

Gene Expression Analysis from the Normal Stomach Cells Treated with a Cancer Inducer *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, MNNG

Dongju Jung^{1,2}, Yoonjung Cho³, Tae Ue Kim³ and Sang-Hee Jeong^{1,2,†}

¹Biomedical Science Institute, ²Department of Biomedical Laboratory Science, College of Life and Health Sciences, Hoseo University, Asan, Chungnam 31499, Korea

³Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Kangwon 26493, Korea

N-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) is a carcinogen made of modified guanine on which alkyl group is added on 6th oxygen. It has been used for inducing different types of cancers experimentally *in vivo* and *in vitro*. Stomach cancer might be the best well established particular cancer induced with MNNG. Comparative analysis of gene expression between normal stomach cell and MNNG-treated stomach cell could give much information to understand cancer formation in stomach. To this end, normal human stomach cells HS738 were treated with DMSO or MNNG. Genetic comparison was conducted with purified RNA from the treated cells for 6 hours or 24 hours. Total 13 genes were selected based on their high induction folds and comprehensible function to cancer formation. Some of the genes were cancer-promoting whereas the others were anti-cancer genes. These results could give important information of genetic changes in stomach cells during MNNG-induced stomach cancer formation.

Key Words: *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), Stomach cancer, Gene expressions

N-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) is a carcinogen made of modified guanine on which alkyl group is added on 6th oxygen. MNNG has been broadly used for inducing cancer formation in normal cells and animals since its cancer-promoting effect on rat stomach was experimentally succeeded (Sugimura, 1967). When MNNG is administered to rats, severe inflammation with erosion appears in two weeks and finally adenocarcinoma of the glandular stomach is developed in weeks 35-72 through atypical and regenerative changes (Yamashita, 2002). Cultured normal cells were also developed significant cancerous formation after MNNG treatment having changes on their

attachment, morphology, ploidy, mucin secretion, and tyrosine phosphorylation without complete malignant formation (Malik, 1997). Thus MNNG is a very useful small molecule for inducing artificial cancerous formation in laboratory animals and cultured cells. In addition to this, genetic analysis is a powerful tool to uncover genes that have functions to many different types of cellular processes and cancer formation (Jin 2015; Jin 2015). Combining the small molecule that have clear function toward cancer formation and whole RNA genetic analysis could give much information to understand molecular genetic processes in cancer generation. Here we analyzed the detailed gene inductions by MNNG

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†Corresponding author: Sang-Hee Jeong, Biomedical Science Institute and Department of Biomedical Laboratory Science, Hoseo University, Asan 31499, Korea.

Tel: +82-41-540-9675, Fax: +82-41-540-9829, e-mail: jeongsh@hoseo.edu

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treatment in normal stomach cells.

For the gene expression analysis, normal human stomach cell line, HS738 were cultured and treated with MNNG for 6 hr or 24 h. DMSO-treated HS738 cells were used as control. The normal stomach cells were able to maintain viability under 50 μ M of MNNG for up to 24 h (Fig. 1). Total RNA was purified with Trizol reagent (Thermo scientific) following manufacturer's instruction. RNA sequencing was conducted in the TheragenEtex (Suwon, South Korea). Briefly, 2 μ g of total RNA was treated with DNase I, and poly (A) mRNA was enriched via purification with Oligo(dT)-labeled magnetic beads. The purified mRNAs were disrupted into short fragments and used as template for synthesizing double-stranded cDNAs. After end-repair and poly (A) addition, the cDNAs were hybridized with sequencing adapters using

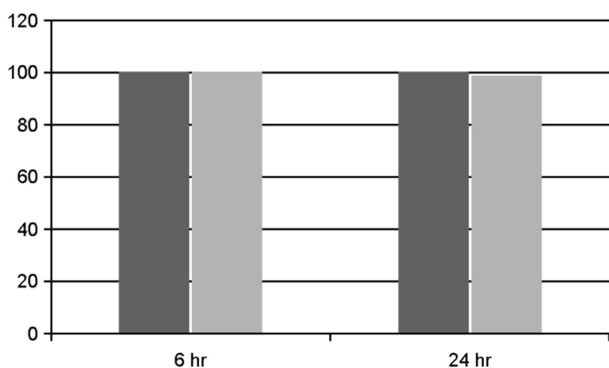


Fig. 1. Cell viability test. Dark columns represent viability of DMSO-treated normal stomach cell line HS738. Grey columns represent viability of HS737 treated with 50 μ M of MNNG, which is dissolved in DMSO. Cell viability tests were conducted using 'EZ-CYTOX' from DoGen South Korea.

the TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA.) for constructing cDNA library. The library was sequenced using a HiSeq 2500 sequencer (Illumina) in rapid run mode. The sequenced cDNA had highly matched with the genes in chromosome (Fig. 2). Cluster generation was performed, followed by 2 \times 100 cycle sequencing reads separated by a paired-end turnaround. Image analysis was performed using the HiSeq control software version 1.8.4. MNNG-induced genes were selected based on expression increase that was higher than 7-folds than that from the DMSO-treated cells after 24 h treatment. The most increased gene was a mitochondrial protein, preY (Table 1). The gene expression was induced 557-folds in HS738 cells by MNNG treatment but it seems that there might be no clear relationship between preY and cancer formation. LEAP2 induction was higher in 6 hr than that in 24 hr. LEAP2 is acronym of 'liver expressed antimicrobial peptide 2'. Although it was highly induced by MNNG, LEAP2 induction seems not have profound contribution to cancer formation. OMG represent 'oligodendrocyte-myelin glycoprotein', which is a Nogo receptor ligand that inhibits neurite outgrowth (Wang, 2002). Like the previous two genes, OMG might not have direct relationship to cancer formation. The following genes are supposed to have some roles to cancer formation of normal stomach cells by MNNG treatment. Carboxypeptidase A3 (CPA3) was reported that it was a novel gene highly induced by histone deacetylase inhibitors in prostate cancer cells (Huang, 1999). BEX2 was reported to be expressed in a subset of primary breast cancers (Naderi, 2007). GDF5 was reported to regulate angiogenesis in a breast cancer cell line (Margheri, 2012). Cysteine protease inhibitor cystatin

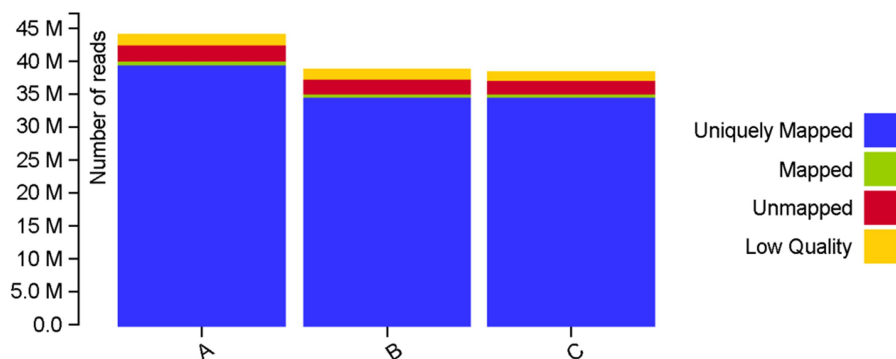


Fig. 2. Assignment of sequenced genes. Total 27,748 gene fragment were sequenced. More than 80% of the sequenced genes were able to be mapped. A indicates DMSO-treated HS738 cells. B indicates HS738 cells treated with 50 μ M MNNG for 6 hr. C indicates HS-738 cells treated with 50 μ M MNNG for 24 hr.

Table 1. Gene expression levels

Gene name	Chromosome	Description	6 h (folds)	24 h (folds)
PYURF	4	Mitochondrial protein, preY	532.6	555.7
LEAP2	5	Liver expressed antimicrobial peptide	52.7	12.1
OMG	17	Oligodendrocyte myelin glycoprotein	2.4	12.1
CPA3	3	Carboxypeptidase 3	13.7	11.2
BEX2	X	Brain-expressed X-linked 2	3.3	9.4
GDF5	20	Growth differentiation factor 5	2.9	9.3
CSTA	3	Cystatin A	5.8	9.2
srGAP3	3	SLIT-ROBO Rho GTPase-activating protein 3	3.1	9.2
BTG2	1	B-cell translocation gene 2	15.6	9.2
IFIT2	10	Interferon-induced protein with tetratricopeptide repeats 2	3.7	7.4
TP53TG1	7	TP53 target gene 1	1.3	7.3
SDPR	2	Serum-deprived response	5.7	7.2
SCN2B	11	Voltage-gated sodium channel, type II	3.5	7.1

The MNNG-induced genes expressed more than 7-folds higher compared with their expression levels in DMSO-treated cells and known for their functions were selected. The genes are listed according to the induction folds in 24 hr.

A (CSTA) was reported that it was a gene deregulated in several skin cancers (Gupta, 2015). srGAP3, a member of the Slit-Robo sub-family of Rho GTPase-activating proteins (Rho GAPs), was known to have a tumor suppressor role in mammary epithelial cells (Lahoz, 2013). BTG2, the B-cell translocation gene 2, is a p53-induced gene having significant anti-proliferative roles for disease progression including human breast cancer (Takahashi, 2011). IFIT2 was reported to contribute to inhibiting migration and metastatic activity of oral squamous cell carcinoma cells (Lai, 2013). TP53TG1 was a gene identified using transgene TP53-containing colon cancer cell line. It was identified as a target gene of TP53 and supposed to respond to cellular damage (Takei, 1998). SDPR was reported to act as a metastasis suppressor in breast cancer (Ozturk, 2016). Expressions of voltage-gated sodium channels in cancers have been known. SCN2B is a voltage-gated Na⁺ channels which was upregulated in carcinomas by potentiating metastasis of the cancers (Fraser, 2014).

Conclusively, we were able to identify genes of which expression were upregulated in normal stomach cells by MNNG treatment. None of the three highly induced genes,

Pre Y, LEAP2 and OMG, seem to be involved in cancer formation in spite of their high induction folds. Some of the genes are able to potentiate cancer formation when its expression was disrupted: GDF5, CSTA, BTG2, IFIT2, TP53TG1, and SDPR. In addition to these, BEX2, srGAP3, and SDPR are tumor suppressor genes whereas VGSCs is a tumor-promoting gene. This result shed light on understanding of gene expressions that contribute to stomach cancer formation by MNNG treatment.

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Conflict of interest

The authors declare that they have no competing interests.

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