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The action of topical application of Vitamin B_{12} ointment on radiodermatitis in a porcine model

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Abstract

Radiodermatitis is an inevitable side effect of radiotherapy in cancer treatment and there is currently no consensus on effective drugs for treating the condition. Vitamin B_{12} is known to be effective for repairing and regenerating damaged skin. However, there are few studies on the use of Vitamin B_{12} for treating radiodermatitis. This study explored the therapeutic efficacy and mechanism of action of Vitamin B_{12} ointment on radiodermatitis. A porcine model of grade IV radiodermatitis was established. The ointment was applied for 12 weeks after which histological staining, transmission electron microscopy, RT-qPCR, western blotting, and gene sequencing were performed for the evaluation of specific indicators in skin samples. After 12 weeks of observation, the Vitamin B_{12} treatment was found to have significantly alleviated radiodermatitis. The treatment also significantly reduced the expression levels of NF-κB, COX-2, IL-6, and TGF-β in the skin samples. The pathways involved in the effects of the treatment were identified by analysing gene expression. In conclusion, Vitamin B_{12} ointment was found to be highly effective for treating radiodermatitis, with strong anti-radiation, anti-inflammatory, and anti-fibrosis effects. It is thus a promising drug candidate for the treatment of severe radiodermatitis.

KEYWORDS

radiation fibrosis, radiation injury, radiodermatitis, radiotherapy, Vitamin B_{12}

Key Messages

- Vitamin B_{12} ointment is highly effective for the treatment of severe radiodermatitis in a porcine model
- Vitamin B_{12} ointment has strong anti-radiation, anti-inflammatory, and anti-fibrosis effects
- Vitamin B_{12} ointment is a promising drug candidate for the treatment of severe radiodermatitis

1 | INTRODUCTION

Radiotherapy is a major anti-cancer treatment and is suitable for most malignant tumours.[1] However, radiodermatitis is an unavoidable side effect of radiotherapy with 95% of patients experiencing different degrees of radiodermatitis during radiotherapy.[2,3] The clinical manifestations of radiodermatitis include skin erythema, desquamation, ulcer necrosis, skin atrophy, fibrosis, oedema, necrosis, fistula formation, and prolonged wound healing. These seriously affect the quality of life of patients and restricts the smooth completion of the treatment plan.[4] Therefore, the development of a specific and effective drug for the treatment of radiodermatitis would have immeasurable economic and social benefits.

Vitamin B_{12}, also known as cobalamin, is a water-soluble vitamin. As an important bioactive substance in vivo, Vitamin B_{12} exists in many forms in vivo. It plays an indispensable role in DNA synthesis, methylation, and maintaining genomic stability, and acts as a cofactor in a variety of biochemical processes. Vitamin B_{12} accelerates the proliferation of granulation tissue and the regeneration of epithelial cells. It can also repair and regenerate damaged skin epithelial cells and vascular endothelial cells and nourish and promote the repair of damaged nerve sheaths.[5-7] In addition, Vitamin B_{12} also has significant antipruritic and analgesic effects.[8,9]

In this study, we used mini fragrant pigs to establish an animal model of grade IV radiodermatitis. We studied the therapeutic efficacy and mechanism of action of a compound Vitamin B_{12} ointment, an original drug with intellectual property rights independently developed by our team, on radiodermatitis. The research results are expected to provide a valuable reference for the clinical application of Vitamin B_{12} ointment in the future.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Three mini fragrant pigs, aged 6 to 7 months were used. The pigs were of both sexes and weighed 15 ± 2 kg and were housed in the standard animal laboratory (22°C) of the experimental animal center of the Basic Medical College of Jilin University. Animal experiments followed the guidelines of the National Institutes of Health on the care and use of experimental animals (NIH Publication No. 8023, revised 1978), and all animals were maintained in accordance with guidelines and requirements for the care and use of laboratory animals of Jilin University.

2.2 | Radiation exposure

Before irradiation, 3% pentobarbital sodium (Merck, Germany) was given by intravenous injection at a dose of 1 mL/kg. Local irradiation was carried out with a medical linear accelerator (23EX, Varian, USA). The irradiation area consisted of three 3 × 3 cm^2 skin areas, with an additional 3 × 3 cm^2 area used as the blank control (Figure 1). The 6 MeV electron beam was irradiated with 75 Gy for a single dose, and the dose rate was 4 Gy/min.

2.3 | Drug application and observation

Four 3 × 3 cm^2 areas were designated as the drug-free, Vitamin B_{12}, triethanolamine, and normal skin groups, respectively. The Vitamin B_{12} and triethanolamine groups received daily applications of Vitamin B_{12} ointment (Jilin Aodong Yanbian Pharmaceutical, China) and Biafine Topical Emulsion scald ointment (Biafine, France), respectively. The skin changes in the irradiated area were observed and recorded weekly. The skin reaction was evaluated by Radiation Therapy Oncology Group acute skin toxicity grading. After 12 weeks of medication and observation, skin samples were taken from the tested areas and analysed as described below.

2.4 | Histopathological staining

Fresh skin tissue samples were fixed in 4% paraformaldehyde (Beijing Chemical Plant, China), dehydrated, embedded in paraffin, and cut into 4 μm slices. Sections were stained with Masson's trichrome staining or haematoxylin and eosin (H&E), and histological changes were observed under a light microscope (CTR600, Leica, Germany). Skin thickness was measured by Image-Pro Plus software 6.0 and calculated from the mean.

2.5 | Immunohistochemical staining

The tissue was paraffin-embedded and sectioned as described above. After deparaffinisation, antigen retrieval, and blocking, the sections were incubated overnight at 4°C with anti-COX-2 (1:200), NF-κB (1:200), and IL-6 (1:200) primary antibodies. This was followed by probing with the appropriate secondary antibodies for 2 hours at room temperature. All antibodies were purchased from Abcam (Cambridge, UK). The colour was developed using DAB

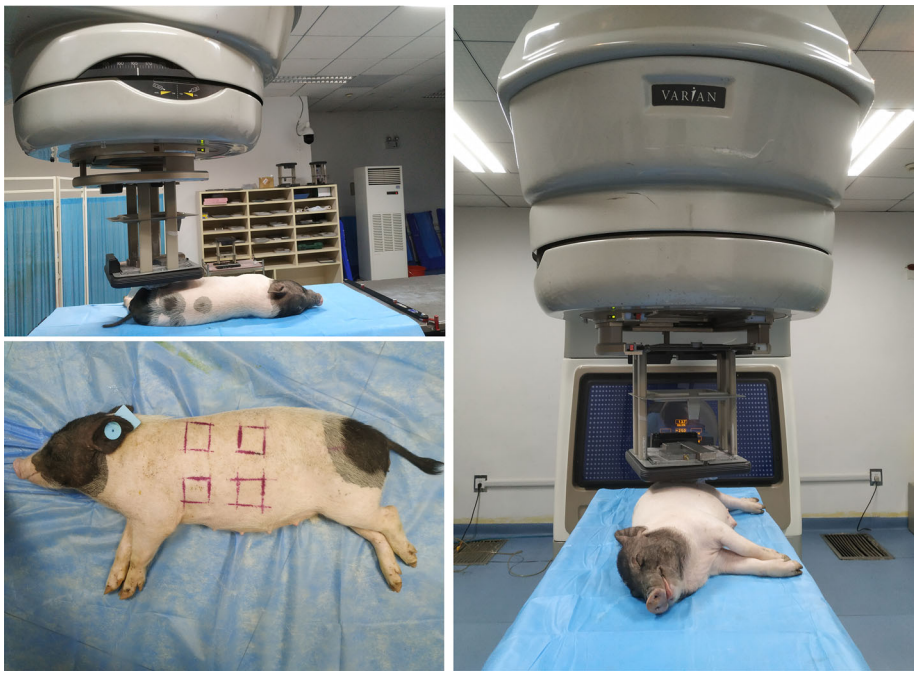

FIGURE 1 The day before the irradiation, the hair was shaved from the tested areas. The skin in the test areas were irradiated with a medical linear accelerator and marked with lines. The four marked areas were divided into drug-free group, triethanolamine group, Vitamin B_{12} group and blank control group.

(3,3′-diaminobenzidine) chromogenic solutions (Absin, China). After counterstaining the nuclei with haematoxylin (Biyuntian, China), the sections were dehydrated, treated with Histo-Clear, sealed with neutral balsam, and examined and imaged under light microscopy.

2.6 | Immunofluorescence staining

Paraffin sections were deparaffinised, antigen retrieved, blocked, and then incubated overnight at 4°C with TGF-β primary antibody (1:300) and horseradish peroxidase-labelled secondary antibody. All the above antibodies were purchased from Abcam. Nuclear staining was performed in the dark by adding DAPI staining solution (Sigma, USA), and sections were sealed with anti-fluorescence quenching sealant. Sections were observed under a fluorescence microscope (TE2000-U, Nikon, Japan), and images were collected.

2.7 | Transmission electron microscopy analysis

The skin tissue samples were fixed in 1% osmic acid (Ted Pella Inc, USA) at room temperature for 2 hours, dehydrated in a gradient of ethanol and acetone, embedded, and cut into 0.5 to 1.0 μm slices. After staining with toluidine blue, uranyl acetate, and lead citrate, the ultrastructure of the tissue was observed and photographed under a transmission electron microscope (Hitachi, Japan).

TABLE 1 Primer sequences used in RT-qPCR analyses

Gene	Primer (5′-3′)	Primer sequence
NF-κB	Forward	CTCCACAAGGCAGCAAATAGACGAG
	Reverse	CGTGGGGCACTTTGTTGAGAGTTAG
COX-2	Forward	GGCTGCCCTTTTACCTCATTCAGTG
	Reverse	TCCAGAGTGGGAAGAGCTTGCATTA
IL-6	Forward	TACCAAAGCACTGATCCAGACCCTG
	Reverse	CTTCATCCACTCGTTCTGTGACTGC
TGF-β	Forward	CCAAGGGCTACCATGCCAATTTCTG
	Reverse	CTGGTTGTACAGAGCCAGGACCTT

2.8 | RT-qPCR

Total RNA was extracted from skin tissue with TRIzol reagent (Tiangen, China) and reverse-transcribed into cDNA with the First Strand cDNA Synthesis Kit (NEB, USA). RT-qPCR was performed with SYBR fluorescent dye (Tiangen, China). The PCR was performed with preset cycles in a fluorescent quantitative PCR apparatus (Applied Biosystems, USA). The primer sequences are shown in Table 1. For data processing, the cycle threshold (CT) values of the target genes and the internal reference gene were obtained after amplification, and the $2^{-\Delta\Delta CT}$ method was used for relative quantitative analysis.

2.9 | Western blotting

The skin tissue was cut into small pieces and homogenised to extract the total protein. The protein concentration was determined by the Bicinchoninic acid (BCA) method, and the buffer was added at the ratio of 4:1. The sample was boiled in boiling water for 15 minutes, then 100 μl mixture was taken out from the solution and placed in a new EP tube for SDS-PAGE electrophoresis (Servicebio, China). The protein bands from the electrophoresis gel were transferred to nitrocellulose membrane (Servicebio, China). After blocking with 5% skimmed milk powder at room temperature for 1 hour, the membranes were incubated with COX-2, NF-κB, IL-6, or TGF-β antibodies overnight at 4°C. After labelling with the secondary antibody, banding diagrams were obtained using a developer. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal reference.

2.10 | Gene sequencing analysis

Following the construction of the cDNA library, high-throughput sequencing was performed by Shanghai Tianhao Biotechnology Co., Ltd. (China) using the Illumina HiSeq 2000 platform. The results were analysed in R (version 4.1.1). The analysis included the compilation of volcano plots and an mRNA clustering heatmap, while functional enrichment of the genes and their associated pathways was conducted by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

2.11 | Statistical analysis

SPSS software (version 17.0, Chicago, IL) was used to analyse the data, and the dose data were expressed as mean ± standard deviation. The *t*-test was used to evaluate statistical differences. Significant differences were shown as $^{*}P < .05$, $^{**}P < .01$.

3 | RESULTS

3.1 | Evaluation of skin appearance and grade of radiodermatitis

The changes in skin appearance and radiodermatitis grade with time after irradiation are summarised in

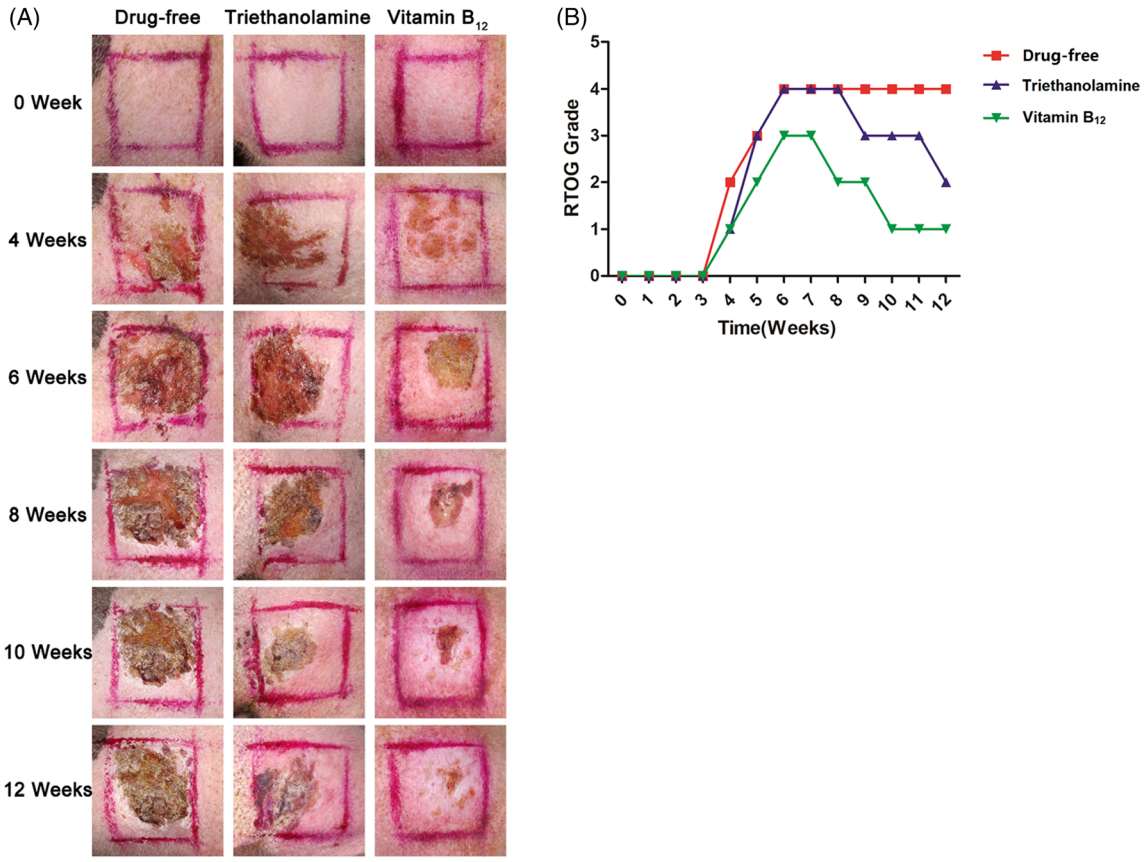

FIGURE 2 Skin changes after irradiation. (A) Skin appearance and (B) grade of radiodermatitis with time after irradiation. The observation time points were 0, 4, 6, 8, 10 and 12 weeks. The skin recovery rate of Vitamin B_{12} group was significantly better than that of other groups.

Figure 2. By the fourth week after irradiation, the drug-free group had developed moist desquamation (grade II radiodermatitis) while the triethanolamine and Vitamin B_{12} groups had developed grade I radiodermatitis. By the sixth week, ulcers (grade IV radiodermatitis) were apparent in the drug-free and triethanolamine groups while the Vitamin B_{12} group had developed grade III radiodermatitis. The radiodermatitis in the Vitamin B_{12} group gradually recovered with a corresponding decrease in the radiodermatitis grade. Gradual recovery was also visible in the triethanolamine group; however, the recovery was slower than that in the Vitamin B_{12} group. However, poor recovery was seen in the drug-free group and the grade IV ulceration continued to develop into a persistent skin injury. By the 12th week, grade IV radiodermatitis was still apparent in the drug-free group, while the dermatitis had reduced to grade II in the triethanolamine group and to grade I in the Vitamin B_{12} group, with only a little dry desquamation visible in the latter, indicating almost complete recovery.

3.2 | Histological analysis

H&E staining showed that the changes in the epidermal layer essentially corresponded to the changes in the appearance of the skin. Scabs were visible in the epidermal layer in the sections from the drug-free group (Figure 3A). The epidermal thickness ranged from high to low in the order of the drug-free group, the triethanolamine group, the Vitamin B_{12} group, and the normal skin group (Figure 3C, $P < .01$). Irradiation-induced epidermal thickening was significantly reduced in the Vitamin B_{12} group compared with the drug-free group. In addition, the subepidermal tissue was denser in the Vitamin B_{12} group compared with the drug-free and triethanolamine groups (Figure 3A). Masson staining showed that there was a large amount of collagen deposition in the drug-free group (Figure 3B), far more than in the Vitamin B_{12} and normal skin groups.

3.3 | Immunohistochemistry and immunofluorescence analysis

Immunohistochemical staining showed low COX-2 protein staining in normal skin tissues and the epidermal layer had almost no staining. In the drug-free group, extensive COX-2 staining was visible in both epidermal and subcutaneous tissue, much higher than in the Vitamin B_{12} group (Figure 4A). Like COX-2,

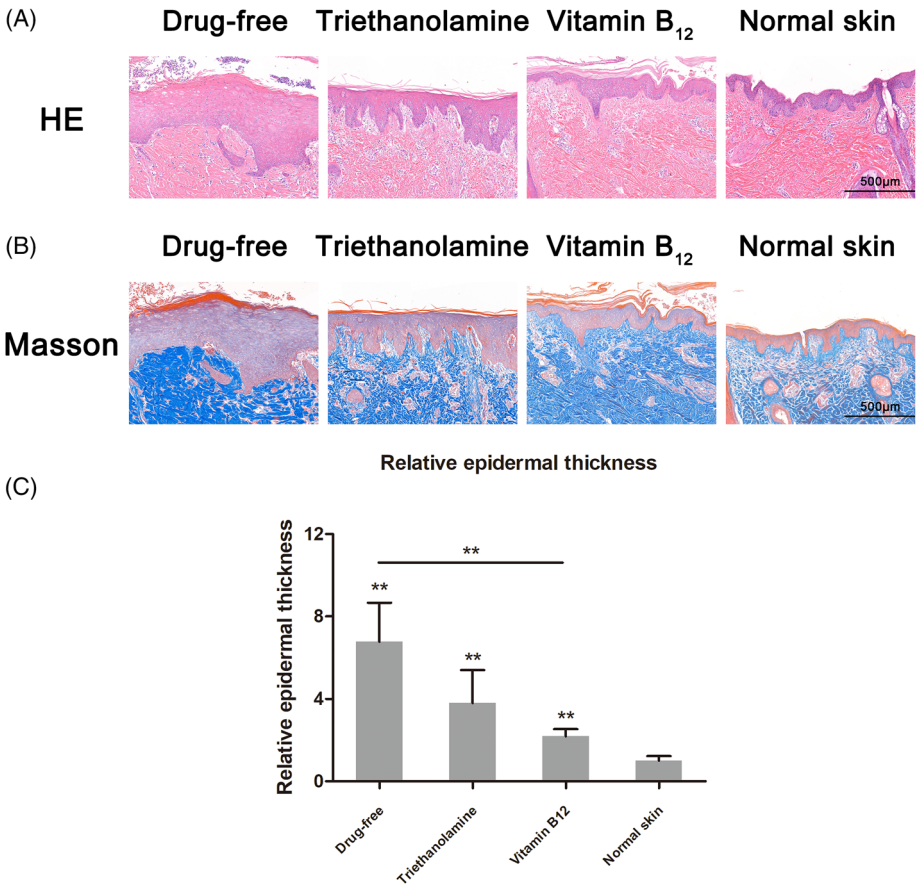

FIGURE 3 Histological changes in the skin in the different groups. (A) H&E staining (×100). (B) Masson staining (×100). (C) Changes in the relative thickness of the epidermis of the skin tissues in each group. *$P < .05$ (n = 5), **$P < .01$ (n = 5)

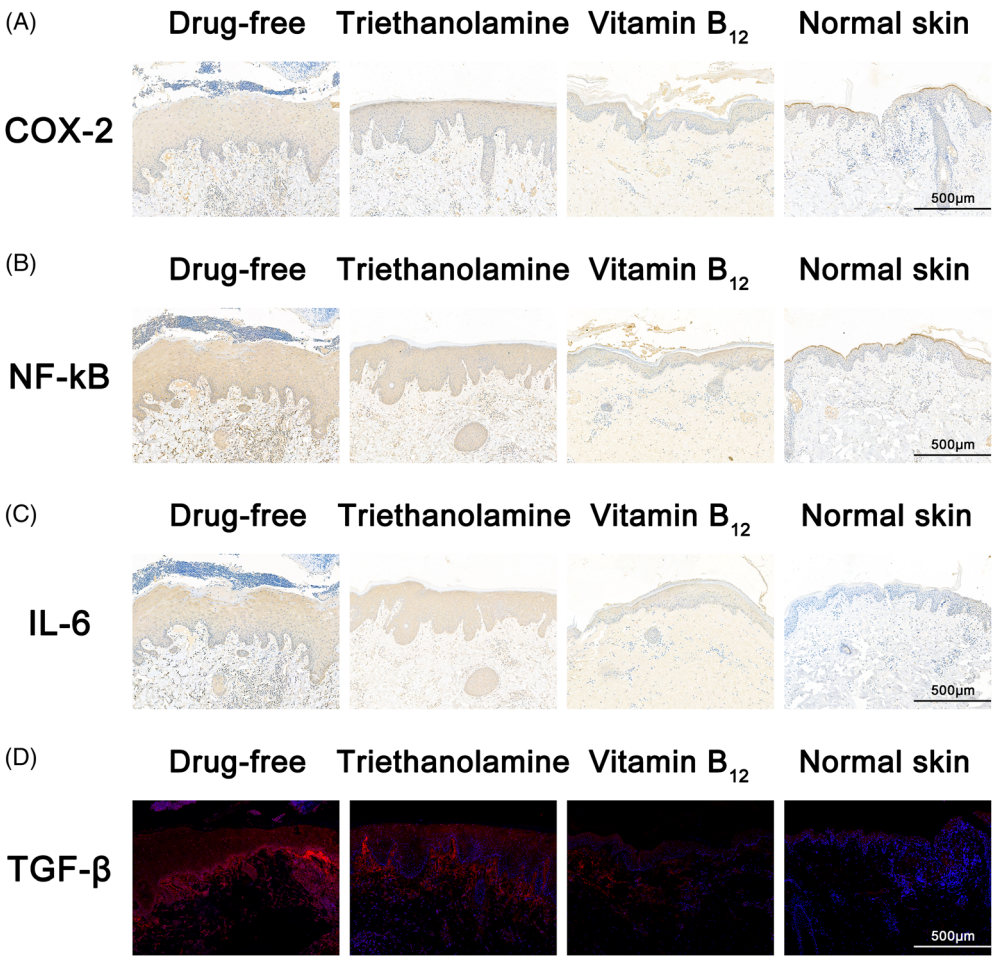

FIGURE 4 Detection of protein expression levels in skin tissue in the different groups. (A) COX-2, detected by immunohistochemistry, (B) NF-κB, detected by immunohistochemistry, (C) IL-6, detected by immunohistochemistry, and (D) TGF-β, detected by immunofluorescence. All magnifications ×100

NF-κB protein is rarely expressed in normal skin tissues. However, NF-κB was highly expressed in both the drug-free and triethanolamine groups, with diffuse staining visible. The Vitamin B_{12} group showed less expression, only slightly higher than that in the normal skin group (Figure 4B). IL-6 was highly expressed in the drug-free and triethanolamine groups, while only expressed at low levels in the Vitamin B_{12} and normal skin groups (Figure 4C). TGF-β expression was highest in untreated skin, ranging (from high to low) in the order of the drug-free group, triethanolamine group, Vitamin B_{12} group, and normal skin group (Figure 4D). These results show that Vitamin B_{12} treatment can effectively reduce the expression levels of COX-2, NF-κB, IL-6, and TGF-β in the skin tissue after irradiation.

3.4 | Transmission electron microscopy

Ultrastructural changes in the skin tissue were observed under transmission electron microscopy. In the transverse sections of normal skin, collagen fibres were arranged in tubular structures with small interfibrillar spaces (Figure 5A). In the longitudinal sections of normal skin, the fibres were regularly arranged and similar in length. The sections of Vitamin B_{12} skin showed a similar appearance to that of normal skin (Figure 5B). In contrast, in the drug-free and triethanolamine groups, the collagen fibres in the transverse sections showed larger interfibrillar spaces and sparse arrangements and were irregularly arranged and dissimilar in length in the longitudinal sections (Figure 5A,B). Examinations of the hemidesmosome and tight junction structures showed a slight reduction in the hemidesmosome numbers in the epidermis of the Vitamin B_{12} group compared with normal skin, with significant reductions in the drug-free and triethanolamine groups (Figure 5C). In addition, the structures of the tight junctions at the dermis-epidermal junction were similar in the Vitamin B_{12} and normal-skin groups but were sparser in the drug-free and triethanolamine groups (Figure 5D).

3.5 | RT-qPCR detection of mRNA expression levels of related molecules in skin tissues

RT-qPCR showed that the mRNA expression of COX-2, NF-κB, IL-6, and TGF-β in normal skin was significantly

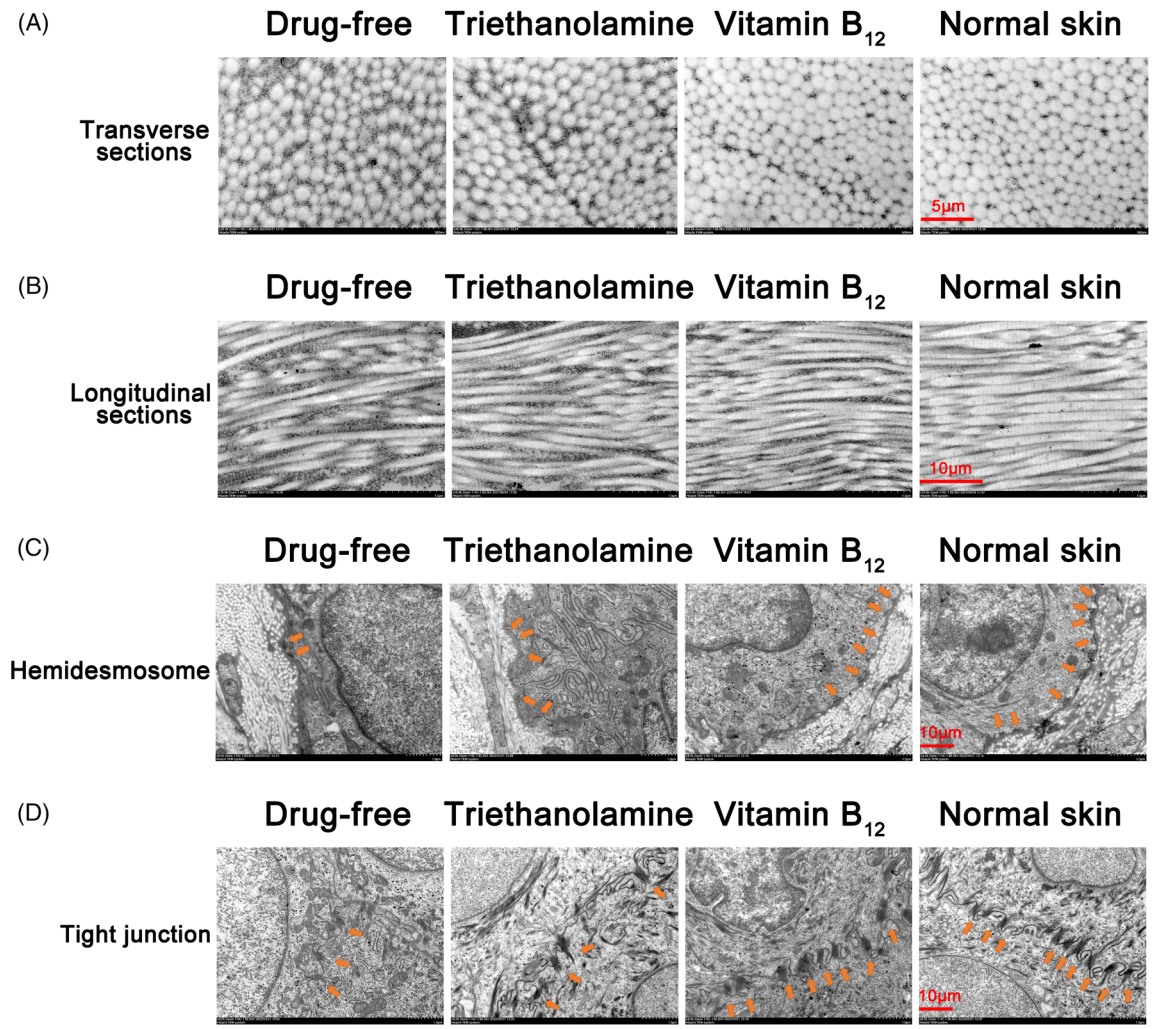

FIGURE 5 Transmission electron micrographs of (A) transverse sections of collagen fibres (×25 000), (B) longitudinal sections of collagen fibres (×15 000), (C) hemidesmosomes (×8000), and (D) tight junctions (×8000). The arrows point to the (C) hemidesmosomes and (D) tight junctions, respectively.

lower than those in the other groups (Figure 6). Compared with the drug-free and triethanolamine groups, the expression of COX-2 and NF-κB in the Vitamin B_{12} group was significantly reduced ($P < .01$) (Figure 6A,B). The expression of IL-6 in the Vitamin B_{12} group was significantly lower than that in the drug-free group ($P < .01$). There was no significant difference in the expression of IL-6 between the triethanolamine group and the Vitamin B_{12} group ($P > .05$) (Figure 6C) while TGF-β mRNA levels in the Vitamin B_{12} group were significantly lower than those in the drug-free and triethanolamine groups ($P < .01$; $P < .05$, respectively) (Figure 6D).

3.6 | Western blotting

The western blotting results are shown in Figure 7. The expression level of COX-2 in the Vitamin B_{12} group was lower than that in the drug-free and triethanolamine groups and only slightly higher than that in the normal-skin group. Both NF-κB and IL-6 protein levels were high in the drug-free group, with only minimal expression in normal skin. The expression of these proteins in the triethanolamine and Vitamin B_{12} groups was intermediate between the above two groups. TGF-β protein expression was highest in the drug-free group, which was much higher than that of both the Vitamin B_{12} and normal skin groups.

3.7 | Gene sequencing analysis

Gene sequencing was performed on samples from the Vitamin B_{12} and drug-free groups. A volcano plot and a heatmap of significantly different mRNA

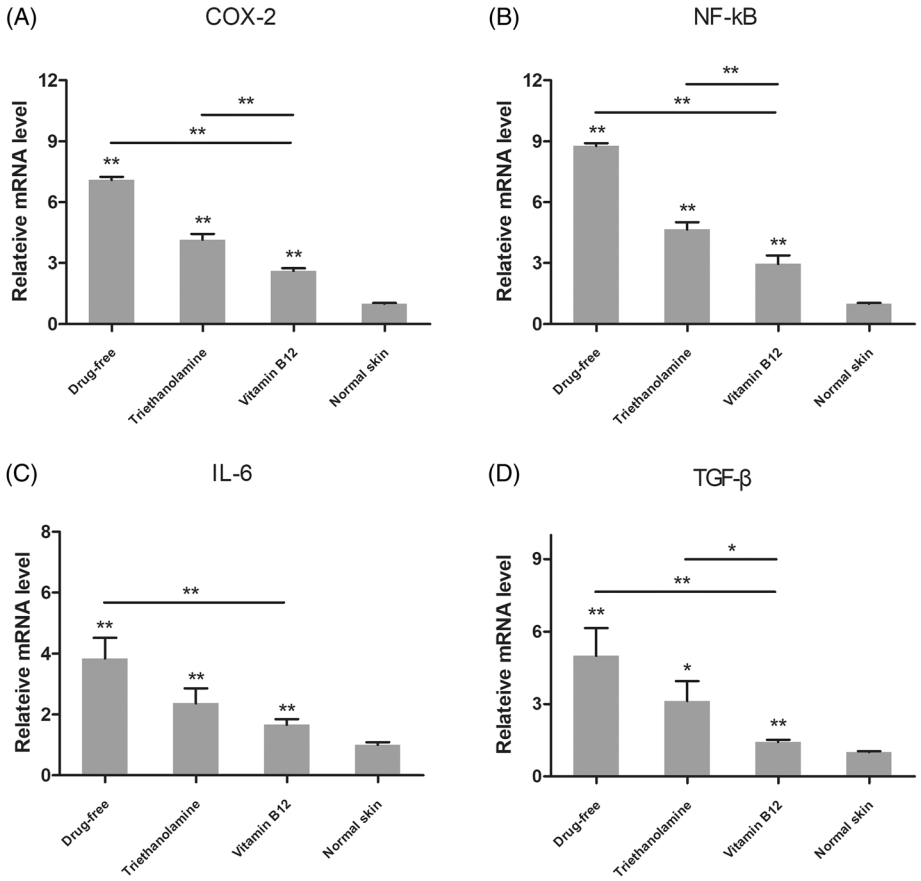

FIGURE 6 mRNA expression levels detected by RT-qPCR in skin tissue in the different groups. (A) COX-2 (B) NF-κB (C) IL-6 (D) TGF-β. *$P < .05$ (n = 3), **$P < .01$ (n = 3)

clusters (Top 50) based on the gene sequencing results are shown in Figure 8. Compared with the drug-free group, the Vitamin B_{12} group had 54 up-regulated genes including CHIA, PPP1R14A, LYZ, SLC5A8, and ADRA1A, and 152 down-regulated genes, including P2RY2, ANKRD1, HP, ABRA, and LBP. The scatter plot demonstrating the KEGG pathway enrichment analysis of the differentially expressed genes between the two groups is shown in Figure 9A. The enriched pathways included 'Calcium signalling', 'Glycolysis/Gluconeogenesis', 'Carbon metabolism', and 'Adenosine monophosphate-activated protein kinase signalling'. The up-and down-regulated genes were mapped to their KEGG pathways. The red and blue boxes represented the up-regulated and down-regulated genes in this pathway, respectively (Supporting information). Next, the 10 most significantly enriched GO functions were selected from the three categories of Biological process (BP), Cellular component (CC), and Molecular Function (MF), respectively (Figure 9B). It can be intuitively seen that the role of Vitamin B_{12} is related to molecular functions and biological processes involving myofibril assembly and the development of muscle cells, tissues, and overall structure. In addition, a directed acyclic graph illustrating the

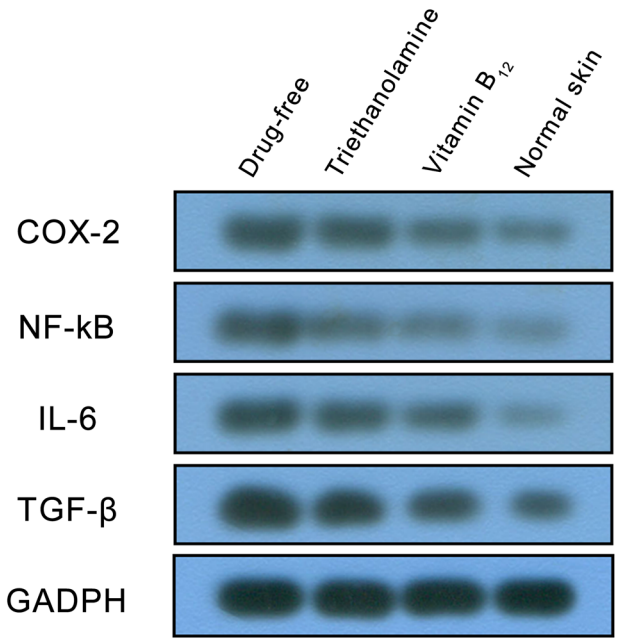

FIGURE 7 Western blot showing COX-2, NF-κB, IL-6, and TGF-β expression in the different groups. GADPH was used as the loading control.

GO enrichment analysis was drawn, from which the interrelationships between differential gene functions could be seen (Figure 10).

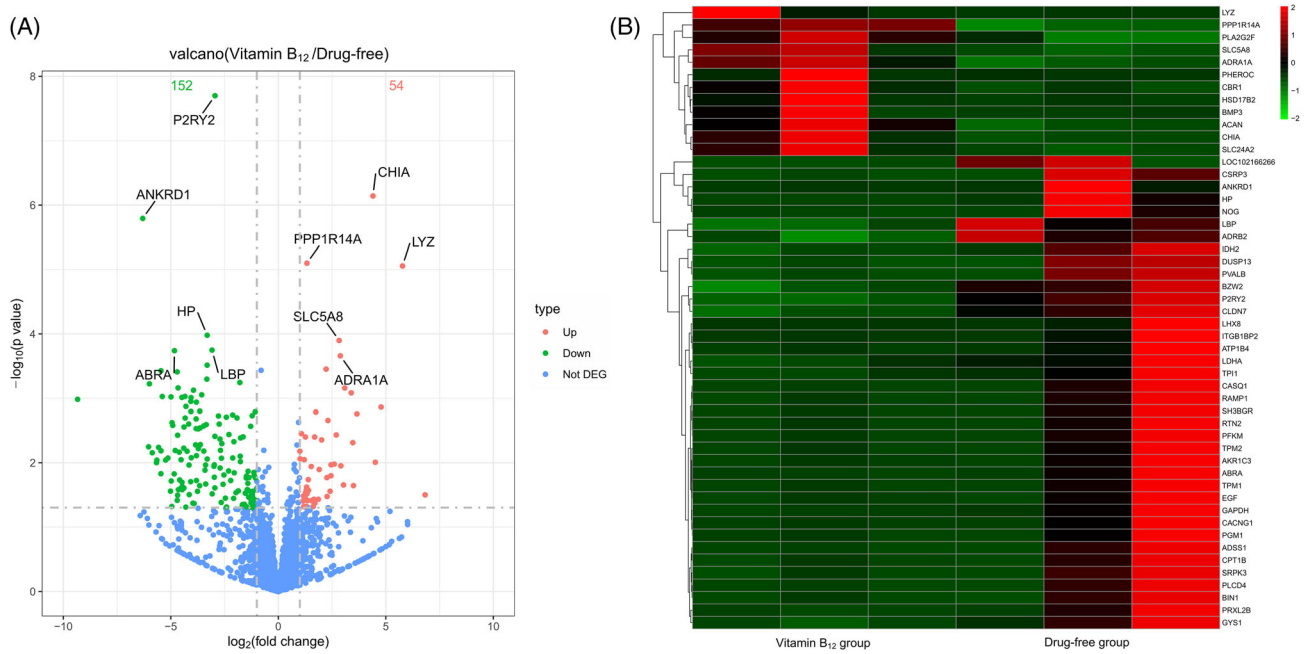

FIGURE 8 (A) Volcano plot and (B) heatmap of significantly different mRNA clusters (Top 50)

4 | DISCUSSION

The Vitamin B_{12} ointment independently developed by our team overcomes the shortcomings of rapid drying and short action time seen with commonly used Vitamin B_{12} aqueous solutions for external use. The ointment allows full absorption of the drugs by the skin during microvascular dilation in the early stage of radiodermatitis. Vitamin B_{12} ointment can protect the skin, reduce external stimulation, prevent ulceration, enhance skin resistance, and effectively repair hair follicles, sebaceous glands, and superficial ulcers. Vitamin B_{12} ointment can not only soften hard and tough skin and restore its local function but also promotes the absorption of pigmentation. In addition, Vitamin B_{12} ointment has excellent analgesic and antipruritic effects, and can effectively relieve skin pain.[10-12]

Mini fragrant pigs are unique to China and have a skin morphology and tissue structure that strongly resembles that of humans. Importantly, the pig skin responds to radiation in a similar manner to human skin, both in terms of time- and dose-dependence.[13,14] Therefore, the mini fragrant pig is an ideal choice for an animal model of radiodermatitis. In the study, the observations of the damaged skin over 12 weeks confirmed the positive therapeutic effect of Vitamin B_{12} ointment on radiodermatitis. Histological staining confirmed that treatment with the ointment slowed the tendency toward epidermal thickening and collagen deposition after irradiation.

The repair of collagen fibres is an important part of the healing process of skin lesions. Transmission electron microscopy observations showed that treatment with the Vitamin B_{12} ointment resulted in a more orderly arrangement and distribution of the collagen fibres in the skin, closely resembling the appearance of normal skin tissue. The hemidesmosome is the primary connecting structure between basal cells and the underlying basement membrane band that connects the epidermis to the dermis. Tight junctions between adjacent keratinocytes can be used to assess the integrity of the epidermis.[15,16] It was also observed that the number of hemidesmosomes and tight junctions in the Vitamin B_{12} group closely resembled those of normal skin and were much higher than seen in the other groups. These ultrastructural observations confirmed that the degree of healing in the dermis and the dermis-epidermal junction in the Vitamin B_{12} group was better than in the other two treated groups.

Ionising radiation can, directly and indirectly, activate NF-κB, and activation of NF-κB can up-regulate transcription of the stress response protein COX-2.[17,18] NF-κB and COX-2 are minimally expressed in normal pig skin tissues but their expression levels increase sharply after irradiation.[19] In addition, COX-2 can stimulate the synthesis of prostaglandins in the inflammatory cascade, ultimately leading to tissue damage.[20] Kim et al demonstrated that curcumin inhibits the expression of COX-2 and NF-κB in skin epithelial cells induced by radiation, coinciding with the relief of skin appearance and histological changes after irradiation.[21] Combined with the

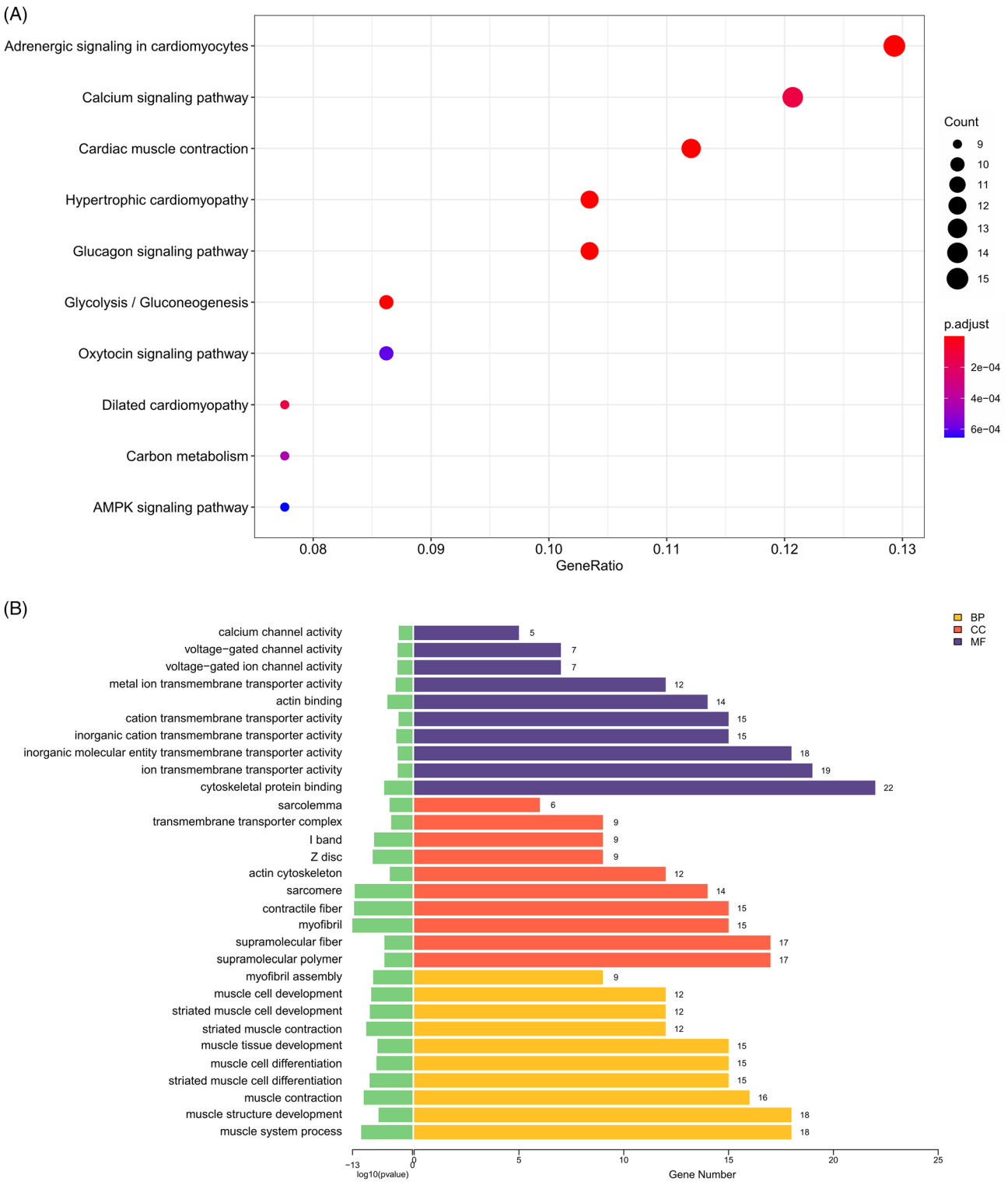

FIGURE 9 (A) KEGG enrichment scatter plot and (B) GO enrichment bar chart for differentially expressed genes

results of this study, it can be speculated that Vitamin B_{12} ointment has a similar effect to curcumin, that is, reducing the expression of pro-inflammatory radiodermatitis-induced factors NF-κB and COX-2 to alleviate the skin histological changes and inflammatory reaction after radiation, to improve the symptoms of radiodermatitis.

IL-6 is one of the key cytokines in the inflammatory response and has a wide range of biological effects.[22] After skin injury, mitogen-activated protein kinase is activated in vivo, and the expression of IL-6 at the wound site is rapidly increased. In addition to its direct activation and toxic effects on vascular endothelial cells and

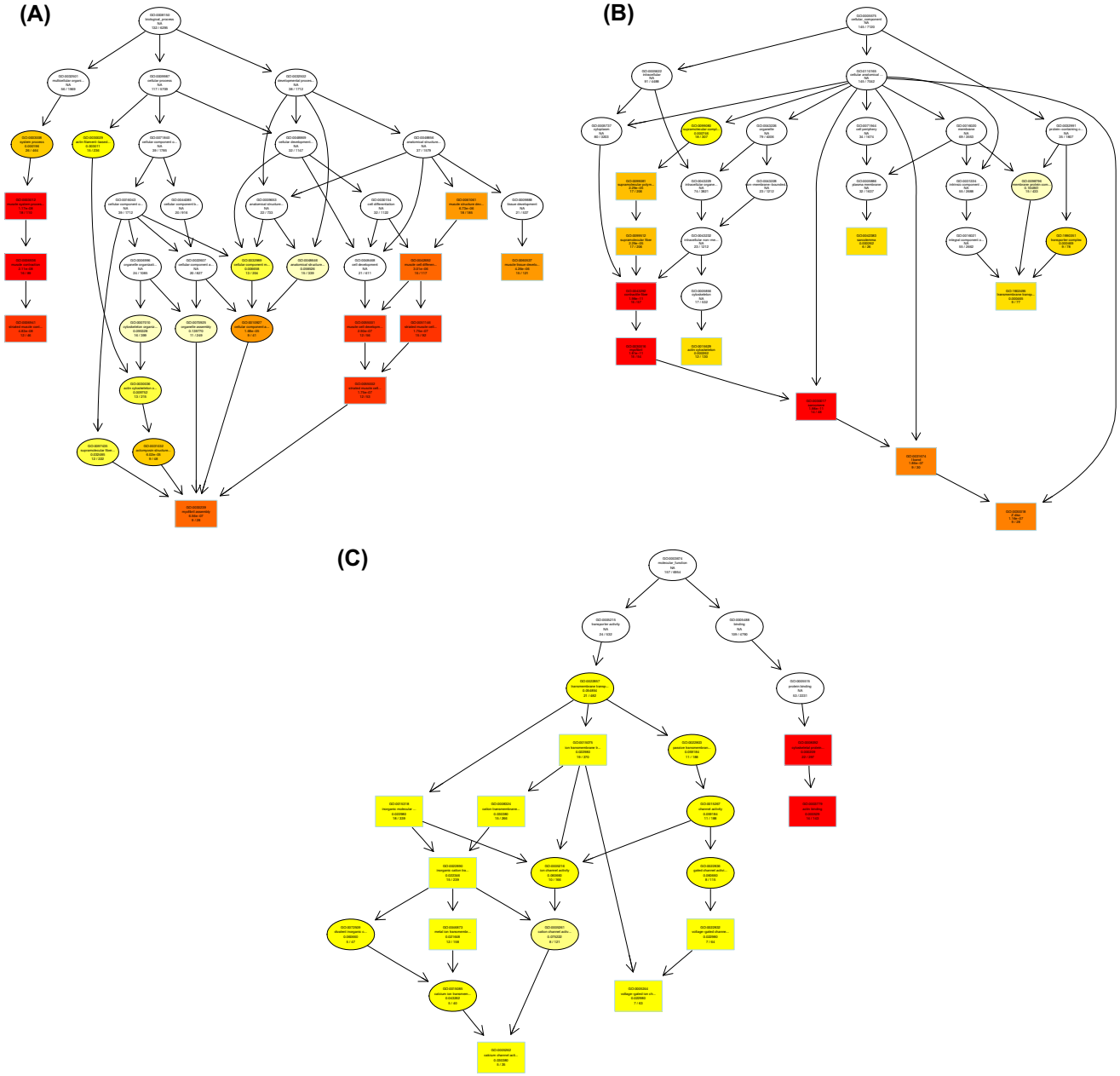

FIGURE 10 GO enrichment-directed acyclic graph of (A) BP classification, (B) CC classification, and (C) MF classification

inflammatory cells, IL-6 mainly induces the synthesis of acute-phase proteins, catalyses and amplifies inflammatory reactions and toxic effects, causing tissue cell damage.[23] The results of the present study confirmed that Vitamin B_{12} can reduce the release of inflammatory cytokine IL-6, reduce the degree of the local inflammatory response, and may promote the regeneration and healing of wounds.

TGF-β plays an important role in radiation-induced fibrosis. In the early stage of endothelial cell injury, cytokines secreted by leukocytes, including TGF-β, infiltrate irradiated tissues, inducing an inflammatory response and early fibrosis.[24] Eosinophils play a direct regulatory role in fibroblast proliferation, collagen synthesis, and lattice contraction mediated by TGF-β.[25] Martin et al confirmed that TGF-β1 is one of the key cytokines leading to early and late radiation-induced fibrosis and is the main switch of skin fibrosis after radiation.[26-28] After skin irradiation, the excessive accumulation of collagen and other extracellular matrix components is an important cause of radiofibrosis. The Masson staining results showed that Vitamin B_{12} ointment treatment can significantly reduce the deposition of collagen fibres in the skin tissue. Therefore, it can be confirmed that Vitamin B_{12} reduces the expression of the radiofibrosis-related factor TGF-β to reduce skin fibrosis after radiation and improve the chronic symptoms of radiodermatitis.

Gene sequencing analysis was performed to further explore the specific molecular mechanism by which Vitamin B_{12} ameliorates radiodermatitis. The results identified a number of up-regulated and down-regulated differentially expressed genes. The findings lay the foundation for further exploration of the key genes and targets involved in the treatment of radiodermatitis. It was apparent from the KEGG pathways enrichment analysis that Vitamin B_{12} ameliorates radiodermatitis through various signalling pathways. GO enrichment showed a strong association between Vitamin B_{12} and myofibrils and the development of muscle cells and structure, which is consistent with the above results showing that Vitamin B_{12} promotes tissue repair. These findings may help to elucidate the role of Vitamin B_{12} in promoting the repair and regeneration of skin tissue in terms of molecular function and mechanisms.

In summary, Vitamin B_{12} ointment was found to have significant anti-radiation, anti-inflammatory, and anti-fibrosis effects, and was shown to be effective for the treatment of severe radiodermatitis. The various signalling pathways and biological processes involved were also analysed. The application of Vitamin B_{12} ointment as a specific drug for radiodermatitis in clinical practice is especially worth looking forward to.

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CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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