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Transcriptome Analysis and the Prognostic Role of NUDC in Diffuse and Intestinal Gastric Cancer

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Abstract

Introduction: There have been few studies about gene differences between patients with diffuse-type gastric cancer and those with intestinal-type gastric cancer. The aim of this study was to compare the transcriptomes of signet ring cell gastric cancer (worst prognosis in diffuse-type) and well-differentiated gastric cancer (best prognosis in intestinal-type); NUDC was identified, and its prognostic role was studied. **Materials and Methods:** We performed next-generation sequencing with 5 well-differentiated gastric cancers and 3 of signet ring cell gastric cancer surgical samples. We performed gene enrichment and functional annotation analysis using the Database for Annotation, Visualization and Integrated Discovery bioinformatics resources. Immunohistochemistry was used to validate NUDC expression. **Results:** Overall, 900 genes showed significantly higher expression, 644 genes showed lower expression in signet ring cell gastric cancer than in well-differentiated gastric cancers, and there was a large difference in adhesion, vascular development, and cell-to-cell junction components between the 2 subtypes. We performed variant analysis and found 52 variants and 30 cancer driver genes, including NUDC. We analyzed NUDC expression in gastric cancer tissue and its relationship with prognosis. Cox proportional hazard analysis identified T stage, N stage, and NUDC expression as independent risk factors for survival ($P < 0.05$). The overall survival of the NUDC-positive group was significantly higher (53.2 $\pm$ 0.92 months) than that of the NUDC-negative group (44.6 $\pm$ 3.7 months) ($P = 0.001$) in Kaplan-Meier survival analysis. **Conclusion:** We found 30 cancer driver gene candidates and found that the NUDC-positive group showed significantly better survival than the NUDC-negative group via variant analysis.

Keywords

stomach neoplasm, signet ring cell, transcriptome sequencing, immunohistochemistry, biomarker

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Introduction

Gastric cancer is the sixth most common cancer worldwide and remains the second leading cause of death by global cancer statistics 2018.[1] The standard of care for stage I stomach cancer is surgery alone, but patients with stage II or III disease undergo surgery with adjuvant chemotherapy. Historically, stomach cancer was microscopically classified by Lauren's classification as intestinal-type gastric adenocarcinoma (IGC) or diffuse-type gastric adenocarcinoma (DGC), and this classification system is still widely used.[2] IGC results in a tumor with maintained gland shape and is often associated with intestinal metaplasia. However, DGC results in a mass or an infiltrating pattern and does not form glands, and tumor cells infiltrate the stroma as single cells or small cell groups.[3]

The etiologies of the 2 types of cancer are different. IGC is believed to develop from the evolution of a precancerous lesion, which was reported by Dr. Correa.[4,5] Chronic gastritis develops into intestinal metaplasia, dysplasia, and adenocarcinoma, and Helicobacter pylori has been reported as a factor. In contrast, there is no hypothesized theory for how DGC develops, but hereditary diffuse-type gastric cancer has been reported to be caused by hereditary E-cadherin (CDH1) deletion in New Zealand.[6] The detection rate of E-cadherin mutations in Maori families with hereditary gastric cancer is reported to be up to 30%, and in individuals with such mutations, most cases of stomach cancer (less than 70%) occur by the age of 80. The incidence rate of signet ring cell gastric cancer (SRC-GC) among DGC cases is known to be approximately 8% to 30%.[7,8] Signet ring cells have a small nucleus on one side, the cytoplasm is full of mucus, and rarely forms a line or coronary structure.[9] Although the Lauren classification is traditionally used, the World Health Organization (WHO) classification is considered more objective. SRC-GC is classified as a positive subtype in the WHO classification.[10] The prognosis of SRC-GC is better than that of other types of gastric cancer when detected and treated early; however, when the cancer is progressive, the cancer cells tend to invade, and lymph node metastasis is generally high, resulting in a poorer prognosis than those of other types of gastric cancer.[7] Gene analysis between the subtypes is needed to identify the phenomena associated with different prognoses of DGC than IGC.

The survival and progression of gastric adenocarcinoma are not only clearly different in terms of gross and molecular subtype; there have been a few studies about gene differences between IGC and DGC.[11] Specifically, there have been few studies about gene differences between patients with diffuse-type gastric cancer and those with intestinal-type gastric cancer. The aim of this study was to compare the transcriptomes of signet ring cell gastric cancer (SRC-GC; worst prognosis in diffuse-type) and well-differentiated gastric cancer (WD-GC, best prognosis in intestinal-type); from our experiments, nuclear distribution protein (NUDC) was identified.

NUDC is related to the nudC gene of the filamentous fungus *Aspergillus nidulans*, which is essential for the movement of nuclei following mitosis and for colony growth. Human NUDC is important in not only normal hematopoietic cells but also malignant hematopoietic precursors.[12,13] NUDC overexpression has been reported in several cancers, but there has been little research on the influence of NUDC on stomach cancer.[14-18] We conducted a survival analysis and investigated the role of NUDC in gastric cancer patients.

Materials and Methods

Ethics Approval

This study was designed and carried out according to the principles of the Declaration of Helsinki (1989). Written consent was obtained from all participants before inclusion in the trial. The study was approved by the Institutional Review Board of Gyeongsang National University Hospital (GNUHIRB 2009-54).

RNA Extraction and Next-Generation Sequencing

We collected surgical stomach cancer tissue samples from 9 patients who underwent surgical operations between 2012 and 2015. Frozen tissue from patients who agreed to preoperative tissue collection was stored in a −70°C deep freezer.

We choose 4 SRC-GC and 5 WD-GC cases. The cancer tissue was cut into 5 μm sections, and H&E staining was performed. H&E-stained slides were used to identify the morphology and location of gastric cancer tissue within normal gastric tissue. Afterward, the remaining tissue was cut at 10-20 μm thickness. We performed tissue collection by laser capture microdissection (LCM; Veritas LCM2110, Molecular Device Corporation, CA, USA) and compared these slides with the H&E slides (Figure 1A-C) according to the manufacturer's recommendations. After tissue collection, we extracted RNA using a Total Purification Kit (Norgene Bioteck Corp, Thorold, Ontario, Canada) according to the manufacturer's recommendations.

We collected 9 surgical samples in total, 6 WD-GC samples and 3 SRC-GC samples, and we found that 1 WD-GC sample was severely degraded. Ultimately, we compared 5 WD-GC samples with 3 SRC-GC samples (Figure 1D-F). We performed transcriptome sequencing, and we compared expression profiles between clinically comparable samples using transcriptome resequencing data. mRNA sequencing was performed with next-generation sequencing technology by Macrogen, Inc. (Seoul, Republic of Korea). We performed gene enrichment and functional annotation analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics resources (https://david.ncifcrf.gov) with the Ingenuity Pathway Analysis system (Qiagen, Hilden, Germany), and we determined the top 10 different genes in terms of the biologic process, cellular component, and molecular function categories and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway differences between SRC-GC and WD-GC (Figure 2).[19]

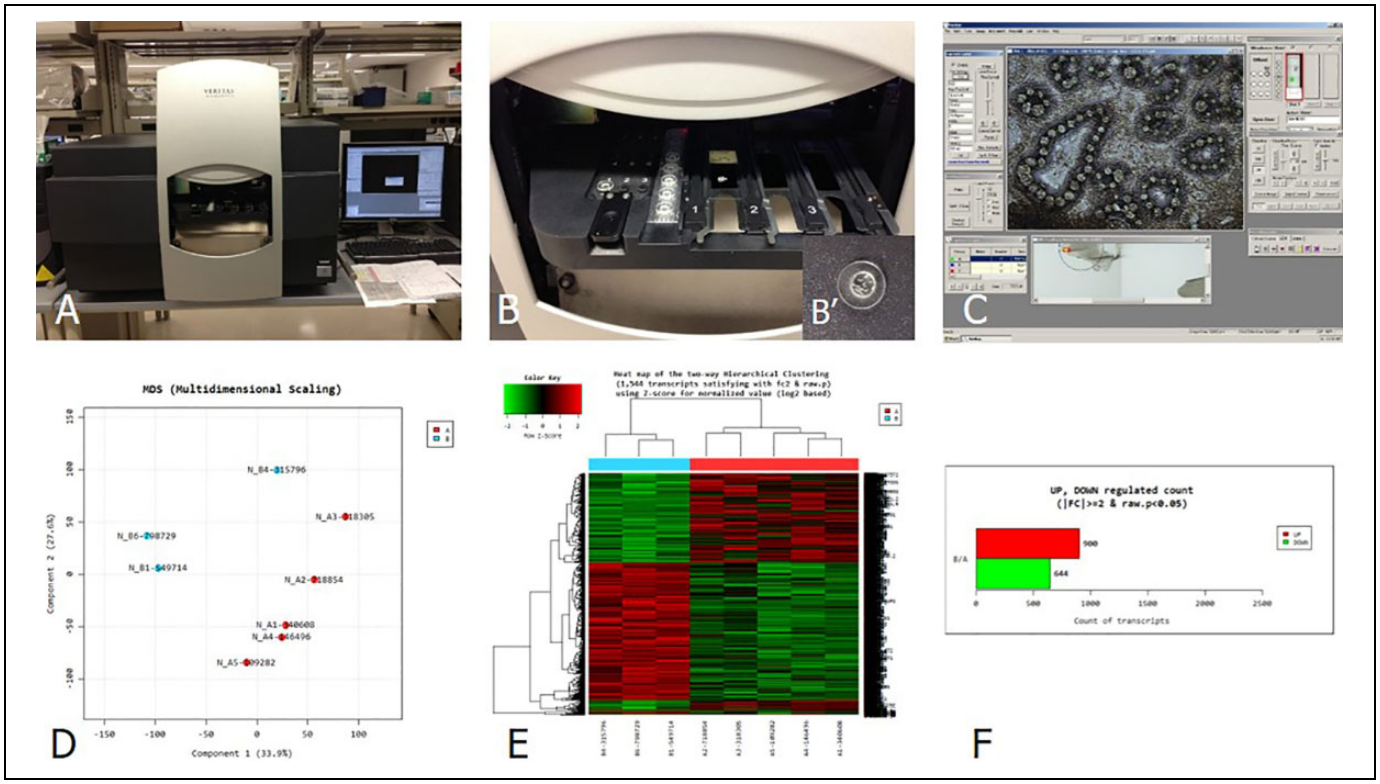

Figure 1. We collected surgical samples by laser capture microdissection (LCM, A-C), and we compared expression profiles using transcriptome resequencing data (D-F). A, LCM machine (Veritas LCM2110). B, Placement of the cap. B', shows each captured cap. C, LCM process. D, Multidimensional scaling plot. E, Heat map for hierarchical clustering. F, Significant count by fold change and *P*-value.

We generated a schematic illustration of the results of the cancer driver variant analysis. We filtered 170,602 variants and 16,460 genes, and we ultimately extracted 52 variants and 30 cancer driver genes. We performed variant analysis and network analysis with Insilicogen Inc. software (Yongin-si, Korea). We found 16,460 genes with 170,602 variants by comparing IGC (A group) and DGC (B group) samples. The genes were filtered by the 1000 Genomes Project, ExAC, NHLBI ESP exomes (with the parameters African American and European American), and Allele Frequency Community (with the parameters common variants and allele frequency below 0.1) resources. Common variants were filtered to include only pathogenic variants (according to the ACMG guidelines classification). We selected more than 2/3 of the samples in the SRC-GCB group and more than 2/5 of the samples in the WD-GC group and filtered the results by biological context (metastasis, signet ring adenocarcinoma or signet ring cell primary gastric adenocarcinoma). Finally, we found cancer driver variants with a frequency greater than 0.01% in the COSMIC and TCGA databases (Figure 3; Table 1).

Validation of NUDC Expression by Tissue Microarray Analysis and Immunohistochemistry

We generated a tissue microarray with surgical samples from 313 patients and collected clinical data from the patients' pathological reports regarding cancer recurrence. In the case of death, the National Statistical Office of the Republic of Korea was commissioned for confirmation.

Immunohistochemistry staining of NUDC was performed using monoclonal anti-rabbit NUDC antibody (1:300; Abcam, Boston, MA, USA). For immunohistochemical staining, unstained slides were treated with 3% H_2O_2 after deparaffinization and rehydration. After heating the slides in a microoven to induce epitope expression, we performed immunohistochemical staining by using the UltraVision LP detection system (Lab Vision Corporation, Fremont, CA, USA). The expression of NUDC was scored by a pathologist blinded to the clinicopathological data. Cytoplasmic reactions were scored according to the percentage of NUDC-positive cells as follows: 0, negative, 1+ (1%-24%), 2+ (25%-49%), and 3+ (50%-74%) (Figure 4).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 24.0 software (IBM Corporation, Somers, NY, USA). Data represents the mean $\pm$ SD. The significance of the difference was determined by χ^2 test, Student's *t*-test, and Cox proportional hazard analysis. The Kaplan-Meier method was used to analyze patient overall survival. Statistical tests were either 2-sided or 1-sided, and $P \leq 0.05$ was considered statistically significant.

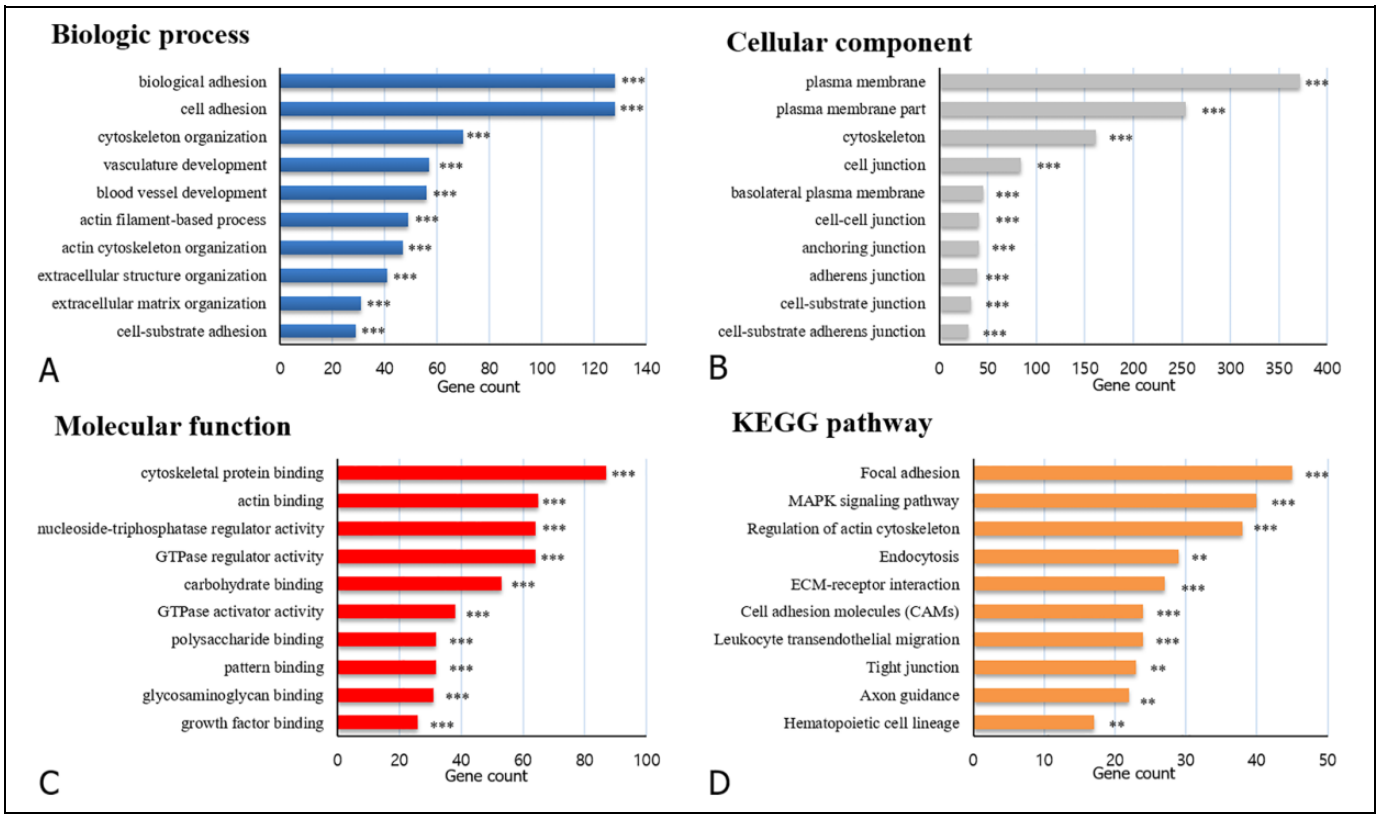

Figure 2. We performed gene enrichment and functional annotation analysis using the DAVID database, and the top 10 differentially expressed genes in the biological process, cellular component, and molecular function categories and the KEGG pathway differences between signet ring cell gastric adenocarcinoma and well-differentiated gastric adenocarcinoma are shown. DAVID, Database for Annotation, Visualization and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes.

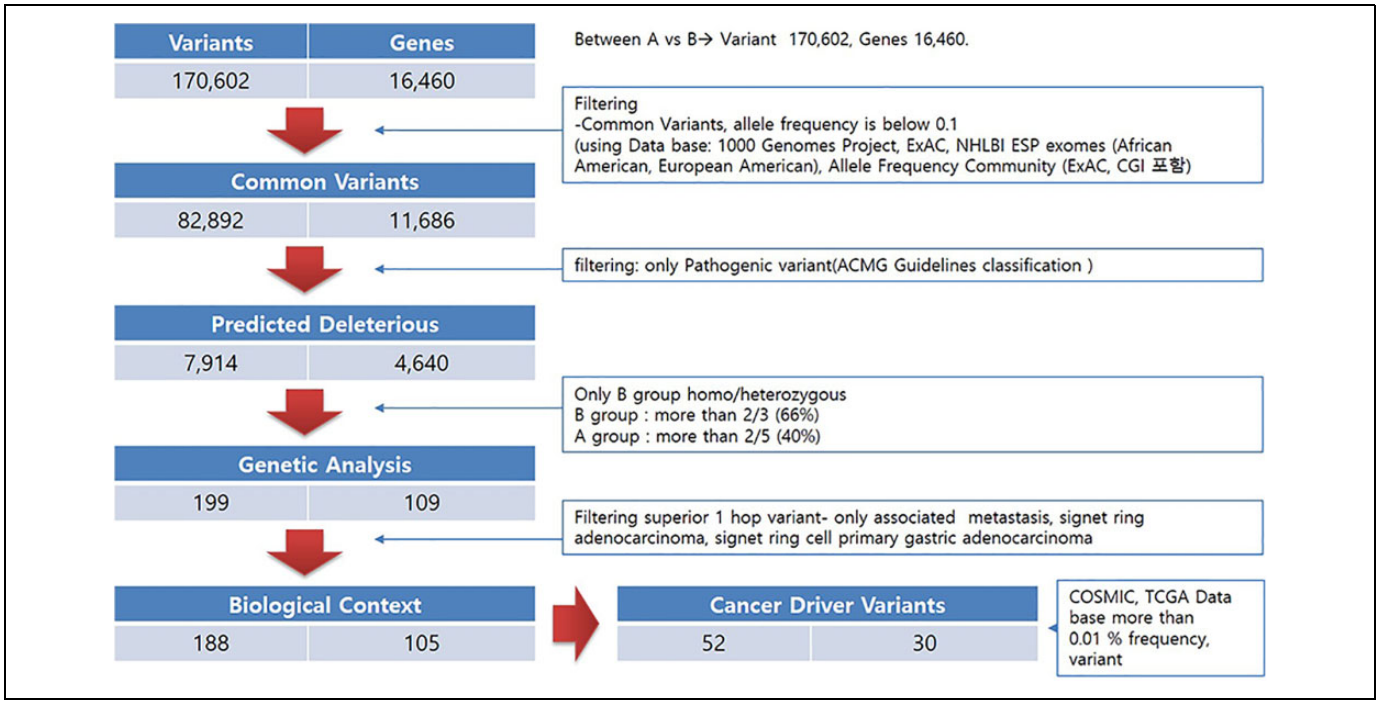

Figure 3. Schematic illustration of the results of the cancer driver variant analysis. We filtered 170,602 variants and 16,460 genes, and we ultimately extracted 52 variants and 30 cancer driver genes.

Table 1. The Important 30 Cancer Driver Genes List Between Signet Ring Cell Type Gastric Adenocarcinoma and Well Differentiated Type Gastric Adenocarcinoma.

Symbol	Entrez gene name	Location	Type(s)	Variant number	SRC-GC (N = 3)	WD-GC (N = 5)
AATF	Apoptosis antagonizing transcription factor	Nucleus	Transcription regulator	2	3	1
ALMS1	ALMS1, centrosome and basal body associated protein	Cytoplasm	Other	1	2	0
CDC14B	Cell division cycle 14B	Nucleus	Phosphatase	1	2	1
CHFR	Checkpoint with forkhead and ring finger domains, E3 ubiquitin protein ligase	Nucleus	Enzyme	1	2	0
DOCK1	Dedicator of cytokinesis 1	Cytoplasm	Other	2	2	0
DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1	Cytoplasm	Peptidase	1	2	0
EIF2AK3	Eukaryotic translation initiation factor 2 alpha kinase 3	Cytoplasm	Kinase	1	2	1
FBLN5	Fibulin 5	Extracellular Space	Other	1	2	0
FLNA	Filamin A	Cytoplasm	Other	2	3	1
H3F3C	H3 histone, family 3C	Other	Other	1	2	0
HLA-C	Major histocompatibility complex, class I, C	Plasma Membrane	Other	1	3	0
HLA-DQA2	Major histocompatibility complex, class II, DQ alpha 2	Plasma Membrane	Transmembrane receptor	4	2	1
HNRNPM	Heterogeneous nuclear ribonucleoprotein M	Nucleus	Other	2	2	1
IFT74	Intraflagellar transport 74	Cytoplasm	Other	1	2	0
LGALS9B	Lectin, galactoside-binding, soluble, 9B	Cytoplasm	Other	2	2	1
MOV10L1	Mov10 RISC complex RNA helicase like 1	Nucleus	Enzyme	1	2	0
MUSK	Muscle, skeletal, receptor tyrosine kinase	Plasma Membrane	Kinase	2	2	0
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	Nucleus	Transcription regulator	1	2	0
NUDC	NudC nuclear distribution protein	Cytoplasm	Other	1	2	0
PDE4D	Phosphodiesterase 4D	Cytoplasm	Enzyme	2	2	0
PHLPP1	PH domain and leucine rich repeat protein phosphatase 1	Cytoplasm	Enzyme	1	2	0
PICALM	Phosphatidylinositol binding clathrin assembly protein	Cytoplasm	Other	1	2	1
PLA2G4A	Phospholipase A2 group IVA	Cytoplasm	Enzyme	1	2	0
PTPRS	Protein tyrosine phosphatase, receptor type S	Plasma Membrane	Phosphatase	2	2	0
RGPD4	RANBP2-like and GRIP domain containing 5	Nucleus	Other	6	3	3
SEMA4D	Semaphorin 4D	Plasma Membrane	Transmembrane receptor	1	2	0
SOS2	SOS Ras/Rho guanine nucleotide exchange factor 2	Cytoplasm	Other	1	2	0
SPAST	Spastin	Nucleus	Enzyme	2	3	1
TBC1D3	TBC1 domain family member 3	Extracellular Space	Other	1	2	1
ZNF267	Zinc finger protein 267	Nucleus	Other	6	2	1

Abbreviations: SRC-GC, signet ring cell gastric cancer; WD-GC, well-differentiated gastric cancer.

Results

Comparison of the Transcriptome Between SRC-GC and WD-GC

We performed next-generation sequencing on 3 SRC-GC and 5 WD-GC samples. We compared the 2 groups with multidimensional scaling and found that SRC-GC and WD-GC samples showed clear differences (Figure 1D), including clear differences in the 2-dimensional heatmap for hierarchical clustering (Figure 1E). We performed statistical analysis, and we found that 900 genes showed significantly higher expression and 644 genes showed lower expression in SRC-GC than in WD-GC (Figure 1F).

To identify differences in gene ontology (GO), we performed gene enrichment and functional annotation analysis using DAVID, and we identified the top 10 different genes in the biological process, cellular component, and molecular function categories and KEGG pathway differences between IGC and DGC, and the results are shown in Figure 2.

For the genes in the biological process category, the genes with the largest differences between SRC-GC and WD-GC were related to biological adhesion and cell adhesion. Cytoskeletal organization, vascular development and blood vessel development were also different between the 2 groups. Regarding the cellular component category, the plasma membrane, plasma membrane part, cytoskeleton, cell junction basolateral plasma

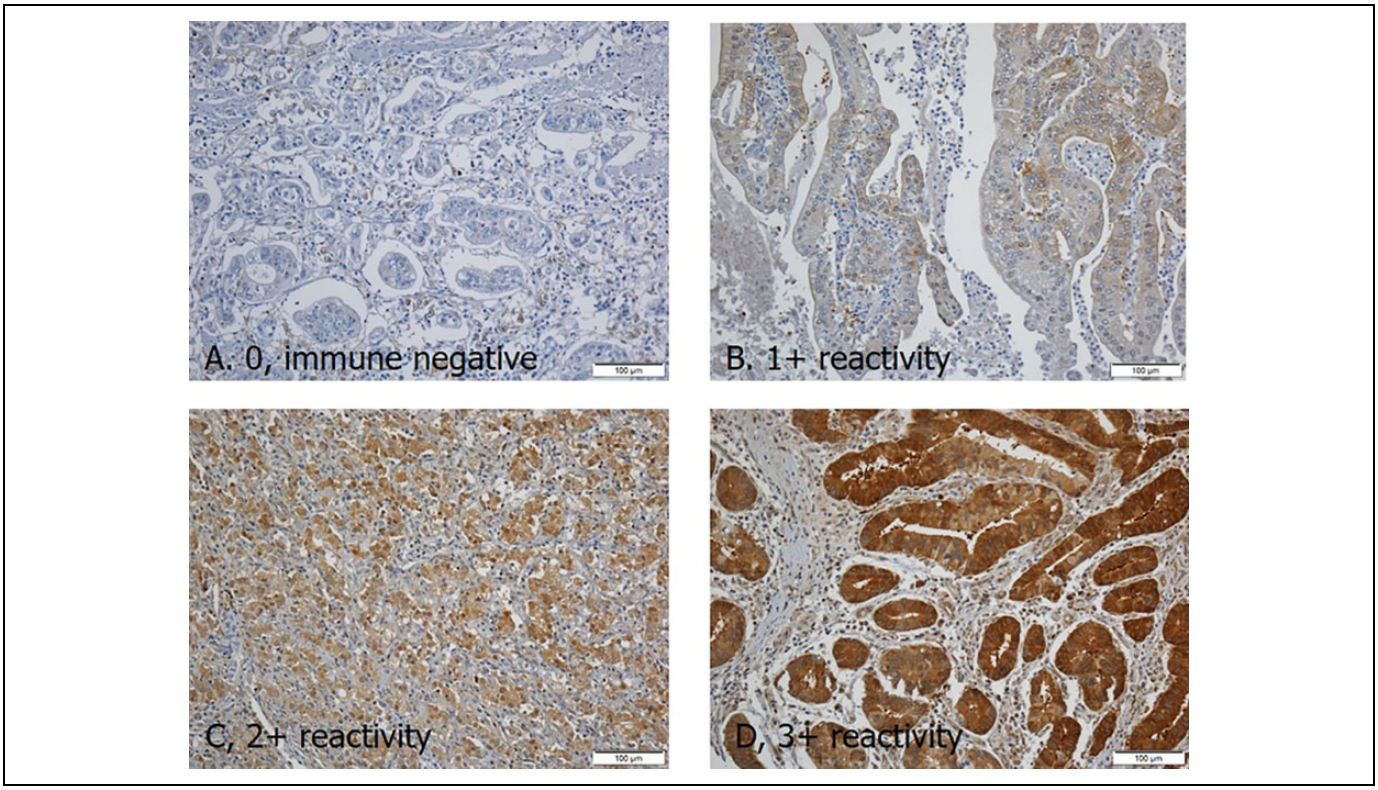

Figure 4. Immunohistochemical analysis of NUDC expression in gastric carcinoma tissues. Staining of NUDC was performed using anti-NUDC antibody cytoplasmic reactions were scored according to the percentage of NUDC-positive cells as follows: 0, negative, 1+ (1%-24%), 2+ (25%-49%), and 3+ (50%-74%). Arrows show representative cells with positive staining for NUDC.

membrane and cell-cell junction terms were significantly different between the 2 groups. Regarding the molecular function category, the cytoskeletal protein binding, actin binding, nucleoside-triphosphate regulator activity, GTPase regulator activity, and carbohydrate binding terms were significantly different between the 2 groups. In the KEGG pathway analysis, the focal adhesion, MAPK signaling pathway, regulation of actin cytoskeleton, endocytosis, ECM-receptor interaction, and cell adhesion molecule terms were significantly different between the 2 groups.

We performed variant analysis between the SRC-GC and WD-GC groups, and we found 170,602 variants among 16,460 genes (Figure 3). We identified which of these variants and genes were associated with pathologic variants with a frequency of more than 0.01% using a public database, and we ended up with 52 variants and 30 genes (Table 1). In terms of intracellular location, 13 of the 30 genes, including ALMS1, DOCK1, NUDC, and PHLPP1, were located in the cytoplasm, 9 genes, including AATF and NFKB, were located in the nucleus, 5 genes, including SEMA4D and MLA-C, were located in the plasma membrane, and 2 genes, FBLN5 and TBC1D3, were located in the extracellular space. In terms of the type of protein encoded by the gene, PPHLPP1, CHFR, PDE4D, PLA2G4A, and MOV10L1 encode enzymes, and EIP2AK3 and MISK encode kinases. AATF and NFKB1 are transition regulators, CDC14B and PTPRS are phosphatases, and DYNC1H1 is a peptidase.

The NUDC-Positive Group Showed Better Survival Than the NUDC-Negative Group

NUDC was identified among the 30 cancer driver genes, and we analyzed NUDC expression in gastric cancer tissue and its relationship with clinical prognosis. We scored NUDC expression in gastric cancer samples (n = 313) using immunohistochemistry, as shown in Figure 2, and we divided samples with a staining score over 1+-3+ into the NUDC-positive group and those with a staining score of 0 into the NUDC-negative group to analyze clinicopathological factors.

The mean age of the patients was 63.0 $\pm$ 10.8 years, and more patients were male than female (M/F: 203/110). According to the WHO classification, the most common phenotype was moderately differentiated (n = 109), followed by poorly differentiated (n = 102), well differentiated (n = 61), and signet ring cells (n = 27). The intestinal type was more common (n = 169) than the diffuse type (n = 61) or mixed type (n = 11). The mean tumor size was 4.5 $\pm$ 2.8 cm, and early T stage (T1/2/3/4: 149/33/95/36), no lymph node metastasis (N0/1/2/3: 18,844/37/41/51), no distant metastasis (M0/1: 311/2), and early TNM stage (stage I/II/III/IV: 166/58/87/2) were the most common characteristics. In terms of NUDC expression, NUDC positivity was more common than NUDC negativity (NUDC 0/1/2/3: 28/113/107/65) (Supplement Table 1).

We performed Cox proportional hazard analysis of clinicopathological features and NUDC expression, and we found that

tumor stage (T1 vs T2-4), lymph node status (N0 vs N ≥ 1), and NUDC expression (negative vs. positive) were independent risk factors for survival ($P < 0.05$). However, there were no differences in age, sex, WHO class, metastasis, or chemotherapy. In terms of tumor stage, the hazard ratio of the T2-4 group was 3.68 times higher than that of the T1 stage group (95% CI, 1.33-10.01, $P = 0.012$). In terms of lymph node status, the hazard ratio of the N ≥ 1 group was 9.22 times higher than that of the N0 group (95% CI 3.8-22.2, $P < 0.001$). In terms of NUDC expression, the hazard ratio of the NUDC-negative group was 2.44 times higher than that of the NUDC-positive group (95% CI 1.27-4.69) (Table 2).

Table 2. Cox Proportional Hazard Analysis by Clinicopathological Features and NUDC Expression by Immunohistochemistry in 313 Gastric Cancer Patients.[a]

Variable	Value	*P* value	Hazard ratio	95% CI
Age	< 70	0.121	Reference	
	≥ 70		1.51	0.89-2.56
Gender	Male	0.147	1.51	0.86-2.66
	Female		Reference	
WHO	classification			
	Differentiated (Well/moderate)	0.27	Reference	
	Undifferentiated (poorly/Signet ring cell type)		1.33	0.79-2.22
Tumor	EGC (T1)	**0.012**	Reference	
	AGC (T2-4)		**3.68**	**1.33-10.01**
Lymph node	No (N0)	**<0.001**	Reference	
	Yes (N ≥ 1)		**9.22**	**3.8-22.2**
Metastasis	No (M = 0)	0.182	Reference	
	Yes (M = 1)		2.78	0.61-12.5
Chemotherapy	No	0.764	Reference	
	Yes		1.12	0.53-2.35
NUDC expression	Negative (score 0)	**0.007**	**2.44**	**1.27-4.69**
	Positive (score 1-3)		Reference	

Abbreviations: NUDC, nuclear distribution protein C; WHO, World Health Organization; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; SRC, signet ring cell carcinoma; LN, lymph node; EGC, early gastric cancer; AGC, advanced gastric cancer.
[a]Values in boldface indicate the significance of the differences determined using the Student's unpaired T-test.

We performed Kaplan-Meier survival analysis according to NUDC expression. Figure 5A shows each overall survival curve according to NUDC expression (negative, 1, 2, and 3 ($P = 0.008$)). The overall survival of the NUDC-positive group was significantly higher (53.2 ± 0.92 months, 95% CI, 51.4-55.0 months) than that of the NUDC-negative group (44.6 ± 3.7 months, 95% CI, 37.3-51.9 months) ($P = 0.001$) (Figure 5B).

Discussion

The aim of this study was to compare the transcriptomes of 2 very different subtypes of gastric cancer (SRC-GC and WD-GC). We found that there was a large difference in adhesion, vascular development, and cell-to-cell junction components, and we also conducted cancer variable analysis. We found 30 cancer driver gene candidates. We found that the NUDC-positive group showed significantly better survival than the NUDC-negative group, as determined by transcriptome variant analysis.

Collecting fresh RNA is difficult during cancer surgery because ischemia has already occurred. Even if the procedure is performed quickly after resection and the surgical procedure mainly uses vessel ligation, RNA degradation is inevitable during surgery.[20] Additionally, most biopsies are performed in advanced cancer, and there is so much inflammation and fibrosis around the tumor mass that it is not easy to distinguish

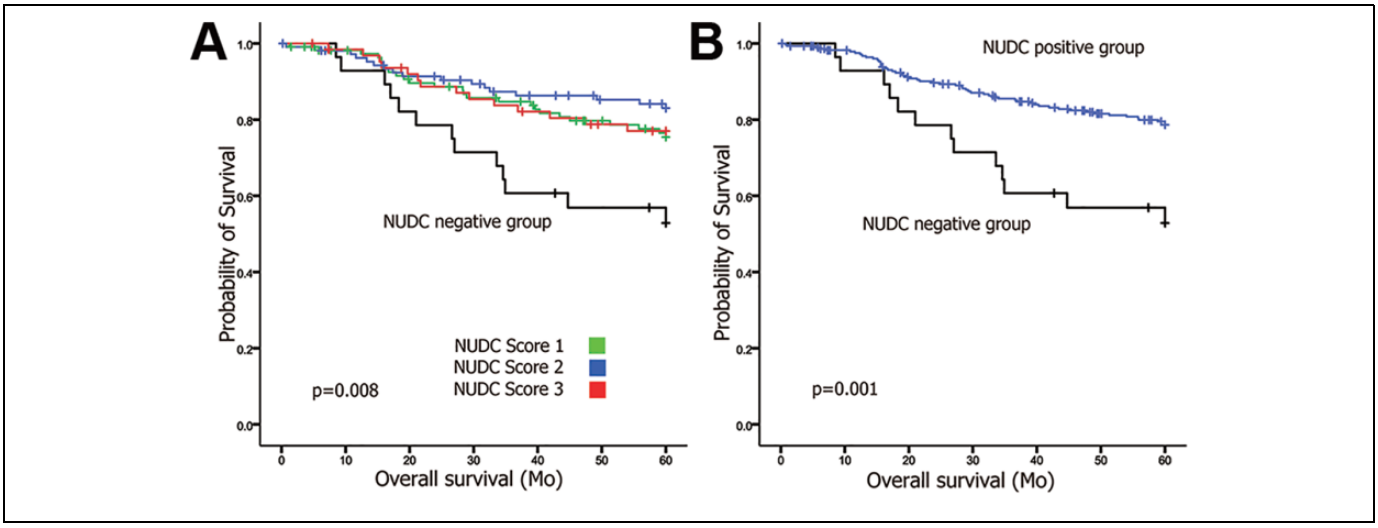

Figure 5. Patients with NUDC overexpression showed longer survival times than patients with NUDC underexpression in the Kaplan-Meier survival analysis. A, Overall survival according to NUDC expression (negative to 3). B, Overall survival of the NUDC-positive and NUDC-negative groups.

between cancer, fibrotic, and inflamed tissue with the naked eye. Therefore, if next-generation sequencing is performed using tissue, at least H&E staining should also be performed to confirm that the tissue is cancerous. We performed LCM, but for the DGC samples, we thought that there might be muscle tissue contamination because diffuse cancers mostly invade nearby tissues, especially gastric muscle.[21] Therefore, there have been few reports studying whole transcriptomes in gastric cancer.

Recently, a transcriptome sequencing study of SRC-GCs vs non-SRC-GCs was reported.[11] The researchers conducted the study to identify key mRNAs and signaling pathways and the transcriptome in SRC-GC, and gene expression was analyzed by comparing 30 SRC-GS samples and 30 non-SRC-GC samples. MAGEA2, MEGA3, MEGA4, MEGS5 (cancer-testis antigens) and regenerating islet derived 1 beta (REG1B) showed differences between the 2 groups. Fifteen KEGG pathways were reported, including the Rap1 signaling pathway and the EGFR tyrosine kinase inhibitor resistance pathway. The endocytosis pathway was also differentially activated between the 2 groups in this study. We found that the terms adhesion, cytoskeleton, vascular development, and blood vessel development were enriched in SRC-GCs compared with WD-GCs in the DAVID analysis. SRC-GCs have some components of DGC, and they are known to have greater adherence to surrounding tissues during invasion and greater inflammation than IGCs. These characteristics are one reason why most samples are identified as advanced stage samples, and advanced cancer has central necrosis with peripheral inflammation. As such, the tumors need to be dissected by LCM. Many molecular function terms, such as cytoskeletal protein, actin, and GTP, can also be related to the invasive and adhesive characteristics of DGCs. On the other hand, IGCs tend to form glands and have limited inflammation and adhesion to surrounding tissues. The concept of our study, which compared transcriptomes, was similar to that of Zhao *et al*'s study,[11] but the control groups were different. They compared SRC-GCs and poorly differentiated GCs, which are both DGCs, but we compared SRC-GCs (DGCs) and WD-GCs (IGCs). Additionally, the number of next-generation sequencing samples was smaller in their study (4 vs 8) than in our study. Therefore, the differences in the control group resulted in differences in the GO analysis and pathway analysis.

Several studies have identified biomarkers in stomach cancer, and markers matching up with the Lauren classification strategy have been reported: CDH1, CDX-2, MSI, and HER2.[22] Of these, HER2 is a unique marker that can be used in the development of targeted agents. The expression of HER2 is more associated with IGC than DGC.[23] We also studied the differentially expressed genes between the 2 groups; however, we thought that variant genes would play an important role in the generation of cancer, so we conducted variant analysis first. For the variant gene analysis, the reliability of the results increases as the algorithm finds and analyzes genes that have been previously studied and published, but finding new biomarker genes is difficult. All 30 candidate genes identified are genes that have been published as biomarkers, and studying whether they are expressed or useful as markers in cancer is very interesting. However, NUDC has been reported in colorectal cancer, but there has been few research in stomach cancer field. Additionally, the recently studied markers were discovered through a comparative study of normal and cancer tissues from multiple organs. However, NUDC was discovered through comparative analysis of 2 subtypes of gastric cancer with quite different prognoses (signet ring cell gastric cancer, worst prognosis in diffuse-type and well-differentiated gastric cancer, best prognosis in intestinal-type).

A mammalian NUDC-like protein has been reported to be important for cell viability and dynein stability (dyneins are a family of cytoskeletal motor proteins that move along microtubules in cells).[24] The depletion of NUDC is reported to reduce end-on microtubule attachments at kinetochores and is one of the causes of chromosomal condensation defects at the metaphase plate.[16] It has been reported that NUDC is required for Plk1 targeting to the kinetochore and chromosomal condensation. Silencing of NUDC using siRNA interferes with proliferating HeLA cells and affects Caenorhabditis elegans, and overexpression of NUDC using adenovirus is known to lead to multinucleation of cells.[25]

In the cancer field, NUDC overexpression has been reported to block prostate cancer cell division, and this strategy has been developed as a targeted therapy.[14] NUDC has been reported to be increased in acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, lung cancer, melanoma, and prostate cancer.[15,17,25,26] In addition, NUDC is reported to increase the proliferation, migration and invasion of renal carcinoma cells.[17] In the TCGA database-associated stomach cancer dataset (TCGA-STAD), the mutation rate of NUDC was 3% (3/440), and the copy number variation (CNV) gain and loss rates were 1.16% (5/432) and 12.73% (55/432), respectively. We found an NUDC variant in 2 of 3 samples of SRC-GC but no variants in 5 WD-GC samples. These NUDC variants in SRC-GCs might be nonfunctional and nonfunctioning of movement of nuclei following mitosis may play an important role not only in driving SRC-GC, but also in progression of stomach cancer. We are planning to study the changes in the function of NUDC according to whether it varies in the future.

The limitation of this study is the small number (8 samples) of samples. We could not analyze the relationship between each NUDC score and survival due to the small number of samples and the changes in function of NUDC according to whether it is variant. However, the cancer tissues were dissected by LCM, which produced a more accurate result. The increased accuracy of LCM was confirmed by IHC analysis in the large cancer cohort. To the best of our knowledge, there are few studies comparing the variants and transcriptomes of SRC-GCs and WD-GCs.

In conclusion, we found 30 gastric cancer driver variants by comparing SRC-GCs and WD-GCs. NUDC was identified as one of the cancer driver variants and could be a potential good prognostic marker in gastric adenocarcinoma.

Authors' Note

Sang-Ho Jeong and Miyeong Park contributed equally to this study. This study was designed and carried out according to the principles of the Declaration of Helsinki (1989). Written consent was obtained from all participants before inclusion in the trial. The study was approved by the Institutional Review Board of Gyeongsang National University Hospital (GNUHIRB 2009-54).

Declaration of Conflicting Interests

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Supplemental Material

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