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Gene expression profiling of *Lucilia sericata* larvae extraction/secretion-treated skin wounds

Erdal Polat [a], İlayda Aksöz [b], Hülya Arkan [b], Ender Coşkunpınar [c], Fahri Akbaş [d], İlhan Onaran [b,*]

[a] Department of Microbiology and Clinical Microbiology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey
[b] Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey
[c] Department of Internal Medicine, Istanbul Medical Faculty, Division of Medical Genetics, Istanbul University, Istanbul, Turkey
[d] Department of Medical Biology, Faculty of Medicine at Bezmialem Vakif University, Istanbul, Turkey

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ABSTRACT

The larvae of *Lucilia sericata* have been successfully used as medicinal maggots in the healing of wounds. The excretion/secretion (ES) products of the larvae have been shown to efficiently debride wounds and help the healing process. The mechanisms underlying ES-induced wound healing are not yet completely understood. One of the intriguing questions is the role of ESs in modulating gene expression at the transcriptional level in the skin wound environment during the healing process. To address this question, a study was conducted in which the ES-induced gene expression profile in wound biopsies and ES-treated wounds of rat skin in comparison with control group was analyzed at the molecular level by monitoring the expression of genes associated with wound healing. The expression levels of 82 genes at 4, 7, and 10 days after wounding were determined using a PCR array system following cDNA synthesis. A comparison from wounds revealed that 38 mRNAs ($\geq$5-fold expression) were differentially expressed in the ES-treated skin. For 27 genes, the multiple-test corrected p-value was statistically significant ($p \leq 0.00061$). The expression pattern of these mRNAs was also altered during a period of 10 days. Many of the upregulated or downregulated mRNAs with therapy were extracellular matrix, cell adhesion-related proteins and growth factors. The genes that have the highest fold change (>1000-fold) were *Col1a2*, *Col4a1*, *Ctsk*, *Ccl7*, *Angpt1*, *Cd40lg*, *Egf* and *Itgb5*. Several of these gene products might play key roles in ES-induced wound healing. These findings may provide new insight for an understanding of the therapeutic potential of ESs for wound healing.

1. Introduction

Maggot debridement therapy (MDT), also called as larval therapy, larvae therapy, biodebridement and biosurgery, has a long history of use in the treatment of chronic and infected wounds. The 1st and 2nd stages of *Lucilia sericata* larvae have been used successfully in the treatment of pathologic conditions such as venous stasis ulcers, decubitus ulcers, temporal mastoiditis, and Fournier's gangrene, and the treatment of necrotic tumor masses and other soft-tissue wounds. Clinical observations and experimental data indicate that the molecules involved in the beneficial effects of maggots are believed to be contained in their excretions/secretions (ESs) (Mumcuoglu et al., 1998; Sherman, 2003; Steenvoorde et al., 2007). The larvae of *L. sericata* help the healing process by the proteolytic digestion of necrotic tissue and disinfection and by the stimulation of granulation tissue formation. In addition, it has been suggested that maggots exhibit other, more direct, mechanisms which contribute to the enhanced healing of wounds (Sherman, 2003; Wollina et al., 2000, 2002). MDT is thus widely used for the treatment of various refractory wounds including diabetic foot and venous ulcers. However, the molecular mechanisms involved in treating non-healing wounds by ESs are not very well understood. Wound healing is a highly complex process involving coordinated interactions between various cellular and biochemical components. Although it has been known that wound healing involves a highly orchestrated multiple-step and events that correlate with the appearance of various cell types in the wound bed during distinct phases of the healing process, many of the limitations in the treatment of pathological condition such as impaired wound healing are based on lack of detailed knowledge of the molecular mechanisms of wound healing.

Presently available evidence indicates that a set of genes is highly regulated at the transcriptional, translational and post translational levels after wound formation (Cooper et al., 2004). The study of the changes in gene expression at the transcriptional level may yield insights into understanding the molecular mechanisms that regulate wound healing and may provide new and more efficient treatment for chronic wounds. Lisa Cooper et al. (2004) revealed that nearly 100

Abbreviations: ESs, excretions/secretions; MDT, maggot debridement therapy; ECM, extracellular matrix.

[*] Corresponding author at: Cerrahpaşa Tıp Fakültesi, Temel Tıp Bilimleri Binası, Tıbbi Biyoloji ABD, Cerrahpasa, 34098 Fatih, Istanbul, Turkey.

E-mail addresses: onaran.il@gmail.com, ilonaran@istanbul.edu.tr (İ. Onaran).

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Table 1
Alphabetical sequence of genes investigated and the fold change in mRNA expression in response to the ES-treated wounds at 4, 7, and 10 days.

Gene symbol	Gene description	Day 4		Day 7		Day 10	
		Fold change	p-Value[a]	Fold change	p-Value[a]	Fold change	p-Value[a]
Atct1	Actin, alpha, cardiac muscle 1	−6.06	0.00033[#]	1.77	0.00245	−9.03	0.00002[#]
Angpt1	Angiopoietin 1	−1.24	0.00415	1180.35	0.00066	−2.95	0.00003[#]
Ccl12	Chemokine (C–C motif) ligand 12	1.51	0.00055[#]	3.26	0.00044[#]	1.14	0.00026[#]
Ccl7	Chemokine (C–C motif) ligand 7	−2.92	0.00040[#]	4269.94	0.00118	22.09	0.00333
Cd40lg	Cd40 ligand	1136.20	0.00258	4.81	0.00892	1365.30	0.00040[#]
Cdh1	Cadherin 1	−2.77	0.00173	3.82	0.00253	−2.06	0.00071
Col14a1	Collagen, type XIV, alpha 1	−2.17	0.00095	2142.38	0.00044[#]	−4.68	0.00045[#]
Col1a1	Collagen, type I, alpha 1	−1.02	0.85245	4.89	0.00004[#]	−17.03	0.00195
Col1a2	Collagen, type I, alpha 2	4870.99	0.00234	–	–	−1590.21	0.00262
Col3a1	Collagen, type III, alpha 1	−2.86	0.00288	4.81	0.08961	464.65	0.00085
Col4a1	Collagen, type IV, alpha 1	1530.73	0.00223	4.81	0.07453	149.60	0.00019[#]
Col4a3	Collagen, type IV, alpha 3	–	–	4.81	0.04679	189.36	0.00008[#]
Col5a1	Collagen, type V, alpha 1	−2.76	0.00049[#]	8.22	0.00205	−6.99	0.00058[#]
Col5a2	Collagen, type V, alpha 2	−2.83	0.00033[#]	−1.01	0.78927	−3.28	0.00109
Col5a3	Collagen, type V, alpha 3	1.18	0.24427	−4.10	0.00128	−17.03	0.00007[#]
Csf2	Colony stimulating factor 2 (granulocyte-macrophage)	2.52	0.00004[#]	−2.33	0.00458	−6.02	0.00016[#]
Csf3	Colony stimulating factor 3 (granulocyte)	−2.22	0.00106	−64.22	0.00114	4.00	0.00050[#]
Ctgf	Connective tissue growth factor	−3.90	0.00082	1.11	0.07206	−2.51	0.00331
Ctnnb1	Catenin, beta 1	−2.03	0.00072	−1.16	0.00049[#]	−2.84	0.00024[#]
Ctsg	Cathepsin G	−2.38	0.00295	1.50	0.00004[#]	−2.51	0.00002[#]
Ctsk	Cathepsin K	−10,369.08	0.00059[#]	1.15	0.00141	−1.67	0.00346
Ctsl1	Cathepsin L1	−2.69	0.00190	1.44	0.00038[#]	−2.84	0.00003[#]
Cxcl1	Chemokine (C-X-C motif) ligand 1	1269.46	0.00029[#]	4.81	0.01495	−20.82	0.00013[#]
Cxcl11	Chemokine (C-X-C motif) ligand 11	−2.80	0.00008[#]	−1.21	0.00067	−1.35	0.00421
Cxcl3	Chemokine (C-X-C motif) ligand 3	−1.53	0.00225	−1.19	0.02071	−11.31	0.00002[#]
Cxcl5	Chemokine (C-X-C motif) ligand 5	−2.03	0.00215	−1.11	0.08066	−9.06	0.00014[#]
Egf	Epidermal growth factor	−2.00	0.37386	4.39	0.10003	−10,015.87	0.00178
Egfr	Epidermal growth factor receptor	−2.14	0.00024[#]	1.26	0.00560	−6.96	0.00006[#]
F13a1	Coagulation factor XIII, A1 polypeptide	−3.31	0.00033[#]	1.38	0.00052[#]	–	–
F3	Coagulation factor III	−3.01	0.00146	1.21	0.00024[#]	−2.17	0.00006[#]
Fga	Fibrinogen alpha chain	−3.47	0.00001[#]	1.24	0.00048[#]	−3.42	0.00063
Fgf10	Fibroblast growth factor 10	−2.20	0.00001[#]	1.29	0.00075	−5.03	0.00039[#]
Fgf2	Fibroblast growth factor 2	−6.13	0.00031[#]	1.29	0.00221	−2.59	0.00106
Fgf7	Fibroblast growth factor 7	−1.22	0.00879	−2.29	0.00027[#]	1.21	0.03942
Hbegf	Heparin-binding EGF-like growth factor	−1.71	0.00272	–	–	40.79	0.00061[#]
Hgf	Hepatocyte growth factor	−1.60	0.00265	2.46	0.00027[#]	−8.03	0.00014[#]
Ifng	Interferon gamma	−3.31	0.00052[#]	1.53	0.00033[#]	−7.70	0.00001[#]
Igf1	Insulin-like growth factor 1	−101.13	0.00062	−85.63	0.00040[#]	−3.18	0.00001[#]
Il10	Interleukin 10	−1.89	0.00027[#]	1.36	0.00112	−4.79	0.00003[#]
Il1b	Interleukin 1 beta	−2.62	0.00033[#]	1.41	0.00202	−1.99	0.00078
Il2	Interleukin 2	−9.32	0.00073	1.33	0.00001[#]	−2.00	0.00074
Il4	Interleukin 4	−2.05	0.00058[#]	1.53	0.00001[#]	−1.87	0.00001[#]
Il6	Interleukin 6	−2.37	0.00001[#]	1.39	0.00064	−1.35	0.00021[#]
Il6st	Interleukin 6 signal transducer	−2.93	0.00004[#]	−1.18	0.00063	−1.87	0.00033[#]
Itga1	Integrin, alpha 1	−1.97	0.00086	1.18	0.00001[#]	−3.96	0.00011[#]
Itga2	Integrin, alpha 2	−2.62	0.00017[#]	−2.87	0.00143	−2.06	0.00004[#]
Itga3	Integrin, alpha 3	−14.17	0.00005[#]	−4.16	0.00076	−4.16	0.00005[#]
Itga4	Integrin, alpha 4	1.32	0.00121	1.41	0.00405	−4.58	0.00004[#]
Itga5	Integrin, alpha 5	−1.55	0.01077	−1.04	0.32313	−8.75	0.00018[#]
Itga6	Integrin, alpha 6	−2.54	0.00001[#]	1.31	0.00068	−1.40	0.00085
Itgb1	Integrin, beta 1	−1.20	0.05178	1.46	0.00135	−5.41	0.00056[#]
Itgb3	Integrin, beta 3	−1.25	0.05183	1.31	0.07540	−1.89	0.00017[#]
Itgb5	Integrin, beta 5	−2.31	0.00729	3.66	0.09727	−5752.61	0.00021[#]
Itgb6	Integrin, beta 6	−2.15	0.00047[#]	1.24	0.00146	−2.58	0.00010[#]
Mapk1	Mitogen activated protein kinase 1	−2.31	0.00067	1.60	0.00057[#]	−3.28	0.00009[#]
Mapk3	Mitogen activated protein kinase 3	−12.30	0.00079	1.28	0.00067	−4.20	0.00001[#]
Mif	Macrophage migration inhibitory factor	−16.06	0.00001[#]	1.47	0.00825	−2.01	0.00155
Mmp1a	Matrix metallopeptidase 1a	−2.07	0.00002[#]	1.95	0.00071	−4.87	0.00008[#]
Mmp2	Matrix metallopeptidase 2	−1.89	0.11587	−1.04	0.89322	−45.89	0.00084
Mmp7	Matrix metallopeptidase 7	1.17	0.07235	−261.38	0.00013[#]	−4.03	0.00002[#]
Mmp9	Matrix metallopeptidase 9	−6.80	0.00071	11.47	0.00118	−2.01	0.00100
Pdgfa	Platelet-derived growth factor alpha polypeptide	1.10	0.44791	1.45	0.15367	−11.20	0.00012[#]
Plat	Plasminogen activator, tissue	−1.18	0.00233	1.25	0.00726	−2.68	0.00160
Plau	Plasminogen activator, urokinase	−1.28	0.00224	1.06	0.00135	−3.69	0.00001[#]
Plaur	Plasminogen activator, urokinase receptor	−1.53	0.00038[#]	1.17	0.00167	−3.81	0.00001[#]
Plg	Plasminogen	−2.02	0.00062	1.27	0.00126	−1.14	0.00110
Pten	Phosphatase and tensin homolog	−1.95	0.00035[#]	−1.08	0.00096	−2.77	0.00070
Ptgs2	Prostaglandin-endoperoxide synthase 2	−1.42	0.00088	1.34	0.00351	–	–
Rac1	Ras-related C3 botulinum toxin substrate 1	−3.71	0.00292	1.07	0.03989	−3.47	0.00015[#]
Rhoa	Ras homolog gene family, member A	−1.08	0.00918	–	–	−3.92	0.00175
Serpine1	Serpin peptidase inhibitor, clade E, member 1	−2.65	0.00062	−1.15	0.04696	−2.17	0.00012[#]
Stat3	Signal transducer and activator of transcription 3	−2.81	0.00036[#]	1.82	0.00033[#]	−1.68	0.00001[#]
Tagln	Transgelin	−1.75	0.00125	1.22	0.00006[#]	−2.16	0.00028[#]
Tgfa	Transforming growth factor alpha	−1.50	0.00026[#]	1.95	0.00183	−2.06	0.00172