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Short Communication

Translation termination and protein folding pathway genes are not correlated in gastric cancer

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Abstract

Background: The eukaryotic release factor 3 (eRF3) has been shown to affect both tubulin and actin cytoskeleton, suggesting a role in cytoskeleton assembly, mitotic spindle formation and chromosome segregation. Also, direct interactions between eRF3 and subunits of the cytosolic chaperonin CCT have been described. Moreover, both eRF3a and CCT subunits have been described to be up-regulated in cancer tissues. Our aim was to evaluate the hypothesis that eRF3 expression levels are correlated with the expression of genes encoding proteins involved in the tubulin folding pathways.
Methods: Relative expression levels of *eRF1*, *eRF3a/GSPT1*, *PFDN4*, *CCT2*, *CCT4*, and *TBCA* genes in tumour samples relative to their adjacent normal tissues were investigated using real time-polymerase chain reaction in 20 gastric cancer patients.
Results: The expression levels of *eRF3a/GSPT1* were not correlated with the expression levels of the other genes studied. However, significant correlations were detected between the other genes, both within intestinal and diffuse type tumours.
Conclusions: *eRF3a/GSPT1* expression at the mRNA level is independent from both cell translation rates and from the expression of the genes involved in tubulin-folding pathways. The differences in the patterns of expression of the genes studied support the hypothesis of genetically independent pathways in the origin of intestinal and diffuse type gastric tumours.
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Keywords: CCT subunits; eukaryotic release factor 3a/G-to-S phase transition 1 (eRF3a/GSPT1); gastric cancer; protein folding pathways; translation termination.

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The eukaryotic release factor 3 (eRF3) has multi-functional properties in eukaryotic cells. The canonical role of eRF3 is to regulate protein synthesis as a GTP-dependent stimulator of eRF1 in the termination step of translation (1). After translation termination, the maturation process of some proteins, such as actins and tubulins, involves interaction of nascent chains with the heterohexameric protein prefoldin (PFDN). Its function is to deliver non-native target proteins to the eukaryotic cytosolic chaperonin for facilitated folding (2). PFDN is a hetero-oligomeric complex assembled from six different proteins which interacts mainly with newly synthesised actins and tubulins, and protects them from aggregation while being transferred to the cytosolic chaperonin CCT (chaperonin containing TCP-1) (3). In mammalian cells, the CCT is a hetero-oligomeric complex with a double-ring-like structure showing eight-fold rotational symmetry of eight different subunits, encoded by a family of related genes designated from Cctα to Cctζ-1. Each subunit of this complex recognises specific target proteins and they collectively modulate ATPase activity (4). CCT has been shown to assist in the folding of actin and tubulin in the presence of ATP in vitro (5) and to bind newly synthesised actin and tubulin in vivo (6). After release from the CCT complex in a quasi-native conformation, tubulins are accepted by cofactors following two different folding pathways: α-tubulin is captured by cofactor B (TBCB), while β-tubulin is captured by cofactor A (TBCA) (7). Then, cofactors E (TBCE) and D (TBCD) capture α- and β-tubulin, respectively. The two pathways converge and α-tubulin, β-tubulin, TBCE and TBCD form a super-complex. Cofactor C (TBCC) interacts with this complex and upon GTP hydrolysis assembled competent α/β-tubulin heterodimers are released (7). A number of studies have demonstrated that the members of the tubulin folding pathway participate in the control of assembly and dynamics of cytoskeleton by controlling heterodimer maturation (8, 9).

An interaction between CCT subunits and eRF3 in human cells was first predicted using a theoretical model (10), and experimentally confirmed by immunoprecipitation (11). Using different organisms as study models, eRF3 has also been shown to affect both tubulin and actin cytoskeleton (12–15), suggesting a role in cytoskeleton assembly, mitotic spindle formation and chromosome segregation. The eRF3 protein is essential for G1 to S phase transition of the cell cycle (16, 17). Interestingly, CCT expression levels also correlate with growth rates in mammalian cultured cells, and the expression of CCT is markedly up-regulated in the early S phase of the cell cycle (9, 18).

In a previous study, we reported that *eRF3a/GSPT1* is up-regulated in 70% of the intestinal histological type gastric tumours (19). In addition, it was reported that the CCTα and CCTβ subunits are up-regulated in hepatocelular carcinomas and in 83% of colonic tumours (20). The identification of the genes and pathways that are altered in gastric tumourigenesis might contribute to improve diagnosis and therapy. In this study, we quantified the mRNA relative levels of expression of a number of components of the translation termination (eRF1 and eRF3a/GSPT1), protein folding (*CCT2* and *CCT4*) and tubulin specific folding pathway (*PFDN4* and *TBCA*) in gastric cancer patients, by real time-polymerase chain reaction using TaqMan probes (Table 1). Our aim was to investigate if the expression of the genes encoding the components of these pathways are correlated and collectively contribute to tumourigenesis.

Freshly frozen samples of gastric tumours were obtained together with a sample from the adjacent non-cancerous tissue from 20 gastric cancer patients. Tissue samples were stored in RNAlater (Ambion, Austin, TX, USA) at –20°C immediately after surgery until RNA extraction. The samples were verified to be gastric cancers by histological examination performed by a pathologist. Tumours were divided into two groups according to their histological types, which differed in epidemiology, aetiology, pathogenesis and behaviour (21): 10 intestinal type carcinomas (well differentiated cells, which retain cell cohesion allowing the formation of glandular structures with sharp margins) and 10 diffuse type carcinomas (containing small undifferentiated cells or small cell clusters with deceptive margins, which invade large areas of the stomach and can lead to early metastasis).

All the genes showed overexpression in a larger fraction of the intestinal type tumours (Figure 1). This is mostly observed in *eRF3a/GSPT1* gene expression, which showed overexpression in 70% of the intestinal type tumours analysed, whereas only 10% of the diffuse type tumours overexpressed the gene (Table 2). Regarding the genes coding for molecular chaperones, the CCT subunits were the most commonly up-regulated, showing overexpression in more than 70% of the intestinal tumours. *PFDN4* and *TBCA* were overexpressed in approximately 60% of the intestinal type samples. Within the diffuse type tumours, the mRNA levels of all the genes analysed were lower than in the intestinal tumours (Figure 1).

The observed up-regulation of *eRF3a/GSPT1* does not correlate with the expression of any of the other genes analysed (Table 3). In accordance with previous results (19), *eRF1* expression, which is the main factor in translation termination, did not correlate with *eRF3a/GSPT1* mRNA levels, presumably because eRF3a is necessary to accomplish other roles in the cell apart from translation termination. Although not statistically significant, it is worth noting that correlations between *eRF3a/GSPT1* and the other genes analysed are mostly negative correlations, especially in the intestinal type tumours (Table 3).

It is known that the transfer of the polypeptide chain from the PFDN to the CCT complex is carried out through a mechanism that implies physical interaction between both complexes and that does not involve the release of the substrate (23). Accordingly, our results revealed strong correlations between *PFDN4* and *CCT2* ($r=1$, $p<0.001$) in the intestinal tumours, and also between *PFDN4* and *CCT4* ($r=0.885$, $p<0.01$) in the diffuse tumours. Also, we observed strong correlations between *CCT2* and *TBCA* in both types of tumours ($r=0.967$, $p<0.001$ in intestinal; $r=0.909$, $p<0.001$ in diffuse tumours), possibly as a consequence of functional interactions between them.

According to immunoprecipitation results, eRF3 interacts with specific subunits of the CCT complex and was found to be localised coincidently with CCTβ

Table 1 Sequences of primers and probes.

Genes	Sequences of primers and probes (5′-3′)
eRF1	F: TGC ATC TAA CAT TAA GTC ACG AGT R: AAC CGC CTT TCA GTC CTG GGA GCC P: TCC ACA GTA TAC AAC CAG ACC ATT
eRF3a/GSPT1	F: CGC CAG GTG CTC CTA AGA AAG R: CAA ATA CAT TAT TTG TCC TCC AAT GGT P: ACT TGC CAG CAT CTA CGT GCC CAA TG
PFDN4	F: TTG GTG ATG TCT TCA TTA GCC ATT R: TTC CAC TCT GGA TTC TAA GGC G P: AAG AAA CGC AAG AAA TGT AAG AAG CAA AGA AAA ATTAG
TBCA	F: TGA GAG CTG AAG ACG GTG AA R: AGA TCC AAA TAT GCG GCT TC P: CTG GAT TCT TGT AGG ATC TCT GCC TGC
CCT2	AoD (Hs00197562_m1, Applied Biosystems, Branchburg, NJ, USA)
CCT4	AoD (Hs00272345_m1, Applied Biosystems)
18S rRNA	TaqMan PDAR (Applied Biosystems)

Gene-specific primers and a TaqMan probe labelled with 5′FAM and 3′MGB were used for all genes. Reactions were performed as described previously (19).

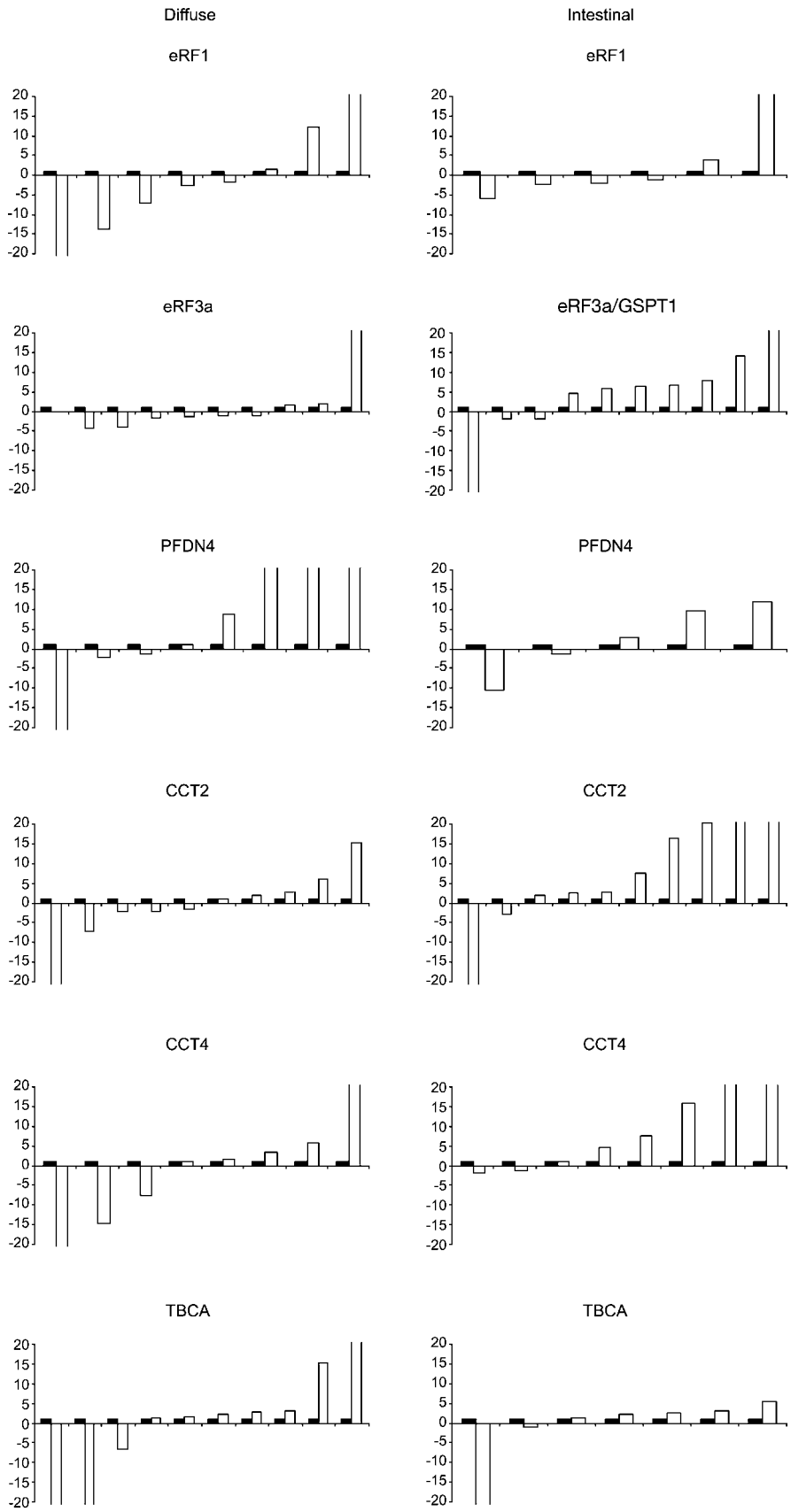

Figure 1 Relative levels of expression of *eRF1*, *eRF3a/GSPT1*, *PFDN4*, *CCT2*, *CCT4*, and *TBCA* genes in tumour samples (open bars) relative to their adjacent normal tissues (closed bars).
Relative quantification of the mRNA levels of each gene (quantity of transcripts in tumour samples relative to normal tissues) was determined using the $\Delta\Delta C_T$ method (22). Briefly, the amount of target was normalised to the endogenous reference gene (*18S rRNA*) and its expression in tumour samples was calculated relative to a calibrator (normal adjacent sample). Final results are expressed as n-fold difference in tumour expression relative to non-cancerous adjacent tissue.

Table 2 Analysis of the mRNA level variations between intestinal and diffuse type tumours.

	Intestinal type			Diffuse type		
	Underexpression	Without variation	Overexpression	Underexpression	Without variation	Overexpression
eRF1	33.33% (2/6)	33.33% (2/6)	33.33% (2/6)	50.00% (4/8)	25.00% (2/8)	25.00% (2/8)
eRF3a/GSPT1	10.00% (1/10)	20.00% (2/10)	70.00% (7/10)	30.00% (3/10)	60.00% (6/10)	10.00% (1/10)
PFDN4	20.00% (1/5)	20.00% (1/5)	60.00% (3/5)	25.00% (2/8)	25.00% (2/8)	50.00% (4/8)
CCT2	20.00% (2/10)	10.00% (1/10)	70.00% (7/10)	40.00% (4/10)	20.00% (2/10)	40.00% (4/10)
CCT4	0.00% (0/7)	28.50% (2/7)	71.50% (5/7)	37.50% (3/8)	25.00% (2/8)	37.50% (3/8)
TBCA	14.30% (1/7)	28.60% (2/7)	57.10% (4/7)	30.00% (3/10)	20.00% (2/10)	50.00% (5/10)

Table 3 Correlations between the mRNA expression levels of the genes.

			Intestinal type tumours					
			eRF1	*eRF3a*	*PFDN4*	*CCT2*	*CCT4*	*TBCA*
Diffuse type tumours	*eRF1*	CC		0.316	0.866	0.490	0.408	0.943
		p		0.541	0.333	0.324	0.495	0.057
	eRF3a/GSPT1	CC	−0.282		−0.395	−0.009	−0.586	−0.075
		p	0.498		0.510	0.098	0.127	0.874
	PFDN4	CC	0.889**	−0.371		1**	0.645	1**
		p	0.003	0.356		–	0.239	–
	CCT2	CC	0.639	0.044	0.639		0.390	0.967**
		p	0.088	0.904	0.088		0.339	0.000
	CCT4	CC	0.748*	−0.218	0.885**	0.68		0.422
		p	0.033	0.604	0.004	0.063		0.405
	TBCA	CC	0.34	−0.104	0.34	0.909**	0.5	
		p	0.41	0.775	0.41	0.000	0.207	

Correlations were determined by the Spearman Rho coefficient using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). CC, correlation coefficient; p, probability: *$p<0.05$; **$p<0.01$.

in particular sites of the cytosol (11). Moreover, analysis of eRF3 and actin expression revealed that both proteins colocalise in the cell periphery (11). However, in our study, *eRF3a/GSPT1* mRNA levels of expression did not correlate with any of the CCT subunits analysed in gastric cancer samples. Our hypothesis of *eRF3a/GSPT1* expression being correlated with the expression levels of *CCT2* and *CCT4* was not verified.

Although *eRF3a/GSPT1* expression at the mRNA level is likely to be independent from both cell translation rates and protein folding pathways, our results revealed up-regulated levels of expression of components of the translation termination and the protein folding pathway in gastric cancer tissues, mainly in the intestinal histological type tumours. These results are in good agreement with previously published studies (19, 20) and support the hypothesis of genetic independent pathways in the origin of intestinal and diffuse type gastric tumours. Understanding eRF3a/GSPT1 gene regulation and its relation with cell cycle progression and cellular proliferation may have prognostic value and potential therapeutic applications.

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