Alteration of the Transcriptional Profile of Human Embryonic Kidney Cells by Transient Overexpression of Mouse TRPM7 Channels

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Key Words

TRPM7 • Microarray • Transcriptome • Differential Expression • Cellular Pathway

Abstract

Background: TRPM7 is a cation channel containing a functional kinase domain. The functional activity of TRPM7 is essential for cell viability and growth, and its expression is up-regulated in certain pathological conditions, such as ischemia. **Methods:** In order to assess the effects of TRPM7 activity on cellular gene expression, inducible HEK293 cell-lines harboring the wild-type mouse TRPM7 and a mutant lacking the kinase domain were established. The wild-type and the non-functional TRPM7 channels were induced transiently and the comparative changes in cellular transcription were investigated. **Results:** From the genome-scale analysis using a human genome expression microarray, we identified 951 genes altered in transcription significantly and specifically by the expression of the functional TRPM7 channel. **Conclusion:** By analyzing the genes differentially expressed by TRPM7, we were able to provide potential target proteins and to delineate the cellular pathways affected by the channel function.

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Introduction

Transient receptor potential (TRP) channels constitute a large and diverse family of proteins that are expressed in many tissues and cell types [1-2]. Belonging to the superfamily of voltage-gated channel proteins, TRP channels contain six transmembrane segments (S1–S6) and a pore-forming region in their structures. Functionally, these channels are categorized as nonselective cation channels, despite a large diversity in their ion permeability and Ca²⁺ selectivity. The family of TRP channels is divided into six subfamilies according to sequence homology rather than ligand function or selectivity [3-5]. As a member of the TRP channel family, TRPM7 (transient receptor potential cation channel, subfamily M, member 7) shows similarity to melastatin (TRPM1), whose down-regulated level of mRNA has been used as a prognosis marker for metastasis with localized melanoma [6]. The TRPM channel subfamily has eight members, TRPM1 to TRPM8. The TRPM7 channel has distinctive characteristics for ion permeation, allowing passage of Ca²⁺ as well as Mg²⁺ and other cations [7]. The expression of TRPM7 is ubiquitous in the brain, spleen, kidney, lung, heart, liver, and microglia [7-8]. Recent reports showed that its expression was also found in various cancer cells

and mast cells [9-12]. TRPM7 is one of only two ion channels containing its own kinase domain in its cytosolic C-terminus [8]. Although the channel kinase belongs to the recently discovered α -kinase family [13], functional kinase activity is not essential for TRPM7 channel activity [14]. Neither phosphotransferase-deficient forms of the kinase domain, nor autophosphorylation of the channel altered channel activity were found [14]. However, a truncation mutant of the kinase domain failed to evoke an ionic current [14-15], and thus the kinase domain of TRPM7 is thought to be a crucial factor for the functional expression of this channel. Although TRPM7 allows permeation of Mg^{2+} , magnesium ions and magnesium-nucleotide block channel activity [7, 16]. Thus, native TRPM7 has been characterized as a magnesium-nucleotide-regulated metal ion current (MagNum).

The passage of Mg^{2+} through TRPM7 is involved in magnesium homeostasis in mammalian cells [17-18]. In addition, the permeation of Ca^{2+} has been associated with Ca^{2+} overload during anoxic cell death [19]. TRPM7 phosphorylates annexin I [20] and regulates cell adhesion by controlling calpain [15]. TRPM7 activity was also related to cytoskeleton activity [21]. It was found that the experimental reduction of TRPM7 expression by either knock-out or knock-down methodology compromised cell growth and proliferation [9, 17]. Alternatively, the overexpression of TRPM7 was shown to exert a harmful influence upon cell viability by regulating cell adhesion [7, 15]. In addition, the expression of TRPM7 was reported to be up-regulated in pathological conditions such as ischemia [22-23].

Since the manipulation of TRPM7 can dramatically affect many different aspects of cellular processes, it can be assumed that cellular transcription may be altered substantially by the regulation of TRPM7 gene expression. In the present study, we investigated the overall changes in transcription profile (or transcriptome) by transient overexpression of the TRPM7 channel. For comparative analysis, two kinds of inducible cell-lines stably expressing the wild-type TRPM7 channel and a deletion mutant lacking the kinase domain were generated. Human whole genome microarrays were used to identify genes affected by TRPM7 overexpression. Replicated microarray analyses revealed 1259 probes that are up-regulated or down-regulated by the overexpression of TRPM7. Further analysis of our data showed that the proteins involved in several key cellular pathways, e.g., cellular growth and proliferation, cell death, cellular morphology, and movement, were affected by TRPM7 channel expression.

Materials and Methods

Two female NZW rabbits were used for immunization experiments. All animals were used and maintained according to regulations provided by the animal ethical committee of the Gwangju Institute of Science and Technology.

Constructs, mutagenesis, and creation of inducible cell-lines

Wild-type murine TRPM7 (gene accession number: AY032951) was modified with a FLAG-tag at its amino terminus as described previously [7] and subcloned into an inducible mammalian expression vector, pcDNA4/TO (Invitrogen, Carlsbad, CA). A deletion mutant of the TRPM7 channel lacking the kinase domain (TRPM7 Δ KD) was generated by the polymerase-chain reaction (PCR). The amplified DNA fragment was substituted for the wild-type TRPM7 gene in the pcDNA4/TO expression vector using Not I and Apa I restriction sites. The final constructs were sequenced to confirm their authenticity. This deletion removed the protein region downstream of position 1,599 including the entire kinase domain of the TRPM7 channel.

Modified HEK293 cells, T-RE_xTM-293 (Invitrogen), were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and an antibiotic, 5 μ g/mL blasticidin (Invitrogen). Cells were cultured in a humidified atmosphere of 5% CO₂ at 37 °C. In order to obtain inducible cell-lines for overexpression, the vectors harboring wild-type TRPM7 and TRPM7 Δ KD, and mTRPM7/pcDNA4-TOTM and mTRPM7₁₋₁₅₉₉/pcDNA4-TOTM, respectively, were transfected into T-RE_xTM-293 cells grown in a six-well plate using Polyfect reagent (Qiagen, Valencia, CA) according to methods recommended by the manufacturer. Cells were grown with 0.5 mg/mL zeocin (Invitrogen) in media replenished every 2 days for selection. The expressions of the wild-type and the mutant TRPM7 in T-RE_xTM-293 cells were suppressed by the Tet repressor until treatment with an inducer, tetracycline.

Electrophysiological recordings and data analyses

The T-RE_xTM-293 cells harboring the wild-type and the kinase-domain-deleted TRPM7 channels were grown on glass coverslips with DMEM medium supplemented with 10% fetal bovine serum, 5 μ g/mL blasticidin, and 0.5 mg/mL zeocin. Expression of TRPM7 and TRPM7 Δ KD was induced for 48 h by adding tetracycline to the culture medium at a final concentration of 1 μ g/mL. The expression of TRPM7 channel proteins was confirmed by immunoblot analysis.

Whole-cell patch-clamp experiments were conducted at 48 h postinduction in a bath solution with the following composition: 135 mM Na-methanesulfonate, 3 mM CaCl₂, 0.5 mM ethylenediaminetetraacetic acid (EDTA), and 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (pH adjusted to 7.2 with NaOH). Intracellular pipette-filling solutions contained 135 mM Cs-methanesulfonate, 10 mM 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 3.67 mM CaCl₂, and 10 mM HEPES (pH adjusted to 7.2 with CsOH). In order to prevent Ca²⁺-release-activated Ca²⁺ channel current, the internal concentration of free Ca²⁺ was buffered with BAPTA

as described above at 100 nM, calculated by using Maxchelator software (Stanford University, CA). After establishment of the whole-cell configuration, a voltage ramp of -120 to 100 mV was delivered from a holding potential of 0 mV for 500 ms. The channel currents were amplified using an Axopatch 200B (Axon Instrument, Foster City, CA), low-pass filtered at 1 kHz using a four-pole Bessel filter, and digitized at a frequency of 10 kHz using a Digidata 1200A (Axon Instruments). Commercial software packages such as Clampex 8.1 (Axon Instrument) and Origin 6.1 (MicroCal, Northampton, MA) were used for the acquisition and analysis of macroscopic data.

Generation of anti-TRPM7 antiserum

Mouse TRPM7-specific anti-serum was raised in rabbits against the peptide mapping at 1385–1555 of mouse TRPM7. Since this region was retained in the TRPM7 Δ KD mutant channel, both the wild-type and the mutant TRPM7 proteins could be probed. Complementary DNA corresponding to residues 1385–1555 was amplified by PCR and subcloned into the pET15b vector using Nde I and Bam HI. His-tagged proteins were expressed in *E. coli* and purified using Ni-NTA column chromatography. Purified protein (~500 μ g) mixed with Freund's adjuvant was administered to the rabbits subcutaneously. After two subsequent boosting, anti-serum was collected and evaluated.

Immunoblot analysis

Cells were lysed in 20 mM HEPES (pH 7.5), 120 mM NaCl, 5.0 mM EDTA, 1.0% Triton X-100, 0.5 mM dithiothreitol, 1.0 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN) on ice for 30 min. The lysates were spun at 12,500 rpm for 25 min to pellet the insoluble material, and the proteins from the supernatant were quantified using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA). After addition of 5X SDS gel loading buffer (250 mM Tris-Cl; pH 6.8, 500 mM dithiothreitol, 10% SDS, 0.5% bromophenol blue, 50% glycerol), the mixture was incubated at 37°C for 15 min. About 30 μ g of protein sample was separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, proteins were blotted onto polyvinylidene difluoride (PVDF) membrane in transfer buffer (39 mM glycine, 48 mM Tris-Cl, 20% methanol, pH 8.3) at 200 mA for 90 min using Mini-PROTEAN III (Bio-Rad). The blots were blocked with 3% bovine serum albumin in 1X Tris-buffered saline with Tween-20 (TBS-T) (20 mM Tris-Cl; pH 7.5, 150 mM NaCl, 0.1% Tween-20 (polyethylenesorbite monolaurate)) at room temperature for 1 h with agitation, washed three times with 1X TBS-T, and then incubated in 10 mL of 1X TBS-T containing the primary antibody (anti-mTRPM serum 1:5,000, anti-FLAG M2 antibody 1:7,500) at room temperature for 90 min. After being washed three times with 1X TBS-T, the membrane was incubated in 5 mL of 1X TBS-T containing the secondary antibody (1:10000, Jackson ImmunoResearch, West Grove, PA) for 45 min. The membrane was washed as above and immersed in ECL Western blotting detection reagents (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The blots were placed on a plastic wrap sheet and exposed to X-ray film (Konica, Tokyo, Japan).

Extraction of total RNA and microarray analyses

Total RNA from inducible cell-lines was extracted for microarray analysis. Expression of TRPM7 and TRPM7 Δ KD was induced by adding tetracycline to the culture medium at 1 μ g/mL. After treatment with tetracycline for 3 days, RNA was extracted. Expression of TRPM7 and TRPM7 Δ KD was confirmed using immunoblotting before extraction of RNA. In order to meet quality standards for RNA in the microarray analysis, RNeasy Mini kit (Qiagen) was used. The purity of the RNA was verified by comparing the integrity of ribosomal RNA (rRNA) using agarose gel electrophoresis and by measuring the absorbance ratio.

RNA samples prepared from cells without tetracycline (control, -Tet) and with tetracycline treatment for 3 days (+Tet) were analyzed. Probe preparation and microarray hybridization were performed by Seoulin Bioscience (Seoul, Korea). Briefly, cDNA was synthesized from total RNA using the One-Cycle cDNA Synthesis Kit (Affymetrix, Santa Clara, CA). Single stranded cDNA was synthesized using Superscript II reverse transcriptase and T7-oligo(dT) primers at 42°C for 1 h. Double stranded cDNA was obtained by using DNA ligase, DNA polymerase I and RNase H at 16°C for 2 h, and followed by T4 DNA polymerase at 16°C for 5 min for gap filling. After clean up with a Sample Cleanup Module (Affymetrix), ds-cDNA was used for *in vitro* transcription. cDNA was transcribed using the GeneChip IVT Labeling Kit (Affymetrix), in the presence of biotin-labeled CTP and UTP. After clean up with a Sample Cleanup Module (Affymetrix), fifteen microgram of labeled cRNA was fragmented from 35 to 200 bp by fragmentation buffer (Affymetrix). Fifteen micrograms of the fragmented cRNA was hybridized to a Human Genome U133 Plus 2.0 Array (Affymetrix). The array image was scanned and analyzed using Genechip operating software (GCOS) (Affymetrix). The preprocessing procedure for the cell intensity files (CEL) and the following microarray analyses were performed using GenPlex software (Istec Inc., Goyang, Korea) to evaluate the expression signals generated by the Affymetrix chip. Global scaling normalization was then performed by using MAS 5.0, after which the normalized data were log-transformed using base 2. Hierarchical clustering was also performed with the centered Pearson's correlation, using these selected genes, based on the complete linkage and distance matrix. A list of genes with an altered expression of a >2-fold increase or decrease between the -Tet and +Tet samples were collected. Genes with statistical significance have *p*-values lower than 0.05 (Welch's *t*-test). The microarray data have been deposited in the Gene Expression Omnibus (GEO) database (GEO accession no. GSE23102).

Data analysis and interpretation

Biological interpretation of genes of interest was conducted from web-based databases, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (<http://www.genome.jp/kegg/>), the Gene Ontology (GO) (<http://www.geneontology.org/>), and the pathway analysis tool, Ingenuity Pathway Analysis (IPA) (Ingenuity® System, www.ingenuity.com). The biological roles of genes of interest were inferred from the KEGG pathway and GO terminologies.

To avoid overestimation of the specific functions, the probe-oriented expression information was converted into gene-oriented expression information. In order to prevent incorrect interpretation of genes that have more than two probes in the microarray chip, we reduced the information of expression from 54,645 to 28,923 probes. The overrepresented KEGG pathways and GO terminologies for the gene set were calculated using a χ^2 test by enrichment analyses with 1,000 run times, and a *p*-value and false discovery rate (FDR) less than 0.05 were considered to be statistically significant.

The analysis using the GO terminology allowed us to categorize the affected genes based on their cellular location and biological processes. The analysis using the KEGG pathway, a collection of pathway maps representing molecular interaction and reaction networks, revealed the cellular processes affected by TRPM7 expression. The IPA program was used to investigate the interactions between the uploaded genes and other genes in the Ingenuity Pathway Knowledge Base and generate a series of networks. Data sets which contain Affymetrix probe set identifiers and fold-changes were uploaded into Ingenuity Pathway Analysis (IPA). The probe sets differentially expressed in control versus +Tet samples were also used for the analysis of canonical pathways, biological functions, and molecular networks. Fisher's exact test was used to estimate the statistical significance. Networks are scored based on the number of focus molecules analyzed and the total number of molecules in the knowledge database that could potentially be included in the networks.

Real-time polymerase chain reaction

Total RNA isolated from inducible cell-lines was reverse-transcribed to first-strand cDNA using M-MLV reverse transcriptase (Invitrogen) and oligo(dT)12–18 primer (Invitrogen). The obtained cDNA was subjected to quantitative real-time PCR in accordance with the manufacturer's instructions. PCR was performed in triplicate using 10 μ L of SYBR Premix ExTaq (Takara, Japan) and 2 μ L of cDNA as a template in 20 μ L of final volume. PCR amplification was preceded by incubation of the mixture for 15 min at 95°C, and 40 cycles of the amplification step consisted of denaturation, annealing, and extension. The denaturation was performed for 30 s at 95°C, annealing at 55°C, and extension for 30 s at 72°C with fluorescence detection after each cycle. After the final cycle, melting-point analyses of all samples were performed within the range of 65–95°C with continuous fluorescence detection. The expression level of α -tubulin was used for normalization.

Results

Generation of inducible cell-lines to identify the TRPM7-specific transcriptional alteration

Mouse TRPM7 was expressed using an inducible system in which the channel gene had been stably integrated into modified HEK 293 cells, T-RE_xTM 293 (see Methods). The expression of the channel protein was

tightly suppressed until the chemical inducer, tetracycline, was added. However, since the treatment of inducer itself could also affect the transcription profile, we established another cell-line harboring a nonfunctional TRPM7 mutant (TRPM7 Δ KD) as a control in which the entire kinase domain at the C-terminus was deleted (Fig. 1A). This kinase domain was proven to be critical for the functional activity of the TRPM7 channel [14–15].

The wild-type and mutant TRPM7 channels were expressed by treating with tetracycline and, after 48 h, were examined by immunoblot analysis using rabbit anti-TRPM7 antiserum. After the induction with tetracycline, new immunoreactive bands appeared in the cell-lines harboring genes for wild-type TRPM7 and TRPM7 Δ KD (Fig. 1B). Such protein bands were observed neither in the parental cell-line (*1st and 2nd lanes from left*), nor in the absence of tetracycline (*3rd lane from left*). The immunoreactive bands detected at 212 and 182 kDa in Figure 1B corresponded to the expected size of the wild-type and mutant channel, respectively, and the level of protein expression was comparable.

The functional activity of the wild-type and mutant channels was investigated by electrophysiological means. At 48 h after treatment with tetracycline, TRPM7 currents were recorded under whole-cell configuration. Since the channel activity of TRPM7 is inhibited by intracellular Mg²⁺, TRPM7 channel activity can be elicited during whole-cell recording by lowering internal Mg²⁺ by dialyzing cells with a pipette solution lacking Mg²⁺ [7]. When the whole-cell configuration was established, cells induced with tetracycline (+Tet) evoked a much larger whole-cell current (*filled circles*) compared with the untreated cells, –Tet (*empty circles*) (Fig. 1C). On the average, initial levels of whole-cell current in +Tet cells were about 8.8 times greater compared with –Tet cells. As the number of sweeps was increased after cell rupture and the inside of the cell was dialyzed progressively, the whole-cell current gradually increased and reached a maximum value at 5 min after whole-cell formation. In Figure 1D, the instantaneous current–voltage (*I–V*) relationship obtained at 300 s after cell rupture is shown for the induced (*black line*) and uninduced (*grey line*) cells, respectively. The outwardly rectifying *I–V* relationship of the whole-cell current closely resembled that of the cloned TRPM7 channel [7–8]. At the time-point of 300 s after whole-cell configuration, +Tet cells showed about a 7.5-fold increase in current level compared with –Tet cells. The ionic current induced by tetracycline was potently blocked by 2-aminoethoxydiphenyl borate (2-APB), a known blocker of TRPM7 (<http://life.gist.ac.kr/mnl/publication/>

Fig. 1. Generation of two inducible cell-lines for microarray analysis. Two kinds of tetracycline-inducible cell-lines were generated to investigate the alteration of transcriptome by TRPM7 channels. **A.** The diagram of the wild-type (WT) TRPM7 channel and a deletion mutant lacking the kinase domain (TRPM7 Δ KD). Each construct contained a Flag-tagged sequence in the N-terminal region. **B.** Selection of inducible cell-line, which expresses the WT (*left*) and TRPM7 Δ KD channel (*right*). Expression of the channel protein (–Tet and +Tet) in candidate cell-lines was analyzed using immunoblotting. As the control, the lysate of untransfected T-REx 293 cell-line was also included (*left* two lanes). A total of 30 μ g of cell lysate was loaded in each lane. **C** and **E.** Time-dependent current density curves of the WT and TRPM7 Δ KD channel. The current traces of the WT TRPM7 channel evoked by treatment with tetracycline (●, $n = 6$) and without tetracycline (○, $n = 5$) were shown. Voltage ramp of –120 to 100 mV from a holding potential of 0 mV was delivered for 500 ms. A current at +90 mV for each sweep was used. The current of the TRPM7 Δ KD channel was also shown (+Tet (●), $n = 10$; –Tet (○), $n = 4$). **D** and **F.** Representative I – V curves of WT and TRPM7 Δ KD channels. Whole-cell patch clamp analyses of currents produced after a break with nominal 0 Mg^{2+} pipette solution. The current traces evoked by a treatment with tetracycline (+Tet) were highlighted with black lines. Grey lines showed the current without tetracycline (–Tet).

supplement.pdf). More than 80% of tetracycline-induced outward currents were blocked by 300 μM 2-APB.

We also analyzed the inducible cell-line of the deletion mutant, TRPM7 Δ KD, by electrophysiological means. Despite the appropriate level of protein expression as evident in Fig. 1B, the TRPM7 Δ KD cells induced with tetracycline, +Tet (filled circles), did not show any additional current compared with the uninduced cells, –Tet (empty circles), at any time-points of whole-cell configuration (Fig. 1E). These levels of whole-cell current were equivalent to the endogenous current usually recorded in the parental HEK293 cells (Fig. 1F). Therefore, these results indicate that the mouse TRPM7 gene was stably integrated in the T-REx 293 cell and that a large number of functional TRPM7 channels can be induced by tetracycline. On the other hand, cells harboring the deletion mutant gene, TRPM7 Δ KD, did not provide any additional ionic currents to the cells despite the appreciable level of protein expression, and thus this cell-line could be utilized as a valid control in subsequent transcriptional profiling experiments.

Selection of differentially expressed genes by microarray analyses

Initially, we determined the concentration of tetracycline and the duration of treatment for the induction of wild-type TRPM7 and TRPM7 Δ KD. The level of protein expression was not changed significantly by the concentration of tetracycline, unless it was higher than 1 $\mu\text{g/mL}$, and reached a maximum level at 24 h after treatment (*data not shown*). For RNA preparation, the wild-type and deletion mutant were always induced for 72 h at 1 $\mu\text{g/mL}$ tetracycline.

Total RNA was prepared separately from three independent cultures of TRPM7 and TRPM7 Δ KD permanent cells before (–Tet) and after (+Tet) the tetracycline treatment. Each RNA sample was analyzed with a human genome expression array, Affymetrix Human Genome U133 Plus 2.0 ChipTM, which contains almost 45,000 probe sets representing more than 39,000 transcripts derived from approximately 33,000 well-substantiated human genes. When the transcriptional profiles were analyzed, many genes were found to be

Fig. 2. Gene expression changes in HEK cells by TRPM7 expression. Differentially expressed genes (DEG) were selected by comparing the microarray data from two different inducible cell-lines which express wild-type TRPM7 (TRPM7) and a TRPM7 deletion mutant lacking the kinase domain (TRPM7ΔKD). A. Hierarchical dendrogram of 40 representative genes that are significantly affected by TRPM7 between –Tet and +Tet. Gene expression changes are shown by the color bar where red indicates an increase and green represents a decrease. B. Differently expressed probes in samples that express the wild-type TRPM7 channel. Shown are the numbers of gene probes significantly altered by the treatment of tetracycline compared to the untreated cells in TRPM7 inducible cells in two different criteria. *Black circle*: statistically significant genes (p -value < 0.05); *grey circle*: fold-change > 2 or < 0.5. C. Differently expressed probes in samples that express the TRPM7ΔKD channel. D. Differently expressed probes that satisfy two criteria, fold-change (>2 or <0.5) and p -value (<0.05) in samples of WT (black circle) and ΔKD mutant (grey circle) TRPM7 channel. E. Volcano plot of differently expressed probes by overexpression of mouse TRPM7. Filled dots: five genes in Venn diagram D, which are affected under conditions of both WT and mutant TRPM7; empty dots: genes regulated by overexpression of WT TRPM7.

expressed differentially in the cells expressing TRPM7 (+Tet) and the control cells (–Tet) in Figure 2. The expression patterns of specific genes were substantially different between –Tet and +Tet cell-lines. Forty representative genes specifically affected by TRPM7 are shown in a hierarchical dendrogram (Fig. 2A). In total, the transcription of 4,632 probes was found to be increased or decreased more than 2-fold (Fig. 2B, *black circle*). The transcriptional changes of 4,143 probes were found to be statistically significant in Welch’s t -test with a p -value smaller than 0.05 (Fig. 2B, *grey circle*). We were able to obtain 1,264 probes that were expressed differentially by TRPM7 expression based on the cut-off criterion of 2-fold differential expression and the statistical significance at $p < 0.05$ (Fig. 2B, *overlapped area*). Table 1 shows an abbreviated list of genes with specifically increased or decreased transcription from mouse TRPM7 overexpression. We repeated identical analyses for the cells expressing the nonfunctional TRPM7 channel,

TRPM7ΔKD (+Tet) and the control cells (–Tet). Based on the criteria of fold-change and t -test, respectively, 2,374 probes (Fig. 2C, *black circle*) and 1,561 probes (Fig. 2C, *grey circle*) were found to be differentially expressed. Combining the two cut-off criteria, the transcription of 112 probes was found to be expressed differentially by the induction of the TRPM7ΔKD channel (Fig. 2C, *overlapped area*). By comparing the differentially expressed genes (DEG) by TRPM7 induction, between induced (+Tet) vs. uninduced cells (–Tet), and those by TRPM7ΔKD, we were able to find five different genes commonly found in both lists (Fig. 2D, *overlapped area*), and these genes were excluded from subsequent analyses. Finally, we were able to generate a list of 1,259 probes of which transcription was altered specifically by TRPM7 expression. These affected genes were plotted against their p -values and the fold-changes, in what is known as a ‘volcano plot’ (Fig. 2E, also *empty circles*). Five genes commonly found in TRPM7 and TRPM7ΔKD samples

Table 1. Partial lists of differentially expressed genes by overexpression of the TRPM7 channel. Microarray analysis discovered genes differentially expressed by TRPM7. All genes selected for the table show a statistically significant (p -value < 0.05) changes in transcription (Welch's t -test). A. Top 20 of the most highly up-regulated gene by TRPM7. The up-regulated gene list shown is a part from a total 631 probes. B. Top 20 of the most highly down-regulated gene by TRPM7. Gene list shown is a part from a total 628 probes.

Probe ID	Fold Change	Gene Title
A		
214502_at	46.737	histone cluster 1, H2bj
243581_at	29.942	Activating transcription factor 6
228202_at	26.306	Phospholamban
219829_at	24.868	integrin beta 1 binding protein (melusin) 2
239738_at	19.423	dachshund homolog 2 (Drosophila)
1554199_at	17.834	protein tyrosine phosphatase, receptor type, O
202411_at	15.999	interferon, alpha-inducible protein 27
239921_at	15.652	similar to matrilin 2 precursor
236562_at	14.337	zinc finger protein 439
203698_s_at	13.856	frizzled-related protein
223952_x_at	12.910	dehydrogenase/reductase (SDR family) member
234994_at	12.745	KIAA1913
235916_at	12.347	yippee-like 4 (Drosophila)
1570253_a_at	11.829	syntaxin 6; Ras homolog enriched in brain like 1
215244_at	11.698	DiGeorge syndrome critical region gene 5 (non-coding)
241510_at	11.078	similar to Phospholipid scramblase 1 (PL scramblase 1)
213451_x_at	10.826	tenascin XB
231402_at	9.637	KIAA0773 gene product
1557438_at	9.399	CDNA clone IMAGE: 5285425
229133_s_at	8.863	Zinc finger protein 397
B		
1556185_a_at	0.028	CDNA clone IMAGE:5260162
240386_at	0.052	Transient receptor potential cation channel, subfamily M, member 1
230300_at	0.055	CDNA FLJ42315 fis, clone TRACH2019661
1559652_at	0.069	Mesoderm induction early response 1, family member 3
235830_at	0.079	5'-nucleotidase domain containing 1
232086_at	0.081	Phosphoinositide-3-kinase, class 3
237363_at	0.091	Chromosome 9 open reading frame 68
239767_at	0.096	CDNA clone IMAGE:5261489
235561_at	0.102	Transcribed locus
244557_at	0.102	Transcribed locus
214713_at	0.107	YLP motif containing 1
237649_at	0.109	COP9 constitutive photomorphogenic homolog subunit 4
241962_at	0.113	5'-nucleotidase domain containing 1
1556123_a_at	0.114	Homo sapiens, clone IMAGE:4863312, mRNA
237083_at	0.116	Transcribed locus
242186_x_at	0.118	latrophilin 3
230154_at	0.120	WW domain containing adaptor with coiled-coil
234827_at	0.120	MRNA; cDNA DKFZp564M0463 (from clone DKFZp564M0463)
234998_at	0.120	CDNA clone IMAGE:5313062
1556211_a_at	0.123	Androgen-induced 1

were clustered at the lower central corners near the cut-off values (Fig. 2E, *filled circles*). Among the total 54,675 probe sets, 631 probe sets were significantly increased and 628 probe sets were significantly decreased, and these 1,259 probes were subjected to subsequent analyses. Finally, we obtained 951 genes which were significantly and specifically affected by the functional TRPM7 after subtracting the duplicated or unannotated probes.

Validation of differentially expressed genes using quantitative PCR analyses

In order to validate the microarray results, we performed quantitative real-time PCR (qRT-PCR) on nine

different genes representing up-regulated, down-regulated, and unchanged genes. The nine genes tested by qRT-PCR were *Atf3*, *Map2*, and *Rgs5* for up-regulated genes, *Sri*, *Flj22222*, and *Klhdc4* for down-regulated genes, and *Mut*, and *Tnks2*, and *Tubal1b* for unchanged genes, respectively. Since the transcription of a tubulin subunit (tubulin alpha1b), a well-known housekeeping gene, was unaltered in the microarray experiment, the corresponding gene, *Tubalb*, was chosen as a reference. A set of specific primers were designed for each gene (<http://life.gist.ac.kr/mnl/publication/supplement.pdf>), and total RNA was prepared in a fashion identical to that of the microarray experiments. Consistent

Fig. 3. Quantitative real-time PCR (qRT-PCR) of representative DEGs. Microarray data were validated with qRT-PCR of nine representative genes. A. qRT-PCR of nine genes: down-regulated, up-regulated, and unchanged genes. SRI, sorcin; FLJ22222, hypothetical protein FLJ22222; KLHDC4, kelch domain containing 4; ATF3, activating transcription factor 3; MAP2, microtubule-associated protein 2; RGS5, regulator of G-protein signaling 5; TUBA1B, alpha tubulin; MUT, methylmalonyl coenzyme A mutase; TNKS2, tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2. B. Correlation between the fold changes in the expression of nine genes by microarray analysis versus qPCR. A statistically significant correlation coefficient was found ($r^2 = 0.901$, $p < 0.0001$).

with the microarray data, a similar expression pattern was observed in the qRT-PCR results for all three groups of genes (Fig. 3A). The data from microarray analysis and qRT-PCR were highly correlated ($r^2 = 0.901$, $p < 0.0001$) (Fig. 3B). Thus, we were able to utilize the data obtained from microarray experiments for subsequent analyses with a certain degree of confidence.

Analyses of microarray data

We conducted biological interpretation of the altered transcriptome using two different web-based databases,

Fig. 4. Bioinformatic analyses of genes affected TRPM7 expression. Analyses were performed on 1,259 probes of which transcription were significantly altered by TRPM7 expression. A. Gene ontology-based analysis was performed for cellular components by using χ^2 -test. Number of genes affected by TRPM7 expression (*light gray*) was compared with number of genes chosen randomly (*dark gray*) in different cellular compartments. Genes expressed in the nucleus were significantly changed ($p = 0.005$, FDR = 0.02). FDR = False Discovery Rate. B. Gene ontology (biological process)-based analysis. Number of genes affected by TRPM7 expression (*light gray*) was compared with number of genes chosen randomly (*dark gray*) in different biological processes. Genes involved in the positive regulation of programmed cell death satisfy the p -value ($p = 0.026$, FDR = 0.14). Data analysis was conducted by using χ^2 -test. C. Analysis based on the KEGG pathway. Number of genes affected by TRPM7 expression (*light gray*) was compared with number of genes chosen randomly (*dark gray*) in cellular pathways. Focal adhesion ($p = 0.000$, FDR = 0.00), fatty acid metabolism ($p = 0.029$, FDR = 0.05), and bile acid biosynthesis ($p = 0.027$, FDR = 0.02) were discovered by χ^2 -test. D. The top 20 functional categories of genes affected by TRPM7. Biofunctions related to TRPM7 channels were identified by using IPA (Ingenuity Pathway Analysis) software.

Table 2. Ten highest scoring networks of differentially expressed genes by TRPM7. Using Ingenuity Pathway Analysis (IPA) software, genetic networks significantly affected by TRPM7 channels were generated. Total 40 networks were discovered and remaining 30 networks are shown in Table S2. Abbreviation of the molecule names is according to the recommendation of HUGO consortium.

	Molecules in Network	Score	Focus Molecules	Top Functions
1	CCT5, CCT8, CDC2L6, CTH, DHX30, E3, ELK4, ELP3, EPHA7, IFRD1, ITGA7, KIAA0090, LHX9, LPP, MAPK8IP3, MYBBP1A, NDFIP2, NEDD4L, NHP2L1, NUMA1, OSBP1, OVGPI, PLEKHO1, PPP2R2D, RIT1, RPL22, RRP9, SKIL, SMURF1, SMURF2, TMBIM4, WDR61	44	31	Genetic Disorder, Metabolic Disease, Cell Morphology
2	AK2, BCAT1, BOP1, CIDEA, DFFA, DFFB, DGKE, FXC1, HLA-B, IGF2BP1, IMPACT, ING1, MARS, METTL13, MINA, MYC, MYO5C, NAP1L1, NR2E1, PAICS, PELO, PERP, PPP6C, RARS, RFX2, SCAMP1, SR140, SUPT3H, TADA3L, TAF12, TPD52, TPP2	44	32	DNA Replication, Recombination, and Repair, Cellular Compromise, Cell Cycle
3	ACSL3, ARL3, ASXL1, CUL7, DNM2, FBXO11, FNTB, FTL, GADD45A, INPP4A, JMY, MKI67, MKI67IP, MPHOSPH6, MRE11A, PHF1, PHF19, PMEP1, PSIP1, PTP4A1, PTP4A2, RAD52, RCHY1, SFRS3, SMC4, SUB1, TBCD, TMSB15A, TP53, TRIO, UPP1, XPA	43	32	Cell Cycle, DNA Replication, Recombination, and Repair, Nucleic Acid Metabolism
4	ADAM12, AOC3, APPBP2, AREG, CD44, CHRNA3, CKMT1B, COPS4, CUL4A, CUZD1, HNRNP, HPSE, ITGB1BP2, KRT10, LAMC1, MADCAM1, MAPKAP1, MLLT3, OLIG2, PCSK5, PHIP, PPM1A, STMN4, SYNCRIP, SYTI1, ULBP2	33	26	Cell-To-Cell Signaling and Interaction, Cell-mediated Immune Response, Cellular Movement
5	ASNS, ATF3, C1R, CEBPG, CREB5, DNAJC10, EFEMP1, FOSL2, HLA-C, IFNAR1, KLRA1, LRP3, MDM4, MR1, MTCH2, NFIL3, NUDT21, PDIA2, PDIA3, PSMA2, SEC62, SEC63, SEC31A, SERP1, SRI, XBP1	33	26	Protein Trafficking, Cell Death, Cell Morphology
6	ATP11B, ATP11C, ATP9B, ATPase, CLEC11A, COX11, CRABP1, CYLD, FOS, GCNT1, HSPD1, KIAA0368, MGAT4A, MRPL20, PSMC2, RFC3, RUVBL2, SFPQ, SMARCE1, SSSCA1, STAM2, UCHL5, USP46, USP13, UTP14A, VPS36	31	26	Cellular Movement, Immune Cell Trafficking, Infectious Disease
7	ARF3, BCL11B, C11ORF30, CBX5, EEF1A1, FGF2, GRIN2C, HIRIP3, HIST1H2BK, HRSP12, LILRA2, LTB, MAGED2, MAX, MTUS1, NR2F2, PDE3B, RAB27A, STC2, SYTL5, TP73, VAMP1, ZNF808	27	23	Nervous System Development and Function, Tissue Morphology, Cellular Development
8	ALDH7A1, APOE, ARG2, CEL, ELL2, GAD2, IL1RAP, IP6K2, LPL, LRRC8C, NR1H3, OTUD7B, PLTP, PNPLA3, S1PR3, SCARB1, SLC37A4, SLC7A1, TAOK2, TFPI, TICAM2, TNFRSF12A, UNC5CL	27	23	Lipid Metabolism, Small Molecule Biochemistry, Molecular Transport
9	ADD2, AKAP9, GAP43, HIF0, HNRPDL, KPNA4, MECP2, NUP153, PIN4, PLN, PPIA, PPIL2, PPP1CB, PPP1R15A, RAB3IP, RAN, RGPD5, SLC8A1, SLC8A3, SNUPN, TNPO1, TPR	26	22	Drug Metabolism, Molecular Transport, Small Molecule Biochemistry
10	ATL3, BTG3, CAV2, CTNND1, DHRS9, FILIP1L, FRZB, GEM, GPX3, HDLBP, KLHL24, LAMB2, MIB1, NDUFA7, NDUFB4, NDUFC2, PCTK1, PGRMC2, PPP2R5C, PTPRO, SOD2, SRD5A1	25	22	Drug Metabolism, Lipid Metabolism, Small Molecule Biochemistry

GO and KEGG Pathway. The biological implication of 1,259 probes affected by TRPM7 expression was investigated by using 1,000 iterative analyses. By using iterative χ^2 -test, we discovered which GO categories or KEGG pathways are statistically over-represented in a set of differentially expressed genes from microarray data (*light grey*). Analysis using a set of genes randomly

chosen (*dark grey*) is also shown in figure as a control. The GO analysis provided two different categories significantly affected by the TRPM7: the genes located in the nucleus (GO:0005634) and the genes involved in cell-death regulation (GO:0043068) (Fig. 4A and 4B). In terms of cellular components, the nucleus met both the significance value (*p*-value) and the false discovery rate

(FDR) in GO analysis. Although the pathway involving positive regulation of programmed cell-death was found to be altered significantly ($p = 0.026$), the value of FDR was 0.14 larger than the usual criterion of <0.05 . When we checked the molecular function of the affected genes in GO, no specific function satisfying the criterion ($p < 0.05$ and $FDR < 0.05$) was revealed. KEGG pathway analysis provided three different cellular pathways significantly affected by TRPM7 expression (Fig. 4C): focal adhesion (KEGG:04510), fatty acid metabolism (KEGG:00071), and bile acid biosynthesis (KEGG:00120).

We then utilized the IPA application to find the genetic network and to assess statistically relevant biofunctions and canonical pathways regulated by TRPM7. Among the 1,259 probes identified in the microarray assay, 1,045 probes were identified in the IPA database. Biological relevance of these 1,045 probes was investigated by categorizing the dataset into biological functions. Functional categories of TRPM7-regulated genes were identified, and the top 20 categories are represented in a pie chart (Fig. 4D). The five top-ranking categories were cell growth and proliferation, skeletal and muscular disorders, cancer, cell death, and metabolic disease. Biofunctions, known to be influenced by TRPM7, were included in the list, e.g., cellular growth and proliferation, cell death, cellular movement, and cell morphology. Further analysis of DEG generated the networks in which the 1,045 probes were linked as foci. In total, 40 networks were generated that contained 10 or more focus genes. The top 10 networks are shown in Table 2, and the remaining 30 networks are shown in supplemental data (<http://life.gist.ac.kr/mnl/publication/supplement.pdf>). Each network score was larger than 9, which showed that the network was not due to random choice and was statistically significant. Networks that have a relatively high score were associated with the following pathways: cell cycle, cell morphology, cell-to-cell signaling and interaction, cellular movement, and cell death. In addition, IPA also identified the cellular pathways that met the fold-change cut-off of 2 and p -value of 0.05 (Fisher's exact test). Table 3 shows the list of 28 pathways recognized. The top 5 pathways altered by TRPM7 expression were ataxia telangiectasia-mutated gene (ATM) signaling, cell cycle, bile acid biosynthesis, arginine and proline metabolism, and p53 signaling. Two pathways, fatty acid metabolism and bile acid biosynthesis, revealed in the KEGG analysis were also found in the IPA analysis of canonical pathways. Since Ca^{2+} is an essential regulator of cell cycle and cell proliferation [24-25], and the down-regulation of TRPM7 expression was shown to inhibit

Canonical Pathways	-log(p-value)
ATM Signaling	3.25
Cell Cycle: G1/S Checkpoint Regulation	2.45
Bile Acid Biosynthesis	2.43
Arginine and Proline Metabolism	2.03
p53 Signaling	2.01
Corticotropin Releasing Hormone Signaling	1.92
Circadian Rhythm Signaling	1.91
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	1.67
Cdc42 Signaling	1.63
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	1.62
Glutamate Metabolism	1.54
Glycerolipid Metabolism	1.53
β-alanine Metabolism	1.53
Retinoic acid Mediated Apoptosis Signaling	1.50
Histidine Metabolism	1.46
Mechanisms of Viral Exit from Host Cells	1.45
RAN Signaling	1.43
Aryl Hydrocarbon Receptor Signaling	1.40
Apoptosis Signaling	1.38
Protein Kinase A Signaling	1.37
Virus Entry via Endocytic Pathways	1.36
Antigen Presentation Pathway	1.34
Protein Ubiquitination Pathway	1.34
Caveolar-mediated Endocytosis Signaling	1.33
Glycine, Serine and Threonine Metabolism	1.33
Alanine and Aspartate Metabolism	1.33
Fatty Acid Metabolism	1.31
Pyruvate Metabolism	1.31

Table 3. List of cellular pathways most affected by TRPM7 expression. IPA analysis of canonical pathways revealed the 28 cellular pathways significantly affected by TRPM7 channels which contain a p -value lower than 0.05 (Fisher's exact test).

cell growth and proliferation [9, 17], the interaction between those affected genes by TRPM7 up-regulation were examined in the cell cycle pathway. As shown in Figure 5, the transcription of several key components of the mammalian cell cycle was significantly altered by TRPM7 expression. These genes, represented in green and red boxes, included GSK3β, TGFβ, c-Myc, Cip1(p21), p53, SCF, Max, and Cyclin D. Among the canonical pathways, other representative signaling pathways, ATM and Cdc42 signaling are also shown in <http://life.gist.ac.kr/mnl/publication/supplement.pdf>.

Discussion

In this study, we examined the changes in transcriptional profiles of HEK293 cells by the overexpression of TRPM7 using microarray technology and analyzed the biological significance using bioinformatical tools. The functional activity of TRPM7 has been linked to important cellular physiology, and the altered expression of this channel was found to affect many key cellular processes. Moreover, TRPM7 was found to be up-regulated in various physiological and pathophysiological conditions.

Fig. 5. Cell-cycle pathway affected by TRPM7 expression. Genes differentially regulated by TRPM7 channels involved in the cell cycle (G1/S check point regulation) are shown in diagram by using IPA software. The cell cycle was visualized as a representative pathway affected by TRPM7. Among various genes in the cell cycle pathway, those affected significantly by TRPM7 expression are highlighted (colored boxes). Red: up-regulated genes; green: down-regulated genes.

Since it was conceivable that the up-regulation of TRPM7 might bring about substantial changes in the expression of many different genes, transcriptional profiling on the genome scale could offer a powerful approach for identifying differentially expressed genes and for discovering cellular pathways influenced by the channel function. We chose HEK293 cells for several reasons. First of all, HEK 293 cells have been widely used as prototypic cells in cell biology research for many years. Derived from primary cultures of human embryonic kidney cells by transformation with adenovirus DNA [26], these cells are easy to handle and to transfect with foreign genes. Secondly, these cells have been used heavily for expressing ion channels, including TRPM7 [7, 17, 27], and for electrophysiological as well as cell biological studies. Thirdly, an inducible expression system based on HEK 293 cells was commercially available. Using this system, we were able to achieve high-level expression of mouse TRPM7 with minimal cytotoxic effects on the channel.

Differentially expressed messages specific for TRPM7 activity were selectively obtained by subtracting those expressed in a nonfunctional TRPM7 in which the C-terminal kinase domain critical for channel function was deleted. When the differentially expressed genes were analyzed using a microarray representing more than 33,000 human genes, we obtained a list of 951 genes of which transcription was either up- or down-regulated significantly and specifically by the expression of functional TRPM7.

The biological meaning of the transcriptome change was interpreted in several aspects using bioinformatics tools. The affected genes were localized in terms of their subcellular compartments and assigned to the cellular pathways involved. We obtained the most informative results from the IPA application with which biofunctions and cellular pathways influenced by TRPM7 activity can be evaluated. The analysis revealed several cellular processes, previously reported to be influenced by TRPM7, such as cellular growth and proliferation, cell

death, cellular movement, and cell morphology (Table 2). In addition, a few different diseases, including skeletal and muscular disorders, cancer, and metabolic disease, were also suggested to be linked to TRPM7 function (Tables 2 and 3).

By placing the up- or down-regulated genes into the corresponding database, we were also able to discover the cellular networks and pathways affected by TRPM7. Intriguingly enough, the cell cycle was ranked second in both the cellular network (Table 2) and canonical pathways (Table 3). As shown in Figure 5, functional interactions were revealed among the genes, including Cyclin D, p53, and Cip21, affected by TRPM7. In human retinoblastoma, elimination of TRPM7 by siRNA markedly decelerated cell proliferation and retarded the G1/S cell cycle [9]. In mast cells, TRPM7-like MagNuM current was shown to be regulated at crucial checkpoints during cell cycle progression [28]. Thus, it remains to be explored whether the effects of TRPM7 on cell proliferation is mediated through these candidate proteins *in vivo*.

Since it is well documented that the dysregulation of the cell cycle, the series of events leading to cell division and duplication, is directly connected to various cancers, TRPM7 may be closely linked to certain types of cancer. In human gastric adenocarcinoma cells, the TRPM7 channel plays an essential role for cell survival and thus suppression of TRPM7-induced cell death in gastric cancer [11]. In addition, blockade of TRPM7 channels by Gd^{3+} and 2-APB or suppression of TRPM7 expression by siRNA inhibited the growth and proliferation of human head and neck carcinoma cells [10]. It has not yet been determined whether the aforementioned genes related to the cell cycle are differentially expressed in these cancer cells.

There are other previous reports corroborating or relating to the transcriptional profiling results presented in this study. Representative pathways that were discovered by microarray analysis are cell adhesion and cellular movement (Fig. 4). In fact, TRPM7-expressing HEK cells showed swelling and detachment of the cells [7]. The overexpression of the TRPM7 channel in HEK-293 cells induced a loss of cell adhesion, an action dependent on calpain, a calcium-dependent protease [15]. In contrast to these results, a low-level expression of TRPM7 increased the level of $[Ca^{2+}]$, and induced cell spreading and adhesion and the formation of focal adhesions by regulating actomyosin contractility [21]. It was also reported that TRPM7 channels play an important

role in the migration of tumor cells as a novel potential-regulator of the Ca^{2+} influx [29]. Extracellular Mg^{2+} and TRPM7 are important for bone formation and resorption by platelet-derived growth factor (PDGF)-induced migration of human osteoblasts [30-31].

TRPM7 channels are also related to ischemic and hypoxic damage. The expression of TRPM7 in the cortex and hippocampus was significantly increased in an ischemic condition via the trkA/PI3K cascade [22-23]. In fact, earlier studies have shown that TRPM7 plays a key role in anoxic cell death, and suppression of TRPM7 could reduce the delayed neuronal death after ischemia [19, 32]. Since TRPM7 channels contribute to the neuronal damage during ischemia by conducting redundant ion influx, which mediates Ca^{2+} overload, some pathways discovered by our study could provide crucial clues to reduce cell death in an ischemic condition.

The relationship between Ca^{2+} ions and gene expression has been investigated in several studies. The manipulation of store-operated calcium entry (SOCE) in HEK293 cells showed a change in several genes that are related to the cell cycle, cell proliferation, apoptosis, hormone, and growth factors [33]. The application of Ca^{2+} ionophore A23187 to the B lymphoblast showed a change in genes that code for cytokines, transcription or growth factor, and proteins of cell metabolism and the cell cycle [34]. Voltage-dependent Ca^{2+} channels are linked to gene expression conveying physiological functions related to circadian rhythms, long-term memory, and neuronal survival [35]. Recently, it was suggested that TRPM7 mediates intracellular zinc accumulation and zinc-induced neurotoxicity and proposed that this channel might be a novel target for neurological disorders caused by Zn^{2+} toxicity [36]. The level of intracellular zinc is known to affect the expression of p53, gadd45, and c-fos in epithelial cells [37], which is also consistent with the microarray data shown in Fig. 5 and <http://life.gist.ac.kr/mnl/publication/supplement.pdf>. In addition, the expression of genes involved in cell division, survival, and adhesion were found to be altered dramatically by the modulation of the amount of zinc ion [38].

In conclusion, we obtained transcriptional profiles of HEK293 cells altered by the overexpression of TRPM7 and revealed the cellular pathways affected by TRPM7 channels. The list of genes and cellular pathways linked to TRPM7 presented in this study will be useful in understanding the physiological function of this unique channel and unraveling the cellular processes involved.

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References

- 1 Clapham DE, Runnels LW, Strubing C: The trp ion channel family. *Nat Rev Neurosci* 2001;2:387-396.
- 2 Clapham DE: Trp channels as cellular sensors. *Nature* 2003;426:517-524.
- 3 Pedersen SF, Owsianik G, Nilius B: Trp channels: An overview. *Cell calcium* 2005;38:233-252.
- 4 Ramsey IS, Delling M, Clapham DE: An introduction to trp channels. *Annu Rev Physiol* 2006;68:619-647.
- 5 Vassort G, Fauconnier J: [transient receptor potential, trp channels: A new family of channels broadly expressed]. *Med Sci (Paris)* 2008;24:163-168.
- 6 Duncan LM, Deeds J, Cronin FE, Donovan M, Sober AJ, Kauffman M, McCarthy JJ: Melastatin expression and prognosis in cutaneous malignant melanoma. *J Clin Oncol* 2001;19:568-576.
- 7 Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, Fleig A: *Ltrpc7* is a *mg*. Atp-regulated divalent cation channel required for cell viability. *Nature* 2001;411:590-595.
- 8 Runnels LW, Yue L, Clapham DE: Trpplk, a bifunctional protein with kinase and ion channel activities. *Science* 2001;291:1043-1047.
- 9 Hanano T, Hara Y, Shi J, Morita H, Umabayashi C, Mori E, Sumimoto H, Ito Y, Mori Y, Inoue R: Involvement of trpm7 in cell growth as a spontaneously activated Ca^{2+} entry pathway in human retinoblastoma cells. *J Pharmacol Sci* 2004;95:403-419.
- 10 Jiang J, Li MH, Inoue K, Chu XP, Seeds J, Xiong ZG: Transient receptor potential melastatin 7-like current in human head and neck carcinoma cells: Role in cell proliferation. *Cancer Res* 2007;67:10929-10938.
- 11 Kim BJ, Park EJ, Lee JH, Jeon JH, Kim SJ, So I: Suppression of transient receptor potential melastatin 7 channel induces cell death in gastric cancer. *Cancer Sci* 2008;99:2502-2509.
- 12 Wykes RC, Lee M, Duffy SM, Yang W, Seward EP, Bradding P: Functional transient receptor potential melastatin 7 channels are critical for human mast cell survival. *J Immunol* 2007;179:4045-4052.
- 13 Ryazanov AG: Elongation factor-2 kinase and its newly discovered relatives. *FEBS Lett* 2002;514:26-29.
- 14 Matsushita M, Kozak JA, Shimizu Y, McLachlin DT, Yamaguchi H, Wei FY, Tomizawa K, Matsui H, Chait BT, Cahalan MD, Nairn AC: Channel function is dissociated from the intrinsic kinase activity and autophosphorylation of trpm7/chak1. *J Biol Chem* 2005;280:20793-20803.
- 15 Su LT, Agapito MA, Li M, Simonson WT, Huttenlocher A, Habas R, Yue L, Runnels LW: Trpm7 regulates cell adhesion by controlling the calcium-dependent protease calpain. *J Biol Chem* 2006;281:11260-11270.
- 16 Kozak JA, Cahalan MD: Mic channels are inhibited by internal divalent cations but not atp. *Biophys J* 2003;84:922-927.
- 17 Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, Kurosaki T, Fleig A, Scharenberg AM: Regulation of vertebrate cellular Mg^{2+} homeostasis by trpm7. *Cell* 2003;114:191-200.
- 18 Ryazanova LV, Rondon LJ, Zierler S, Hu Z, Galli J, Yamaguchi TP, Mazur A, Fleig A, Ryazanov AG: Trpm7 is essential for Mg^{2+} homeostasis in mammals. *Nat Commun* 2010;1:109.
- 19 Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W, MacDonald JF, Tymianski M: A key role for trpm7 channels in anoxic neuronal death. *Cell* 2003;115:863-877.
- 20 Dorovkov MV, Ryazanov AG: Phosphorylation of annexin i by trpm7 channel-kinase. *J Biol Chem* 2004;279:50643-50646.
- 21 Clark K, Langeslag M, van Leeuwen B, Ran L, Ryazanov AG, Figdor CG, Moolenaar WH, Jalink K, van Leeuwen FN: Trpm7, a novel regulator of actomyosin contractility and cell adhesion. *EMBO J* 2006;25:290-301.
- 22 Zhao L, Wang Y, Sun N, Liu X, Li L, Shi J: Electroacupuncture regulates trpm7 expression through the trka/pi3k pathway after cerebral ischemia-reperfusion in rats. *Life Sci* 2007;81:1211-1222.
- 23 Jiang H, Tian SL, Zeng Y, Li LL, Shi J: Trka pathway(s) is involved in regulation of trpm7 expression in hippocampal neurons subjected to ischemic-reperfusion and oxygen-glucose deprivation. *Brain Res Bull* 2008;76:124-130.
- 24 Means AR: Calcium, calmodulin and cell cycle regulation. *FEBS Lett* 1994;347:1-4.
- 25 Berridge MJ, Bootman MD, Lipp P: Calcium—a life and death signal. *Nature* 1998;395:645-648.
- 26 Graham FL, Smiley J, Russell WC, Nairn R: Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 1977;36:59-74.
- 27 Runnels LW, Yue L, Clapham DE: The trpm7 channel is inactivated by pip(2) hydrolysis. *Nat Cell Biol* 2002;4:329-336.
- 28 Tani D, Monteilh-Zoller MK, Fleig A, Penner R: Cell cycle-dependent regulation of store-operated i(crac) and mg^{2+} -nucleotide-regulated magnum (trpm7) currents. *Cell calcium* 2007;41:249-260.

- 29 Chen JP, Luan Y, You CX, Chen XH, Luo RC, Li R: Trpm7 regulates the migration of human nasopharyngeal carcinoma cell by mediating Ca^{2+} influx. *Cell calcium* 2010;47:425-32.
- 30 Abed E, Moreau R: Importance of melastatin-like transient receptor potential 7 and cations (magnesium, calcium) in human osteoblast-like cell proliferation. *Cell Prolif* 2007;40:849-865.
- 31 Abed E, Moreau R: Importance of melastatin-like transient receptor potential 7 and magnesium in the stimulation of osteoblast proliferation and migration by platelet-derived growth factor. *Am J Physiol Cell Physiol* 2009;297:C360-368.
- 32 Sun HS, Jackson MF, Martin LJ, Jansen K, Teves L, Cui H, Kiyonaka S, Mori Y, Jones M, Forder JP, Golde TE, Orser BA, Macdonald JF, Tymianski M: Suppression of hippocampal trpm7 protein prevents delayed neuronal death in brain ischemia. *Nat Neurosci* 2009;12:1300-1307.
- 33 Zagranichnaya TK, Wu X, Danos AM, Villereal ML: Gene expression profiles in hek-293 cells with low or high store-operated calcium entry: Can regulatory as well as regulated genes be identified? *Physiol Genomics* 2005;21:14-33.
- 34 Kozian D, Proulle V, Nitsche A, Galitzine M, Martinez MC, Schumann B, Meyer D, Herrmann M, Freyssinet JM, Kerbiriou-Nabias D: Identification of genes involved in Ca^{2+} ionophore a23187-mediated apoptosis and demonstration of a high susceptibility for transcriptional repression of cell cycle genes in b lymphoblasts from a patient with scott syndrome. *BMC Genomics* 2005;6:146.
- 35 Deisseroth K, Mermelstein PG, Xia H, Tsien RW: Signaling from synapse to nucleus: The logic behind the mechanisms. *Curr Opin Neurobiol* 2003;13:354-365.
- 36 Inoue K, Branigan D, Xiong ZG: Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels. *J Biol Chem* 2010;285:7430-7439.
- 37 Fanzo JC, Reaves SK, Cui L, Zhu L, Wu JY, Wang YR, Lei KY: Zinc status affects p53, gadd45, and c-fos expression and caspase-3 activity in human bronchial epithelial cells. *Am J Physiol Cell Physiol* 2001;281:C751-757.
- 38 Liu CG, Zhang L, Jiang Y, Chatterjee D, Croce CM, Huebner K, Fong LY: Modulation of gene expression in precancerous rat esophagus by dietary zinc deficit and replenishment. *Cancer Res* 2005;65:7790-7799.