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

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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 CREBFRF were identified as significantly increased after *H. pylori*, but significantly decreased with kimchi and NEO1, CLDN8, KLRG1, and IGBP1 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.(1,2) 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) 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.(6,7) 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”).(8,9)

Under the hypothesis that dietary intervention of fermented cpkgimchi can prevent *H. pylori*-associated gastric cancer in mice model, long-term intervention of cpkgimchi 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 inhibition of IL-6/IL-6R/JAK.(10-12) 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, *CagA*+ and VacA 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 10⁵ 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 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 ground into a

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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 epikimchi extracts were 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% CO₂. 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. The 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. The RC (Read Count) data were processed based on quantile normalization method using EdgeR within R (R development Core Team, 2016) using Bioconductor. Gene classification was based on searches done by DAVID (https://david.abcc.ncifcrf.gov/) and Medline databases (http://www.ncbi.nlm.nih.gov/). Pathway analysis was performed on differentially expressed genes based on the Kyoto Encyclopedia of Genes and Genome (KEGG) pathway databases.

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, 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. 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/F2G/F2P/PPP1R15A/C/EBP/DDIT3, PERK-mediated unfolded protein response like DDIT3/ASNS, genes showing response to toxic substance like CTH/F2G/F2P/PPP1R15A, apoptosis genes like CHAC1/DDIT3/BIRC3/PPP1R15A/PPTPRH, TNF signaling pathway genes like CEBPB/FOS/BIRC3, and mitogen activated protein kinase (MAPK) signaling pathway genes like DDIT3/FOS/SOS1/F2G. 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
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.
AGS cells infected with *H. pylori* vs AGS cells infected with *H. pylori* and kimchi

| Genes category          | % of Total significant |
|-------------------------|------------------------|
| Aging                   | 2.68%                  |
| Apoptosis               | 1.92%                  |
| Angiogenesis            | 1.57%                  |
| Cell cycle              | 1.61%                  |
| Cell death              | 1.44%                  |
| Cell migration          | 1.61%                  |
| DNA repair              | 1.99%                  |
| Extracellular matrix    | 1.71%                  |
| Immune response         | 1.69%                  |
| Inflammatory response   | 1.63%                  |
| Inflammation            | 1.62%                  |
| Secretion               | 1.45%                  |
| Cell differentiation    | 0.43%                  |

**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-DBP1, cell cycle regulator genes like SPDYC/SPDY4, defense response to gram-positive bacterium like ACP5/LTA/SSCS5D, 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 (CREB3) as ER stress genes afforded *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

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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

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.
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*, 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 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 aberant phenotype arising during *H. pylori*-associated

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

| 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              |
| VTN1        | 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              |
| CNRIP       | 1            | 1.241     | 1.104              |
| FOS         | 1            | 1.467     | 1.370              |
| Dlk4        | 1            | 1.449     | 1.376              |
| C3orf58     | 1            | 1.226     | 1.190              |
| BTG1        | 1            | 1.233     | 1.189              |
| PTPRN       | 1            | 1.689     | 1.382              |
| FLJ13156    | 1            | 1.313     | 1.231              |
| TMEM30C     | 1            | 916.191   | 6.804              |
| LACTBL1     | 1            | 983.274   | 6.804              |
| FGFR1       | 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              |
| FOCD-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              |
| ESPN1       | 1            | 1.321     | 1.125              |
| SLC25A36    | 1            | 1.117     | 1.081              |
| PSCP        | 1            | 1.126     | 1.088              |
| RIOK3       | 1            | 1.121     | 1.077              |
| TCL22D3     | 1            | 1.391     | 1.280              |
| PCP2        | 1            | 1.504     | 1.218              |
| PER1        | 1            | 1.149     | 1.215              |
| SLC1A4      | 1            | 1.143     | 1.103              |
| PKC2        | 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              |
| CEBP8       | 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).
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

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.
Fig. 3. continued.
Table 2. Lists of KRGS (Kimchi Response Gene Signature)

| 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              |
| ACPS       | 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              |
| ACPS       | 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              |
| CAP52      | 1            | 0.432     | 0.605              |
| LRRQ1      | 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              |
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 \textit{H. pylori}, especially \textit{VacA} positive, -induced chronic inflammation and IM may promote neoplastic transformation through apoptosis of parietal cells and activation of ER

| Genes          | Non-infected | \(H.\) \textit{pylori} | \(H.\) \textit{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                          |
| SGGC           | 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                          |
| LIN01600       | 1            | 0.249                   | 0.382                          |
| FLJ53360       | 1            | 0.247                   | 0.482                          |
| SLC4A3         | 1            | 0.244                   | 1.129                          |
| LIN01391       | 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                          |
| ZBP2           | 1            | 0.013                   | 0.710                          |
| HLA-DPB1       | 1            | 0.012                   | 0.825                          |
| LIN01088       | 1            | 0.013                   | 0.954                          |
| MYBP2          | 1            | 0.015                   | 1.519                          |
| MMP28          | 1            | 0.017                   | 1.333                          |
| TTC22          | 1            | 0.011                   | 0.604                          |
| IGF8P1         | 1            | 0.011                   | 0.940                          |
| SGCE           | 1            | 0.018                   | 0.886                          |
| LIN00612       | 1            | 0.012                   | 1.304                          |
| FTCDNL1        | 1            | 0.011                   | 1.308                          |
| MLX1PL         | 1            | 0.595                   | 1.059                          |
| LIN01508       | 1            | 0.593                   | 0.729                          |
| SNORA59A       | 1            | 0.448                   | 0.700                          |
| SHANK2-AS3     | 1            | 0.467                   | 0.970                          |
| LIN01058       | 1            | 0.461                   | 0.659                          |
| FLJ16779       | 1            | 0.494                   | 0.702                          |
| CDH15          | 1            | 0.556                   | 0.842                          |
| LY6G6E         | 1            | 0.412                   | 0.518                          |
| CAP52          | 1            | 0.432                   | 0.605                          |
| LRR1Q1         | 1            | 0.456                   | 0.793                          |
| ZNF572         | 1            | 0.560                   | 0.697                          |
| LIN00886       | 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                          |
| SGGC           | 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 \textit{H. pylori} infected vs \textit{H. pylori} + kimchi (significantly decreased after \textit{H. pylori}, but significantly increased after \textit{H. pylori} in the presence of kimchi, \(p<0.05\)).
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 SLC7A11, 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, IGFBP1, genes in antioxidant, cell adhesion, cell defense, and regeneration.
**H. pylori**-associated chronic atrophic gastritis (excess ER stress/apoptosis)

**H. pylori**-associated oncogenic inflammation (increased oxidative stress/inflammation)

**H. pylori**-associated retarded regeneration (attenuated cytoprotection)

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**Kimchi intake** (cancer preventive kimchi)

**through transcriptomic profiling,**

Genes for ER stress
- CHAC1, FGF21, ASNA, CTH, CREBRF

Genes for oxidative stress
- C-Fos, TXNIP, PTPRN, SLC7a11

Genes for inflammatory angiogenesis
- DLL4, FGF18

Genes for cytoprotection
- NEO1, CLDN8, KLRG1, IGFBP1

Rejuvenation, Restoration, Cancer prevention

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

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, *H. pylori* 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/SPDY4E, 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.\(^{40}\) 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,\(^{41-53}\) 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

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\(^{*}\)See online.  [https://doi.org/10.3164/cjn.20-116](https://doi.org/10.3164/cjn.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. 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, skin damages, but importantly KLRG1 impaired regulatory T cell competitive fitness in the gut. However, these two genes, NEO1 and NKRGL1, 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 *H. pylori* infection. As shown in Supplementary Fig. 1 and 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. 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*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, and *Weissella cibaria*.

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, among which we have applied AD, WAD, and iBMS. However, we showed bar graph as shown in Supplementary Fig. 3 and Supplementary 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.

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| 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 |
| RNAsq        | RNA sequencing |
| UPR          | unfolded protein response |

**Conflict of Interest**

No potential conflicts of interest were disclosed.

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