Identification of candidates for driver oncogenes in scirrhous-type gastric cancer cell lines

Eirin Sai1 | Yoshiyuki Miwa2 | Reina Takeyama3,4 | Shinya Kojima3,4 | Toshihide Ueno3,4 | Masakazu Yashiro5 | Yasuyuki Seto2 | Hiroyuki Mano3,4

Abstract
Scirrhous-type gastric cancer (SGC) is one of the most intractable cancer subtypes in humans, and its therapeutic targets have been rarely identified to date. Exploration of somatic mutations in the SGC genome with the next-generation sequencers has been hampered by markedly increased fibrous tissues. Thus, SGC cell lines may be useful resources for searching for novel oncogenes. Here we have conducted whole exome sequencing and RNA sequencing on 2 SGC cell lines, OCUM-8 and OCUM-9. Interestingly, most of the mutations thus identified have not been reported. In OCUM-8 cells, a novel CD44-IGF1R fusion gene is discovered, the protein product of which ligates the amino-terminus of CD44 to the transmembrane and tyrosine-kinase domains of IGF1R. Furthermore, both CD44 and IGF1R are markedly amplified in the OCUM-8 genome and abundantly expressed. CD44-IGF1R has a transforming ability, and the suppression of its kinase activity leads to rapid cell death of OCUM-8. To the best of our knowledge, this is the first report describing the transforming activity of IGF1R fusion genes. However, OCUM-9 seems to possess multiple oncogenic events in its genome. In particular, a novel BORCS5-ETV6 fusion gene is identified in the OCUM-9 genome. BORCS5-ETV6 possesses oncogenic activity, and suppression of its message partially inhibits cell growth. Prevalence of these novel fusion genes among SGC awaits further investigation, but we validate the significance of cell lines as appropriate reagents for detailed genomic analyses of SGC.

KEYWORDS
BORCS5-ETV6, CD44-IGF1R, fusion kinase, scirrhous-type gastric cancer, tyrosine kinase inhibitor

INTRODUCTION

Despite the advent of screening technologies for the upper digestive tract, gastric cancer remains the third leading cause of cancer-related deaths, and almost 1 million people die of this disease worldwide every year.1,2 In particular, scirrhous-type of gastric cancer (SGC) is one of most intractable cancer subtypes, with a 5-year survival rate of only 11%-16%.3,4 SGC is characterized by rapid growth and infiltration of poorly differentiated or signet-ring cell-type cancer cells with marked surrounding fibrosis.3-7 SGC
often lacks apparent mucosal lesions, making it difficult to detect in the early stages.

A wide range of genomic/epigenomic analyses has been conducted to identify therapeutic targets for gastric cancer. By combining whole exome sequencing (WES) and RNA-sequencing (RNA-seq) datasets, for instance, The Cancer Genome Atlas team proposed to divide gastric cancer into 4 subtypes: (i) Epstein-Barr virus-positive type, with a CpG island methylator phenotype and frequent PIK3CA nonsynonymous mutations; (ii) microsatellite instability-positive type, with a high mutation burden; (iii) chromosomal instability-positive type with frequent mutations within CDH1, RHOA and ARID1A. The GS type is often associated with diffuse-type gastric cancer that significantly overlaps with SGC.

Somatic mutations within RHOA were also reported in 14%-25% of diffuse-type gastric cancer. Such RHOA mutants are presumed to act in a dominant-negative manner to the wild-type protein, and, thereby, promote cell survival. It is, however, unclear if these RHOA mutations are enriched in SGC.

Genome-wide mutation screening with next-generation sequencers (NGS) for SGC is severely hampered by low tumor contents by the markedly increased fibrous tissues in a given specimen. SGC cell lines have, therefore, useful resources to identify their transforming genes. FGFR2 gene was, for instance, shown to become amplified and oncogenic in an SGC cell line, KATO-III. MET amplification is also reported to be present in some SGC cell lines. To identify potential therapeutic targets in SGC, here we have conducted WES and RNA-seq for 2 SGC cell lines, OCUM-8 and OCUM-9. Interestingly, we revealed novel transforming fusion genes, CD44-IGF1R from the former cell line and BORCS5-ETV6 from the latter.

2 | MATERIALS AND METHODS

2.1 | Cell lines

Human embryonic kidney (HEK) 293T cells and mouse 3T3 fibroblasts were obtained from the ATCC (https://www.atcc.org). SGC cell lines, KATO-III and NUGC4, were purchased from Japanese Collection of Research Bioresources (http://cellbank.nibiohn.go.jp/english/). OCUM-1, -2M, -8,-9 and -12 were established by M.Y. All cell lines were maintained in DMEM-F12 medium supplemented with 10% FBS and 2 mmol/L glutamine (all from Invitrogen).

2.2 | Next-generation sequencer analyses

Genomic DNA was isolated from each cell line and subjected to enrichment of exonic fragments with a SureSelect Human All Exon Kit v5 (Agilent) followed by nucleotide sequencing with the HiSeq2500 platform (Illumina) using the paired-end option. Bioinformatics analyses were conducted as reported previously. Nonsynonymous mutations were called only when ≥10% of reads corresponded to the mutations at the positions with a total coverage of ≥20.

Total RNA was isolated from each cell line with the use of an RNeasy Mini Kit (Qiagen) and was subjected to RNA-seq using a NEBNext Ultra Directional RNA Library Prep Kit (New England BioLabs). Relative expression level (fragments per kilobase of exon per million reads mapped, FPKM) of genes were calculated with the Cufflinks pipeline (http://cole-trapnell-lab.github.io/cufflinks/), and fusion genes were searched with deFuse.

2.3 | Cloning of fusion genes

A full-length cDNA of CD44-IGF1R was recovered from the RNA of OCUM-8 cells by RT-PCR with the following primers: 5’-TTTCGCTCGGACACCATGGACAC-3’ and 5’-GATCCAGAGATCATGTCCTG-3’.

Genomic PCR or RT-PCR for the CD44-IGF1R fusion point was conducted with the following primers: 5’-TGGACAAGTTTTGGTGGCACGCAG-3’ and 5’-CAGCCTACAATGACTTCAGTGCC-3’ or the cDNA primers (5’TTCGCTCGGACACCATGGACAC-3’ and 5’-GGCAGAGCGATGATCAGATGGATG-3’), respectively. Similarly, genomic PCR or RT-PCR for GAPDH was conducted with the genomic primers (5’-GTCAATGGGTGTGAACCAGAAG-3’ and 5’-TCTCATAACATGACTTCCTCCAC-3’), or the cDNA primers (5’-GTCAGTGGTGGACCTGACCT-3’ and 5’-TGAACCTGACAAAGTGTTG-3’), respectively. The cDNA of the kinase-dead mutant for CD44-IGF1R was generated with a Site-Directed Mutagenesis Kit (Invitrogen).

Full-length cDNAs of BORCS5-ETV6 and the wild-type ETV6 were PCR-amplified using the following primers: 5’-CGTTTTCTGTTCACCATCTGTCCTGGCCTC-3’ and 5’-GGACTGGTTGGTCTCCACATTCTG-3’ for the former gene, and 5’-CTCGCTGTGAGACATGTCTGGACAC-3’ and 5’-GGACTGGTTGGTCTCCACATTCTG-3’ for the latter. The genomic rearrangement for the BORCS5-ETV6 fusion in OCUM-9 was PCR-amplified with a long-range PCR enzyme (Takara LA Taq, Takara Bio) from the genomic DNA with the following primers: 5’-GACCGTGACCCACACATTAG-3’ and 5’-TTTTGGAGGAGATGCTGCACAT-3’ or the cDNA primers (5’-AGCGTCACCCCCCACACATTAG-3’ and 5’-TTTTGAGAGGAGATGCTGCACAT-3’), respectively. The cDNA of the kinase-dead mutant for CD44-IGF1R was generated with a Site-Directed Mutagenesis Kit (Invitrogen).

2.4 | Functional assay

The cDNA of each gene was ligated into the pMX retroviral vector (Cell Biolabs), and the recombinant vectors were introduced together with an ecotropic packaging plasmid (Takara Bio) into HEK293T cells.
to obtain infectious virus particles. For the focus formation assay, 3T3 cells ($2 \times 10^5$) were infected with ecotropic recombinant retroviruses and cultured for 2 weeks in DMEM–F12 supplemented with 5% calf serum (Invitrogen). For the in vivo tumorigenicity assay, 3T3 cells ($5 \times 10^5$) expressing each gene were inoculated subcutaneously into nude mice.

To quantitate the copy number of *IGF1R* or *MET*, a part of *IGF1R* or *MET* gene was PCR-amplified with the *IGF1R*-specific primers (5′-GTCCTGTAGGTGGAGGT-3′ and 5′-CCCTTGGCAACTCTCTCATA-3′) or the *MET*-specific primers (5′-GCAGAACAAGCTCTCA-3′ and 5′-CCCTTGGCAACTCTCTCATA-3′), respectively, by using a droplet digital PCR system (ddPCR; QX200, Bio-rad) with the internal control of RPP30 gene amplified with the following primers: 5′-GATTTGGACCTGCGAGCG-3′ and 5′-GCCGTGTCTCCACAAATG-3′.

For immunoblot analyses, cell lysates were obtained from each cell line with the lysis buffer (1% NP-40, 50 mmol/L Tris–HCl, 150 mmol/L NaCl, 1 mmol/L NaF and 1 mmol/L Na$_3$VO$_4$). Fifteen micrograms of cell lysates were separated through SDS-PAGE, and probed with antibodies to IGF1R (#9750), phosphorylated IGF1R (#3918), MET (#8198S) or phosphorylated MET (#3077, all from Cell Signaling Technology).

3.1 Genomic analyses of OCUM-8

Whole exome sequencing of the genome of OCUM-8 and -9 was conducted with the mean coverage of 154× and 137×, respectively. More than 96% of exome regions were sequenced at >20× coverage in both cell lines. Messenger RNA was also extensively sequenced with NGS for OCUM-8 and -9, yielding the total nucleotides of 38 and 41 gigabases, respectively.

As shown in Table S1, after excluding nucleotide changes present in our in-house database for normal variations of human genome, we identified a total of 72 nonsynonymous mutations that are present in both exome and RNA-seq datasets of OCUM-8 (with a threshold of total coverage of ≥50× and of the mutation ratio of ≥25% in both datasets). Many of them have not been associated with gastric cancer nor recurrently reported in the COSMIC database version 87 (https://cancer.sanger.ac.uk/cosmic). An exception was the mutation for TP53 (E271K) found 37 times in the COSMIC database. The clinical relevance of this mutation is, however, obscure because it is assessed as “Uncertain significance” in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) as of March 2019.

3.2 Discovery of a novel fusion gene, CD44-IGF1R

Because amplification of FGFR2 gene and augmentation of tyrosine kinase activity in its protein product plays an essential role in the proliferation of a SGC cell line, KATO-III, we next analyzed chromosome copy number alterations (CNA) in OCUM-8. The high coverage of our WES analysis enabled us to infer detailed CNV, revealing focal amplification of 11p13 and 15q26 loci in OCUM-8 as well (Figure 1C). Increased in OCUM-8 as well (Figure 1C).

To examine if this genomic alteration produces fusion proteins, we searched for fusion mRNAs among the RNA-seq dataset of OCUM-8. Interestingly, in-frame fusion transcripts were, indeed, found between CD44 and IGF1R and between IRS2 and EHF in OCUM-8 (Table S2). By using RT-PCR, we obtained a full-length CD44-IGF1R fusion cDNA from OCUM-8. Nucleotide sequencing of the PCR product revealed that exon 1 of CD44 becomes fused to exon 12 of IGF1R (Figures S1, S2A). Analyses on OCUM-8 genomic DNA further confirmed that chromosome 11 is disrupted at a position 1499 bp-downstream to CD44 exon 1, and further ligated to a

2.5 Knockdown experiments with shRNA

Nucleotides corresponding to a shRNA against ETV6 were synthesized based on the shRNA (hs-sh-9528) from DECIPHER project (http://www.decipherproject.net), and inserted into the pMKO.1-GFP vector, which was a gift from Dr William Hahn (Addgene plasmid #10676; http://n2t.net/addgene:10676; RRID:Addgene_10676). The resultant pMKO.1-shETV6-GFP plasmid was transfected into HEK293 cells, and the culture supernatant containing the recombinant retrovirus was used to transfect into HEK293 cells, and the culture supernatant containing the recombinant retrovirus was used to infect OCUM-9 cells for 2 days. Real-time RT-PCR was conducted with a primer set (5′-TATAGAAATGTCCTAGCGGCT-3′ and 5′-TATAGAAATGTCCTAGCGGCT-3′) for ETV6, and with another primer set (5′-CAAGCTGGTGTGACTGCT-3′ and 5′-CAAGCTGGTGTGACTGCT-3′) for GAPDH.

2.6 Accession code

The raw sequencing data have been deposited in the Japanese Genotype-Phenotype Archive (JGA, http://trace.ddbj.nig.ac.jp/jga), which is hosted by DDBJ, under the accession number JGAS00000000179.
nucleotide at 917 bp-upstream to exon 12 of IGF1R in chromosome 15 (Figures 1D, S2B, S3). Importantly, the expression of CD44-IGF1R gene is driven by the promoter of CD44 that is known to be abundantly expressed in cancer stem cell fractions.\(^{17}\)

### 3.3 CD44-IGF1R is a transforming kinase and therapeutic target in OCUM-8

The CD44-IGF1R transcript can encode a chimeric protein of 561 amino acid residues with a predicted molecular weight of 63,386, consisting of the signal peptide of CD44 and the transmembrane and the intracellular domains of IGF1R (Figure 2A). Because fusion proteins of the IGF1R tyrosine kinase domain have rarely been reported, we assessed its transforming activity by using mouse 3T3 fibroblasts.

In the focus formation assay, as demonstrated in Figure 2B, expression of human wild-type CD44 did not induce transformed foci. Forced expression of the wild-type IGF1R induced some phenotypic changes in 3T3 cells but did not yield fully transformed, piled up foci. In contrast, however, induction of CD44-IGF1R resulted in marked transformation in 3T3, as did that of EML4-ALK. In contrast, the kinase-dead mutant of CD44-IGF1R, CD44-IGF1R(KM) in which the lysine residue at amino acid position 227 is replaced with a methionine, failed to produce such transformation, indicating that CD44-IGF1R exerts its oncogenic potential through its elevated tyrosine kinase activity.

The transforming potential of CD44-IGF1R was further confirmed by the nude mouse tumorigenicity assay (Figure 2C). Mouse 3T3 cells expressing each gene were inoculated subcutaneously into nude mice. The cells expressing CD44-IGF1R but not CD44-IGF1R(KM) produced subcutaneous tumors in all injection sites, confirming the oncogenic potential of CD44-IGF1R in vivo.

We further investigated if CD44-IGF1R is a therapeutic target for OCUM-8. First, overexpression of the CD44-IGF1R protein in OCUM-8 was confirmed by an immunoblot analysis. Total cell lysates prepared from OCUM-1, KATO-III, OCUM-8 and OCUM-9 were examined with antibodies to the IGF1R protein, revealing that broad bands of 65-90 kDa were detected with the antibody only in OCUM-8 (Figure 2D, left panel). OCUM-8 cells were then incubated with linsitinib, a selective inhibitor against IGF1R tyrosine kinase activity.\(^{18}\) As shown in the right panel of Figure 2D, immunoblot examination with antibodies to tyrosine-phosphorylated IGF1R revealed that phosphorylation of CD44-IGF1R became decreased with linsitinib in a dose-dependent manner, while the protein amount of CD44-IGF1R was stable with the treatment.

We next investigated whether the enzymatic activity of CD44-IGF1R was essential for the survival of OCUM-8 cells. As demonstrated in Figure 2E, linsitinib strongly and rapidly inhibited the viability of OCUM-8 but did not affect that of CD44-IGF1R-negative OCUM-9 and KATO-III cells.
Met amplification in OCUM-9

Genome profiles of OCUM-9 were also investigated. As depicted in Table S3, a total of 73 nonsynonymous mutations were detected in OCUM-9 with the same threshold from our WES and RNA-seq data. Interestingly, RHOA(L57V) found in OCUM-9 has been already shown to be associated with gastric cancer.9 While the Leu-to-Val substitution at amino acid position 57 was demonstrated to be loss-of-function, its relevance to gastric cancer remains elusive.

As in the case of OCUM-8, we assessed CNV in the genome of OCUM-9. Focal amplification was detected in chromosomes 2p, 7q and 12p (Figure 3A). Because the 7q locus contains the MET gene, we further evaluated the copy number of MET with ddPCR among the control cells, KATO-III, NUGC4 and OCUM-1, -2M, -8, -9 and -12 cell lines (Figure 3B). MET is highly amplified in OCUM-9 (copy number = 31.4), whereas its amplification (copy number ≥ 4) was not observed in the other samples. The expression level of MET was further evaluated by the RNA-seq dataset, revealing its overexpression in OCUM-9 cells (Figure 3C).

Immunoblot analyses with antibodies to MET among OCUM-1, KATO-III, OCUM-8 and OCUM-9 confirmed the overexpression of MET protein (Figure 3D, left panel). In OCUM-9 cells, MET is highly tyrosine-phosphorylated, but such phosphorylation becomes suppressed by the treatment with crizotinib,19 an inhibitor for MET tyrosine kinase activity (Figure 3D, right panel). Next, OCUM-9 cells were cultured in the presence of crizotinib, showing that crizotinib rapidly inhibited cell viability in a dose-dependent manner (Figure 3E). Crizotinib, in contrast, did not affect that of OCUM-8 and KATO-III. In OCUM-9, MET protein-tyrosine kinase is, thus, a suitable therapeutic target.
3.5 Other oncogenic alterations in OCUM-9

As shown in Table S2, OCUM-9 has an in-frame fusion gene between BORCS5 (also known as LOH12CR1) and ETV6 (Table S2), both of which are localized in the focal amplification locus at chromosome 12p13 (Figure 3A). Based on the estimated copy number gain, this amplicon likely contains ETV6, BCL2L14, LRP6, MANSC1 and BORCS5 (Figure 4A). In accordance with the copy number gain, RNA-seq revealed that these genes are abundantly expressed in OCUM-9 cells but not in the other SGC cell lines. It should be noted that genes outside this amplicon are not aberrantly expressed in OCUM-9.

Furthermore, the BORCS5-ETV6 fusion transcript was detected only in OCUM-9 (Figure S4A), and the genomic rearrangement leading to this fusion was further confirmed by genomic analyses (Figure S4B,C).

The predicted BORCS5-ETV6 message ligates the amino-terminal 20 amino acids of BORCS5 to the majority of ETV6 protein (Figures 4B, S5). To assess the transforming ability of the BORCS5-ETV6 fusion gene, we conducted the 3T3 focus formation assay. As shown in Figure 4C, both the wild-type ETV6 and BORCS5-ETV6 carry an oncogenic ability when abundantly expressed. Given the high expression of BORCS5-ETV6 in OCUM-9, this fusion gene likely contributes to OCUM-9 scirrhous cancer development. Indeed, shRNA-mediated knockdown of ETV6 message partially suppressed the growth of OCUM-9 cells (Figure S6).

Because the focal amplification in chromosome 2p contains the MYCN gene, a well-known oncogene, we further examined this amplicon in detail. Copy number estimation reveals that these genes are overexpressed only in OCUM-9 cells.

4 DISCUSSION

Here we have examined oncogenic genomic events in 2 scirrhous-type cancer cell lines, OCUM-8 and OCUM-9, and found CD44-IGF1R fusion-type oncogene in the former. IGF1R codes for a receptor for type I insulin-like growth factor, which is a transmembrane-type tyrosine kinase. Both IGF1 and IGF2 can bind to and activate the enzymatic potential of IGF1R, and thereby induce a plethora of intracellular signalings. Stimulation of IGF1R can lead to, for instance,
**FIGURE 4** Other oncogenic events of OCUM-9. A, Genes at chromosome 12p13.2 to 12p13.1 are schematically demonstrated, and the genes amplified in OCUM-9 are shown in magenta. The table further demonstrates the expression level (in FPKM) of these genes in KATO-III, NUGC4, OCUM-1, OCUM-8, OCUM-9 and OCUM-12. B, The BORCS5-ETV6 fusion cDNA can encode a protein with the amino-terminal 20 amino acids of BORCS5 and the carboxyl-terminal 397 amino acids of ETV6. The coiled-coil, Pointed and ETS DNA-binding domains are indicated. C, Mouse 3T3 cells were infected with an empty virus (Mock) or recombinant retrovirus expressing wild-type ETV6 or BORCS5-ETV6 fusion gene, and cultured for 7 d. Scale bar, 100 μm. D, Genes at chromosome 2p24.3 to 2p24.2 are schematically shown, and the genes amplified in OCUM-9 are shown in magenta. The expression level of these genes is demonstrated as in (A).
IGF1R has been shown to be a potential oncogene in cancer. Furthermore, IGF1R may play an essential role in acquiring resistance to anti-cancer agents.

Although IGF1R overexpression was reported to be a biomarker for drug response or poor outcome, rearrangements of IGF1R gene have rarely been reported. Kekeeva et al. discovered a fusion transcript of IGF1R-TTC23 in bladder cancer that encodes the amino-terminal extracellular region of IGF1R fused to TTC23 whose function is yet unknown. Piarulli et al. reported on a patient with an ALK-negative inflammatory myofibroblastic tumor carrying FN1-IGF1R fusion transcript. Both groups identified IGF1R fusion candidates from RNA-seq data but did not confirm the corresponding genomic rearrangements nor examine the transforming ability of the protein products. It should be noted, however, that while the predicted protein product of IGF1R-TTC23 seems not to carry any enzymatic activities, FN1-IGF1R should retain the intracellular tyrosine kinase domain of IGF1R.

In this manuscript, we have validated the genomic rearrangement that fuses the CD44 and IGF1R loci. Because the copy number of both genes is markedly increased (Figure 1A), complex rearrangements involving an amplicon containing CD44-IGF1R may take place in the OCUM-8 genome. CD44 is a cell-surface marker for cancer stem cells, and is, indeed, highly expressed in our SGC cell lines (data not shown). In addition, CD44 is involved in the invasion process of SGC cells through the CD44-RAC1 pathway. The promoter of CD44 should be, therefore, highly active in SGC cells, and may be an ideal partner for IGF1R to be in-frame fused. CD44-IGF1R exerts a marked transforming ability in 3T3 cells both in vitro and in vivo, and suppression of its activity in OCUM-8 induces rapid cell death, implying that CD44-IGF1R is an essential growth driver for this cell line. To search for CD44-IGF1R in SGC specimens, we further conducted RNA-seq and RT-PCR analyses on formalin-fixed paraffin-embedded tissues from 75 patients with SGC but failed to detect any IGF1R fusions among the clinical specimens (data not shown).

In the Tumor Fusion Gene Data Portal (https://tumorfusionportal.com) where gene fusions can be searched among the data of The Cancer Genome Atlas project, 9 IGF1R fusion transcripts are reported as of June 2019 (3 of them are in-frame). Similarly, the cBioPortal database (http://www.cbioportal.org) contains 7 fusion transcripts involving IGF1R, and 1 of them is found in stomach adenocarcinoma. Neither database contains the CD44-IGF1R fusions. IGF1R may, therefore, directly participate in carcinogenesis at a low frequency.

ETV6 belongs to the ETS family of transcription factors and is known to be fused to potential oncogenes, such as RUNX1 and NTRK3. ETV6 fusion to BORCS5 has not been reported yet. The focus formation assay revealed that BORCS5-ETV6 has a transforming ability as the wild-type ETV6, although the shape of foci generated by the former seems to be distinct from the latter (Figure 4C). BORCS5 is a component of the BLOC1-related complex, and functions to regulate lysosome localization.

Because BORCS5 contains an amino-terminal myristoylation signal, the predicted BORCS5-ETV6 may have an ability to anchor to lipid layer and, thereby, exert oncogenic roles partially different from the wild-type ETV6. The dependency of OCUM9 to BORCS5-ETV6 for growth further supports the important role of this novel fusion gene in carcinogenesis (Figure S6). Although no fusion transcripts for BORCS5 are present in the Tumor Fusion Gene Data Portal, 8 BORCS5 fusions are reported in the cBioPortal (none of them are BORCS5-ETV6).

In addition to BORCS5-ETV6, however, there are other oncogenic genomic alterations in OCUM-9. The MET tyrosine kinase gene is highly amplified in OCUM-9, and suppression of its enzymatic activity led to rapid cell death (Figure 3A, B, E). Furthermore, MYCN is markedly amplified and expressed in the same cell line (Figures 3A and 4D). Importantly, amplification of MYCN is frequently found in neuroblastoma, and the forced expression of MYCN in dopamine β-hydroxylase-expressing cells in mice is sufficient to induce tumors resembling human neuroblastoma. It is, therefore, likely that multiple oncogenic events contribute to the generation of OCUM-9.

We here reported novel oncogenic alterations in gastric cancer, IGF1R-fusion and ETV6-fusion genes, prevalence of which awaits further investigation. Detailed examination of other SGC cell lines may further decipher the molecular mechanisms underlying SGC and provide us therapeutic targets in this highly intractable malignancy.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest for this article.

ORCID

Masakazu Yashiro https://orcid.org/0000-0003-5743-7228
Hiroyuki Mano https://orcid.org/0000-0003-4645-0181

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SUPPORTING INFORMATION

Additional supporting information may be found in the Supporting Information section at the end of the article.

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