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Polyglycine expansions in eRF3/GSPT1 are associated with gastric cancer susceptibility

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Gastric cancer remains a major cause of death in the developed countries, and a large percentage is still genetically unexplained. Because of their major role in cell survival, mutations in translation factors and altered expression of these genes have been associated with cancer development. Apart from its role in translation termination, the eukaryotic translation release factor 3 (eRF3) is involved in several critical cellular processes, such as cell cycle regulation, cytoskeleton organization and apoptosis. The aim of this study was to evaluate *eRF3*/*GSPT1* gene as a potential genetic susceptibility associated locus for gastric cancer, analysing a stable GGC expansion in exon 1 encoding a polyglycine tract in the N-terminal domain of the protein. DNA was obtained from 139 patients with gastric cancer and from 100 individuals of a healthy control population. The GGC expansion was amplified by PCR and the number of repeats determined by genotyping in an automatic sequencer. There are five known alleles encoding from 8 to 12 glycines. The most common allele encodes 10 glycines. The 12-Gly allele was detected exclusively in the cancer patients (allelic frequency = 5%). Regardless of the genotype, patients with the 12-Gly allele had a 20-fold increased risk for gastric cancer. We also detected a single-base alteration in the gene (*G274T*) although no correlation with cancer development has been found. Thus, our results show that the GGC expansion may have a potential role in regulating eRF3/GSPT1 expression and/or changing the protein function that can lead to gastric cancer development.

Introduction

Because of its heterogeneity and multiple molecular alterations in pathogenesis, the prognosis of gastric cancer remains poor and a large percentage of tumours remain genetically unexplained (1). Search for new biomarkers of gastric cancer becomes imperative.

Trinucleotide repeat disorders are the most abundant forms of repeat expansion diseases (2). Several exonic trinucleotide repeats have been associated with oncological pathologies, either conferring a protective effect or associated with elevated risk for disease (3). The best-known examples are the androgen receptor (*AR*) gene polymorphisms, a CAG and a GGC trinucleotide repeats in exon 1, the length of which have been linked to prostate cancer survival and breast cancer risk (4). However, the molecular mechanisms underlying the polyglutamine and polyglycine length modulation of AR function have not been totally elucidated; results from different groups working with different racial–ethnic populations are often inconclusive and sometimes even conflicting (5). Furthermore, any causal relation between the presence of a particular allele and the development of cancer has been reported.

The importance of the translation machinery in cancer development (6–8) is now widely recognized. Somatic mutations and changes in the gene expression pattern of initiation, elongation and termination translation factors have been described associated with different types of cancer (9–12).

The eukaryotic release factor 3 (eRF3) has multifunctional properties in eukaryotic cells. Beside its role in translation termination (13,14), this protein was also reported to be involved in cell cycle regulation (15,16), mRNA decay (17,18), recycle of ribosomes (19) and apoptosis (20).

The N-terminal domain of eRF3 contains a polyglycine expansion encoded by a stable (GGC)*n* tract in *eRF3*/*GSPT1* exon 1 gene. There are five known alleles which encode 8, 9, 10, 11 and 12 glycines. This domain of eRF3 was shown to lack critical regions for eRF1 binding, thus not being essential to its role at the termination step of protein synthesis (21). The N-domain of the protein interacts with the poly-A binding protein (PABP) during translation reinitiation, which binds the poly(A) tail of mRNA and also associates with the eukaryotic initiation factor (eIF) 4G (17,22).

The aim of this study was to evaluate *eRF3*/*GSPT1* gene as a potential genetic susceptibility associated locus for gastric cancer, analysing a stable GGC expansion in exon 1 encoding a polyglycine tract in the N-terminal domain of the protein. As it is involved in essential cellular processes, modifications in the N-terminal domain of this protein might be of critical relevance.

Materials and methods

We analysed the GGC repeat expansion in exon 1 of *eRF3*/*GSPT1* in 139 individuals with gastric cancer (113 adenocarcinomas and 26 mucocellular carcinomas), and compared with a control population of 100 healthy blood donors, after informed consent was obtained.

DNA was extracted from peripheral blood using a phenol–chloroform extraction (23). DNA from formalin-fixed paraffin-embedded tissues was extracted using chelex resins (24). The DNA from fresh tissue was extracted using the DNeasy Tissue Kit (Quiagen, Hilden, Germany) according to the manufacturer's protocol.

The (GGC)*n* fragment was PCR amplified using a 6′-FAM labelled forward primer (5′-CATTTCTCGCTCTCTGTCCAC-3′) and a non-labelled reverse primer (5′-CTGGTCCCAGCAGTCAGG-3′) (25), being genotyped in an ABI 310 sequencer and analysed with GeneScan 3.7 software (ABi, Branchburg, NJ, USA). Reaction conditions for the fragment amplification were 2.5 mM $MgCl_2$; (Fermentas, Hanover, MD, USA) 2.5 mM dNTPs (Fermentas, Hanover, MD); 2× Enhancer solution (Invitrogen, Carlsbad,

Abbreviations: *AR*, androgen receptor; eIF, eukaryotic initiation factor; eRF3, eukaryotic translation release factor 3; PABP, poly-A binding protein.

Table I. SSCP primers sequence

Primer	Sequence	Fragment size (bp)
0 F	ggtggttgggtgggaggag	212
0 R	ggacagagagcgggaaatgg	
1a F	cagcagcagcagcgactc	208
1a R	gaaggacggcacgaactc	
1b F	gtccacgccgccgagttc	181
1b R	ccgcacccctacgccatg	
2 F	gattgaggcttgtgtcgtgttatt	231
2 R	caaacagtatctagctttacacaccatat	
3 F	aggaacagtcattgtgtgaaggtaat	258
3 R	tcgtaacttacataatgcaccttaaatctt	
4a F	aggaaatggaactacatatgcaaactt	409
4a R	caggtagctaaaaaggatgttctatgttt	
4b F	catgggagcacaaagaagaaataag	261
4b R	caggtagctaaaaaggatgttctatgttt	
5 F	ggatttatgccccaaattcatatg	256
5 R	cctgttttatctcctttgtgtgactgta	
6 F	tctgacgttgaaattttcgacaat	253
6 R	ttaacaggctccaaaagcactaagt	
7 F	aaaactctgatgttcttaggaatattgttc	271
7 R	ctaacaaatatacagatggactggaaatg	
8 F	gaaggccaaggagtaaactgtttc	256
8 R	aagtcgtacaataacaaaaccatcctta	
9a F	taaggatggttttgttattgtacgacttt	251
9a R	tccgactgctctttgagatttg	
9b F	tcactttatgccctgctcag	133
9b R	taagtaatttaagtaataacatccttttcc	
10 F	tttatgttgacttcctttaatggcttt	207
10 R	aactagaaacaaaatgcacaacaatga	
11 F	atacagcaccaagaagtctgaaac	168
11 R	tcaataaatagataggaaacagcataacc	
12 F	tatgttgcgtaatgcagtgaatgt	271
12 R	cattaaaacccactatgacaacaacaa	
13 F	accctaaggctaaagataagaatgtg	143
13 R	cttaaactcactgttatttccacctc	
14 F	ttggcattattctgattttctcatactct	272
14 R	tgaagatgaccaaagctaggtatcct	
15a F	ggtttggcagtaaagctagttaattagtaa	267
15a R	aaataagagaaggcggtgtgaagta	
15b F	cctacttcacaccgccttctc	425
15b R	gctaatgcaacaatgttacatccag	

Primer numbers correspond to the exon to which each primer belongs. Fragment 0 belongs to 5′ UTR. Small letters indicate exons that had to be amplified in two fragments. All primers were designed using Primer3 Software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

CA, USA); *Taq* Polymerase (Fermentas, Hanover, MD) 0.035 U/μl and the primers' concentrations were 0.4 μM. Cycling conditions were as follows: an initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 62°C for 50 s and 72°C for 1 min. At the end, there was a final extension step at 72°C for 5 min.

Direct DNA sequencing of each detected allele was performed to confirm the GGC repeat expansion.

Moreover, a mutation screening was carried out in all eRF3/GSPT1 exons, exon–intron junctions and regulatory regions by PCR–SSCP using the primers listed in Table I. All reactions were done according to the following conditions: 2.5 mM $MgCl_2$ (Fermentas, Hanover, MD); 2.5 mM dNTPs (Fermentas, Hanover, MD); 0.033 U/μl *Taq* Polymerase (Fermentas, Hanover, MD) and 0.2 μM of the primer concentration. Some fragments required the addition of Enhancer solution 2× concentrated (fragments 0, 1a and 1b). Cycling conditions were an initial denaturation step at 95°C for 7 min, followed by 35 cycles at 95°C for 2 min, 55°C for 1 min and 72°C for 1 min. At the end, there was a final extension step at 72°C for 10 min.

The SSCP was carried under standard conditions as described in the literature (23).

All statistical analyses were carried out using SPSS version 12.0.

Results

Five alleles were detected, which encode 8, 9, 10, 11 and 12 glycines; the 10-Gly allele being the most common in the Portuguese population and the 12-Gly allele only found in the cancer population.

Genotypic frequencies were in Hardy–Weinberg equilibrium in the control population (Fisher's χ^2 $P > 0.05$), but not in the gastric cancer population (Fisher's χ^2 $P = 0.0024$). Our results revealed significant differences between the gastric cancer and control populations ($\chi^2 = 15.486$; $P = 0.002$), the 12-Gly allele exclusive of the gastric cancer population (allelic frequency = 5%). A total of 8% of the patients studied presented the 12-Gly allele, meaning that it was found in homozygoty in two cases. LOH was investigated and not detected in these cases. Patients with the 12-Gly allele had a 20-fold increased risk for gastric cancer (OR = 19.933; 95% CI = 1.179–333.94; $P = 0.001$) (Figure 1).

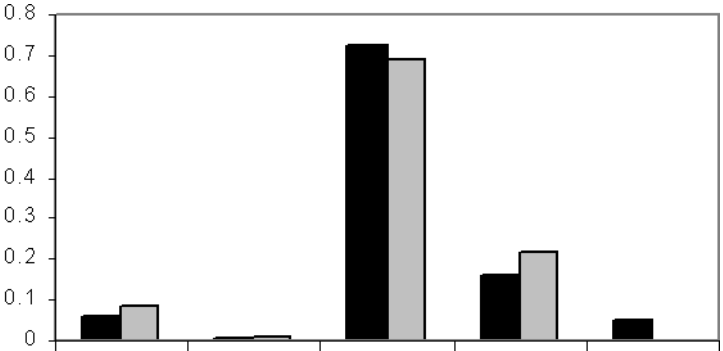
Significant differences were also observed in allelic frequencies between gastric adenocarcinomas and mucocellular carcinomas ($\chi^2 = 34.881$; $P = 0.001$), the 12-Gly allele being more frequent in the latter (allelic frequency = 3.1 versus 13.5%). All the individuals that presented the 12-Gly allele had gastric cancer independent of their genotype (Figure 2), although only 12/12, 12/11 and 12/10 genotypes were detected. Susceptibility for gastric cancer was shown not to be associated with any particular genotype (see Figure 2).

We further investigated the presence of additional genetic variability in *eRF3*/*GSPT1* gene that could be linked with the polyglycine expansion polymorphism. The *eRF3*/*GSPT1* gene of selected individuals who carried each allele was extensively scanned by PCR–SSCP followed by direct sequencing when adequate. We detected a thymine deletion in a stretch of 10 thymines in intron 5 (g24528–24529delT), adjacent to the intron–exon junction, that might have influence in the splicing process. Also, a synonymous nucleotide substitution in exon 14 was detected (G1755C).

Additionally, a nucleotide substitution G274T in exon 1, resulting in an amino acid change from Gly to Cys at codon 92 was detected. We screened this mutation in a group of gastric cancer patients and control individuals, and found no significant differences in the frequencies between both groups ($\chi^2 = 0.04$; $P > 0.05$) (Table II). Odds ratio (OR) analysis failed to reveal any risk/protective effect associated with either allele. Moreover, the mutation did not segregate with any of the alleles of the polyglycine expansion. Although genetic databases revealed that this Gly is a conserved amino acid at codon 92 of *eRF3*/*GSPT1*, our results provided evidence to support that this is a polymorphism rather than a mutation (Table III).

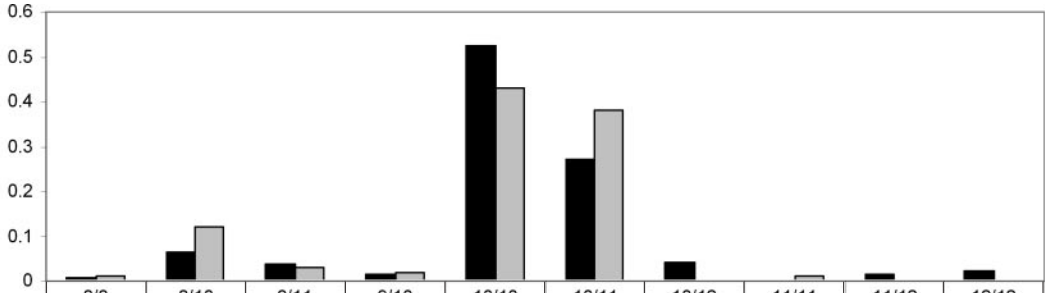
Discussion

Herewith we show, for the first time, a strong association between a polymorphic trinucleotide repeat in a translation factor and gastric cancer susceptibility. In addition, we found a direct correlation between the presence of the 12-Gly allele and gastric cancer. The molecular pathogenic pathway leading to the cancer phenotype is still not elucidated. It can involve a dominant gain of function effect by shifting the protein's conformation, thus altering its function or achieving toxicity. This could have an effect on eRF3 functions namely at different levels of translation regulation (13,14,17,19), in cytoskeleton organization, affecting chromosome segregation and cytokinesis (26–28), or in apoptosis (20). Loss of function can also occur, by interfering with transcription, RNA processing or translation efficiency. Depletion of eRF3 has



	8	9	10	11	12
Cases (N=278)	0.058	0.007	0.723	0.162	0.05
Controls (N=200)	0.085	0.01	0.69	0.215	0
OR 95% CI	0.687 0.16-2.79	0.687 0.09-4.93	Ref	0.719 0.44-1.11	19.933 1.17-336.94
χ^2 p	0.721	1		0.184	0.001

Fig. 1. Allelic frequencies of the eRF3 polyglycine repeat length alleles in the gastric cancer patients and control individuals. Frequencies and odds ratio analysis of the eRF3 repeat length polymorphism (Gly, glycine; OR, odds ratio; CI, confidence interval; *P*, *P*-value).



	8/8	8/10	8/11	9/10	10/10	10/11	10/12	11/11	11/12	12/12
Cases (N=139)	0.007	0.065	0.036	0.014	0.525	0.273	0.043	0	0.014	0.022
Controls (N=100)	0.01	0.12	0.03	0.02	0.43	0.38	0	0.01	0	0
OR 95% CI	0.589 0.03-9.66	0.147 0.06-1.31	4.143 0.29-82.16	0.589 0.00-4.34	Ref	0.589 0.38-1.09	7.694 0.43-139.9	0.197 0.08-4.90	2.959 0.18-63.09	4.143 0.29-82.16
P (χ^2)	1	0.074	0.552	0.63		0.1	0.089	0.376	0.533	0.552

Fig. 2. Genotypic frequencies of the eRF3 polyglycine repeat length alleles in the gastric cancer patients and control individuals. Frequencies and odds ratio analysis of the eRF3 repeat length polymorphism (Gly, glycine; OR, odds ratio; CI, confidence interval; *P*, *P*-value).

Table II. Odds ratio results for the polymorphism G274T in gastric cancer patients when compared with a healthy control population

	g	t
Gastric cancer ($N = 15$)	0.43	0.57
Controls ($N = 22$)	0.45	0.55
OR	Ref	1.09
95% CI		0.43–2.77

χ^2 not significant ($P > 0.05$).
OR, odds ratio; CI, confidence interval.

Table III. Amino acid conservation at position 92 throughout several organisms

Organism	Partial eRF3 protein sequence
Homo sapiens	A A E F V P S F L R **G** – P A A P P P P A G
Pan troglodytes	A A E F V P S F L R **G** – P A A P P P P A G
Canis familiaris	A A E F V P S F L R **G** – P A Q P Q T P A A
Mus musculus	A A E F V P S F L R **G** – P A Q P P L S P A
Rattus norvegicus	A A E F V P S F L R **G** – P A Q P P L S P A
Oryctolagus cuniculus	A A E F V P S F L R **G** – P A P P P A P A G
Gallus gallus	A A E F V P S F L R **G** A P A P G L P P P S

Polymorphic amino-acid in bold.

been recently shown to increase the read-through in HeLa cells to the same extent as eRF1 depletion does, which can lead to a pool of aberrant polypeptides in the cell (29,30). Also, although not usual, downregulation of translation factors associated with malignancies have been reported (31). Due to the presence of CpG islands, aberrant DNA methylation patterns may also occur leading to the inactivation of gene expression, being the variation in the number of GGC elements causal for the variability in the transcription level of the gene. A CCG expansion has been proposed as the mechanism responsible for the hypermethylation of the repeats and the adjacent CpG-rich promoter in *FMR1* gene, preventing the binding of transcription factors and consequently leading to transcription

repression. Subsequent to this expansion, *FMR1* is silenced causing fragile X syndrome (32). In the human *AR*, it was suggested that a hairpin stability caused by GGC expansions could interfere with translation, and lead to protein decrease (33). As well, a short polyalanine expansion in *PABP2* gene causes nuclear filament inclusions in skeletal muscle fibres, responsible for oculopharyngeal muscular dystrophy (34).

Most of the studies done so far on the role of genetic variants' contribution to gastric cancer highlighted individual variations in cancer risk, associated with specific variant alleles of different genes (polymorphisms) that are present in a significant proportion of the normal population. In this study, we report the presence of a specific allele directly associated with gastric cancer development. Importantly, the molecular mechanism associated with the presence of the 12-Gly allele in *eRF3*/*GSPT1* remains to be elucidated.

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References

1. Zheng,L., Wang,L., Ajani,J. and Xie,K. (2004) Molecular basis of gastric cancer development and progression. *Gastric Cancer*, **7**, 61–77.
2. Siyanova, EIu. and Mirkin,S.M. (2000) Expansion of trinucleotide repeats. *Mol. Biol.* **35**, 208–223.
3. Tran,N., Bharaj,B.S., Diamandis,E.P., Smith,M., Li,B.D. and Yu,H. (2004) Short tandem repeat polymorphism and cancer risk: influence of laboratory analysis on epidemiologic findings. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 2133–2140.
4. Ferro,P., Catalano,M.G., Dell'Eva,R., Fortunati,N. and Pfeffer,U. (2002) The androgen receptor CAG repeat: a modifier of carcinogenesis? *Mol. Cell Endocrinol.*, **193**, 109–120.
5. Li,C., Gronberg,H., Matsuyama,H., Weber,G. *et al.* (2003) Difference between Swedish and Japanese men in the association between AR CAG repeats and prostate cancer suggesting a susceptibility-modifying locus overlapping the androgen receptor gene. *Int. J. Mol. Med.*, **11**, 529–533.
6. Clemens,M.J. and Bommer,U.A. (1999) Translational control: the cancer connection. *Int. J. Biochem. Cell Biol.*, **31**, 1–23.
7. Caraglia,M., Budillon,A., Vitale,G., Lupoli,G., Tagliaferri,P. and Abbruzzese,A. (2000) Modulation of molecular mechanisms involved in protein synthesis machinery as a new tool for the control of cell proliferation. *Eur. J. Biochem.*, **267**, 3919–3936.
8. Rajasekhar,V.K. and Holland,E.C. (2004) Postgenomic global analysis of translational control induced by oncogenic signalling. *Oncogene*, **23**, 3248–3264.
9. Frazier,M.L., Inamdar,N., Alvula,S., Wu,E. and Kim,Y.H. (1998) Few point mutations in elongation *factor-1gamma* gene in gastrointestinal carcinoma. *Mol. Carcinog.*, **22**, 9–15.
10. Rothe,M., Ko,Y., Albers,P. and Wernert,N. (2000) Eukaryotic initiation factor 3 p110 mRNA is overexpressed in testicular seminomas. *Am. J. Pathol.*, **157**, 1597–1604.
11. Anand,N., Murthy,S., Amann,G. *et al.* (2002) Protein elongation factor *EEF1A2* is a putative oncogene in ovarian cancer. *Nat. Genet*, **31**, 301–305.
12. Malta-Vacas,J., Aires,C., Costa,P., Conde,A.R., Ramos,S., Martins,A.P., Monteiro,C. and Brito,M. (2005) Differential expression of the eukaryotic releasing factor 3 (eRF3/GSPT1) in gastric cancer. *J. Clin. Pathol.*, **58**, 621–625.
13. Zhouravleva,G., Frolova,L., Le Goff,X., Le Guellec,R., Inge-Vechtomov,S., Kisselev,L. and Philippe,M. (1995) Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. *EMBO J.*, **14**, 4065–4072.
14. Frolova,L., Le Goff,X., Zhouravleva,G., Davydova,E., Philippe,M. and Kisselev,L. (1996) Eukaryotic polypeptide chain release factor eRF3 is an eRF1- and ribosome-dependent guanosine triphosphatase. *RNA*, **2**, 334–341.
15. Kikuchi,Y., Shimatake,H. and Kikuchi,A. (1988) A yeast gene required for the G1-to-S transition encodes a protein containing an A-kinase target site and GTPase domain. *EMBO J.*, **7**, 1175–1182.
16. Hoshino,S., Miyazawa,H., Enomoto,T., Hanaoka,F., Kikuchi,Y., Kikuchi,A. and Ui,M. (1989) A human homologue of the yeast GST1 gene codes for a GTP-binding protein and is expressed in a proliferation-dependent manner in mammalian cells. *EMBO J.*, **8**, 3807–3814.
17. Hoshino,S., Hosoda,N., Araki,Y., Kobayashi,T., Uchida,N., Funakoshi,Y. and Katada,T. (1999) Novel function of the eukaryotic polypeptide-chain releasing factor 3 (eRF3/GSPT) in the mRNA degradation pathway. *Biochemistry*, **64**, 1367–1372.
18. Hosoda,N., Kobayashi,T., Uchida,N., Funakoshi,Y., Kikuchi,Y., Hoshino,S. and Katada,T. (2003) Translation termination factor eRF3 mediates mRNA decay through the regulation of deadenylation. *J. Biol. Chem.*, **278**, 38287–38291.
19. Uchida,N., Hoshino,S., Imataka,H., Sonenberg,N. and Katada,T. (2002) A novel role of the mammalian GSPT/eRF3 associating with poly(A)-binding protein in Cap/Poly(A)-dependent translation. *J. Biol. Chem.*, **277**, 50286–50292.
20. Hedge,R., Srinivasula,S.M., Datta,P. *et al.* (2003) The polypeptide chain-releasing factor GSPT1/eRF3 is proteolytically processed into an IAP-binding protein. *J. Biol. Chem.*, **278**, 38699–38706.
21. Merkulova,T.I., Frolova,L.Y., Lazar,M., Camonis,J. and Kisselev,L.L. (1999) C-terminal domains of human translation termination factors eRF1 and eRF3 mediate their *in vivo* interaction. *FEBS Lett.*, **443**, 41–47.
22. Cosson,B., Berkova,N., Couturier,A., Chabelskaya,S., Philippe,M. and Zhouravleva,G. (2002) Poly(A)-binding protein and eRF3 are associated *in vivo* in human and *Xenopus* cells. *Biol. Cell*, **94**, 205–216.
23. Sambrook,J. and Russel,D. (2001) *Molecular Clonning, A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
24. Coombs,N., Gough,A. and Primrose,N. (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucleic Acids Res.*, **27**, e12.
25. Riggins,G.J., Lokey,L.K., Chastain,J.L., Leiner,H.A., Sherman,S.L., Wilkinson,K.D. and Warren,S.T. (1992) Human genes containing polymorphic trinucleotide repeats. *Nat. Genet*, **2**, 186–191.
26. Basu,J., Williams,B.C., Li,Z., Williams,E.V. and Goldberg,M.L. (1998) Depletion of a *Drosophila* homolog of yeast Sup35p disrupts spindle assembly, chromosome segregation, and cytokinesis during male meiosis. *Cell Motil. Cytoskeleton*, **39**, 286–302.
27. Borchsenius,A.S., Tchourikova,A.A. and Inge-Vechtomov,S.G. (2000) Recessive mutations in *SUP35* and *SUP45* genes coding for translation release factors affect chromosome stability in *Saccharomyces cerevisiae*. *Curr. Genet.*, **37**, 285–291.
28. Valouev,I.A., Kushnirov,V.V. and Ter-Avanesyan,M.D. (2002) Yeast polypeptide chain release factors eRF1 and eRF3 are involved in cytoskeleton organization and cell cycle regulation. *Cell Motil. Cytoskeleton*, **52**, 161–173.
29. Janzen,D.M. and Geballe,A.P. (2004) The effect of eukaryotic release factor depletion on translation termination in human cell lines. *Nucleic Acids Res.*, **32**, 4491–4502.
30. Chauvin,C., Salhi,S., Le Goff,C., Viranaicken,W., Diop,D. and Jean-Jean,O. (2005) Involvement of human release factors erf3a and erf3b in translation termination and regulation of the termination complex formation. *Mol. Cell. Biol.* **25**, 5801-5811.
31. Martin,M.E., Perez,M.I., Redondo,C., Alvarez,M.I., Salinas,M. and Fando,J.L. (2000) 4E binding protein 1 expression is inversely correlated to the progression of gastrointestinal cancers. *Int. J. Biochem. Cell Biol.*, **32**, 633–642.
32. Grabczyk,E., Kumari,D. and Usdin,K. (2001) Fragile X syndrome and Friedreich's ataxia: two different paradigms for repeat induced transcript insufficiency. *Brain Res. Bull.*, **56**, 367–373.
33. Ding,D., Xu,L., Menon,M., Reddy,G.P. and Barrack,E.R. (2005) Effect of GGC (glycine) repeat length polymorphism in the human androgen receptor on androgen action. *Prostate*, **62**, 133–139.
34. Brais,B., Bouchard,J.P., Xie,Y.G. *et al.* (1998) Short GCG expansions in the PABP2 gene cause oculopharyngeal muscular dystrophy. *Nat. Genet.*, **18**, 164–167.

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