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CRISPR/Cas9-mediated editing of GABRR2 gene in RGC-5 cells induces random exon deletion, exon splicing and new exon recruitment

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Highlights

►CRISPR/Cas9 induces random deletions in the target region of GABRR2 gene.

►Both big and small indels can lead to unexpected high probability of exon truncation/skipping and exon recruitment.

►It is the first observation of exon recruitment by CRISPR/Cas9-mediated GABRR2 gene editing.

Keywords: CRISPR-Cas9; GABRR2; RGC-5; Exon skipping; Exon recruitment

Introduction

CRISPR/Cas9 is a powerful gene-editing system for efficient genome manipulations by using single-guide RNA (sgRNA) that targets the complementary sequence to generate double-strand DNA breaks[1-3]. sgRNA and nuclease Cas9 form a nuclease complex that hybridizes with genomic DNA containing a NGG protospacer adjacent motif (PAM). This system has increased the accessibility of gene editing by greatly simplifying the design of editing reagents. Repair of the DNA damage often introduces small deletions or insertions that disrupt the target gene and thereby knock out its function. CRISPR/Cas9 has been widely used to generate cell and animal lines with specific gene function being disrupted or knockout[4-8]. Applying the genome surgery to study gene function and to correct disease-associated genetic mutations highlights therapeutic applications in curing genetic disorders.

Exon skipping is a consequence of RNA splicing cause cells to “skip” over faulty or misaligned sections of genetic code, leading to a truncated but still functional protein despite the genetic mutation. CRISPR/Cas9-based genome editing can induce mutations in genome that has high probability to cause exon truncation, deletion or splicing. The accidents that mutations induced exon skipping were usually in the cases of the insertion of a large gene cassette or several nucleotides within exon[7,9-11]. Exon skipping caused by deletion mutations was seldom reported [12]. Screening mutations in diploid cells is difficult because of two copies of chromosome. It is an important task for determining genotypes in diploid cells, systematically analyzing the mutations in two alleles and detecting the real mRNA isoforms in diploid cells

subjected to CRISPR/Cas9 editing before using these diploid cells in gene function research.

Retinal ganglion cell (RGC) is a type of neuron located near the inner surface of the retina, RGC degeneration is the leading cause of irreversible blindness worldwide. GABRR2 is a member of the rho subunit family which forms ligand-gated chloride channel on retinal ganglion cell membrane, and it has a significant impact on visual information processed by the retina to the brain. In this study, we applied CRISPR/Cas9 to edit the GABRR2 gene in mouse retinal ganglion cells (RGC-5) to study what exactly happened in two alleles and what real mRNA isoforms formed in diploid cells subjected to CRISPR/Cas9 editing. A single sgRNA was employed to generate double-strand DNA breaks in the target locus. We used PCR sequencing for single clone validation of CRISPR/Cas9-mediated genomic editing in diploid cells. The indels and the corresponding effects at the target locus were further studied at genomic and RNA levels. Our results show that CRISPR/Cas9 induces random deletions in the target region, and both big and small indels can lead to unexpected high probability of exon truncation/skipping and exon recruitment phenomena. It is the first observation of exon recruitment by CRISPR/Cas9-mediated gene editing.

Materials and Methods

Generation of sgRNA targeting constructs

GABRR2 protein was encoded by 9 exons with total 1473 base pairs of nucleotides. The commercial anti-GABRR2 antibody recognized the peptide encoded by exon4.

Thus, the sgRNAs targeting exon5 or exon6 region of the GABRR2 gene will be a good choice. Moreover, the sgRNAs targeting the middle region of GABRR2 gene will induce the termination of protein translation. In this study, four sgRNAs were designed based on the high alignment scores and parameter values of NCBI nuclotide BLAST by inputting the latest 12bp guide RNA sequence plus 3bp PAM sequence. The 15bp guide sequences which can only match GABRR2 exon5 or exon6 regions are selected for the final experiment to exclude the off-target effect of CRISPR/Cas9. The sgRNAs were synthesized by Invitrogen (Beijing, China), and the guiding sequences and target regions were depicted in **Figure. 1A**. Oligos of sgRNAs were annealed and sub-cloned into BbsI-digested pX330 (addgene#42230) and the resulting constructs were confirmed by DNA sequencing.

T7E1 cleavage

RGC-5 cells were cultured in DMEM medium (Jinuo Biotech, Hangzhou, Zhejiang, China) containing 15% FBS (Gibco, Grand Island, NY, USA) and Penn/Strep solution (Jinuo Biotech, Hangzhou, Zhejiang, China). Cells grew in 6-well plates with cell density around 40% were transfected with 5μg of Cas9/sgRNA construct using Lipo6000 according to the manufacturer's instructions (Beyotime Biotech, Shanghai, China). After 72 hours, genomic DNA was isolated, and PCR was performed to amplify the genomic sequence spanning exon5 and exon6 of the GABRR2 gene using 5'-gcaagtgtggacggttaggagc-3' (mEx5-F) and 5'-cggatggtccacaccctatg-3' (mEx6-R). PCR conditions were composed of one initial step of 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 90 seconds, and

finally single step of 72°C for 10 minutes. PCR products were analyzed by electrophoresis on 1% agarose gels. After purification, the PCR products were digested using T7E1 enzyme (NEB, Beijing, China).

Generation of single cell clones

After transfected with Cas9/sgRNA-4 expression plasmids as described above, individual RGC-5 cells were transferred to wells, firstly in 96-well plates and then 6-well plates, and grown to confluence. Genomic DNA was isolated from individual clones and PCR was performed using mEx5-F/mEx6-R primers as described above. For clone validation, the PCR products were used for DNA sequencing. For DNA further genotype assay, the PCR products were cloned into pMD19-T vectors and sequenced.

Total RNA extraction and RT-PCR

Total RNA in each single clone was extracted using RNAiso Plus kit (Takara, Beijing, China) and cDNA was synthesized by reverse transcription using Takara 1st strand cDNA Kit (Takara, Beijing, China). The GABRR2 cDNA sequence spanning from exon3 to exon8 was amplified using 5'-ctgtaggtgtggacgtgcagg-3' (E3-F) and 5'-agctgacccagaggtagatgtcc-3' (E8-R). The primers and their target regions were highlighted in **Figure. 1B.** PCR was performed using Tag polymerase under following conditions: 5 minutes at 94℃, 35 cycles of 94℃ for 30 seconds, 60℃ for 30 seconds and 72℃ for 90 seconds, and finally 72℃ for 10 minutes. The PCR products were cloned into pMD19-T vectors and sequenced.

GABRR2 DNA, cDNA and protein sequence analysis

The DNA sequence results obtained from TA clones conducted by genomic PCR and cDNA PCR were compared with published C57BL/6J mouse chromosome 4 genome sequence (NCBI reference sequence: NC_000070.6) and mouse GABRR2 cDNA (clone MGC:67750; NCBI reference sequence: BC057957.1) to identify clones with deletions, insertions or exon skipping. The GARRR2 amino acid sequence was predicted from the cDNA and compared to the NCBI protein sequence (NP_032102.2). Sequence analysis was performed with chromas software and multiple sequence alignments were performed using DNAMAN software (Lynnon software platform).

Western blot analysis

Protein lysates from cultured cells were prepared in TBS buffer (100 mM Tirs-Cl, 0.9 % (w/v) NaCl) with proteinase inhibitors (Beyotime Biotechnology, Shanghai, China). Proteins were separated on 10% SDS-PAG and then transferred onto nitrocellulose membrane, after washing with TTBS buffer (100 mM Tirs-Cl, 0.9 % (w/v) NaCl, 0.1 % (v/v) Tween 20) the membrane was probed with 1:1000 anti-GABRR2 antibody (ab83223, abcam company, China) or 1:1000 anti-Actin antibody(CST 8457). The anti-GABRR2 antibody recognized the peptide corresponding to a region within the internal sequence amino acids (144-193) that was encoded by exon4 (SNKSMTFDGR LVKKIWVPDV FFVHSKRSFT HDTTTDNIML RVFPDGHVLY).

Results

CRISPR/Cas9-mediated editing of GABRR2 gene

We have designed four different single-guide RNAs targeting exon5 and exon6 of the mouse GABRR2 gene. T7 Endonuclease I (T7EI) cleavage assay was used to evaluate the efficacy of Cas9/sgRNAs. Cas9-induced breaks will leave a variety of mutations, and there will always be some wild type sequence remaining. When we amplify the target region, denature and renature the PCR products, there will be mismatches at the target site. T7EI endonuclease cleaves double-stranded DNA at positions of mismatches. As shown in T7E1 cleavage assay (Figure. 2), two DNA bands were detected in sgRNA-1, sgRNA-2 and sgRNA-4, indicating that these sgRNAs edited the genomic DNA. We chose to use Cas9/sgRNA-4 in final experiments based on T7E1 cleavage data, and this plasmid was transformed into RGC-5 cells to generate single clones.

Abstract

CRISPR/Cas9 and its variations provide an efficient tool for targeted genome editing. CRISPR/Cas9-based genome editing can induce mutations in genome that has high probability to cause exon truncation or deletion. However, screening mutations in diploid cells is difficult because of two copies of chromosome. It is an important task for determining genotypes in diploid cells subjected to editing before using these cells in gene function study. In this study, we applied CRISPR/Cas9 to edit the GABRR2 gene in mouse retinal ganglion cells to study what exactly happened in two alleles and what real mRNA isoforms formed in diploid cells. A single sgRNA was employed to generate double-strand DNA breaks. PCR sequencing was used for single clone validation in diploid cells subjected to editing. The indels and the corresponding effects at the target locus were further studied at genomic and RNA levels. We observed that CRISPR/Cas9 induces random deletions in the target region of GABRR2 gene, and both big and small indels can lead to unexpected high probability of exon truncation/skipping. In addition, random deletions in genomic region recruited introns to generate new "exon". It is the first observation of exon recruitment by CRISPR/Cas9-mediated GABRR2 gene editing. The observations may offer a reference for the future gene splicing study.

We obtained 10 single cell clones by the limiting dilution cloning method. The genomic DNA was purified from individual clones, and the genomic region spanning exon5 and exon6 of the GABRR2 gene was amplified using PCR. As illustrated in **Figure. 3A**, a 750-bp PCR band was detected from clones 1, 2, 5, 7-10, while there were two bands from clones 3, 4 and 6.

For clone validation, single-band PCR products were purified and directly sequenced. Sequence analysis with chromas software (Figure. 3B) shows sequence disruptions around the sgRNA target region in clones 1, 2, 7, 9 and 10, indicating that an unequal genome editing occurred in two alleles in these clones. For the clones 3, 4 and 6, both DNA bands were purified separately and sequenced. The data suggest that these alleles were subjected to unequal genomic editing, with single sgRNA inducing deletions of 200bp to 400 bp in one allele. The sequences from clones 5 and 8 were not disrupted. The sequence results reveal that clone 8 is wild-type. However, clone 5 contained a deletion of 56bp in the target region in both alleles.

Genomic DNA and mRNA sequence analysis of individual clones

The GABRR2 gene resides on chromosome 4. Since RGC-5 cells are diploid and exhibit two copies of chromosome 4, above easy validation can only prove which single clone underwent genome editing. To further understand what exactly happened in two alleles, PCR products spanning exon5 and cxon6 of the GABRR2 gene in all clones were subcloned into pMD19-T vector and sequenced. To analyze the effects at the mRNA level, complimentary DNA (cDNA) was prepared from total RNA in all

clones and used as a PCR template to determine the coding region spanning from exon3 to exon8 of the GABRR2 gene. The changes in the genomic DNA and cDNA are demonstrated in **Figure 4**.

Clone 1 exhibited unequal modifications (Figure. 4A). Deletions of 2bp and 4bp in the targeting sequence were detected in two alleles, respectively. Analysis of cDNA sequence reveals that 2bp and 4bp deletions corresponding to the genomic defection occurred in exon5 and exon7 was spliced out.

Clone 2 had a deletion of 2bp in the targeting sequence in one allele and a large deletion of 79bp in another allele. The latter deletion was composed of 60bp in the last part of exon5 and 19bp in the initial part of intron5 (**Figure. 4**B). When the GABRR2 cDNA sequence was examined, the corresponding 2bp deletion in exon5 was observed but no other deletion or mutation was detected in this variant. Interestingly, the GABRR2 mRNA showed splicing of exon5 and also contained an insertion from the initial 18bp sequence of intron7 into exon7 to form a new "exon7".

In clone 3, deletions of 318bp in one allele and 34bp in another in the target region were observed (**Figure. 4C**), corresponding to the two apparent DNA bands (**Figure. 3A**). The 318bp deletion consists of the last 71bp sequence of exon5, the whole intron5 (186bp long) and the first 61bp of exon6. This genotype was reflected in the mature mRNA with 71bp in exon5 and 61bp in exon6 being missing. In the 34bp deletion, the missing part was composed of the last 27bp part of exon5 and 7bp sequence (GTAATGA) of intron5. Deletion of these parts altered the excision of

intron5. Consistently, the last 27bp of exon5 was absent in the mature mRNA. Furthermore, the remaining 179bp in intron5 became a new "exon" and was incorporated into the mature mRNA between exon5 and exon6.

Clone 4 contained deletions of 18bp and 487bp surrounding the target region in two alleles, respectively (**Figure. 4D**). For the larger deletion, the last 226bp of intron4, the whole exon5 (83bp long) and the first 178bp of intron5 were deleted. While in the short deletion, the last 18bp of exon5 was removed. At the mRNA level, both exon5 and exon6 were spliced out in the mature mRNA. In addition, another mRNA isoform was discovered, in which part of exon5 (the last 62bp) and the whole exon6 was lost, and a new "exon" of 150bp long appeared and linked to exon7. This 150bp new "exon" was originally from intron6 with the inner site from 36bp to 185bp.

Clone 5 contained a deletion of 56bp around the target region in two alleles (**Figure. 4E**). The 56bp deletion was composed of 34bp in the last part of exon5 and 22bp in the initial part of intron5. Both exon5 and exon6 were spliced out in the mature mRNA.

In clone 6, deletions of 60bp and 257bp in the target region were observed (**Figure. 5A**), consistent with the two apparent PCR products (Figure.3A). The 60bp deletion resulted in loss of the last 30bp of exon5 and 30bp of intron5. Such a deletion gave rise to splicing out of exon5 and exon7 in the mature mRNA. The 257bp deletion in the second allele removed the last 152bp of intron4, the whole exon5 (83bp) and the

first 22bp of intron5, leading to generation of a new mRNA isoform without exon4, exon5, exon6 and exon7.

Clone 7 exhibited unequal modifications within the targeting area with deletions of 1bp in one allele and 7bp in the second allele (**Figure. 5B**). At the mRNA level, one splicing isoform resulting from the 7bp deletion in exon5 and, in this isoform, exon7 was spliced out. We also obtained a different splice isoform without exon5, exon6 and exon7.

Deletions of 1bp and 2bp within the targeting sequence were detected in clone 9 (**Figure. 5D**). The 1bp deletion in exon5 corresponding to the genomic defection was observed in the mature mRNA, in which exon7 was spliced out. While the 2bp deletion corresponding to the genomic defection was not observed in the mRNA, an isoform with deletion of the last 21bp of exon5 and the whole exon7 was detected.

Deletions of 2bp and 4bp within the targeting sequence were seen in clone 10 (**Figure.5E)**. The 2bp deletion generated a new "exon7" in the mature mRNA, resulting from insertion of 18bp originally located in intron7 immediately after exon7. The exon7 was spliced out in the mature mRNA as a result of the 4bp deletion.

Protein expression pattern analysis

To confirm the effects of CRISPR/Cas9 editing on the GABRR2 protein expression, western blotting was performed by using anti-GABRR2 antibody recognizing the peptide sequence (144-193) that was encoded by exon4. This is the only anti-

GABRR2 antibody tagged epitope in the current market. Unfortunately, this commercial antibody is not specific for GABRR2 (data not shown).

CRISPR/Cas9-mediated exon splicing or frameshifts were therefore predicted by translating cDNA sequences using DNAMAN software. The wild-type GABRR2 protein is 490 amino acid residues long with a molecular weight of 57 kDa. As shown in Figure 6, both exon splicing and deletion resulting from CRISPR/Cas9-mediated gene editing could generate premature translation of the GABRR2 protein. Different mRNA deletion or splicing isoforms could produce proteins with various lengths ranging from 355 amino acids (clone 1), 332 amino acids (clone 5) and 320 amino acids (clone 4) to 123 amino acids (clone 6).

Discussion

CRISPR/Cas9 and its variations provide an efficient and convenient tool for targeted genome editing in mammalian cells, rodent embryo, zebrafish, drosophila, plants and bacteria[1,4,7,8,13,14]. These DNA editing strategies have been widely used for gene knock-out and knock-in, mutation, targeted correction and even chromosome elimination and inversion[15-19], highlighting promising therapeutic applications.

DNA damage and mutation have also been shown to regulate exon skipping[20,21]. The accidents that CRISPR/Cas9-mediated mutations induced exon skipping were reported usually in the cases of the insertion mutations. An early study reported exon skipping due to insertions of a large gene cassette[10]. An insertion of seven nucleotides in exon3 of pycr1a gene resulted in exon skipping in zebrafish[7].

Moreover, Single nucleotide insertion within exon3 of FLOT1 gene induced random splicing of several exons in Hela cells[22]. In contrast, the exon skipping accident induced by the deletion mutations was rarely reported, a study using pairs of sgRNAs to cut whole exon14 out of MET gene in HEK293 cells caused exon14 splicing out in matured mRNA corresponding to the genomic defection [21]. In addition, a recent study focusing on CRISPR/Cas9-mediated β-catenin gene editing shows small deletions or insertions that partially alters exon splicing or unexpected larger deletions[12]. Above cases had recently been summarized in a review[11]. In the current study, we provide evidence that single sgRNA induced random exon deletions in the target region in the GABRR2 gene in RGC-5 cells. A high frequency of such exon deletions in the genome causes skipping of one or several exons in the mature mRNA.

The main defect of CRISPR/Cas9 gene editing is the off-target effect. The specificity of sgRNA is the key factor controlling the off-target effect. In this study, the latest 12bp guide RNA sequence plus 3bp PAM sequence were used in BLAST assay. The 15bp guide sequences which can only match GABRR2 exon5 or exon6 regions are selected for the experiment to exclude the off-target effect of CRISPR/Cas9 on GABRR2. In the current study, we focused on the effect of genomic editing in exon5 by analyzing the mRNA sequence from exon3 to exon8. The strategy screening several exons upstream and downstream of the targeted exon enables us to obtain enough sequence information for analyzing mRNA isoforms induced by CRISPR/Cas9. We showed that removal of large DNA fragments in the genome using

CRISPR/Cas9 unexpectedly initiated recruitment of adjacent introns to form new "exon" in the mature mRNA. Such exon recruitment was detected in four clones in the current study. Deletions initiated exon recruitment which occurs randomly in any intron regions of the genome. For example, in clones 2 and 10, a deletion of 18bp of intron7 was incorporated into the mature mRNA. For clone3, deletion of 34bp, composed of the last 27bp of exon5 and 7bp of intron5, altered excision of intron5 or mRNA editing. Moreover, in clone 4, 150bp sequence within intron6 was recruited and incorporated into the mature mRNA. Our results provide evidence on the function of intron in genome, intron sequences could be used as a resource for formation of new exons. To our knowledge, this is the first observation of such exon recruitment by CRISPR/Cas9-mediated gene editing. Such new "exon" could be translated to form unexpected proteins that are not endogenously expressed by cells and confer new functions. Therefore, one should analyze the results from CRISPR/Cas9-mediated gene editing with caution.

CRISPR/Cas9 has been exploited to study alternative splicing of the titin gene by precisely deleting the MEX5 exon in the murine genome[23]. In our observation, all clones analyzed show different genome editing patterns. For example, deletions, as small as 1-4bp, have such far-reaching effects on distant exons, and the exon 7 is especially vulnerable to indels. A novel exon splicing enhancer or sequence module residing around the sgRNA-4 cut site was supposed to be involved into the regulation of exon 7 usages. Surprisingly, many of the mutations caused unpredictable splicing patterns. In clone 7 and 9, the same 1bp deletion amazingly results in two different

splicing defects in two separate clones. It looks like the exon splicing phenomenon occurred randomly in the cells. However, no answer can be offer for these questions. Further studies are required to provide a better understanding of the exon splicing mechanism.

Conclusions

PCR sequencing was employed for the validation of gene editing in RGC-5 diploid cells, it revealed that CRISPR/Cas9-mediated GABRR2 gene editing can result in random exon splicing, exon deletions and exon recruitment. The observations in this study may offer a reference for the future gene splicing study and it could also be useful for future meta studies.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Figure legends

Figure 1. CRISPR-Cas9-Mediated GABRR2 Gene Editing in Mouse RGC5 cells.

(A) Schematic illustration of the sgRNA targeting site in the GABRR2 gene. The sgRNA guiding sequences are labeled in yellow. Protospacer adjacent motif (PAM) is marked in green. Primers for PCR to amplify the GABRR2 genomic sequence spanning exon5 and exon6 are marked by red arrow. Exon5 and exon6 are indicated in gray halftones. (B) Schematic representation of the primers target sequences in exon3 and exon8. These primers were used on cDNA amplification and sequencing. Red lines illustrate the locations of E3-F and E8-R primers. Exon3 and exon8 are indicated in gray halftones.

Figure 2. T7E1 cleavage assay on PCR products from different cell clones subjected to Cas9-sgRNA modification. M, DNA markers; 1, wild-type RGC5 cell without sgRNA/Cas9 modification; 2, sgRNA-1; 3, sgRNA-2; 4, sgRNA-3; 5, sgRNA-4. Arrows indicate the T7E1 cleavage bands resulting from sgRNA-mediated editing.

Figure 3. Validation of GABRR2 gene editing in different RGC-5 cell clones. (A) Electrophoresis assay on PCR products amplifying the genomic region spanning exon5 and exon6 of the GABRR2 gene in different cell clones. The numbers represent the PCR products from the related clones. (B) DNA sequencing of the PCR products confirms the status of CRISPR/Cas9 targeted genome region in different clones. Clone 8 is wild-type. Deletion of DNA in clone 5 confirmed CRISPR/Cas9-mdiated editing in two alleles. The red lines show the sequence disruptions, indicating genomic editing in two alleles.

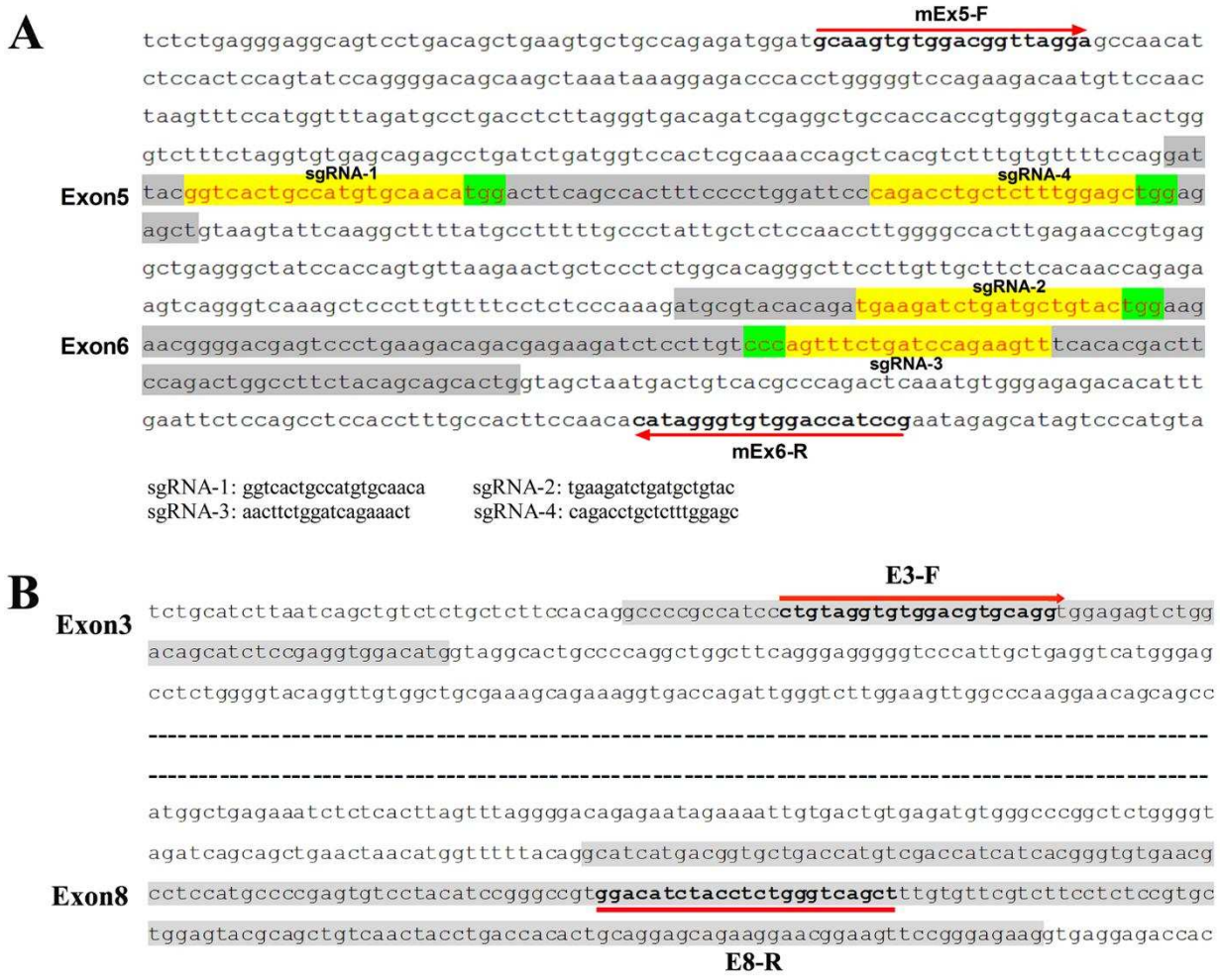
Figure 4. Schematic illustration and analysis of GABRR2 isoforms in clones 1-5. Genomic DNA and cDNA sequences were determined by subcloning of PCR products and sequencing.The genomic DNA and cDNA sequencing maps are attached behind the schema. The cutting cassette showed the deletions in DNA or cDNA.

Figure 5. Schematic illustration and analysis of GABRR2 isoforms in clones 6-10. Genomic DNA and cDNA were used as PCR templates, TA cloning and DNA sequencing were to confirmed the isoforms of GABRR2 in clones 6-10.The

sequencing maps are attached behind the schema. The cutting cassette showed the deletions in DNA or cDNA.

Figure 6. GABRR2 protein expression pattern in single cell clones. Different exon splicing or deletion isoforms are predicted to generate truncated stop of GABRR2 proteins by analyzing the RT-PCR products spanning exon3 to exon8 of GABRR2. The stop code in each cDNA sequence is marked by red line. The partial cDNA sequences and lenth of amino acids are showed in different clones.

Figr-1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sgRNA-3: aacttctggatcagaaact
sgRNA-4: cagacctgctctttggagc
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E3-F
Exon3
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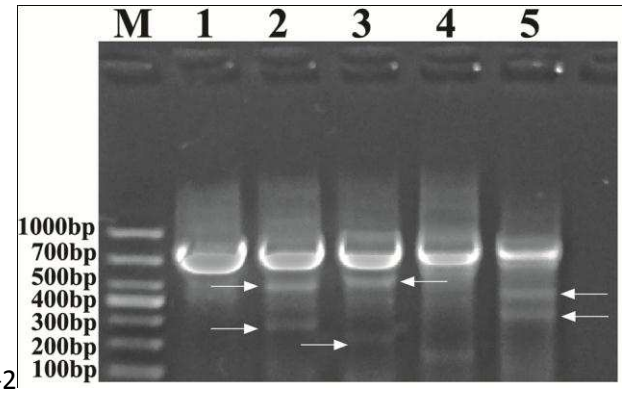
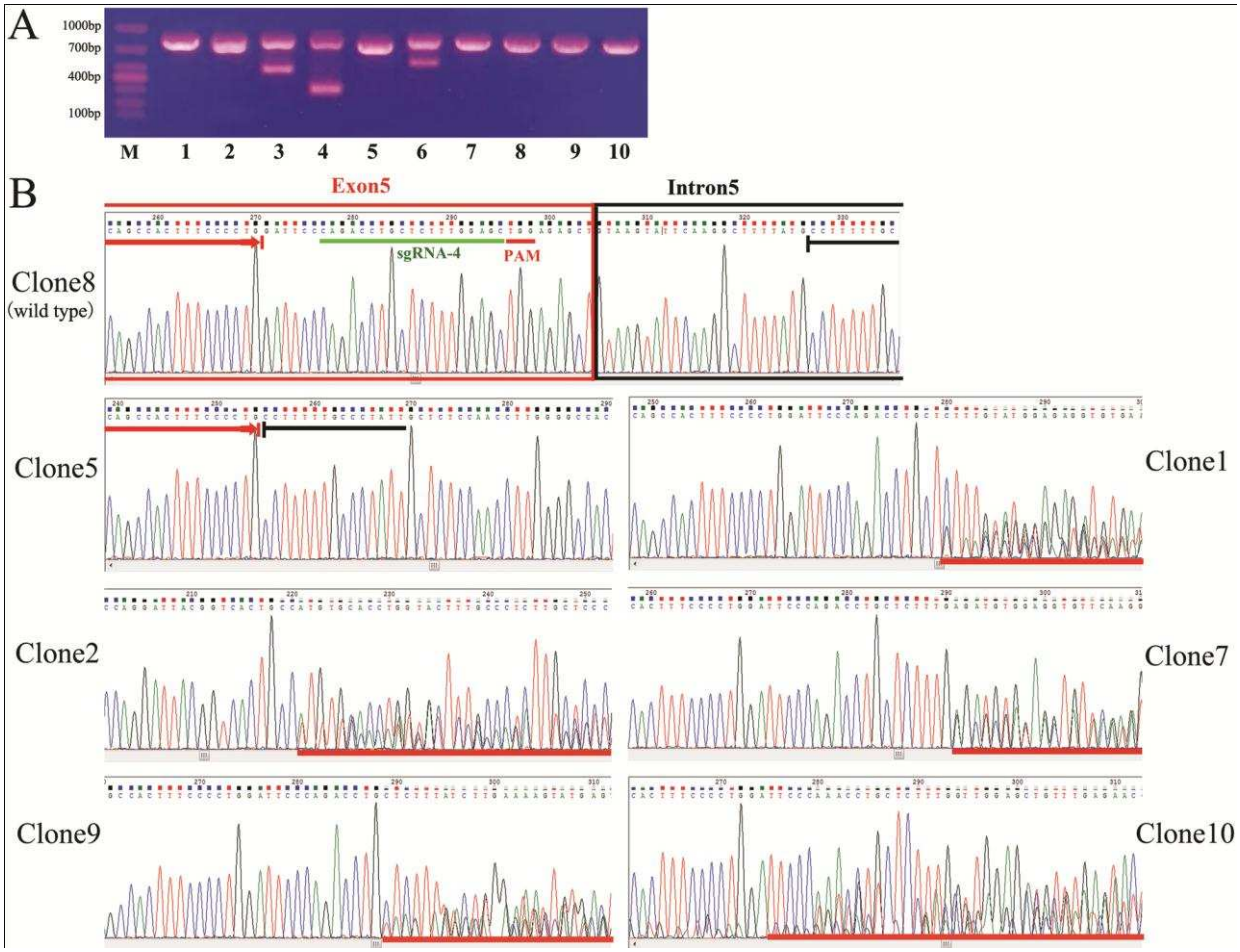
E8-R



Figr-2

Figr-3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700bp
400bp
100bp
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B
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Intron5
sgRNA-4
PAM
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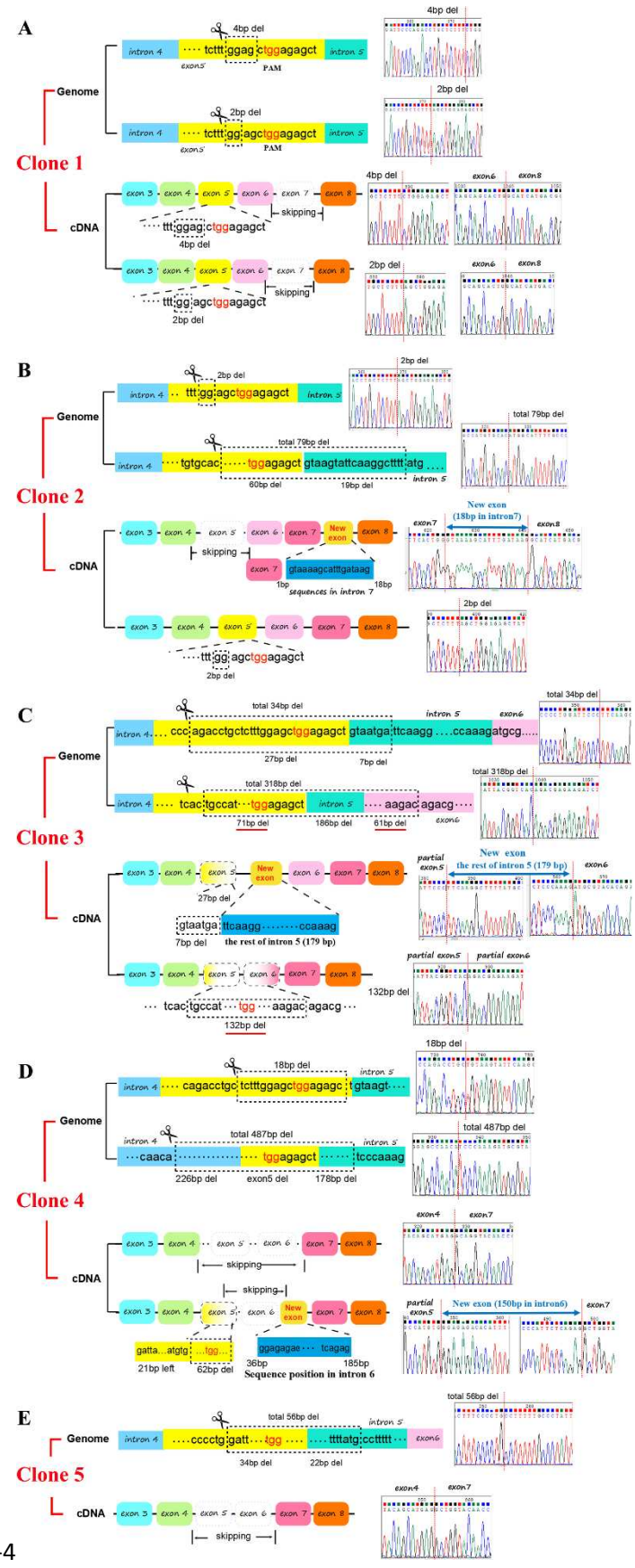
(wild type)
Clone5
Clone1
Clone2
Clone7
Clone9
Clone10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PAM
skipping
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(18bp in intron7)
sequences in intron 7
C
Clone 3
total 34bp del
total 318bp del
partial exon5
partial exon6
the rest of intron 5 (179 bp)
D
Clone 4
18bp del
total 487bp del
226bp del
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Sequence position in intron 6
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Clone 5
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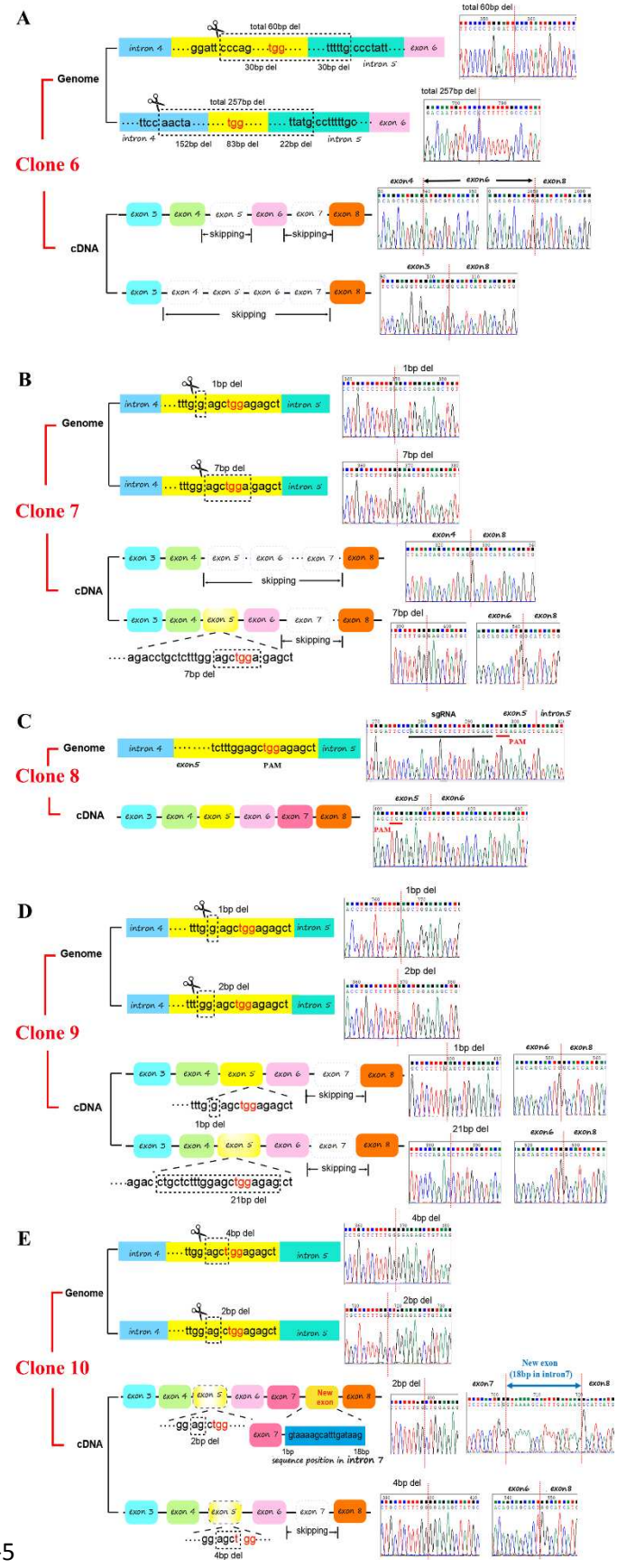
Figr-4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cDNA
B
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Clone 7
cDNA
C
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D
Genome
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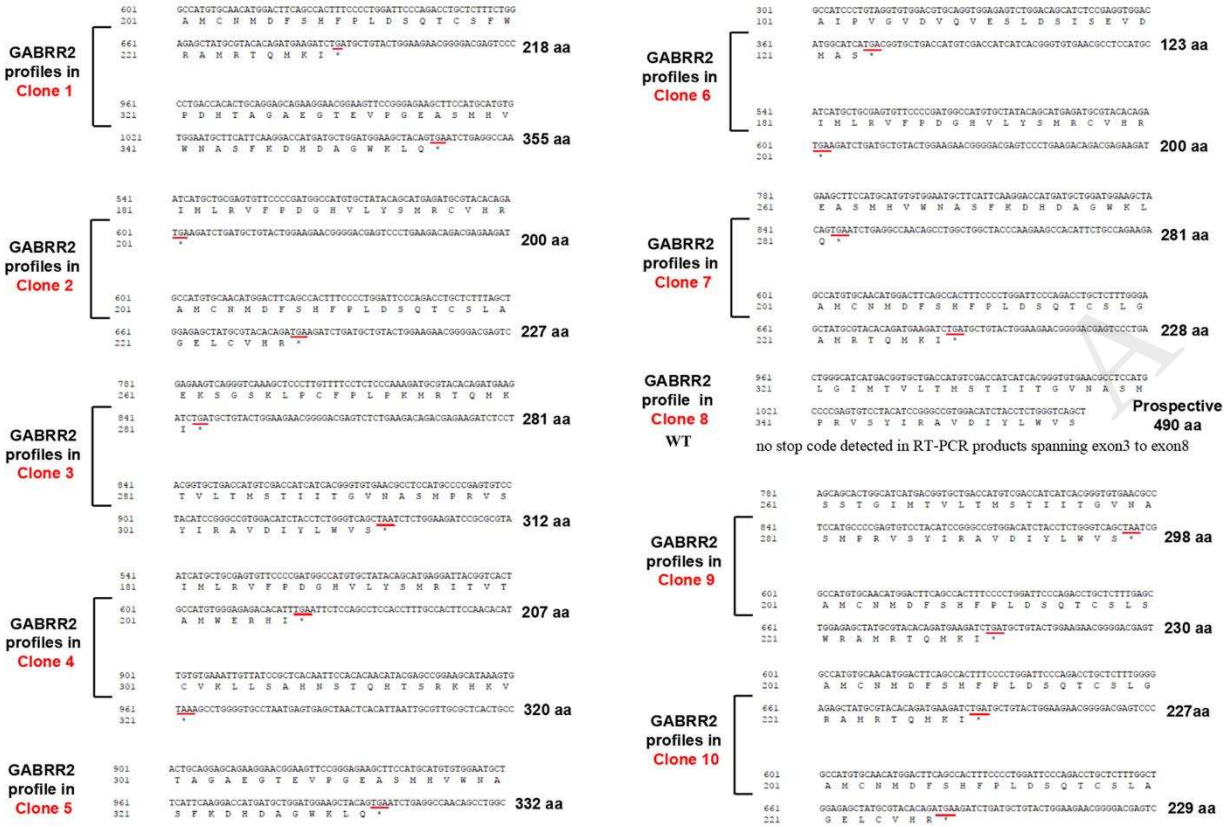
E
Genome
Clone 10
cDNA

Figr-5

Figr-6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GABRR2 profiles in Clone 2
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227 aa
GABRR2 profiles in Clone 3
281 aa
312 aa
GABRR2 profiles in Clone 4
207 aa
320 aa
GABRR2 profile in Clone 5
332 aa
GABRR2 profiles in Clone 6
123 aa
200 aa
GABRR2 profiles in Clone 7
281 aa
228 aa
GABRR2 profile in Clone 8
WT
Prospective 490 aa
no stop code detected in RT-PCR products spanning exon3 to exon8
GABRR2 profiles in Clone 9
298 aa
230 aa
GABRR2 profiles in Clone 10
227aa
229 aa