NOTES

A novel aberrant splicing of $G_{s\alpha}$ transcript in human leukemia cell lines

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Abstract The α subunit of the stimulatory G protein, $G_{s\alpha}$, is involved in the stimulation of adenylate cyclase pathway of signal transduction. The various $G_{s\alpha}$ mRNAs are generated either by alternative splicing or by using alternative promoter. A novel aberrant splicing of $G_{s\alpha}$ transcript in human leukemia cell lines is reported. The entire coding region of $G_{s\alpha}$ gene obtained, referred to as $G_{s\alpha Leu}$, was amplified by PCR with cDNA from Jurkat human leukemia cDNA library or the reverse-transcribed first strand cDNA from human leukemia cell line HL-60 as a template. The result of DNA sequencing indicated that, compared with originally reported $G_{s\alpha}$, $G_{s\alpha Leu}$ has two in-frame deletions, one in amino acid residues 23—249 and the other in amino acid residues 254—260, suggesting that $G_{s\alpha Leu}$ may play an important role in leukemia. The complete coding region of $G_{s\alpha Leu}$ was inserted downstream of the thrombin site of pGEX-2T fusion protein expression vector and expressed in the form of GST/$G_{s\alpha Leu}$ at high level, which was then purified to be electrophoretically pure by one-step affinity chromatography. The expression of $G_{s\alpha Leu}$ lays foundation for the study of its function.

Keywords: $G_{s\alpha}$, splicing, leukemia, cloning and expression.

G proteins are a family of guanine-nucleotide binding proteins that transduce extracellular signals into cellular responses. These proteins are heterotrimers consisting of α, β and γ subunits. It is the α subunit that binds guanine nucleotides and is unique to each G protein; the β and γ subunits form a tightly but noncovalently associated dimer that functions as a single unit and are similar in each G protein. The α subunit associates with β and γ subunits to form an inactive protein complex of the GDP-bound α subunit. The agonist-activated receptor catalyzes the exchange of GTP for GDP on the α subunit. The binding of GTP leads to dissociation of G protein from receptor, and of α subunit from βγ, thus activating G protein. Hydrolysis of bound GTP to GDP by intrinsic GTPase activity of the α subunit allows reassociation into the inactive trimer. Although the β γ dimer plays an important role in modulating effector activity, the function of a G protein is primarily determined by its α subunit. To date, 16 different mammalian α subunit genes have been cloned. Among these, $G_{s\alpha}$ gene, which encodes a protein activating the adenylate cyclase pathway of signal transduction, was found to be involved in a number of human disorders. Mutations in the $G_{s\alpha}$ gene were discovered in human tumors. In addition to the multiple mutations, there are now several examples of alternative splicing, including aberrant splicing, of $G_{s\alpha}$ transcript. Here we report a novel aberrant splicing of $G_{s\alpha}$ transcript in human leukemia cell lines.

1 Materials and methods

Plasmid pUC18 was purchased from Sino-American Biotechnology Company. The pQE30 and pGEX-2T plasmids were stored in our laboratory. The former contained *E. coli* phage T5 promoter and two lac operators, and the latter contained tac promoter, glutathione S-transferase (GST) gene encoding a 26 ku protein and a thrombin site. The pQE30/$G_{s\alpha Leu}$ plasmid (in this study, the gene that we obtained was termed $G_{s\alpha Leu}$), which can be digested with BamHI and KpnI to produce the complete coding region of $G_{s\alpha Leu}$, was constructed in our laboratory. *E. coli* DH5α and JM103 were stored in our laboratory. Human leukemia cDNA library from Jurkat cell line was purchased from Clontech (Catalogue number

HL4015AB, 95/96). Human leukemia cell line HL-60 was a kind gift from Wang Lisheng at Beijing Institute of Radiation Medicine. PolyA Ttract System 1 000 kit, purchased from Promega, was used to isolate mRNA. Bulk GST Purification Modules from Pharmacia is a kit for purification of proteins.

HL-60 human leukemia cells were grown in RPMI1640 medium supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 at 37℃. mRNA isolation from HL-60 cells was performed using magnetic particles according to the instructions of the PolyATtract System 1000 kit.

Extraction of plasmid, digestion, cloning, identification and so on were performed as described in reference [8].

PCR amplification with cDNA from human leukemia cDNA library as a template was performed as follows. The upstream primer used for amplifying the complete coding region of $G_{s\alpha}$ gene is 5′-GCGAGCTCATGGGCTGCCTCGGGAACAGTAAG-3′, and contains SacI site and initiation codon. The downstream primer is 5′-CGGGTACCTTAGAGCAGCTCGTACTGACGAAG-3′, and contains KpnI site and stop codon. Following denaturation of cDNA at 94℃ for 5 min, Taq or Vent DNA polymerase was added. Thirty cycles of amplification, consisting of denaturation at 94℃ for 1 min, annealing at 51℃ for 1 min and elogation at 72℃ for 1.5—2 min, were followed by a final extension reaction at 72℃ for 7 min.

RT-PCR was carried out as follows. First strand cDNA was synthesized from HL-60 mRNA with the downstream primer mentioned above. The first strand cDNA synthesis reaction mixture was used for amplification of the entire coding region of $G_{s\alpha}$ gene. The amplification reaction was carried out using reaction conditions as described above.

DNA sequencing was performed using the Sanger dideoxy chain termination method.

$G_{s\alpha Leu}$ was expressed using the GST gene fusion expression system. *E. coli* cells harboring recombinant plasmid were grown to an OD600 of about 0.6 in LB medium at 30℃, and then induced for 3 h by the addition of IPTG to a final concentration of 0.1 mmol/L. The cells were harvested by centrifugation and washed once by PBS. The cell pellet was suspended in PBS. The suspended cells were disrupted using a sonicator, and then centrifuged at 12 000 r/min for 10 min at 4℃. The supernatant was used for the purification of GST/$G_{s\alpha Leu}$ fusion protein by affinity chromatography according to the instructions of the Bulk GST Purification Module.

2 Results

(ⅰ) PCR amplification of the complete coding region of human $G_{s\alpha}$ gene. The human $G_{s\alpha}$ gene is a split gene composed of 13 exons and 12 introns extending about 20 kb. The length of the complete coding region of the $G_{s\alpha}$ gene is about 1.2 kb. The $G_{s\alpha}$ are expressed in a variety of tissues and cell lines, although the relative expression differs among them. We performed PCR amplification of $G_{s\alpha}$ using cDNAs derived from Jukart human leukemia library and human leukemia cell line HL-60 (not less than 3 separate experiments). The primers used for the PCR amplification were specific for $G_{s\alpha}$ based on GenBank searching. As shown in fig. 1, a clear band of about 500 bp smaller than that of normal $G_{s\alpha}$ gene was obtained in each PCR reaction.

(ⅱ) Cloning and sequencing of the PCR products. To determine whether the PCR products that we obtained are $G_{s\alpha}$ gene, the 500-bp products were recovered from agarose gel and digested with SacI and KpnI. The digested fragment was then ligated to plasmid pUC18 digested with SacI and KpnI. *E. coli* DH5α was transformed with the above construct. Transformants were screened by DNA digestion analysis and PCR. The resulting recombinant was designated DH5α(pUC18/$G_{s\alpha Leu}$). Fig. 2 shows the result of a typical restriction analysis of the recombinant plasmid. Sequencing of the inserted cDNA showed that $G_{s\alpha Leu}$ is different from the previously reported normal $G_{s\alpha}$ gene in the coding region (fig. 3). The $G_{s\alpha Leu}$ has two in-frame deletions, one in amino acid residues 23—249 (corresponding to partial sequence of exons 1—10) and the other in amino acid residues 254—260 (corresponding to partial sequence of exon 10). Based on the previously reported data, various $G_{s\alpha}$ exons are shown in table 1. The result indicates that $G_{s\alpha Leu}$ is a novel polypeptide of 160 amino acids.

(ⅲ) Cloning and expression of $G_{s\alpha Leu}$. pQE30/$G_{s\alpha Leu}$ was digested with KpnI, flushed with T4 DNA polymerase and cleaved with BamHI. The DNA fragment containing the complete coding region of $G_{s\alpha Leu}$

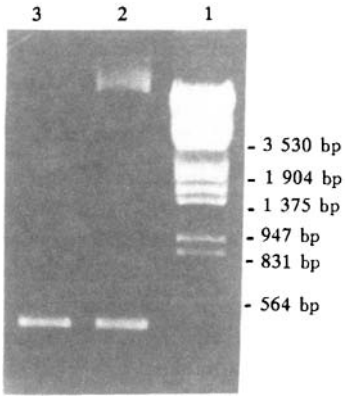

Fig. 1. Agarose gel electrophoresis of the PCR products. 1, λDNA + EcoR Ⅰ + Hind Ⅲ marker; 2, PCR amplification with cDNA library from Jurkat cells as a template; 3, PCR amplification with the first strand cDNA reverse-transcribed from HL-60 as a template.

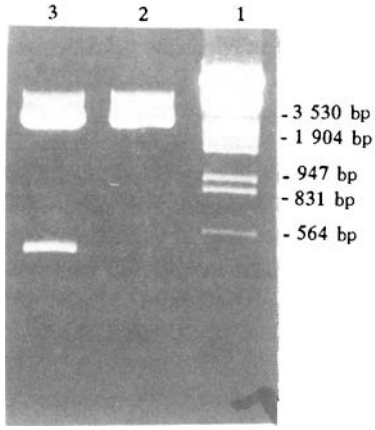

Fig. 2. Restriction analysis of recombinant plasmid pUC18/G_{saLeu}. 1, λDNA + EcoRI + HindⅢ marker; 2, pUC18 + SacI + KpnI; 3, pUC18/G_{saLeu} + SacI + KpnI.

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ATG GGC TGC CTC GGG AAC AGT AAG ACC GAG GAC CAG CGC AAC GAG
Met Gly Cys Leu Gly Asn Ser Lys Thr Glu Asp Gln Arg Asn Glu
GAG AAG GCG CAG CGT GAG GCC AGC AGC AGC TAC AAC CAG ACC AAC
Glu Lys Ala Gln Arg Glu Ala Ser Ser Ser Tyr Asn Gln Thr Asn
CGC CTG CAG GAG GCT CTG AAC CTC TTC AAG AGC ATC TGG AAC AAC
Arg Leu Gln Glu Ala Leu Asn Leu The Lys Ser Ile Trp Asn Asn
AGA TGG CTG CGC ACC ATC TCT GTG ATC CTG TTC CTC AAC AAG CAA
Arg Trp Leu Arg Thr Ile Ser Val Ile Leu Phe Leu Asn Lys Gln
GAT CTG CTC GCT GAG AAA GTC CTT GCT GGG AAA TCG AAG ATT GAG
Asp Leu Leu Ala Glu Lys Val Leu Ala Gly Lys Ser Lys Ile Glu
GAC TAC TTT CCA GAA TTT GCT CGC TAC ACT ACT CCT GAG GAT GCT
Asp Tyr Phe Pro Glu Phe Ala Arg Tyr Thr Thr Pro Glu Asp Ala
ACT CCC GAG CCC GGA GAG GAC CCA CGC GTG ACC CGG GCC AAG TAC
Thr Pro Glu Pro Gly Glu Asp Pro Arg Val Thr Arg Ala Lys Tyr
TTC ATT CGA GAT GAG TTT CTG AGG ATC AGC ACT GCC AGT GGA GAT
Phe Ile Arg Asp Glu Phe Leu Arg Ile Ser Thr Ala Ser Gly Asp
GGG CGT CAC TAC TGC TAC CCT CAT TTC ACC TGC GCT GTG GAC ACT
Gly Arg His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp Thr
GAG AAC ATC CGC CGT GTG TTC AAC GAC TGC CGT GAC ATC ATT CAG
Glu Asn Ile Arg Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln
CGC ATG CAC CTT CGT CAG TAC GAG CTG CTC TAA
Arg Met His Leu Arg Gln Tyr Glu Leu Leu  *
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Fig. 3. Nucleotide and deduced amino acid sequences of G_{saLeu}. *, Stop codon.

was recovered and ligated into pGEX-2T digested with BamHI and SmaI. *E. coli* JM103 was transformed with the above construct. The resulting recombinant plasmid was designated 2T/G_{saLeu}. *E. coli* JM103 cells harboring the 2T/G_{saLeu} plasmid were grown at 30℃ and induced by IPTG. As shown in fig. 4, JM103(2T/G_{saLeu}) expressed a 44 ku GST/G_{saLeu} fusion protein as expected. The expression product

Table 1 Comparison of exons of various $G_{s\alpha}$

Names[a)]	Exons													
	1	2	3	CAG	4	5	6	7	8	9	10	11	12	13
$G_{s\alpha}$-1	+	+	+	−	+	+	+	+	+	+	+	+	+	+
$G_{s\alpha}$-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
$G_{s\alpha}$-3	+	+	−	−	+	+	+	+	+	+	+	+	+	+
$G_{s\alpha}$-4	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Ishikawa *et al*.	+[1]	+	+	?	+	+	+	+	+	+	+	+	+	+
Swaroop *et al*. 1	+[2]+[3]	+	+	−	+	+	+	+	+	+	+	+	+	+
Swaroop *et al*. 2	+[2]+[3]	+	−	−	+	+	+	+	+	+	+	+	+	+
Swaroop *et al*. 3	+[2]	+	+	−	+	+	+	+	+	+	+	+	+	+
Swaroop *et al*. 4	+[2]	+	−	−	+	+	+	+	+	+	+	+	+	+
Ali *et al*. 1	+	+	+	+	+[a]	−	−	−	−	−	+[b]	+	+	+
Ali *et al*. 2	+	+	+	+	+	+	+	+	+[c]	−	+[d]	+	+	+
G_{astro}	+[4]	+	+	−	+	+	+	+	+	+	+	+	+	+
Crawford *et al*.	+	+	+	+[5]	−	−	−	−	−	−	−	−	−	−
$G_{s\alpha Leu}$	+[e]	−	−	−	−	−	−	−	−	−	+[f]	+	+	+

a) Sequence names or author names. +[1], +[2], +[3], +[4], Various corresponding exons; +[a], +[b], +[c], +[d], +[e], +[f], partial sequences of corresponding exons; ?, not mentioned in the published paper.

was purified to be electrophoretically pure by one-step affinity chromatography, laying foundation for functional study of $G_{s\alpha Leu}$.

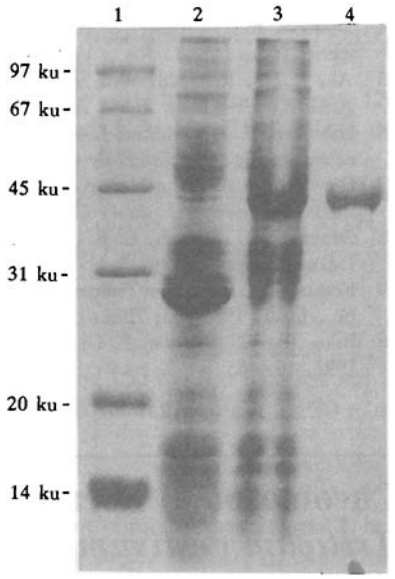

Fig. 4. SDS-PAGE analysis of $G_{s\alpha Leu}$. 1, Protein molecular weight marker; 2, JM103 harboring plasmid pGEX-2T; 3, JM103 harboring plasmid 2T/$G_{s\alpha Leu}$; 4, purified GST/$G_{s\alpha Leu}$ fusion protein.

3 Discussion

Originally four forms of $G_{s\alpha}$ ($G_{s\alpha}$-1 to -4) were isolated and shown to be splicing products of a single gene, resulting from alternative use of exon3 and (or) the use of trinucleotide CAG[9] (table 1). Each of these various $G_{s\alpha}$ is capable of stimulating adenylate cyclase. Conventionally, $G_{s\alpha}$-1 is called $G_{s\alpha}$. Further diversity is created by alternative splicing or by the use of alternative promoter. Ali *et al*. identified the truncated $G_{s\alpha}$ transcripts in a human glioblastoma cell line, HS683, and in an SV40-transformed human astroglial cell line, SVG by RT-PCR[5]. The truncated $G_{s\alpha}$ transcripts, with deletions in the central region of the molecule, seem to have originated due to aberrant splicing within exonic sequences, which did not conform to the consensus GT/AG splice signals. In one of the abnormally spliced transcripts dinucleotides AA and GG were present at the donor and acceptor splice sites respectively, and in the other the donor and acceptor sites were GT and TG respectively, which also occurred in the originally isolated $G_{s\alpha}$. We report here the presence of a truncated $G_{s\alpha}$ cDNA with in-frame deletions, different from those reported by Ali, in human leukemia cell lines Jurkat and HL-60. It has been confirmed that mutations at codons 201 and 227 can cause tumor formation. To determine whether such oncogenic mutations occurred in leukemia, Baker *et al*. analyzed DNA from leukemia cells from 59 patients by PCR[10]. The result showed that no mutations were detected in the amplified $G_{s\alpha}$ gene. However, our result indicated that, in human leukemia cell lines Jurkat and HL-60, aberrant splicing occurred between exons 1 and 10 and within exon 10, thus causing deletions of these exons. In the former case, AA, identical to that reported by Ali, and CC were present at the donor and acceptor sites respectively; in the latter case, the sequences of the donor and acceptor sites are AA, also identical to that reported by Ali *et al*., and AC respectively. We conclude from the above results that the abnormal $G_{s\alpha}$ occurred in leukemia, at least in some kinds of leukemia, at the step of splicing of $G_{s\alpha}$ transcript, so Baker *et al*. could not detect the mutation of $G_{s\alpha}$ gene.

It has been reported that the amino-terminal 29 residues are involved in binding of α subunit of G_s to βγ subunits and the carboxyl-terminal 160 residues are required for interactions of $G_{s\alpha}$ with receptors and effector enzymes. The $G_{s\alpha Leu}$ that we obtained contains the amino-terminal 22 residues and the carboxyl-terminal 134 residues, and between them is four amino acid residues (SerSerSerTyr), similar to flexible linkers, suggesting that the $G_{s\alpha Leu}$ may retain some aspects of function of normal $G_{s\alpha}$. Deletion of GTPase activity site between exons 8 and 9 in $G_{s\alpha Leu}$ may lead to a constitutive activation of adenylate cyclase. This activation increases cAMP formation in cells, thereby stimulating proliferation of these cells. This may be one of the main factors in causing leukemia. The functional significance of $G_{s\alpha Leu}$ in leukemia remains to be defined.

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References

1 Spiegel, A. M., The molecular basis of disorders caused by defects in G proteins, *Horm. Res.*, 1997, 47: 89.
2 Barlier, A., Pellegrini-Bouiller, I., Caccavelli, L. *et al.*, Abnormal transduction mechanisms in pituitary adenomas, *Horm. Res.*, 1997, 47: 227.
3 Ishikawa, Y., Bianchi, C., Nadal-Ginard, N. *et al.*, Alternative promoter and 5′ exon generate a novel $G_{s\alpha}$ mRNA, *J. Biol. Chem.*, 1990, 265: 8458.
4 Swaroop, A., Agarwal, N., Gruen, J. R. *et al.*, Differential expression of novel $G_{s\alpha}$ signal transduction protein cDNA species, *Nucl. Acid. Res.*, 1991, 19: 4725.
5 Ali, I. U., Reinhold, W., Salvador, C. *et al.*, Aberrant splicing of $G_{s\alpha}$ transcript in transformed human astroglial and glioblastoma cell lines, *Nucl. Acid. Res.*, 1992, 20: 4263.
6 Habecker, B. A., Martin, J. M., Nathanson, N. M., Isolation and characterization of a novel cDNA which identifies both neural-specific and ubiquitously expressed $G_{s\alpha}$ mRNAs, *J. Neurochem.*, 1993, 61: 712.
7 Crawford, J. A., Mutchler, K. J., Sullivan, B. E. *et al.*, Neural expression of a novel alternatively spliced and polyadenylated $G_{s\alpha}$ transcript, *J. Biol. Chem.*, 1993, 268: 9879.
8 Sambrook, J., Fritsch, E. F., Maniatis, T., *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, 1989.
9 Kozasa, T., Itoh, H., Tsukamoto, T. *et al.*, Isolation and characterization of the human $G_{s\alpha}$ gene, *Proc. Natl. Acad. Sci. USA*, 1988, 85: 2081.
10 Baker, B. W., Norton, J. D., Analysis of mutations in the G_s protein α subunit gene in human leukemia, *Leuk. Res.*, 1992, 16: 485.

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