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Effects of four polyphenols on mouse wound healing and the gene expression profile of resveratrol action

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Summary. Studies have demonstrated the potent effects of polyphenols on cutaneous wound healing. However, the molecular mechanisms underlying polyphenol activity are incompletely understood. Herein, mice were experimentally wounded, intragastrically treated with four polyphenols, resveratrol, tea polyphenols, genistein, and quercetin; and monitored for 14 days. Resveratrol was the most effective compound, promoting wound healing starting at day 7 after wounding, by enhancing cell proliferation and reducing apoptosis and subsequently promoting epidermal and dermal repair, collagen synthesis and scar maturation. RNA sequencing was performed in control and resveratrol-treated tissues on day 7 after wounding. Resveratrol treatment upregulated 362 genes and downregulated 334 genes. Gene Ontology enrichment analysis showed that differentially expressed genes (DEGs) were associated with different biological processes (keratinization, immunity, and inflammation), molecular functions (cytokine and chemokine activities), and cellular components (extracellular region and matrix). Kyoto Encyclopedia of Genes and Genomes pathway analysis indicated that DEGs were predominantly enriched in inflammatory and immunological pathways, including cytokine-cytokine receptor interaction, chemokine signaling, and tumor necrosis factor (TNF) signaling. These results show that resveratrol accelerates wound healing by promoting keratinization and dermal repair and attenuating immune and inflammatory responses.

Key words: Mouse, Wound healing, Polyphenols, Resveratrol, RNA sequencing

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Introduction

Wound healing is crucial for restoring skin integrity and function after skin injury. Wound healing involves four consecutive and overlapping stages: hemostasis, inflammation, proliferation, and remodeling (Gurtner et al., 2008; Eming et al., 2014). These complex events involve the coordinated effort of macrophages, fibroblasts, keratinocytes, and endothelial cells, and are tightly regulated by a complex signaling network involving growth factors, cytokines, and chemokines (Gurtner et al., 2008; Eming et al., 2014). Poor coordination among cells or cytokines due to metabolic disorders, immune dysfunction, microbial infection, or dysregulation of cellular signaling can lead to chronic wounds or excessive scarring (Qing, 2017).

Polyphenols are a large family of natural plant compounds widely distributed in vegetables, fruits, and seeds. Important dietary sources of polyphenols are onions (flavonols); tea, apples, and red wine (flavonols and catechins); citrus fruits (flavanones); soy (isoflavones); berries and cherries (anthocyanidins); and cacao and grape seeds (proanthocyanidins) (Manach et al., 2004). These compounds have attracted increasing attention because of their antioxidant, anti-inflammatory, anti-aging, anti-fibrosis, and anti-cancer properties (Manach et al., 2004; Nichols and Katiyar, 2010).

Plant polyphenols have promising effects on wound healing. For instance, genistein, a soybean-derived isoflavone, accelerates normal and diabetic wound healing owing to its antioxidant and anti-inflammatory activity, and modulating estrogen receptor signaling (Emmerson et al., 2010; Park et al., 2011; Tie et al., 2013; Coma et al., 2021). Quercetin, an abundant dietary flavonoid, accelerates wound healing by modulating the inflammatory response (Jangde et al., 2018; Choudhary et al., 2020; Fu et al., 2020; Kant et al., 2020, 2021). Tea polyphenols are the most active and beneficial compounds in tea and promote diabetic wound healing and skin repair (Qin et al., 2010, 2013; Khan and Mukhtar, 2018; Chen et al., 2020; Xu et al., 2021). Resveratrol, a non-flavonoid polyphenolic compound

found in more than 72 plant species, including grapes, peanuts, and blueberries, accelerates normal and diabetic wound healing. Resveratrol decreases oxidative stress and inflammation, increases cell proliferation, prevents aging, and promotes angiogenesis (Subedi et al., 2017; Zhao et al., 2017; Huang et al., 2019; Kaleci and Koyuturk, 2020; Zhao et al., 2020; Kamolz et al., 2021; Liu et al., 2021; Zhou et al., 2021; Hecker et al., 2022).

These studies demonstrate the potential therapeutic effects of plant polyphenols on wound healing but have limitations. First, the type of formulation (e.g., topical or encapsulated into hydrogels or nanoparticles) and the treatment model (e.g., *in vivo* or *in vitro*) varied among studies. Second, the therapeutic effects of these polyphenolic compounds are promising but were not examined together. Third, although the modulation of signaling pathways may underlie the therapeutic effects, the molecular mechanisms underlying these effects have not been identified.

This study compared the effects of four polyphenols using a mouse cutaneous wound model. RNA sequencing and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of differentially expressed genes (DEGs) were performed to determine the molecular mechanisms underlying resveratrol activity.

Materials and methods

Mice, wound creation, and polyphenol treatment

Eight-week-old wild-type C57/BL6J male mice were from the Experimental Animal Center of Southern Medical University (Guangzhou, China). All experiments were approved by the Institutional Review Board of Guangzhou Red Cross Hospital (2020-107-01), and were performed in accordance with institutional guidelines and regulations.

Wounds were created as described in our previous study (Hu et al., 2020). Briefly, mice were anesthetized, and the back skin was shaved, treated with a lotion to remove the remaining hair, and cleaned with alcohol. Using a sterile 4 mm biopsy punch, two bilateral full-thickness wounds were created on the skin, one on each side. Wounds were photographed on days 0, 3, 7, and 10 after wounding. Wound diameter was measured using Photoshop CS6 software to calculate the wound area. The percentage of wound closure at each time point was calculated using the formula [1 - (post-treatment wound area/initial wound area)]×100. Mice were euthanized on days 7 and 14 after wounding (six mice for each timepoint for each group), and wound tissue specimens were collected for further analysis.

Mice were randomly divided into five groups and treated intragastrically via a 50 mm intragastric needle with the following polyphenols (purity >99%; Aladdin Company; Shanghai, China): resveratrol (50 $mg \cdot kg^{-1} \cdot d^{-1}$), genistein (160 $mg \cdot kg^{-1} \cdot d^{-1}$), tea polyphenols (100 $mg \cdot kg^{-1} \cdot d^{-1}$), and quercetin (50 $mg \cdot kg^{-1} \cdot d^{-1}$). These treatment doses were based on our previous study which demonstrated that they effectively preserved the ovarian follicles in aging rats (Chen et al., 2010). The compounds were dissolved in saline. The control group was treated with saline. After given polyphenols for 14 consecutive days, mice were subjected to wounding surgery, and then continuously given polyphenols every day until euthanasia for specimen collection.

Hematoxylin and eosin staining

Wound tissue specimens were fixed in 4% paraformaldehyde and processed using paraffin wax and standard methods. Three wound tissue cross sections (3 μm, taken 100 μm apart) were stained with hematoxylin and eosin (H&E) (Cat.HHS16, HT110116, Sigma-Aldrich) for histomorphological evaluation. The length of the epithelial tongues denotes the newborn epithelial layers extending beyond the wound edges divided by the original wound diameter (Chen et al., 2015). The area of granulation tissue was quantified by measuring the dermal area using the Image J software (1.52a, National Institutes of Health, USA), and the results are expressed as mm^2 (Ketomaki et al., 2019).

Van Gieson collagen staining and collagen maturity assessment

Wound collagen synthesis was assessed using van Gieson staining (Cat. DC0046, Nanjing Jiancheng Bioengineering, Nanjing, China). The maturity of collagen fibers was scored according to our previous report (Hu et al., 2020) and the following criteria: 0, pale pink color; 1, pink color; 2, red color; 3, deep red color.

Immunofluorescence

Wound cross-sections were dewaxed in xylene and rehydrated through a graded ethanol series. Tissue sections were blocked in 5% goat serum/ phosphate buffered solution for 20 min and incubated with primary antibodies overnight at 4°C and with Alexa Fluor 594-conjugated secondary antibodies (111-585-003, Jackson Immunoresearch; West Grove, PA, USA). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI, P36935, Invitrogen; Carlsbad, CA, USA). Samples were imaged using a laser-scanning confocal microscope (FluoView FV1000, Olympus; Tokyo, Japan). The following primary antibodies were used: Ki-67 (1:100; 9129, Cell Signaling Technology, Shanghai, China), CD3 (1:50; ab5690, Abcam, Shanghai, China), CD20 (1:50; ab64088, Abcam, Shanghai, China), CD31 (1:100; 3528, Cell Signaling Technology, Shanghai, China), F4/80 (1:50; sc-377009, Santa Cruz Biotechnology, Dallas, TX, USA). The Ki-67 (indicating proliferating cells) level was scored as the number of cells with positive signals in the wound area. CD31 marks vessels, which are categorized as microvessels (diameter <15 μm) and mature vessels (diameter >15 μm), and they are quantified as their sum number in the area of dermis (Sorg et al., 2007; Lee et al., 2015). The

intensities of CD3, CD20 and F4/80 were analyzed using the Image J software.

Cell apoptosis assay

Cell apoptosis was evaluated in wound cross-sections by the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay using the DeadEnd Fluorometric TUNEL System (G3250, Promega, Madison, WI, USA). TUNEL-positive cells were observed under a FluoView FV1000 confocal microscope. The TUNEL level was scored as the number of cells with positive signals in the wound area.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA from wound tissues was purified using the Trizol reagent (Invitrogen) and reverse-transcribed to cDNA using the Hifair™ II 1st Strand cDNA Synthesis Kit (Yeasen Biotech; Shanghai, China), and cDNA was amplified and quantified using the StepOne Plus Real-Time PCR System (Applied Biosystems; Waltham, MA, USA) and the Hieff® qRT-PCR SYBR Green Master Mix (Yeasen Biotech). Gene expression was normalized to *Gapdh* using the $2^{-\Delta\Delta Ct}$ method. Assays were performed in triplicate. The primer sequences are listed in Table 1.

RNA sequencing and bioinformatic analysis

Total RNA was extracted from tissues using Trizol reagent on day 7 after wounding. After quality control, RNA sequencing was performed on an Illumina NovaSeq platform (Novogene Biotech, Beijing, China). Differential expression analysis was performed using the DESeq2 R package version 1.20.0. DESeq2 provides statistical procedures for measuring differential gene expression from digital data using a model based on negative binomial distributions. P-values were adjusted using Benjamini and Hochberg's approach to control the false discovery rate. Genes with an adjusted p-value of less than 0.05 were considered differentially expressed.

GO enrichment analysis of DEGs was performed using the clusterProfiler R package, in which gene length bias was corrected. GO terms with corrected *P* values of less than 0.05 were considered significantly enriched. KEGG is a database derived from large datasets generated by genome sequencing and other high-throughput technologies and is used to analyze high-level functions of biological systems (http://www.genome.jp/kegg). KEGG pathway enrichment analysis of DEGs was performed using the clusterProfiler R package. The Reactome database combines data on biochemical reactions and signaling pathways of human model species. Reactome pathways with *P*-values of less than 0.05 were considered significantly enriched.

Statistics

All experiments were performed in triplicate. Data are expressed as the mean ± SEM. Differences in categorical variables were analyzed using the Chi-square test, and differences in continuous variables were analyzed using Student's t-test for two groups and the ANOVA test for more than two groups (SPSS 13.0, Chicago, IL, USA). A *P* value of less than 0.05 was considered statistically significant.

Results

Effects of four polyphenols on wound healing

We compared the effects of four plant polyphenols on wound healing during 10 days. Wound closure was faster in resveratrol-treated mice, as revealed by the progressive and significant decrease of wound width (Fig. 1A,C), reaching ~50% on day 7, and ~90% on day 10 after wounding vs. ~60% in the control group ($P<0.01$) (Fig. 1B). Genistein and tea polyphenols also improved wound healing, but showed evident effects after day 10 (Fig. 1). Unfortunately, quercetin had no positive effect on wound healing. Consistent to the wound healing routine, resveratrol and genistein obviously accelerated follicle regeneration and hair growth around the wound area (Fig. 1A), in line with the positive roles of hair growth on wound closure. These results suggested that among the four tested polyphenolic compounds, resveratrol was the most effective in accelerating wound healing.

Resveratrol increases cell proliferation and decreases cell apoptosis in wounds

Next, we assessed cell proliferation and apoptosis in control and resveratrol-treated wounds. Immunofluorescence showed that Ki-67-positive signals were significantly higher in the resveratrol group, with

Table 1. Primer sequences for quantitative reverse transcription polymerase chain reaction.

Gene	Forward primer (5'→ 3' sequences)	Reverse primer (5'→ 3' sequences)
Il-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
Il-19	CTCCTGGGCATGACGTTGATT	GCATGGCTCTCTTGATCTCGT
Il-24	GAGCCTGCCCAACTTTTTGTG	TGTGTTGAAGAAAGGGCCAGT
Cxcl1	ACTGCACCCAAACCGAAGTC	TGGGGACACCTTTTAGCATCTT
Cxcr2	GCTCTGACTACCACCCAACCTTG	AGAAGAGCAGCTGTGACCTGCTG
Ccl7	CCTGGGAAGCTGTTATCTTCAA	TGGAGTTGGGGTTTTCATGTC

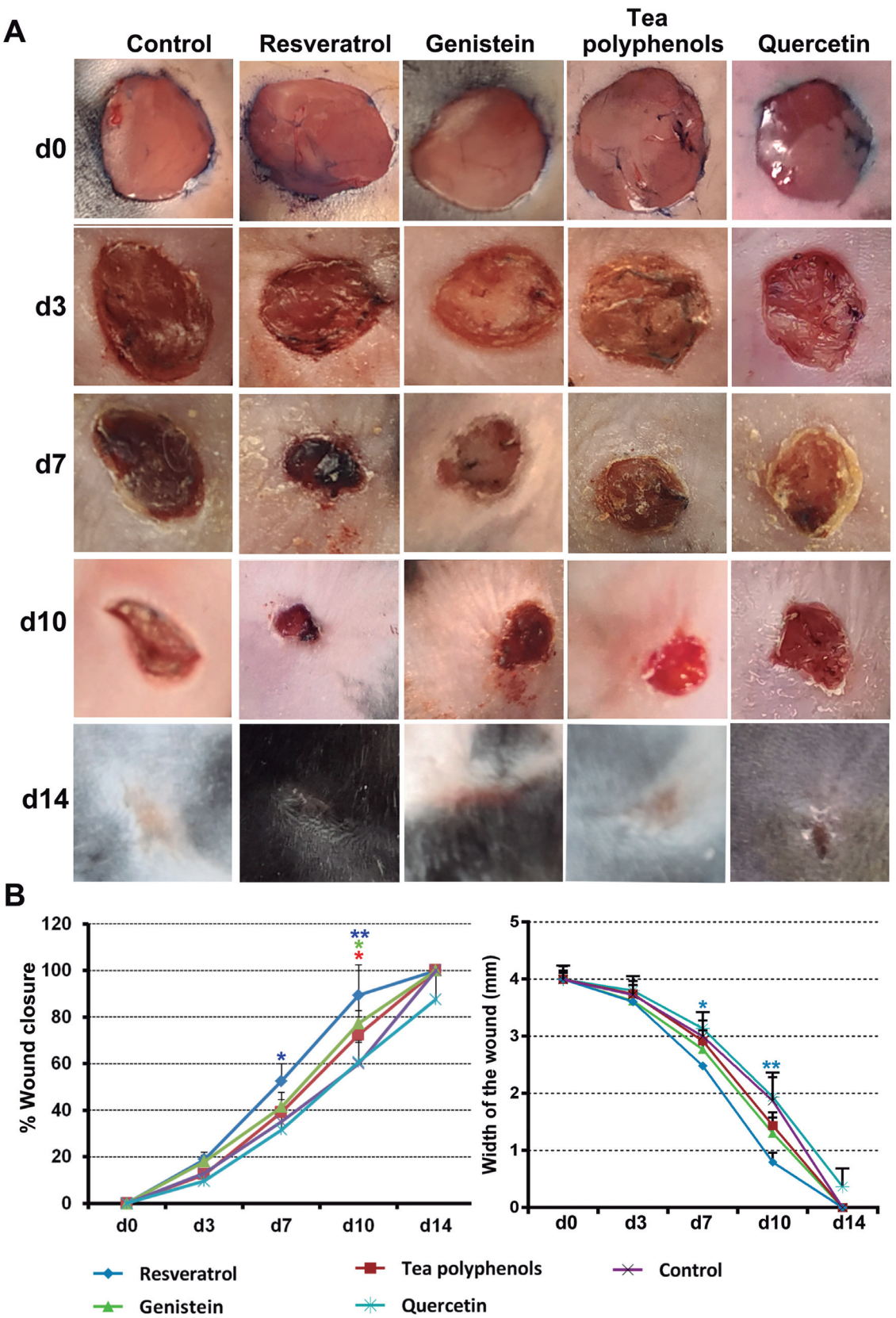

Fig. 1. Comparison of the effects of four polyphenols on mouse wound healing. **A.** Kinetics of wound closure in mice treated with saline (control), resveratrol, tea polyphenols, genistein and quercetin, respectively. Wounds were photographed on days 0, 3, 7, 10 and 14 after wounding. Shown are representative images for wounds at indicated time-points from each group. **B.** Quantification of the wound healing in **A** as percentage wound closure (left) and width of the wounds (right). *, $P<0.05$; **, $P<0.01$. n=6 for each group.

positive signals uniformly distributed in the epidermis and dermis, indicating that resveratrol promoted cell proliferation both in the epidermis and dermis (Fig. 2). Both genistein and tea polyphenols increased Ki-67-positive signals in the epidermis but not in the dermis. Consistent with the wound healing, Ki-67 signals in the epidermis but not in the dermis were decreased in quercetin-treated wounds (Fig. 2). TUNEL assay showed that resveratrol remarkably decreased TUNEL signals both in the epidermis and dermis, indicating that resveratrol protected cells from apoptosis (Fig. 3). Genistein showed similar effects (Fig. 3). Tea polyphenols decreased TUNEL signals in the epidermis. However, quercetin had no detectable effect on apoptosis (Fig. 3). These data suggest that resveratrol accelerates wound healing by enhancing cell proliferation while preventing apoptosis in the dermis and epidermis. Therefore, this compound was chosen for further analysis.

Resveratrol promotes epidermal development and collagen synthesis

We next monitored the wound healing in resveratrol

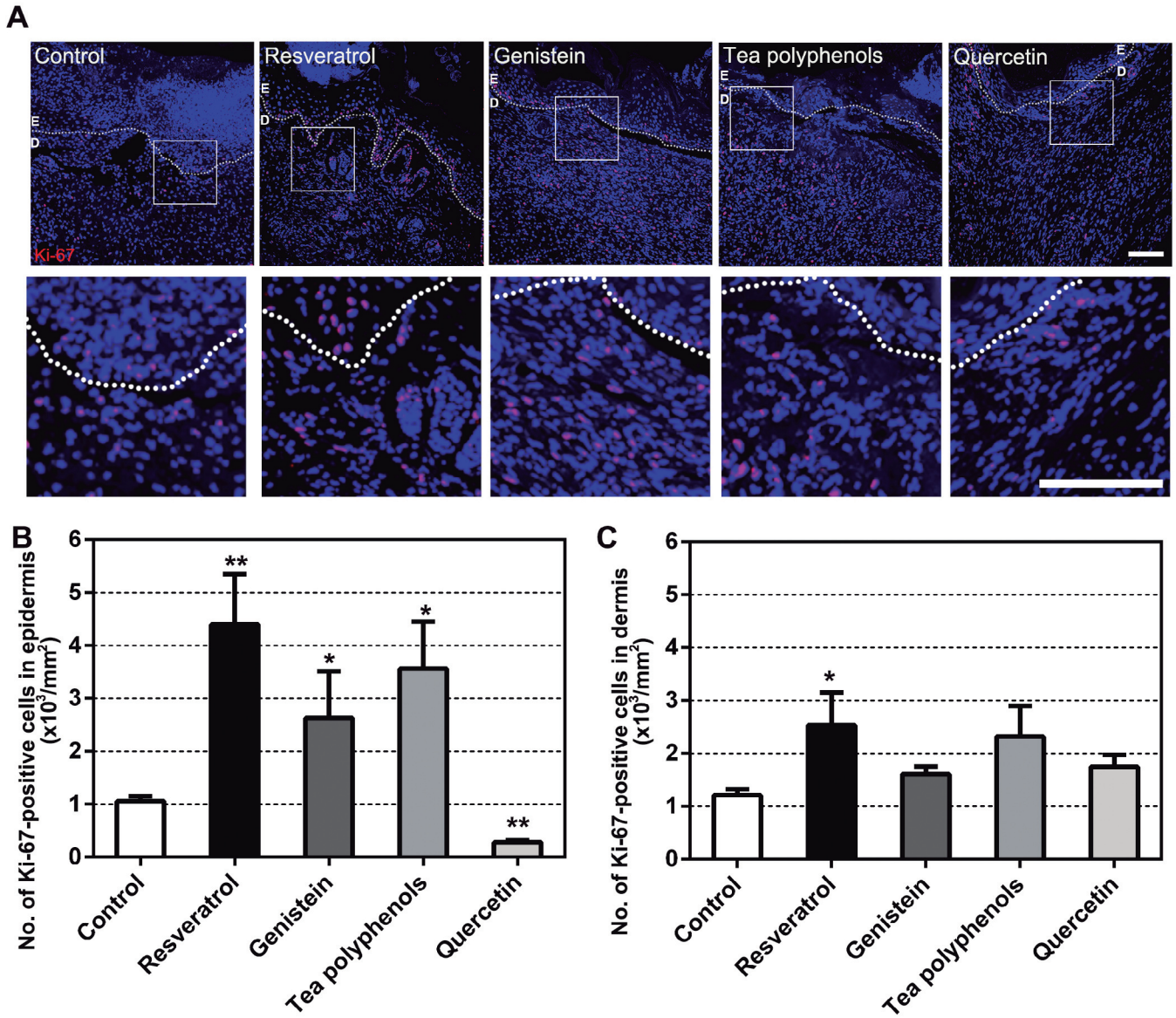

Fig. 2. Cell proliferation revealed by Ki-67 staining. **A.** Immunofluorescent staining of Ki-67 (red) in day 7 wound sections of each group. Nuclei were counterstained with DAPI. Lower panels are the magnifications of the boxes in upper panels. White dash lines indicate epidermal and dermal layers. E, epidermis, D, dermis. **B, C.** Quantification of the Ki-67 signals in epidermis **(B)** and dermis **(C)**. Bars denote the number of Ki-67-positive cells normalized to the wound areas. Three sections (taken 100 μm apart) from each mouse were assessed. *, $P<0.05$; **, $P<0.01$. n=6 for each group. All samples were from control and resveratrol-treated wounds on day 7 following wounding. Scale bars: upper panels, 200 μm; lower panels, 100 μm.

group more precisely. H&E staining showed that at day 7, resveratrol-treated wounds showed clear and continuous multi-cell layers in epidermis, and the scab layers could be separated, indicating a complete epidermal closure of wounding. In sharp contrast, no clear epidermal boundary was observed in the controls, and the epidermal and scab layers were visually indistinguishable from each other (Fig. 4A,B). Moreover, the length of the epidermal tongues and the size of the granular tissues, two of which are important for wound closure, were remarkably increased in day 7 resveratrol wounds (Fig. 4A-D), consistent with the data of Ki-67 staining. Besides, the decrease of microvessels while increase of mature vessels indicated that maturation of scar tissue was accelerated (Fig. 4A,B,E,F). By day 14, the histomorphological appe-

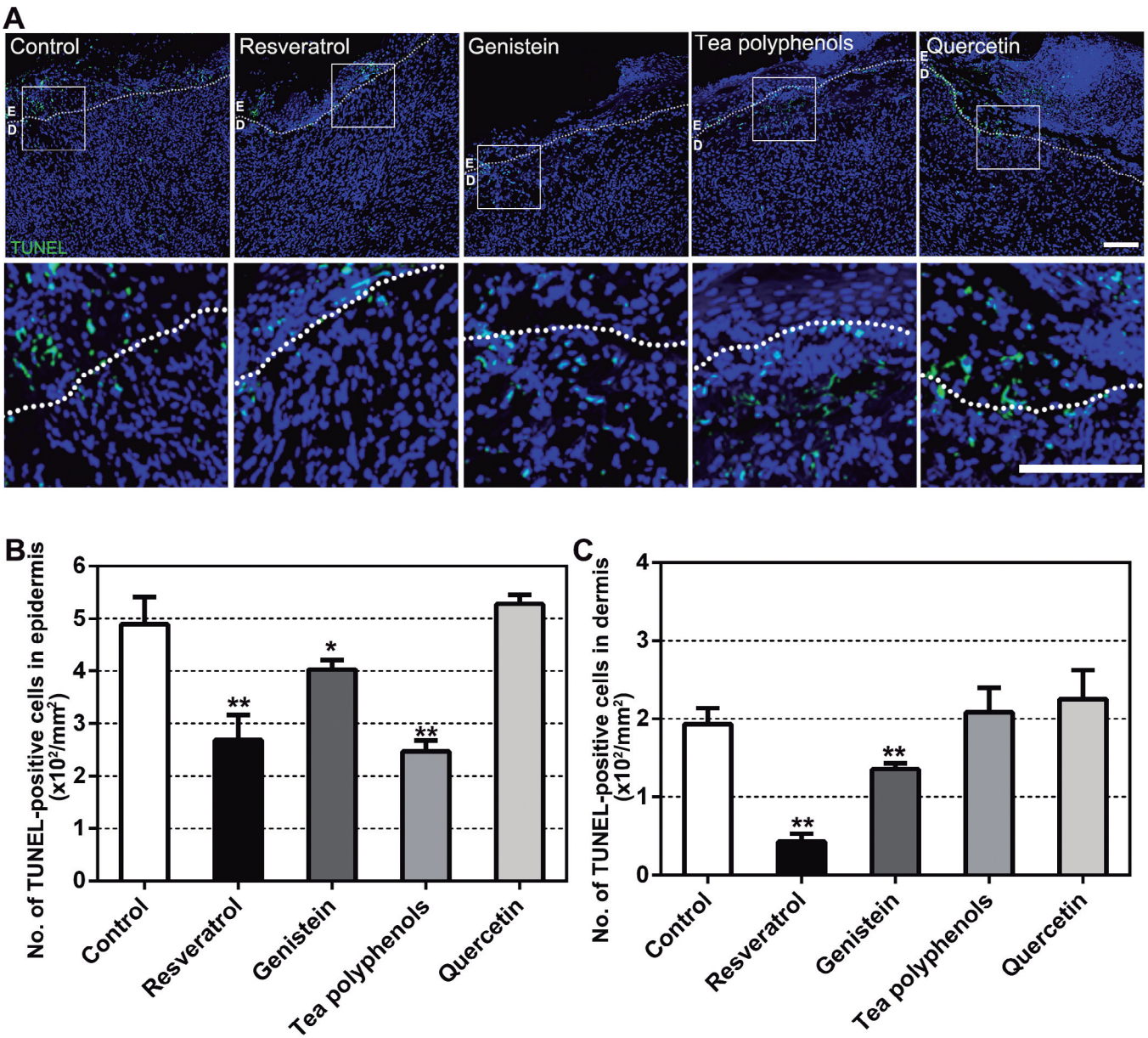

Fig. 3. Cell apoptosis revealed by TUNEL staining. **A.** Fluorescent TUNEL staining (green) in day 7 wound sections of each group. Nuclei were counterstained with DAPI. Lower panels are the magnifications of boxes in upper panels. White dash lines indicate epidermal and dermal layers. E, epidermis, D, dermis. **B, C.** Quantification of the TUNEL signals in epidermis **(B)** and dermis **(C)**. Bars denote the number of TUNEL-positive cells normalized to the wound areas. Three sections (taken 100 μm apart) from each mouse were assessed. *, $P<0.05$; **, $P<0.01$. ns, not significant. n=6 for each group. All samples were from control and resveratrol-treated wounds on day 7 following wounding. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling. Scale bars: upper panels, 200 μm; lower panels, 100 μm.

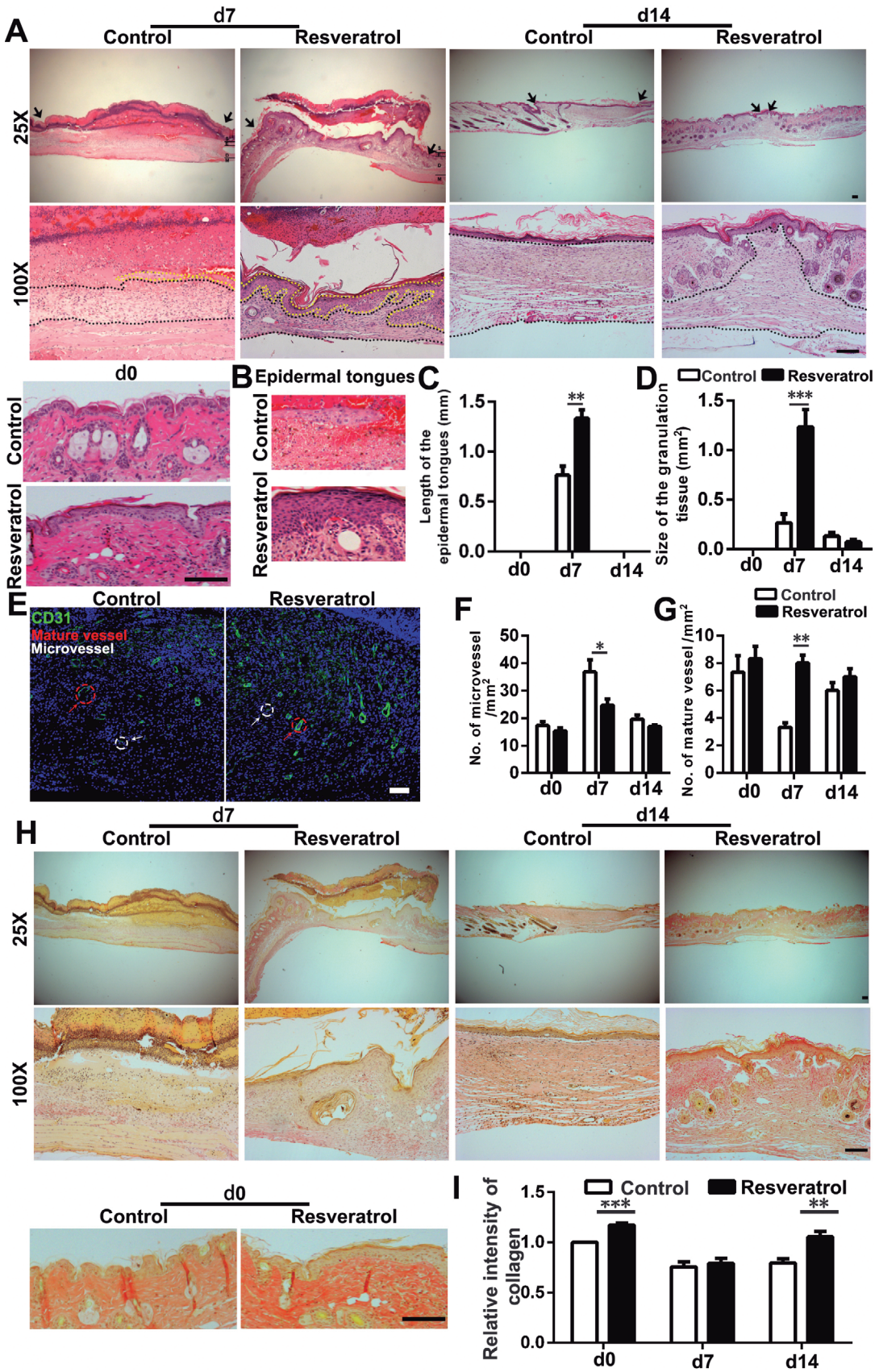

Fig. 4. H&E and van Geoson staining. **A.** H&E staining showed the progressive histomorphological changes in resveratrol-treated and control wounds. Arrows indicate wound area. E, epidermis, D, dermis, M, muscle. Black dot lines outline the area of granular tissue, yellow dot lines outline the area of epidermal tongue. **B.** Representative images showing epidermal tongue. **C, D.** Quantifications of the length of epidermal tongues **(C)** and the size of granular tissues **(D)**. **E.** Immunofluorescence of CD31 indicating microvessel (red circle and arrows) and mature vessel (white circle and arrows). **F, G.** Quantifications of the No. of micovessels **(F)** and the No. of mature vessels **(G)**. **H.** van Geoson staining revealed the collagen deposition in resveratrol-treated and control wounds. **I.** Collagen fibers scoring. Samples were from control and resveratrol-treated wounds on day 0, 7 and 14 following wounding. *, $P<0.05$; **, $P<0.01$. ***, $P<0.001$. n=6 for each group. Scale bars: 100 μm.

arance and cell archietecture of resveratrol-treated wound tissues at day 14 was almost identical to those at day 0 immediately before wounding (Fig. 4A). These findings suggested that resveratrol accelerated wound closure jointly by promoting cell proliferation, enhancing contraction of the wound and granular tissue formation.

To further evaluate the influence of resveratrol on matrix remodeling, we performed van Gieson staining to assess the collagen synthesis. Treatment with resveratrol for 7 days did not affect collagen synthesis in the wound area, but that for 14 days significantly increased collagen deposition in dermis (Fig. 4G,H). These results cumulatively demonstrated that resveratrol promotes epidermal and dermal repair and collagen synthesis to accelerate wound closure.

Comparison of gene expression profiles between control and resveratrol-treated wounds and functional enrichment analysis

To elucidate the biological functions and pathways associated with the effects of resveratrol, RNA sequencing in combination with GO enrichment and KEGG analyses were performed in control and resveratrol-treated tissues on day 7 after wounding, a critical time-point for wound healing as at this time both epidermal and dermal cells maintain high proliferation activity, and remodeling of extracellular matrix is increasingly active. The expression of DEGs was visualized using volcano plots. Resveratrol treatment upregulated 334 genes and downregulated 362 genes (Fig. 5A). GO enrichment analysis revealed that DEGs were associated with several biological processes (keratinization, keratinocyte differentiation, epidermal development, inflammatory responses, immune system process, chemotaxis, and immune responses), molecular functions (structural molecule activity, cytokine, and chemokine activity), and cellular components (extracellular region and matrix, and plasma membrane) (Fig. 5B), in good agreement with the finding of H&E staining that resveratrol promoted epidermal thickening (Fig. 4). KEGG pathway enrichment analysis indicated the enrichment of inflammation and immunity-related pathways, including cytokine-cytokine receptor interaction, chemokine signaling, and TNF signaling pathways (Fig. 5C). These data suggest that resveratrol accelerates wound healing by inducing keratinization and modulating immune and inflammatory responses.

The expression of inflammatory molecules is decreased in resveratrol-treated wounds

The effect of resveratrol on inflammatory responses was assessed by quantifying the levels of inflammatory and immune factors by qRT-PCR. In line with RNA

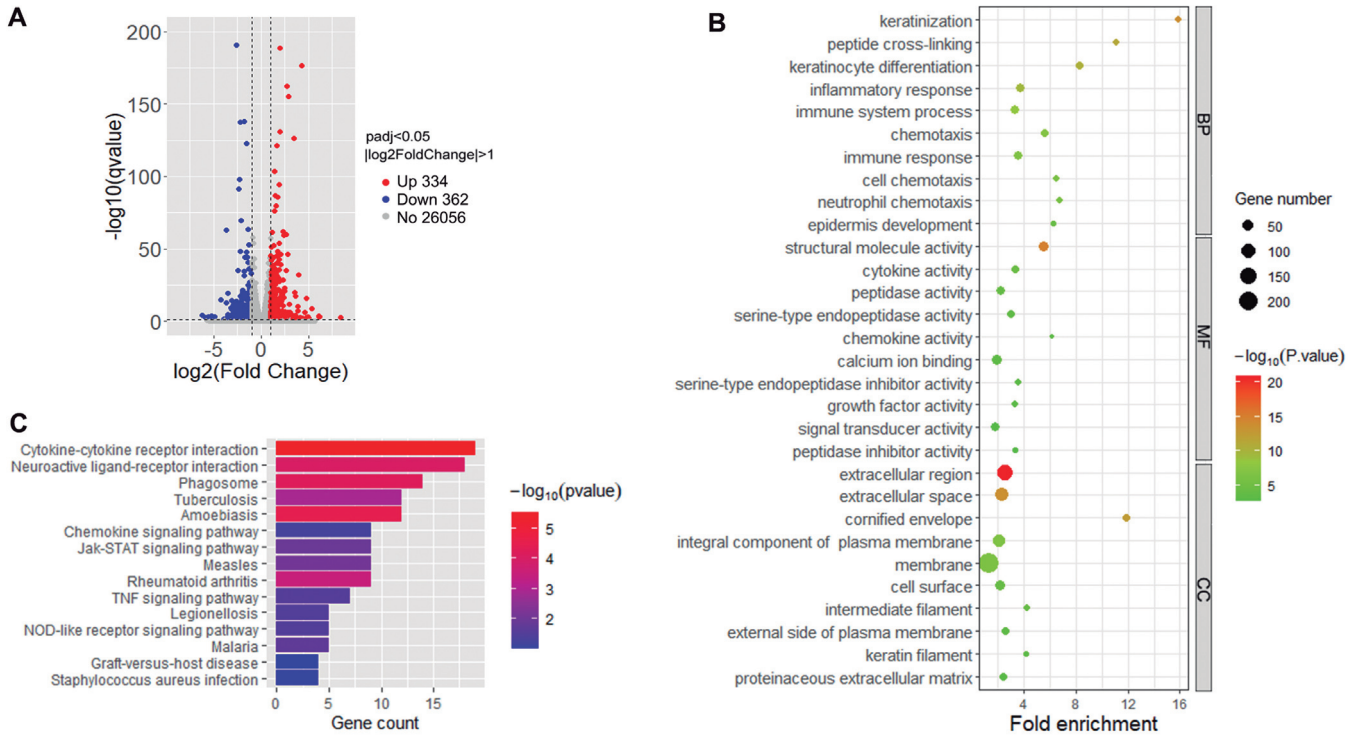

Fig. 5. RNA sequencing of control and resveratrol-treated wounds and functional enrichment analyses. **A.** volcano map showing the number of DEGs in resveratrol-treated group compared with control group: 334 upregulated and 362 downregulated. **B.** The most significant GO enrichment and KEGG pathways of the DEGs **(C)**. All samples were from control and resveratrol-treated wounds on day 7 following wounding.

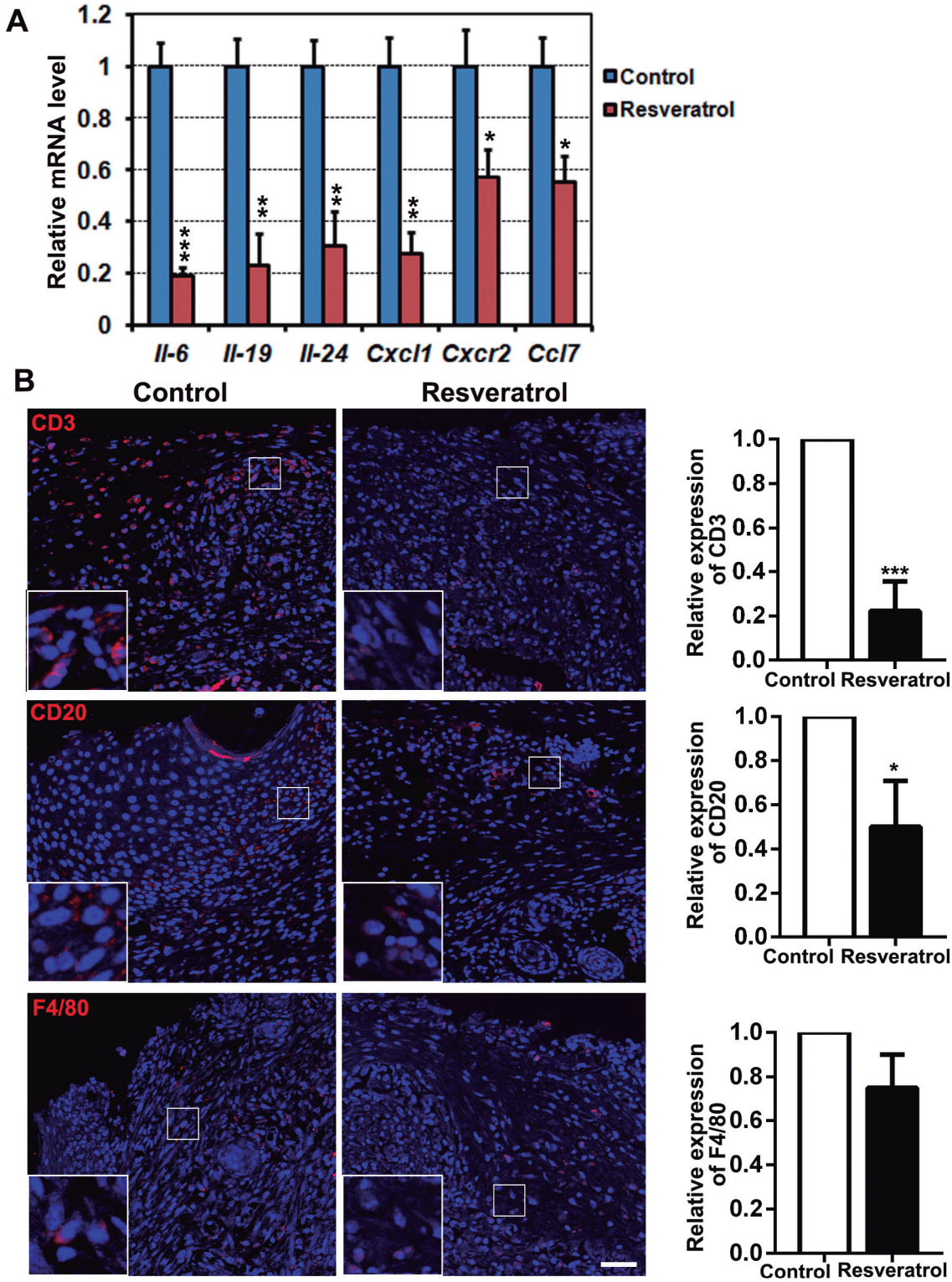

Fig. 6. Reduced pro-inflammatory cytokines production and immune cell infiltration in resveratrol-treated wounds. **A.** PCR analyses of the messenger RNA level of target pro-inflammatory cytokines. *, *P*<0.05; **, *P*<0.01. ***, *P*<0.001. n=3 for each group. Il-6, Interleukin-6; Il-19, Interleukin-19; Il-24, Interleukin-24; Cxcl1, chemokines (C-X-C motif) ligand 1; Cxcr2, chemokines (C-X-C motif) receptor 2; Ccl7, chemokine (C-C motif) ligand 7. **B.** Immunofluorescent staining of the indicated immune cell markers. Nuclei were counterstained with DAPI. Magnifications are shown on the lower left panel. Quantifications are shown on the right panel. CD3, cluster of differentiation 3, a T lymphocyte marker; CD20, cluster of differentiation 20, a B lymphocyte marker; F4/80, a macrophage marker. All samples were from control and resveratrol-treated wounds on day 7 following wounding. Scale bar: 100 μm.

sequencing data, qRT-PCR results showed that resveratrol treatment significantly decreased the messenger RNA (mRNA) levels of several molecules involved in immune and inflammatory responses, including pro-inflammatory cytokines *Il-6,* Il-19, and *Il-24*, chemokines (C-X-C motif) ligand 1 (*Cxcl1*) and its receptor *Cxcr2*, and chemokine (C-C motif) ligand 7 (*Ccl7*) (Fig. 6A). In turn, the fluorescence signal of the T lymphocyte marker CD3 and the B lymphocyte marker CD20 decreased in the resveratrol-treated group, indicating that resveratrol decreased immune cell activity (Fig. 6B). However, the level of the macrophage marker F4/80 remained unchanged (Fig. 6B). These results validate RNA sequencing data and demonstrate that resveratrol promotes wound healing by reducing the infiltration of immune cells in wound tissue and the production of pro-inflammatory cytokines.

Discussion

To our knowledge, this study is the first to compare the efficacy of resveratrol, tea polyphenols, genistein, and quercetin on wound healing. Our results support previous summaries regarding the significant effects of resveratrol, genistein and tea polyphenols on cutaneous wound healing (Park et al., 2011; Khan and Mukhtar, 2018; Hecker et al., 2022). The conflicting results on the efficacy of quercetin may be attributed to differences in wound models (normal or diabetic), treatment method (intragastric, topical, or nanoparticle), and dosing (Jangde et al., 2018; Choudhary et al., 2020; Fu et al., 2020; Kant et al., 2020, 2021; Badhwar et al., 2021). We found that resveratrol was the most effective compound, with significant repair on day 7 after wounding and ~90% wound closure on day 10. Furthermore, resveratrol increased cell proliferation, decreased apoptosis, therefore promoted epidermal and dermal repair and matrix remodeling to accelerate wound healing and scar maturation.

Resveratrol accelerates common and diabetic wound healing by decreasing inflammation, oxidation and aging, and promoting cell proliferation and angiogenesis (Subedi et al., 2017; Zhao et al., 2017; Huang et al., 2019; Kaleci and Koyuturk, 2020; Zhao et al., 2020; Liu et al., 2021; Zhou et al., 2021). However, wound healing is a complex process, and these previous studies evaluated distinct cell types and biochemical events, underscoring the need to systematically assess global molecular changes induced by resveratrol in wounds. The results of RNA sequencing combined with GO enrichment and KEGG analysis showed that DEGs were enriched in biological processes associated with keratinization, immunity, and inflammation.

A previous study involving primary human epidermal keratinocytes reported that the anti-inflammatory and wound healing effects of resveratrol depended more on epidermal growth factor receptor signaling than on antioxidant and free radical scavenging activity (Pastore et al., 2012). The chemokines analyzed were CXCL8/interleukin 8, CCL2/monocyte chemotactic protein-1, and CXCL10/interferon γ-inducible protein 10 kDa (Pastore et al., 2012). In line with these findings, qRT-PCR results showed that resveratrol decreased the expression of Il-6, Il-19, Il-24, Cxcl1/Cxcr2, and Ccl7 (Fig. 6B). Moreover, sequencing data provided evidence that resveratrol downregulated the expression of pro-inflammation molecules and pathways. Importantly, evidence supporting our results came from both *in vitro* (Subedi et al., 2017) and *in vivo* (Zhao et al., 2020) studies, as well as a review article (Pignet et al., 2021), together highlighting the mechanism of inhibiting inflammatory pathways. In addition, an *in vivo* study in wounding rats infected with extra pathogens showed that resveratrol reduced the infiltration of mast cells and increased the infiltration of lymphocytes and macrophages in wound tissues. The infiltration of neutrophils varied depending on the type of pathogens (Shevelev et al., 2020). Conversely, our immunofluorescence results showed that resveratrol decreased the infiltration of CD3-positive and CD20-positive lymphocytes, whereas the number of F4/80-positive macrophages remained unchanged (Fig. 6B). The conflicting results may be due to differences in the protocols used to create wounds and in the treatments on wounds. In addition, the most enriched biological process, molecular function, and cellular component ("wound healing," "metalloendopeptidase activity, and "proteinaceous extracellular matrix," respectively) indicated the presence of significant changes in the dermis. These GO terms, in combination with the findings from HE and van Geoson stainings, suggest that resveratrol promotes the repair of epidermis and dermis to accelerate wound closure. Since resveratrol is rich in daily food, a dietary supplement of resveratrol would be an economic and effective way to treat wounds, including diabetic wounds. However, our results indicated an increased collagen deposition in the resveratrol-treated wounds at day 14 after wounding, suggesting that the treatment dose or time need further monitoring.

In summary, we assessed the effects of four polyphenols on mouse wound healing and observed that resveratrol was the most effective compound. Resveratrol enhanced cell proliferation and reduced apoptosis, thus promoting epidermal development and matrix remodeling. RNA sequencing showed that DEGs were enriched in processes associated with keratinization, extracellular matrix, and immunity. The results of qRT-PCR and immunofluorescence demonstrated that resveratrol accelerated wound healing by synergistically promoting keratinization and dermal remodeling and attenuating immune and inflammatory responses.

Conflict of interest. The authors have nothing to disclose.

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