Biochemical and Biophysical Research Communications 380 (2009) 11–16

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Regulation and functional role of eEF1A2 in pancreatic carcinoma

Haixia Cao, Qi Zhu *, Jia Huang, Baiwen Li, Su Zhang, Weiyan Yao, Yongping Zhang

Department of Gastroenterology, Rui Jin Hospital, Shanghai Jiaotong University School of Medicine, No. 197 Rui Jin Er Road, Shanghai 200025, China

ARTICLE INFO

Article history:
Received 25 December 2008
Available online 10 January 2009

Key words:
eEF1A2
Protein translation
Pancreatic cancer
Cell growth and invasion

ABSTRACT

Pancreatic cancer typically has an unfavourable prognosis due to late diagnosis and a lack of therapeutic options. Thus, it is important to better understand its pathological mechanism and to develop more effective treatments for the disease. Human chromosome 20q13 has long been suspected to harbour oncogenes involved in pancreatic cancer and other tumours. In this study, we found that eEF1A2, a gene located in 20q13, was significantly upregulated in pancreatic cancer. Little or no expression of eEF1A2 was detected in normal human pancreatic and chronic pancreatitis tissues, whereas increased eEF1A2 expression occurred in 83% of the pancreatic cancers we studied. Furthermore, using in vitro and in vivo model systems, we found that overexpression of eEF1A2 promoted cell growth, survival, and invasion in pancreatic cancer. Our data thus suggest that eEF1A2 might play an important role in pancreatic carcinogenesis, possibly by acting as a tumour oncogene.

Introduction

Pancreatic cancer is one of the most aggressive malignancies, with 5-year survival rates averaging less than 5% [1,2]. Despite great advances in the characterization of molecular alterations commonly present in pancreatic cancer, improved understanding of its molecular basis is required to identify new markers for early diagnosis and provide potential targets for therapeutic intervention. Alteration of the expression of genes involved in cell proliferation or apoptosis can cause abnormal cell growth and malignant transformation. Although most studies have focused on the transcriptional regulation of gene expression, recent studies have suggested that dysfunctional protein translation may also contribute to cancer development. In particular, the protein elongation factor eEF1A2 has been identified as an important player in many different human tumours [3–7]. However, little is known about the role of eEF1A2 in pancreatic cancer.

eEF1A2 is a housekeeping gene and one of the two isoforms of protein elongation factor eEF1A. In contrast to eEF1A1, which is expressed ubiquitously, eEF1A2 is normally present only in the heart, brain and muscle tissues [8–10]. The classical role for these proteins involves regulation of ribosomal polypeptide elongation by binding of amino-acylated tRNA and facilitation of its recruitment to the ribosome [11]. In addition to its central role in protein translation, eEF1A2 also has some non-canonical functions such as cytoskeleton modification [12–14] and targeting of proteins for degradation [15], as well as involvement in the heat shock response [16], apoptosis [17] and phophatidylinositol signalling [18].

Recent studies have shown that eEF1A2 is upregulated in a subset of ovarian, lung, breast and hepatocellular tumours, suggesting an important role for this molecule in oncogenesis [3–7]. Expression of wild-type eEF1A2 was found to transform rodent fibroblasts and increase their tumourigenicity in nude mice [3]. In lung cancer, overexpression of eEF1A2 was correlated with increased Ki-67 expression and poor prognosis [4]. These observations implicate eEF1A2 in oncogenesis, and led us to hypothesize that eEF1A2 is also involved in pancreatic cancer.

In the present study, we investigated the expression of eEF1A2 in normal and pathological pancreatic tissues, as well as in pancreatic cancer cells, and examined the effect of eEF1A2 expression on pancreatic cancer cell growth, proliferation and motility using in vitro and in vivo assays.

Materials and methods

Tumour samples and cell lines. All investigations described in this study were performed after informed consent was obtained, according to the guidelines of the Institutional Review Board and the ethical committee of Rui Jin Hospital. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissues were de-waxed in xylene and rehydrated in graded alcohols. After antigen retrieval and blocking of non-specific reactions, sections were incubated with eEF1A2 (1:200, a gift from Helen Newbery, Edinburgh), PCNA (1:2000, Cell Signalling Technology, Danvers, MA) or CD31 antibodies (1:100, Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight.

* Corresponding author.
E-mail address: zhuqi@medmail.com.cn (Q. Zhu).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.12.171

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA from cultured cells or human pancreatic tissue samples was isolated using the RNeasy RNA isolation kit (Qiagen, Valencia, CA) following the manufacturer's protocol. RNA was quantitated spectrophotometrically and 2 μg of RNA were used to make cDNA using Thermoscript RT-PCR Reaction System (Invitrogen,Carlsbad,CA) per manufacturer's protocol. Each PCR reaction was carried out in 25 μl mixture. The amplification products were electrophoresed on 1.2% agarose gels. Primers used for RT-PCR are as follows: eEF1A2 primers forwards 5′-tcgaatctccctctggaagtt-3′, reversed 5′-cttgaaccacggcatgttg-3′; eEF1A1 primers forwards 5′-aacattdtcgtcattggaca-3′ reversed 5′-acttgctggtctcaaatttc-3′.

Western blot analysis. Anti-EF1a antibody was purchased from Upstate Biotechnology (Charlottesville,VA) and anti-ß-actin antibody was acquired from Sigma (St. Louis, MO). Protein samples (20 μg) were subjected to SDS gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes using a Bio-Rad wet transfer system. Immunoreactive bands were detected using an enhanced chemiluminescence detection system (ECL, Amersham Pharmacia Biotech, Piscataway, NJ, USA), according to the manufacturer's instructions.

Construction and transfection of expression plasmids. EEF1A2 full length cDNA was cloned into a pDC316 carrier plasmid to generate pDC316-EEF1A2. The plasmid pDC316-EEF1A2 and the skeleton plasmid pBHG-fiber5/35 were co-transfected into HEK293 cells using Polyfect (Qiagen, Hilden, Germany). The co-transfection yielded the recombinant Ad5/F35-EEF1A2 plasmid. Successful recombination was confirmed by observation of cytotoxicity as well as by PCR. The control recombinant plasmid Ad5/F35-GFP (green fluorescent protein) was obtained by the same method. SW1990 cells, which express relatively low levels of endogenous eEF1A2, were transfected with Ad5/F35-EEF1A2 or with the control Ad5/F35-GFP plasmid.

Cell viability assessment. Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye according to the standard protocol. The absorbance of the samples was measured using a microplate reader at 570 nm. Additionally, cell proliferation was determined by counting cells with a Coulter Counter.

Colony formation assay. A soft agar colony formation assay was used to assess the anchorage-independent growth ability of cells. For each well in a six-well plate, 1.0×10^3 cells were mixed with 1 mL DMEM medium containing 10% fetal bovine serum and 0.3% agar and then plated on a 0.6% agar base. Colonies with diameters larger than 50 μm were counted at 15 days after plating.

Cell cycle analysis by fluorescence activated cell sorting (FACS). Cells seeded in six well plates were harvested and washed with cold PBS, centrifuged at 1200 rpm and stored overnight in 70% ethanol at 4 °C. The following day, cells were washed with cold PBS and resuspended in 200 μl 1.12% sodium citrate containing RNase A (250 μg/ml) for 30 min at room temperature. Thereafter, cells were stained with 50 μg/ml propidium iodide in 1.12% sodium citrate solution. The stained cells were analyzed for DNA content at 448 nm by flow cytometry in a FACScan device equipped with a 15-mW argon ion laser.

Cell migration and invasion assay. Cell migration and invasion assays were performed using transwell chambers with 8 μm pores (Chemicon). The lower chambers of the transwell plates were filled with 500 μl medium containing 10% fetal bovine serum as a chemoattractant. The cell suspension (300 μl) was then added to the upper chamber, and plates were incubated at 37 °C for 24 and 48 h. Cells that migrated to the lower surface of the polycarbonate

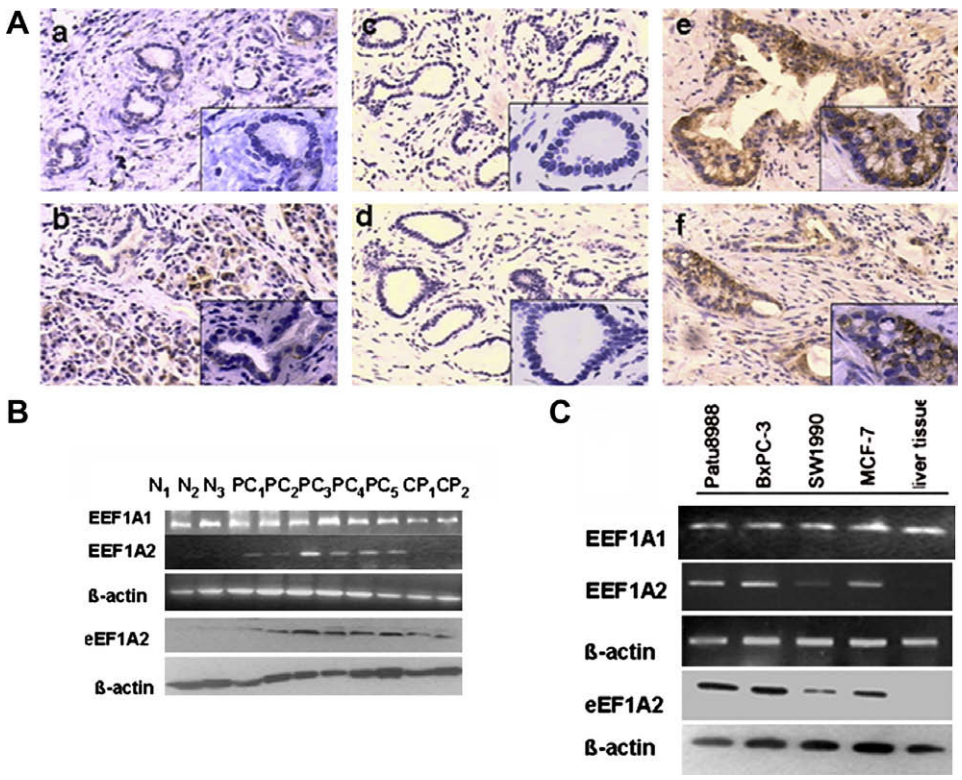

Fig. 1. eEF1A2 is overexpressed in pancreatic cancer. (A) Immunohistochemical analysis of eEF1A2 expression in two representative samples of normal human pancreas (a and b), chronic pancreatitis (c and d), and pancreatic ductal carcinoma (e and f) tissues using low-power image (×100) and representative high-power microscopy images (×200). (B) RT-PCR and western blotting were performed on human pancreatic tissues including those from normal pancreas (N,10), chronic pancreatitis (CP,6) and pancreatic cancer (PC,20). (C) eEF1A1 and eEF1A2 mRNA levels and protein expression was analyzed in pancreatic cancer cells. MCF-7 cells served as a positive control and normal human liver tissues served as a negative control.

membrane were stained with haematoxylin. The cells that migrated to the lower surface were quantified by counting 10 randomly selected microscope fields at ×400 magnification.

In vitro wound healing assay. Wounds were mechanically generated by scratching with a sterile pipette tip. Cells were washed with PBS, then cultured in complete culture medium. Photographs were taken at 0, 12 and 24 h after wound generation. Cells that migrated into the wounded area were counted and the distance of migration from the cellular monolayer to the wounded area during this time period was also subsequently measured.

Cell adhesion assay. The cell adhesion assay (Chemicon) was performed according to the manufacturer's protocol. Ninety-six well plates were coated with type I collagen, type II collagen, type IV collagen, fibronectin, laminin, tenascin or vitronectin, as well as bovine serum albumin as a control. The attached cells were stained with cresyl violet, rinsed with distilled water, solubilized in acetic acid, and quantified using a microtiter plate reader at 570 nm.

Nude mouse subcutaneous xenograft model. Balb/c nude mice were maintained under specific pathogen-free conditions in the Shanghai Experimental Animals Centre of Chinese Academy of Sciences, and their care was in accordance with institutional guidelines. To determine the effect of eEF1A2 gene overexpression on in vivo growth, 1×10^6 SW1990 cells were implanted subcutaneously in nude mice. Two weeks after tumour cell inoculation, mice were divided randomly into 3 groups (5 mice/group). Each animal in the three groups was injected with 1×10^8 PFU (plaque-forming units) of Ad5/F35-GFP, Ad5/F35-EEF1A2 or control PBS every three days for 24 days. Tumour dimensions and body weights were recorded every three days. Tumour volume (V) was estimated from tumour length (l) and width (w) using the formula $V = lw^2\pi/6$. PCNA staining was used to evaluate cell proliferation, and positively stained cells were expressed as the percentage of total tumour cells in the five higher magnification fields (×200). *A* CD31 polyclonal antibody was used to assess micro-vessel density (MVD) in tumours according to the procedure previously described by Weidner et al. [19].

Statistical analysis. Each experiment was repeated at least three times. Differences in mean values were evaluated using ANOVA tests. All statistical analyses were performed using SPSS 11.0 software (Chicago, IL, USA). $p < 0.05$ was considered to be statistically significant.

Results

eEF1A2 was upregulated in pancreatic cancer

eEF1A2 expression was analyzed by immunohistochemistry in thirteen normal pancreatic tissues, eight chronic pancreatitis tissue samples and thirty-five pancreatic cancer tissues. The staining pattern indicated strong expression of eEF1A2 in 29/35 (83%) of pancreatic cancer tissues (Fig. 1A). In contrast, normal pancreatic ducts and chronic pancreatitis samples were both negative for eEF1A2 staining in most biopsies (Fig. 1A). When eEF1A2 was expressed, the staining was specific and granular in the cytoplasm. No expression of eEF1A2 was demonstrated in islets.

To further assess the potential role of eEF1A2 in pancreatic cancer, its expression was examined by RT-PCR and western blot analysis in ten samples of normal pancreas, six with chronic pancreatitis and twenty samples of pancreatic adenocarcinoma as well as three pancreatic caner cell lines. eEF1A2 mRNA and protein expression was increased in pancreatic cancers, but reduced or not present in normal pancreatic and chronic pancreatitis samples (Fig. 1B). In addition, eEF1A2 was also overexpressed in BxPC-3 and PaTu8988 cell lines, while little or no expression was detected in

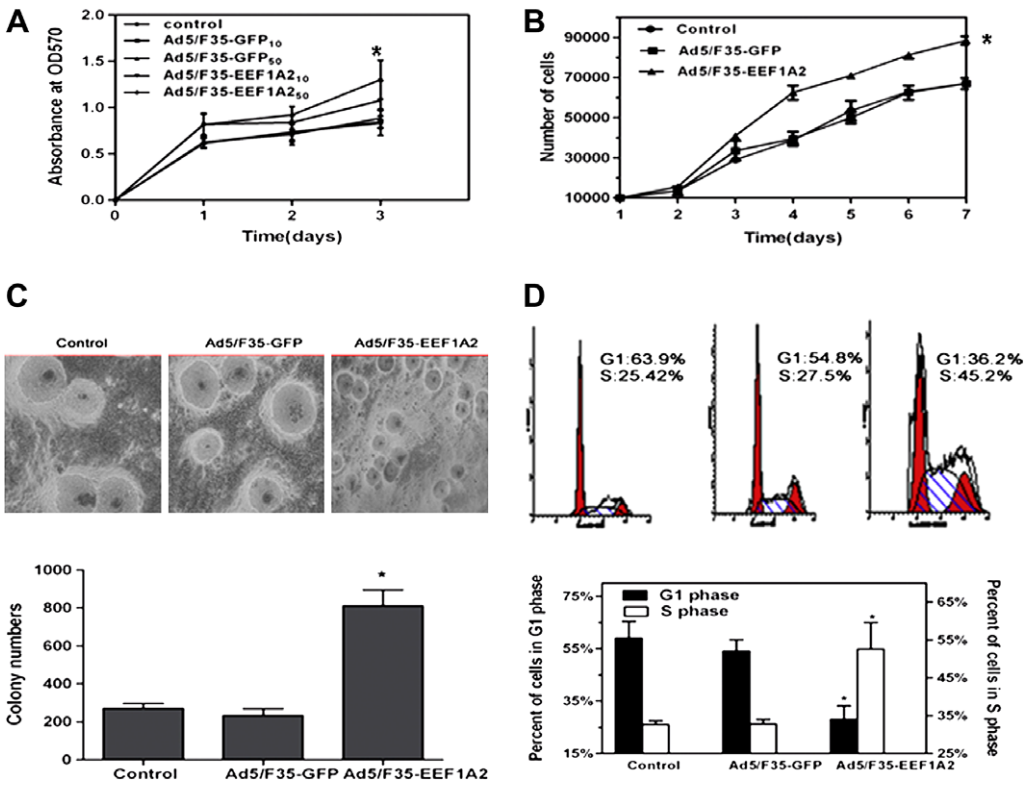

Fig. 2. eEF1A2 promoted growth, proliferation and S-phase entry in SW1990 cells. (A) Growth of SW1990 cells transduced with adenovirus was measured by MTT assay. (B) The cell growth curve was obtained by counting cell numbers per well on each day for a week. (C) Colony formation assay was performed using SW1990 cells transduced with adenovirus for 48 h. (D) Ad5/F35-EEF1A2 transduction increased the S-phase population in SW1990 cells after 48 h. $^*p < 0.05$.

SW1990 cell lines (Fig. 1C). There was no difference of eEF1A1 expression between normal control and pancreatic cancer in either cell line or tissue samples.

eEF1A2 overexpression enhanced cell growth and proliferation

To address the function of eEF1A2 in pancreatic cancer development, we successfully constructed the recombinant adenovirus plasmid Ad5/F35-EEF1A2. SW1990 cells expressing relatively low levels of endogenous eEF1A2 were transfected with full-length eEF1A2 cDNA. After transfection of eEF1A2 cDNA, we verified its overexpression in SW1990 cells by RT-PCR and western blotting and compared the expression levels to those of cells transfected with control vectors (Supplementary Fig. 1). The results also demonstrated that the eEF1A1 expression level was not influenced in the cells infected with Ad5/F35-EEF1A2 (Supplementary Fig. 1). When compared to mock-transfected cells, eEF1A2 overexpression significantly enhanced the proliferation of SW1990 cells as determined by both MTT assay (Fig. 2A) and cell counting (Fig. 2B). As shown in Fig. 2C, overexpression of eEF1A2 also led to increased colony formation in a soft agar assay. These results suggest that EEF1A2 plays an important role in the regulation of pancreatic cell proliferation.

To further determine whether the cell cycle distribution was changed by overexpression of eEF1A2 in SW1990 cells, we used flow cytometry to analyze the populations of cells entering different phases of the cell cycle at 48 h after virus infection. Transfection of eEF1A2 resulted in an increased number of cells entering the S phase, with a corresponding decrease in the number of cells in the G1 growth phase (Fig. 2D).

eEF1A2 promoted cell motility, invasion and adhesion

Increased cell motility, invasion and adhesion are key characteristics of tumour progression. To determine whether eEF1A2 plays a role in cell metastasis, we performed matrigel invasion, transwell migration and wound healing assays. Cell invasion was increased for eEF1A2-transfected cells when compared to parental or GFP-transfected cells (Fig. 3A). Using the transwell assay, we further observed that eEF1A2-transfected cells migrated faster than parental cells, and this difference particularly striking between 24 h and 48 h after transfection (Fig. 3B). Cell migration was also detected using the wound healing assay with similar results. Quantification of the track area revealed a 50% increase in cell motility for cells transfected with eEF1A2 as compared to control cells (Fig. 3C). These results show that eEF1A2 expression increased cell migration and invasion in pancreatic cancer cells in vitro.

Furthermore, to assess the levels of cell adhesion with various extracellular matrices, we performed an adhesion assay using an array of ECM proteins. Cells transfected with eEF1A2 showed high-

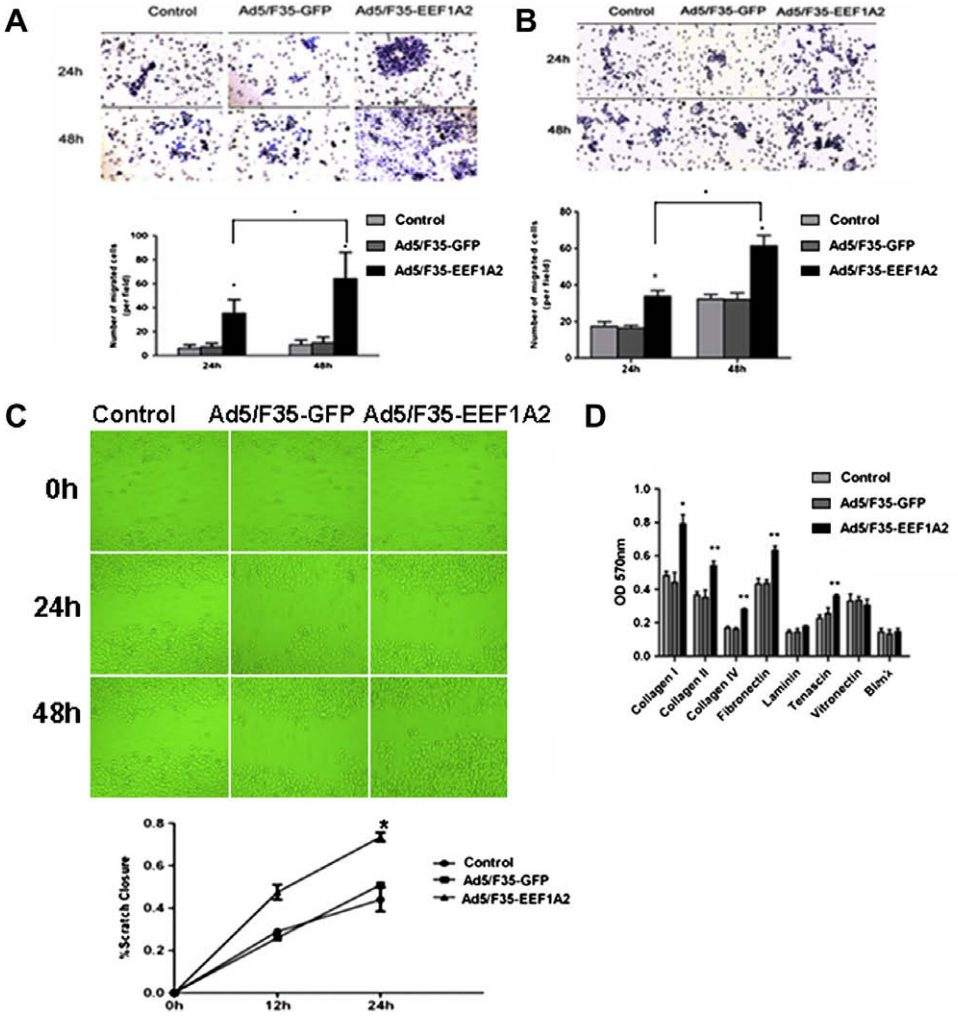

Fig. 3. Effect of eEF1A2 on cell migration, invasion and adhesion. (A) Invasive potential of cells across Matrigel-coated transwell chambers. (B) Cell motility assay was performed using transwell chambers. (C) In vitro wound healing assay. Representative photographs (×100) at 0, 12, and 24 h after wound initiation are shown. Percent wound closure (lower) was assessed by measuring the distance between the wound edges. (D) SW1990 cells with or without eEF1A2 transfection were assayed for adhesion to ECM-coated substrates. $^{*}p < 0.001$ $^{**}p < 0.05$.

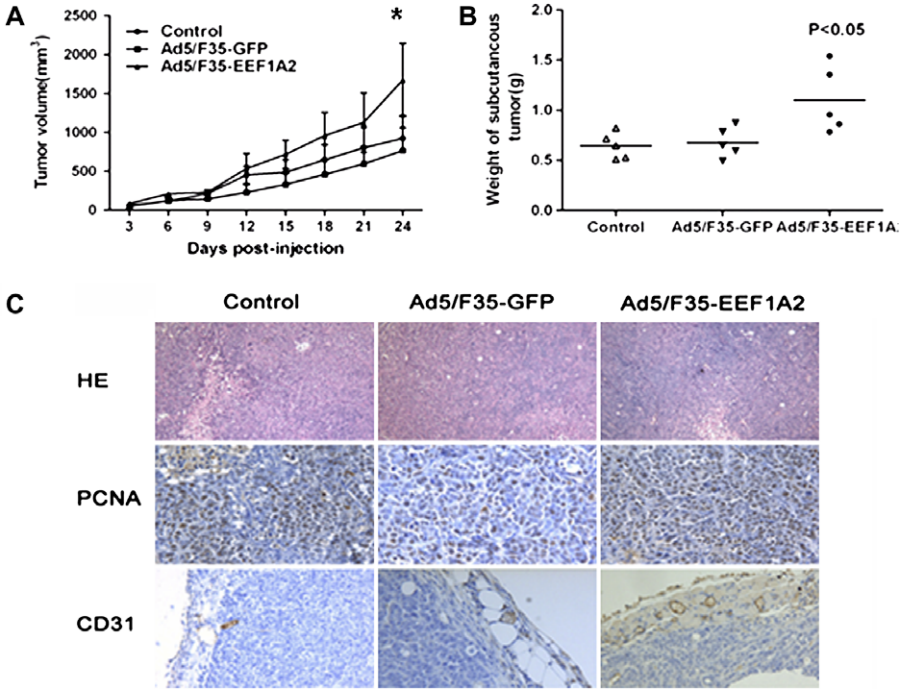

Fig. 4. The effect of eEF1A2 expression on tumour growth in vivo. (A) Tumour sizes every two days in each group ($n = 5$). (B) Weights of dissectable tumours in each group ($n = 5$). (C) Representative H&E staining and histological staining of anti-PCNA and anti-CD31 antibodies (×200) $^{*}p < 0.05$.

er adherence than parental cells in wells coated with collagen I (Fig. 3D). There was no significant difference in adherence between cells transfected with GFP and eEF1A2 on surfaces coated with laminin or vitronectin, whereas there were weak differences in cell adhesion for other collagens, fibronectin and tenascin (Fig. 3D). Overall, these data suggest that eEF1A2 expression partially enhanced cellular adhesion to extracellular matrices.

eEF1A2 promoted pancreatic cancer growth in a nude mouse model with subcutaneous xenografting

We further analyzed the role of eEF1A2 on tumour growth in vivo using an immunodeficient nude mouse model. There was a dramatic increase in tumour volume in Ad5/F35-EEF1A2 groups, as compared to the control groups (Fig. 4A). As shown in Fig. 4B, tumour weight was also significantly increased in Ad5/F35-EEF1A2 groups. These results suggest that overexpression of eEF1A2 promoted pancreatic cancer progression. Furthermore, immunohistochemical analysis of the harvested tumours suggested that eEF1A2 overexpression was associated with increased tumour cell proliferation (PCNA reactivity) and angiogenesis (micro-vessel density, MVD). As shown in Fig. 4C, the percentage of PCNA-positive cells in the Ad5/F35-EEF1A2 group was 87.9%, while the percentages of PCNA-positive cells in the Ad5/F35-GFP and PBS groups were 60.32% and 55.26%, respectively ($p < 0.05$). Immunostaining with the endothelial cell marker CD31 revealed a significant increase in vascularization in the Ad5/F35-EEF1A2 group (20.32 ± 5.21) as compared to the Ad5/F35-GFP (6.05 ± 1.25) or PBS (5.78 ± 1.81) groups ($p < 0.05$).

Discussion

eEF1A2 is a protein elongation factor that recruits amino-acylated tRNA to the ribosome during the elongation phase of translation [20]. In the present study, we examined the expression of eEF1A2 in pancreatic cancer tissues as well as in normal pancreas and chronic pancreatitis tissues. eEF1A2 was upregulated in a high proportion of pancreatic cancer tissues and pancreatic cancer cell lines, while there was little expression in normal pancreas or chronic pancreatitis tissues. Immunohistochemical staining showed cytoplasmic localization of eEF1A2 in human pancreatic cancer ductal epithelium cells. All the evidence we got from our experiments demonstrated that it is eEF1A2 instead of eEF1A1 that plays a important role in pancreatic cancer. There may be two possibilities to explain the difference between our results and Dr. Ohnami's [21]. First, the elevation of eEF1A2 and eEF1A1 maybe related to the specific subtype of pancreatic cancer. Second, the different ethnic background may cause the elevation of different isoform of eEF1A. However, the mechanisms of eEF1A2 overexpression require further investigation. Anand et al. and Grassi et al. have reported that overexpression of eEF1A2 does not completely depend on the genomic status of this locus [3,7], suggesting the existence of alternative mechanisms.

Other investigators have suggested at least two possible mechanisms: (1) eEF1A2 specifically up-regulates proteins that activate cell growth, or (2) that increased eEF1A2 expression causes an overall increase in protein translation resulting in increased cell proliferation. The effect of eEF1A2 on apoptosis remains controversial. It has previously been shown that eEF1A2 may protect against apoptosis or induce apoptosis upon serum deprivation [22–25]. The ability of eEF1A2 to shorten microtubules and bind to F-actin [4] has raised the possibility that eEF1A2-derived cytoskeletal alterations may facilitate tumourigenesis. eEF1A2 has also been reported to activate tyrosine phosphorylation [26] and phosphotidylinositol-4-kinase, [27] indicating a role in the control of phosphatidylinositol signalling, actin remodelling, cell motility and ubiquitination [28].

We previously established the clinical relevance of eEF1A2 in patients with pancreatic cancer. Our findings suggest that eEF1A2 promotes pancreatic tumorigenesis by enhancing cell growth, proliferation and motility in vitro and in vivo. This tumourigenic function of eEF1A2 was also shown in hepatocellular and ovarian tumours by Anand et al. and Grassi et al. [3,7]. These data are consistent

with the results of Amiri et al. [18], who reported that eEF1A2 expression made cells more motile and invasive in vitro. This finding in nude mice was further strengthened by the transformation of fibroblasts by ectopic expression of wild-type EEF1A2 and the tumourigenic growth of these cells when xenografted into mice [3]. In addition, our data also suggest that overexpression of eEF1A2 is associated with neo-angiogenesis. Angiogenesis has been proposed as a prognostic factor for tumours that require it for growth.

In summary, our results suggest that eEF1A2 protein is upregulated in pancreatic cancers, and that this overexpression could facilitate cell growth, invasion, and adhesion by increasing PCNA expression and promoting angiogenesis. eEF1A2 may, therefore, potentially serve as a tumour oncogene in the pathogenesis of pancreatic cancers.

Acknowledgments

We thank Professor Jianqing Ding for valuable comments on the manuscript. This research was supported in part by the Natural Sciences Foundation of China (30670941), and by Shanghai Natural Science Fund (04ZR14071).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.12.171.

References

[1] D. Li, K. Xie, R. Wolff, J.L. Abbruzzese, Pancreatic cancer, Lancet 363 (2004) 1049–1057.
[2] Y.H. Shaib, J.A. Davila, H.B. El-Serag, The epidemiology of pancreatic cancer in the United States: changes below the surface, Aliment Pharmacol. Ther. 24 (2006) 87–94.
[3] N. Anand, S. Murthy, G. Amann, M. Wernick, L.A. Porter, I.H. Cukier, C. Collins, J.W. Gray, J. Diebold, D.J. Demetrick, J.M. Lee, Protein elongation factor EEF1A2 is a putative oncogene in ovarian cancer, Nat. Genet. 31 (2002) 301–305.
[4] R. Li, H. Wang, B.N. Bekele, Z. Yin, N.P. Caraway, R.L. Caraway, R.L. Katz, S.A. Stass, F. Jiang, Identification of putative oncogenes in lung adenocarcinoma by a comprehensive functional genomic approach, Oncogene 25 (2005) 2628–2635.
[5] G. Kulkarni, D.A. Turbin, A. Amiri, S. Jeganathan, M.A. Andrade-Navarro, T.D. Wu, D.G. Huntsman, J.M. Lee, Expression of protein elongation factor eEF1A2 predicts favorable outcome in breast cancer, Breast Cancer Res. Treat. 102 (2007) 31–41.
[6] V.A. Tomlinson, H.J. Newbery, N.R. Wray, J. Jackson, A. Larionov, W.R. Miller, J.M. Dixon, C.M. Abbott, Translation elongation factor eEF1A2 is a potential oncoprotein that is overexpressed in two-thirds of breast tumours, BMC Cancer 5 (2005) 113.
[7] G. Grassi, B. Scaggiante, R. Farra, B. Dapas, F. Agostini, D. Baiz, N. Rosso, C. Tiribelli, The expression levels of the translational factors eEF1A 1/2 correlate with cell growth but not apoptosis in hepatocellular carcinoma cell lines with different differentiation grade, Biochimie 89 (2007) 1544–1552.
[8] S.M. Knudsen, J. Frydenberg, B.F. Clark, H. Leffers, Tissue-dependent variation in the expression of elongation factor-1 alpha isoforms: isolation and characterisation of a cDNA encoding a novel variant of human elongation-factor 1 alpha, Eur. J. Biochem. 215 (1993) 549–554.
[9] S. Lee, A.M. Francoeur, S. Liu, E. Wang, Tissue-specific expression in mammalian brain, heart, and muscle of S1, a member of the elongation factor-1 alpha gene family, J. Biol. Chem. 267 (1992) 24064–24068.
[10] D.M. Chambers, J. Peters, C.M. Abbott, The lethal mutation of the mouse wasted (wst) is a deletion that abolishes expression of a tissue-specific isoform of translation elongation factor 1alpha encoded by the Eef1a2 gene, Proc. Natl. Acad. Sci. 95 (1998) 4463–4468.
[11] G.J. Browne, C.G. Proud, Regulation of peptide-chain elongation in mammalian cells, Eur. J. Biochem. 269 (2002) 5360–5368.
[12] J. Condeelis, Elongation factor 1 alpha, translation and the cytoskeleton, Trends Biochem. Sci. 20 (1995) 169–170.
[13] M. Bektas, R. Nurten, Z. Gurel, Z. Sayers, E. Bermek, in: Interactions of eukaryotic elongation factor 2 with actin: a possible link between protein synthetic machinery and cytoskeleton, FEBS Lett. 356 (1994) 89–93.
[14] S.R. Gross, T.G. Kinzy, Translation elongation factor 1A is essential for regulation of the actin cytoskeleton and cell morphology, Nat. Struct. Mol. Biol. 12 (2005) 772–778.
[15] C.M. Chung, C. Man, Y. Jin, C. Jin, X.Y. Guan, Q. Wang, T.S. Wan, A.L. Cheung, S.W. Tsao, Amplification and overexpression of aurora kinase A(AURKA) in immortalized human ovarian epithelial(HOSE) cells, Mol. Carcinog. 43 (2005) 165–174.
[16] I. Shamovsky, M. Ivannikov, E.S. Kandel, D. Gershon, E. Nudler, RNA-mediated response to heat shock in mammalian cells, Nature 440 (2006) 556–560.
[17] S. Ejiri, Moonlighting functions of polypeptide elongation factor 1: from actin bundling to zinc finger protein R1-associated nuclear localization, Biosci. Biotechnol. Biochem. 66 (2002) 1–21.
[18] A. Amiri, F. Noei, S. Jeganatham, G. Kulkarni, D.E. Pinke, J.M. Lee, eEF1A2 activates Akt and stimulates Akt-dependent actin remodelling, invasion and migration, Oncogene 26 (2007) 3027–3040.
[19] N. Weidner, J.P. Semple, W.R. Welch, Tumour angiogenesis and metastasis – correlation in invasive breast carcinoma, N. Engl. J. Med. 324 (1991).
[20] J.W. Hershey, Translational control in mammalian cells, Annu. Rev. Biochem. 60 (1991) 717–755.
[21] S. Ohnami, N. Matsumoto, M. Nakano, et al., Identification of genes showing differential expression in antisense K-ras-transduced pancreatic cancer cells with suppressed tumorigenicity, Cancer Res. 59 (1999) 5565–5571.
[22] L.B. Ruest, R. Marcotte, E. Wang, Peptide elongation factor eEF1A-2/S1 expression in cultured differentiated myotubes and its protective effect against caspase-3-mediated apoptosis, J. Biol. Chem. 277 (2002) 5418–5425.
[23] S. Talapatra, J.D. Wagner, C.B. Thompson, Elongation factor-1 alpha is a selective regulator of growth factor withdrawal and ER stress-induced apoptosis, Cell Death Differ. 9 (2002) 856–861.
[24] R. Chang, E. Wang, Mouse translation elongation factor eEF1A-2 interacts with Prdx-I to protect cells against apoptotic death induced by oxidative stress, J. Cell Biochem. 100 (2007) 267–278.
[25] L.I. Slobin, The role of eucaryotic factor Tu in protein synthesis. The measurement of the elongation factor Tu content of rabbit reticulocytes and other mammalian cells by a sensitive radioimmunoassay, Eur. J. Biochem. 110 (1980) 555–563.
[26] G. Panasyuk, I. Nemazanyy, V. Felonenko, B. Negrutskii, A.V. Elskaya, A2 isoform of mammalian translation factor eEF1A displays increased tyrosine phosphorylation and ability to interact with different signalling molecules, Int. J. Biochem. Cell Biol. 40 (2008) 63–71.
[27] W. Yang, W. Burkhart, J. Cavallius, W.C. Merrick, W.F. Boss, Purification and characterization of a phosphatidylinositol 4-kinase activator in carrot cells, J. Biol. Chem. 268 (1993) 392–398.
[28] H. Gonen, C.E. Smith, N.R. Siegel, C. Kahana, W.C. Merrick, K. Chakraburtty, A.L. Schwartz, A. Ciechanover, Protein synthesis elongation factor EF-1 alpha is essential for ubiquitin-dependent degradation of certain N alpha-acetylated proteins and may be substituted for by the bacterial elongation factor EF-Tu, Proc. Natl. Acad. Sci. 91 (1994) 7648–7652.