenium on immune function of spleen in mice

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ARTICLE INFO

Keywords:
Selenium
Mice spleen
Serum cytokines
Proteome

ABSTRACT

The effects of selenium on serum cytokines, immune cell composition and spleen proteome in mice are still unclear. In this study, male mice were fed with selenium-containing diets for eight weeks, and serum cytokines were assayed using Luminex liquid suspension chip. The spleen was obtained for histological morphology, immunohistochemistry and proteome sequencing. The results indicated that the spleen index and histological structure of the spleen were reduced and damaged with low and high selenium. Serum cytokines were regulated by selenium. Additionally, compared with medium selenium, there were 180 and 189 differential proteins with high and low selenium, respectively. The differential proteins were mainly associated with metabolic processes, regulation of response to oxidative stress, cell maturation and differentiation, cytokines and receptor binding, and were verified using Western blotting. In conclusion, it was suggested that low and high selenium affected the secretion of cytokines and impaired the immune function of the spleen.

1. Introduction

As an essential element for humans and animals, selenium plays a biological function in the form of selenoprotein, which is closely related to the development of many diseases, such as diabetes, cardiovascular disease, cancer, immune defence and inflammatory disorders (Avery & Hoffmann, 2018; Fairweather-Tait et al., 2011; Rayman, 2012; Roman et al., 2014; Xia et al., 2021b). It was reported that selenium deficiency in humans is associated with mortality risk from Acquired immune deficiency syndrome (AIDS) (Avery & Hoffmann, 2018) and COVID-19 (Moghaddam et al., 2020). And selenium deficiency can induce kidney (Li et al., 2020), liver (Tang et al., 2020) and spleen (Li et al., 2021) damage by regulating inflammation in pigs, inducing immune damage to the spleen by heat shock protein (Khoso et al., 2016), cytokine (Khoso et al., 2019), endoplasmic reticulum stress (Zhang et al., 2020a) and NF-κB signalling pathway (Zhang et al., 2021b; Zhang et al., 2020a) in chickens. Selenium supplementation can reduce the level of inflammatory cytokines in sows during gestation (Mou et al., 2020; Mou et al., 2021), inhibit inflammation of mouse mastitis induced by *Staphylococcus aureus* (Zhang et al., 2019), reduce levels of pro-inflammatory cytokines caused by *B. subtilis* in the chicken intestine (Yang et al., 2021). Additionally, excessive selenium supplementation is harmful to health (Lv et al., 2021; Rayman, 2020; Wang et al., 2016a; Wang et al., 2016b; Grotto et al., 2018), such as immune injury of the spleen and thymus in chicken, and hypertension in rats.

It was shown that selenium can regulate T helper (Th) cell differentiation, regulatory T helper cell phenotype, B-cell numbers and antibody production, adherence and migration of leukocytes, and phagocytosis of macrophages in mice (Avery & Hoffmann, 2018; Huang et al., 2012). Recently, it was suggested that selenium could regulate the immune response of dendritic cells (DC) in chicken, mice and humans (Sun et al., 2017; Sun et al., 2018; Xia et al., 2021a; Zhang et al., 2021a). The potential mechanisms of selenium involved in the regulation of immune cell function include production and species of reactive oxygen species, calcium and redox signalling, extracellular signal-regulated kinase (ERK), hypoxia-inducible factor 1α (HIF-1α) and NF-κB pathways (Avery & Hoffmann, 2018; Li et al., 2020; Mou et al., 2021; Yang et al., 2021; Zhang et al., 2021b; Zhang et al., 2020a; Zhang et al., 2021a). However, the study of selenium on the serum cytokines and immune cell composition in the spleen of mice is still not comprehensive.

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https://doi.org/10.1016/j.jff.2021.104914
Received 16 September 2021; Received in revised form 6 December 2021; Accepted 23 December 2021
Available online 24 December 2021

Src family kinases, such as Fyn and Lyn, play an important role in immune cells, including perforin-dependent cryptococcal killing by natural killer (NK) cells (Oykhman et al., 2013), Fcγ receptor-mediated mast cell function (Paranjape et al., 2020), proliferation and antibody production of B cells (Zhang et al., 2021c), T-cell activation and effector function (van der Donk et al., 2021). Reduced expression of major histocompatibility complex (MHC) class II regulatory factor (Rfx1) activates $CD14^+$ monocytes in coronary artery disease patients (Du et al., 2019), promotes the differentiation of primary $CD4^+$ T-cells into Th17-cells in autoimmune diseases (Zhao et al., 2018) and is associated with poor prognosis of hepatocellular carcinoma (Liu et al., 2018). Bruton's tyrosine kinase (Btk) plays an essential role in the development and functions of B cells, has different functions in macrophages and DC and is associated with malignancies and autoimmune diseases (Pal Singh et al., 2018; Rip et al., 2018). Whether selenium affects the expression of these essential proteins in mouse spleen is unknown. Recently, it was reported that the transcriptome and proteome of the chicken spleen (Zhang et al., 2020a, 2020b), metabolome and transcriptome of mice liver and brain were affected by selenium deficiency (Yim et al., 2019). Additionally, the proteome of mice intestine were affected by sodium selenite and selenomethionine (Zhai et al., 2019). However, the effect of selenium on the proteome of mice spleen remains unclear.

So, in this study, mice were fed diets containing different concentrations of sodium selenite for eight weeks. The spleen index, histological morphology, immune cell composition and serum cytokines were evaluated. Moreover, bioinformatics analysed changes in the spleen proteome and some essential proteins associated with immune function were identified using Western blotting.

2. Materials and methods

2.1. Mouse model

Forty-five male mice (C57BL/6J) aged three weeks were bought and fed in Guizhou Laboratory Animal Engineering Technology Centre. After feeding with normal diets containing 0.15 mg/kg selenium for one week, they were then randomly divided into low, medium and high selenium groups, fed diets *ad libitum* with purified ingredients (Table 1) containing 0.08, 0.25 or 1 mg/kg selenium (as a form of sodium selenite)

Table 1
Diet compositions.

	Low Se Diet (g/kg)	Medium Se Diet (g/kg)	High Se Diet (g/kg)
Casein	220	same	same
L-cystine	3	same	same
Corn Starch	387.5	same	same
Maltodextrin	132	same	same
Sucrose	100	same	same
Cellulose	50	same	same
Soybean Oil	60	same	same
Choline Bitartrate	2.5	same	same
Vitamin Mix[1]	10	same	same
Mineral Mix[2]	35	same	same
Sodium Selenite (1% Se)	0.008 (Se)	0.025 (Se)	0.1 (Se)

[1] Vitamin mix compositions (g): vitamin A, 0.8; vitamin D, 0.25; vitamin E, 15; vitamin K, 0.075; vitamin C, 0; vitamin B1, 0.6; vitamin B2, 0.6; vitamin B6, 0.7; vitamin B12, 2.5; D-calcium pantothenate, 1.6; niacin, 3; folic acid, 0.2; biotin, 0.02; sucrose, 974.655; In total, 1000.

[2] Mineral mix compositions (g): calcium carbonate, 357, potassium dihydrogen phosphate, 196; potassium citrate, 70.78; sodium chloride, 74; Potassium sulfate, 46.6; magnesia, 24; ferric citrate, 6.06; zinc carbonate, 1.65; sodium silicate, 1.45; manganese carbonate, 0.63; copper carbonate, 0.3; chromium potassium sulfate, 0.275; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; lithium chloride, 0.0174; potassium iodate, 0.01; ammonium molybdate, 0.00795; ammonium vanadate, 0.0066; sucrose, 221.03625; In total, 1000.

(Beijing Keao Xieli Feed Co., Ltd.) for eight weeks as described by Huang et al., 2012 and Hoffmann et al., 2010. The drinking water for the mice was purified water and *ad libitum*. All animal protocols were approved by the Institutional Animal Care and Use Committee at Guizhou Medical University.

2.2. Spleen index

The mice were anaesthetised and killed after eight weeks, and the spleens were obtained and washed with phosphate buffer saline. The spleen index was calculated as spleen weight (mg)/mouse weight (g).

2.3. Hematoxylin-eosin staining

The spleen was fixed with 4% formaldehyde, dehydrated with graded ethanol solutions, soaked in xylene and embedded in paraffin. Sections were prepared and stained with hematoxylin-eosin (HE), then observed using a Nikon Eclipse E100 microscope (Japan).

2.4. Immunohistochemistry

Paraffin sections of the spleen were dewaxed followed by antigen retrieval, incubated with 3% BSA for 30 min at room temperature, and then incubated overnight with primary antibodies at 4 °C, including CD11c (1:200, GB11059), CD3 (1:700, GB111337), CD4 (1:800, GB11064), CD20 (1:500, GB11540) and F4/80 (1:1000, GB11027) collected from Servicebio, followed by HRP-labelled secondary antibodies for 50 min at room temperature. Diaminobenzidine was used for colour development and haematoxylin was added for redyeing. Finally the slices were dehydrated and sealed, and then observed under an optical microscope (Nikon, Japan). At least three fields were randomly selected for each section in each group, and the average optical were analysed using Image-Pro Plus 6.0 (Media Cybernetics, USA).

2.5. Cytokine detection

Serum samples were obtained by centrifugation at 10,000 rpm for ten minutes, and the concentrations of interleukin-1α (IL-1α), IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, Eotaxin, granulocyte colony-stimulating factor (G-CSF), interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) were determined using the Luminex liquid suspension chip (M60009RDPD) on a Bio-Plex MAGPIX System (Bio-Rad) by Wayen Biotechnologies (Shanghai) Inc.

2.6. Proteome sequencing

The proteome sequencing of the spleen was conducted using the 4D label-free quantitative (4D-LFQ) proteomics by Hangzhou Jingjie Biotechnology Co., Ltd. Briefly, spleen proteins were extracted with urea lysate containing protease inhibitors and then enzymolysed using trypsin. The enzymatic hydrolysate was separated in an UPLC system and analysed using TIMSTOF PRO mass spectrometry (Thermo, USA). After that the MS/MS data were processed using the Maxquant search engine (v.1.6.6.0).

2.7. Bioinformatics

Bioinformatics enrichment analysis was conducted to identify the function of differential proteins. The differential protein was defined as $>$ 1.2-fold and $p < 0.05$, and subcellular localisation was conducted using CELLO (http://cello.life.nctu.edu.tw/). Gene ontology (GO) analysis was conducted using InterProScan (http://www.ebi.ac.uk/interpro/), and the KEGG pathway enrichment was conducted using KEGG Mapper (http://www.kegg.jp/kegg/mapper.html), and $p < 0.05$ is considered significant.

2.8. Western bolt

The protein extracts of the spleen were prepared in RIPA buffer and Western blotting was conducted. The antibodies include Fyn (sc-434), Lyn (sc-7274), granzyme A (sc-33692), Rfx1 (sc-374270), Btk (sc-28387), CD74 (sc-6262), Rab27b (sc-517602) and Gapdh (sc-47724) obtained from Santa Cruz and CD40 (AF5336) obtained from Affinity.

2.9. Statistical analysis

Statistical analysis was conducted by origin 9.0 (Originlab, USA), and all data were presented as means ± standard deviation. All experiments were repeated at least thrice, and analysis of variance was applied to determine statistically significant differences between groups, *p*-values less than 0.05 indicated statistical significance.

3. Results

3.1. Selenium can affect spleen index, histological morphology and immune cell composition in mice

The results in Fig. 1A indicated that the spleen index was markedly reduced with low and high selenium compared with medium selenium. HE staining demonstrated that the lymphocytes were closely arranged and regular in shape. The number of white medulla and red medulla lymphocytes was abundant in all three groups (Fig. 1B). Differently, there were several extramedullary haematopoietic cells in the low selenium group (black arrow), and a few apoptotic bodies in the germinal centre (green arrow), brown-yellow pigmentation (red arrow) and some extramedullary haematopoietic cells (yellow arrow) in the high selenium group. Additionally, the immune cell composition was detected (Fig. 1C, 1D), the $CD11c^+$ DC and $CD3^+$ T-cells were significantly decreased in the low selenium group, and $CD3^+$ T-cells were significantly reduced in the high selenium group. The $CD20^+$ B-cells in the low selenium group were significantly increased.

3.2. Selenium can affect the content of serum cytokines in mice

To further verify the role of selenium in the immune response of mice, the content of serum cytokines was evaluated (Fig. 2). It was shown that, compared with the medium selenium group, the contents of IL-3, IL-12p70, Eotaxin, G-CSF and IFN-γ were reduced, IL-5 and IL-17A were increased in low and high selenium groups. IL-1α was increased, IL-4, IL-6 and IL-10 were reduced in the low selenium group, but IL-6 and TNF-α were increased in the high selenium group.

3.3. Identification of differential proteins

Additionally, the proteome sequencing of the spleen was conducted using 4D-LFQ. The results in Fig. 3 indicated that 44 proteins were up-regulated and 96 proteins were down-regulated with high selenium compared with those of low selenium, 87 proteins were up-regulated and 93 proteins were down-regulated with high selenium compared with those of medium selenium, 57 proteins were up-regulated and 132 proteins were down-regulated with medium selenium compared with those of low selenium. The proteins that changed the most include aldehyde dehydrogenase (Aldh1a7), ATP-binding cassette sub-family C member 9 (Abcc9), glutathione peroxidase 3 (Gpx3), peroxisomal

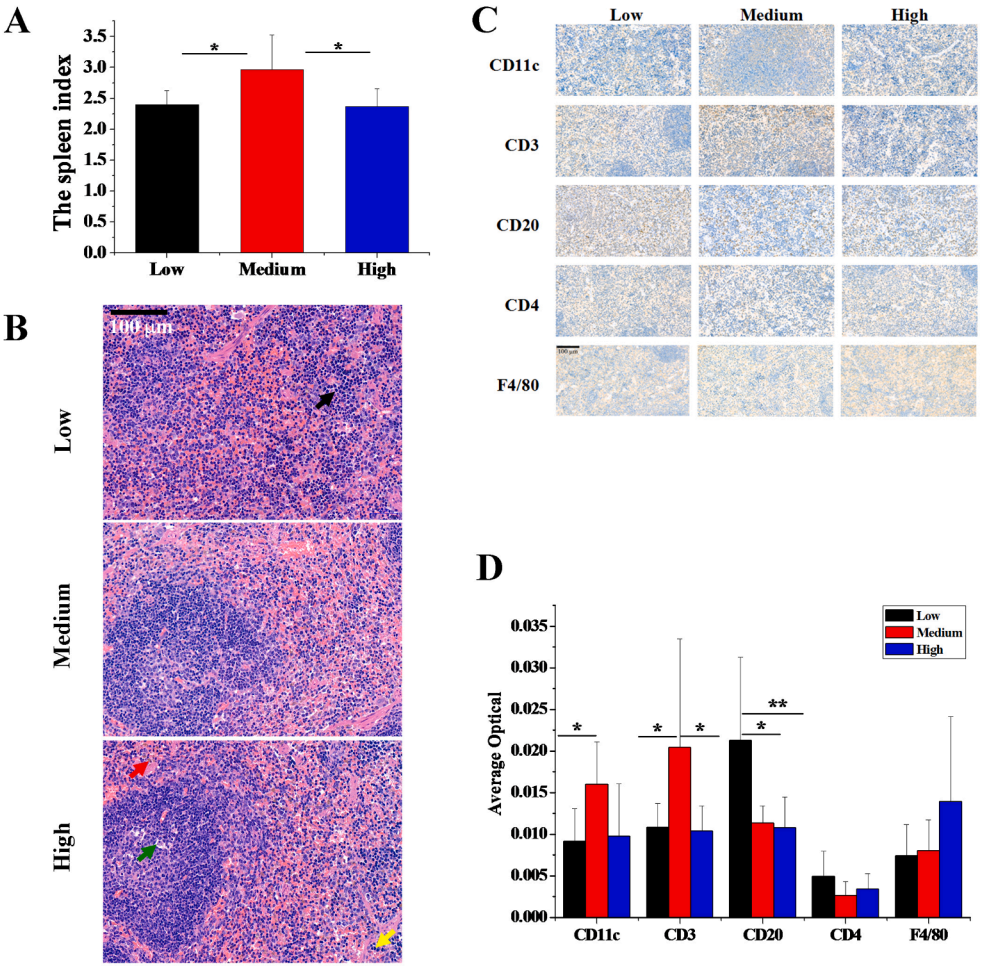

Fig. 1. Effect of selenium on spleen index, histological morphology, and immune cell composition in mice. Forty-five male mice were divided into low (0.08 mg/kg), medium (0.25 mg/kg) or high (1 mg/kg) selenium groups; after eight weeks, the spleen index (A) of anaesthetised mice was analysed (n = 7). (B) Histological morphology of spleen was performed by HE staining and examined by a microscope (n = 3), plenty of extramedullary haematopoietic cells were observed in low selenium group (black arrow), and a few apoptotic bodies in the germinal centre (green arrow), brown-yellow pigmentation (red arrow) and a small amount of extramedullary haematopoietic cells (yellow arrow) appeared in high selenium group. (C) $CD11c^+$ DC, $CD3^+$ T cell, $CD4^+$ T cell, $CD20^+$ B-cell, and $F4/80^+$ macrophage in spleen were analysed using immunohistochemistry, three fields were randomly selected for each section in each group, and (D) the average optical were analysed using Image-Pro Plus (n = 3). Data are presented as the mean ± standard deviation. $^*p < 0.05$, compared with medium selenium; $^{**}p < 0.01$, compared with high selenium.

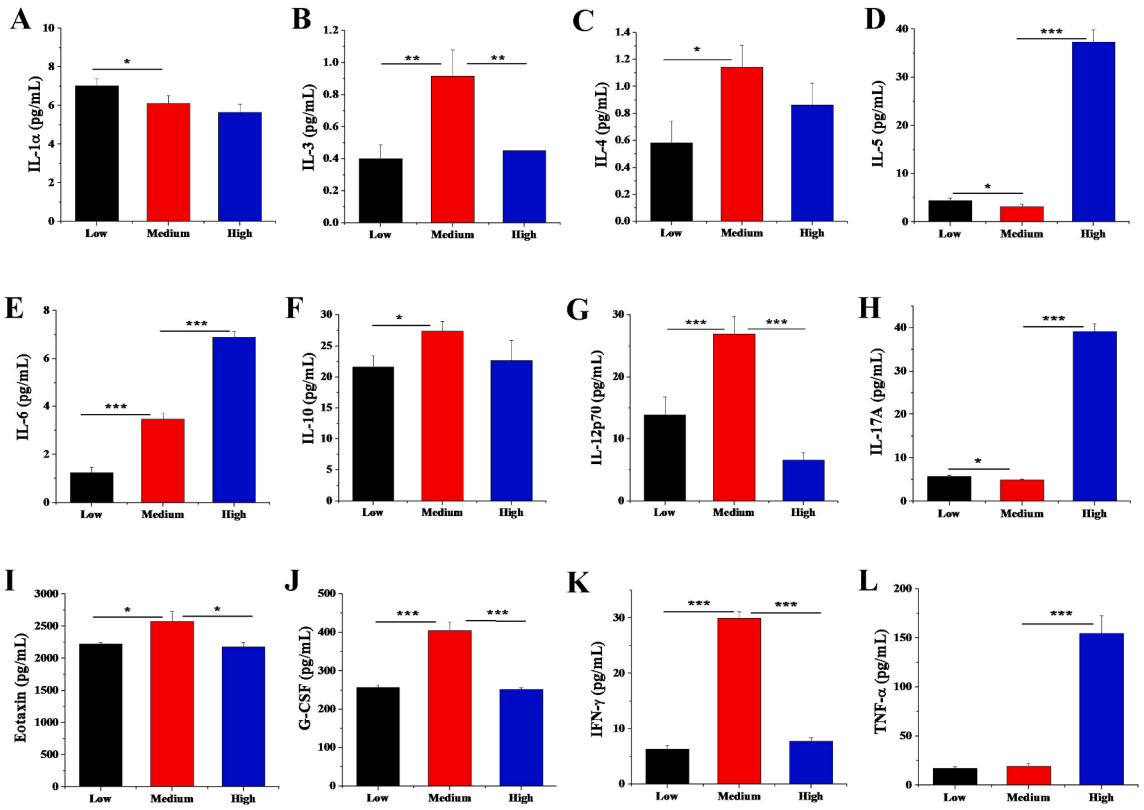

Fig. 2. Effect of selenium on the content of serum cytokines in mice. Serum samples were collected, and then the levels of (A) interleukin-1α (IL-1α), (B) IL-3, (C) IL-4, (D) IL-5, (E) IL-6, (F) IL-10, (G) IL-12p70, (H) IL-17A, (I) Eotaxin, (J) G-CSF, (K) IFN-γ and (L) TNF-α were determined using the Luminex liquid suspension chip on a Bio-Plex MAGPIX System (n = 6). Data are presented as mean ± standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with medium selenium.

biogenesis factor 3 (Pex3) and so on.

3.4. Subcellular location of differential proteins

The results in Fig. 4 suggested that the differential proteins were located in the cytoplasm, nucleus, mitochondria, extracellular and plasma membrane in the spleen with high selenium compared to low selenium, those of high selenium compared to medium selenium were nucleus, cytoplasm, mitochondria, plasma membrane and extracellular. And that of medium selenium compared with low selenium were cytoplasm, nucleus, mitochondria, extracellular and plasma membrane, differently, there were differential proteins located in the endoplasmic reticulum.

3.5. GO analysis of differential proteins

GO categorisation analysed functional information of differential proteins. The primary biological process (Fig. 5A) includes the metabolic process of porphyrin containing compound, peptide, amide, neutral lipid, glycolipid, monosaccharide and hexose, protein transport and organelle localisation, organisation of chromatin, lysosome and lytic vacuole, regulation of response to oxidative stress, cell maturation and differentiation, regulation of IL-1, IL-8 and IL-12, showing that selenium may affect the function of immune cells in the spleen by regulating metabolism.

Among the cellular compartment (Fig. 5B), mainly include organelle membrane and plasma membrane, lysosomes, vacuoles, Golgi transport complex, respiratory chain complex I, ubiquitin ligase complex, cation channel complex, receptor complex and MHC protein complex, suggesting that selenium may regulate the changes of the complex related to immune function by affecting the complexes on the membrane.

For molecular function (Fig. 5C), including peroxidase activity, oxidoreductase activity, deaminase activity, isomerase activity, cytokine receptor activity, transmembrane receptor activity, cytokine binding, peptide antigen binding, T cell receptor binding, CD8 receptor binding, CD4 receptor binding and structural constituent of the cytoskeleton, indicating that selenium may affect the binding of cytokines and receptors by regulating enzyme activity.

3.6. KEGG analysis of differential proteins

KEGG pathway enrichment (Fig. 6) showed that selenium might affect metabolic pathways, such as protein processing in the endoplasmic reticulum, cysteine and methionine metabolism, pentose and glucuronate interconversions, galactose metabolism, amino sugar and nucleotide sugar metabolism, synthesis and degradation of ketone bodies, butanoate metabolism, purine metabolism, biotin metabolism, retinol metabolism and so on. And selenium may regulate signalling pathways, including IL-17, NF-κB, Notch, HIF-1 and TNF signalling pathways. Additionally, selenium may regulate Th1 and Th2 cell differentiation, FcγR-mediated phagocytosis, cytokine-cytokine receptor interaction and primary immunodeficiency. Additionally, selenium might be associated with inflammatory bowel disease, autoimmune thyroid disease, type 1 diabetes mellitus and viral infection.

3.7. Verification of differential proteins by Western blot

Based on proteomic analysis of the differential proteins, several proteins associated with immune function were selected for validation. The results in Fig. 7 indicated that, compared with the medium selenium group, the protein levels of Lyn, granzyme A, CD74 and Rab27b were reduced in the low selenium group, and Fyn, Lyn, granzyme A, Rfx1, Btk and CD74 were increased in high selenium group. In contrast, CD40 and Rab27b in the high selenium group were decreased. Additionally,

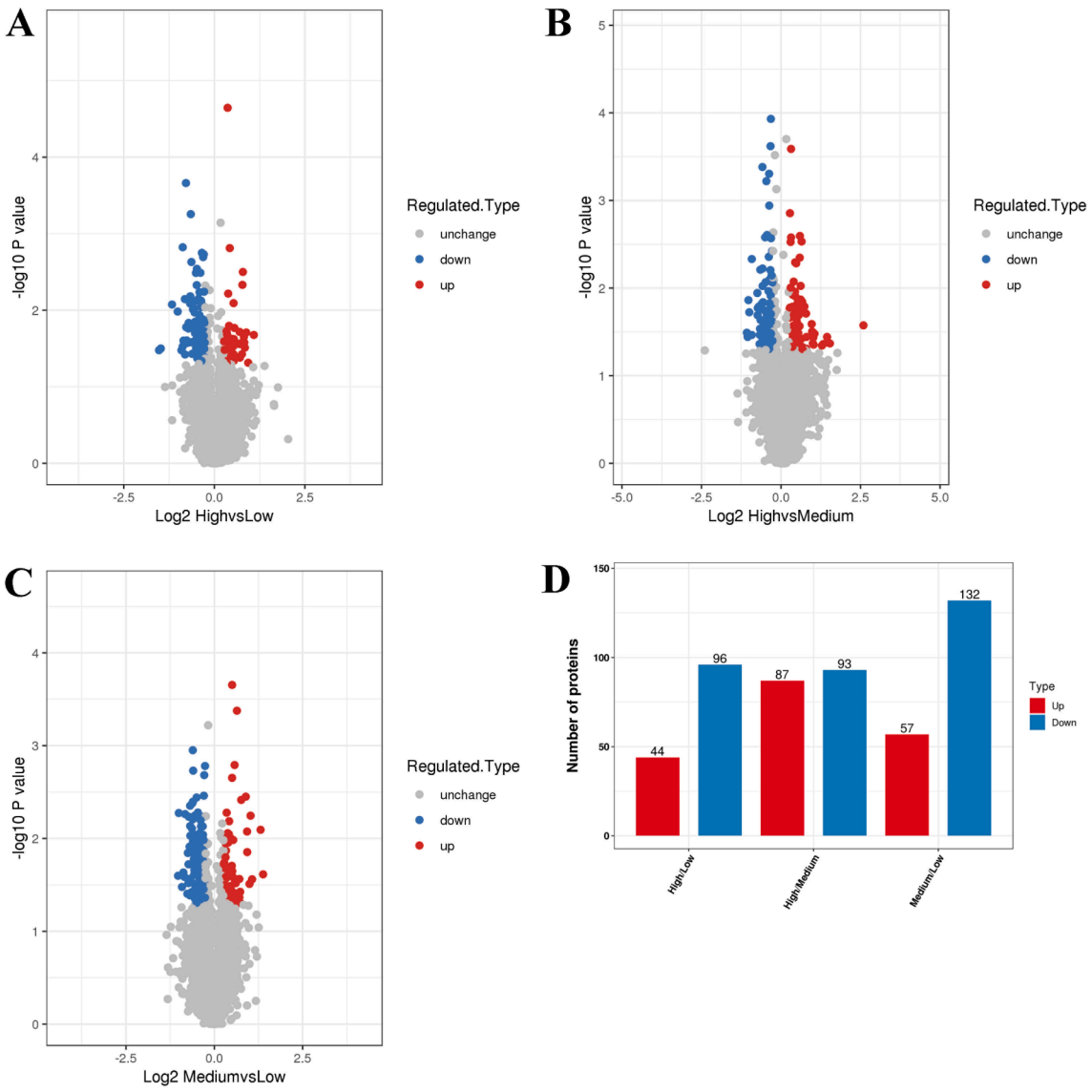

Fig. 3. Effect of selenium on differential protein in mice spleen. The proteome sequencing of the spleen was conducted using 4D label-free quantitative (4D-LFQ) proteomics, and the differential proteins were identified with a significant difference at $p < 0.05$ (n = 3). Quantitative volcano maps of differentially expressed proteins were shown as (A) High/Low, (B) High/Medium, (C) Medium/Low, and (D) the number of differential proteins was displayed.

selenoproteins sensitive to selenium, such as Gpx1 and selenoprotein K (SELENOK) were also detected, the results indicated that Gpx1 and SELENOK were increased with the increase of selenium concentration in mice spleen (Supplemental Fig. 1).

4. Discussion

Previous studies showed that dietary selenium could affect the immune function of the spleen in pigs (Li et al., 2021) and chickens (Khoso et al., 2016; Khoso et al., 2019; Wang et al., 2016a; Zhang et al., 2021b; Zhang et al., 2020a; Zhang et al., 2020b), and selenomethionine can affect the number of B cells and T-cells in the spleen of mice (Vega et al., 2007). Additionally, the spleen index of chickens was reduced with a selenium-deficient diet (Wang et al., 2018b), and the spleen weight of mice was reduced after phenylhydrazine treatment under selenium deficiency condition (Liao et al., 2018), both of which resulted in spleen injury. Consistently, our results showed that the mice spleen index was reduced in the low and high selenium groups, and the histological structure of the spleen was also damaged, which may cause impaired immune function of the spleen and the inability to initiate the normal immune response. The effects of selenium on immune cells in mice were previously studied with or without stimulation (Huang et al., 2012). Our previous study found that the function of DC prepared from mice with low or high selenium was impaired (Zhang et al., 2021a), which is consistent with the reduction in the number of $CD11c^+$ DC in this study. Differently, low and high selenomethionine reduced $CD19^+$ B-cell numbers in female mice (Vega et al., 2007), while low sodium selenite increased $CD20^+$ B-cell numbers in male mice in this study. Organic (Vega et al., 2007) and inorganic selenium had a similar effect on increasing the numbers of $CD4^+$ T-cells, but there was no effect on $F4/80^+$ macrophages. However, the effects of selenium on various immune cell subtypes *in vivo* require more study.

Previous studies suggested that selenium can affect cytokine secretion of immune organs and cells (Li et al., 2021; Huang et al., 2012; Sun et al., 2018). Consistent with previous findings, our study showed that selenium could affect the secretion of serum cytokines in mice. IL-1β was increased in the blood and spleen of a pig under selenium deficiency condition (Tang et al., 2020; Li et al., 2021), in agreement with our findings for IL-1α. Selenium deficiency decreased IL-6 in the spleen of chicken (Khoso et al., 2019; Zhang et al., 2021b), while it was increased

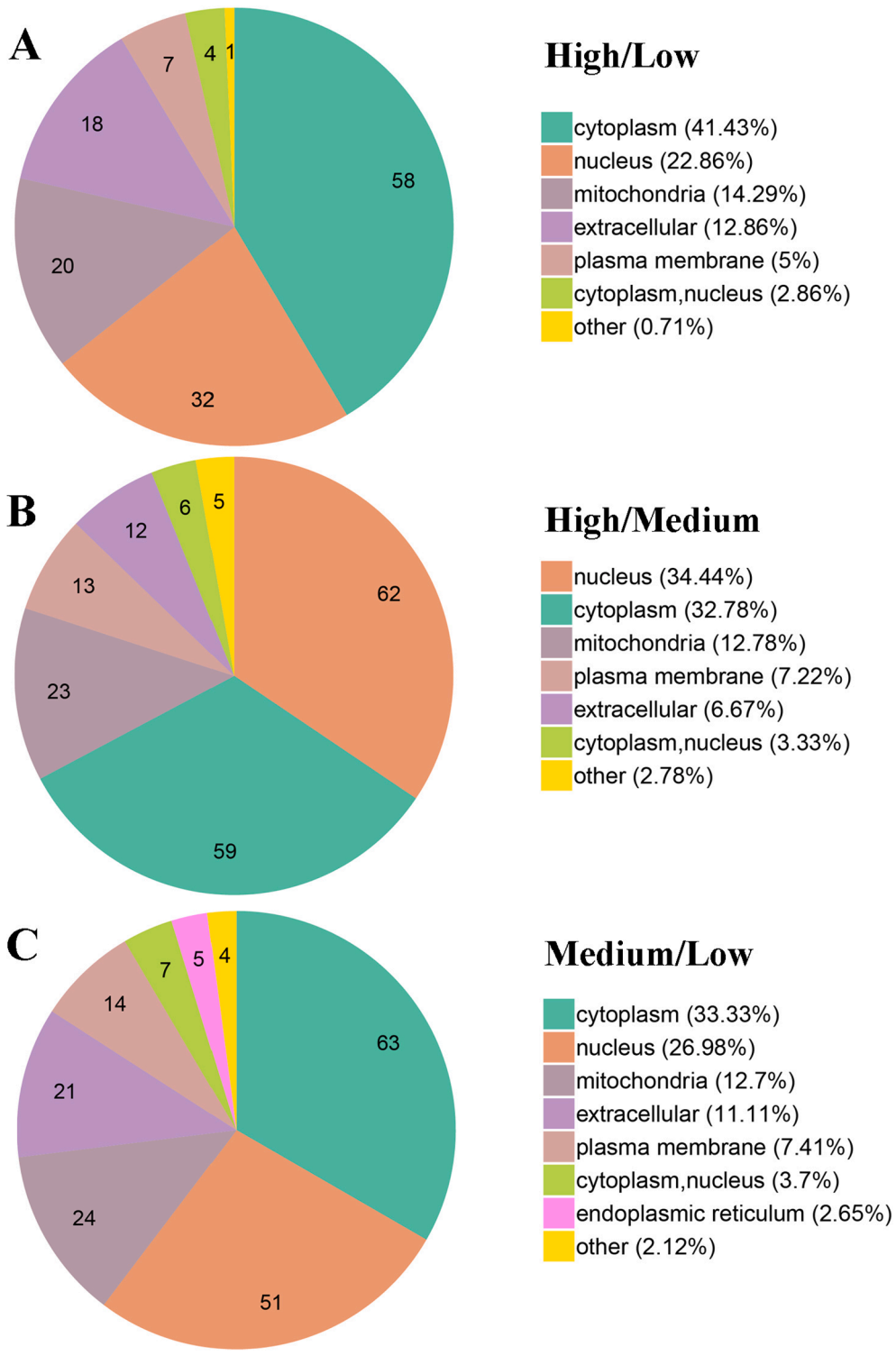

Fig. 4. Effects of selenium on proteins of different suborganelles in mice spleen. Subcellular location of differential proteins was analysed, and showed as (A) High/Low, (B) High/Medium and (C) Medium/Low (n = 3).

in the renal, blood and spleen of pigs (Li et al., 2020; Tang et al., 2020; Li et al., 2021). IL-10 in the renal and spleen of pigs were decreased (Li et al., 2020; Li et al., 2021). In this study, low selenium reduced IL-6 and IL-10 in the serum of mice, while high selenium increased IL-6 content. Moreover, the reduction in IL-12p70 was consistent with the decline in the number and impaired function of DC (Zhang et al., 2021a). IL-17 was reduced in the spleen of chicken (Khoso et al., 2019) and increased in the renal and spleen of pigs (Li et al., 2020; Li et al., 2021). Our study indicated that IL-17A was increased with low and high selenium. Additionally, IL-3, IL-4, IL-5, Eotaxin, G-CSF, IFN-γ and TNF-α were also affected in this study. Altogether, selenium deficiency leads to pathological changes and inflammatory injury in pig spleen, kidney, liver and chicken spleen. Our results suggested that low and high selenium can affect the composition of immune cells by regulating the secretion of cytokines, induces inflammatory injury and impaired immune response in mice spleen, or inflammatory damage in other organs.

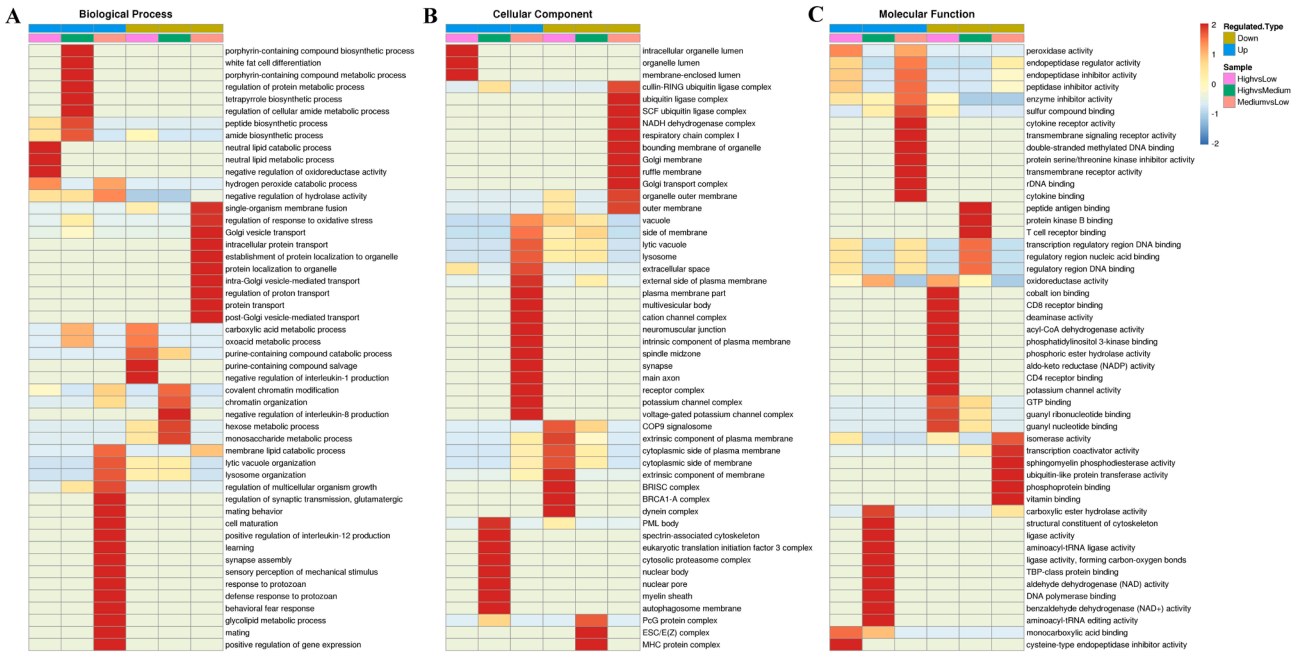

Fig. 5. Gene Ontology analysis of differential proteins in the spleen of mice fed with selenium. Functional information of differential proteins were analysed by GO categorisations, (A) biological process, (B) cellular compartment and (C) molecular function (n = 3).

Proteomic analysis showed that selenium could regulate cell maturation and differentiation, and affect the secretion of IL-1, IL-8 and IL-12 in the spleen, consistent with the above results. Furthermore, changes in the receptor complex and MHC complex affected the binding of cytokines, antigens and receptors. Recent reviews have indicated that changes in redox status can regulate immune responses, including cytokine secretion, activation and suppression of immune cells (Mullen et al., 2020; Sun et al., 2020). Our study showed that the impairment of the immune function of the spleen was related to oxidative stress regulation and changes in oxidoreductase activity. It was also suggested that the redox was involved in the metabolic regulation of immune cells (Sun et al., 2020; Griffiths et al., 2017). Effective immunotherapy can affect lipid metabolism and ketone body metabolism in mitochondria and enhance the killing effect of T-cells by MHC-related proteins (such as CD74) and antigen-presenting proteins (Harel et al., 2019). Additionally, methionine metabolism affected tumour therapy in mice (Gao et al., 2019). Interestingly, selenium affected lipid metabolism, ketone body metabolism and methionine metabolism in the spleen of mice, and antigen binding, MHC complex was changed, indicating that selenium influenced the immune function of the spleen in mice by regulating metabolism, and it provides new insights into the role of selenium in cancer therapy. It was shown that the NF-κB pathway was involved in the regulation of immune function in chickens and sow (Mou et al., 2021; Yang et al., 2021; Zhang et al., 2021b; Zhang et al., 2020a). HIF-1 played an important role in DC, T-cells, B cells and macrophages (Liu et al., 2019; Cho et al., 2019; Burrows et al., 2020; Stothers et al., 2018). Moreover, Notch and TNF signalling were also important in the immune system (Vanderbeck & Maillard, 2021; Holbrook et al., 2019). In this study, NF-κB, HIF-1, Notch and TNF signalling pathways were all involved in regulating spleen immune function by selenium in mice. Altogether, selenium regulated the immune function of the spleen through redox state, metabolism and signalling pathways.

KEGG analysis showed changes in the expression of H-2 class I histocompatibility antigen (H2-K1) and H-2 class II histocompatibility antigen (H2-Aa) in the spleen under high selenium condition might cause inflammatory bowel disease, autoimmune thyroid disease, type 1 diabetes mellitus and viral infection. And Btk and tumour necrosis factor receptor superfamily member 5 (CD40) was associated with primary immunodeficiency in low selenium. H2-K1 and H2-Aa were the components of MHC-I and MHC-II (Mangold et al., 2017; Stables et al., 2011), and defects in them caused impaired antigen presentation (Yamamoto et al., 2020; Jurewicz & Stern, 2019). The decrease in Btk and CD40 in B-cell resulted in the imbalance of B-cell and T cell interaction (Rip et al., 2018), CD40/CD40L (CD40 ligand) pathway was critical for germinal centre responses and the activation of T-cells, DC, macrophages, NK cells and granulocytes (Karnell et al., 2019). FcγRs were involved in antigen uptake, processing and presentation, associated with autoimmune diseases (Junker et al., 2020), and this study showed that FcγR-mediated phagocytosis was influenced by selenium. Our results indicated that immune diseases caused by low and high selenium were associated with impaired antigen presentation and B cell-T cell interaction imbalance.

It was suggested that Aldh1a7, an isoform of aldehyde dehydrogenase, is a marker for stem cells (Muzio et al., 2012), the increased Aldh1a7 in the high selenium group might regulate the activity of spleen stem cells. Loss of Abcc9, a component of potassium channels, induced fatigability and cardiac dysfunction in mice (Smeland et al., 2019). Significantly down-regulated in low and high selenium groups, indicating that the spleen function was impaired. Gpx3 was reduced with low selenium, but Pex3 was increased, which is essential for peroxisomal membrane biogenesis and detoxification (Jansen & van der Klei, 2019). Consistent with previous findings (Lee et al., 2020; Verma et al., 2011), increasing of selenium upregulated the levels of Gpx1 and SELENOK. Moreover, Gpx1 and SELENOK were involved in the regulation of immune cell function (Huang et al., 2012).

Other important proteins that had changed include B-cell lymphoma/leukaemia 11A (Bcl11a), B-cell receptor-associated protein 29 (Bcap29), Ig alpha chain C region, interferon regulatory factor 2(Itf2), gamma interferon-inducible lysosomal thiol reductase (Ifi30), perforin 1 (Prf1) and so on. Additionally, Fyn, Lyn, granzyme A, Rfx1, CD74 and Rab27b was also influenced by selenium. It was shown that Fyn and Lyn were essential to perforin-dependent killing of NK cells (Oykhman et al., 2013), and granzyme A played an important role in cytotoxic cells (van Daalen et al., 2020). Apoptosis of the germinal centre with high selenium may be related to Fyn, Lyn and granzyme A elevation. Rfx1 participated in autoimmune disease (Zhao et al., 2018), hepatitis B virus

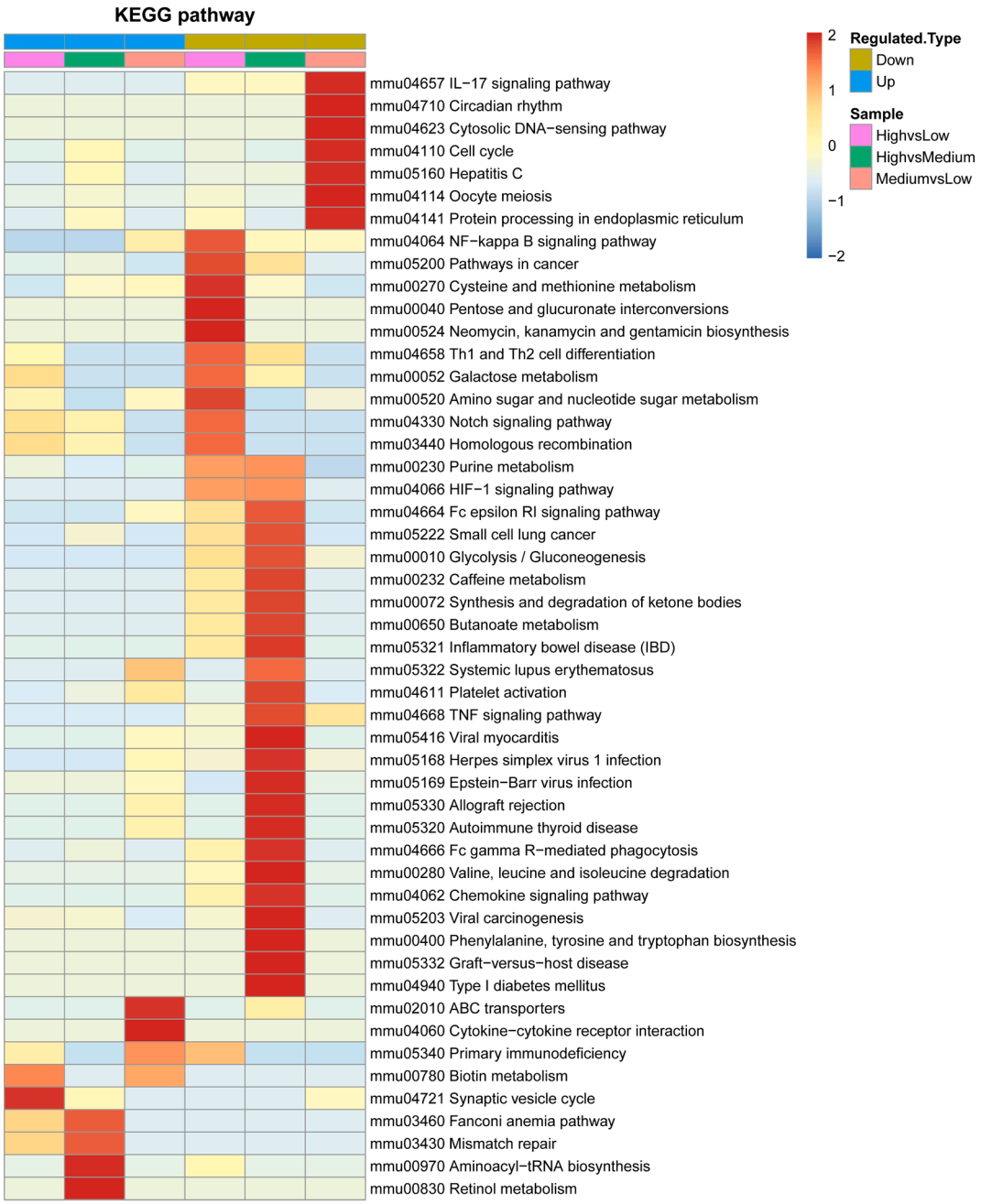

Fig. 6. KEGG analysis of differential proteins in the spleen of mice fed with selenium. The KEGG pathway enrichment of differential proteins was conducted by KEGG Mapper, the number and name of signalling pathways are on the right side of the image (n = 3).

replication (Wang et al., 2018a), and was a target for cancer therapy (Issac et al., 2021), which may be related to viral infection, immune diseases and spleen damage in this study. Rab27b was critical for neutrophil chemotaxis, mast cell exocytosis and exosome secretion (Singh et al., 2014; Prashar et al., 2017; Ostrowski et al., 2010), this might result in impaired immune function and cytokine secretion in mice with low or high selenium. In brief, this study confirmed that various proteins that play essential roles in immune function were regulated by selenium.

Previous studies showed that histone modification (Wang et al., 2018c), protein post-translational modifications (Liu et al., 2016; Hu & Sun, 2016), autophagy (Deretic, 2021) and DNA damage (Uchihara et al., 2021) were involved in regulating immune cell function. Interestingly, our results found that proteins related to DNA repair, histone acetylation and methylation, protein deacetylation and ubiquitination, and autophagies were also regulated by selenium levels. The damage of spleen immune function caused by low and high selenium in this study also may be related to these factors, and it provides a reference for further study on the effects of selenium on immune function.

5. Conclusions

In conclusion, our study showed that low and high selenium affected the secretion of cytokines and impaired the immune function of the spleen by regulating the redox state, metabolism and signalling pathways in mice, and confirmed that some proteins related to immune cell function and immune diseases were regulated by selenium.

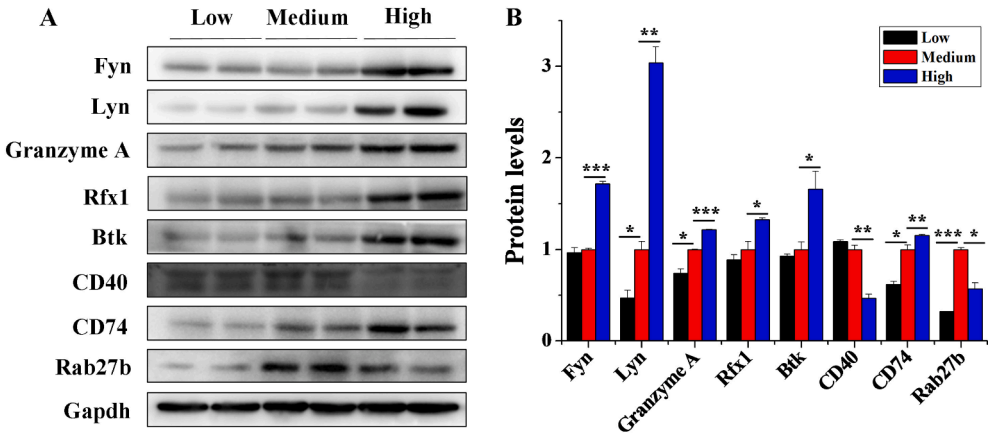

Fig. 7. Verification of differential proteins using Western blotting. (A) Protein levels of Fyn, Lyn, granzyme A, Rfx1, Btk, CD40, CD74 and Rab27b in the spleen of mice fed with selenium were detected using Western blotting, (B) the density values relative to Gapdh were analysed (n = 4). Data are presented as mean ± standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with medium selenium.

Ethics statement

This study was approved by the Institutional Animal Care and Use Committee of Guizhou Medical University.

CRediT authorship contribution statement

Xin Zhang: Methodology, Writing – original draft, Data curation. **Liangliang Zhang:** Methodology, Writing – original draft, Data curation. **Kaide Xia:** Methodology, Writing – original draft, Data curation. **Jie Dai:** Data curation. **Jiangtao Huang:** Data curation. **Yun Wang:** Data curation. **Guiming Zhu:** Data curation. **Zuquan Hu:** Data curation. **Zhu Zeng:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Yi Jia:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (No. 21867007 and No. 21561006), Key Laboratory of Infectious Immune and Antibody Engineering of Guizhou Province (No. KY[2020]012), Guizhou Provincial Natural Science Foundation (No. [2019]1258 and No. LH[2016]7372), and Opening fund of Hubei Key Laboratory of Bioinorganic Chemistry & Materia Medica (No. BCMM202002).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104914.

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