Invited Paper

Global gene expression in human skin tissue induced by intense terahertz pulses

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(Received March 10, 2018)

Abstract: Terahertz (THz) radiation has been gaining increasing interest for medical applications due to the potential of pathological contrast for diagnostic applications. However, the interaction mechanism with biological systems implies that intense THz pulses can induce significant non-thermal biological effects, and these have been observed at the molecular, cellular, and tissue level of organization. To investigate the biological processes that are dysregulated by exposure to intense THz pulses, the global differential gene expression profile in skin tissue models was measured. Furthermore, gene ontology analysis identified biological processes that are significantly over-represented by the set of THz-affected genes. In total, 1,681 genes were found to be differentially expressed by exposure to intense THz pulses, and the down-regulated genes were significantly associated with several biological processes related to epidermal differentiation, suggesting the potential for THz exposure to dysregulate/inhibit differentiation or skin function in exposed tissue. The intensities required for the onset of potentially dysregulatory effects are much higher than intensities currently utilized in technologies for diagnostic medical applications.

Keywords: Terahertz, Gene expression, Bioinformatics

doi: 10.11906/TST.028-033.2018.03.03

1. Introduction

Terahertz (THz) frequencies couple strongly to low-frequency molecular vibration and stretching/twisting modes of hydrogen bond networks, which are found in water, all proteins, and DNA [1]. This interaction with biological systems and sensitivity to water content and molecular structure has led to the development of imaging/spectroscopy technologies exploiting diagnostic contrast between diseased and normal tissue [2-4]. However, THz interactions with biological systems are not entirely benign: THz exposures have been shown to non-thermally alter gene expression at the transcript and protein level [5], induce cellular responses against wound stimuli in mouse skin [6], affect cell differentiation in mammalian cells [7], or cause severe forms of

genotoxic stress at the DNA [8] or chromosome [9] level. This implies the potential for exposure risks for which safety regulations do not currently exist, or novel diagnostic/therapeutic modalities for which a characterization of the tissue response is necessary. In this work, we analyze the biological effects of extended THz exposure in human skin tissue models by measuring changes to the global gene expression profile, and we use the identified differentially expressed genes to predict likely downstream phenotypic effects via gene ontology (GO) analysis.

2. Experimental methods

Intense picosecond-duration THz pulses were generated by optical rectification of infrared (800 nm) laser pulses in $LiNbO_3$ and detected by electro-optic sampling in GaP [10]. Plots of the THz waveform and corresponding frequency spectrum are shown in Figure 1.

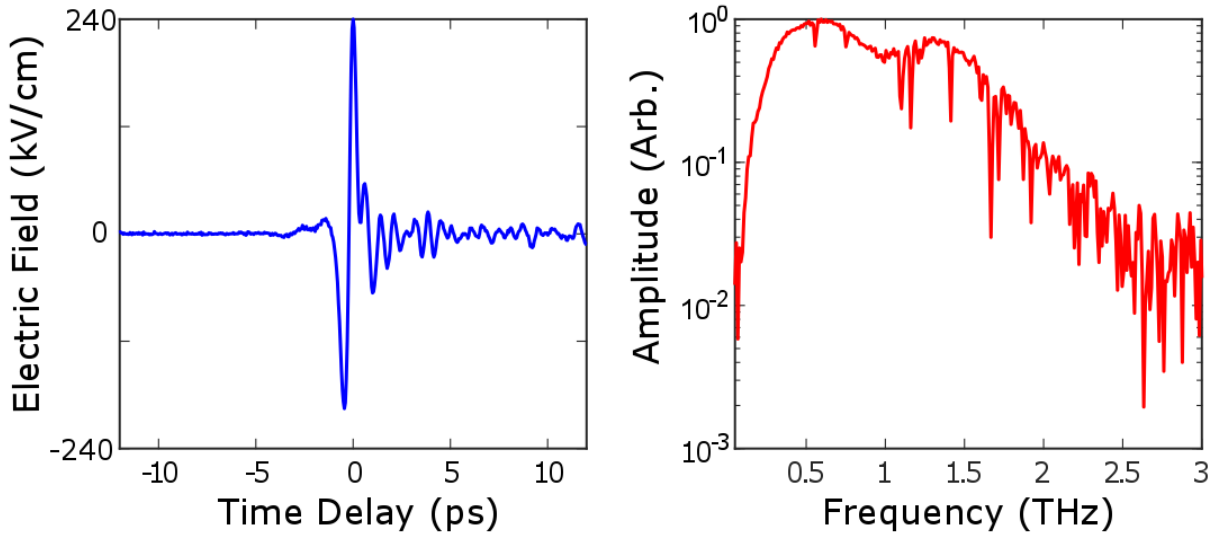

Fig. 1 Waveform and amplitude spectrum of the THz pulse used for exposures.

A THz pulse train (1 *kHz* repetition rate, 2.4 μJ/pulse) was focused onto a ~2 mm^2 region on the surface of human skin tissue models for 10 minutes. Each sample was then incubated (37°C, 5% CO_2) for 30 minutes, snap-frozen in liquid nitrogen, and stored at -80°C. Unexposed control samples went through the same process, but with the THz beam fully blocked. Each set of exposure parameters were repeated in quadruplicate.

The skin models used in this study are 3D co-cultures of dermal fibroblasts in a collagen matrix and epidermal keratinocytes (https://www.mattek.com/product-category/tissue-models/epidermft/). The exposed portions of the tissues were excised, and transcript concentrations were measured for each sample using the Illumina HumanHT-12 v4 microarray platform as per the manufacturer's instructions (Illumina, San Diego, CA, USA). Differences between the controlled

and exposed tissues were quantified, and significant differences between the two experimental conditions were identified. Genes for which $|log_2(FC)| > 0.58$ and $p < 0.05$ were considered significantly differentially expressed, where FC is the fold-change of the exposed measurement relative to control, and p is the associated p-value, adjusted for multiple hypothesis testing.

Gene ontology (GO) analysis identified biological processes that are most significantly associated with the list of genes that are differentially expressed by intense THz pulses. GO terms and associated gene sets were acquired from the Gene Ontology Consortium (geneontology.org). The statistical over-representation is quantified by the odds-ratio (OR) and the corresponding p-value determined by Fisher's exact test and adjusted for multiple hypothesis testing. Terms with $OR > 1$ and $p < 0.01$ were considered statistically significantly over-represented.

3. Results

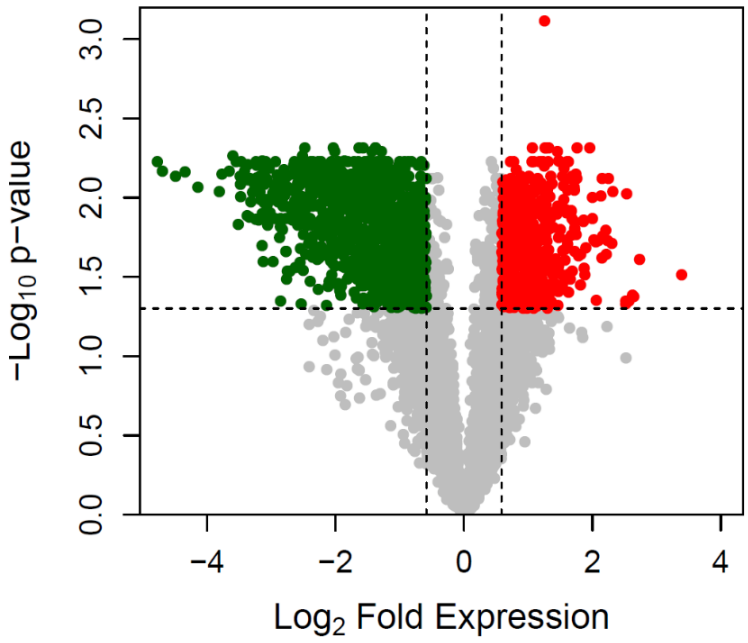

Fig. 2 Volcano plot showing the global differential gene expression induced by intense THz pulses in human tissue models. Black lines indicate conventionally-defined significance thresholds, where $|log_2(FC)| > 0.58$ and $p < 0.05$.

The volcano plot (Fig. 2) shows the magnitude and statistical significance of the differential gene expression profile induced by exposure to intense THz pulses. It was found that 1681 genes met the criteria to be considered significantly differentially expressed, with 1088 genes downregulated (green) and 593 genes upregulated (red). Many of the differentially expressed genes were found to correspond to central proteins that regulate a diverse set of important cellular functions such as inflammatory response, division, differentiation, motility, and cell death.

GO analysis identified 25 biological processes that are significantly over-represented by the set of THz-downregulated genes. The ORs and p-values of a subset of significant GO terms are shown in Table 1.

Tab. 1 Biological processes over-represented by THz-downregulated genes

GO ID	GO Term	Odds Ratio	p-value
GO:0008544	epidermis development	9.35	3.72E-25
GO:0043588	skin development	9.15	6.05E-22
GO:0030216	keratinocyte differentiation	11.91	4.09E-19
GO:0031424	keratinization	25.41	1.02E-18
GO:0009913	epidermal cell differentiation	9.87	1.02E-18
GO:0030855	epithelial cell differentiation	4.69	8.55E-15
GO:0018149	peptide cross-linking	19.70	1.82E-12
GO:0061436	establishment of skin barrier	42.61	3.58E-08
GO:0033561	regulation of water loss via skin	28.40	2.56E-07
GO:0042742	defense response to bacterium	6.25	1.88E-05
GO:0050891	multicellular organismal water homeostasis	10.94	2.88E-05
GO:0030104	water homeostasis	10.33	4.14E-05
GO:0010951	negative regulation of endopeptidase activity	4.24	4.95E-05
GO:0010466	negative regulation of peptidase activity	4.01	1.08E-04
GO:0045103	intermediate filament-based process	13.87	2.04E-04
GO:0045104	intermediate filament cytoskeleton organization	13.87	2.04E-04
GO:0052548	regulation of endopeptidase activity	3.12	2.63E-04
GO:0045861	negative regulation of proteolysis	3.40	4.10E-04
GO:0052547	regulation of peptidase activity	2.86	1.11E-03
GO:0031581	hemidesmosome assembly	23.00	1.95E-03
GO:0051346	negative regulation of hydrolase activity	2.85	2.00E-03
GO:0042303	molting cycle	5.80	2.25E-03
GO:0042633	hair cycle	5.80	2.25E-03
GO:0007586	digestion	5.45	3.53E-03
GO:0048730	epidermis morphogenesis	15.33	6.13E-03

5. Discussion

As expected, even though this analysis quantified differential expression of all detectable genes in the human genome, the identified terms in Table 1 are closely related to the specific system under study (i.e., skin). Many terms that broadly describe processes that take place in the multiple layers of skin (*skin development, establishment of skin barrier, regulation of water loss via skin, multicellular organismal water homeostasis, molting cycle, hair cycle*) were among the most significant GO terms identified. Specialized processes that only occur in the keratinocytes of the epidermal layer (*keratinocyte differentiation, keratinization, epidermal cell differentiation, epithelial cell differentiation, epidermis morphogenesis*) were also significantly over-represented, which may be due to the majority of the energy being absorbed on the superficial layers in the

highly attenuating aqueous environment.

Additionally, processes that specifically regulate the epidermal differentiation processes (*peptide cross-linking, regulation of peptidase activity, intermediate filament-based process, hemidesmosome assembly*) were identified. These effects are consistent with the previously observed effect of THz exposure to skin, which localized the effect to the "epidermal differentiation complex" [5].

The differential gene expression profile presented was induced by intense THz pulses with pulse energies of 2.4 μJ and peak fields of 240 *kV/cm*. These correspond to peak and average intensities of 79 MW/cm^2 and 74 mW/cm^2, respectively. The low average powers associated with the pulse train correspond to a temperature increase that was measured to be <1*K*, and is negligible in terms of biological response. Genes that encode for proteins related to heat regulation were not significantly upregulated, and there were no biological processes corresponding to thermal stress response identified in the GO analysis.

Significant changes as defined by conventional significance thresholds (black dashed lines in Fig. 2) were not observed for 1.5 μJ pulse energy (187 *kV/cm*) or lower. This indicates that THz technologies that are intended for human exposures have intensities that are significantly lower than the intensities required to induce significant modification to gene expression. For example, the Teraview medical imaging system utilizes THz pulses with peak and average powers of ~100 *W* and 100 *nW*, respectively. These are roughly ~5 orders of magnitude lower than the powers required to induce significant effects on gene expression dynamics observed in this experiment [11].

6. Conclusions

In this work, measurements of global differential gene expression profiles were scrutinized by gene ontology analysis in order to predict likely potential phenotypic endpoints induced by exposure to intense THz pulses. Based on this gene expression profile, statistically over-represented biological processes were identified. The majority of processes that are highly correlated to the list of THz-pulse-affected genes regulate development and maintenance of skin. Terms that are localized to the superficial regions of skin (epidermis, epithelial tissue, keratinocyte-based processes) were also calculated to have highly significant over-representation in the list of THz-affected genes. These data predict that epidermal differentiation is predominantly affected, and may be suppressed by extended exposure to intense THz pulses, consistent with previously performed studies [5]. Our analysis has also uncovered previously unknown gene-level mechanisms that may be responsible for effects observed in other THz-biological studies.

Acknowledgements

We acknowledge support from NSERC, CFI, and the AITF Strategic Chairs Program, and technical assistance from Beipei Shi, Greg Popowich, Matt Reid, Rocio Rodriguez-Juarez, Rommy Rodriguez-Juarez, Andrey Golubov, and Yaroslav Ilnytskyy.

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