MEBT/MEBO 对小鼠烧伤创面组织中 K19 表达的影响及作用机制
Effects and Action Mechanism of MEBT/MEBO on the Expression of K19 in Burn Wounds of Mouse

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　　【摘要】　目的　探究烧伤创疡再生医疗技术（moist exposed burn therapy/moist exposed burn ointment，MEBT/MEBO）对烧伤创面组织中角蛋白 19（keratin 19，K19）表达的影响及可能的作用机制。方法　选取 15 只健康雄性昆明小鼠，按照随机数表法随机选取 5 只作为对照组，10 只建立深 II 度烫伤模型后随机分为模型组与治疗组，其中治疗组小鼠创面采用湿润烧伤膏进行处理、模型组小鼠创面采用生理盐水进行处理、对照组小鼠背部脱毛后不做任何处理，1 周后取皮肤或创面组织并通过免疫组织化学染色、蛋白质印迹（Western blot）及定量聚合酶链反应（quantitative polymerase chain reaction，Q-PCR）等技术检测自噬相关蛋白 Beclin-1、LC3 及 K19 的表达水平。结果　治疗第 1 ~ 7 天，治疗组小鼠创面清洁，并始终保持湿润状态，且随着治疗时间的延长逐渐呈现上皮化状态；模型组小鼠创面逐渐加深，并出现感染征象。治疗 1 周后，免疫组织化学染色法及 Western blot 法检测结果显示，3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平对比，治疗组 > 模型组 > 对照组，P 均 < 0.01，差异具有统计学意义；除 Western blot 法检测结果显示治疗组与模型组小鼠创面组织中 LC3 表达水平对比，P > 0.05 外，其余各检测结果均显示小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平两两对比，P < 0.01，差异具有统计学意义。Q-PCR 技术检测结果显示，3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平对比，治疗组 > 模型组 > 对照组，P < 0.01，差异具有统计学意义；3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平两两对比，P 均 < 0.01，差异具有统计学意义。结论　MEBT/MEBO 促进烧伤创面愈合可能与增加创面组织中 K19 的表达有关，而诱导自噬相关蛋白的高表达可能是其具体机制之一。
　　【关键词】　烧伤创疡再生医疗技术；烧伤；创面；角蛋白 19；自噬相关蛋白；LC3；Beclin-1
　　【文章类型】　论著

　　【Abstract】　**Objective**　To explore the effects and possible action mechanism of MEBT/MEBO（moist exposed burn therapy/moist exposed burn ointment）on the expression of keratin 19（K19）in burn wounds. **Methods**　Fifteen healthy male Kunming mice were selected as experimental subjects，of which，5 mice were randomly assigned into a control group according to the random number table，and the other 10 mice were randomly divided into a model group and a treat-

ment group after the models of deep second degree burn wounds were established. The wounds in the treatment group were managed with MEBO while the wounds in the model group were managed with normal saline. In contrast, no intervention was given to the mice in the control group after their back hair was removed. One week later, skin or wound tissues were taken in all subjects, and the expression levels of autophagy-related proteins including Beclin-1, LC3 and K19 were tested with methods such as immunohistochemical staining, Western blot, quantitative polymerase chain reaction (Q-PCR) and etc. **Results**　During the treatment duration of day $1-7$, the wound surface was clean and always at a moist status in the treatment group, gradually presenting epithelialization with time, while the wounds in the model group deepened in depth gradually, with signs of infection. One week after treatment, the results of immunohistochemical staining and Western blot showed statistically significant differences as the expression levels of Beclin-1, LC3 and K19 in mouse skin or burn wounds were respectively compared among the three groups, with highest levels in the treatment group, then the model group and lowest in the control group, all $P < 0.01$. Except the Western blot results showed no statistically significant difference when the LC3 expression level in wound tissues was compared between the treatment group and the model group ($P > 0.05$), all the other pairwise comparisons in terms of the expression levels of Beclin-1, LC3 and K19 in skin or wound tissues among the three groups showed statistically significant differences based on the other test results, $P < 0.01$. According to the Q-PCR test results, the comparison of K19 mRNA expression levels in mouse skin or wound tissues among the three groups showed statistically significant difference, with highest level in the treatment group, and then the model group and lowest in the control group, $P < 0.01$. The pairwise comparisons of K19 mRNA expression levels in skin or wound tissues among the three groups all showed statistically significant difference, $P < 0.01$. **Conclusion**　The effect of MEBT/MEBO in promoting burn wound healing may be related to the increased expression of K19 in wound tissues, and inducing the high expression of autophagy related proteins may be one of its specific mechanisms.

【**Key words**】　MEBT/MEBO; Burns; Wounds; Keratin-19; Autophagy-related protein; LC3; Beclin-1

角蛋白（keratin）主要用于维持细胞结构的完整性和稳定性，其中角蛋白 19（keratin 19，K19）阳性表达的表皮干细胞可增殖、分化为皮肤各层组织细胞予以修复创面。部分研究显示，在烧伤创疡再生医疗技术（moist exposed burn therapy/moist exposed burn ointment，MEBT/MEBO）治疗深度烧伤创面的过程中，K19 阳性表达的干细胞可发生从无到有并不断增加的变化[1-2]，但具体机制尚不明确。为此，笔者于本研究中观察了 MEBT/MEBO 对小鼠烧伤创面组织中 K19、自噬相关蛋白 LC3 及 Beclin-1 表达的影响，并分析了可能的作用机制，以期为 MEBT/MEBO 的临床应用提供理论依据。

1　材料与方法

1.1　实验动物

随机选取 15 只体重为 19～21 g 的 SPF 级健康雄性昆明小鼠作为受试对象。所有小鼠均由湖北省实验动物研究中心提供，饲养环境清洁，室温（25.0 ± 3.0）℃，自由进食进水。本研究经中南大学湘雅三医院动物伦理委员会批准，符合动物实验的伦理学要求。

Keratin is mainly for maintaining the integrity and stability of cell structure, while epidermal stem cells containing the positively expressed keratin 19 (K19) can proliferate and differentiate into skin tissues of all layers, and further to repair skin wounds. Some studies have showed that during the treatment course of deep burn wounds with moist exposed burn therapy/moist exposed burn ointment (MEBT/MEBO), the number of stem cells with positively expressed K19 presented a change from nothing to something and continued to increase[1-2], for which, however, the exact mechanism remains unclear. To this end, this study observed the effects of MEBT/MEBO on the expression of K19, and autophagy-related proteins LC3 and Beclin-1 in mouse burn wounds, and also analyzed the possible action mechanism, with a view to providing theoretical basis for the clinical application of MEBT/MEBO.

1. Materials and methods

1. 1. Experimental animals

Fifteen SPF healthy male Kunming mice, weighing $19-21$ g, were randomly selected as subjects. All subjects, provided by Hubei Experimental Animal Research Center, are kept in a clean environment and at room temperature (25.0 ± 3.0)℃, with free access to food and water. This study was approved by the Animal Ethics Committee of The Third Xiangya Hospital of Central South University and was in line with the ethical requirements of animal experiments.

1. 2 主要试剂与药物

免疫组织化学 SP 试剂盒及 DAB 显色试剂盒，LC3 （bs-8878R）、K19 （bs-2190R） 及 GAPDH（bs-2188R）抗体：北京博奥森生物技术有限公司生产；Beclin-1 （sc-48341） 抗体：Santa Cruz Biotechnology 公司生产；湿润烧伤膏：汕头市美宝制药有限公司生产，国药准字 Z20000004。

1. 3 模型建立与分组

随机选取 10 只小鼠，采用 4% 水合氯醛（400 mg/kg，即 0.2 mL/20 g）腹腔注射麻醉及背部脱毛备皮后，将自制的带有 2 cm × 2 cm 孔洞的木板置于备皮处，并经木板孔洞将备皮区置于 （92.0 ± 1.0）℃的热水中浸泡 20 s，建立面积约 20% TBSA 的烫伤创面 （经烫伤组织病理切片证实为深Ⅱ度烫伤）；烫伤后立即予以腹腔注射生理盐水抗休克 （20 mL/kg，即 0.4 mL/20 g）。小鼠苏醒后能够正常进食进水即表明建模成功。建模成功后，按照随机数表法随机分为模型组与治疗组，每组 5 只；其余 5 只未建模的小鼠背部脱毛后作为对照组。

1. 4 局部处理

对照组：正常进食进水，备皮处不做任何处理。

治疗组：正常进食进水，创面均匀涂抹湿润烧伤膏，每 2 h 换药 1 次，连续治疗 1 周。治疗过程中，保证创面始终处于湿润状态；每次换药前拭净创面分泌物及残余药膏。

模型组：正常进食进水，创面采用生理盐水冲洗，每 2 h 冲洗 1 次，连续冲洗 1 周。

1. 5 样本采集

定时观察并拍照记录治疗组与模型组小鼠的创面变化。治疗 1 周后，取对照组小鼠的皮肤组织及治疗组与模型组小鼠的创面组织均分为 3 份，1 份用于免疫组织化学染色检测，2 份置于 −80 ℃冰箱内保存备用。

1. 6 免疫组织化学染色法检测 Beclin-1、LC3 及 K19 的表达水平

待所有标本采集完毕，取新鲜组织进行石蜡包埋、切片、脱蜡及 3% H_2O_2 处理 10 min、PBS 冲洗 5 min 后行微波修复；修复后，滴加正常山羊血清并于 37 ℃环境下封闭 10 min，然后分别相应加入 Beclin-1 抗体、LC3 抗体及 K19

1. 2. Main reagents and drugs

Immunohistochemical SP kit and DAB Substrate kit；antibodies of LC3（bs-8878R），K19（bs-2190R）and GAPDH（bs-2188R）are manufactured by Beijing Bioss Bio-technology Co.，Ltd.；Beclin-1 （sc-48341）antibody by Santa Cruz Biotechnology；Moist Exposed Burn Ointment（MEBO）by Shantou MEBO Pharmaceutical Co.，Ltd.（SFDA Approval No.：Z20000004）.

1. 3. Modeling and Grouping

Anesthetize 10 mice selected randomly from the 15 subjects by intraperitoneal injection of 4% Chloral Hydrate（400 mg/kg，i. e.，0.2 mL/20 g），remove their back hair to prepare skin，put the self-made plank with a 2 cm × 2 cm hole onto the prepared skin，and then soak the prepared skin through the plank hole into hot water（92.0 ± 1.0）℃ for 20 s to establish a scald wound of about 20% TBSA （identified as deep second degree scalds according to scalding tissue pathological sections）. Immediately after the scalding injury，inject normal saline intraperitoneally to prevent subsequent shock（20 mL/kg，i. e.，0.4 mL/20 g）. The models were established successfully if the mice can have water and food normally when they wake up. After successful modeling，the 10 mice were assigned into a model group and a treatment group according to the random number table，5 in each group，while the left 5 mice only with back hair removal were included into the control group.

1. 4. Local management

Control group：eat and drink normally，no intervention to the prepared skin.

Treatment group：eat and drink normally，apply a layer of MEBO onto the wound surface，change dressing every two hours continuously for one week. During the treatment course，keep the wound surface moist all the time，and remove the secretion and residual drug on the wound surface before each dressing change.

Model group：eat and drink normally，rinse the wound surface with normal saline every two hours continuously for one week.

1. 5. Sample collection

Regularly observe and record the wound changes in the treatment group and the model group. One week after treatment，collect skin tissue sample of each mouse in the control group，wound tissue sample of each mouse in the treatment group and the model group. Each tissue sample is divided evenly to three samples，one is used for the immunohistochemical staining and the other two are stored in −80℃ refrigerator for later use.

1. 6. Test the expression levels of Beclin-1，LC3 and K19 by the immunohistochemical staining

After sample collection，take the fresh tissue sections to undergo paraffin embedding，slicing and de-waxing and 3% H_2O_2 treatment for 10 min and PBS rinsing for 5 min，followed by microwave repair. After repairing，normal goat serum was added into the sections，and

抗体（1∶200）4 ℃孵育过夜；孵育过夜后，PBS 冲洗 3 次（每次 5 min），加入二抗孵育 10 min；二抗孵育后，PBS 冲洗 3 次（每次 5 min），加入 HRP 标记复合物（三抗）孵育 10 min；三抗孵育后，PBS 冲洗 3 次（每次 5 min），然后依次进行 DAB 显色，苏木素复染，酒精脱水，二甲苯透明，中性树胶封片；每张切片于显微镜下随机选取 5 个高倍视野检测 LC3、K19 及 Beclin-1 的平均光密度，并取其均值进行统计学分析。

1.7　Western blot 法检测 Beclin-1、LC3 及 K19 的表达水平

待所有标本采集完毕，取 1 份备用组织进行 RIPA 裂解、匀浆机研磨，提取总蛋白，并经 SDS 聚丙烯酰胺凝胶电泳分离及转膜后，TBST 洗膜 3 次；洗膜后，5% 脱脂奶粉封闭，4 ℃孵育相应目的抗体（LC3、K19、BECN-1，1∶1000或 GADPH 1∶2000）过夜；孵育过夜后，TBST 洗膜 3 次，室温孵育 HRP-抗兔二抗（1∶5000）；孵育二抗后，TBST 洗膜 3 次，ECL 显影。

1.8　Q-PCR 技术检测 K19 mRNA 的表达水平

待所有标本采集完毕，取 1 份备用组织按照试剂盒说明书中的 Trizol 法提取总 RNA，并检测质量及浓度（A260∶A280在 1.8 ~ 2.1 之间视为 RNA 质量合格）后，置于 - 80 ℃冰箱内保存备用。

按照逆转录试剂盒说明书以总 RNA 为模板逆转录合成 cDNA 后进行 RT-q PCR 检测，并采用荧光定量统计软件进行分析，结果以待测基因与内参基因 GAPDH 的表达水平比值表示。其中 K19 引物长度为 138 bp，序列为上游-CAGATAAGCAAGACCGAAG，下游-CAGCTGGACTCCATAACG；β-actin 引物序列为上游-GAGGGAAATCGTGCGTGAC，下游-CTGGAAGGTGGACAGTGAG。

1.9　统计学处理

采用 GraphPad Prism 5 统计软件对所得数据进行统计学分析，其中计量资料以（$\bar{x} \pm s$）表示，采用单因素方差分析或 q 检验，均以 $P < 0.05$ 为差异具有统计学意义。

keep them blocked for 10 min at 37℃, and then add Beclin-1 antibody, LC3 antibody and K19 antibody（1∶200）in turn, incubate at 4℃ for overnight followed by PBS wash three times（5 min each）, and then add secondary antibody to incubate for 10 min. After second incubation, rinse the sections with PBS for 3 times（5 min each）, add HRP labeled complex（third antibody）to incubate for 10 min followed by PBS wash for 3 times（5 min each）. Proceed sequentially with DAB stain, Hematoxylin counterstain, ethanol dehydration, transparency with xylene and mounting with neutral gum. Randomly select 5 high power fields in each section under microscope to detect the mean optical density of LC3, K19 and Beclin-1, and calculate the mean value for statistical analysis.

1.7. Test the expression levels of Beclin-1, LC3 and K19 by the Western blot method

After sample collection, take out one prepared tissue sample to undergo RIPA lysis and Homogeniser grinding for the extraction of total proteins. The total proteins were separated by using SDS-polyacrylamide gel electrophoresis and transferred onto a membrane, followed by TBST wash three times. After washing, use 5% nonfat dry milk to block, incubate the targeted antibodies（LC3, K19, BECN-1, 1∶1000 or GADPH, 1∶2000）at 4 ℃ for overnight. And then, do TBST wash three times and incubate HRP-anti-rabbit secondary antibody（1∶5000）at room temperature, followed by TBST wash again for three times and ECL development.

1.8. Test the expression level of K19 mRNA with the Q-PCR technique

After sample collection, take out the other prepared tissue sample to extract the total RNA with the Trizol method as specified in the kit manual and store the extracted total RNA in a refrigerator of - 80℃ for later use after its quality and density have been detected qualified（A260: A280 ranging between 1.8 - 2.1 is regarded as right RNA quality）.

According to the kit manual, generate cDNA by reverse transcription with the total RNA as template to proceed with the RT-q PCR test. Quantitative fluorescence statistical software was used for analysis, of which the results were expressed as the ratio of measured gene expression level to the expression level of the reference gene GAPDH. The length of K 19 primer was 138bp, with upstream sequence as -CAGATAAGCAAGACCGAAG and the downstream as -CAGCTGGACTCCATAACG, while the upstream sequence of β-actin primer was -GAGGGAAATCGTGCGTGAC and the downstream sequence was -CTGGAAGGTGGACAGTGAG.

1.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5, in which the measurement data was expressed as（$\bar{x} \pm s$）and analyzed with the single factor variance analysis or q test. $P < 0.05$ was considered as statistically significant.

2　结果

2.1　大体观创面愈合情况

治疗组：治疗第 1 ~ 7 天，创面清洁，并始终保持湿润状态，且随着治疗时间的延长逐渐呈现上皮化状态；模型组：治疗第 1 ~ 7 天，创面逐渐加深，并出现感染征象（图 1）。

2. Results

2. 1. Overview on wound healing condition

Treatment group：during the treatment duration of day 1 – 7, the wounds were clean and always moist, presenting epithelialization gradually with time. Model group：during the treatment duration of day 1 – 7, the wounds gradually deepened in depth and showed signs of infection（Fig. 1）.

治疗组
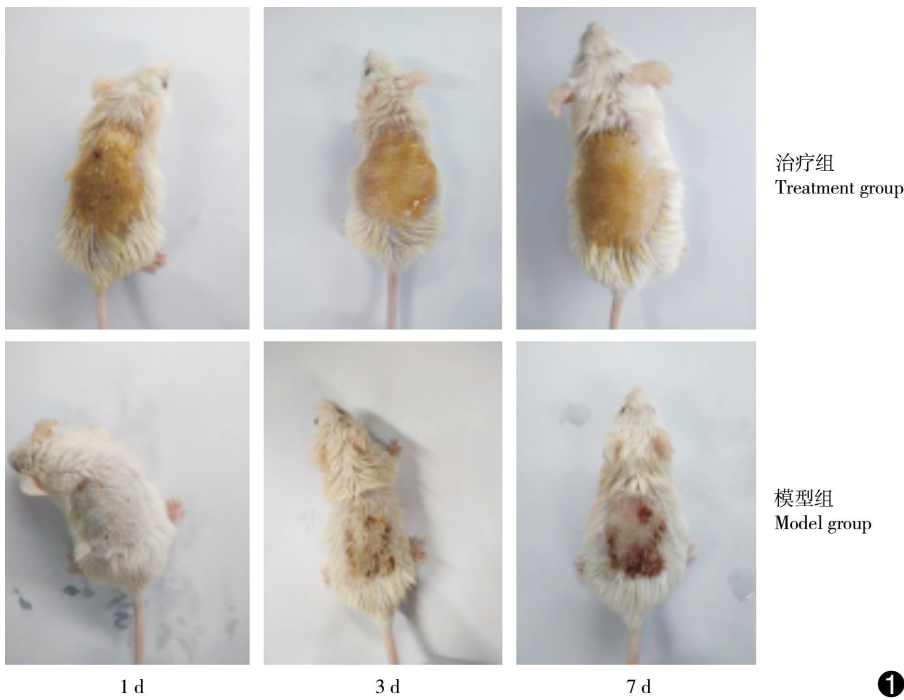Treatment group

模型组
Model group

1 d　　　　3 d　　　　7 d　　❶

图 1　治疗组与模型组小鼠创面愈合情况典型图
Fig. 1　Typical pictures of mouse wound healing condition in the treatment group and the model group

2.2　免疫组织化学染色法检测结果

免疫组织化学染色法检测 Beclin-1、LC3 及 K19 的结果显示，3 组小鼠皮肤或创面组织细胞内均可见棕黄色颗粒分布，且以治疗组小鼠创面组织细胞内分布最多（图 2）；Beclin-1、LC3 及 K19 平均光密度值对比，治疗组 > 模型组 > 对照组，$P < 0.01$，差异具有统计学意义；3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 平均光密度值两两对比，P 均 < 0.01，差异具有统计学意义（图 3，表 1）。

2.3　Western blot 法检测结果

Western blot 法检测 Beclin-1、LC3 及 K19 的结果显示，3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平对比，治疗组 > 模型组 > 对照组，$P < 0.01$，差异具有统计学意义；除治疗组与模型组小鼠创面组织中 LC3

2. 2. Results of the immunohistochemical staining

The immunohistochemical staining of Beclin-1, LC3 and K19 showed that there were brown granules scattering in the skin or wound tissue cells of the three groups, with the most in the treatment group（Fig. 2）. The comparison of mean optical density of Beclin-1, LC3 and K19 among the three groups showed statistically significant difference（treatment group > model group > control group, $P < 0.01$）. The pairwise comparison of mean optical density of Beclin-1, LC3 and K19 among the three groups all showed statistically significant differences, $P < 0.01$（Fig. 3, Table 1）.

2. 3. Results of the Western blot analysis

According to the Western blot analysis, the expression levels of Beclin-1, LC3 and K19 in skin or wound tissues of the three groups showed a pattern of the treatment group being higher than the model group, with the latter being higher than the control group, presenting a statistically significant difference, $P < 0.01$. The comparison of LC3 expression levels between the treatment group and the model
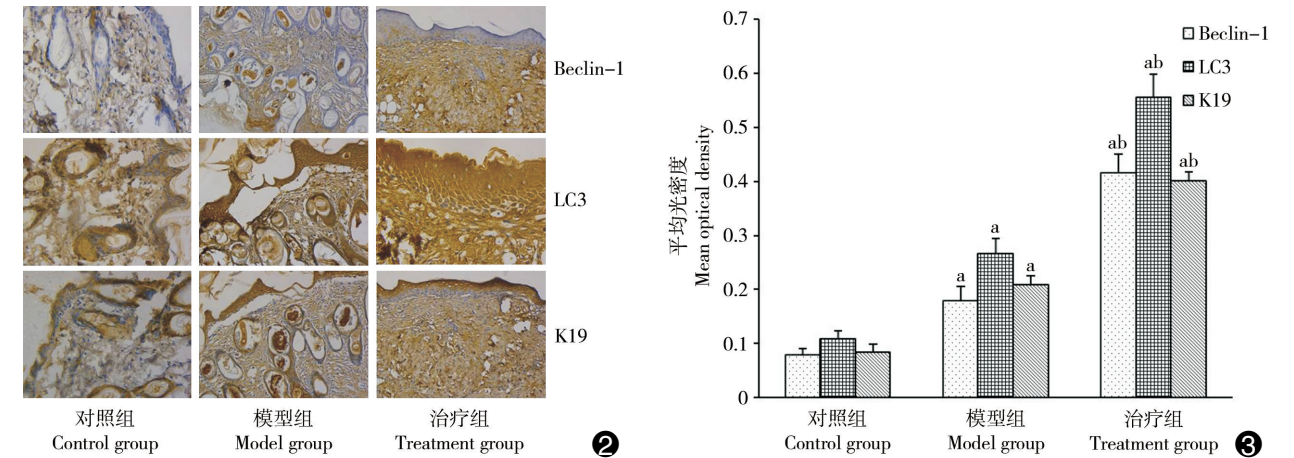
图 2　3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 免疫组织化学染色结果典型图；图 3　免疫组织化学染色
法检测 Beclin-1、LC3 及 K19 平均光密度直方图（a 表示与对照组对比 $P<0.01$，b 表示与模型组对比 $P<0.01$）
Fig. 2　The typical pictures of the immunohistochemical staining results of Beclin-1，LC3 and K19 in the three groups；
Fig. 3　The histogram of mean optical density of Beclin-1，LC3 and K19 detected by the immunohistochemical staining
（a represents comparison with the control group，$P<0.01$；b represents comparison with the model group，$P<0.01$）

表 1　3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 平均光密度值对比 $(\bar{x}\pm s)$

Table 1　Comparison of mean optical density of Beclin-1，LC3 and K19 in skin or wound tissues of the three groups $(\bar{x}\pm s)$

组别 Group	例数 Number of cases	Beclin-1	LC3	K19
治疗组 Treatment group	5	0.416 ± 0.034^{ab}	0.555 ± 0.044^{ab}	0.401 ± 0.029^{ab}
模型组 Model group	5	0.178 ± 0.028^{a}	0.266 ± 0.029^{a}	0.209 ± 0.015^{a}
对照组 Control group	5	0.078 ± 0.012	0.108 ± 0.015	0.083 ± 0.016
F 值 F value		216.996	256.741	290.968
P 值 P value		<0.01	<0.01	<0.01

注：3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 平均光密度值对比，P 均 <0.01，差异具有统计学意义。3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 平均光密度值两两对比，其中与对照组对比，$^{a}P<0.01$，差异具有统计学意义；与模型组对比，$^{b}P<0.01$，差异具有统计学意义

Note：Statistically significant differences were observed when the mean optical densities of Beclin-1，LC3 and K19 in skin or wound tissues of the three groups were compared，all $P<0.01$. The mean optical densities of Beclin-1，LC3 and K19 in skin or wound tissues of the three groups were compared in pairs，in which the comparisons with the control group and with the model group all showed statistically significant differences（respectively $^{a}P<0.01$ and $^{b}P<0.01$）

表达水平对比，$P>0.05$ 外，其余各组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平两两对比，P 均 <0.05，差异具有统计学意义（图 4，表 2）。

2.4　Q-PCR 技术检测结果

　　Q-PCR 技术检测 K19 mRNA 的结果显示，3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平对比，治疗组 > 模型组 > 对照组，$P<0.01$，差异具有统计学意义；3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平两两对比，P 均 <0.01，差异具有统计学意义（图 5，表 3）。

group showed no statistically significant difference，$P>0.05$. Except that，all the other pairwise comparisons of the expression levels of Beclin-1，LC3 and K19 among the three groups showed statistically significant differences，all $P<0.05$（Fig. 4，Table 2）.

2.4. Results of the Q-PCR technique

　　According to the detection results of the Q-PCR technique，the expression level of K19 mRNA in skin or wound tissues in the three groups showed a pattern of the treatment group being higher than the model group，with the latter being higher than the control group，presenting a statistically significant difference，$P<0.01$. The pairwise comparisons of K19 mRNA expression levels among the three groups all showed statistically significant differences，all $P<0.01$（Fig. 5，Table 3）.
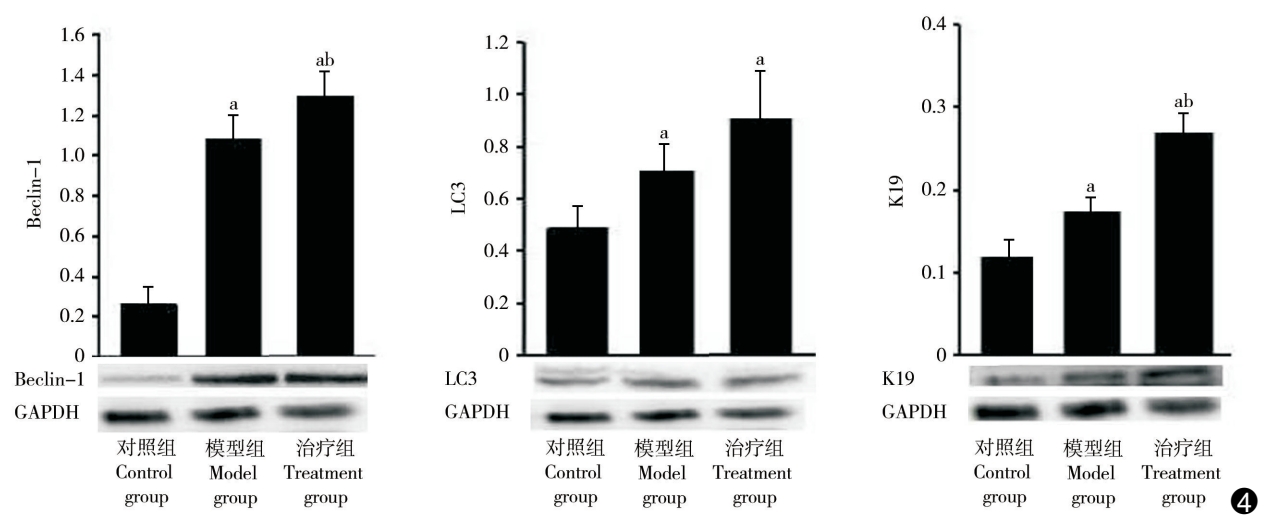
图 4　Western blot 法检测 Beclin-1、LC3 及 K19 表达水平直方图（a 表示与对照组对比 $P < 0.05$，b 表示与模型组对比 $P < 0.05$）

Fig. 4　The histogram of the expression levels of Beclin-1, LC3 and K19 according to the Western blot analysis (a represents comparison with the control group, $P < 0.05$; b represents comparison with the model group, $P < 0.05$)

表 2　3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平对比 $(\bar{x} \pm s)$

Table 2　Comparison of the expression levels of Beclin-1, LC3 and K19 in skin or wound tissues of the three groups $(\bar{x} \pm s)$

组别 Group	例数 Number of cases	Beclin-1	LC3	K19
治疗组 Treatment group	5	1.295 ± 0.126^{ab}	0.928 ± 0.167^{a}	0.271 ± 0.025^{ab}
模型组 Model group	5	1.088 ± 0.112^{a}	0.706 ± 0.101^{a}	0.173 ± 0.017^{a}
对照组 Control group	5	0.259 ± 0.088	0.485 ± 0.083	0.118 ± 0.022
F 值 F value		124.667	9.675	64.446
P 值 P value		< 0.01	< 0.01	< 0.01

注：3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平对比，P 均 < 0.01，差异具有统计学意义。3 组小鼠皮肤或创面组织中 Beclin-1、LC3 及 K19 表达水平两两对比，其中与对照组对比，[a]$P < 0.05$，差异具有统计学意义；与模型组对比，[b]$P < 0.05$，差异具有统计学意义

Note：Statistically significant differences were observed when the expression levels of Beclin-1, LC3 and K19 in skin or wound tissues were compared among the three groups, all $P < 0.01$. The expression levels of Beclin-1, LC3 and K19 in skin or wound tissues were compared in pairs among the three groups, in which statistically significant differences were observed in comparisons respectively with the control group ([a]$P < 0.05$) and with the model group ([b]$P < 0.05$)

3　讨论

　　研究表明，角蛋白是表皮细胞的主要结构蛋白，可在细胞内形成广泛的网状结构，对表皮具有重要的保护作用，其中 K19 是表皮干细胞的特异性标志物，其单克隆抗体被广泛应用于表皮干细胞的检测[3-4]。此外，K19 还可通过促进细胞外基质的降解或细胞移动提高细胞的转移能力，进而促进创面的再生修复[5-8]。有

3. Discussion

　　Studies have indicated that keratin, as the main structural protein of epidermal cells, can form a wide mesh structure in cells, playing an important role of protecting epidermis. Keratin 19 (K19) is a specific marker of epidermal stem cells, and its monoclonal antibody has been widely applied in the detection of epidermal stem cells[3-4]. Moreover, K19 can improve cell migration by accelerating the degradation of extracellular matrices, or cell movement, and further promote the regenerative repair of wounds[5-8]. Some studies have confirmed
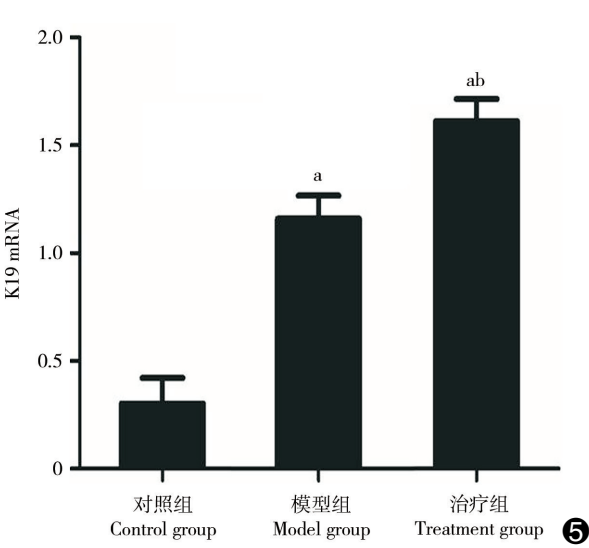
图 5　Q-PCR 技术检测 K19 mRNA 表达水平直方图（a 表示与对照组对比 $P < 0.01$，b 表示与模型组对比 $P < 0.01$）

Fig. 5　The histogram of K19 mRNA expression levels tested by the Q-PCR technique（a represents comparison with the control group，$P < 0.01$；b represents comparison with the model group，$P < 0.01$）

表 3　3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平对比 $(\bar{x} \pm s)$

Table 3　Comparison of K19 mRNA expression level in skin or wound tissues among the three groups $(\bar{x} \pm s)$

组别 Group	例数 Number of cases	K19 mRNA
治疗组 Treatment group	5	1.610 ± 0.103^{ab}
模型组 Model group	5	1.160 ± 0.107^{a}
对照组 Control group	5	0.303 ± 0.118
F 值 F value		183.786
P 值 P value		< 0.01

注：3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平对比，$P < 0.01$，差异具有统计学意义。3 组小鼠皮肤或创面组织中 K19 mRNA 表达水平两两对比，其中与对照组对比，$^{a}P < 0.01$，差异具有统计学意义；与模型组对比，$^{b}P < 0.01$，差异具有统计学意义

Note：Statistically significant difference was observed when the K19 mRNA expression levels in skin or wound tissues were compared among the three groups，$P < 0.01$. The expression levels of K19 mRNA in skin or wound tissues were compared in pairs among the three groups，in which statistically significant differences were observed in comparisons respectively with the control group（$^{a}P < 0.01$）and with the model group（$^{b}P < 0.01$）

研究证实，MEBT/MEBO 治疗深度烧伤创面的过程中，K19 阳性表达干细胞可出现从无到有、从少到多，再从多到少、从少到无的变化规律，但具体机制尚不明确。

笔者在临床研究中发现，烧伤创面应用 MEBT/MEBO 治疗后，创面坏死组织可无损伤地液化排除，作用温和无刺激，并可减轻局部炎症反应，同时对残存细胞具有保护作用，这与作为程序性细胞死亡机制及细胞自我防御机制的自噬介导的细胞代谢的动态平衡及自我保护[9-13]具有异曲同工之处，而已有研究证实，MEBT/MEBO 治疗的创面修复主要依赖于 K19 阳性表达的表皮干细胞的分化、增殖、迁移及融合，且 K19 还可抑制创伤造成的炎症反应[14]。因此，在本研究中，笔者对比观察了 MEBT/MEBO 对小鼠烧伤创面组织中自噬相关蛋白 Beclin-1、LC3 及 K19 表达的影响。结果显示，治疗 1 周后，3 组小鼠皮肤或创面组织中 Beclin-1、LC3、K19 及 K19 mRNA 表达水平对比，治疗组 > 模型组 > 对照组，P 均 < 0.01，差异具有统计学意义；除 Western blot 法检测结果中显示治疗组与模型组小鼠创面组织中 LC3 表达水平对比，$P > 0.05$ 外，其余各检测结果

that in the treatment of deep burns with MEBT/MEBO, the stem cells with the positively expressed K19 presented a change from nothing to something, from less to more, and then gradually decreasing to nothing, for which the exact mechanism is still not clear.

In clinical studies, the authors found that after the application of MEBT/MEBO on burn wounds, the necrotic tissues on wound surface can liquefy and be removed without damage to residual tissues, being very mild and non-irritating, and also the local inflammatory responses can be relieved and residual cells can be protected well, which realized the same satisfactory results as the dynamic balance and self-protection of autophagy-mediated cell metabolism as a mechanism of procedural cell death and cellular self-defense, though in different approaches [9-13]. And also, studies have confirmed that in wound treatment with MEBT/MEBO, the wound repair mainly depends on the differentiation, proliferation, migration and fusion of K19-positively expressed epidermal stem cells and, moreover, K19 can inhibit the inflammatory responses caused by traumas [14]. Thus, in this study, the effects of MEBT/MEBO on the expression of autophagy-related proteins such as Beclin-1, LC3 and K19 were investigated in mouse burn wounds. The results showed that after one week of treatment, the expression levels of Beclin-1, LC3 and K19 in mouse skin or wound tissues presented a statistically significant difference among the three groups, with the treatment group being higher than the model group and the latter being higher than the control group, $P < 0.01$. No statistically significant difference was observed

中均显示小鼠皮肤或创面组织中 Beclin-1、LC3、K19 及 K19 mRNA 表达水平两两对比，$P < 0.01$，差异具有统计学意义。即 MEBT/MEBO 可明显提高小鼠烧伤创面组织中自噬相关蛋白 Beclin-1、LC3 及 K19 的表达水平，促进创面的愈合。由此推断，MEBT/MEBO 治疗的创面组织中 K19 的表达水平明显升高可能与自噬相关[15-19]。

　　综上所述，MEBT/MEBO 促进烧伤创面的愈合与提高创面组织中 K19 的表达水平有关，且诱导自噬相关蛋白的高表达可能是其作用机制之一。因本研究设计限制，其对自噬相关通路的调控机制仍需进一步深入研究探讨。

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when the LC3 expression levels were compared between the treatment group and the model group according to the Western blot method ($P > 0.05$), while based on all the other detection results the pairwise comparisons of the expression levels of Beclin-1, LC3, K19 and K19 mRNA among the three groups showed statistically significant differences, all $P < 0.01$. This means, MEBT/MEBO can markedly improve the expression levels of autophagy-related proteins including Beclin-1, LC3 and K19 in mouse burn wounds, and promote wound healing. It can be concluded that the significantly elevated expression level of K19 in wound tissues treated with MEBT/MEBO may be associated with autophagy [15-19].

To sum up, MEBT/MEBO in promoting the healing of burn wounds is related to improving the expression level of K19 in wound tissues, of which inducing the high expression of autophagy-related proteins may be one mechanism. In this study, due to design limitations, the regulatory mechanism of MEBT/MEBO in autophagy-related signaling pathways remains to be further explored in the future.

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