Ibrahim *Egyptian Journal of Forensic Sciences* (2018) 8:29
https://doi.org/10.1186/s41935-018-0059-5

Egyptian Journal of Forensic Sciences

ORIGINAL ARTICLE Open Access

Human skin identification using specific gene marker at different storage temperatures

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Abstract

Background: Late cornified envelope 1D (LCE1D), skin-specific mRNA, is used in forensic researche on human skin cell identification. Before using (LCE1D) in criminal casework, the impact of storage conditions on its expression should be measured and assessed. To detect the effects of storage temperature and duration, skin swabs were collected from six volunteers and stored at room temperature (25 °C) and warmer temperature (40 °C) for 5 days.

Results: The (LCE1D) expressions, detected via Real Time PCR, were significantly diminished by increased storage duration. While there were also decreased (LCE1D) expressions among warmer temperature samples, this difference was not statistically significant.

Conclusion: Our results suggest that forensic investigators should consider sample collection time prior to result interpretation.

Keywords: Forensic genetics, LCE1D mRNA, Human skin, Storage, RT- PCR

Background

Many methods have been used to identify the cellular origin of biological stains. Forensic biology laboratories commonly use messenger RNA (mRNA), microRNAs (miRNAs), DNA methylation and microbial markers for cellular origin identification (Goray et al., 2010). Identification of tissue-specific (mRNA) markers is an emergent technique in the field (Visser et al., 2011).

The outer epithelial layer of human skin is continuously developed and renewed by differentiating basal layer stem cells (Tadeu and Horsley, 2014). Accordingly, every day humans shed significant numbers of cells that contain DNA, epidermal proteins and mRNA. RNA can be isolated up to 180 days with high stability despite the presence of tissue ribonucleases (Zubakov et al., 2009; Visser et al., 2011).

Late cornified envelope (LCE) gene clusters are located within the epidermal differentiation complex (EDC) on chromosome 1. The LCE cluster contains multiple conserved genes that encode stratum corneum proteins (Jackson et al., 2005). LCE genes, including late cornified envelope 1D (LCE1D), are abundant and highly sensitive skin biomarkers. Molecular methodologies have been developed in order to measure LCE1D expressions and identify the cutaneous origin of forensic samples (Hanson et al., 2012). The LCE1D gene in skin swabs can be detected up to 21 days after collection (Abd El Razik et al., 2015). However, the impact of storage conditions on its expression has to be analyzed and assessed. Hence, this study aimed to evaluate the impact of storage duration and temperature on cutaneous LCE1D mRNA expression using real time reverse transcriptase PCR (RT- PCR).

Methods

Prior to conducting this experiment, the following minimal necessary sample was calculated using G-Power calculator: two groups of different temperatures, 6 measurements over time, an 80% power (beta = 0.20), a 95% level of significance (alpha 0,05) and an effect size of 0.5. The minimal sample size required to reject the null-hypothesis was six.

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Upon the receipt of informed written consent from the six healthy participants, a pre-moistened sterile cotton swab (sterile Millipore water) was used to collect the participants' palmar skin cells. Volunteers with skin diseases were excluded (Alessandrini et al., 2003). Six skin swabs were collected from each participant: 3 from each hand (Visser et al., 2011). The participants' shedder statuses were nearly impossible to determine (Phipps and Petricevic, 2007). However, some known factors increased the amount of skin markers available for transfer (e.g. preventing hand washing prior to the collection and applying the cotton swab with pressure and friction) (Goray et al., 2010).

These swabs were divided into two groups; Group 1 was stored at 25 °C and Group 2 was stored at 40 °C. LCE1D mRNA expression levels were detected after 0, 1, 2, 3, 4 and 5 days. Measuring the LCE1D mRNA expression levels required the following three steps, according to Hanson et al. (2012):

1. RNA extraction from samples using SV Total RNA Isolation System (Z3100), promega, Madison, USA.

Intact RNA isolation requires four essential steps: the effective disruption of cells, the denaturation of nucleoprotein complexes, the inactivation of endogenous ribonuclease (RNase) activity and the removal of contaminating DNA and proteins. All these steps were performed according to the manufacturer's protocol.

2. Conversion of RNA into cDNA using PCR Reverse Transcription System (A5001), promega, Madison, USA.

The extracted RNA was reverse transcribed into cDNA. Its yield and quality were determined at wavelength 260 nm using a UV2300 spectrophotometer (Indiamart, India).

3. Detection and quantification of the amplified genes using real time PCR Brilliant III SYBR® Green QRT-PCR Master Mix (600886), Stratagene Division, Agilent, Europe.

The amplified gene was determined via real time quantitative PCR (qPCR). The real time- PCR reaction mixture was 50 μl and consisted of 25 μl SYBR Green Mix (2×), 0.5 μl kidney cDNA, 2 μl primer pair mix (5 pmol/ μl each primer), and 22.5 μl H_2O.

The used primers' sequences were as follows (Bauer et al. 2003):

LCE1D Forward 5-CCTGTGCTGCCTGTGACT-3
Reverse5-GGCACTTAGGGGACATTT-3
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
Forward 5-TGATGACATCAAGAAGGTG GTGAAG-3
Reverse 5-TCCTTGGAGGCCATGTGGGC CAT-3

The PCR program set up was as follows: 1 cycle for 2 min. at 50 °C, 1 cycle for 10 min. at 95 °C, 1 cycle for 15 s at 95 °C, then 30 s at 60 °C followed by 30 s at 72 °C repeated 40 times and 1 cycle for 10 min. at 72 °C. The real time- PCR results were analyzed according to step one applied biosystem software. The normalized LCED (dCt) value of each sample was calculated as the difference between the Ct value of GAPDH (internal housekeeping gene) and LCE1D (Visser et al., 2011).

Statistical methods

The data were coded and analyzed using the Statistical Package for Social Sciences(SPSS) version 21. The data were summarized using mean and standard deviation. Comparisons between values measured after 0, 1, 2, 3, 4 and 5 days were conducted using paired t-test and Repeated Measures ANOVA. *P*-values less than 0.05 were considered statistically significant.

Results

LCE1D mRNA expression levels measured in our samples, are presented in Table 1 and Fig. 1. The Group 1 (swabs kept at 25 °C), LCE1D expression levels were significantly higher (*P*-value < 0.05) than Group 2 (swabs kept at 40 °C), especially for the first storage duration (1 day) (0.85 ± .14). Furthermore, significant differences between LCE1D expression and storage duration were observed in both groups.

Our repeated analysis of variances ANOVA indicated significant evidence that storage duration affected LCE1D expression with a Wilks' Lambda of 0.010, F = 1.43, and P 0.000. In our pairwise comparisons, the

Table 1 LCE1D in swab samples at different time interval and temperatures

Skin markers Duration	LCE1D Mean ± SD	
	Group 1 (25 °C)	Group 2 (40 °C)
0	1.06 ± .02	1.06 ± .02
1 day	.85 ± .14	.77 ± .12
2 days	.56 ± .15	.52 ± .21
3 days	.53 ± .08	.46 ± .09
4 days	.49 ± .12	.34 ± .1
5 days	.32 ± .1	.22 ± .05
P value	0.025	0.000

P value < 0.05 is significant

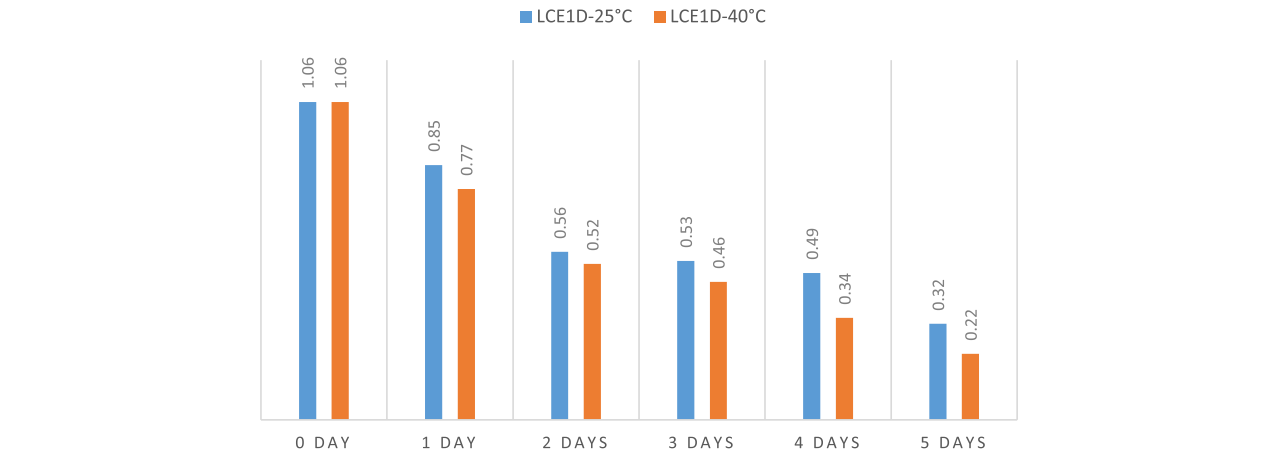

Fig. 1 Mean late cornified envelope gene 1D (LCE1D) values in all swab samples for different temperatures and storage durations

difference between the pairs (0, 2/0, 3/0, 4/0, 5/1, 3 and 1, 5) was also significant (*P*-value < 0.05) Table 2.

LCE1D showed higher expression at 25 °C and relatively low expression at 40 °C. However, the LCE1D expression of the studied groups was not significantly different (*P*-value > 0.05) Table 3.

Discussion

Due to the high skin specificity of LCE1D and its limited cross-reactivity with other human tissues, Hanson et al., 2012 used LCE1D for cutaneous tissue identification. The analysis of time-dependent degraded nucleic acids became a focus of clinical medicine as well as forensic sciences (Bergboer et al., 2011). That being said, the quantitative information on LCE gene family expression in humans is limited (Sampaio-Silva et al., 2013). Therefore, this study was conducted to evaluate the effect of storage temperature and duration on LCE1D expression.

In the present study, the detected LCE1D expression (up to five storage days) concurred with Abd El Razik et al. (2015). They reported that the LCE1D gene could be detected in skin swabs for up to 21 storage days, and its expression decreased significantly in a time-dependent manner. In contrast to our findings, Bergboer et al. (2011)

Table 2 Pairwise comparison of storage durations

Storage duration	Parameter	0 day	1 day	2 days	3 days	4 days	5 days
0	*P*-value	–	.5	.01*	.003*	.001*	.001*
95% Confidence Interval	Lower bound	–	−.14	.137	.242	.299	.415
	Upper bound	–	.48	.773	.805	.661	.969
1 day	*P*-value	.5	–	.57	.03*	.3	.02*
95% Confidence Interval	Lower bound	−.484	–	−.250	.032	−.172	.088
	Upper bound	.141	–	.816	.672	.789	.952
2 days	*P*-value	.01*	.6	–	1	1	1
95% Confidence Interval	Lower bound	−.773	−.816	–	−.523	−.340	−.324
	Upper bound	−.137	.250	–	.660	.390	.797
3 days	*P*-value	.003*	.03*	1	–	1	.142
95% Confidence Interval	Lower bound	−.805	−.672	−.660	–	−.375	−.048
	Upper bound	−.242	−.032	.523	–	.288	.384
4 days	*P*-value	.001*	.3	1	1	–	.096
95% Confidence Interval	Lower bound	−.661	−.789	−.390	−.288	–	−.035
	Upper bound	−.299	.172	.340	.375	–	.458
5 days	*P*-value	.001*	.022*	1	.14	.096	–
95% Confidence Interval	Lower bound	−.969	−.952	−.797	−.384	−.458	–
	Upper bound	−.415	−.088	.324	.048	.035	–

**P* value < 0.05 is significant

Table 3 Statistical significance of LCE1D values in swab samples kept at 25 °C and 40 °C

Storage condition		LCE1D Mean ± SD	95% confidence interval for means		*P* value
			Lower Bound	Upper Bound	
Temperature	25 °C	0.6 ± .3	0.5	0.7	0.07
	40 °C	0.5 ± .3	0.4	0.6	

P value < 0.05 is significant

reported that some LCE family genes were induced at the end of storage duration. They assumed that their methodology, storage media and irradiation could affect the expression levels of the LCE genes (Sampaio-Silva et al., 2013). Moreover, the skin cells in our study were exposed to disruption and isolation at collection time. The isolated skin cells were deprived from the ideal medium for maintained mRNA expression. Moreover, the surrounding condition could have increased the ribonuclease enzymatic activity and hastened the ribonucleic acid degradation (Isom et al., 1987; Probst et al., 2006).

We studied the effect of two storage temperatures on LCE1D expression. However, our findings did not show any statistical significant difference. Similarly, Bergboer et al. (2011) and Sampaio-Silva et al. (2013) found a high expression of LCE gene in skin samples when kept at room temperature (21 °C ± 2). Another study by Lv et al. (2014) found varied RNA expression and degradation behaviors and suggested that environmental factors could play a role in gene expression. Nevertheless, our study provided evidence that minimal change in temperature did not disturb LCE1D gene expression. Our results augmented the findings of Yang et al. (2016), who also reported that different storage temperatures could activate or suppress different genes. However, such temperature changes did not show any significant alterations in gene expression.

Conclusion

Reverse transcription polymerase chain reaction (RT -PCR) is a useful technique to analyze molecular biomarkers with forensic implications. Identifying the cellular origin and collection time of forensic samples using LCE1D mRNA marker is a complex technology that requires an improved understanding of the dynamics of mRNA degradation. The skin marker LCE1D was detected for up to 5 storage days after the sample collection time. Moreover, the storage temperature did not significantly affect LCE1D gene expression.

Although these findings were promising, the current study had some limitations. For instance, we only used one mRNA marker. We preferred to focus on a specific skin marker to detect its degradation pattern as recommended by Lv et al. (2014), who demonstrated that each RNA had a specific degradation pattern.

Recommendation

We recommend further analysis of various LCE gene family expression from different skin sites using a lager sample size to augment the results of this preliminary study before using the LCE gene family in a forensic context.

Abbreviations

LCE1D: Late cornified envelope 1D; mRNA: messenger RNA

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

Presented in the main paper.

Authors' contributions

SFI research idea, plan, data collection, and writing of manuscript. The author read and approved the final manuscript.

Ethics approval and consent to participate

The research protocol was approved by the Ethical committee - Faculty of Medicine, Cairo University.

Consent for publication

Not applicable.

Competing interests

The author declares that he/she has no competing interests.

Publisher's Note

Received: 27 July 2017 Accepted: 14 March 2018
Published online: 21 March 2018

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