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Detection of Target Genes of FOXA Transcription Factors Involved in Proliferation Control

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Abstract—To reveal the mechanism of tumor-suppressing activity of FOXA proteins in liver, a search for potential target genes of these transcription factors involved in proliferation control was carried out. In the first step, we have used data from the literature concerning gene expression in mouse liver (high content of FOXA proteins) and kidney (FOXA expression is absent) obtained by hybridization on microchips. A search for FOXA binding sites in regulatory regions of forty differentially expressing genes involved in proliferation control was carried out using the computer method SITECON. Eleven genes containing clusters of potential FOXA sites incorporating 3-6-fold repeats of TTTG were revealed. The FOXA-specific interaction with such microsatellite sites was confirmed by gel-retardation technique using the GST-fused protein containing the DNA-binding domain of FOXA2. Six genes containing clusters of confirmed binding sites—*Cul2*, *Cdc73*, *Ptk*, *Pdcd*, *Creb*, and *Ppp2r5d*—were selected. The effect of hepatocarcinogen orthoaminoazotoluene (OAT), which lowers the FOXA activity, on expression of these genes was studied by the real-time PCR. OAT was shown to increase sharply the level of mRNA of the *Cul2* and *Cdc73* genes.

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The development and normal vital activity of a multicellular organism requires strictly controlled changes in the balance of differentiation, proliferation, and apoptosis processes in its organs and tissues. In this connection, study of the involvement in proliferation and/or apoptosis control of transcription factors responsible for differentiation and functioning of certain cell types is of particular interest. The best studied in this respect is C/EBPα, which plays an important role in differentiation of granulocytes, monocytes, hepatocytes, and adipocytes, as well as in providing for normal functioning of lung, liver, and mammary cells [1, 2]. C/EBPα mutations or a decrease in its expression were shown to contribute significantly to malignant transformation of these cells [3-6]. In contrast, artificial enhancement of C/EBPα expression results in cell phenotype normalization [7]. Studying the mechanism of the C/EBPα antiproliferative effect has shown that this transcription factor stimulates expression of a gene encoding one of main regulators of the cell cycle, p21 inhibitor of cyclin-dependent kinases, which results in cell division arrest [8]. C/EBPα also appeared to be able to form a triple complex with Rb and E2F4, blocking expression of E2F target genes, which also results in arrest of proliferative processes [9]. Another example of a tissue-specific transcription factor with pronounced antiproliferative properties is MyoD, which maintains differentiated state of muscle cells and regulates myoblast division [10, 11]. Stimulation of MyoD expression in a number of normal and transformed cell lines results in arrest of proliferation of these cells [12]. Among MyoD target genes is $p57^{kip2}$, which is also an inhibitor of cyclin-dependent kinases [13].

One of the C/EBPα target genes in lung tumor cells is a gene encoding FOXA2 transcription factor (formerly known as HNF3β). Stimulation of FOXA2 expression in these cells, like stimulation of C/EBPα expression, resulted in significant growth inhibition, apoptosis, and the loss of clone-forming activity, which suggested that FOXA2 is a tumor suppressor in lung tissue [14]. Our data suggest a similar role of the FOXA family transcription factors in liver. We have shown that activity of these factors, necessary for differentiation of hepatocytes [15], decreases in response to hepatocarcinogens only rather

Abbreviations: GST) glutathione-S-transferase; IPTG) isopropyl-β-D-thiogalactopyranoside; OAT) orthoaminoazotoluene; TTR) transthyretin.

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than to their non-carcinogenic analogs, and is observed only in animal species (or lines), in which this compound produces tumors in liver [16, 17]. This proposal is also supported by data on the FOXA3 (formerly known as HNF3γ) involvement in inhibition of hepatocyte proliferation [18].

To elucidate the mechanism of tumor-suppressing activity of FOXA proteins in liver, potential target genes of these transcription factors were sought whose products may be involved in proliferation and/or apoptosis control in this organ.

MATERIALS AND METHODS

Reagents. [α-^{32}P]dATP was from Amersham (Great Britain); X-ray film from Retina (Germany); oligonucleotides from Biosset (Russia); Klenow fragment of *E. coli* DNA polymerase I from SibEnzim (Russia); DEAE-81 paper from Whatman (Great Britain); Tris, glutathione, glutathione-agarose, HEPES, spermine, spermidine, bromophenol blue, and isopropyl-β-D-thiogalactopyranoside (IPTG) from Sigma (USA); acrylamide, bis-acrylamide, and SDS from BioRad (USA); EGTA and EDTA from Fluka (USA); sucrose and glycerol from Serva (Germany). Other reagents were produced in Russia and were of special purity or chemically pure grades.

Oligonucleotides. Double-stranded oligonucleotides correspond to predicted FOXA binding sites (CDC40 [–4168] 5′-cagtTTCTTCACTCCTTTTGTTTGTGGCACACAG-3′, CDC73 [+1302] 5′-cagtAAATTTTTTTTTGTTTGTTTGTTTTTTTCA-3′, CFB [–3581] 5′-cagtTGTAGTTTTTGTTTGTTTGTTTGTTTTTTG-3′, ULK2 [–1970] 5′-cagtGTGTTTGTTTGTTTGTTTGTTTGTTTGGTA-3′) and to the known FOXA-site from mouse transthyretin gene promoter (TTR [–111] 5′-cagtCGAGTTGACTAAGTCAATAATCAGAATCAGTCG-3′) [19]; the second strand is not shown, the lowercase letters indicate nucleotides forming after annealing protruding ends that were completed using Klenow fragment of DNA polymerase I in the presence of [α-^{32}P]dATP to obtain labeled probes; beside the gene name (in parentheses) there is the site position relative to the transcription site.

Animals. Twelve-day-old male mice of ICR line bred in the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, were used in this work.

Production of recombinant protein. To obtain the glutathione-S-transferase (GST)-fused protein, PCR on mouse genomic DNA was used to produce a fragment corresponding to the DNA-binding domain of FOXA2 protein (primers 5′-GCGGAATTCCGCTCGGGACCCCAGACGTA and 5′-GCGCTCGAGTCCCCGAGCTGAACCTGA) and containing at its ends sites for restriction endonucleases (*Xho*I, *EcoR*I), and then the fragment was cloned into plasmid pGex-4T-3 (Amersham). Primary structure of the insert and the presence of an unbroken reading frame were confirmed using an ABI 3100 automatic sequencer (Applied Biosystem, Germany). Strain BL21 of *Escherichia coli* was used for expression of the GST-fused protein. Transformed cells were grown to optical density A_{600} = 0.3-0.4 at 37°C, IPTG was added to 0.1 mM, then the cells were grown for an additional 3 h at 30°C. Then cells were sedimented by centrifugation for 10 min at 1000*g*, suspended in buffer I (25 mM Hepes, pH 7.8, 150 mM KCl, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM dithiothreitol (DTT), 10% glycerol), and sonicated (22 kHz, 70 W) for 1 min. The cell debris was sedimented by centrifugation at 11,000*g* for 15 min. The supernatant was passed slowly through 20 μl glutathione-agarose equilibrated in buffer I, then the glutathione-agarose was washed with 200 μl buffer I. The protein was eluted with 60 μl of buffer I containing 10 mM reduced glutathione, and the eluate was divided into aliquots and frozen at –70°C.

Preparation of extracts of liver cell nuclei and the gel retardation technique. Nuclear extracts were obtained as described in [16]. All procedures were carried out on ice as quickly as possible. To study the effect of carcinogens, orthoaminoazotoluene (OAT) was dissolved in olive oil and injected intraperitoneally in a dose of 22.5 mg per 100 g of body weight 4 h prior to sacrifice. Control animals obtained only the solvent.

Reactive mixture (8 μl) contained 1 ng unlabeled oligonucleotide and 4 μg protein of nuclear extract or recombinant protein, preliminarily incubated with sonicated DNA of salmon sperm (1 μg per 5 μg protein) for 10 min at 0°C in buffer I. In the case of competitive analysis, the reactive mixture additionally contained 1 or 10 ng of appropriate unlabeled oligonucleotide. After incubation at 4°C for 15 min, the mixture was analyzed by electrophoresis in 5% polyacrylamide gel in 0.5-fold TBE buffer (89 mM Tris-borate, 89 mM H_3BO_3, 2 mM EDTA). The gel was dried, and the binding pattern was visualized either on a phosphoimager (Molecular Imager FX Pro Plus; BioRad) or on an X-ray film.

PCR in real time. Four hours before sacrifice animals obtained an injection of OAT as described above. RNA was isolated using the RNeasy Mini kit (Qiagen, USA) according to the manufacturer's protocol, and cDNA was obtained from 1-3 μg of total RNA by reverse transcription using 40 ng of primer $d(T)_{18}$ and 2000 activity units of RNA-dependent DNA polymerase MoMLV in 30 μl of buffer containing 20 mM Tris-HCl, pH 8.3, 2 mM $MgCl_2$, 5 mM DTT, 100 mM KCl, 100 μM of each of deoxyribonucleoside triphosphates, and 15-20 activity units of RNasin. The reaction was carried out for 1 h at 42°C and stopped by addition of 2 μl 50 mM EDTA. The real-time PCR was carried out using TaqMan Universal PCR master mix (Applied Biosystem) and TaqMan

Primers and TaqMan probes used for the real-time PCR

Gene	Primers	TaqMan probe
Cdc731	5'-catctgctcgtaagactcaaactc 5'-cgtgacccttttcttctgatttgg	5'-tgcagcccagcctgtacctagacc
Creb3l31	5'-gataccctgtacccggaggag 5'-gccagcagcttcttctcatcc	5'-tcgccctcccgcttcaacctcact
Ptk2b1	5'-ccagcactccgatgacatcc 5'-tgacccgtctacccatcaaatg	5'-actggtcgctctgaggccctcgtc
Ppp2r5d1	5'-catctcctctctctccttcattcg 5'-agccactggaacaagacaatcc	5'-acctgccaccgctgactccaactg
Cul21	5'-gttggcatatgttcctgagtaagg 5'-ccacagcatcattcagcaagag	5'-acctgcccttctgcttctccgcct
Pdcd81	5'-tctaccttccttccatctttcagc 5'-ttcctcaatacctcagcaactgg	5'-ccgagtccatcaacttccgccgct

probes and primers (Medigen, Russia) (table) in accordance with the recommended protocol. The "housekeeping" genes *GAPDH* and *18S* rRNA (Applied Biosystems kits) were used as an endogenous control. Amplification was carried out on an ABI 7300 amplifier (Applied Biosystems). The results of the real-time PCR were calculated using the ABI Prism7000sds1.2.3 program (Applied Biosystems) in automatic regime.

Elaboration of a method for FOXA-site recognition. The training sample was based on the published data for nineteen FOXA binding sites detected by footprinting with DNase I. The method for recognition of potential FOXA binding sites was based on the SITECON algorithm using analysis of a set of conserved context-dependent conformational and physicochemical properties determined for short regions of the DNA sequence alignment [19]. The algorithm puts forward the level of conformational similarity as a measure of the analyzed sequence likeness to the training sample. To evaluate the underprediction error, the jack-knife approach was used, which is based on the alternate removal of sequences from the training sample and their following recognition. Errors of overprediction were determined by recognition of binding sites in a random sequence of 100,000 bp generated by random mixing the nucleotides in the original training sample. Based on these data, the conformational similarity level of 96% (underprediction error 0.61, overprediction error 1/8326 bp) was chosen for searching potential FOXA sites.

RESULTS AND DISCUSSION

It is known that genes of all three FOXA family proteins are actively expressed in the liver of adult mice, whereas their expression is absent from the kidney [20]. Owing to this, the results of analysis of gene expression in these organs of adult mice, obtained by hybridization on microchips and published in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database, were used for primary selection of possible FOXA target genes. A total of 686 genes, the expression level of which in liver significantly differed from that in kidneys, were selected from this database. It is reasonable to expect that such a sample is enriched with FOXA target genes. Then, based on analysis of data from the literature, 40 genes associated with apoptosis and cycle control were chosen from the selected 686 genes. Nucleotide sequences of these genes and adjacent 5′-flanking 5000 bp regions were extracted from the GenBank/EMBL databases.

For searching for potential binding sites of FOXA proteins in these genes, the computer method SITECON was used [21]. Since each regulatory region of known FOXA target genes usually contains several nearby located FOXA sites [22, 23], we considered as potential target genes only those in sequences of which clusters of two and more FOXA sites were detected (no less than two sites per 50 bp). There appeared 15 such genes. In 11 of these, the 3-6-link repeats of TTTG tetranucleotides were detected within clusters. To date, no such microsatellites have been found among FOXA protein binding sites. Since in most supposed target genes FOXA sites were organized as TTTG repeats, we studied binding to FOXA of oligonucleotides corresponding to such sites by the DNA retardation in the gel. We used in experiments the GST-fused protein synthesized by *E. coli* cells and containing the DNA-binding FOXA2 domain (GST-DBDFOXA2), which provides for recognition of the FOXA protein binding sites [24]. As seen in Fig. 1, oligonucleotides containing the 3-6-link TTTG repeats efficiently competed for binding to the recombinant protein with the known FOXA site of the mouse transthyretin gene promoter. At

the same time, the two-link repeat present in a number of predicted sites did not provide for the repeat-incorporating oligonucleotide binding to GST-DBDFOXA2. So, we have discovered a new type of sites for the FOXA protein binding that are arranged as three and more links of TTTG motif.

There are already a number of works pointing to the ability of some transcription factors to bind certain microsatellite repeats in regulatory regions of genes, causing alteration of transcription activity of the latter [25-28]. In this case, it was shown for some proteins like p53 binding to the TGYCC pentanucleotide repeats that the number of repeats directly correlates with the level of transcription activation of p53 target genes [27]. In contrast to this, no such situation was observed in our experiments on FOXA binding to different length microsatellites (Fig. 1).

To study a possible effect of FOXA on expression of the found potential target genes, we used a fact that we found earlier—a decrease in the activity of these transcription factors in liver after treatment by OAT of mouse lines sensitive to its hepatocarcinogenic effect [29]. In this work 12-day-old mice of ICR line, in which a single injection of OAT in 100% of cases stimulated tumor growth in liver, were used as an experimental model. Data in Fig. 2 show that treatment with OAT of 12-day-old mice results in almost complete inhibition of the DNA-binding activity of FOXA proteins.

The use of the real-time PCR for studying the effect of OAT on expression of six genes containing clusters of microsatellite FOXA sites, such as *Creb3l3* (cAMP responsive element binding protein 3-like 3), *Ppp2r5d* (protein phosphatase 2, regulatory subunit B (B56), delta isoform), *Cdc73* (V cell division cycle 73, the Paf1/RNA polymerase II complex component, homolog (*Saccharomyces cerevisiae*)), *Cul2* (Cullin2), *Ptk2b* (protein tyrosine kinase 2 beta), and *Pdcd8* (programmed cell death 8), showed that genes *Cul2* and *Cdc73* exhibited the most pronounced response to the hepatocarcinogen injection to 12-day-old mice of ICR line (Fig. 3). Expression of the *Cul2* gene increased 10-fold, and that of *Cdc73* increased fivefold. Changes in expression of the other genes were significantly less pronounced. The *Cul2* gene product, cullin 2, is a component of E3 ubiquitin-protein-ligase complex. It was shown that in early embryogenesis of *Caenorhabditis elegans* cullin 2 is involved in meiosis II (transition from metaphase to anaphase), cyclin B breakdown, chromosome condensation, and degradation of CCCH-digital proteins that define dorso-ventral polarity. The E3 complex containing cullin 2 is responsible for inhibition of CKI-1 inhibitor belonging to the family of cyclin-dependent kinases, which results in CDK activation and makes possible the cell transition from G1 to S-phase, thus promoting cell proliferation [30-33]. It was shown that the *Cul2* gene expression was increased in a number of human uveal melanoma cell lines, which allows one to consider it as a tumor marker [34].

The product of the *Cdc73* (cell division cycle 73) gene in yeasts and mammals is a component of the

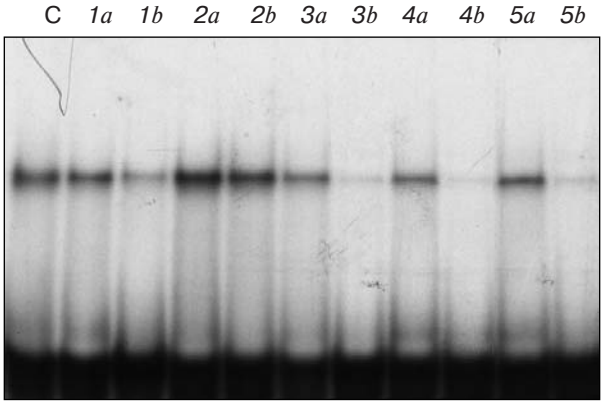

Fig. 1. Interaction of recombinant protein containing the FOXA2 DNA-binding domain (GST-DBDFOXA2) with oligonucleotides corresponding to predicted FOXA sites and incorporating 2- (*2*), 3- (*3*), 4- (*4*), and 6-fold (*5*) TTTG repeat. C, GST-DBDFOXA2 binding to the FOXA site of mouse transthyretin (TTR) gene promoter; *1-5*) GST-DBDFOXA2 binding to FOXA site of the TTR gene in the presence of competitors: *1*) TTR; *2*) predicted FOXA site from *Cdc40* gene; *3*) from *Cdc73* gene; *4*) from *Cfb* gene; *5*) from *Ulk2* gene. *a*) The probe-to-competitor ratio was 1 : 1; *b*) the competitor was in 10-fold excess.

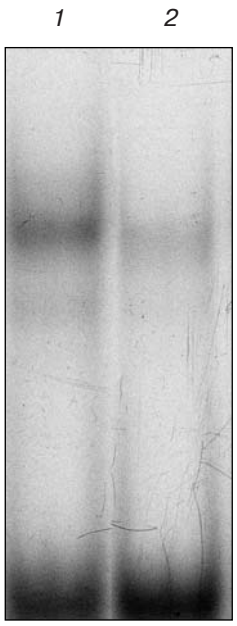

Fig. 2. Effect of OAT on DNA-binding activity of FOXA proteins in liver of 12-day-old mice of ICR line. The results of one of three independent experiments are shown, in each of them livers of three control (*1*) and three OAT-treated (*2*) animals were used. The labeled probe is the oligonucleotide corresponding to the FOXA site of the mouse transthyretin gene promoter.

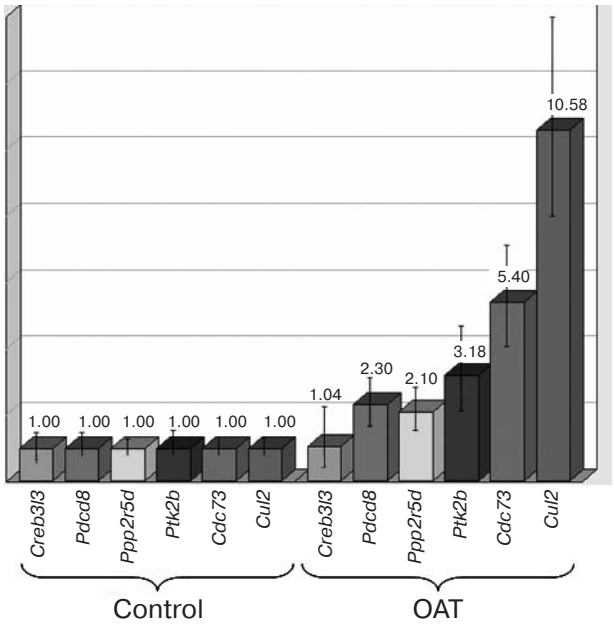

Fig. 3. Change in the expression of potential FOXA target genes in liver in response to OAT. The "housekeeping" gene *GAPDH* was used as internal control. The pattern is changed a little with *18S* rRNA gene as internal control.

Paf1/RNA polymerase II complex [35, 36]. Disturbance of the function of this protein is known to be associated with the development of parathyroid and kidney tumors [37, 38]. It was shown on fibroblast cell cultures that overexpression of this gene exhibits a pronounced antiproliferative and proapoptotic effects [39]. On the other hand, it was found that in cell lines 293FT and COS7 expressing large T-antigen of SV40 virus, Cdc73 is able to enhance cell growth, thus providing for the cell cycle transition to S phase [40]. It is also known that in humans the increased expression of the other component of the Paf1/RNA polymerase II complex, namely of Paf1, also results in induction of cell growth and emergence of tumors [41].

Thus, the data are indicative of a possible involvement of HNF3 proteins in cell division regulation, and the decrease in their activity in response to carcinogens may cause distortion of processes associated with cell division and development and so be responsible for following development of tumors in liver.

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REFERENCES

1. Ramji, D. P., and Foka, P. (2002) *Biochem. J.*, **365**, 561-575.
2. Schrem, H., Klempnauer, J., and Borlak, J. (2004) *Pharmacol. Rev.*, **56**, 291-330.
3. Halmos, B., Huettner, C. S., Kocher, O., Ferensci, K., Karp, D. D., and Tenen, D. G. (2002) *Cancer Res.*, **62**, 528-534.
4. Nerlov, C. (2004) *Nat. Rev. Cancer*, **4**, 394-400.
5. Wang, G. L., Iakova, P., Wilde, M., Awad, S., and Timchenko, N. A. (2004) *Genes Dev.*, **18**, 912-925.
6. Gery, S., Tanosaki, S., Bose, S., Bose, N., Vadgama, J., and Koeffler, H. P. (2005) *Clin. Cancer Res.*, **11**, 3184-3190.
7. Halmos, B., Basseres, D. S., Monti, S., D'Alo, F., Dayaram, T., Ferensci, K., Wouters, B. J., Huettner, C. S., Golub, T. R., and Tenen, D. G. (2004) *Cancer Res.*, **64**, 4137-4147.
8. Cha, H. H., Cram, E. J., Wang, E. C., Huang, A. J., Kasler, H. G., and Firestone, G. L. J. (1998) *Biol. Chem.*, **273**, 1998-2007.
9. Iakova, P., Awad, S. S., and Timchenko, N. A. (2003) *Cell*, **16**, 495-506.
10. Weintraub, H. (1993) *Cell*, **75**, 1241-1244.
11. Lassar, A., and Munsterberg, A. (1994) *Curr. Opin. Cell Biol.*, **6**, 432-442.
12. Crescenzi, M., Fleming, T. P., Lassar, A. B., Weintraub, H., and Aaronson, S. A. (1990) *Proc. Natl. Acad. Sci. USA*, **21**, 8442-8446.
13. Vaccarello, G., Figliola, R., Cramerotti, S., Novelli, F., and Maione, R. (2006) *J. Mol. Biol.*, **356**, 578-588.
14. Halmos, B., Basseres, D. S., Monti, S., D'Alo, F., Dayaram, T., Ferenczi, K., Wouters, B. J., Huettner, C. S., Golub, T. R., and Tenen, D. G. (2004) *Cancer Res.*, **12**, 4137-4147.
15. Schrem, H., Klempnauer, J., and Borlak, J. (2002) *Pharmacol. Rev.*, **54**, 129-158.
16. Merkulova, T. I., Kropachev, K. Y., Timofeeva, O. A., Vasiliev, G. V., Ilnitskaya, S. I., Levashova, Z. B., Kobzev, V. F., and Kaledin, V. I. (2003) *Biochemistry (Moscow)*, **66**, 520-528.
17. Merkulova, T. I., Kropachev, K. Y., Timofeeva, O. A., Vasiliev, G. V., Levashova, Z. B., Ilnitskaya, S. I., Kobzev, V. F., Pakharukova, M. Yu., Bryzgalov, L. O., and Kaledin, V. I. (2005) *Mol. Carcinogenesis*, **4**, 223-232.
18. Nakamura, T., Mura, T., Saito, K., Ohsawa, T., Akiyoshi, H., and Sato, K. (1998) *Biochem. Biophys. Res. Commun.*, **253**, 352-357.
19. Lai, E., Prezioso, V. R., Smith, E., Litvin, O., Costa, R. H., and Darnell, J. E., Jr. (1990) *Genes Dev.*, **4**, 1427-1436.
20. Kaestner, K. H., Hiemisch, H., Lucow, B., and Schutz, G. (1994) *Genomics*, **20**, 377-385.
21. Oschepkov, D. Y., Vityaev, E. E., Grigorovich, D. A., Ignatieva, E. V., and Khlebodarova, T. M. (2004) *Nucleic Acids Res.*, **32**, 208-212.
22. Merkulova, T. I., Merkulov, V. M., and Mitina, R. L. (1997) *Mol. Biol.* (Moscow), **4**, 714-725.
23. Schoneveld, O. J., Gaemers, I. C., and Lamers, W. H. (2004) *Biochim. Biophys. Acta*, **1680**, 114-128.
24. Overdier, D. G., Porcella, A., and Costa, R. H. (1994) *Mol. Cell Biol.*, **4**, 2755-2766.
25. Maueler, W., Bassili, G., Arnold, R., Renkawitz, R., and Epplen, J. T. (1999) *Gene*, **226**, 9-23.
26. Albanese, V., Biguet, N. F., Kiefer, H., Bayard, E., Mallet, J., and Meloni, R. (2001) *Hum. Mol. Genet.*, **10**, 1785-1792.
27. Contente, A., Dittmer, A., Koch, M. C., Roth, J., and Dobbelstein, M. (2002) *Nat. Genet.*, **30**, 315-320.

28. Iglesias, A. R., Kindlund, E., Tammi, M., and Wadelius, C. (2004) *Gene*, **341**, 149-165.
29. Kropachev, K. Y., Kaledin, V. I., Kobsev, V. F., Plisov, S. Y., Levashova, Z. B., and Merkulova, T. I. (2001) *Mol. Carcinogenesis*, **31**, 10-15.
30. Feng, H., Zhong, W., Punkosdy, G., Gu, S., Zhou, L., Seabolt, E. K., and Kipreos, E. T. (1999) *Nat. Cell Biol.*, **1**, 486-492.
31. De Renzo, C., Reese, K. J., and Seydoux, G. (2003) *Nature*, **424**, 685-689.
32. Liu, J., Vasudevan, S., and Kipreos, E. T. (2004) *Development*, **131**, 3513-3525.
33. Sonneville, R., and Gonczy, P. (2004) *Development*, **131**, 3527-3543.
34. Zuidervaart, W., van der Velden, P. A., Hurks, M. H., van Nieuwpoort, F. A., Out-Luiting, C. J. J., Singh, A. D., Frants, R. R., Jager, M. J., and Gruis, N. A. (2003) *Brit. J. Cancer*, **89**, 1914-1919.
35. Yart, A., Gstaiger, M., Wirbelauer, C., Pecnik, M., Anastasiou, D., Hess, D., and Krek, W. (2005) *Mol. Cell. Biol.*, **25**, 5052-5060.
36. Rozenblatt-Rosen, O., Hughes, C. M., Nannepaga, S. J., Shanmugam, K. S., Copeland, T. D., Guszczynski, T., Resau, J. H., and Meyerson, M. (2005) *Mol. Cell Biol.*, **25**, 612-620.
37. Bradley, K. J., Cavaco, B. M., Bowl, M. R., Harding, B., Cranston, T., Fratter, C., Besser, G. M., Conceicao Pereira, M., Davie, M. W., Dudley, N., Leite, V., Sadler, G. P., Seller, A., and Thakker, R. V. (2006) *Clin. Endocrinol.*, **64**, 299-306.
38. Juhlin, C., Larsson, C., Yakoleva, T., Leibiger, I., Leibiger, B., Alimov, A., Weber, G., Hoog, A., and Villablanca, A. (2006) *Endocr. Relat. Cancer*, **13**, 509-523.
39. Lin, L., Czapiga, M., Nini, L., Zhang, J. H., and Simonds, W. F. (2007) *Cancer Res.*, **5**, 183-193.
40. Iwata, T., Mizusawa, N., Taketani, Y., Itakura, M., and Yoshimoto, K. (2007) *Oncogene*, **26**, 6176-6183.
41. Moniaux, N., Nemos, C., Schmied, B. M., Chauhan, S. C., Deb, S., Morikane, K., Choudhury, A., Vanlith, M., Sutherlin, M., Sikela, J. M., Hollingsworth, M. A., and Batra, S. K. (2006) *Oncogene*, **25**, 3247-3257.