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Original article

Regulation of cell cycle and differentiation markers by pathogenic, non-pathogenic and opportunistic skin bacteria

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ABSTRACT

Skin is the first line of defense against the physical, chemical and the biological environment. It is an ideal organ for studying molecular responses to biological infections through a variety of skin cells that specialize in immune responses. Comparative analysis of skin response to pathogenic, non-pathogenic, and commensal bacteria would help in the identification of disease specific pathways for drug targets. In this study, we investigated human breast reduction skin responses to *Cutibacterium acnes (C. acnes)*, *Staphylococcus aureus (S. aureus)*, *Staphylococcus epidermidis (S. epidermidis)*, and TLR1/2 agonist using Affymetrix microarray chips. The Pam3CSK4 solution and bacterial cultures were prepared and inoculated in steel rings, that were placed on the acetone treated epidermis in a petri dish. After 24 h incubation, 8 mm punch biopsies were taken from the center of the ring, and RNA was extracted. The genome-wide expression was then analyzed using Affymetrix HG-133A gene chip microarray. We found that the *C. acnes* and *S. aureus* boosted the production of extracellular matrix components and attenuated the expression of differentiation markers. The above responses were mediated through the TLR2 pathway. Skin also responded to *S. aureus* and *C. acnes* by inducing the genes of the cell cycle machinery; this response was not TLR2-dependent. *S. aureus* induced, whereas *C. acnes* suppressed the genes associated with apoptosis; this was also not TLR2-dependent. Moreover, *S. epidermis* apparently did not lead to changes in gene expression. We conclude that the breast reduction skin is a very useful model to study the global gene expression in response to bacterial treatments.

1. Introduction

Skin response to fight against foreign antigens is highly dependent on its immune system, which could be innate (promote cutaneous inflammation) or adaptive (promotes memory responses) immune response (Ruff et al., 2020). The commensal microbes reside on skin areas where temperature, moisture, and pH is suitable for their growth and contribution to cutaneous innate immunity (Callewaert et al., 2020). Keratinocytes, the main type of the epidermis acting as a semi-permeable barrier, play a significant role in the host's defense system, providing both a physical and immunological barrier against infection. Keratinocytes express a wide range of innate immune receptors such as toll-like receptors (TLRs), NOD-like receptors (NLRs), and Rig-like receptors (RLRs), which recognize pathogen associated molecular patterns (PAMPs), collectively called pattern recognition receptors (PRRs). In addition to the keratinocytes, other cutaneous and subcutaneous cells, such as Langerhans cells, dendritic cells (DCs), mast cells, lymphocytes, plasma cell, natural killers (NKs), and fibroblasts also express PRRs and participate in the innate immune response against pathogenic microbes (Wang and Li, 2020; Chieosilapatham et al., 2021). Furthermore, the production of

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pro-inflammatory cytokines (IL-17, IL-21, IL-22, IL-26) by TH17 cells also play an important role in skin immunity. Antimicrobial peptides (AMPs), an effector of innate immunity present on keratinocytes can inactivate or kill a wide range of microorganisms either by membrane disruption or chemotaxis of leukocytes such as memory T cells and DCs. A recent finding has shown that disruption of the skin barrier and pro-inflammatory cytokines presence showed a role in stimulating keratinocytes, which as a result induce AMPs expression. For example, IL-17 and IL-22 induce AMPs production from keratinocytes, and IL-21 and IL-22 contribute to wound healing by inducing epidermal proliferation (Cua and Tato, 2010). Hence, these defense mechanisms are expressed on the healthy upper keratinocytes layers, which is important for modulating the survival of microbial pathogen at the surface of the skin.

A dramatic increase of antibiotic resistance strains has become a major issue for the pharmaceutical industry and a universal health challenge (Iwu et al., 2020), specifically methicillin-resistant *Staphylococcus aureus* (Lee et al., 2018). Identification of molecular/signaling pathways regulated by various bacterial strains will provide understanding of the pathogen's behaviors.

Historically, many studies have been performed *in vitro* to investigate molecular responses of keratinocytes to bacterial infections (Krishna and Miller, 2012; Mak et al., 2012). However, apart from their non-human character, animal skin models have been proven ineffective for reproducible molecular responses of bacterial infection for an extended period of time (Popov et al., 2014). To our best knowledge, we are the first group to analyze the human skin responses to commensals mimicking the real environment. For this, we have used Affymetrix microarray chips to investigate the human breast reduction skin responses to different bacterial strains including opportunistic pathogen '*Cutibacterium acnes* (*C. acnes*)', pathogen '*Staphylococcus aureus* (*S. aureus*)' commensal '*Staphylococcus epidermidis* (*S. epidermidis*)', and Toll-like receptors1/2 (TLR1/2) agonist (Pam3CSK4).

2. Materials and methods

2.1. Preparation of bacterial cultures

Three bacterial cultures (*C. acnes, S. aureus, and S. epidermidis*) were incubated for 2 h before the experiment at 37 °C for growth recovery.

2.2. Provenance and preparation of human skin

Fresh human skin was provided within a few hours after breast reduction surgery was performed by the Translational Research Core of the NYU Langone Medical Center. The subcutis, adipose, and as much as possible of the dermis was removed using surgical scissors and a scalpel. The skin was then placed in a large petri dish with the epidermis side up on ~ 3 mm thick wad of autoclaved paper towels thoroughly soaked in DMEM medium (Fig. 1A). An adequate amount of DMEM was added to keep the samples fed from below, through the paper towel cushion, for the length of the experiment, supplementing as necessary (Vangipuram et al., 2013).

To introduce the reagents atop the epidermis, we used steel cloning rings 1 cm diameter, 0.7 cm deep, generously glopped with sterile vaseline on the bottom rim to prevent leakage. To unseal the epidermal lipid barrier and allow agents access to keratinocytes, 1 mL of acetone was poured into each steel ring and was removed after 1 min. This process was repeated three times with 1 min interval between each treatment. The remaining acetone was allowed to evaporate until the epidermis seemed dry. Next, the skin was treated with different gram-positive bacteria including *C. acnes, S. aureus,* and *S. epidermidis*, as well as with Pam3CSK4 (an agonist of TLR1/2, 300 ng/mL). As a control, sterile DMEM medium was poured into one of the rings.

The skin was incubated with bacteria for 24 h and at 37 °C in 5% CO_2 incubator. The next day, samples from the rings were streaked onto agar plates to confirm the gross colony phenotype of the applied bacteria, as well as the sterility of the control and the Pam3CSK4 rings (Fig. 1B). From the middle of each ring, a 6 mm punch biopsy was taken. The skin biopsies were stored in RNA later at −20 °C to stabilize the RNA until RNA extraction.

2.3. RNA extraction

Qiagen RNeasy Mini Kit was used to extract RNA from skin biopsies stored in RNA later. All steps were performed at 4 °C and for centrifugation Eppendorf Centrifuge 5415 was used. For RNA extraction from skin biopsies, reagents provided with the kit were prepared as follows. Firstly, β-Mercaptoethanol (20 μL) was dispensed in RLT buffer (1 mL) and stored at 4 °C. The working solution of RPE buffer was prepared by adding 4 mL of ethanol (95%) in 1 mL RPE buffer, mixed gently, and stored at 4 °C. The RNase-free DNase provided by Qiagen, was used for on-column

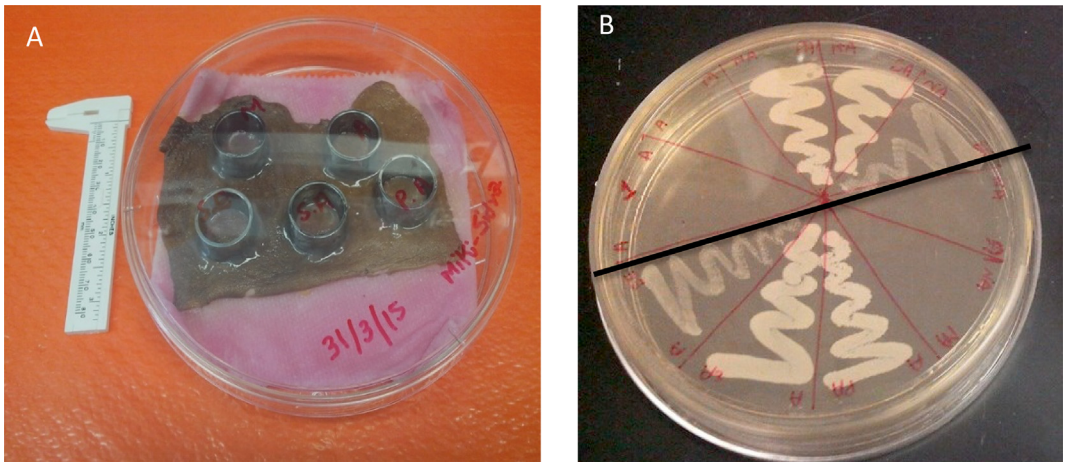

Fig. 1. Human breast reduction skin challenged with different bacterial strains and TLR1/2 agonist. **A:** Human skin was treated with DMEM media, TLR1/2 agonist (Pam3CSK4) and concentrated cultures of *C. acnes, S. aureus and S. epidermidis* and incubated for 24 h. **B:** Liquid from cloning rings was streaked on LB agar plate for contamination check.

DNA digestion. DNase stock solution was prepared by injecting 550 μL RNase-free water into the DNase vial using a sterile RNase-free needle and syringe. The stock solution was mixed gently by inversion and 50 μL aliquots were prepared to store at −20 °C for future use. Before use, DNase aliquot was defrosted at room temperature and 350 μL RDD buffer (provided in kit) was added to prepare 400 μL DNase working solution for on-column DNA digestion.

Skin biopsies were homogenized using lysing kits containing ceramic lysis beads (zirconium oxide) of 2.8 mm and 5.0 mm in 2 mL reinforced tubes (CKMix50-R, Bertin Corp). The MINILYS homogenizer (Bertin Technologies) was used to grind and disrupt skin biopsies (6 mm) using high energy 3D acceleration of lysis beads in lysing kits containing 700 μL cell lysis RLT buffer. QIAshredder spin columns (Qiagen) were used for rapid homogenization of skin tissue lysates. In single-use spin columns, 700 μL tissue lysate was dispensed and centrifuged at 10500 rpm for 3 min. The column was then removed and the collection tube containing flow-through was capped and used for the next step. The 70% ethanol was added to an equal volume of tissue lysate (700 μL) and mixed properly by pipetting. The tissue lysate (700 μL) was immediately transferred to an RNeasy Mini spin column placed in a 2 mL collection tube and centrifuged at 11000 rpm for 15sec in a microcentrifuge. The column-bound DNA was digested by the on-column digestion technique. First, RNeasy column bound RNA was washed with 350 μL RW1 buffer by centrifugation at 10500 rpm for 15sec. The flow-through was discarded and 80 μL DNase solution was directly transferred to RNeasy column membrane and incubated at room temperature (25 °C) for 15 min to ensure DNA digestion. After incubation, 350 μL RW1 buffer was dispensed in the column, centrifuged at 10500 rpm for 15sec, and the flow-through was discarded to wash bound RNA, RPE buffer (500 μL) was added to RNeasy spin columns, centrifuged at 10500 rpm for 15sec and the flow-through was discarded. This

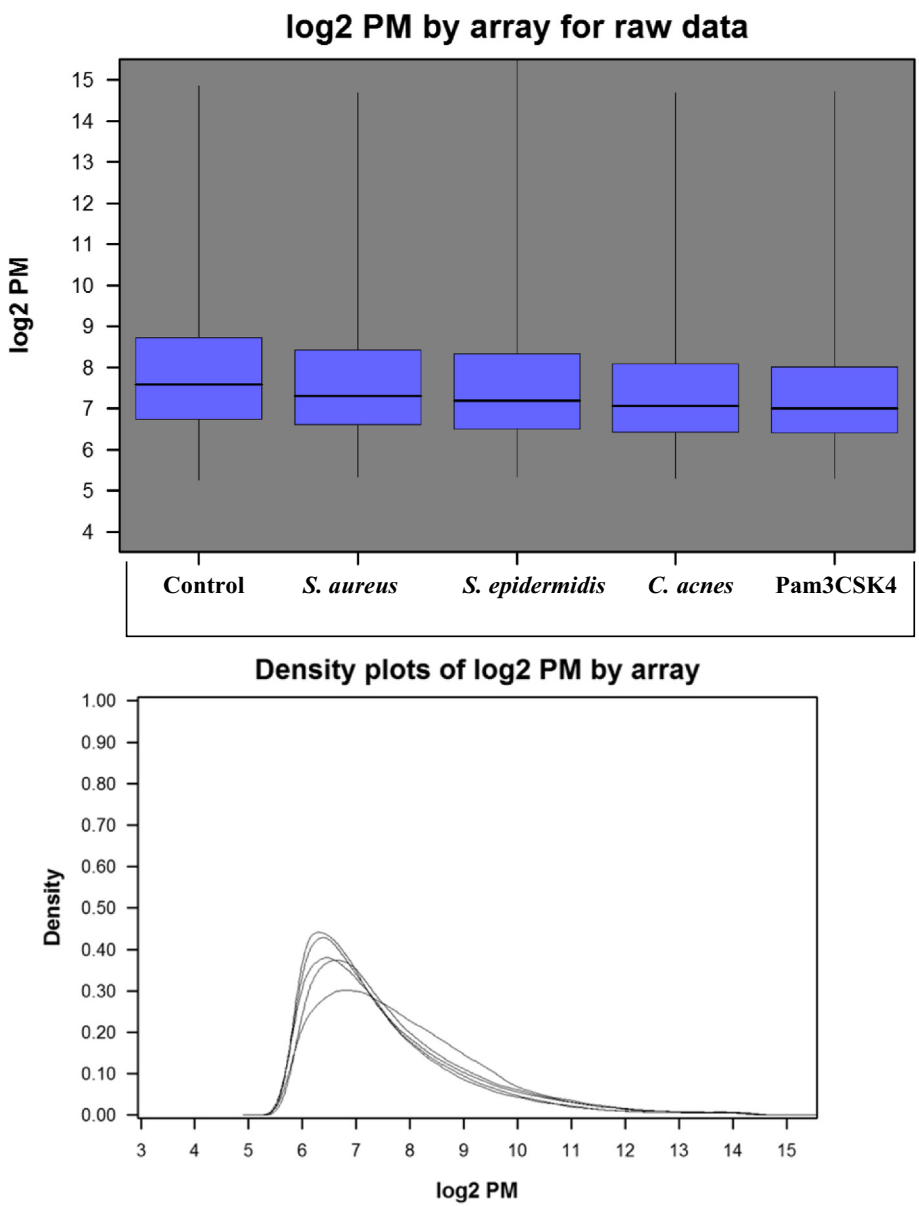

Fig. 2. Box plot and density plot of skin biopsies microarray data using RMAExpress.

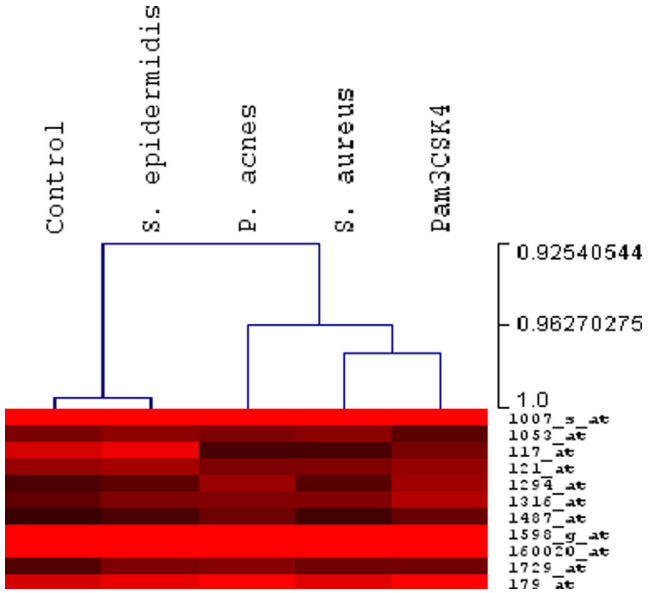

Fig. 3. Cluster analysis of bacterial strains and Pam3CSK4 challenged skin biopsies microarray data using multiple expression viewer software.

step was repeated with 2 min centrifugation. The RNeasy spin column was transferred to a new collection tube and centrifuged at 12000 rpm for 1 min to dry the column membrane. Then RNeasy spin column was transferred to a new 1.5 mL collection tube. To elute RNA, 40 μL RNase-free water was directly added to the spin columns and centrifuged at 10500 rpm for 1 min. The collection tube containing RNA solution was capped and stored at 20 °C for microarrays. Initially, RNA isolation was confirmed by running 7 μL RNA solution on 1.5% agarose gel and viewed on Biorad Gel Doc EZ imager. The RNA samples were submitted for processing by Genome Technology Center of the NYU Langone Medical Center microarray core facility. The concentration and quality of RNA were then checked with the NanoDrop method before hybridization to microarrays.

2.4. Microarray analysis

Microarray analysis was performed using AffymetrixGPL571 HG-U133A_2 microarray chips. The raw data was processed using RMAExpress to verify the quality of microarray data and the log2-transformed values were saved in excel sheets. The hierarchical clustering was obtained using Multiple expression Viewer (MeV) software [http://mev.tm4.org/]. For the gene set enrichment (GSE) analyses, we used the algorithms from the Broad Institute (Subramanian et al., 2005). With this approach, we compared our microarray results with the various gene sets available online, including gene ontology categories, pathway data, and previously characterized transcriptional analyses, as suggested by the Broad Institute staff.

For the GSE analyses, we used the log2 transformed transcriptional microarray data that was arranged in excel sheets. From the 22,278 genes, we first removed the unexpressed genes and those with unreliably low measured values by deleting with maximal expression in any sample not reaching the cut-off value 6, leaving a total of 12,409 genes retained for further analysis. For each comparison, genes with a 2-fold or better difference of expression were considered differentially expressed and selected for further analysis using DAVID software [http://david.abcc.ncifcrf.gov/]. The Venn diagrams were obtained using online resources [http://bioinfogp.cnb.csic.es/tools/venny/index.html].

3. Results

Microarray analysis was performed using Affymetrix microarray chip (GPL571 [HG-U133A_2]). The raw data received in CEL

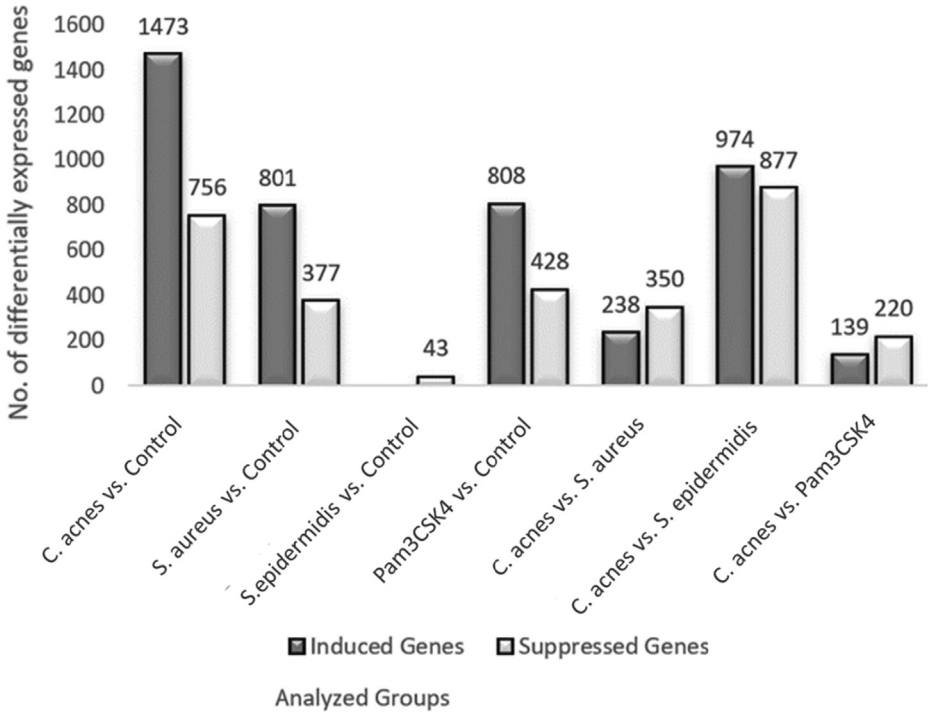

Fig. 4. Induced and suppressed genes in human skin challenged with different bacteria and Pam3CSK4.

files was processed using RMAExpress and log2 transformed data was saved in excel sheets. The box and density plots were also acquired to analyze the quality of microarray data (Fig. 2). The box plot presented that microarray data was symmetrically distributed in all chips. Similarly, the density plot indicated the uniform distribution of signals across the microarray chips. This means that the analyzed RNAs and hybridizations were of high quality. Hierarchical cluster analysis examined the relationship between the isolates by grouping bacterial isolates with similar gene expression profiles (Eisen et al., 1998). Here, the hierarchical cluster was obtained using MeV software. As shown in Fig. 3, samples from control and *S. epidermidis*-treated skin was located on a single branch of the dendrogram, whereas the samples of *C. acnes*, *S. aureus,* and Pam3CSK4 were located on the other branch. Furthermore, the differential expression of genes was more similar between *S. aureus*- and Pam3CSK-treated skin biopsies than with the *C. acnes*-treated ones. The log2 transformed transcriptional microarray data for 22,278 genes was arranged and labeled in excel sheets. A total of 12,409 expressed genes with a minimum cut-off value of 6 were selected for analysis. The microarray data was compared in the following groups; 1) *C. acnes vs.* Control; 2) *S. aureus vs.* Control; 3) *S. epidermidis vs.* Control; 4) Pam3CSK4 *vs.* Control; 5) *C. acnes vs. S. aureus* 6) *C. acnes vs. S. epidermidis*; 7) *C. acnes vs.* Pam3CSK4. The number of induced and suppressed genes in each group is presented in Fig. 4. For each comparison gene with 2-fold change were selected for analysis using DAVID software. The top ten ontological categories obtained for each comparison are presented in Tables 1–7.

C. acnes is a gram-positive human skin commensal, however infected pilosebaceous units present increased concentration of *C. acnes* which then modifies skin immunity leading to acne progression (Li et al., 2014). The top ten clusters of induced or suppressed gene ontologies in human breast reduction skin biopsies infected with *C. acnes* are listed in Tables 1 and 2(a-d). Genes shown to be strongly upregulated by microarray were mostly related to the cell cycle including microtubule organization, chromosome arrangement, DNA replication, mitotic cell cycle, and regulation of cell cycle (Table 1c). Besides, extracellular matrix proteins (collagen and laminins), macrophages, and T-cells specific chemokines were found to be upregulated. We also observed the upregulation of genes involved in vasculature development and blood vessel development. The top cluster suppressed by *C. acnes* included ontological categories as "ectoderm development" and "keratinocytes differentiation" (ES 9.81). Interestingly, the genes represented keratinocytes differentiation makers (Table 1d). Also, the genes for apoptosis, apoptosis regulation, phagocytosis, and adaptive immunity were downregulated. Overall, *C. acnes* primarily induced keratinocytes division in the infected human skin while suppressing keratinocytes differentiation.

S. aureus is a major cause of skin, soft tissues invasive, and life-threatening infections. We analyzed the differential expression of skin infected with concentrated *S. aureus* culture. The clusters of induced and suppressed gene ontologies found in skin biopsies challenged with *S. aureus* are given in Tables 2a and 2e(a–e). Among the top ten induced clusters "extracellular region part" and "extracellular region" were the most frequent ontological categories. Most of the genes present in induced clusters were from the extracellular matrix including collagen, laminin, integrin, metallopeptidases, insulin growth factor, tenascin, fibronectin, and thrombospondin. Also, chemoattractant for monocytes, basophils, T-cells and inflammatory cytokines including IL-6, IL-8, selectin E were also upregulated. The genes for collagen metabolism, ecto-

Table 1a,b
Top 10 clusters of induced and suppressed gene ontologies in *C. acnes*-challenged *vs.* control skin biopsy.

Sr.	a) *C. acnes* challenged skin: Induced		Sr.	b) *C. acnes* challenged skin: Suppressed	
	Gene Ontologies	p-Value		Gene Ontologies	p-Value
1	**ES 9.66**		**1**	**ES 9.81**	
	spindle	4.68E-13		ectoderm development	7.75E-15
	microtubule cytoskeleton	8.54E-11		keratinocyte differentiation	3.53E-10
2	**ES 9.63**		**2**	**ES 5.42**	
	extracellular matrix part	1.58E-12		Neg. R. of apoptosis	1.10E-06
	ECM-receptor interaction	8.07E-10		anti-apoptosis	5.33E-06
3	**ES 8.67**		**3**	**ES 4.96**	
	cell cycle	1.03E-10		vesicle	3.49E-06
	mitosis	3.50E-10		cytoplasmic vesicle	1.17E-05
4	**ES 7.58**		**4**	**ES 4.47**	
	chromosome	3.05E-10		cell fraction	1.26E-05
	chromosomal part	4.09E-09		insoluble fraction	1.27E-05
5	**ES 6.80**		**5**	**ES 4.16**	
	proteinaceous ECM	7.41E-11		R. of apoptosis	7.35E-06
	ECM	4.50E-10		R. of PCD	9.73E-06
6	**ES 6.67**		**6**	**ES 4.07**	
	DNA metabolism	1.34E-08		sterol metabolism	1.63E-06
	cellular response to stress	5.32E-07		cholesterol metabolism	2.38E-06
7	**ES 5.36**		**7**	**ES 3.59**	
	vasculature development	6.60E-08		GTP binding	1.68E-04
	blood vessel development	9.43E-08		guanyl nucleotide binding	2.64E-04
8	**ES 5.35**		**8**	**ES 3.39**	
	nuclear lumen	1.33E-07		plasma membrane part	1.40E-05
	organelle lumen	2.87E-07		intrinsic to plasma membrane	1.83E-03
9	**ES 4.93**		**9**	**ES 2.96**	
	cytoskeleton organization	4.12E-07		Res. to molecule of bacterial origin	4.83E-04
	actin filament-based process	5.06E-05		Res. to LPS	7.65E-04
10	**ES 4.88**		**10**	**ES 2.85**	
	R. of cell cycle	2.21E-09		Pos. R. of signal transduction	3.36E-05
	R. of mitotic cell cycle	4.62E-06		R. of I-kB/NF-kB cascade	2.94E-03

ES, Enrichment score; ECM, extracellular matrix; R. Regulation; Pos. R., Positive regulation; Neg. R., Negative regulation; Res., Response; PCD, Programmed cell death; LPS, Lipopolysaccharide

derm development, and glycosaminoglycan binding were also found in these clusters. Principally, *S. aureus* induced cell division, LPS processing, and chemotaxis. In Tables 2b clusters of gene ontologies suppressed by *S. aureus* in breast reduction skin include "epidermis development" and "keratinocytes differentiation". The genes present in this cluster were similar to the keratinocytes differentiation genes suppressed by *C. acnes*. Furthermore, gene ontologies for processes in plasma membrane, vesicle-mediated transport, and cholesterol metabolism were downregulated. Moreover, genes for positive regulation of the cell cycle, anti-apoptosis, chemical homeostasis, signal transduction were also downregulated. In summary, *S. aureus* induced cell cycle and innate immunity genes which facilitate bacterial infection while suppressed differentiation and bacterial metabolism genes and processes to increase *S. aureus* survival and evade skin immunity. Importantly, the results of the experiment of human skin challenged with different gram-positive bacterial strains revealed that *C. acnes* and *S. aureus* significantly induced cell cycle genes while suppressing keratinocytes differentiation. Besides, *C. acnes,* and *S. aureus* significantly suppressed Golgi and endoplasmic reticulum (ER) specific bacterial components processing genes (Tables 1 and 2).

The gene regulation with *S. epidermidis*, a skin commensal, was very similar to the untreated one as it apparently did not induce any genes, even though it suppressed few membrane receptor genes as represented by the low ES values (Table 3). Interestingly, differentially expressed genes in Pam3CSK4-challenged cells were similar to those in *C. acnes*- and *S. aureus*-challenged cells, except that cell cycle genes were not induced and adaptive immunity genes were stimulated (Table 4). This finding suggests that *C. acnes* and *S. aureus* induced skin cells proliferation genes through the receptors other than or in addition to TLR1/2.

The comparison of differential expression between *C. acnes*- and *S. aureus*-challenged cells showed that, in contrast to the *C. acnes, S. aureus* significantly induced innate immunity system together with cell division genes and suppressed bacterial components processing genes more strongly than *C. acnes* (Table 5). This finding may explain the pathogenic behavior of *S. aureus*. The *C. acnes vs. S. epidermis* comparison was not significantly different from *C. acnes vs.* control comparison (Tables 1 and 6). Finally, a comparison of differential expression in *C. acnes- vs.* Pam3CSK4-challenged cells indicated that cell cycle and apoptosis genes were prominently induced by *C. acnes* whereas Pam3CSK4 induced innate immunity and wounding response genes similar to the changes in *S. aureus*-challenged cells (Table 7).

4. Discussion

Skin has a major role in host defense, providing both a physical and immunological barrier against infection. The factors that initiate keratinocyte signaling in the presence of a substantial skin microbiome consisting of both commensal and pathogenic flora are not completely understood. In this study, we have explored human breast reduction skin response to pathogenic (*C. acnes*

Table 1c,d
Full list of genes found in gene ontologies extracelluar matrix part" and "ectoderm development.

c) Extracellular matrix genes			d) Ectoderm development		
	Gene Symbol	Gene Name		Gene Symbol	Gene Name
	EFEMP2	EGF-ECM protein 2		ALOX12B	arachidonate 12-lipoxygenase
	TIMP3	TIMP inhibitor 3		C1orf68	chromosome 1 ORF68
	AGRN	agrin		CALML5	calmodulin-like 5
C	COL1A1	collagen, type I, alpha 1	**D**	CDSN	corneodesmosin
C	COL1A2	collagen, type I, alpha 2		CST6	cystatin E/M
C	COL3A1	collagen, type III, alpha 1		elf3	E74-like factor 3 (epithelial-specific)
C	Col4a1	collagen, type IV, alpha 1		emp1	epithelial membrane protein 1
C	col4a2	collagen, type IV, alpha 2	**D**	ereg	epiregulin
C	COL4A5	collagen, type IV, alpha 5		Fabp5F	fatty acid binding protein 5-like2
C	Col5a1	collagen, type V, alpha 1	**D**	Flg	filaggrin
C	Col5a2	collagen, type V, alpha 2	**D**	IVL	involucrin
C	COL6A1	collagen, type VI, alpha 1		JAG1	jagged 1 (Alagille syndrome)
C	Col6a3	collagen, type VI, alpha 3		KLK5	kallikrein-related peptidase 5
C	COL7A1	collagen, type VII, alpha 1		KLK7	kallikrein-related peptidase 7
C	Col15a1	collagen, type XV, alpha 1		Krt16	keratin 16
C	COL18A1	collagen, type XVIII, alpha 1		KRT17	keratin 17
	DST	dystonin	**D**	KRT2	keratin 2
	Fbn1	fibrillin 1		KRT6A	keratin 6A
	fn1	fibronectin 1		KRT6B	keratin 6B
	Hspg2	heparan sulfate proteoglycan 2	**D**	LCE2B	late cornified envelope 2B
L	LAMA4	laminin, alpha 4	**D**	LOR	loricrin
L	LAMB1	laminin, beta 1		OVOL1	ovo-like 1
L	lamb2	laminin, beta 2 (laminin S)	**D**	ppl	periplakin
L	lamb4	laminin, beta 4		Psen1	presenilin 1
L	LAMC1	laminin, gamma 1	**D**	S100A7	S100 calcium binding protein A7
	LUM	lumican		SCEL	sciellin
	MFAP5	microfibrillar associated protein 5		SFN	stratifin
	MMRN2	multimerin 2		SPINK5	serine peptidase inhibitor
	nid1	nidogen 1	**D**	Sprr1a	small proline-rich protein 1A
	SPARC	cysteine-rich secreted protein	**D**	Sprr1b	small proline-rich protein1B
	TNC	tenascin C	**D**	SPRR2B	small proline-rich protein 2B
	TNXA,B	tenascin XA & B		TCHH	trichohyalin
	Tff3	trefoil factor 3 (intestinal)	**D**	Tgm1	transglutaminase1 (epidermal typeI)
			D	TGM3	transglutaminase3
				Tgm5	transglutaminase 5
				tp63	tumor protein p63
				UGCG	UDP-glucose glucosyltransferase

and *S. aureus*) and nonpathogenic bacteria (*S. epidermidis*) as well as TLR1/2 agonist Pam3CSK4, to better understand the mechanism of skin infection (O'Shaughnessy and Brown, 2015; Wickersham et al., 2017).

C. acnes is a dominant member of the skin microbiota, which leads to pathogenesis once colonized in follicles. *S. aureus* is commonly found on the skin and in the upper respiratory tract, but it can become an opportunistic pathogen causing infection. While exploring the skin responses to these bacteria, we found that *C. acnes* and *S. aureus* adopt two supporting strategies to evade the host immune system. Firstly, it dominantly upregulated the genes and processes that are involved in mitotic cell division. The upregulated cell cycle results in increased production of nutrients, which could be used in bacterial own growth (Bohnsack and Hirschi, 2004).

TLR's are an important class of the innate immunity system which recognize structurally conserved molecules derived from microbes. TLR1-6 and −9 have been identified in keratinocyte, while TLRs 2–5, −7, −9 and −10 are expressed in melanocytes (Burns and Yusuf, 2014). The role of TLR2 in cell proliferation has been well established. *C. acnes* and *S. aureus* interaction with the host is mainly mediated by TLR2 receptor recognition. *C. acnes* envelop proteins including GroEL, lipoglycans, Dnak and peptidoglycans act as a ligand for TLR2 (Su et al., 2017; Nagy et al., 2005; Kim et al., 2002). TLR2 makes heterodimers with TLR1 or TLR6 receptors activating downstream signaling pathway. Predominantly, recognition of the live/heat killed bacteria is mediated by the TLR2/6 heterodimers. The recognition of PAMPS or DAMPs by TLR2 on human keratinocytes activate Myeloid differentiation primary-response 88 (MyD88) dependent signaling pathways and cellular responses that lead to the release of cytokines and chemokines subsequently increasing chances of skin cells survival and proliferation (Burns and Yusuf, 2014).

Secondly, we found that *C. acnes* and *S. aureus* suppressed cell differentiation as a secondary process to avoid host immunity (Tables 1 and 2). Similarly, Choi et al. (2018) showed that *C. acnes* derived vesicles increased keratinocytes proliferation and dysregulated epidermal differentiation. Whereas Akaza et al. (2009) investigating the expression of keratinocyte differentiation-specific markers, keratins, and pro-inflammatory cytokines in normal human epidermal keratinocytes (NHEK) exposed to *C. acnes in vitro*. They found that *C. acnes* significantly affects the expression of inflammatory and differentiation markers in keratinocytes (Akaza et al., 2009). Likewise, *S. aureus* toxins based on inhibition of the epidermal cells differentiation have been investigated by multiple research groups. Such as Munro et al. (2010) showed that *S. aureus* toxins assist in infection by inhibiting epidermal cell differentiation (Munro et al., 2010). Epidermal cell differentiation inhibitors known as EDIN and EDIN-like factors, a group of toxins targeting RhoA master regulator of the actin cytoskeleton, may confer virulence properties on *S. aureus* (Messad et al., 2013). Thus, inhibition of cell differentiation is another important strategy adopted by the bacteria for infection.

In contrast to our findings, Duckney et al. (2013) found that none of the tested species of *S. epidermidis* and *C. acnes* were able to alter the expression of keratinocyte differentiation or expression markers and inflammatory response even when tested at high concentrations on reconstructed human epidermis topically, while topical *S. aureus* induced a weak reaction. When these bacteria were added to the medium, all of the tested species

Table 2a,b
Top 10 clusters of induced and suppressed gene ontologies in *S. aureus*-challenged *vs.* control skin biopsy.

Sr.	a) *S. aureus* challenged skin: Induced		Sr.	b) *S. aureus* challenged skin: Suppressed	
	Gene Ontologies	p-Value		Gene Ontologies	p-Value
1	**ES 11.86**		**1**	**ES 8.60**	
	extracellular region part	1.26E-17		epidermis development	6.62E-14
	extracellular region	1.96E-10		keratinocyte differentiation	2.97E-09
2	**ES 7.70**		**2**	**ES 4.21**	
	cell cycle process	2.16E-09		cell fraction	9.80E-08
	cell division	2.27E-09		insoluble fraction	2.54E-05
3	**ES 6.62**		**3**	**ES 3.71**	
	polysaccharide binding	3.07E-08		plasma membrane part	1.52E-05
	pattern binding	3.07E-08		intrinsic to plasma membrane	2.98E-04
4	**ES 6.58**		**4**	**ES 2.08**	
	blood vessel development	8.57E-10		cholesterol metabolic process	1.71E-03
	vasculature development	1.61E-09		sterol metabolic process	2.91E-03
5	**ES 6.45**		**5**	**ES 2.01**	
	proteinaceous ECM	3.35E-13		cell–cell junction	5.27E-04
	ECM-receptor interaction	3.91E-08		Tight junction	1.65E-03
6	**ES 6.08**		**6**	**ES 1.88**	
	skeletal system development	5.51E-09		Res. to endogenous stimulus	4.12E-03
	bone development	7.76E-06		Res. to organic substance	4.68E-03
7	**ES 5.73**		**7**	**ES 1.85**	
	ECM organization	2.53E-08		cytoplasmic vesicle	2.04E-03
	collagen fibril organization	1.14E-04		vesicle	3.43E-03
8	**ES 4.78**		**8**	**ES 1.77**	
	cell migration	5.85E-06		extracellular space	9.03E-03
	cell motion	7.01E-06		extracellular region part	1.75E-02
9	**ES 4.38**		**9**	**ES 1.72**	
	membrane-enclosed lumen	6.22E-07		R. of cell migration	1.34E-03
	nuclear lumen	2.33E-05		R. of locomotion	3.42E-03
10	**ES 3.88**		**10**	**ES 1.59**	
	Res. to organic substance	1.31E-06		IL-1 receptor antagonist activity	4.94E-03
	Res. to endogenous stimulus	4.16E-04		FGFR antagonist activity	4.94E-03

ECM, extracellular matrix; R. Regulation; Res., Response; PCD, Programmed cell death; FGFR, Fibroblast growth factor receptor Extracellular matrix genes induced and suppressed in *S. aureus* challenged *vs.* control skin biopsy

Table 2c
Full list of genes found in gene ontologies extracelluar matrix part and "ectoderm development"

c) Extracellular matrix genes			
Induced		Induced	
Gene Symbol	Gene Name	Gene Symbol	Gene Name
HTRA1	HtrA serine peptidase 1	DKK3	dickkopf homolog 3
SPARCL1	SPARC-like 1	Fbn1	fibrillin 1
TIMP1	TIMP metallopeptidase inhibitor 1	FGL2	fibrinogen-like 2
TIMP3	TIMP metallopeptidase inhibitor 3	fn1	fibronectin 1
ada	adenosine deaminase	Flrt3	fibronectin transmembrane 3
apod	apolipoprotein D	FBLN1	fibulin 1
BGN	biglycan	FBLN2	fibulin 2
bchE	butyrylcholinesterase	FBLN5	fibulin 5
Ctsk	cathepsin K	FSTL1	follistatin-like 1
CCL19	chemokine ligand 19	gpX3	glutathione peroxidase 3
Ccl2	chemokine ligand 2	IGF2INSINS	insulin-like growth factor2
ccl21	chemokine ligand 21	igfbp4	insulin-like growth factor4
CXCL1	chemokine ligand 1	IGFBP5	insulin-like growth factor5
CXCL10	chemokine ligand 10	IGFBP6	insulin-like growth factor6
CXCL12	chemokineligand 12	igfbp7	insulin-like growth factor7
CXCL2	chemokine ligand 2	ICAM1	intercellular adhesion molecule 1
Cxcl3	chemokine ligand 3	IL6	interleukin 6
clu	clusterin	IL8	interleukin 8
COL1A1	collagen, type I, alpha 1	lamb2	laminin, beta 2
COL1A2	collagen, type I, alpha 2	lamb4	laminin, beta 4
COL3A1	collagen, type III, alpha 1	LAMC1	laminin, gamma1
Col4a1	collagen, type IV, alpha 1	LGALS1	lectin
col4a2	collagen, type IV, alpha 2	LEPR	leptin receptor
COL4A5	collagen, type IV, alpha 5	LIF	leukemia inhibitory factor
Col5a2	collagen, type V, alpha 2	LUM	lumican
COL6A1	collagen, type VI, alpha 1	lox	lysyl oxidase
COL6A2	collagen, type VI, alpha 2	MGP	matrix Gla protein
Col6a3	collagen, type VI, alpha 3	Mmp1	matrix metallopeptidase1
Col15a1	collagen, type XV, alpha 1	Mmp2	matrix metallopeptidase2
CSF3	colony stimulating factor3	Mmp28	matrix metallopeptidase 28
cfd	complement factor D	MFAP5	microfibrillar associated protein 5
CFH	complement factor H	mfap4	microfibrillar-associated protein 4
CTGF	connective tissue growth factor	nid1	nidogen 1
DCN	decorin	postn	periostin, osteoblast specific factor
Dpt	dermatopontin	PLAT	plasminogen activator, tissue
Induced		**Suppressed**	
Gene Symbol	**Gene Name**	**Gene Symbol**	**Gene Name**
PECAM1	platelet/endothelial cell adhesion	fxyd6	ion transport regulator 6
PTN	pleiotrophin	ADM	adrenomedullin
PCYOX1	prenylcysteine oxidase 1	Apcs	amyloid P component
PCSK5	proprotein convertase	BTC	betacellulin
SPARC	secreted protein cysteine-rich	CCL22	chemokine ligand 22
SELE	selectin E	CHI3L1	chitinase 3-like1
SEMA3C	semaphorin 3C	CHI3L2	chitinase 3-like2
srgn	serglycin	F3	coagulation factorIII
SERPINE2	serpin peptidase inhibitor E	csf1	colony stimulating factor1
SERPING1	serpin peptidase inhibitorG	ereg	epiregulin
Spon2	spondin 2, ECM protein	hmox1	heme oxygenase1
stc1	stanniocalcin 1	IDE	insulin-degrading enzyme
TNC	tenascin C	IL1F5	interleukin 1 family
TNXATNXB	tenascin XB&A	IL1F7	interleukin 1 family
Thbs1	thrombospondin 1	Il1f9	interleukin 1 family
TFPI	tissue factor pathway inhibitor	KLK5	kallikrein-related peptidase 5
Tgfbr3	TGF beta receptor III	PRSS8	protease, serine, 8
TNFSF10	TNF lignand superfamily10	SLURP1	secreted protein
VCAN	versican	sorD	sorbitol dehydrogenase
		TNXATNXB	tenascin XB & A
		TGFA	transforming growth factorα
		Vash1	vasohibin 1

Full list of genes found in gene ontologies "response to organic substance" and "ectoderm development" from comparison of *S. aureus* challenged *vs.* control skin biopsy

Table 2e,d
Full list of genes found in gene ontologies extracelluar matrix part" and "ectoderm development.

d) Response to Organic substance			
Induced			Induced
Gene Symbol	Gene Name	Gene Symbol	Gene Name
ADAM10	metallopeptidase domain 10	ID2	inhibitor of DNA binding 2
BAIAP2	BAI1-associated protein 2	Id3	inhibitor of DNA binding 3
bchE	butyrylcholinesterase	IDH1	isocitrate dehydrogenase1
BCL2	B-cell CLL/lymphoma 2	IGF2	insulin-like growth factor 2
BTG2	BTG family, member 2	igfbp7	insulin-like growth factor 7
C1s	complement component 1	IL6	interleukin 6
CASP1	apoptosis-related cysteine peptidase	irak3	IL-1 receptor-associated kinase 3
Casp3	apoptosis-related cysteine peptidase	KLF10	Kruppel-like factor 10
CASP8	apoptosis-related cysteine peptidase	LEPR	leptin receptor
Ccl2	chemokine ligand 2	LONP2	lon peptidase 2, peroxisomal
CCNA2	cyclin A2	lox	lysyl oxidase
CFB	complement factor B	MGP	matrix Gla protein
COL1A1	collagen, type I, alpha 1	NR4A2	nuclear receptor subfamily 4A2
COL3A1	collagen, type III, alpha 1	pdgfra	PDGF alpha polypeptide
COL6A2	collagen, type VI, alpha 2	Pik3r1	PI3K, regulatory subunit 1 (alpha)
Colec12	collectin sub-family member 12	ptch1	patched homolog 1
CYP1A1	cytochrome P4501A1	PTGS2	prostaglandin-endoperoxide synthase2
CYP1B1	cytochrome P4501B1	rhoqRHOQP2	ras homolog gene familyQ
cyr61	cysteine-rich angiogenic inducer	SELE	selectin E
DDIT3	DNA-damage-inducible transcript 3	SERPINH1	serpin peptidase inhibitorH
Dnajb4	DnaJ (Hsp40) homolog	SMAD1	SMAD family member 1
Egr1	early growth response 1	socs2	suppressor of cytokine signaling 2
Egr2	early growth response 2	TAF9	TAF9 RNA polymerase II
EIF2AK2	translation initiation factor	Tgfbr3	transforming growth factorBR3
eif2ak3	translation initiation factor	Thbs1	thrombospondin 1
eno2	enolase 2	TIMP3	TIMP metallopeptidase inhibitor 3
Fas	TNF receptor superfamily	TXNIP	thioredoxin interacting protein
GNG11	G protein gamma 11		
GRB10	growth factor receptor-bound protein 10		
id1	inhibitor of DNA binding 1		

e) Ectoderm development				
Suppressed			**Gene Symbol**	**Gene Name**
Gene Symbol	**Gene Name**		ALOX12B	arachidonate 12-lipoxygenase
ABCG1	ATP-binding cassette		C1orf68	C1 ORF 68
ADCY7	adenylate cyclase 7		CALML5	calmodulin-like 5
ADM	adrenomedullin	D	CDSN	corneodesmosin
BCL2L1	BCL2-like 1		CST6	cystatin E/M
CCNE1	cyclin E1		elf3	E74-likefactor 3 (epithelial-specific)
Cd24CD24L4	CD24 molecule	D	ereg	epiregulin
CGA	glycoprotein hormones		Fabp5	fatty acid binding protein 5-like2
DUSP1	dual specificity phosphatase 1	D	Flg	filaggrin
HMGCS1	HMG-Coenzyme A synthase 1	D	IVL	involucrin
hmox1	heme oxygenase1		KLK5	kallikrein-related peptidase 5
IRS1	insulin receptor substrate 1		KLK7	kallikrein-related peptidase 7
irs2	insulin receptor substrate 2		KRT17	keratin 17
ME1	malic enzyme 1	D	KRT2	keratin 2
PRSS8	Serine protease 8	D	LCE2B	late cornified envelope 2B
SLC18A2	solute carrier family 18	D	LOR	loricrin
Sort1	sortilin 1		OVOL1	ovo-like 1(Drosophila)
		D	ppl	periplakin
		D	S100A7	S100 calcium binding protein A7
			SCEL	sciellin
			SPINK5	serine peptidase inhibitor
		D	SPRR2B	small proline-rich protein 2B
		D	Tgm1	transglutaminase1
		D	TGM3	transglutaminase3
			UGCG	UDP glucosyltransferase

D, Differentiation.

Table 3
Top 10 clusters of suppressed gene ontologies in *S. epidermidis* challenged *vs.* control skin biopsy.

Sr.	*S. epidermidis* challenged skin: Suppressed	
	Gene Ontologies	p-Value
1	**ES 1.67**	
	icosanoid receptor activity	1.59E-04
	prostanoid receptor activity	1.59E-04
2	**ES 1.60**	
	homeostatic process	4.54E-03
	Signaling by GPCR	6.77E-02
3	**ES 1.53**	
	Regulation of locomotion	7.84E-03
	Regulation of cell migration	5.05E-02
4	**ES 1.27**	
	ECM. structural constituent	5.81E-04
	extracellular region	4.43E-02
5	**ES 1.20**	
	membrane fraction	4.33E-02
	insoluble fraction	4.94E-02
6	**ES 0.91**	
	R. of locomotion	7.84E-03
	anti-apoptosis	7.16E-02
7	**ES 0.75**	
	cell projection	2.48E-02
	neuron projection	4.91E-02
8	**ES 0.56**	
	cell death	1.98E-01
	death	2.01E-01
9	**ES 0.33**	
	metal ion binding	4.01E-01
	cation binding	4.14E-01
10	**ES 0.23**	
	phosphorylation	5.18E-01
	phosphorus metabolic process	6.28E-01

GPCR, G-protein coupled receptors; ECM, Extracellular matrix.

induced inflammatory responses and keratinocyte cell death with species-specific potency. *C. acnes* and *S. epidermidis* induced specific alterations in the expression of keratinocyte differentiation and proliferation markers whereas *S. aureus* induced complete keratinocyte cell death suggesting a barrier reparation response. In our study, the skin permeability was increased by three times washings with acetone. In contrast to the findings from Duckney et al. (2013), we found that *S. epidermidis* suppressed only a few of the genes with very low enrichment scores. Moreover, not even a single gene was induced in comparison to the control experiment.

We further explored, whether *C. acnes* and *S. aureus* induced the cell proliferation and suppressed differentiation merely through TLR2 and TLR1/6 dimers or there are some other receptors for complete infection Pam3CSK4. Pam3CSK4 is a TLR1/2 agonist that activates inflammatory cytokines *via* the Myd88 dependent signaling pathway. Interestingly, Pam3CSK4 mediated upregulated genes were very similar to the *C. acnes* and *S. aureus* except for cell cycle process genes. Nevertheless, among downregulated processes, the apoptotic process was the only one not suppressed by the Pam3Csk4. These evidences show that these bacteria adopt additional pathways to elicit these responses.

TLR receptors other than TLR1/2 involvement in bacterial infection have been explored by various research groups. Although TLR5 is found to be activated by flagellin, a ligand not found on *S. aureus* and *C. acnes* surface, its involvement in cell proliferation is recognized. Moreover, its ligands and functions need to be further explored. Hoste et al. (2015) found that the combination of bacteria, chronic inflammation, and wounding cooperate to trigger skin cancer in a mouse model in which constitutive epidermal extracellular-signal-regulated

Table 4
Top ten clusters of induced and suppressed gene ontologies in Pam3CSK4 challenged *vs.* control skin biopsy.

Sr.	a) Pam3CSK4 challenged skin: Induced		Sr.	b) Pam3CSK4 challenged skin: Suppressed	
	Gene Ontologies	p-Value		Gene Ontologies	p-Value
1	**ES 11.45**		**1**	**ES 8.48**	
	extracellular region part	6.52E-18		ectoderm development	1.22E-14
	extracellular region	6.86E-10		keratinocyte differentiation	2.86E-08
2	**ES 7.90**		**2**	**ES 4.08**	
	vasculature development	2.09E-11		sterol metabolism	3.38E-06
	blood vessel development	4.49E-11		Metabolism of lipids and lipoproteins	3.88E-06
3	**ES 7.89**		**3**	**ES 3.94**	
	proteinaceous ECM	3.47E-16		nuclear envelope-ER network	2.49E-05
	collagen	1.40E-11		endoplasmic reticulum	2.59E-05
	PDGF binding	3.68E-08	**4**	**ES 2.98**	
4	**ES 7.31**			membrane-bounded vesicle	1.29E-04
	cell motion	7.62E-09		vesicle	2.72E-04
	cell migration	1.63E-08	**5**	**ES 2.52**	
5	**ES 7.09**			desmosome	3.76E-06
	R. of locomotion	1.09E-08		apical junction complex	7.89E-05
	Pos. R. of locomotion	2.85E-07	**6**	**ES 2.49**	
6	**ES 5.17**			insoluble fraction	1.54E-04
	skeletal system development	5.44E-08		membrane fraction	1.63E-04
	bone development	2.95E-05	**7**	**ES 2.48**	
7	**ES 4.98**			fatty acid metabolic process	1.97E-04
	pattern binding	2.10E-06		icosanoid biosynthetic process	3.36E-02
	glycosaminoglycan binding	7.81E-06	**8**	**ES 2.47**	
8	**ES 4.95**			Res. to organic substance	2.77E-04
	Res. to wounding	4.36E-09		Res. to hormone stimulus	1.10E-02
	defense Res.	1.20E-04	**9**	**ES 2.12**	
	inflammatory Res.	2.76E-03		peptide cross-linking	2.75E-03
9	**ES 3.38**			amino-acyl transferase activity	9.00E-03
	vesicle lumen	9.47E-07	**10**	**ES 1.96**	
	Hemostasis	4.50E-04		lysosome organization	2.37E-03
10	**ES 3.31**			vacuole organization	1.31E-02
	chemotaxis	1.50E-06			
	taxis	1.50E-06			

R, Regulation; Pos. R., Positive Regulation; Res, Response; ECM, Extracellular matrix

Table 5
Top ten clusters of induced and suppressed gene ontologies in *C. acnes vs. S. aureus* challenged skin biopsy.

Sr.	a) *C. acnes vs. S. aureus:* Induced	Sr.	b) *C. acnes vs. S. aureus:* Suppressed		
	Gene Ontologies	p-Value		Gene Ontologies	p-Value
1	**ES 3.63**		**1**	**ES 9.07**	
	cell adhesion	3.54E-05		inflammatory Res.	1.04E-12
	biological adhesion	3.62E-05		Res. to wounding	1.19E-12
2	**ES 3.23**		**2**	**ES 8.35**	
	actin cytoskeleton	4.12E-04		Res. to molecule of bacterial origin	3.81E-10
	cytoskeletal protein binding	4.27E-04		Res. to bacterium	1.96E-09
3	**ES 2.82**		**3**	**ES 6.65**	
	cytoskeleton	1.99E-04		extracellular region part	5.45E-09
	non-membrane-bounded organelle	5.13E-03		extracellular space	1.49E-07
4	**ES 2.35**		**4**	**ES 6.49**	
	contractile fiber part	8.69E-06		Res. to organic substance	5.13E-19
	actin cytoskeleton	4.12E-04		Res. to endogenous stimulus	4.68E-09
5	**ES 2.09**		**5**	**ES 5.62**	
	plasma membrane part	6.49E-06		blood vessel development	4.76E-07
	integral to plasma membrane	8.08E-03		vasculature development	6.80E-07
6	**ES 1.83**		**6**	**ES 4.75**	
	cardiac muscle tissue development	8.32E-04		ectoderm development	1.17E-07
	VCMC differentiation	6.99E-03		epithelial cell differentiation	3.76E-05
7	**ES 1.75**		**7**	**ES 4.06**	
	Neg. R. of cell migration	6.27E-03		Pos. R. of N. compound metabolism	5.64E-08
	R. of cell migration	6.42E-03		Pos. R. of cellular biosynthesis	7.49E-08
8	**ES 1.63**		**8**	**ES 3.86**	
	adherens junction	2.71E-03		R. of apoptosis	2.98E-06
	anchoring junction	4.80E-03		Neg. R. of apoptosis	2.33E-05
9	**ES 1.42**		**9**	**ES 3.77**	
	Vascular smooth muscle contraction	3.10E-03		Pos. R. of cell communication	8.26E-06
	Cytoskeletal R. by Rho GTPase	7.45E-03		Pos. R. of signal transduction	2.57E-05
10	**ES 1.40**		**10**	**ES 3.69**	
	cell migration	2.72E-02		polysaccharide binding	2.86E-05
	cell motion	4.47E-02		pattern binding	2.86E-05
12	**ES 1.24**		**11**	**ES 3.43**	
	death	4.85E-02		Pos. R. of locomotion	1.48E-06
	apoptosis	4.89E-02		Pos. R. of cell migration	4.42E-06
13	**ES 1.02**		**14**	**ES 2.53**	
	extracellular region part	4.03E-02		epidermal cell differentiation	3.10E-04
	extracellular space	7.00E-02		keratinocyte differentiation	1.19E-03

R. Regulation; Pos. R., Positive regulation; Neg. R., Negative regulation; Res., Response; VCMC, ventricular cardiac muscle cell; N, Nitrogen; C., Cellular

kinase-MAP-kinase signaling results in epidermal inflammation and skin wounding induces tumors. These findings were further confirmed by antibiotic treatment inhibits, whereas injection of flagellin induces, tumors in a TLR-5-dependent manner. TLR-5 is also involved in chemical-induced skin carcinogenesis in wild-type mice. TLR5 on human keratinocytes by its ligand, flagellin, resulted in the production of TNFα, IL-8, and the antimicrobial peptides, human β-defensins 2 and 3 (hBD2 and hBD3) (Miller, 2008). TLR5 is present on the epithelium in skin and initiates a signaling cascade that leads to the activation of immunomodulators and inflammatory molecules in MyD88 dependent pathway (McInturff et al., 2005). It seems that more functional roles of TLR5 are waiting to be revealed in addition to recognizing the bacterial flagellin. Many open questions regarding TLR5 beyond its recognition of flagellin remain to be answered (Yang and Yan, 2017). Thus, TLR5 may be involved in inducing the *C. acnes* and *S. aureus* mediated responses.

5. Conclusion

Microarray global expression analysis is a useful tool to investigate the effects of bacterial infection on host genome expression. To the best of our knowledge, we are the first group to show that breast reduction skin is a very useful model to study the global gene expression in response to bacterial treatments. While these gene ontologies are highly important to understand the human molecular responses to pathogenic and non-pathogenic bacteria, we should be aware that these are only the preliminary study on gene expression responses to bacterial infections *in vitro* and need further validation.

Table 6
Top ten clusters of suppressed gene ontologies in *C. acnes vs. S. epidermidis* challenged skin biopsy.

Sr.	a) *C. acnes vs. S. epidermidis:* Induced		Sr.	b) *C. acnes vs. S. epidermidis:* Suppressed	
	Gene Ontologies	p-Value		Gene Ontologies	p-Value
1	**ES 9.36**		**1**	**ES 8.75**	
	cell division	9.32E-11		ectoderm development	6.53E-14
	mitosis	1.66E-10		keratinocyte differentiation	1.81E-08
2	**ES 8.48**		**2**	**ES 6.96**	
	non-membrane-bounded organelle	1.78E-11		cell fraction	3.07E-08
	microtubule cytoskeleton	1.89E-09		insoluble fraction	6.86E-08
3	**ES 7.42**		**3**	**ES 6.38**	
	extracellular region part	9.33E-12		vesicle	6.39E-08
	extracellular matrix	3.87E-10		cytoplasmic vesicle	8.31E-08
4	**ES 6.90**		**4**	**ES 4.71**	
	spindle	2.94E-11		anti-apoptosis	1.32E-05
	microtubule cytoskeleton	1.89E-09		R. of cell death	1.62E-05
5	**ES 5.81**		**5**	**ES 4.56**	
	chromosome	3.91E-08		sterol metabolism	3.71E-07
	chromosomal part	1.04E-07		cholesterol metabolism	2.30E-06
6	**ES 5.44**		**6**	**ES 4.36**	
	cell migration	6.71E-07		guanyl nucleotide binding	8.18E-06
	cell motion	4.97E-06		guanyl ribonucleotide binding	8.18E-06
7	**ES 5.22**		**7**	**ES 4.13**	
	extracellular matrix part	3.83E-12		R. of cell death	1.62E-05
	proteinaceous ECM	1.23E-10		R. of apoptosis	2.06E-05
8	**ES 4.93**		**8**	**ES 3.45**	
	cytoskeleton organization	4.83E-07		lipid biosynthesis	1.57E-05
	actin cytoskeleton organization	5.63E-05		fatty acid biosynthesis	1.64E-04
9	**ES 4.04**		**9**	**ES 2.97**	
	vasculature development	2.18E-06		ribonucleotide binding	3.40E-05
	blood vessel development	3.92E-06		purine ribonucleotide binding	3.40E-05
10	**ES 3.82**		**10**	**ES 2.90**	
	R. of cell motion	3.18E-07		cytoskeleton	5.32E-05
	R. of locomotion	3.30E-06		non-membrane-bounded organelle	6.19E-03

ECM, extracellular matrix; R. Regulation

Table 7
Top ten clusters of suppressed gene ontologies in *C. acnes- vs.* Pam3CSK4-challenged skin biopsy.

Sr.	a) *C. acnes vs.* Pam3CSK4: Induced		Sr.	b) *C. acnes vs.* Pam3CSK4: Suppressed	
	Gene Ontologies	p-Value		Gene Ontologies	p-Value
1	**ES 2.14**		**1**	**ES 4.25**	
	cytoskeletal part	2.29E-04		ectoderm development	1.22E-08
	cytoskeleton	2.34E-03		keratinocyte differentiation	1.22E-04
2	**ES 2.09**		**2**	**ES 2.70**	
	striated muscle tissue development	1.67E-04		Res. to organic substance	2.91E-07
	muscle tissue development	2.18E-04		Res. to endogenous stimulus	9.90E-04
3	**ES 2.00**		**3**	**ES 2.50**	
	contractile fiber part	1.26E-04		Res. to oxygen levels	2.58E-04
	contractile fiber	1.83E-04		Res. to hypoxia	1.02E-03
4	**ES 1.81**		**4**	**ES 2.42**	
	cytoskeleton organization	1.94E-04		R. of cell proliferation	4.68E-05
	actin cytoskeleton	1.06E-02		Neg. R. of apoptosis	3.10E-03
5	**ES 1.68**		**5**	**ES 2.22**	
	R. of neuron differentiation	2.22E-03		Neg. R. of molecular function	5.84E-04
	R. of neurogenesis	5.72E-03		Neg. R. of TF activity	1.88E-03
6	**ES 1.58**		**6**	**ES 2.21**	
	blood circulation	9.13E-03		apoptosis	4.88E-03
	circulatory system process	9.13E-03		death	5.02E-03
7	**ES 1.38**		**7**	**ES 2.20**	
	Neg. R. of cell motion	9.15E-04		Pos. R. of cell migration	8.34E-05
	R. of cell motion	2.13E-03		Pos. R. of locomotion	1.53E-04
8	**ES 1.36**		**8**	**1.93**	
	neuron projection	9.19E-03		Res. to wounding	1.46E-03
	cell soma	2.86E-02		defense Res.	2.94E-02
9	**ES 1.15**			inflammatory Res.	3.82E-02
	cell death	5.85E-02	**9**	**ES 1.88**	
	programmed cell death	5.89E-02		cell fraction	3.70E-03
10	**ES 1.12**			microsome	1.60E-02
	Neg. R. of Res. to stimulus	3.15E-02	**10**	**ES 1.80**	
	Neg. R. of Res. to external stimulus	4.63E-02		kinase binding	4.25E-03
				protein kinase binding	2.58E-02

R. Regulation; Pos. R., Positive regulation; Neg. R., Negative regulation; Res., Response.

CRediT authorship contribution statement

Sidra Younis: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Farah Deeba:** Writing – review & editing, Software. **Rida Fatima Saeed:** Writing – review & editing, Software. **Ramzi A. Mothana:** Writing – review & editing, Software, Funding acquisition. **Riaz Ullah:** Writing – review & editing, Software, Funding acquisition. **Muhammad Faheem:** Writing – review & editing, Software. **Qamar Javed:** Supervision, Funding acquisition. **Miroslav Blumenberg:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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