A novel mechanism in maggot debridement therapy: protease in excretion/secretion promotes hepatocyte growth factor production

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Honda K, Okamoto K, Mochida Y, Ishioka K, Oka M, Maesato K, Ikeè R, Moriya H, Hidaka S, Ohtake T, Doi K, Fujita T, Kobayashi S, E Noiri. A novel mechanism in maggot debridement therapy: protease in excretion/secretion promotes hepatocyte growth factor production. Am J Physiol Cell Physiol 301: C1423–C1430, 2011. First published August 31, 2011; doi:10.1152/ajpcell.00065.2011.—Maggot debridement therapy (MDT) is effective for treating intractable wounds, but its precise molecular mechanism, including the association between MDT and growth factors, remains unknown. We administered MDT to nine wounds of lower extremities because they did not respond to conventional therapies. Significant increases of hepatocyte growth factor (HGF) levels were observed in femoral vein blood during 48 h of MDT (P < 0.05), but no significant change was found for vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGF-β1), or tumor necrosis factor-α (TNF-α). We conducted NIH-3T3 cell stimulation assay to evaluate the relation between HGF and protease activity in excretion/secretion (ES) derived from maggots. Compared with the control group, HGF was significantly higher in the 0.05 μg/ml ES group (P < 0.01). Furthermore, protease inhibitors suppressed the increase of HGF (P < 0.05). The HGF expression was increased in proportion to the ES protein concentration of 0.025 to 0.5 μg/ml. In fact, ES showed stronger capability of promoting HGF production and less cytotoxicity than chymotrypsin or bromelain. HGF is an important factor involved in cutaneous wound healing. Therefore, these results suggest that formation of healthy granulation tissue observed during MDT results from the increased HGF. Further investigation to identify molecules enhancing HGF expression by MDT will contribute greatly to drug target discovery for intractable wound healing therapy.

MATERIALS AND METHODS

Patients with intractable ulcers of lower limbs. During May 2007–October 2008, nine patients (66.3 ± 11.8 yr, 5 male and 4 female) with intractable wounds of the lower extremities received MDT in Shonan Kamakura General Hospital. The diagnosis was peripheral arterial disease (n = 7), cholesterol crystal embolism (n = 1), and venous ulcer (n = 1). Eight of nine patients had diabetes mellitus. Three were receiving maintenance dialysis for end-stage renal disease. None of the wounds had responded to conventional therapies including bone marrow transplantation and epithelial progenitor cell transplantation for more than a month. The average period between development of symptoms and initiation of the first session of MDT was longer than 6 mo.

Maggot debridement therapy. We ordered 100–400 sterile larvae of Lucilia sericata larvae from Biotherapy Medical (Shiga, Japan). The number of maggots was determined as 10 larvae per square centimeter of wound area.

Live maggots were put on the wound and covered with wet gauze. Then the affected limb was wrapped in a finely textured stocking. The treatment time for one session of MDT was 48 h. All maggots were removed after 48 h.

Measurement of HGF in blood samples. Immediately before and after each MDT session, blood samples were taken from the femoral vein of the affected limb. All blood samples were subsequently analyzed by SRL (Tokyo, Japan). HGF was measured using an ELISA kit (HGF Otsuka ELISA kit; Otsuka Pharmaceutical, Tokyo, Japan). It was undetectable under 0.3 ng/ml. The serum samples were obtained with informed consent following IRB-approved guidelines of the Shonan Kamakura General Hospital.

Several mechanisms, including induction of fibroblast migration by excretion/secretion (ES) of maggots, have been proposed (20). They consist mainly of necrotic tissue removal, healthy granulation promotion, and antimicrobial activity (3, 34). Although ES is apparently related to promotion of healthy granulation, its precise molecular mechanism remains unknown including the association between MDT and growth factors. This report describes the increase of hepatocyte growth factor (HGF) in blood samples taken from the femoral vein of the affected limb during one session of MDT. HGF is known as an important factor that is involved in cutaneous wound healing (10). Therefore, these results described below enable us to consider that formation of healthy granulation tissue observed during MDT results from increased HGF. Particularly examining protease in ES, we additionally investigated that proteolytic activity influenced ES action on HGF production. Further identification of the molecule that enhances HGF expression by MDT will contribute greatly to drug target discovery for intractable wound healing therapy.

RECENTLY, THE NUMBERS OF PATIENTS WITH INTRACTABLE WOUNDS SUCH AS DIABETIC GANGRENE AND ISCHEMIC ULcers OF THE LOWER EXTREMITIES HAVE BEEN INCREASING, PRESENTING ENORMOUS IMPLICATIONS FOR HEALTHCARE RESOURCES AND COSTS (19). IN MANY CASES OF CRITICAL LIMb ISCHEMIA, APPROPRIATE MANAGEMENT INCLUDING REVAScULIZATION FAIL TO ACHIEVE WOUND HEALING (9, 22). MAGGOT DEBRIDEMENT THERAPY (MDT) HAS BEEN DEMONSTRATED AS EFFECTIVE FOR TREATING INTRACTABLE WOUNDS (6). IN FACT, MDT, WHICH REEMERGED AS A TREATMENT IN THE EARLY 1990s, HAS A FAR LONGER HISTORY OF USE THAN ANTIBIOTICS. AS EARLY AS 1931, BAER REPORTED THAT MAGGOT DEBRIDEMENT THERAPY (MDT) IS EFFECTIVE FOR TREATING INTRACTABLE WOUNDS, BUT ITS PRECISE MECHANISM, INCLUDING THE ASSOCIATION BETWEEN MDT AND GROWTH FACTORS, REMAINS UNKNOWN. WE ADMINISTERED MDT TO NINE WOUNDS OF LOWER EXTREMITIES BECAUSE THEY DID NOT RESPOND TO CONVENTIONAL THERAPIES. SIGNIFICANT INCREASES OF HEPATOCYTE GROWTH FACTOR (HGF) LEVELS WERE OBSERVED IN FEMORAL VEIN BLOOD DURING 48 H OF MDT (P < 0.05), BUT NO SIGNIFICANT CHANGE WAS FOUND FOR VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF), BASIC FIBROBLAST GROWTH FACTOR (bFGF), TRANSFORMING GROWTH FACTOR-β1 (TGF-β1), OR TUMOR NECROSIS FACTOR-α (TNF-α). WE CONDUCTED NIH-3T3 CELL STIMULATION ASSAY TO EVALUATE THE RELATION BETWEEN HGF AND PROTEASE ACTIVITY IN EXCRETION/SECRETION (ES) DERIVED FROM MAGGOTS. COMPARED WITH THE CONTROL GROUP, HGF WAS SIGNIFICANTLY HIGHER IN THE 0.05 μG/ML ES GROUP (P < 0.01). FURTHERMORE, PROTEASE INHIBITORS SUPPRESSED THE INCREASE OF HGF (P < 0.05). THE HGF EXPRESSION WAS INCREASED IN PROPORTION TO THE ES PROTEIN CONCENTRATION OF 0.025 TO 0.5 μG/ML. IN FACT, ES SHOWED STRONGER CAPABILITY OF PROMOTING HGF PRODUCTION AND LESS CYTOTOXICITY THAN CHYMOTRYPSIN OR BROMELAIN. HGF IS AN IMPORTANT FACTOR INVOLVED IN CUTANEOUS WOUND HEALING. THEREFORE, THESE RESULTS SUGGEST THAT FORMATION OF HEALTHY GRANULATION TISSUE OBSERVED DURING MDT RESULTS FROM THE INCREASED HGF. FURTHER INVESTIGATION TO IDENTIFY MOLECULES ENHANCING HGF EXPRESSION BY MDT WILL CONTRIBUTE GREATLY TO DRUG TARGET DISCOVERY FOR INTRACTABLE WOUND HEALING THERAPY.

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Fibroblast cell culture. Mouse embryonic fibroblast (NIH-3T3) cells were grown in 100-mm tissue culture dishes (Falcon; BD Bioscience Discovery Labware, Bedford, MA), containing standard cell culture medium comprising Dulbecco’s Modified Eagle’s Medium (DMEM; Invitrogen) and 10% fetal bovine serum (FBS; Sigma). For experiments, NIH-3T3 cells whose passage numbers were 5–10 were trypsinized and transferred to fresh cell culture medium. Cells were incubated at 37°C in a 5% (vol/vol) CO₂ humidified atmosphere.

ES collection. Lucilia sericata larvae that had been hatched and kept in sterile conditions (Biotherapy Medical) were used for 48 h as MDT. The ES were collected from the third-instar larvae. Briefly, 20 larvae were washed in 10 ml of sterile phosphate-buffered saline solution (PBS, pH 7.3; Invitrogen) for 48 h at 37°C to recover ES products. The collected ES was concentrated ~40-fold in Amicon Ultra 4 (Millipore, Billerica, MA). The protein concentration was determined using protein colorimetric assay (Bio-Rad Laboratories, Hercules, CA), based upon the manufacturer’s protocol.

NIH-3T3 cells stimulation assay (excretion/secretion of maggots). Approximately 1 × 10⁴ NIH-3T3 cells were seeded in 12-well plates. They were incubated for 8 h at 37°C in a 5% (vol/vol) CO₂ humidified atmosphere. After incubation, each well was washed with PBS. Fresh medium containing ES, DMEM, 0.5% FBS, and penicillin-streptomycin-cysteine, and serine protease inhibitor, and 0.683 mol/g protein aprotinin, serine protease inhibitor that inhibits trypsin and phenylmethylsulfonyl fluoride, serine protease inhibitor. They were incubated for 12 h at 37°C in a 5% (vol/vol) CO₂ humidified atmosphere. Then, RNA and cell lysate were obtained from each well.

Quantitative real-time PCR. RNA was extracted from the samples in the wells using the phenol-chloroform method. For reverse transcription, an ImProm-II reverse transcription system (Promega, Madison, WI) was used. Then, quantitative real-time PCR was performed to detect HGF (forward primer, 5’-CCCTATGCGAAGGACAGAA-3’ and reverse primer, 5’-CGCCGTCTCTGATACACC-3’) and β-actin (forward primer, 5’- CGCAACACTGGGATTTGCAT-3’ and reverse primer, 5’- TTCTCCCTTGATGTCAAGC-3’). The expressions of HGF and β-actin in each sample were quantified in separate tubes with the respective primers. PCR conditions were the following: denaturation at 95°C for 10 s with subsequent annealing at 54°C for 30 s and extension at 72°C for 30 s. Based on SYBR Green I fluorescence, the amplification was conducted (LightCycler480; Roche Diagnostics, Tokyo, Japan) according to the standard protocol for the device. The HGF mRNA expression was normalized by β-actin.

Measurement of HGF in NIH-3T3 cell lysate. Cell lysate was collected 12 h after initiation of NIH-3T3 cell stimulation with ES or PBS. Cell lysate was centrifuged at 10,000 g for 10 min. The clear supernatant was used for ELISA. HGF was measured using a HGF Otsuka ELISA kit.

NIH-3T3 cells stimulation assay (chymotrypsin and bromelain). Another experiment was conducted to evaluate whether protease promoted HGF production. In the place of ES, commercially available proteases were used: α-chymotrypsin (Sigma), serine protease and bromelain (Sigma), and cysteine protease. Cytotoxic concentrations of these proteases were confirmed in the preliminary work. Therefore, they were added at lower concentrations than the others, respectively. Detailed procedures were the same as those described above. Chymotrypsin and bromelain were added instead of ES this time.

Immunofluorescence and confocal microscopy. NIH-3T3 cells were grown on coverslips, treated as described in the previous section, washed in PBS, and fixed in 2% PFA (Electron Microscopy Sciences; Fort Washington, PA) for 10 min. Fixed cells were permeabilized in 0.2% Triton X-100 (Sigma) in PBS-2% bovine serum albumin for 15 min and then incubated at room temperature for 60 min with anti–signal transducer and activator of transcription 3 (STAT3) antibodies (Cell Signaling Technology; Danvers, MA). Cells were rinsed and incubated with appropriate secondary antibodies for 60 min. Cells were washed three times in PBS and once in water. Then they were mounted in TO-PRO-3 iodide-containing mounting medium (Invitrogen). All images were acquired using a confocal laser scanning microscope (LSM510; Carl Zeiss).

Statistical analysis. In clinical study, HGF levels were expressed as median. They were evaluated using the Mann-Whitney U-test. In the in vivo study, results were expressed as means ± SE. Student’s t-test was used to compare results. Differences were considered significant when P < 0.05.

Fig. 1. Changes of five parameters immediately before and after one session of maggot debridement therapy (MDT) (n = 9). A: hepatocyte growth factor (HGF). B: vascular endothelial growth factor (VEGF). C: basic fibroblast growth factor (bFGF). D: transforming growth factor-β1 (TGF-β1). E: tumor necrosis factor-α (TNF-α). The lower detection limits of HGF and bFGF are, respectively, 0.3 and 10 ng/ml.

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RESULTS

These nine patients received MDT because other therapies were ineffective. The average number of MDT sessions was 2.00 ± 0.47. All patients showed clinical improvements such as the reduction of necrotic tissue and the formation of healthy granulation tissue. Adverse effects were not observed except for analgesic-controllable pain.

In the clinical investigation, significant elevation of HGF levels was observed during 48 h of MDT (median N.D. vs. 0.37 ng/ml, Mann-Whitney U-test, \(P = 0.035\); Fig. 1). We measured four other cytokines: vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGF-β1), and tumor necrosis factor-α (TNF-α). For these four cytokines, no significant change was found during one session of MDT (Fig. 1). The HGF value was increased or unchanged, except in one patient. No association of changes was found between HGF and the other four values. In the short term, removal of necrotic tissue and growth of healthy granulation tissue were observed among all the patients irrespective of the degree of HGF increase. However, the patient who eventually received major amputation during MDT showed an abrupt decrease of the plasma HGF level. All other patients except one patient who was lost to follow up were able to avoid major amputation after MDT.

First, zymography was performed to examine protease activity in ES. Figure 2 depicts three protease bands, with molecular sizes of 44, 45, and 52 kDa, which were identifiable on the zymogram. Among them, the band of 52 kDa had the strongest protease activity. The respective protease activities of the 44-, 45-, and 52-kDa bands were calculated as 5.0, 3.1, and 40.8 nU (the values were estimated by protease activity of collagenase). The activity was inhibited sufficiently by PI cocktail. Proteases in ES were serine and/or cysteine proteases in light of the composition of PI cocktail. Furthermore, Fig. 3 shows that serine protease was predominant in ES.

Second, NIH-3T3 cells stimulation assay was performed to evaluate both larval ES effects of enhancing HGF expression and association between ES protease activity and HGF expression. Compared with a control group, HGF mRNA levels of NIH-3T3 cells were increased significantly in 0.05 µg/ml ES group (92% increase vs. control, \(P < 0.01\); Fig. 4). This increase of HGF was suppressed by PIs (ES group vs. ES + PI group, \(P < 0.05\); Fig. 4). In preliminary experiments, the degree of both protease inhibition and cytotoxicity determined the applied volume of PIs.

HGF production in cell lysate was measured using ELISA. In the ES group, HGF was elevated significantly compared with the control group (152% increase vs. control, \(P < 0.01\); Fig. 5). As described before, PIs inhibited this increase of HGF because of the addition of ES. These results demonstrate that the larval ES induced fibroblast cells to produce HGF.

NIH-3T3 cell stimulation assay was performed at various protein concentrations ranging from 0.025 to 0.5 µg/ml. HGF expression was increased relative to the ES protein concentration (Fig. 6). Morphological abnormality was absent even at the protein concentration of 0.5 µg/ml.

**Fig. 2.** Zymogram showing staining of excretion/secretion (ES) and ES added to protease inhibitors (PI). At protein volume of 0.05 µg, ES showed a high activity of protease. Three protease bands with molecular sizes of 44, 45, and 52 kDa were identified using zymography. Protease activity was suppressed by protease inhibitor cocktail.

**Fig. 3.** Zymogram staining of ES and ES added to serine protease inhibitor (SPI), 0.683 mol/g prot phenylmethylsulfonyl fluoride. Most protease activity in ES at a protein volume of 0.025 µg was inhibited by phenylmethylsulfonyl fluoride (SPI).

**Fig. 4.** Action of protease inhibitor on ES. Increased hepatocyte growth factor (HGF) mRNA expression induced by larval ES was inhibited by PI (**\(P < 0.05\), *\(P < 0.05\)).
Third, for assessing protease action to promote HGF production, commercially available proteases, chymotrypsin, and bromelain were used in NIH-3T3 cell stimulation assay. Chymotrypsin is a serine protease, whereas bromelain is a cysteine protease, which is of clinical use for wound care. Each volume of the proteases was determined according to the protease activities. Increased HGF expression was confirmed in both chymotrypsin and bromelain groups (Fig. 7). Furthermore, chymotrypsin induced stronger HGF production than bromelain did. However, each protease showed cytotoxicity against fibroblast cells at a low concentration.

Comparison of HGF levels in cell lysate between ES, chymotrypsin, and bromelain is shown in Fig. 8. Amounts of proteases were estimated according to their proteolytic activity. The HGF values are higher in the ES group than in the chymotrypsin or bromelain group.

STAT3 is known to act downstream of c-MET, HGF receptor, to control c-MET-dependent tubulogenesis (4), wound healing (39), invasion (11), anchorage-independent growth, and tumorigenic growth in nude mice (48). At the same time, STAT3 acts directly on the HGF promoter region (21). Confocal microscopic examination presented in Fig. 9 shows that stimulation with ES for 3 h led to STAT3 nuclear translocation.

DISCUSSION

Reportedly, HGF plays a crucial role in cutaneous wound healing (10). A predominant cell type in the processes of tissue repair is the skin fibroblast (24). In fact, HGF is synthesized and secreted predominantly by fibroblasts (14, 15) in vivo as pro-HGF (16). This factor can stimulate the motility, migration, and morphological changes of many cell types, including endothelial cells, epithelial cells, melanocytes, and keratinocytes (15), where activation of the inactive molecule occurs via the protease response to tissue damage (16). However, gene transfer therapy in critical limb ischemia using intramuscular injection of HGF plasmid was insufficient to prevent major
amputation (32, 36, 40). Improvement by the HGF plasmid therapy was observed only for rest pain and ischemic ulcer size (32, 40). We considered the possibility that these unexpected results were obtained partly because exogenous HGF administered by intramuscular injection was less effective than endogenous HGF. Endogenous HGF is involved in enhancing cutaneous wound healing processes, including reepithelialization, neovascularization, and granulation tissue formation (46). In contrast to our clinical results, HGF elevation in serum was not observed in any clinical trial of HGF plasmid therapy against CLI (18, 32, 40). Actually, HGF administration might be insufficient for wound healing because proteases are necessary for its activation. HGF elevation in MDT without its direct administration and nuclear accumulation of STAT3 indicated that this therapy induced both production and activation of endogenous HGF. In fact, MDT is a promising treatment option as an endogenous HGF therapy.

Pleiotropic effects of protease have been reported in other species, although little is known about the proteases of *Lucilia sericata*. The effect includes immunological action as well as angiogenesis. Five serine proteases contribute to immune systems through regulation of the activation of the Toll pathway in *Drosophila* (25). Antimicrobial effects of the serine proteases were observed in response to Gram-positive bacterial and fungal infections. According to one report (3), ES derived from maggots showed an antibacterial effect against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), although it showed a weaker effect against Gram-negative bacteria (1, 23, 44). The similar antimicrobial activities of *Lucilia sericata* and *Drosophila* suggest that serine protease plays a key role in MDT immunologically. In this report, we particularly examined another aspect of the pleiotropic effect: ES action on HGF production.

![Confocal sections of NIH-3T3 cells stimulated with ES for 0 and 3 h and stained for signal transducer and activator of transcription 3 (STAT3) and nuclei. Stimulation with ES led to STAT3 nuclear translocation (arrowhead), although it was inhibited by PI. Scale bar: 10 μm.](image)
Zymography shown in Fig. 2 depicts that ES had high proteolytic activity and that it contains serine and/or cysteine proteases. In addition, Fig. 3 shows that the heterogeneous material consists mainly of serine protease. Results show that HGF secretion is increased by fibroblast cells with ES stimulation. Fibroblast cells appear at an early stage after tissue injury and increase during the progression of wound healing. HGF is also secreted by mesenchymal cells and granulocytes. Although heterogeneous proteases contained in ES might act as an HGF inducer, some different cells might secrete HGF as its producer. C-MET is distributed in basal keratinocytes of the wound edge, suprabasal layers of hyperplastic epidermal wound edges, small vessels, mononuclear infiltrating cells within the papillary dermis, and granulation tissue, although dermal tissue in the center of the ulcer not covered by epithelium revealed only weak staining for c-MET (8). Signaling associated with HGF stimulus is regarded as delivered in multiple pathways. Furthermore, the distribution of c-MET in a skin ulcer (8) resembles healthy granulation that occurs immediately after MDT in the sense that stronger expression is observed at the ulcer edge (Fig. 10).

However, commercially available proteases, chymotrypsin, and bromelain also enhanced HGF production. Chymotrypsin is a well-known serine protease of the digestive system. Bromelain is a cysteine protease extracted from pineapple plants. It is clinically available for the chemical debridement of burns. However, this protease has recently been a focus of attention for its anti-inflammatory effect via reduction in neutrophil migration (7, 13). Our data suggest that the two proteases show characteristics of cytotoxic action despite inadequate ability to enhance HGF production. In contrast, ES showed a stronger capability than these proteases to promote HGF production and less cytotoxicity. Medical agents are permitted considering the balance between effectiveness and adverse effects. Therefore, ES can contribute to drug discovery as other established medicinal substances can.

According to the former experiment using cultured rabbit proximal tubule cells, the half-maximal mitogenic effect of HGF is $6 \times 10^{-11}$ M (17). This concentration is nearly equivalent to 6 ng/ml, which was much higher than that of our clinical data. In contrast, serum HGF in human acute hepatitis and normal controls were reported respectively as 0.45 and 0.27 ng/ml (43). Our data are similar to this clinical result in terms of concentration. A subtle elevation of HGF might be sufficient in vivo because it is activated specifically at the site of tissue injury (30). In addition, the effect of ES is amplified by a positive feedback loop, as described below.

Figure 9 showed that proteases contained in ES derived from maggots were involved in STAT3 activation. The biological effect of STAT3 transcriptional activity requires entry into the nucleus. Therefore, cytoplasmic-nuclear translocation of STAT3 is crucial (38). STAT3, which is known as a downstream transcript factor of c-MET, also increases HGF promoter activity (21). Considering c-MET as a delayed early response gene (5), ES promotes HGF production via a positive feedback loop of HGF/c-MET/STAT3 (Fig. 11).

HGF has been shown to function after conversion to the active form. HGF activator (HGFA), a serine protease, reportedly activates HGF efficiently in vitro (31). Other proteases such as urokinase, tPA, factor XII, factor XI, plasma kallikrein, and endothelin-1 have also been shown to activate HGF in vitro (32, 33). However, the biological relevance of these in vitro observations has not been fully established.

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matriptase, and hepsin are also known to be related to the activation from pro-HGF to HGF (26, 27, 33, 35, 42). In addition, the inactive form of HGFA (pro-HGFA) is converted to its mature form by thrombin and plasma kallikrein (41). It is probable that ES obtained from maggots act at the site either from pro-HGF to HGF or from pro-HGFA to HGF. It can also be presumed that a heterogeneous property of ES is associated with both conversions. Additive effects of multiple substances in ES might increase HGF production without showing apparent cytotoxicity. Because the expression of c-MET stimulated by HGF increases HGF itself through c-SRC and STAT3 that bind to the HGF promoter (5, 45), activation of either pro-HGF, pro-HGFA, or both by larval ES can result in increased expression of endogenous HGF mRNA. ES is reportedly involved in cytokines other than HGF. Alimentary secretions of maggot increase the growth rates of both epithelial growth (32) factor (EGF)-stimulated and interleukin-6 (IL-6) stimulated fibroblasts (37). Binding of EGF to EGFR enhance HGF production with or without c-SRC/STAT3 activation (12), whereas IL-6 promotes HGF transcription and generation of the latent protein (28, 29, 47). Consequently, both are involved in increasing HGF expression.

In summary, this report is the first providing new insight into the mechanism relating HGF and excretion/secretion in maggot debridement therapy. Results showed that proteases in larval ES promote HGF production and that blood samples show a significant endogenous HGF increase during a single session of maggot debridement therapy. Our data suggest that elevation of HGF production contributes to the promotion of healthy granulation tissue in the therapy. Further identification of the molecules that enhance HGF expression by maggot debridement therapy will contribute greatly to drug target discovery for intractable wound healing therapy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

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