Proto-oncogenic isoform A2 of eukaryotic translation elongation factor eEF1 is a target of miR-663 and miR-744

A Vislovukh, G Kratassiouk, E Porto, N Grailevska, C Beldiman, G Pinna, A El'skaya, A Harel-Bellan, B Negrutskii, and I Groisman

State Key Laboratory on Molecular and Cell biology, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kiev 03680, Ukraine; Universite´ Paris Sud, Laboratoire Epigenetique et Cancer, Formation de Recherche en Evolution, 3377 Gif-Sur-Yvette, France and Centre National de la Recherche Scientifique (CNRS), Gif-Sur-Yvette, France

Background: Eukaryotic translation elongation factor 1A2 (eEF1A2) is a known proto-oncogene. We proposed that stimulation of the eEF1A2 expression in cancer tissues is caused by the loss of miRNA-mediated control.

Methods: Impact of miRNAs on eEF1A2 at the mRNA and protein levels was examined by qPCR and western blot, respectively. Dual-luciferase assay was applied to examine the influence of miRNAs on 3'UTR of EEF1A2. To detect miRNA-binding sites, mutations into the 3'UTR of EEF1A2 mRNA were introduced by the overlap extension PCR.

Results: miR-663 and miR-744 inhibited the expression of luciferase gene attached to the 3'UTR of EEF1A2 up to 20% and 50%, respectively. In MCF7 cells, overexpression of miR-663 and miR-744 reduced the EEF1A2 mRNA level by 30% and 50%. Analogous effects were also observed at the eEF1A2 protein level. In resveratrol-treated MCF7 cells the upregulation of mir-663 and mir-744 was accompanied by downregulation of EEF1A2 mRNA. Both miRNAs were able to inhibit the proliferation of MCF7 cells.

Conclusion: miR-663 and miR-744 mediate inhibition of the proto-oncogene eEF1A2 expression that results in retardation of the MCF7 cancer cells proliferation. Antitumour effect of resveratrol may include stimulation of the miR-663 and miR-744 expression.
suggested the possibility of the regulation occurring at the post-transcriptional level. We hypothesised microRNA involvement in the regulation of A1/A2 expression.

MicroRNAs are widely involved in the post-transcriptional regulation of gene expression in both animals and plants (Lagos-Quintana et al., 2001; Reinhart et al., 2002). In mammals, miRNAs are considered to control ~60% of the protein coding genes (Friedman et al., 2009), participating in the regulation of almost every cellular process, including cancerogenesis (Ambros, 2004). In this paper, we describe the involvement of microRNAs in the regulation of A2, the proto-oncogenic isoform of eEF1A. Our bioinformatic search highlighted several miRNA-binding sites in the 3′-UTR of EEF1A2 mRNA, particularly miR-663 and miR-744.

The existence of miR-744 was initially predicted by Berezikov et al. (2006) and later confirmed by Landgraf et al. (2007). However, the literature data about the function of this miRNA appeared only in the last few years. It was shown that miR-744 directly targets transforming growth factor beta-1 (TGF-β1) (Martin et al., 2011). MiR-744 is regarded as a potential oncomarker, as it was found to be stably present in mouse serum (Mi et al., 2012) and was shown to be significantly upregulated in the serum of patients with gastric cancer (Song et al., 2012).

Much more is known about miR-663, which is shown to be involved in cancer progression and to have tumour-suppressor properties. Thus, the miR-663 induces mitotic catastrophe growth arrest in human gastric cancer cells (Pan et al., 2010). Also, miR-663 stimulates differentiation of the acute myeloid leukaemia cell line HL-60, and has been proposed to be potentially useable for the treatment of acute myeloid leukaemia (Bazel, Switzerland). Cells were incubated on a rotating wheel at +4 °C for 20 min. Lysates were centrifuged and supernatant was stored at −80 °C. Proteins were separated on a NuPAGE Novex 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, USA) and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA) in NuPAGE Transfer Buffer (Invitrogen). Membranes were developed using Super Signal West Dura Extended Duration Substrate (Pierce, Rockford, IL, USA).

**Mutagenesis.** Site-specific mutagenesis of miRNA-binding sites was performed by Overlap Extension PCR (Higuchi et al., 1988). In each case, a restriction site was inserted instead of a miRNA-binding site. Primers used for PCR were: universal forward primer 5′-GCTTAGACC GGCGGACGACCTTCGACAGCCGCGGACCGCCGCGCCC-3′ universal reverse primer 5′-GCTCTAGAGAGCGTGGCGAGCGCTGGGC-3′; E663 forward 5′-GGGCGGCGGACGAAATATACCCGGGCCCGGG-3′, E663 reverse 5′-GGCGGGGCGGGGTATTTTATTATTTGCGGCGG-3′; H663 forward 5′-CGCCCCGCAACAAGGTAGCCGGATGT-3′, H663 reverse 5′-ACATGGCCCGGAAGCTTGGTGGGAGCGACCTTGG-3′; K663 forward 5′-GGGAGGGCGGAAGCTTGGTGGGAGCGACCTTGG-3′, K663 reverse 5′-GGCGGGCGGAAGCTTGGTGGGAGCGACCTTGG-3′. Proto-oncogene eEF1A2 is targeted by miR-744 and miR-663 miRNAs. This suggests that the downregulation of the putative oncogene eEF1A2 via microRNA-mediated pathways may be one of the mechanisms responsible for the inhibitory effect of resveratrol on the breast cancer progression.

**MATERIALS AND METHODS**

**Cell lines, plasmids, antibodies and resveratrol treatment.** T47D, LNCaP and DU145 cells were cultured in RPMI 1640 (Sigma, St Louis, MO, USA), while A549, HEK293 and MCF7 cells were cultured in DMEM (Sigma) growth medium. Both media contained 10% FBS (Sigma) and 1% penicillin/streptomycin (Sigma). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

The 3′-UTR of the EEF1A2 mRNA was cloned from human leukocyte DNA and inserted into the pSICHECK-2 reporter vector (Promega, Madison, WI, USA). A pCDNA3.1 vector expressing the eEF1A2 ORF was a kind gift of Charlotte R Knudsen (Department of Molecular Biology, University of Aarhus, Gustav Wieds vej 10 C, 8000 Århus C, Denmark).

Antibeta-actin antibodies were from Santa Cruz (Santa Cruz Biotechnologies, Santa Cruz, CA, USA). In-house anti-eEF1A2 polyclonal antibodies were produced as described (Kolesanova et al., 2013).

For resveratrol treatment, 100 μM resveratrol (in DMSO) (Sigma) was added to the MCF7 cells. After 24 h of incubation, cells were collected, and total RNA was extracted using TRI reagent (Sigma).

**Western blot analyses.** Total cell lysates were prepared in RIPA lysis buffer supplemented with a protease inhibitor cocktail (Roche, Basel, Switzerland). Cells were incubated on a rotating wheel at +4°C for 20 min. Lysates were centrifuged and supernatant was stored at −80°C. Proteins were separated on an 8% SDS-PAGE gel (Sigma). Cells were incubated on a rotating wheel at +4°C for 20 min. Lysates were centrifuged and supernatant was stored at −80°C. Proteins were separated on a NuPAGE Novex 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, USA) and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA) in NuPAGE Transfer Buffer (Invitrogen). Membranes were developed using Super Signal West Dura Extended Duration Substrate (Pierce, Rockford, IL, USA).

**Quantitative PCR.** Total RNA was isolated using TRI reagent (Sigma). One microgram of RNA was used for cDNA synthesis with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Each reaction was performed in a mix of 20 μl reaction mixture containing 1 μl cDNA, 2 × SYBR Green PCR Master Mix (Applied Biosystems) and 0.3 μM of each forward and reverse primer. For miRNA quantification, total RNA was transcribed with a TaqMan Reverse transcription kit (Applied Biosystems), and then amplified using TaqMan Universal PCR Master Mix II. The primers for eEF1A2 qPCR were described earlier (Bosutti et al., 2007). Primers for beta-actin were: forward primer 5′-GGCCGAAATGCTGCGGTCACATT-3′, reverse primer 5′-GATGGAGTTGAAGGGTCTTGGG-3′. MicroRNA primers were purchased from Applied Biosystems. Quantitative PCR was quantified with an ABI PRISM 7500 real-time PCR system.

www.bjcancer.com | DOI:10.1038/bjc.2013.243
**RESULTS**

Identification of miRNAs that potentially target the eEF1A2 mRNA. Several algorithms that take into account conservation of the seed site, distribution through the 3'-UTR, and antisense RNA-binding site accessibility, were applied to identify miRNA that could potentially target the eEF1A2 mRNA. We used TargetScan 5.2, PITA, miRanda and microT v.3.0 programs to find miRNA-seeding sites in the 3'-UTR of eEF1A2 mRNA (Lewis et al, 2005; Kertesz et al, 2007; Betel et al, 2008; Maragkakis et al, 2009). Different algorithms returned different sets of potential miRNA seed sites. However, the most common and the highest scoring microRNAs were: miR-661, miR-663, miR-675 and miR-744 (Figure 1A).

To verify which of predicted miRNAs can regulate eEF1A2 expression, a dual-luciferase assay based on the firefly luciferase reporter gene attached to the eEF1A2 3'-UTR was applied. Reporter DNA construct was co-transfected with miRNA precursor to the HeLa cells. As shown in Figure 1B, only miR-663 and miR-744 were able to significantly reduce the reporter activity. The effect of co-transfection with miR-663 and miR-744 was similar to the effect of miR-744 alone in luciferase test, suggesting the absence of cooperative action of these miRNAs on eEF1A2 mRNA.

Determination of actual miRNA-binding sites in the 3'-UTR of eEF1A2 mRNA. TargetScan 5.2 predicted multiple sites for both miR-663 and miR-744 in the 3'-UTR of eEF1A2 mRNA (Figure 2A). Consequently, to distinguish between true and false positive sites, we mutated predicted target sites by replacing them with the HindIII, EcoRV or KpnI restriction enzyme sites. As shown in Figure 2B, mutations in the region that contains three miR-663 and two overlapping miR-744 sites (E663) did not influence the reporter gene expression. However, a mutation of each of the two downstream miR-663-binding sites (H663 and K663) resulted in the upregulation of luciferase expression. An increase in the reporter gene expression was also observed when a mutation was introduced in the miR-744-binding site (H744). The results suggest that the eEF1A2 mRNA has two binding sites for miR-663, one of which was predicted as being conserved, and one binding site for miR-744.

miR-663 and miR-744 miRNAs decrease eEF1A2 expression at both mRNA and protein levels. In order to choose a model cell line for our study, we evaluated the expression levels of EEF1A2 mRNA in different cell lines where it had been detected by earlier investigations (Tomlinson et al, 2005). Among those tested, the MCF7 breast cancer epithelial cell line was found to express the greatest amount of eEF1A2 (Figure 3A). Importantly, miR-663 and miR-744 expression was also detected in the MCF7 cell line (Figure 3B).
Transfections of MCF7 cells with miR-663 and miR-744 downregulated EEF1A2 mRNA levels by 30% and 50%, respectively (Figure 4A). This treatment also led to significant decreases in endogenous eEF1A2 protein level (Figure 4B). Analogous effects were observed when the cells were transfected with siRNA against EEF1A2 mRNA (Figures 4A and C).

Effect of miRNA-mediated eEF1A2 downregulation on MCF7 cell proliferation and migration. Previous data showed the possibility of the eEF1A2 input to the cell migration and proliferation processes (Cao et al., 2009; Li et al., 2010). Therefore, it is important to elucidate whether miRNA-dependent downregulation of eEF1A2 may have an impact on these parameters in cancer cells.

Indeed, overexpression of mir-663 and mir-744 downregulated the proliferation of MCF7 cells (Figure 5A). Importantly, treatment of MCF7 cells with the anti-eEF1A2 siRNA resulted in a similar effect on the MCF7 cell proliferation (Figure 5A). To prove that the observed effect of microRNAs on cell proliferation is mediated by eEF1A2, we overexpressed the eEF1A2 ORF before the miRNA transfection. The overexpression of eEF1A2 abolished the antiproliferative effect of the miRNAs (Figure 5B). These data suggest that the EEF1A2 mRNA is one of the targets of miR-663 and miR-744 responsible for the effect on MCF7 cell proliferation. The same trend of proliferation inhibition was observed for lung cancer A549 cells transfected with miRNAs 663, 744 and siRNA against eEF1A2 (Supplementary Figure 1A). Importantly, contribution of cell death into the inhibitory effects observed was rather small (Supplementary Figures 1B and C).

The effect of miRNA-dependent eEF1A2 downregulation on cell migration was examined in a wound-healing assay. Interestingly, despite the intense inhibition of cell migration by the miR-663 and miR-744 treatment, we did not observe any changes in cell migration when cells were treated with siRNA against eEF1A2 (Supplementary Figure 2). Perhaps, eEF1A2 is not involved in the cell migration process, in which case the effects of miR-663 and miR-744...
contrary to the irrelative miRNA inhibitors. Thus, resveratrol miR-663 and miR-744 upregulated the EEF1A2 mRNA level, EEF1A2 mRNA level (Figure 6C). As expected, the inhibitors of Under those conditions, the resveratrol had no effect on the miR-744 and miR-663 inhibitors before the resveratrol treatment. specificity of the effect, MCF7 cells were transfected with the specific, low toxicity therapy for each specific case (De Palma and Hanahan, 2012). Here, we describe a mechanism of eEF1A2 regulation by two miRNA, miR-663 and miR-744, the loss of which can result in the development of cancer. miR-663 and, miR-744 are well-known oncosuppressor miRNAs. MicroRNA-744 amount was found to be significantly upregulated in the serum of patients with gastric cancer (Song et al, 2012). There are only two confirmed targets of this miRNA: the oncogene TGF-β, negative regulation, (Martin et al, 2011) and cyclin B1, positive regulation (Huang et al, 2012). In contrast, miR-663 was found to participate in a large number of cellular processes, including tumour genesis. Peculiarly, miR-663 is involved in mitotic catastrophe growth arrest in human gastric cancer cells (Pan et al, 2010), the same type of cancer in which miR-744 is involved. Recently, it has been proposed that miR-744 could be an appropriate target for anticancer treatments of the haematological malignancies (Jian et al, 2012). Interestingly, in lung cancer, mir-663 was discovered to negatively influence the TGF-β1, P53, Bax and Fas expression (Liu et al, 2011). Furthermore, the miR-663 gene was found to be downregulated via methylation in the samples of human hepatocellular carcinoma and breast cancer, as well as in the K-562 leukaemia cell line (Lehmann et al, 2008; Potapova et al, 2011; Yang et al, 2012). There is also one reported example of the opposite effect, where miR-663 stimulated the proliferation and tumorigenesis of nasopharyngeal carcinoma (Yi et al, 2012). However, the list of direct targets of microRNAs miR-633 and miR-744 is far from complete. The best known target of both miRNAs that could be related to their oncosuppressive properties
is TGF-β1 (Liu et al., 2011; Martin et al., 2011). Here, we show that microRNAs miR-663 and miR-744 have a negative impact also on eEF1A2, which is the key component of the translation elongation apparatus. This protein reveals oncogenic properties in some cancer tissues (Anand et al., 2002; Tomlinson et al., 2005). The overexpression of eEF1A2 has been shown to induce the filopodia formation and cell invasion/migration, potentially through cytoskeleton remodelling, in an Akt-and PI3K-dependent manner (Amiri et al., 2007; Jeganathan and Lee, 2007; Li et al., 2010). Eukaryotic translation elongation factor 1A2 possesses antiapoptotic properties, possibly due to the direct interaction with Prdx1 (Chang and Wang, 2007). In addition, the ectopic expression of eEF1A2 altered the three-dimensional morphogenesis of MCF10A cells by influencing the phosphatidylinositol localisation (Pinke and Lee, 2011).

Importantly, the correlation between the low level of eEF1A2 (data not shown) and high level of mir-744 expression (Landgraf et al., 2007) was observed in MCF10 breast, A549 lung and Jurkat leukaemia cells. On the opposite, in the heart tissue where the expression of eEF1A2 is quite high (Lee et al., 1992), the expression of mir-744 was absent (Landgraf et al., 2007). Unfortunately, it is not possible to track the same correlation for miR-663 as no data about its expression are available in MirZ database (Hausser et al., 2009). We postulate that the abnormal occurrence of the eEF1A2 isoform in cancer tissue that is not usually found in normal one is caused, at least partially, by the loss of a microRNA-mediated silencing mechanism. The data on epigenetic inactivation of miR-663 in breast cancer are consistent with this hypothesis (Lehmann et al., 2008).

Our data show that resveratrol may control eEF1A2 expression post-transcriptionally. Resveratrol is a natural phytoalexin, and is found in significant quantities in grapes and, consequently, in red wine. Resveratrol is found to potentially prevent pathologies such as obesity and type 2 diabetes, and possesses cardioprotective and oncosuppressive properties (Jang et al., 1997; Vang et al., 2011). Many authors explain 'French Paradox' by regular intake of red wine that is rich in resveratrol and other polyphenols (Ferrieres, 2004; Lippi et al., 2010; Hengst and Yun, 2012). The first evidence of the oncosuppressive action of resveratrol was reported about 15 years ago (Jang et al., 1997). Since then, numerous studies have shown the ability of resveratrol to suppress the development of different human cancers (Vang et al., 2011). However, the mechanism of resveratrol's antitumour activity is not yet revealed. It is known that miR-663 is upregulated during resveratrol treatment, directly targeting the known proto-oncogenes JunB and JunD, as well as TGF-β1 (Tili et al., 2010a,b); see Tili and Michaille (2011) for review.

Figure 6. Effect of resveratrol on EEF1A2 mRNA and mir-663/744 expression level. MCF7 cells were treated with 100 μM of resveratrol. After 24 h of incubation, levels of microRNAs (A) and eEF1A2 (B) were assayed by qPCR. Treatment of MCF7 cells with DMSO was used as control. (C) Before the resveratrol treatment, MCF7 cells were transfected with mir-663 and mir-744 inhibitors. Transfection with irrelevant (irr.) anti-mir, was used as control. Error bars represent s.d. values. *P<0.05 vs control. n = 3. Student’s t-test.
Here, we have shown for the first time that resveratrol elevates, by at least two-fold, the expression level of miR-744. Recently, resveratrol has been shown to upregulate also miR-663 (Tili et al, 2010a). Thus, the resveratrol-induced upraise in miR-663 and miR-744 can control the proliferation of cancer cells via the inhibition of proto-oncogene eEF1A2.

In conclusion, it is yet unknown which mechanism determines exclusive tissue-specific expression of the eEF1A2 isoform in mammalian organisms. We believe miR-744 and/or miR-663 may be among the factors contributing to this issue.

ACKNOWLEDGEMENTS

We are grateful to Linda Pritchard for critical reading of the manuscript. AV is thankful to FEBS and French Embassy in the Ukraine for short visiting fellowships. Travel support for BN was provided by the PICS and GDRI programs.

REFERENCES

Ambros V (2004) The functions of animal microRNAs. Nature 431(7006): 350–355.
Amiri A, Noei F, Jeganathan S, Kulkarni G, Pinke DE, Lee JM (2007) eEF1A2 is a putative oncogene in ovarian cancer. J Biol Chem 282(1): 267–278.
Berezikov E, van Tetering G, Verheul M, van de Belt J, van Laake L, Vos J, (Tili E) (2012) Upregulation of cyclin B1 by miRNA and its implications in cancer. Nucleic Acids Res 40(4): 1695–1707.
Bosuttì A, Sciagianti B, Grassi G, Guarnieri G, Biolo G (2007) Overexpression of the elongation factor 1A1 relates to muscle proteinosis and proapoptotic p66ShcA gene transcription in hypercatabolic trauma patients. Metabolism 56(12): 1629–1634.
Cao H, Zhu Q, Huang J, Li B, Zhang S, Yao W, Zhang Y (2009) Regulation of eEF1A2 by miR-663 and miR-744. J Exp Pathol 214(2): 267–278.
Chang R, Wang E (2007) Mouse translation elongation factor eEF1A-2 interacts with Prdx-I to protect cells against apoptotic death induced by oxidative stress. J Cell Biochem 100(2): 87–93.
Chang R, Wang E (2007) Mouse translation elongation factor eEF1A-2 interacts with Prdx-I to protect cells against apoptotic death induced by oxidative stress. J Cell Biochem 100(2): 87–93.

We are grateful to Linda Pritchard for critical reading of the manuscript. AV is thankful to FEBS and French Embassy in the Ukraine for short visiting fellowships. Travel support for BN was provided by the PICS and GDRI programs.
Pan J, Hu H, Zhou Z, Sun L, Peng L, Yu L, Liu J, Yang Z, Ran Y (2010) Tumor-suppressive mir-663 gene induces mitotic catastrophe growth arrest in human gastric cancer cells. Oncol Rep 24(1): 105–112.

Pan J, Ruest LB, Xu S, Wang E (2004) Immuno-characterization of the switch of peptide elongation factors eEF1A-1/EF-1alpha and eEF1A-2/S1 in the central nervous system during mouse development. Brain Res Develop Brain Res 149(1): 1–8.

Pinke DE, Kalloger SE, Francetic T, Huntsman DG, Lee JM (2008) The prognostic significance of elongation factor eEF1A2 in ovarian cancer. Gynecol Oncol 108(3): 561–568.

Pinke DE, Lee JM (2011) The lipid kinase PI4KIIIbeta and the eEF1A2 oncogene co-operate to disrupt three-dimensional in vitro acinar morphogenesis. Exp Cell Res 317(17): 2503–2511.

Potapova A, Albat C, Hasemeier B, Haeussler K, Lamprecht S, Suerbaum S, Kreipe H, Lehmann U (2011) Systematic cross-validation of 454 sequencing and pyrosequencing for the exact quantification of DNA methylation patterns with single CpG resolution. BMC Biotechnol 11: 6.

Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. Genes & Develop 16(13): 1616–1626.

Song MY, Pan KF, Su HJ, Zhang L, Ma JL, Li JY, Yuasa Y, Kang D, Kim YS, You WC (2012) Identification of serum microRNAs as novel non-invasive biomarkers for early detection of gastric cancer. PLoS One 7(3): e33608.

Tili E, Michaille JJ (2011) Resveratrol, MicroRNAs, Inflammation, and Cancer. J Nucleic Acids 2011: 102431.

Tili E, Michaille JJ, Adair B, Alder H, Limagne E, Taccioli C, Ferracin M, Delmas D, Latruffe N, Croce CM (2010a) Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD. Carcinogenesis 31(9): 1561–1566.

Tili E, Michaille JJ, Alder H, Volinia S, Delmas D, Latruffe N, Croce CM (2010b) Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFbeta signaling pathway in SW480 cells. Biochem Pharmacol 80(12): 2057–2065.

Tomlinson VA, Newbery HJ, Bergmann JH, Boyd J, Scott D, Wray NR, Sellar GC, Gabra H, Graham A, Williams AR, Abbott CM (2007) Expression of eEF1A2 is associated with clear cell histology in ovarian carcinomas: overexpression of the gene is not dependent on modifications at the EEF1A2 locus. British J Cancer 96(10): 1613–1620.

Tomlinson VA, Newbery HJ, Wray NR, Jackson J, Larionov A, Miller WR, Dixon JM, Abbott CM (2005) Translation elongation factor eEF1A2 is a potential oncoprotein that is overexpressed in two-thirds of breast tumours. BMC Cancer 5: 113.

Vang O, Ahmad N, Baile CA, Baur JA, Brown K, Ciszar A, Das DK, Delmas D, Gottfried C, Lin HY, Ma QY, Mukhopadhyay P, Nalini N, Pezzuto JM, Richard T, Shukla Y, Suriy Y, Szekeres T, Szkudelski T, Walle T, Wu JM (2011) What is new for an old molecule? systematic review and recommendations on the use of resveratrol. PLoS One 6(6): e19881.

Yang Y, Wang LL, Li YH, Gao XN, Liu Y, Yu L (2012) Effect of CpG island methylation on microRNA expression in the k-562 cell line. Biochem Genet 50(1–2): 122–134.

Yi C, Wang Q, Wang L, Huang Y, Li L, Liu L, Zhou X, Xie G, Kang T, Wang H, Zeng M, Ma J, Zeng Y, Yun JP (2012) MiR-663, a microRNA targeting p21(WAF1/CIP1), promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. Oncogene 31(41): 4421–4433.

Zhao S, Fernald RD (2005) Comprehensive algorithm for quantitative real-time polymerase chain reaction. J Comput Biol 12(8): 1047–1064.

This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License.

Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)