



HAL
open science

Effects of Radiofrequency Radiation on Gene Expression: A Study of Gene Expressions of Human Keratinocytes From Different Origins

Catherine Martin, Frédéric Percevault, Kate Ryder, Etiam Sani,
Jean-Christophe Le Cun, Maxim Zhadobov, Ronan Sauleau, Yves Le Dréan,
Denis Habauzit

► To cite this version:

Catherine Martin, Frédéric Percevault, Kate Ryder, Etiam Sani, Jean-Christophe Le Cun, et al.. Effects of Radiofrequency Radiation on Gene Expression: A Study of Gene Expressions of Human Keratinocytes From Different Origins. *Bioelectromagnetics*, 2020, 41 (7), pp.552-557. 10.1002/bem.22287 . hal-02930148

HAL Id: hal-02930148

<https://hal.science/hal-02930148>

Submitted on 9 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3 **Effects of radiofrequency radiation on gene expression: a study of gene**
4 **expressions of human keratinocytes from different origins**
5
6

7 Catherine Martin¹, Frederic Percevault¹, Kate Ryder¹, Etiam Sani¹, Jean-Christophe Le Cun²,
8 Maxim Zhadobov², Ronan Sauleau², Yves Le Dréan¹, and Denis Habauzit^{1*}
9

10
11
12
13
14 ¹ Univ Rennes, Inserm, EHESP, Institut de Recherche en Santé, Environnement et Travail
15 (IRSET) – UMR_S 1085, F-35000 Rennes, France

16
17 ² Univ Rennes, CNRS, Institut d'Électronique et de Télécommunication de Rennes (IETR),
18 UMR 6164, F-35000 Rennes, France
19

20
21
22
23
24 ***Corresponding author:**
25

26
27 Dr Denis Habauzit, Transcription, Environment and Cancer Group, Institute for Research on
28 Environmental and Occupational Health (IRSET), Inserm UMR1085, 9 Avenue du Prof. Léon
29 Bernard, 35043 Rennes Cedex, France. Tel: (+33) 2 23 23 50 95; E-mail:
30
31

32 habauzit.denis@gmail.com
33
34
35

36
37
38
39 Conflict of interest: none
40
41

42
43 Running title: Effects of MMW radiation on gene expression
44
45

46
47
48 Grant sponsor: French National Program for Environmental and Occupational Health, Anses (EST-2016-
49 2-RF-02)
50
51

1
2
3
4
5
6 Keywords: millimeter waves; 60-GHz band; keratinocytes; gene expression; keratinocyte
7
8 origins
9
10

11 12 13 14 15 **Introduction** 16

17
18
19 The answers to questions related to the biological effects of radiofrequency radiation
20 exposure on organisms are pending. In bioelectromagnetic experiments, one of the main
21 difficulties is that the results found in the literature, even those obtained with equivalent
22 models, are poorly reproducible. This poor reproducibility may be due to the use of various
23 biological material and exposure systems, varying exposure durations and, most of the time,
24 varying frequencies. For instance, focusing on the impact of the electromagnetic field on gene
25 expression, we found that the 8 most recent publications used microarrays based on 5
26 different models (mouse, rat, human, Drosophila, and chicken models); 8 different
27 frequencies, from intermediary frequencies to radiofrequencies; and 3 data processing
28 methods (no statistical tests, simple tests, and false discovery rate (FDR) statistical test)
29 [Andocs et al., 2016; Fragopoulou et al., 2018; Habauzit et al., 2020; Kim et al., 2016;
30 Lamkowski et al., 2018; Manta et al., 2017; Woelders et al., 2017; Yeh et al., 2015]. Together,
31 the differences in these parameters make it difficult not only to compare individual
32 experiments but also to replicate them.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52
53 For decades, evaluations of the potential effects of electromagnetic waves have focused on
54 several pathways that could be involved in cancer promotion, such as DNA damage and
55 oxidative stress pathways [Saliev et al., 2019; Vijayalaxmi and Prihoda, 2012]. The research
56
57
58
59
60

1
2
3 performed to date has intensively explored whole-genome gene expression modification
4
5 under radiofrequency exposure [Leszczynski et al., 2012; Leszczynski, 2014]. In particular,
6
7 genomic high-throughput approaches have enabled whole-genome gene expression to be
8
9 screened under both exposure and control conditions, but such approaches have failed to
10
11 produce clear agreements.
12
13

14
15
16 Our group has performed several microarray experiments to evaluate the effects of millimeter
17
18 waves (MMWs) at approximately 60 GHz on gene expression [Habauzit et al., 2014; Le
19
20 Quément et al., 2012; Soubere Mahamoud et al., 2016]. We have used microarray approaches
21
22 with primary cultures of human keratinocytes as the main targets of MMWs. First, we have
23
24 found that when MMWs are applied alone in athermic conditions and when microarrays are
25
26 analyzed with a false discovery rate (FDR) filter, the MMWs have no effect on gene expression
27
28 [Habauzit et al., 2014; Le Quément et al., 2012; Soubere Mahamoud et al., 2016]. However,
29
30 when MMWs are applied at an incident power density (IPD) of 20 mW/cm² (the current
31
32 International Commission on Non-Ionizing Radiation Protection (ICNIRP) upper limit for local
33
34 exposure of the general public [Ahlbom et al., 1998]) in association with other stressors (heat
35
36 or metabolic stresses, for instance), the results from the same analysis (with FDR-filtered data)
37
38 reveal a slight effect of the MMWs on gene expression with 7 and 6 modified genes [Habauzit
39
40 et al., 2014; Soubere Mahamoud et al., 2016]. Among the modified genes, 3 genes exhibit
41
42 changes sufficiently reproducible for study: *ADAMTS6*, *IL7R*, and *NOG*. The aim of this study
43
44 was to evaluate the universality of the expression modifications of these genes in other
45
46 primary cultures of keratinocytes and a cell line.
47
48
49
50
51
52
53
54
55
56
57
58

59 **Materials and methods**

60

Cell cultures

Four cell types were used. The first cell type corresponded to a pool of primary human keratinocytes isolated from 3 neonatal foreskins and was called HEK_3N (Invitrogen, Saint-Aubin, France). The second cell type, called HEK_1N, consisted of primary human keratinocytes from a single donor of neonatal foreskin (Invitrogen, Saint-Aubin, France). The third primary culture (NHEK_3N) was derived from 3 pools of donated neonatal foreskin (Lonza, Levallois-Perret, France). All primary keratinocytes were cultured and exposed as previously described [Habauzit et al., 2014] and were used when they were between passages 4 and 9. Briefly, cells were cultured onto collagen IV-coated plates (Becton Dickinson, Franklin Lakes, NJ) in supplemented keratinocyte serum-free medium (SFM) (Gibco, Carlsbad, CA) with antibiotics (Invitrogen, Saint-Aubin, France). The derived keratinocyte cell line (HaCaT) was used and maintained in culture in Dulbecco's modified Eagle medium (Gibco/Life Technologies, Saint-Aubin, France) supplemented with 10% fetal calf serum (FCS), 1% antibiotics, and 1% L-glutamine as previously described [Le Quément et al., 2014]. The HaCaT cells were used when they were between passages 5 and 10.

Exposure system and experimental setup

The exposure conditions were optimized numerically to maximize the homogeneity of the specific absorption rate (SAR) distribution within the cell monolayer, as detailed previously [Zhadobov et al., 2012]. The IPD was determined numerically and then validated by thermal measurement. The average and peak SAR over the cell monolayer were 594 W/kg and 1233 W/kg, respectively, and corresponded in near-field conditions (2.5 cm between the horn antenna and the plate bottom) to an average IPD of 20 mW/cm². The exposure system and

1
2
3 conditions have been detailed previously [Zhadobov et al., 2012]. Briefly, the $2 \cdot 10^5$ cells, in
4 one well of the 6-well plate, were placed in a MEMMERT UNE400 incubator (Memmert,
5 Schwabach, Germany) adapted for electromagnetic exposure. The inside of the incubator was
6 covered with absorbent material (ECCOSORB HR-1/2"-MB, Emerson & Cuming, Westerlo,
7 Belgium) with a reflectivity below -20 dB around 60 GHz. This limits the maximum reflections
8 to less than 1% in respect to the incident field. Due to the properties of MMWs, cell exposures
9 were performed from the bottoms of the wells (Fig. S1). The cells were exposed in the
10 corresponding culture medium supplemented with 10 mM HEPES for primary keratinocyte
11 cultures and 4.6 mM HEPES for HaCaT cells for 3 h. For each experiment 4 ml of medium was
12 used. Note that the volume does not influence either the SAR or the IPD [Orlacchio et al.,
13 2019]. Two exposure conditions (unexposed (Sham) and MMW-exposed (Expo)) were used
14 under the same cell culture conditions. All cells were exposed in the same incubator with the
15 generator on or off. The experiments were replicated between 3 and 6 times.

37 *RNA extraction and RT-QPCR analysis*

38 RNA was extracted with a NucleoSpin RNA kit (Macherey-Nagel, Hoerd, France) and then
39 quantified by a NanoDrop 8000 spectrophotometer (NanoDrop Technology, Cambridge, UK).
40 Five hundred nanograms of RNA was reverse-transcribed with an iScript kit (Bio-Rad, Hercules,
41 CA) according to the supplier's instructions. All primers used are presented in Table 1. Two
42 housekeeping genes (*TBP* and *GAPDH*) were used for normalization. Quantitative polymerase
43 chain reaction (QPCR) was performed on a Bio-Rad CFX 384-well apparatus with SYBR Green
44 Supermix (Bio-Rad, Hercules, CA). The results were analyzed using the $\Delta\Delta CT$ method.

59 *Statistical analysis*

1
2
3 Statistical analyses for the comparison of conditions were performed using a one-tailed Mann-
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
at $p\text{-value} \leq 0.05$ was considered statistically significant.

Results

Effect of MMWs on gene expression in keratinocytes

ADAMTS6, *IL7*, and *NOG* gene expression in primary keratinocyte cultures was modified by treatment with growth factors, especially Interleukin-1 (IL1) and Epidermal Growth Factor (EGF). These data confirmed that the expression of these genes was sensitive enough to be modulated, as shown in Figure S2 (supplementary data). For the exposure experiments, 4 cellular models of the exact same cell type, human keratinocytes, were used. Among these cell models, 3 primary cultures were obtained from different donors, and one culture was based on a keratinocyte-derived cell line (HaCaT). Although the cell sources were different, the basal expression of *ADAMTS6*, *IL7R*, and *NOG* was similar in the four keratinocyte models (data not shown). Figure 1 shows the variations in *ADAMTS6*, *IL7R*, and *NOG* expression levels. Three different expression profiles were observed after MMW exposure. The first profile indicated that *ADAMTS6* expression was downregulated in HEK_3N cells, with a fold change of 0.5 with associated $p\text{-value}$ of 0.057 (Fig. 1A). The second profile did not show any treatment effects, as illustrated by lack of differences in *ADAMTS6* expression levels in the sham and expo cells (similarly, no differences were found for *IL7R* and *NOG* expression; Fig. 1B-C) in the HEK_1N cells. The third *ADAMTS6* profile was characterized by upregulated gene expression in the expo NHEK_3N primary culture cells and the HaCaT cells with associated $p\text{-}$

1
2
3 *value* of 0.1, findings that corresponded to data previously obtained. Together, these three
4
5 profiles indicate that each biological material shows a specific sensitivity, even when the
6
7 exposure treatment and conditions are exactly the same. This finding was obtained regardless
8
9 of whether *ADAMTS6* expression was downregulated, not affected, or upregulated. The same
10
11 expression patterns were also observed for *IL7R* and *NOG* (Fig. 1B-C). Among these profiles,
12
13 no significant differences were found.
14
15
16
17
18
19

20 21 Discussion

22
23
24
25
26 In our previous microarray experiments, 3 differentially expressed genes were identified, and
27
28 one year later, the findings were confirmed by results from independent experiments based
29
30 on the same cellular model [Habauzit et al., 2014]. These data indicated that MMWs
31
32 significantly upregulate *ADAMTS6*, *IL7R*, and *NOG* expression. These previously published data
33
34 [Habauzit et al., 2014] indicated a specific MMW effect independent of a heat-associated
35
36 MMW response. In this study, we aimed to determine whether the gene regulation observed
37
38 was specific to the type of cell used or whether it reflected a more general regulation that
39
40 could be found regardless of the cell type. Therefore, experiments were replicated with three
41
42 different primary cultures and one cell line. We first tested several growth factors to ensure
43
44 that these genes could be modulated. These controls validated the RT-QPCR method used to
45
46 evaluate the MMWs' effects on gene expression. Then, the duplicated experiments in the
47
48 keratinocyte models showed 3 different expression patterns (downregulation, no effect, and
49
50 upregulation after both treatments), suggesting that the specific sensitivity observed may
51
52 depend more on the model used than on the general cell sensitivity to MMW exposure. This
53
54
55
56
57
58
59
60

1
2
3 observation raises two problems of paramount importance in the field of
4
5 bioelectromagnetism: the reproducibility of the results and impact of the biological model.
6
7

8 Findings of bioelectromagnetic studies are difficult to compare because of the
9
10 multiplicity of exposure systems, frequencies, treatment durations, models, and technologies
11
12 required for biological assessment and statistical analysis. Attention is often concentrated on
13
14 the variations in exposure conditions and wavelengths to explain differences between
15
16 bioelectromagnetic studies. Consequently, the impact of the biological material is often
17
18 underestimated. This problem and related problems linked to biological models have started
19
20 to be illustrated by more general studies in published papers [Lai, 2018; Yakymenko et al.,
21
22 2016].
23
24
25
26
27
28

29 The present study shows that the biological model exerts a strong influence on the
30
31 data obtained, which may at least partly explain the heterogeneity of the reported results in
32
33 the field. It is important to emphasize that these experiments were conducted at the same
34
35 frequency (60.4 GHz), in the same exposure system, and for the same exposure duration. In
36
37 addition, we used 4 cellular models that were all presumed to represent the same biological
38
39 entity (human keratinocytes) and therefore were expected to react in a similar manner.
40
41 Surprisingly, we found 3 different expression profiles despite using identical exposure
42
43 conditions. The statistically significant effect in our first study (based on one primary culture
44
45 pool) was not reproduced when the biological material was changed, although all the models
46
47 were composed of keratinocytes (primary cultures sourced from one to three donors
48
49 randomly obtained from suppliers and a cell line). We conclude that the biological material
50
51 caused great variability in the cellular response. Two possibilities that are not mutually
52
53 exclusive may explain such observations. First, it cannot be ruled out that the observed
54
55
56
57
58
59
60

1
2
3 variations were reflective of random fluctuations not related to the treatment. Considering
4
5 their cost, most in vitro studies are repeated a limited number of times. In these cases, the
6
7 limits of the statistical tests are reached, and false positives can sometimes emerge; for
8
9 instance, a *p-value* of 0.05 indicates a 5% probability of the data representing a false positive.
10
11
12 Second, the variability in the biological response to exposure could have been associated with
13
14 the state or history of the biological material. It is well known that heterogeneous gene
15
16 expression among cellular subclones can be the result of epigenetic modification or somatic
17
18 mutation. Mutations are very rare events; in contrast, epigenetic modifications are common
19
20 and relatively more sensitive to the environment. These modifications are essential for cell
21
22 adaptation and responses to external variations, and they constitute a form of memory that,
23
24 in turn, may influence how cells respond to environmental stimuli [Park et al., 2014; Veith et
25
26 al., 2016]. Thus, it is possible that the different expression profiles observed reflect epigenetic
27
28 modifications specific to the history/state of each cellular model that we used. If sensitivity to
29
30 the electromagnetic field depends on epigenetic memory, then these results raise questions
31
32 about inter-individual sensitivity to stimulation in the human population.
33
34
35
36
37
38
39

40 Although our past and present results are divergent, it is very likely that the
41
42 observations were accurate each time. However, they reflected the situation at the moment
43
44 of the experiment, which depended on the sensitivity and dynamism of the models used. We
45
46 are aware that this study focused on only three genes and that the results may therefore not
47
48 be representative of all expected results in bioelectromagnetism studies. Moreover, whether
49
50 these results can be translated to other radiofrequencies and/or biological models, such as
51
52 brains, remains unclear. This question is valid, and the uncertainties are illustrated by the
53
54 many contradictory results described in the literature. Some publications in the field have
55
56 started to introduce quality criteria [Simkó et al., 2016; Vijayalaxmi, 2016]. Among all the key
57
58
59
60

1
2
3 points considered in these publications, validation of the effects in other cell types (different
4
5 models or equivalent models) should be the main criterion for the identification of clear and
6
7 reproducible effects of radiofrequency exposure.
8
9

10
11 In conclusion, our data demonstrate that in the four keratinocyte cell types, 3 different
12
13 expression patterns (down regulation, upregulation, and no effect) were observed, despite
14
15 their exposure having been the same in all regards. Additional studies will be necessary to
16
17 identify the molecular and cellular origins of such variability in exposure sensitivity.
18
19

20
21 For additional information, see Online Supplementary Materials on the publisher's website.
22
23

24 25 **Legends**

26 27 Table Legend

28
29
30 Table 1: List of primers used to validate the identified differentially expressed genes
31
32

33 34 Figure Legends

35
36 Fig. 1. *ADAMTS6* (A), *IL7R* (B), and *NOG* (C) expression in different primary cultures and a cell line. The
37
38 results are expressed as median, and the error bars represent the interquartile range. In Figure 1A, the
39
40 replicate numbers are indicated in brackets. The associated *p-value* is indicated between each
41
42 comparison.

43
44 Fig. S1. Illustration of the exposure system used for all the detailed experiments.
45
46

47
48 Fig. S2. Gene expression of *ADAMTS6* (A), *IL7R* (B), and *NOG* (C) under stimulation of Tumor Necrosis
49
50 Factor (TNF- α , 50 ng/ml), Fibroblast Growth Factor (FGF, 10 ng/ml), Leukemia Inhibitory Factor (LIF, 10
51
52 ng/ml), Epidermal Growth Factor (EGF, 10 ng/ml), Interleukin-1 (IL-1, 50 ng/ml), and Transforming
53
54 Growth Factor (TGF- β , 10 ng/ml). The results are the means from two independent experiments \pm
55
56 SEMs.
57
58

59 60 **References**

Ahlbom A, Bergqvist U, Bernhardt JH, Cesarini JP, Grandolfo M, Hietanen M, Mckinlay AF,

Repacholi MH, Sliney DH, Stolwijk JA. 1998. Guidelines for limiting exposure to time-

1
2
3 varying electric, magnetic, and electromagnetic fields (up to 300 GHz). *Health Phys*
4
5 74:494–521.
6
7

8 Andocs G, Rehman MU, Zhao Q-L, Tabuchi Y, Kanamori M, Kondo T. 2016. Comparison of
9
10 biological effects of modulated electro-hyperthermia and conventional heat
11
12 treatment in human lymphoma U937 cells. *Cell Death Discov* 2:16039.
13
14

15 Fragopoulou AF, Polyzos A, Papadopoulou M-D, Sansone A, Manta AK, Balafas E,
16
17 Kostomitsopoulos N, Skouroliakou A, Chatgialiloglu C, Georgakilas A, Stravopodis DJ,
18
19 Ferreri C, Thanos D, Margaritis LH. 2018. Hippocampal lipidome and transcriptome
20
21 profile alterations triggered by acute exposure of mice to GSM 1800 MHz mobile
22
23 phone radiation: An exploratory study. *Brain Behav* 8:e01001.
24
25
26

27 Habauzit D, Le Quément C, Zhadobov M, Martin C, Aubry M, Sauleau R, Le Dréan Y. 2014.
28
29 Transcriptome analysis reveals the contribution of thermal and the specific effects in
30
31 cellular response to millimeter wave exposure. *PLoS ONE* 9:e109435.
32
33
34

35 Habauzit D, Nogue G, Bourbon F, Martin C, Del Vecchio F, Maunoir-Regimbal S, Poyot T,
36
37 Valente M, Jaoui R, Crouzier D, Le Dréan Y, Debouzy JC. 2020. Evaluation of the effect
38
39 of chronic 94 GHz exposure on gene expression in the skin of hairless rats in vivo.
40
41
42 *Radiat Res* 193:351–358.
43
44

45 Kim JH, Huh YH, Kim HR. 2016. Induction of autophagy in the striatum and hypothalamus of
46
47 mice after 835 MHz radiofrequency exposure. *PLoS ONE* 11:e0153308.
48
49

50 Lai H. 2018. A Summary of Recent Literature (2007–2017) on Neurobiological Effects of
51
52 Radio Frequency Radiation. In: Markov, M, editor. *Mobile Communications and*
53
54 *Public Health*. Boca Raton, Florida: CRC Press, p. 187-222.
55
56

57 Lamkowski A, Kreitlow M, Radunz J, Willenbockel M, Sabath F, Schuhn W, Stiemer M, Fichte
58
59 LO, Dudzinski M, Böhmelt S, Ullmann R, Majewski M, Franchini V, Eder S, Rump A,
60

- 1
2
3 Port M, Abend M. 2018. Gene expression analysis in human peripheral blood cells
4
5 after 900 MHz RF-EMF short-term exposure. *Radiat Res* 189:529–540.
6
7
8 Le Quément C, Nicolas Nicolaz C, Zhadobov M, Desmots F, Sauleau R, Aubry M, Michel D, Le
9
10 Dréan Y. 2012. Whole-genome expression analysis in primary human keratinocyte
11
12 cell cultures exposed to 60 GHz radiation. *Bioelectromagnetics* 33:147–158.
13
14
15 Le Quément C, Nicolaz CN, Habauzit D, Zhadobov M, Sauleau R, Le Dréan Y. 2014. Impact of
16
17 60-GHz millimeter waves and corresponding heat effect on endoplasmic reticulum
18
19 stress sensor gene expression. *Bioelectromagnetics* 35:444–451.
20
21
22 Leszczynski D. 2014. Radiation proteomics: a brief overview. *Proteomics* 14:481–488.
23
24
25 Leszczynski D, de Pomerai D, Koczan D, Stoll D, Franke H, Albar JP. 2012. Five years later: the
26
27 current status of the use of proteomics and transcriptomics in EMF research.
28
29
30 *Proteomics* 12:2493–2509.
31
32
33 Manta AK, Papadopoulou D, Polyzos AP, Fragopoulou AF, Skouroliakou AS, Thanos D,
34
35 Stravopodis DJ, Margaritis LH. 2017. Mobile-phone radiation-induced perturbation of
36
37 gene-expression profiling, redox equilibrium and sporadic-apoptosis control in the
38
39 ovary of *Drosophila melanogaster*. *Fly (Austin)* 11:75–95.
40
41
42 Orlacchio R, Zhadobov M, Alekseev SI, Nikolayev D, Sauleau R, Le Page Y, Le Dréan Y. 2019.
43
44 Millimeter-Wave Heating in in vitro studies: effect of convection in continuous and
45
46 pulse-modulated regimes. *Bioelectromagnetics* 40:553–568.
47
48
49 Park LK, Maione AG, Smith A, Gerami-Naini B, Iyer LK, Mooney DJ, Veves A, Garlick JA. 2014.
50
51 Genome-wide DNA methylation analysis identifies a metabolic memory profile in
52
53 patient-derived diabetic foot ulcer fibroblasts. *Epigenetics* 9:1339–1349.
54
55
56 Saliev T, Begimbetova D, Masoud A-R, Matkarimov B. 2019. Biological effects of non-ionizing
57
58 electromagnetic fields: two sides of a coin. *Prog Biophys Mol Biol* 141:25–36.
59
60

1
2
3 through down-regulation of insulin-like growth factor 2 in a neuropathic pain model.

4
5 Int J Mol Sci 16:27156–27170.

6
7
8 Zhadobov M, Sauleau R, Augustine R, Le Quément C, Le Dréan Y, Thouroude D. 2012. Near-

9
10 field dosimetry for in vitro exposure of human cells at 60 GHz. Bioelectromagnetics

11
12
13 33:55–64.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only

Table 1: List of primers used to validate the identified differentially expressed genes

Gene Symbol	Forward	Reverse	RefSeq #
ADAMTS6	ACATCAATCCTCTTCTCTGGG	TTCAAGTTCTGCAGTCGAGC	NM_197941.4
IL7R	GACGCATGTGAATTTATCCAGCAC	CATACATTGCTGCCGTTGGAG	NM_002185.5
NOG	AAGCAGCGCCTAAGCAAGAAGC	AATGTCTGCGACCACAGCCACATC	NM_005450.6
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG	NM_002046
TBP	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA	NM_003194

For Review Only

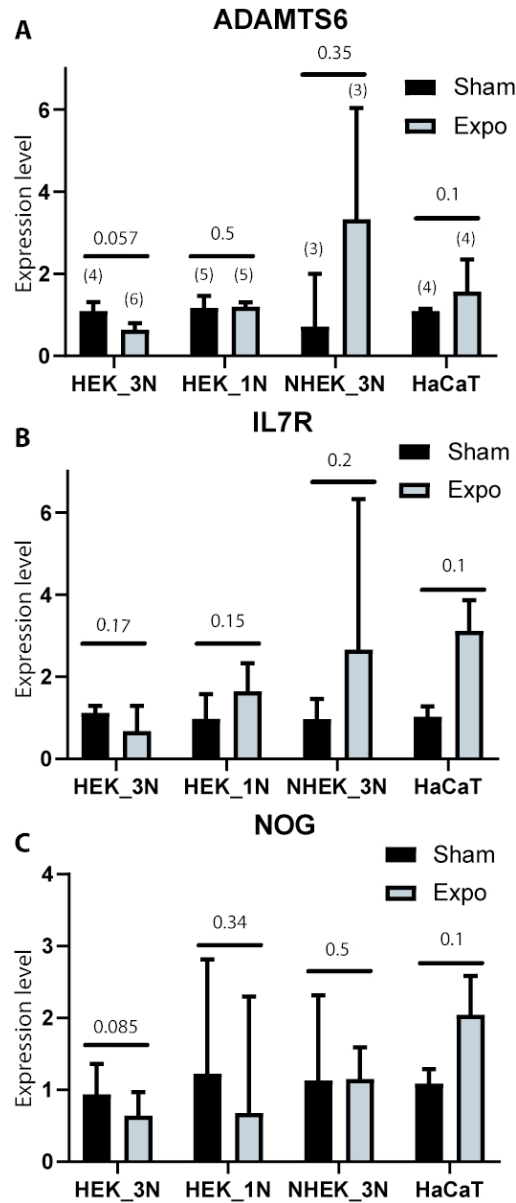


Fig. 1: ADAMTS6 (A), IL7R (B), and NOG (C) expression in different primary cultures and a cell line. The results are expressed as median, and the error bars represent the interquartile range. In Fig. 1A, the replicate numbers are indicated in brackets. The associated p-value is indicated between each comparison.