Original Article

Transcriptome profiling implicated in beneficiary actions of kimchi extracts against *Helicobacter pylori* infection

Jong Min Park,[1] Young Min Han,[2] Ji Young Oh,[3] Dong Yoon Lee,[3] Seung Hye Choi,[3] and Ki Baik Hahm[4,5,*]

[1]Daejeon University School of Oriental Medicine, Daejeon, 34520, Korea
[2]Seoul Center, Korea Basic Science Institute, Seoul, 02456, Korea
[3]CJ Food Research Center, Suwon, 16471, Korea
[4]CHA Cancer Preventive Research Center, CHA Bio Complex, Pangyo, 13497, Korea
[5]Medpacto Research Institute, Medpacto, Seoul, 06668, Korea

(Received 14 July, 2020; Accepted 15 November, 2020; Published online 27 March, 2021)

Dietary intervention to prevent *Helicobacter pylori* (*H. pylori*)-gastric cancer might be ideal because of no risk of bacterial resistance, safety, and rejuvenating action of atrophic gastritis. We have published data about the potential of fermented kimchi as nutritional approach for *H. pylori*. Hence recent advances in RNAseq analysis lead us to investigate the transcriptome analysis to explain these beneficiary actions of kimchi. gastric cells were infected with either *H. pylori* or *H. pylori* plus kimchi. 943 genes were identified as significantly increased or decreased genes according to *H. pylori* infection and 68 genes as significantly changed between *H. pylori* infection and *H. pylori* plus kimchi ($p<0.05$). Gene classification and Medline database showed DLL4, FGF18, PTPRN, SLC7A11, CHAC1, FGF21, ASAN, CTH, and CREBRF were identified as significantly increased after *H. pylori*, but significantly decreased with kimchi and NEO1, CLDN8, KLRG1, and IGFBP1 were identified as significantly decreased after *H. pylori*, but increased with kimchi. After KEGG and STRING-GO analysis, oxidative stress, ER stress, cell adhesion, and apoptosis genes were up-regulated with *H. pylori* infection but down-regulated with kimchi, whereas tissue regeneration, cellular anti-oxidative response, and anti-inflammation genes were reversely regulated with kimchi ($p<0.01$). Conclusively, transcriptomes of *H. pylori* plus kimchi showed significant biological actions.

***Key Words*: *H. pylori*, RNAseq, transcriptome, biomarker, kimchi**

Researchers unanimously agreed that RNA sequencing (RNAseq) and bioinformatics analysis has introduced exciting opportunities to either biomedical or clinical researches as the "go-to technology" producing high-throughput gene expression profiling, more sensitive than microarray platform, very robust, rather precise, and highly quantitative,[1–3] by which RNAseq analysis is being used to study the dynamics and complexity of eukaryotic transcriptomes, giving new biological insights into the active genome, fulfilling "seeing is believing".[3]

Kimchi is a traditional Korean fermented healthy food featured with profuse probiotics through lactic acid fermentation initiated by red pepper, high levels of phytochemicals, vitamins, minerals, and dietary fibers.[4] Though kimchi is a salt containing food, can be misunderstood as procarcinogen food, a well-designed case-control study revealed kimchi significantly decreased the risk of gastric cancer.[5–7] Therefore, as experienced in western countries that yogurt and other fermented milk products had been important sources for probiotics, fermented kimchi is defined as "probiotic food" in Korea because it contains myriad types of probiotic lactobacillus, *L. plantarum* as a representative probiotic strain, in which red pepper powder played significant contribution to fermentation.[8,9] Our research group have published that kimchi can be improved into cancer preventive food factor through adding more gradients, so called "cancer preventive kimchi" (abbreviated "cpkimchi").[10,11]

Under the hypothesis that dietary intervention of fermented cpkimchi can prevent *H. pylori*-associated gastric cancer in mice model, long-term intervention of cpkimchi showed significant preventive effects of *H. pylori*-induced gastric cancer either through the rejuvenation of *H. pylori*-associated precancerous atrophic gastritis or blocking gastric tumorigenesis, for instances, STAT3 inhibition, 15-PGDH induction, and inhition of IL-6/IL-6R/JAK.[11–14] In order to explore transcriptome analysis implicated in these cancer preventive actions of kimchi, in the current study, using RNAseq and bioinformatic analysis, we explored gene profiling analyses to document the beneficiary mechanisms of kimchi extracts against *H. pylori* infection and we could discover the contributing core genes to explain cancer preventive actions of kimchi.

Material and Methods

***H. pylori* culture.** *H. pylori* strain ATCC 43504 (American Type Culture Collection, *Cag*A+ and *Vac*A s1-m1 type's strain) was used. *H. pylori* were cultured at 37°C in BBL Trypticase soy (TS) agar plate with 5% sheep blood (TSAII; BD Biosciences, Franklin Lakes, NJ) under microaerophilic condition (BD GasPaK EZ Gas Generating Systems, BD Biosciences) for 3–5 days. The bacteria were harvested in clean TS broth, centrifuged at 3000 × *g* for 5 min, and resuspended in the broth at a final concentration of 10^9 colony-forming units (CFUs)/ml.

Kimchi preparation and extracts for *in vitro* experiment. Kimchi preparation was based on the standardized kimchi recipe (skimchi) in CJ Food Research Center, Suwon, Korea. First of all, skimchi is made of birned baechu cabbage (a kind of Chinese cabbage), red pepper powders, garlic, ginger, anchovy juice, sliced redish, green onion, some sugar, then fermented for some periods yielding lactobacillus like *L. plantarum*. In addition to these ingredients necessary for skimchi production, additional supplements such as mustard leaf, Chinese pepper, pear, mushroom, and sea tangle juice instead of anchovy juice were included in cancer preventive kimchi (cpkimchi). In order to treat to cell, kimchi was freeze-dried and grounded into a

*To whom correspondence should be addressed.
E-mail: hahmkb@hotmail.com

doi: 10.3164/jcbn.20-116

fine powder. The kimchi powder underwent an extraction process with 20 times of methanol by stirring overnight. Finally, the kimchi methanol extracts were concentrated by heat evaporation (Büchi Rotavapor RE 111; BÜCHI, Flawil, Switzerland) and stored at 4°C. skimchi and cpkimchi extracts was dissolved into 2.5 mg/ml in order to execute *in vitro* experiment.

Cell culture. AGS cells were purchased from ATCC (Manassas, VA), where the cells were properly stored and routinely authenticated (including DNA fingerprinting). After resuscitation in our lab, all the cells were used no longer than 6 months. AGS cells were cultured in RPMI-1640 medium (Gibco BRL, Gaithersburg, MD). All mediums supplemented with 10% fetal bovine serum (Gibco BRL) at 37°C in 5% CO_2. AGS cells were pretreated with kimchi (5 μg/ml) for 1 h and stimulated with *H. pylori* [100 multiplicity of infection (MOI)] for 24 h. The control group was loaded with the same concentration of the dissolving media of DMSO.

RNA isolation and RNAseq. Total RNA was isolated using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA). RNA quality was assessed by Agilent 2100 bioanalyzer using the RNA 6000 Nano Chip (Agilent Technologies, Amstelveen, The Netherlands), and RNA quantification was performed using ND-2000 Spectrophotometer (Thermo Inc., Wilmington, DE). For control and test RNAs, the construction of library was performed using QuantSeq 3'mRNA-Seq Library Prep Kit (Lexogen, Inc., Vienna, Austria) according to the manufacturer's instructions. In brief, each 500 ng total RNA were prepared and an oligo-dT primer containing an Illumina-compatible sequence at its 5'end was hybridized to the RNA and reverse transcription was performed. After degradation of the RNA template, second strand synthesis was initiated by a random primer containing an Illumina-compatible linker sequence at its 5'end. The double-stranded library was purified by using magnetic beads to remove all reaction components. The library was amplified to add the complete adapter sequences required for cluster generation. The finished library is purified from PCR components. High-throughput sequencing was performed as single-end 75 sequencing using NextSeq 500 (Illumina, Inc., San Diego, CA).

Data analysis and pathway analysis. QuantSeq 3'mRNA-Seq reads were aligned using Bowtie2.[15] Bowtie2 indices were either generated from genome assembly sequence or the representative transcript sequences for aligning to the genome and transcriptome. The alignment file was used for assembling transcripts, estimating their abundances and detecting differential expression of genes. Differentially expressed gene were determined based on counts from unique and multiple alignments using coverage in Bedtools.[16] The RC (Read Count) data were processed based on quantile normalization method using EdgeR within R (R development Core Team, 2016) using Bioconductor.[17] Gene classification was based on searches done by DAVID (http://david.abcc.ncifcrf.gov/) and Medline databases (http://www.ncbi.nlm.nih.gov).[18] Pathway analysis was performed on differentially expressed genes basted on the Kyoto Encyclopedia of Genes and Genome (KEGG) pathway databases.[19]

Reverse transcription-polymerase chain reaction. After treatment, the medium was removed by suction, and cells were washed with Dulbecco's PBS twice. RiboEX (500 μl; GeneAll, Seoul, Korea) was added to plates that were incubated for 10 min at 4°C. RiboEX was harvested and placed in a 1.5-ml tube, and 100 μl of chloroform was added and gently mixed. After incubation for 10 min in ice, samples were centrifuged at 10,000 × *g* for 30 min. Supernatants were extracted and mixed with 200 μl of isopropanol, and mixtures were incubated at 4°C for 1 h. After centrifuging at 13,000 × *g* for 30 min, the pellet was washed with 70% (v/v) ethanol. After allowing the ethanol to evaporate completely, the pellet was dissolved in 100 μl of diethylene pyrocarbonate–treated water (Invitrogen). cDNA was prepared using a reverse transcriptase originating from Murine-Moloney leukemia virus (Promega, Madison, WI), according to the manufacturer's instructions. The polymerase chain reaction (PCR) primers used are shown in Supplemental Table 1*. PCR was performed using over 30 cycles of 94°C for 20 s, 58°C for 30 s, and 72°C for 45 s. Oligonucleotide primers were purchased from Bioneer (Seoul, Korea).

Statistical analysis. The data are represented as mean ± SD of the experiments. Either Student's *t* test or a two-way analysis of variance with a post-hoc test was performed to determine the differences between the groups using a commercially available program (SPSS 12 for Windows; SPSS Inc., Chicago, IL). The level of significance was 0.05.

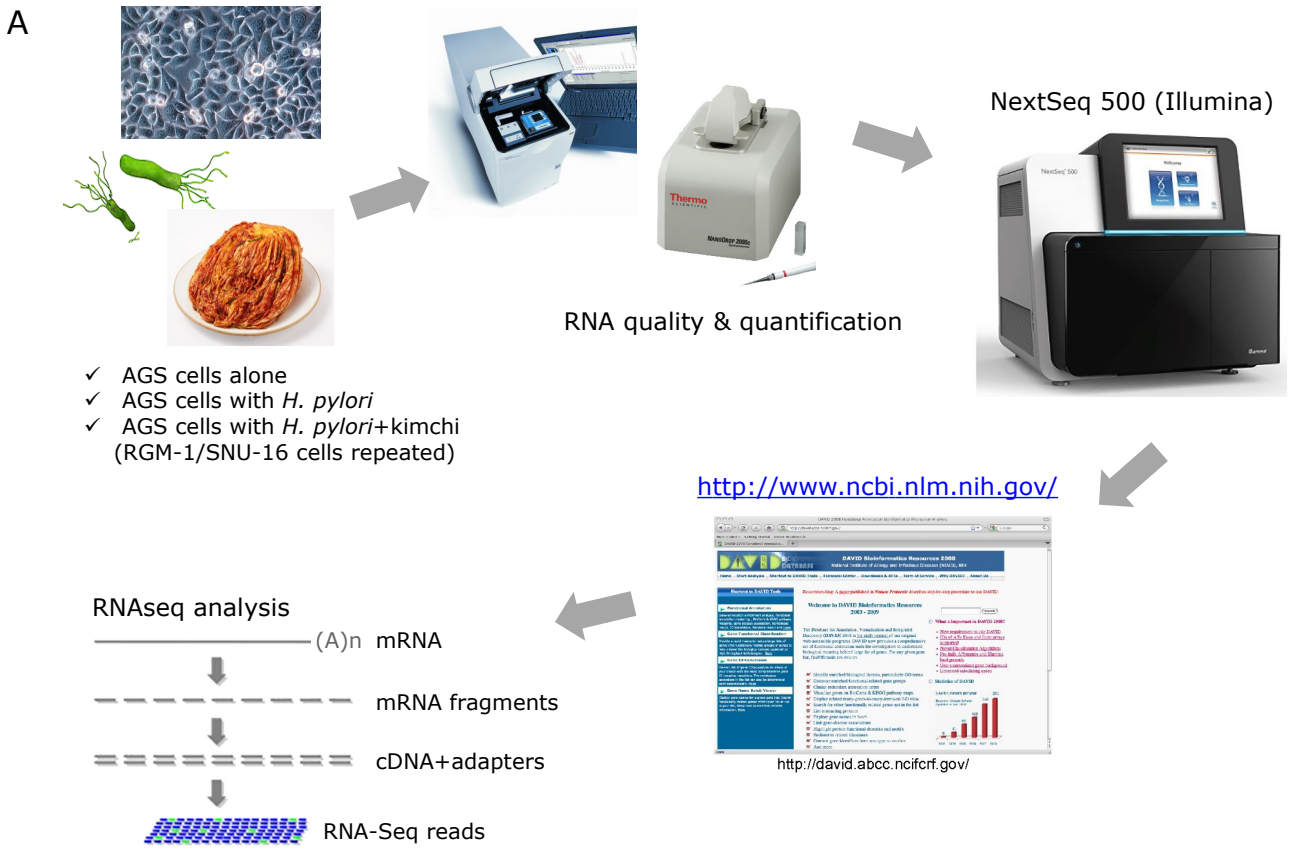
Results

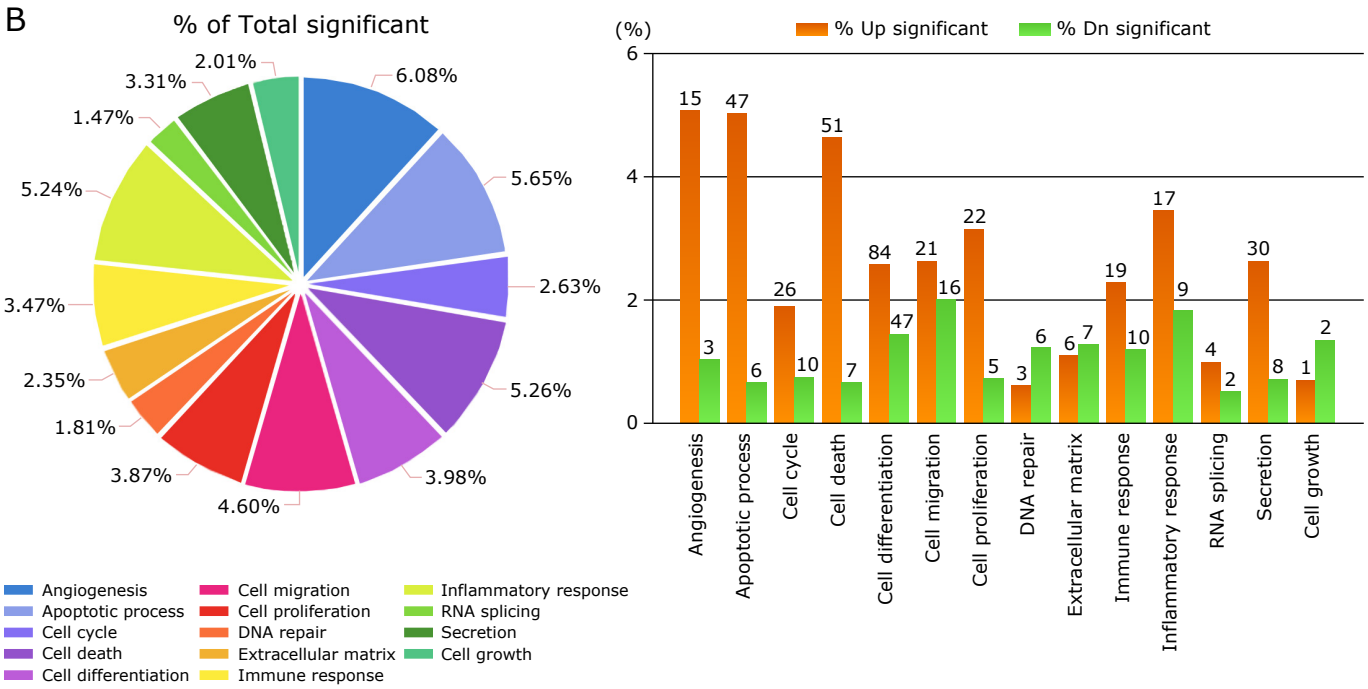
RNAseq analysis. Previous study under the title that dietary intake of fermented kimchi prevented colitis-associated cancer,[20] fermented kimchi prevented *H. pylori*-associated gastric cancer via rejuvenation of chronic atrophic gastritis, and prevention of *H. pylori*-associated gastric cancer with kimchi consistently showed the efficient cancer preventive effect of dietary intake of fermented kimchi on *H. pylori*-associated gastric tumorigenesis.[10] Therefore, using recent advances in precision medicine, in this study, we performed RNAseq analysis in control cells, cells infected with *H. pylori*, and cells infected with *H. pylori* in the presence of extracts from fermented kimchi (Fig. 1A), for which detailed methods are described above. As results, 943 genes were revealed to be significantly different between AGS cells alone and AGS cells infected with *H. pylori* (100 MOI, 48 h) (Fig. 1B) and 68 genes were revealed to be significantly different between AGS cells infected with *H. pylori* (100 MOI) and AGS cells infected with *H. pylori* in the presence of fermented kimchi extracts (Fig. 1C). All of discovered genes were categorized according to gene function and status of gene expression, up and down and the analyses were all repeated in triplicate manner, of which analysis was repeated with pooling of samples as validation manner.

Genes category significantly different that elevated with *H. pylori* infection, but significantly decreased with kimchi administration. Figure 2A showed heatmap comparing the genes, which were significantly increased after *H. pylori* infection, whereas significantly decreased in the presence of kimchi, signifying that transcriptome analysis revealed the genes implicated in antagonizing genes associated with *H. pylori* infection. As summarized in Fig. 2A, they were genes engaged in intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress like CEBPB/CHAC1/DDIT3/PPP1R15A, IL-6 signaling pathway genes like CEBPB/FOS/SOS1, genes implicated in endoplasmic reticulum stress (ER stress) like CHOP-C/EBP complex (CEBPB/DDIT3), endoplasmic reticulum unfolded protein response (UPR) including CTH/FGF21/PPP1R15A/CEBPB/DDIT3, PERK-mediated unfolded protein response like DDIT3/ASNS, genes showing response to toxic substance like FOS/ASNS/SLC7A11, apoptosis genes like CHAC1/DDIT3/BIRC3/PPP1R15A/PTPRH, TNF signaling pathway genes like CEBPB/FOS/BIRC3, and mitogen activated protein kinase (MAPK) signaling pathway genes like DDIT3/FOS/SOS1/FGF21. Using KEGG analysis, a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies, representational transcriptomic presentations of discovered genes were shown in Fig. 2B (apoptosis related-), Fig. 2C (TNF signaling related-), Fig. 2D (cancer pathway related-), and

*See online. https://doi.org/10.3164/jcbn.20-116

doi: 10.3164/jcbn.20-116





AGS cells vs AGS cells infected with *H. pylori*
943 genes (p<0.05)

Fig. 1. RNAseq analysis in normal, *H. pylori* infection, and *H. pylori* under kimchi treatment. (A) *In vitro* AGS cells infected with *H. pylori*, and infected with *H. pylori* in the presence of kimchi, triplicate repeated, NextSeq 500 (Illumina). (B) Gene analyzed according to significantly changed after *H. pylori*, 943 genes included within p<0.05. (C) Gene analyzed according to significantly changed after *H. pylori* in the presence of kimchi extracts, 68 genes included within p<0.05. See color figure in the on-line version.

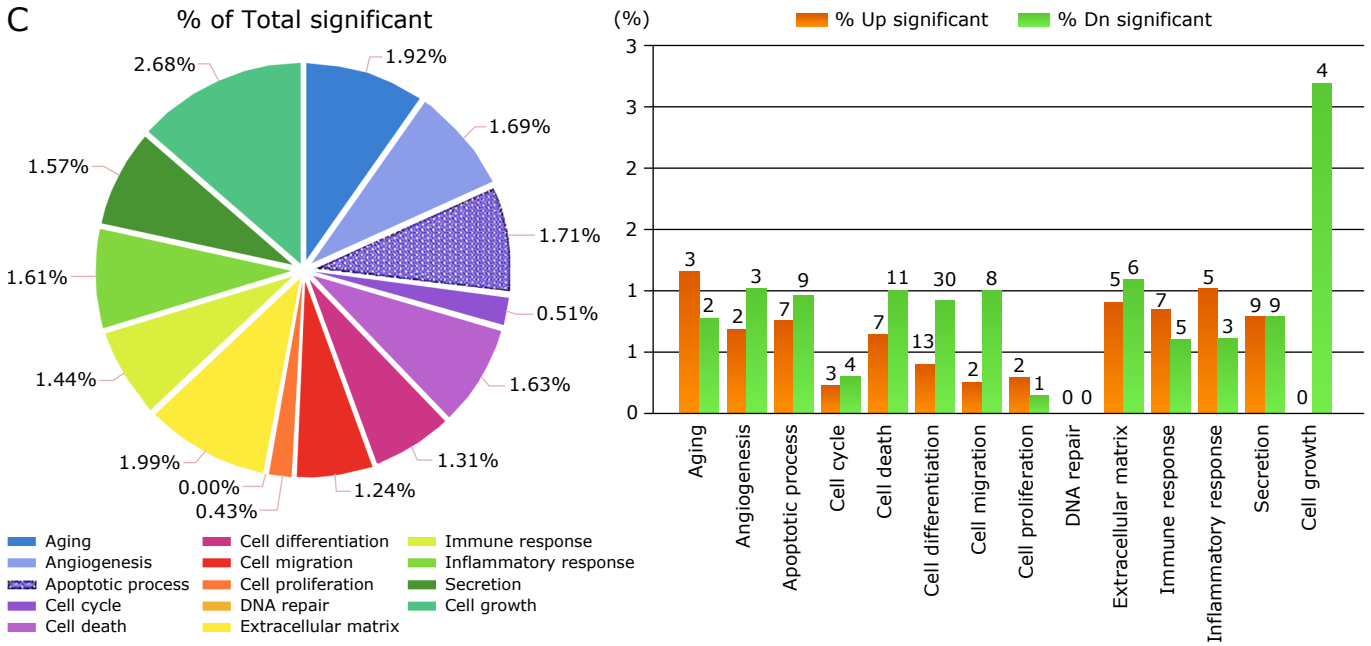


Fig. 1. continued.

Fig. 2E (PI3K/AKT signaling related-) were identified, signals elevated after *H. pylori* infection, but significantly down-regulated with kimchi extract administration in the presence of *H. pylori* infection. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analysis, a database that comprises established and predicted protein interactions to predict protein interactions among differentially expressed genes, was shown in Supplemental Fig. 1A* (inflammatory and oxidative stress related-), Supplemental Fig. 1B* (intrinsic apoptotic signaling pathway in response to ER stress related-), and Supplemental Fig. 1C* (CHOP-C/EBP complex related). Among discovered genes, core lists of gene, lists of genes called "Kimchi Response Gene Signature" (KRGS) were included in Table 1; List of genes showing significant difference between non-infected vs *H. pylori* infected vs *H. pylori* + kimchi (significantly increased after *H. pylori*, but significantly decreased after *H. pylori* in the presence of kimchi, $p<0.05$).

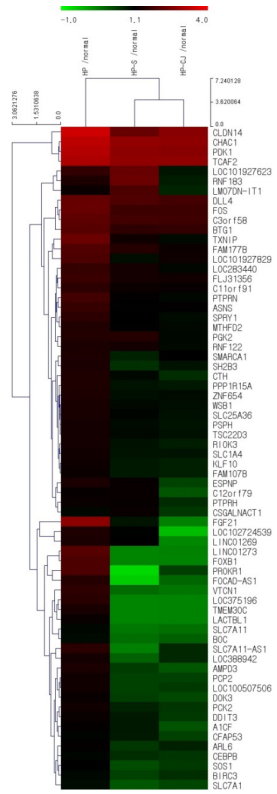
Genes category significantly different that decreased with *H. pylori* infection, but significantly increased with kimchi administration. Figure 3A showed heatmap comparing the genes, which were significantly decreased after *H. pylori* infection, whereas significantly increased in the presence of kimchi, signifying that transcriptome analysis revealed the genes implicated in compensating genes associated with *H. pylori* infection. As summarized in Fig. 3A, they were genes engaged in chemokine receptor family like CCR4/CCR7/ACKR4, positive regulation of interferon-gamma production like SLAMF6/LTA/HLA-DPB1, cell cycle regulator genes like SPDYC/SPDYE4, defense response to gram-positive bacterium like ACP5/LTA/SSC5D, chemokine receptor activity gene like CCR4/ACKR4, and cytokine-cytokine receptor interaction genes like CCR4/CCR7/TNFRSF8/LTA. Using KEGG analysis, representational transcriptomic presentations of discovered genes were shown in Fig. 3B (cytokine-cytokine receptor interaction related-), Fig. 3C (endocrine related-), and Fig. 3D (MAPK signaling pathway related-) were identified, signals decreased after *H. pylori* infection, but significantly up-regulated with kimchi extract administration in the presence of *H. pylori* infection. STRING analysis was shown in Supplemental Fig. 2*. Among discovered genes, core lists of gene, lists of genes called "KRGS" were included in Table 2; List of genes showing significant difference between non-infected vs *H. pylori* infected vs *H. pylori* + kimchi (significantly decreased after *H. pylori*, but significantly increased after *H. pylori* in the presence of kimchi ($p<0.05$).

GO analysis. KRGS can be summarized as follows; GO analysis shown in Fig. 4A showed *H. pylori* triggered significant bursts of oxidative stress genes, ER stress, increased cell adhesion genes, and inflammatory angiogenesis in gastric epithelial cells, but these genetic explosion was significantly attenuated with kimchi extract, concluding these transcriptomes were categorized as KRGS implicated in down-regulated genes with kimchi to ameliorate *H. pylori*-associated damaging. Based on transcriptomic analysis shown in Table 1, some genes were validated with RT-PCR; delta-like protein 4 (DLL4) and fibroblast growth factor 18 (FGF18) as biomarker for angiogenic factors to promote *H. pylori*-inflammation (Fig. 4B), 4 members of FOS, FOSB, FOSL1, and FOSL2 (FOS), thioredoxin interacting protein 2 (TXNIP), protein tyrosine phosphatase receptor type N (PTPRN), and cystine/glutamate antiporter (SLC7A11) as oxidative stress imposed by *H. pylori* infection (Fig. 4C), ChaC glutathione specific gamma-glutamylcyclotransferase 1 (CHAC1), fibroblast growth factor 21 (FGF21), Asparagine synthetase glutamine-hydrolyzing (ASNS), cystathione gamma-lyase (CTH), and CREB3 regulatory factor (CREBRF) as ER stress genes afforded with *H. pylori* infection (Fig. 4D). On the other hand, GO analysis shown in Fig. 4E showed that *H. pylori* infection led to significant disturbance of tissue regeneration, cellular defense system, and antioxidative defense, but kimchi administration led to significant cytoprotection in this matter. Based on transcriptomic analysis shown in Table 2, some genes were validated with RT-PCR; neogenin 1 (NEO1), claudin8 (CLDN8), killer cell lectin-like

*See online. https://doi.org/10.3164/jcbn.20-116

doi: 10.3164/jcbn.20-116

A



Genes category different significantly between *H. pylori* infection ↑ and kimchi ↓
(68 genes, $p<0.05$)

* Intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress → CEBPB/CHAC1/DDIT3/PPP1R15A
* IL-6 signaling pathway → CEBPB/FOS/SOS1
* CHOP-C/EBP complex → CEBPB/DDIT3
* Endoplasmic reticulum unfolded protein response (UPR) → CTH/FGF21/PPP1R15A
* Response to endoplasmic reticulum stress (ER stress) → CEBPB/DDIT3/PPP1R15A
* Response to toxic substance → FOS/ASNS/SLC7A11
* PERK-mediated unfolded protein response → DDIT3/ASNS
* Apoptosis → CHAC1/DDIT3/BIRC3/PPP1R15A/PTPRH
* TNF signaling pathway → CEBPB/FOS/BIRC3
* MAPK signaling pathway → DDIT3/FOS/SOS1/FGF21

B

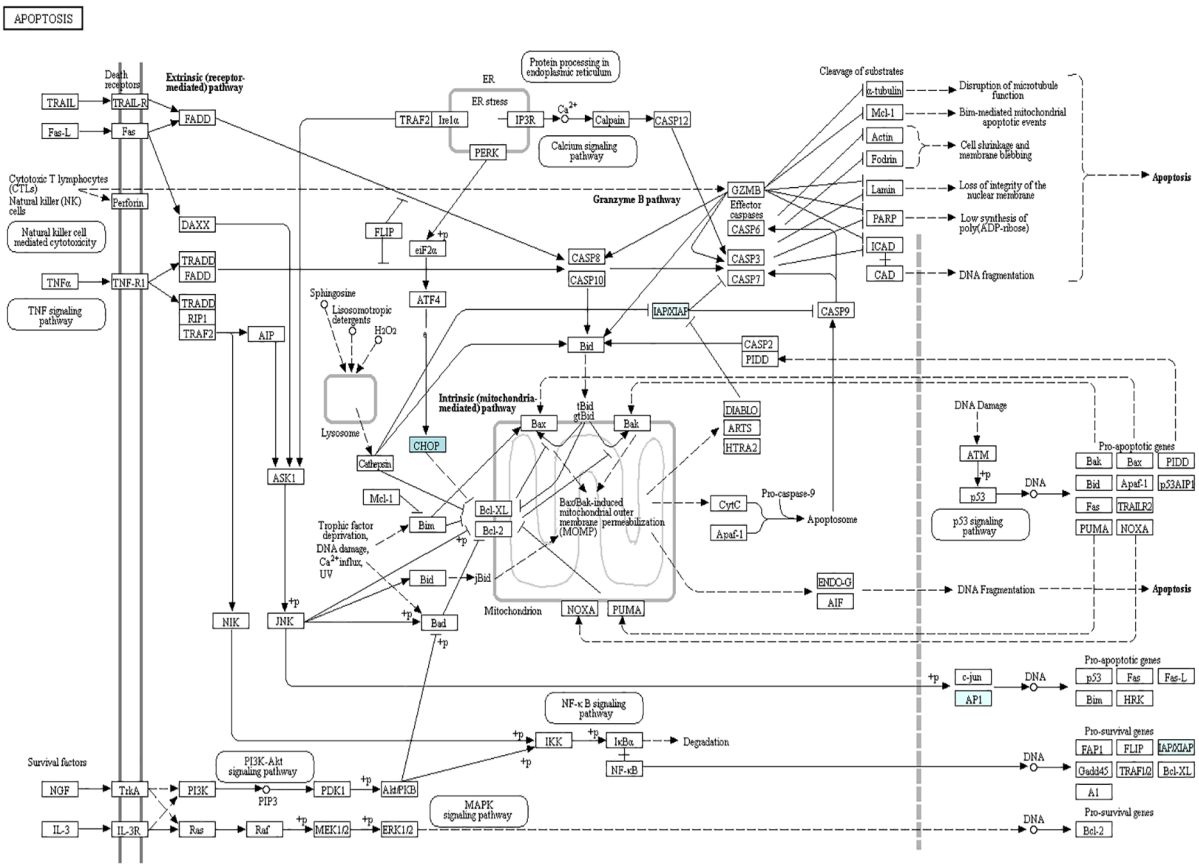


Fig. 2. (A) Heatmap showing significant trend like decreased with *H. pylori* infection, but increased with co-treatment of kimchi, description of gene category. (B–E) KEGG analysis. See color figure in the on-line version.

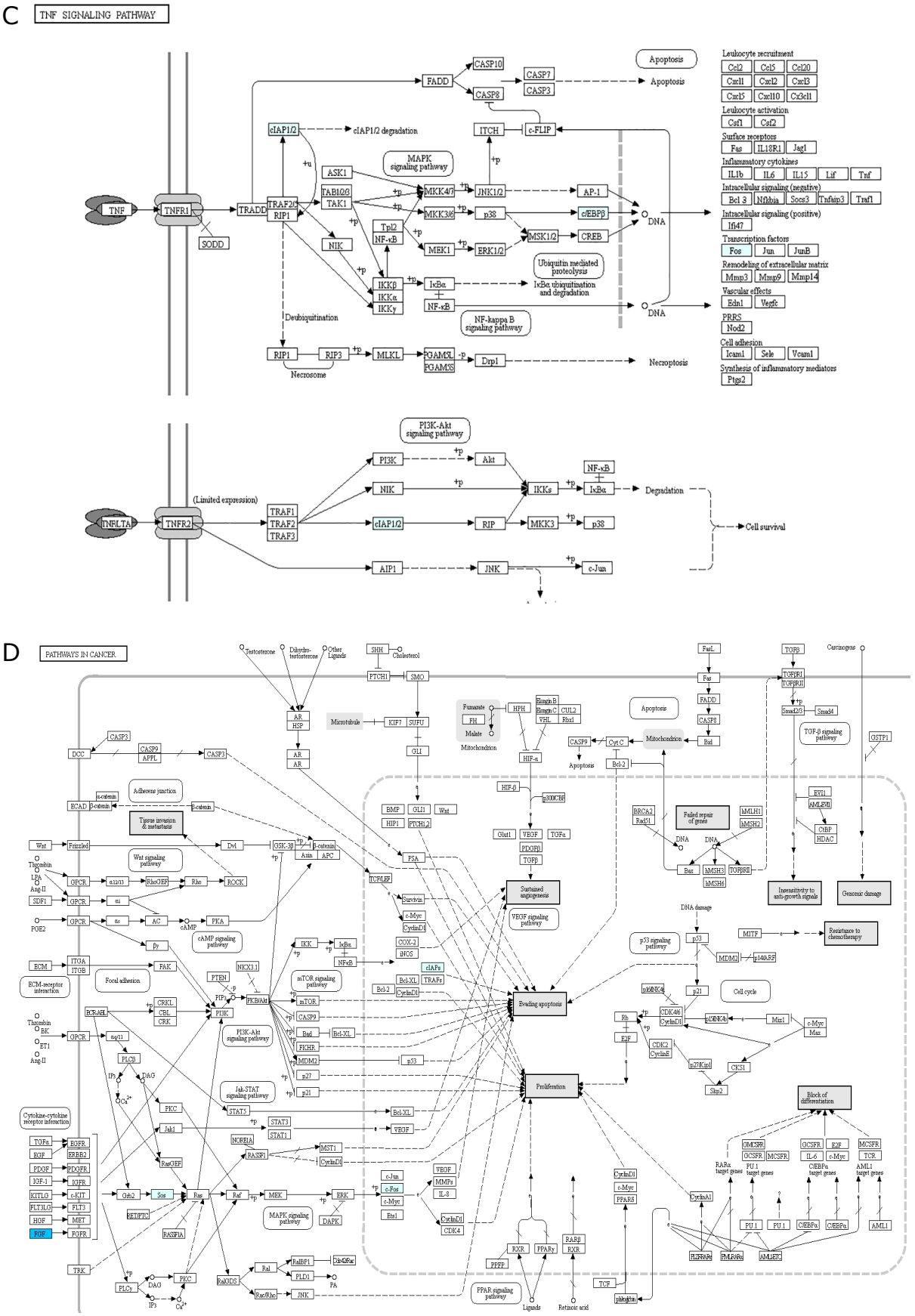


Fig. 2. continued.

doi: 10.3164/jcbn.20-116

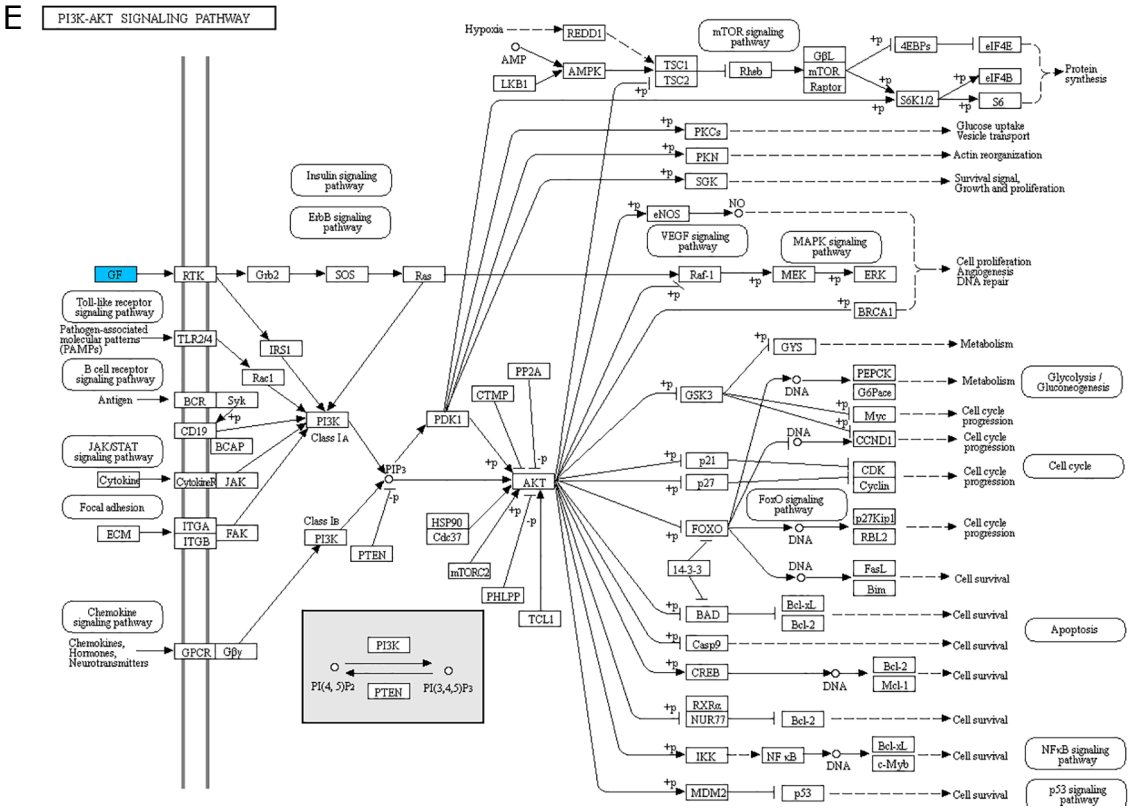


Fig. 2. continued.

receptor G1 (KLRG1), and insulin-like growth factor-binding protein 1 (IGFBP1) (Fig. 4E).

Discussion

In the current study, we conducted gene profiling analyses of *H. pylori*-infected AGS cells influenced with kimchi extracts administration through RNAseq analysis and determined that ER stress/oxidative stress/apoptosis as well as tissue regeneration-related genes played a crucial role in dietary contribution of kimchi against *H. pylori* infection after bioinformatic analysis (Fig. 4). Among genetic influences after *H. pylori* infection, we could isolate genes which were elevated after *H. pylori*, but significantly decreased with kimchi and genes which were significantly decreased after *H. pylori* infection, but significantly increased with kimchi ($p<0.01$). The fact that previous publication that dietary intake of kimchi, especially fermented kimchi, significantly prevented *H. pylori*-associated gastric cancer via the rejuvenation of precancerous atrophic gastritis was also be explained by similar findings drawn from the current investigation increases the significance of current transcriptome analysis.[10] NGS technology has revolutionized genetic and biomedical research.

RNAseq is the use of NGS technology to sequence cDNA (reversed transcribed from RNA) in order to obtain information about RNA. Compared to microarray technology, RNAseq technology offers several obvious advantages. First, RNAseq analysis allows the detection of all isoforms of a gene, even novel ones. Microarray, on the other hand, relies purely on previous knowledge regarding genes to design probes for detection, thus it cannot be used for novel detection. Second, the resolution of microarray usually stays at the gene and exon level, but the resolution of RNAseq can reach the level of a single nucleotide, which allows detection of single nucleotide variance and structural variants, such as small insertions, deletions, alternative splicing, and gene fusion.[21–23] These RNAseq analyses had several advantages over microarrays, to be very robust, more sensitive, highly quantitative, highly reproducible, and highly accurate. Therefore, the findings from the current investigation highlight the implication of dietary, non-microbial, nutritional intervention can be of potential trial for troublesome *H. pylori* infection beyond simple eradication. We clearly can explain how kimchi can fight against *H. pylori* infection, relieving ER stress, decreasing oxidative stress, alleviating apoptosis, mitigating oncogenic inflammation, and affording rejuvenating and cancer preventive actions in chronic *H. pylori* infection. Conclusively, gene classification DAVID and Medline database showed genes like DLL4, FGF18, PTPRN, SLC7A11, CHAC1, FGF21, ASAN, CTH, and CREBRF were identified as significantly increased after *H. pylori*, but significantly decreased with kimchi, whereas genes like NEO1, CLDN8, KLRG1, and IGFBP1 were identified as significantly decreased after *H. pylori*, but significantly increased with kimchi.

Detailly inspected with description for discovered gene, genes for ER stress. Subsequent apoptosis, and oxidative stress were all identified as genes which are elevated after *H. pylori* infection, but significantly attenuated in the presence of kimchi (Fig. 2, Supplemental Fig. 1*, and Table 1); ER stress genes including CEBPB/CHAC1/DDIT3/PPP1R15A, IL-6 signaling pathway genes like CEBPB/FOS/SOS1, genes implicated in ER stress complex like CEBPB/DDIT3, UPR genes including CTH/FGF21/PPP1R15A/CEBPB/DDIT3l/ASNS, genes showing response to toxic substance like FOS/ASNS/SLC7A11, TNF signaling pathway genes like CEBPB/FOS/BIRC3, and MAPK signaling pathway genes like SOS1/FGF21. Intestinal metaplasia (IM) is an abeerant phenotype arising during *H. pylori*-associated

*See online. https://doi.org/10.3164/jcbn.20-116

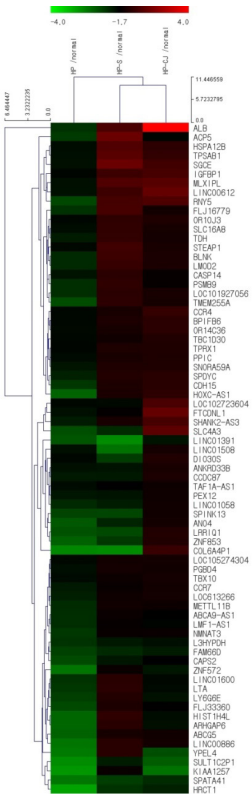
Table 1. Lists of KRGS (Kimchi Response Gene Signature)

	Average of normalize RC (log_2)		
Genes	Non-infected	*H. pylori*	*H. pylori* + kimchi
CLDN14	1	3.759	3.132
FAM177B	1	2.522	1.954
PROKR1	1	3.872	1.850
LOC101927623	1	2.889	2.031
VTCN1	1	3.399	1.183
PGK2	1	2.083	1.756
C12orf79	1	2.750	1.580
CHAC1	1	1.418	1.351
PDK1	1	1.393	1.331
TCAF2	1	1.462	1.412
TXNIP	1	1.241	1.104
FOS	1	1.467	1.370
DLL4	1	1.449	1.376
C3orf58	1	1.226	1.190
BTG1	1	1.233	1.189
PTPRN	1	1.689	1.382
FLJ31356	1	1.313	1.231
TMEM30C	1	916.191	6.804
LACTBL1	1	983.274	6.804
FGF21	1	461.62	6.808
LINC01273	1	491.887	6.807
FOXB1	1	744.837	6.805
LMO7DN-IT1	1	251.773	162.552
ASNS	1	1.155	1.102
C11orf91	1	1.725	1.538
SPRY1	1	1.199	1.123
SLC7A11-AS1	1	1.259	1.126
FOCAD-AS1	1	1.876	1.146
MTHFD2	1	1.123	1.085
PPP1R15A	1	1.125	1.078
CTH	1	1.175	1.087
ZNF654	1	1.151	1.105
SMARCA1	1	1.402	1.308
RNF183	1	1.929	1.564
SH2B3	1	1.159	1.105
RNF122	1	1.236	1.172
AMPD3	1	1.291	1.078
ESPNP	1	1.321	1.125
SLC25A36	1	1.117	1.081
PSPH	1	1.126	1.088
RIOK3	1	1.121	1.077
TSC22D3	1	1.391	1.280
PCP2	1	1.504	1.218
PER1	1	1.149	1.215
SLC1A4	1	1.143	1.103
PCK2	1	1.126	1.063
KLF10	1	1.135	1.089
PTPRH	1	1.339	1.205
FAM107B	1	1.103	1.070
DDIT3	1	1.129	1.067
DOK3	1	1.206	1.085
ARL6	1	1.166	1.117
A1CF	1	1.736	1.259
SOS1	1	1.095	1.054
CFAP53	1	1.213	1.115
CEBPB	1	1.104	1.066
CSGALNACT1	1	1.223	1.138
BIRC3	1	1.134	1.092
SLC7A11	1	1.113	1.019
BOC	1	1.331	1.101
SLC7A1	1	1.085	1.043

List of genes showing significant difference between non-infected vs *H. pylori* infected vs *H. pylori* + kimchi (significantly increased after *H. pylori*, but significantly decreased after *H. pylori* in the presence of kimchi, $p<0.05$).

doi: 10.3164/jcbn.20-116

A



Genes category different significantly between *H. pylori* infection ↓ and kimchi ↑
(172 genes, $p<0.05$)

* Chemokine receptor family → CCR4/CCR7/ACKR4
* Positive regulation of interferon-gamma production → SLAMF6/LTA/HLA-DPB1
* Cell cycle regulator → SPDYC/SPDYE4
* Defense response to gram-positive bacterium → ACP5/LTA/SSC5D
* Chemokine receptor activity → CCR4/ACKR4
* Cytokine-cytokine receptor interaction → CCR4/CCR7/TNFRSF8/LTA

B

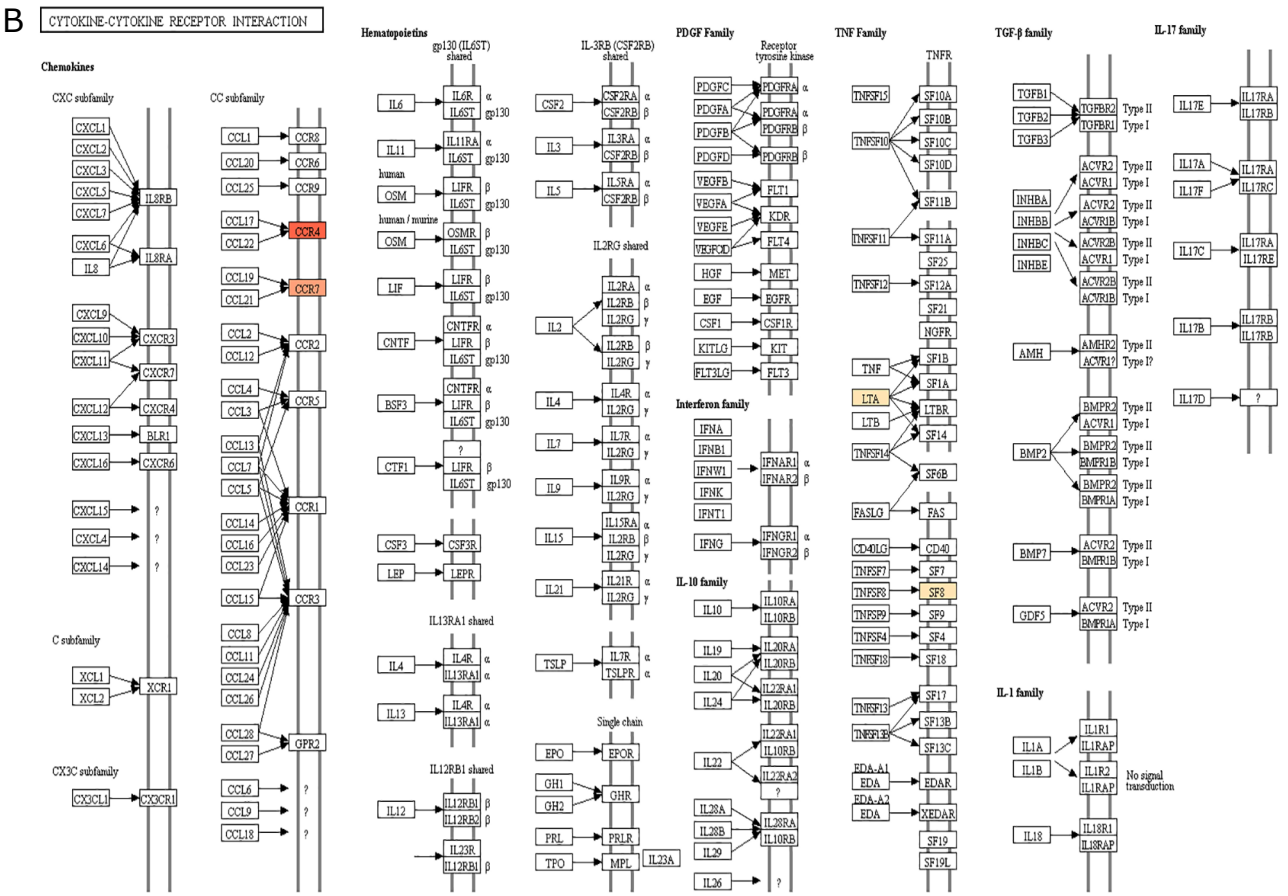


Fig. 3. (A) Heatmap showing significant trend like elevated with *H. pylori* infection, but decreased with co-treatment of kimchi, description of gene category. (B–D) KEGG analysis. See color figure in the on-line version.

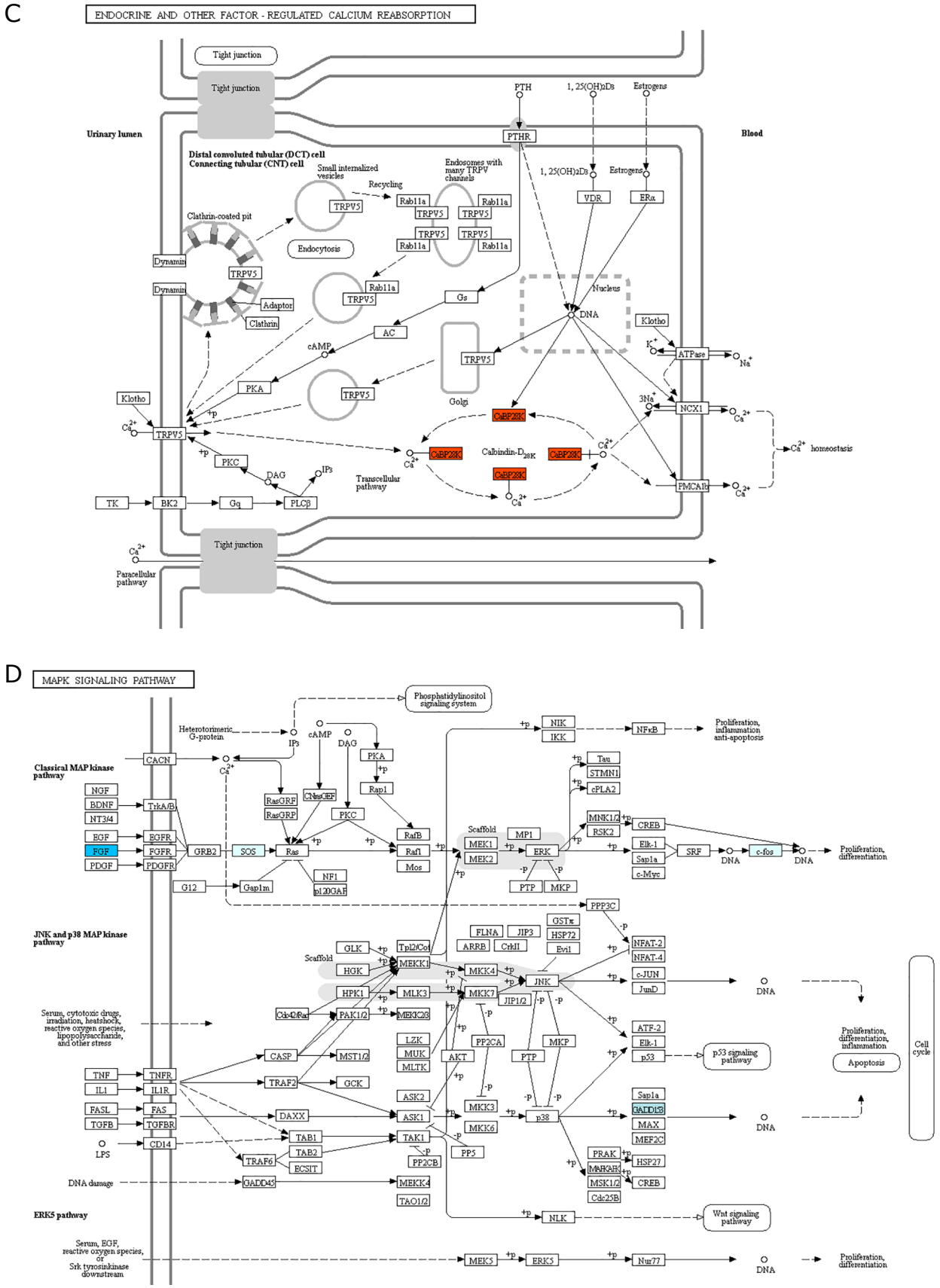


Fig. 3. continued.

doi: 10.3164/jcbn.20-116

Table 2. Lists of KRGS (Kimchi Response Gene Signature)

	Average of normalize RC (log_2)		
Genes	Non-infected	*H. pylori*	*H. pylori* + kimchi
TPRX1	1	0.006	0.449
BPIFB6	1	0.008	0.585
CCR4	1	0.008	0.724
OR10J3	1	0.008	0.447
OR14C36	1	0.007	0.592
ANKRD33B	1	0.008	0.397
ABCA9-AS1	1	0.006	0.200
LTA	1	0.008	0.194
NMNAT3	1	0.006	0.335
SPINK13	1	0.006	0.441
TDH	1	0.012	0.380
LMOD2	1	0.011	0.479
ALB	1	0.045	3.246
ACP5	1	0.014	0.224
RNY5	1	0.013	1.034
TMEM255A	1	0.011	0.524
ZNF853	1	0.012	0.635
ABCG5	1	0.010	0.499
YPEL4	1	0.011	0.158
COL6A4P1	1	0.011	0.863
GYG2P1	1	0.009	0.675
MTVR2	1	0.007	0.418
CLDN8	1	0.009	0.567
SYCP1	1	0.006	0.317
SPDYE4	1	0.005	0.343
CLEC9A	1	0.008	0.589
HEPACAM2	1	0.008	0.532
OLIG2	1	0.007	0.493
SEBOX	1	0.005	0.472
MAS1L	1	0.008	0.456
TDH	1	0.012	0.380
LMOD2	1	0.011	0.479
ALB	1	0.045	3.246
ACP5	1	0.014	0.224
RNY5	1	0.013	1.034
TMEM255A	1	0.011	0.524
ZNF853	1	0.012	0.635
ABCG5	1	0.010	0.499
YPEL4	1	0.011	0.158
COL6A4P1	1	0.011	0.863
GYG2P1	1	0.009	0.675
MTVR2	1	0.007	0.418
CLDN8	1	0.009	0.567
SYCP1	1	0.006	0.317
SPDYE4	1	0.005	0.343
CLEC9A	1	0.008	0.589
HEPACAM2	1	0.008	0.532
OLIG2	1	0.007	0.493
SEBOX	1	0.005	0.472
MAS1L	1	0.008	0.456
MLXIPL	1	0.595	1.059
LINC01508	1	0.593	0.729
SNORA59A	1	0.448	0.700
SHANK2-AS3	1	0.467	0.970
LINC01058	1	0.461	0.659
FLJ16779	1	0.494	0.702
CDH15	1	0.556	0.842
LY6G6E	1	0.412	0.518
CAPS2	1	0.432	0.605
LRRIQ1	1	0.456	0.793
ZNF572	1	0.560	0.697
LINC00886	1	0.460	0.756
SULT1C2P1	1	0.495	0.566
TSPAN32	1	0.273	0.718

Table 2. continued.

Genes	Average of normalize RC (log_2)		
	Non-infected	*H. pylori*	*H. pylori* + kimchi
LOC102724434	1	0.269	0.881
SPATA3-AS1	1	0.246	0.457
C11orf53	1	0.241	0.448
RHBDL3	1	0.210	0.549
SGCG	1	0.202	0.909
CCM2L	1	0.201	0.374
SPDYC	1	0.258	0.713
BLNK	1	0.273	0.617
METTL11B	1	0.285	0.525
LMF1-AS1	1	0.251	0.384
PSMB9	1	0.251	0.464
LINC01600	1	0.249	0.382
FLJ33360	1	0.247	0.482
SLC4A3	1	0.244	1.129
LINC01391	1	0.163	0.366
ANO4	1	0.139	0.569
HOXC-AS1	1	0.267	0.807
HIST1H4L	1	0.244	0.477
ARHGAP6	1	0.277	0.493
SPATA41	1	0.204	0.405
KIAA1257	1	0.140	0.235
HRCT1	1	0.299	0.560
ZPBP2	1	0.013	0.710
HLA-DPB1	1	0.012	0.825
LINC01088	1	0.013	0.954
MYBPH	1	0.015	1.519
MMP28	1	0.017	1.333
TTC22	1	0.011	0.604
IGFBP1	1	0.011	0.940
SGCE	1	0.018	0.886
LINC00612	1	0.012	1.304
FTCDNL1	1	0.011	1.308
MLXIPL	1	0.595	1.059
LINC01508	1	0.593	0.729
SNORA59A	1	0.448	0.700
SHANK2-AS3	1	0.467	0.970
LINC01058	1	0.461	0.659
FLJ16779	1	0.494	0.702
CDH15	1	0.556	0.842
LY6G6E	1	0.412	0.518
CAPS2	1	0.432	0.605
LRRIQ1	1	0.456	0.793
ZNF572	1	0.560	0.697
LINC00886	1	0.460	0.756
SULT1C2P1	1	0.495	0.566
LOC283585	1	0.278	1.021
TSPAN32	1	0.273	0.718
SPATA3-AS1	1	0.246	0.457
C11orf53	1	0.241	0.448
RHBDL3	1	0.210	0.549
SGCG	1	0.202	0.909
CCM2L	1	0.201	0.374
SPDYC	1	0.258	0.713
BLNK	1	0.273	0.617
METTL11B	1	0.285	0.525
LMF1-AS1	1	0.251	0.384

List of genes showing significant difference between non-infected vs *H. pylori* infected vs *H. pylori* + kimchi (significantly decreased after *H. pylori*, but significantly increased after *H. pylori* in the presence of kimchi, $p<0.05$).

carcinogenesis mostly associated with chronic atrophic gastritis (CAG), during which UPR activated by ER stress is prominently observed,[24] leading to conclusion that the UPR induction in the milieu of *H. pylori*, especially *Vac*A positive, -induced chronic inflammation and IM may promote neoplastic transformation through apoptosis of parietal cells and activation of ER

doi: 10.3164/jcbn.20-116

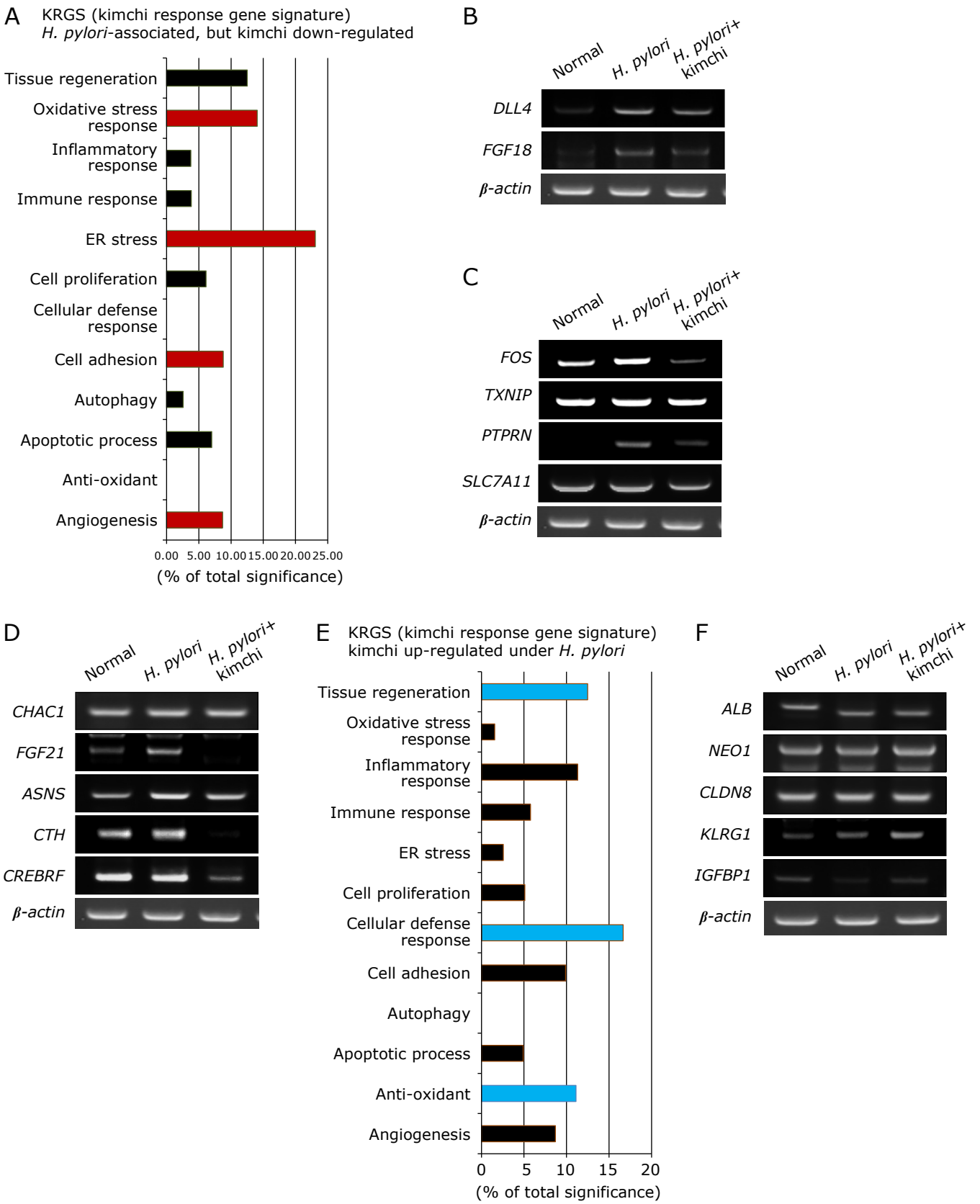


Fig. 4. KRGS. (A) GO analysis of the enrichment of differentially expressed genes between *H. pylori*-associated up-regulated genes, but down-regulated with kimchi GO, gene ontology. (B) RT-PCR for DLL4 and FGF18, gene for angiogenesis. (C) RT-PCR for *FOS*, *TXNIP*, *PTPRN*, and *SLG7A11*, gene for ER stress. (D) RT-PCR for *CHAC1*, *FGF21*, *ASNS*, *CTH*, and *CREBRF*, gene for oxidative stress. (E) GO analysis of the enrichment of differentially expressed genes between *H. pylori*-associated down-regulated genes, but up-regulated with kimchi GO, gene ontology. (F) RT-PCR for *ALB*, *NEO1*, *CLDN8*, *KLRG1*, *IGFBP*, genes in antioxidant, cell adhesion, cell defense, and regeneration.

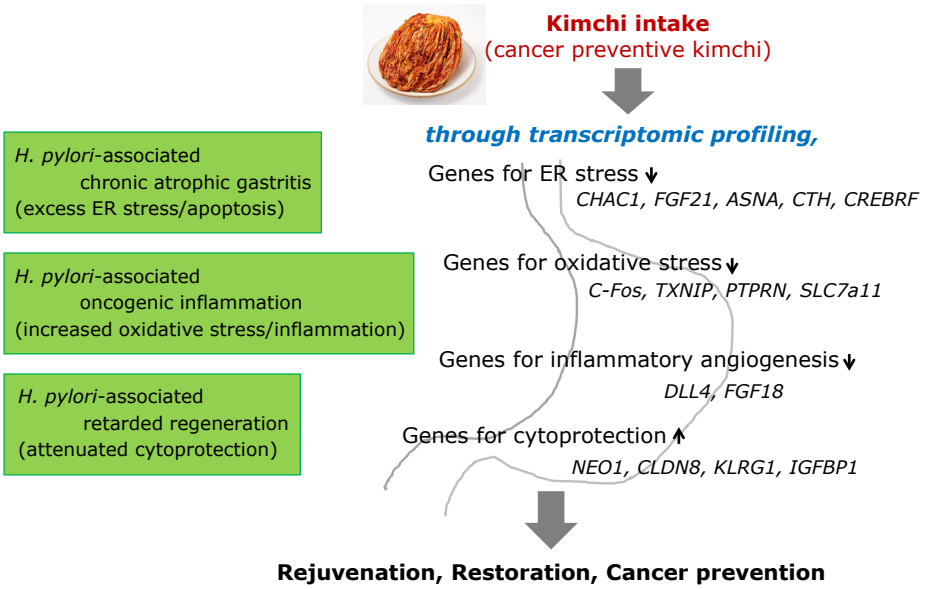


Fig. 5. Schematic presentation showing the core genes implicated in juvenating action as well as cancer preventive action of kimchi against *H. pylori* infection.

stress.[25,26]

During *H. pylori*-associated ER stress activation, in the current study, we have found core two genes, one was that CHAC 1 gene, cation transport regulator 1, of which overexpression in gastric parietal cells may cause the *H. pylori*-induced somatic mutations, especially mutagenic p53, that contribute to the development of gastric cancer and the other was DDIT3 as target gene in ER stress relevant to *H. pylori* infection because these were significantly regulated with kimchi administration.[27–29] As much as ER stress, oxidative stress contributed to *H. pylori*-carcinogenesis, which was influenced with kimchi administration. RNAseq analysis significantly showed the intervention of oxidative stress in *H. pylori* infection and mitigation of oxidative stress by kimchi. Oxidative stress relevant to pathogenesis of *H. pylori* infection and associated gastric carcinogenesis, for instance, *H. pylori*-induced DNA damages, oncogenic activation via oxidative stress, mitochondrial damages, Nrf2-mediated autophagy, and inflammasome activation, had been proved in many publications, by which antioxidants like vitamin C, vitamin E, and rebamipide had been tried for either enhancing eradication rate or relieving gastric inflammation in animals and human trial.[30–37] The fact that reactive oxygen species and reactive nitrogen species produced by *H. pylori* damage the host cells and can result in DNA damage, while *H pylori* has wisely evolved to keep damaging response, while blunting the host's efforts to kill the bacteria led to the host response that dietary intake of kimchi can serve host to prevent gastric cancer. Conclusively, since oxidative stress in *H. pylori* infection is defined as an imbalance between excessive production of reactive oxygen and nitrogen species and depletion of antioxidative system to eliminate the reactive intermediates, kimchi significantly enriched antioxidative defense system against chronic *H. pylori* infection, imposing the importance of adaptive cellular mechanisms involved in disease blocking of *H. pylori*-associated gastric carcinogenesis.[38]

Genes for chemokine receptor family, cell cycle regulator genes, chemokine receptor activity gene, genes for regeneration and cytoprotection were identified as genes which are decreased after *H. pylori* infection, but significantly increased in the presence of kimchi (Fig. 3, Supplemental Fig. 2*, and Table 2), in detail, chemokine receptor family like CCR4/CCR7/ACKR4, cell cycle regulator genes like SPDYC/SPDYE4, defense response to gram-positive bacterium like ACP5/LTA/SSC5D, chemokine receptor activity gene like CCR4/ACKR4, and cytokine-cytokine receptor interaction genes like CCR4/CCR7/TNFRSF8/LTA. Using KEGG analysis, representational transcriptomic presentations of discovered genes were shown in Fig. 3B (cytokine-cytokine receptor interaction), Fig. 3C (endocrine related-), and Fig. 3D (MAPK signaling pathway related-) were identified as signals decreased after *H. pylori* infection, but significantly up-regulated with kimchi extract administration in the presence of *H. pylori* infection. Among these, we have high concerns of genes implicated in tissue regeneration, cellular defense response, and antioxidative response including NEO1, CLDN8, KLRG1, and IGFBP-1 (Fig. 4F). Though unlimited upregulation for tissue regeneration catties risk of carcinogenesis, for instance upregulation of IGFBP-1 is associated with gastric cancer cell migration,[39–41] retarded and deranged healing after *H. pylori* infection render vulnerability to gastric carcinogenesis. Kimchi provide optimal regenerating condition.[10] Next, claudin18 (CLDN18) is good marker for beneficiary role of kimchi against *H. pylori* infection since CLDN18 has been identified as important regulator in gastric cancer, as exemplified that loss of CLDN18 promotes progressive neoplasia development.[42–44] We speculate these tight junction proteins might play to improve *H. pylori*-associated functional dyspepsia in addition to carcinogenesis.[45–47] Conclusively, since tight junction disruption is closely associated with either dysregulation of gastric mucosal barrier or gastric carcinogenesis,[48] kimchi afforded anticipating food factor to keep tight junction protein, CLDN18.

Neogenin 1 (NEO1) gene was not reported regarding *H. pylori* infection, it has been implicated in CNS development and nerve regeneration,[49,50] for instance, regeneration for peripheral neuropathy with pain or spinal cord injury,[51–53] tissue regeneration,[54] optic nerve regeneration,[55] renal regeneration,[56] pancreas regeneration,[57] and vertebrate development.[58] though some reports showed the association between NEO1 and apoptosis.[59] Though not reported regarding the association

*See online. https://doi.org/10.3164/jcbn.20-116

doi: 10.3164/jcbn.20-116

between *H. pylori* infection and regeneration relevant to NEO 1 yet, after our study, we can speculate NEO1 can be possible target of kimchi against *H. pylori*-associated functional dyspepsia or other gastric pathologies, necessitating deeper investigation, hinted from publication by Liu *et al.*[60] that repulsive guidance molecule b as a ligand for NEO 1 is a target for autosomal dominant polycystic kidney disease. Killer cell lectin-like receptor G1 (KLRG1) gene is drawn for the first time in *H. pylori* infection, but gene basis seems to be enough value explaining the association between *H. pylori* infection and nutrient supplementation as beneficiary contribution because KLRG1 imposed positive vaccine response against Ebola virus,[61] skin damages,[62] pediatric sepsis,[63] but importantly KLRG1 impaired regulatory T cell competitive fitness in the gut.[64] However, these two genes, NEO1 and NKRG1, should be further investigated, even though we have identified in the current study.

As listed in Table 1 and Table 2, individual gene can be possible biomarker denoting the contributory role against *H. pylori* infection. STRING analysis, a database that comprises established and predicted protein interactions demonstrated the strongest interactions between genes related to regulation of kimchi against *H. pylori* infection.[65] As shown in Supplemental Fig. 1* and Supplemental Fig. 2*, we can formulate STRING analysis as for KRGS. Though transcriptome analysis was done in the current study, kimchi is also famous for abundant probiotics with fermentation process as evidenced that non-fermented kimchi did not affect these cancer preventive outcomes compared to fermented kimchi.[20] Kimchi is a traditional Korean fermented side-dish containing diverse strains of lactic acid bacilli such as *Lactobacillus plantarum*, *L. acidophilus*, *L. curvatus*, *L. brevis*, *L. sakei*, *Leuconostocmesenteroides*, *Enterococcus faecium*, and *Weissellacibaria*.

As the limitation of the current study, though we have several *in vivo* results showing beneficial outcome of kimchi intake against *H. pylori* as well as other inflammation-based cancer model, all genes identified through current transcriptome analysis should be validated or documented with additional *in vitro* documentation. Cell line used in the current study was AGS gastric cancer cells, but we repeated the current analysis in other cells, RGM-1 as non-transformed cells and SNU-16 cells, after which fundamental transcriptomes as shown in Fig. 4 were similar (data not shown). In order to compare current displayed results with the ones obtained using some of the standard methods for detecting differentially expressed genes (DEG) and up-/down-regulated genes, diverse analysis methods including Welch *t*-statistic, fold change, rank products, average difference (AD), weighted average difference (WAD), moderated *t*-statistic, intensity-based moderated *t*-statistic (iBMS), significance analysis of microarrays, and area under the ROC curve (AUC) has been applied,[66,67] among which we have applied AD, WAD, and iBMS. However, we showed bar graph as shown in Supplemental Fig. 3* and Supplemental Fig. 4* either to facilitate the understanding of profiling or to avoid too many WAS arrow plots of identified data similar to volcano plots in manuscript.

Conclusively, our and other investigators strongly suggested that the diet may play a critical role in defining the final outcome of *H. pylori* infection particularly if certain intake of dietary components is continued for a long time. However, despite a recent surge in research related to the role of dietary ingredients, well-designed, large-scale clinical trials are required to give clinical benefits. In this effort, the current high throughput analysis, RNAseq transcriptome profiling, provide definite advantage and usefulness.

Author Contributions

Study concept and design: JMP and KBH; acquisition of data: JMP, YMH, DYL, SHC analysis and statistical analysis: JMP and KBH; interpretation of data: JYO and KBH; drafting of manuscript: KBH.

Acknowledgments

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (116015-03-1-CG000).

Abbreviations

CAG	chronic atrophic gastritis
DEG	differentially expressed genes
ER stress	endoplasmic reticulum stress
KEGG	Kyoto Encyclopedia of Genes and Genome
KRGS	Kimchi Response Gene Signature
MAPK	mitogen activated protein kinase
MOI	multiplicity of infection
STRING	Search Tool for the Retrieval of Interacting Genes/ Proteins
RNAseq	RNA sequencing
UPR	unfolded protein response

Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 Guo Y, Zhao S, Li CI, Sheng Q, Shyr Y. RNAseqPS: a web tool for estimating sample size and power for RNAseq experiment. *Cancer Inform* 2014; **13 (Suppl 6)**: 1–5.
2 Cloonan N, Grimmond SM. Transcriptome content and dynamics at single-nucleotide resolution. *Genome Biol* 2008; **9**: 234.
3 Avital G, Hashimshony T, Yanai I. Seeing is believing: new methods for *in situ* single-cell transcriptomics. *Genome Biol* 2014; **15**: 110.
4 Han KJ, Lee JE, Lee NK, Paik HD. Antioxidant and anti-inflammatory effect of probiotic *Lactobacillus plantarum* KU15149 derived from Korean homemade diced-radish kimchi. *J Microbiol Biotechnol* 2020; **30**: 591–598.
5 Woo HD, Park S, Oh K, *et al*. Diet and cancer risk in the Korean population: a meta-analysis. *Asian Pac J Cancer Prev* 2014; **15**: 8509–8519.
6 Kim HJ, Kim MK, Chang WK, Choi HS, Choi BY, Lee SS. Effect of nutrient intake and *Helicobacter pylori* infection on gastric cancer in Korea: a case-control study. *Nutr Cancer* 2005; **52**: 138–146.
7 Kim HJ, Chang WK, Kim MK, Lee SS, Choi BY. Dietary factors and gastric cancer in Korea: a case-control study. *Int J Cancer* 2002; **97**: 531–535.
8 Shukla S, Park JH, Chung SH, Kim M. Ochratoxin A reduction ability of biocontrol agent *Bacillus subtilis* isolated from Korean traditional fermented food Kimchi. *Sci Rep* 2018; **8**: 8039.
9 Chun BH, Kim KH, Jeon HH, Lee SH, Jeon CO. Pan-genomic and transcriptomic analyses of *Leuconostoc mesenteroides* provide insights into its genomic and metabolic features and roles in kimchi fermentation. *Sci Rep* 2017; **7**: 11504.
10 Jeong M, Park JM, Han YM, *et al*. Dietary prevention of *Helicobacter pylori*-associated gastric cancer with kimchi. *Oncotarget* 2015; **6**: 29513–29526.
11 Kang BK, Cho MS, Park DS. Red pepper powder is a crucial factor that influences the ontogeny of *Weissella cibaria* during kimchi fermentation. *Sci Rep* 2016; **6**: 28232.
12 Balic JJ, Saad MI, Dawson R, *et al*. Constitutive STAT3 serine

*See online. https://doi.org/10.3164/jcbn.20-116

phosphorylation promotes *Helicobacter*-mediated gastric disease. *Am J Pathol* 2020; **190**: 1256–1270.
13 Ishii Y, Shibata W, Sugimori M, *et al*. Activation of signal transduction and activator of transcription 3 signaling contributes to *Helicobacter*-associated gastric epithelial proliferation and inflammation. *Gastroenterol Res Pract* 2018; **2018**: 9050715.
14 Park JM, Han YM, Oh JY, *et al*. Fermented kimchi rejuvenated precancerous atrophic gastritis via mitigating *Helicobacter pylori*-associated endoplasmic reticulum and oxidative stress. *J Clin Biochem Nutr* 2021; **69**: 158–170.
15 Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012; **9**: 357–359.
16 Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 2010; **26**: 841–842.
17 Gentleman RC, Carey VJ, Bates DM, *et al*. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004; **5**: R80.
18 Huang DW, Sherman BT, Tan Q, *et al*. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol* 2007; **8**: R183.
19 Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 2017; **45**: D353–D361.
20 Han YM, Kang EA, Park JM, *et al*. Dietary intake of fermented kimchi prevented colitis-associated cancer. *J Clin Biochem Nutr* 2020; **67**: 263–273.
21 Liu X, Li N, Liu S, *et al*. Normalization methods for the analysis of unbalanced transcriptome data: a review. *Front Bioeng Biotechnol* 2019; **7**: 358.
22 Posey JE. Genome sequencing and implications for rare disorders. *Orphanet J Rare Dis* 2019; **14**: 153.
23 Narrandes S, Xu W. Gene expression detection assay for cancer clinical use. *J Cancer* 2018; **9**: 2249–2265.
24 Baird M, Woon Ang P, Clark I, *et al*. The unfolded protein response is activated in *Helicobacter*-induced gastric carcinogenesis in a non-cell autonomous manner. *Lab Invest* 2013; **93**: 112–122.
25 Zhu P, Xue J, Zhang ZJ, *et al*. *Helicobacter pylori* VacA induces autophagic cell death in gastric epithelial cells via the endoplasmic reticulum stress pathway. *Cell Death Dis* 2017; **8**: 3207.
26 Akazawa Y, Isomoto H, Matsushima K, *et al*. Endoplasmic reticulum stress contributes to *Helicobacter pylori* VacA-induced apoptosis. *PLoS One* 2013; **8**: e82322.
27 Ogawa T, Wada Y, Takemura K, *et al*. CHAC1 overexpression in human gastric parietal cells with *Helicobacter pylori* infection in the secretory canaliculi. *Helicobacter* 2019; **24**: e12598.
28 Wada Y, Takemura K, Tummala P, *et al*. *Helicobacter pylori* induces somatic mutations in TP53 via overexpression of CHAC1 in infected gastric epithelial cells. *FEBS Open Bio* 2018; **8**: 671–679.
29 Kim JM, Kim JS, Kim N, Ko SH, Jeon JI, Kim YJ. *Helicobacter pylori* vacuolating cytotoxin induces apoptosis via activation of endoplasmic reticulum stress in dendritic cells. *J Gastroenterol Hepatol* 2015; **30**: 99–108.
30 Matsuoka K, Nishiumi S, Yoshida M, Kodama Y. Effects of *Helicobacter pylori* on the glutathione-related pathway in gastric epithelial cells. *Biochem Biophys Res Commun* 2020; **526**: 1118–1124.
31 Fu L, Xie C. A lucid review of *Helicobacter pylori*-induced DNA damage in gastric cancer. *Helicobacter* 2019; **24**: e12631.
32 Benitez J, Marra R, Reyes J, Calvete O. A genetic origin for acid-base imbalance triggers the mitochondrial damage that explains the autoimmune response and drives to gastric neuroendocrine tumours. *Gastric Cancer* 2020; **23**: 52–63.
33 Sokolova O, Naumann M. Crosstalk between DNA damage and inflammation in the multiple steps of gastric carcinogenesis. *Curr Top Microbiol Immunol* 2019; **421**: 107–137.
34 Paik JY, Lee HG, Piao JY, *et al*. *Helicobacter pylori* infection promotes autophagy through Nrf2-mediated heme oxygenase upregulation in human gastric cancer cells. *Biochem Pharmacol* 2019; **162**: 89–97.
35 Shi Y, Wang P, Guo Y, Liang X, Li Y, Ding S. *Helicobacter pylori*-induced DNA damage is a potential driver for human gastric cancer AGS cells. *DNA Cell Biol* 2019; **38**: 272–280.
36 Kumar S, Dhiman M. Inflammasome activation and regulation during *Helicobacter pylori* pathogenesis. *Microb Pathog* 2018; **125**: 468–474.
37 Butcher LD, den Hartog G, Ernst PB, Crowe SE. Oxidative stress resulting from *Helicobacter pylori* infection contributes to gastric carcinogenesis. *Cell Mol Gastroenterol Hepatol* 2017; **3**: 316–322.
38 Díaz P, Valenzuela Valderrama M, Bravo J, Quest AFG. *Helicobacter pylori* and gastric cancer: adaptive cellular mechanisms involved in disease progression. *Front Microbiol* 2018; **9**: 5.
39 Luo C, Sun F, Zhu H, *et al*. Insulin-like growth factor binding protein-1 (IGFBP-1) upregulated by *Helicobacter pylori* and is associated with gastric cancer cells migration. *Pathol Res Pract* 2017; **213**: 1029–1036.
40 Ustundag Y, Sahin H, Ilikhan S, Dogan BG, Kokturk F, Kar F. *Helicobacter pylori* eradication does not change circulating insulin-like growth factor 1 and insulin-like growth factor binding protein 3 levels in patients with and without precancerous gastric lesions. *Am J Med Sci* 2013; **346**: 381–384.
41 Pham TM, Fujino Y, Kikuchi S, *et al*. A nested case-control study of stomach cancer and serum insulin-like growth factor (IGF)-1, IGF-2 and IGF-binding protein (IGFBP)-3. *Eur J Cancer* 2007; **43**: 1611–1616.
42 Yasui W, Sentani K, Motoshita J, Nakayama H. Molecular pathobiology of gastric cancer. *Scand J Surg* 2006; **95**: 225–231.
43 Nardone G, Compare D. Epigenetic alterations due to diet and *Helicobacter pylori* infection in gastric carcinogenesis. *Expert Rev Gastroenterol Hepatol* 2008; **2**: 243–248.
44 Hagen SJ, Ang LH, Zheng Y, *et al*. Loss of tight junction protein claudin 18 promotes progressive neoplasia development in mouse stomach. *Gastroenterology* 2018; **155**: 1852–1867.
45 Lee JY, Kim N, Choi YJ, *et al*. Expression of tight junction proteins according to functional dyspepsia subtype and sex. *J Neurogastroenterol Motil* 2020; **26**: 248–258.
46 Taki M, Oshima T, Li M, *et al*. Duodenal low-grade inflammation and expression of tight junction proteins in functional dyspepsia. *Neurogastroenterol Motil* 2019; **31**: e13576.
47 Nakayama I, Shinozaki E, Sakata S, *et al*. Enrichment of CLDN18-ARHGAP fusion gene in gastric cancers in young adults. *Cancer Sci* 2019; **110**: 1352–1363.
48 Caron TJ, Scott KE, Fox JG, Hagen SJ. Tight junction disruption: *Helicobacter pylori* and dysregulation of the gastric mucosal barrier. *World J Gastroenterol* 2015; **21**: 11411–11427.
49 Dun XP, Parkinson DB. Role of Netrin-1 signaling in nerve regeneration. *Int J Mol Sci* 2017; **18**: 491.
50 De Vries M, Cooper HM. Emerging roles for neogenin and its ligands in CNS development. *J Neurochem* 2008; **106**: 1483–1492.
51 Schubert AL, Held M, Sommer C, Üçeyler N. Reduced gene expression of netrin family members in skin and sural nerve specimens of patients with painful peripheral neuropathies. *J Neurol* 2019; **266**: 2812–2820.
52 Mothe AJ, Tassew NG, Shabanzadeh AP, *et al*. RGMa inhibition with human monoclonal antibodies promotes regeneration, plasticity and repair, and attenuates neuropathic pain after spinal cord injury. *Sci Rep* 2017; **7**: 10529.
53 Yamashita T, Mueller BK, Hata K. Neogenin and repulsive guidance molecule signaling in the central nervous system. *Curr Opin Neurobiol* 2007; **17**: 29–34.
54 Schlegel M, Körner A, Kaussen T, *et al*. Inhibition of neogenin fosters resolution of inflammation and tissue regeneration. *J Clin Invest* 2018; **128**: 4711–4726.
55 Schnichels S, Heiduschka P, Julien S. RGMA and neogenin protein expression are influenced by lens injury following optic nerve crush in the rat retina. *Graefes Arch Clin Exp Ophthalmol* 2012; **250**: 39–50.
56 Wang W, Reeves WB, Ramesh G. Netrin-1 increases proliferation and migration of renal proximal tubular epithelial cells via the UNC5B receptor. *Am J Physiol Renal Physiol* 2009; **296**: F723–F729.
57 De Breuck S, Lardon J, Rooman I, Bouwens L. Netrin-1 expression in fetal and regenerating rat pancreas and its effect on the migration of human pancreatic duct and porcine islet precursor cells. *Diabetologia* 2003; **46**: 926–933.
58 Shin GJ, Wilson NH. Overexpression of repulsive guidance molecule (RGM) a induces cell death through Neogenin in early vertebrate development. *J Mol Histol* 2008; **39**: 105–113.
59 Tassew NG, Mothe AJ, Shabanzadeh AP, *et al*. Modifying lipid rafts promotes regeneration and functional recovery. *Cell Rep* 2014; **8**: 1146–1159.
60 Liu J, Wang W, Liu M, *et al*. Repulsive guidance molecule b inhibits renal cyst development through the bone morphogenetic protein signaling pathway. *Cell Signal* 2016; **28**: 1842–1851.
61 Bowyer G, Sharpe H, Venkatraman N, *et al*. Reduced Ebola vaccine

doi: 10.3164/jcbn.20-116

responses in CMV$^+$ young adults is associated with expansion of CD57$^+$KLRG1$^+$ T cells. *J Exp Med* 2020; **217**:e20200004.
62 Seidel JA, Vukmanovic-Stejic M, Muller-Durovic B, *et al*. Skin resident memory CD8$^+$ T cells are phenotypically and functionally distinct from circulating populations and lack immediate cytotoxic function. *Clin Exp Immunol* 2018; **194**: 79–92.
63 Li Y, Li Y, Bai Z, Pan J, Wang J, Fang F. Identification of potential transcriptomic markers in developing pediatric sepsis: a weighted gene co-expression network analysis and a case-control validation study. *J Transl Med* 2017; **15**: 254.
64 Meinicke H, Bremser A, Brack M, Schrenk K, Pircher H, Izcue A. KLRG1 impairs regulatory T-cell competitive fitness in the gut. *Immunology* 2017; **152**: 65–73.
65 Yang M, Li H, Li Y, Ruan Y, Quan C. Identification of genes and pathways associated with MDR in MCF-7/MDR breast cancer cells by RNA-seq analysis. *Mol Med Rep* 2018; **17**: 6211–6226.
66 Silva-Fortes C, Amaral Turkman MA, Sousa L. Arrow plot: a new graphical tool for selecting up and down regulated genes and genes differentially expressed on sample subgroups. *BMC Bioinformatics* 2012; **13**: 147.
67 Kadota K, Nakai Y, Shimizu K. A weighted average difference method for detecting differentially expressed genes from microarray data. *Algorithms Mol Biol* 2008; **3**: 8.