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DIFFERENTIAL EXPRESSION AND BIOINFORMATICS ANALYSIS OF MIR-184 IN PREGNANT AND NON-PREGNANT GOAT OVARIES

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ABSTRACT

Ovarian follicular development and hormone secretion are complex and coordinated biological processes which alter during pregnancy. Ovarian function is tightly regulated by a multitude of genes, and may also be regulated by specific miRNAs. It is necessary to identify the differentially expressed miRNAs in the ovaries of pregnant and non-pregnant mammals, in order to fully understand the role of miRNA-mediated post-transcriptional regulation in mammalian reproduction. In an effort to uncover miR-184 importance in goats, we examined the differential expression pattern in the ovaries of 5 pregnant and 5 non-pregnant goats and gene ontology (GO) term enrichment of predicted target genes to determine the global biological functions of miR-184. Quantitative real-time PCR (q-PCR) analysis showed that miR-184 was expressed in the ovaries of pregnant goats, but was not expressed in the ovaries of non-pregnant goats. Moreover, thirty-eight target genes of miR-184 were predicted and 10 functional categories of GO terms were significantly overrepresented among the predicted miR-184 target genes. These categories can be broadly defined as regulation of development process and cellular biosynthetic process. Our results demonstrated that miR-184 was involved in various biological processes including pregnancy, developmental and cellular biosynthetic processes.

Key words: miR-184 expression, GO term enrichment, ovaries, pregnant goat.

INTRODUCTION

Micro RNAs (miRNAs), a group of single-stranded non coding small 21-24 nucleotide (nt) RNAs, are involved in diverse aspects of eukaryotic biology including reproduction, development, pathogenesis, cell proliferation, apoptosis and lipo metabolism by pairing to mRNAs, which mainly results in target-specific post-transcriptional repression (Ambros 2004; Esau *et al*. 2004; Inoue 2007; Baley and Li 2012).

Recent researches have indicated that miR-184 was closely related to some human diseases such as Rett syndrome (Nomura *et al*. 2008), Squamous cell carcinoma of the tongue (Wong *et al*. 2008) and Neuroblastoma (Chen and Stallings 2007; Foley *et al*. 2010). Mir-184 was a highly conserved miRNA that was expressed in the female germline and had assumed control over multiple steps in oogenesis and early embryogenesis (Iovino *et al*. 2009). However, differential expression and specific function of miR-184 in goat ovaries remain largely unknown. Pregnancy is a complex reproductive process, which is tightly regulated by various endocrine factors and a large number of genes. The ovaries play an important role during pregnancy. There are significant differences in the activity and endocrine characteristics of the ovary during pregnancy and non-pregnancy (Judd *et al*. 1974; Richards 1994; Stephens and Moley 2009). In the non-pregnant phase, ovulation is normal and estrogen secretion dominates, whereas ovulation is temporarily suspended during pregnancy and progesterone secretion gradually increases to maintain pregnancy.

In the present study, we analyzed the expression level of miR-184 in pregnant and non-pregnant goat ovaries and predicted its target genes. We also calculated GO term enrichment of the targets of miR-184 and examined its biological roles in goat reproduction and development.

MATERIALS AND METHODS

Ovaries collection and total RNA isolation: Five pregnant and 5 non-pregnant Anhui White goats (a Chinese indigenous breed) that were 2-year-old were obtained from the College of Animal Science and Technology in Anhui Agricultural University. The animals were allowed access to feed and water ad libitum under normal conditions and were sacrificed humanely to minimize suffering. The ovaries of these goats were collected and used to isolate total RNA using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The quality of the total RNA was checked using a 1% agarose gel and the samples were stored at -80°C until analysis.

Real-time quantitative PCR analysis: One microgram of total RNA from each sample was reverse-transcribed into cDNA using the miScript Reverse Transcription Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's instructions. After incubation at 37°C for 1 h and deactivation at 95°C for 10 min, the mix was used as the template for q-PCR. Q-PCR was performed using standard protocols on the Roche Light Cycler 480 II Real-Time PCR Detection System (Roche; LC480 II, Basel, Switzerland). In each assay, 1 µl of cDNA was added to 19 µl of mix containing 10 µl 2×SYBR green Super Real PreMix (TIANGEN, Beijing, China), 0.4µl of a miR-184 specific primer (5'-TGGACGGAGAACT GATAAGG-3'), 0.4µl of an universal reverse primer (Qiagen) and 8.2 µl RNase free H_2O. The reaction was amplified in a 96-well optical plate at 95°C for 15 min, followed by 40 cycles of 95 and 60°C for 10 s and 30 s, respectively. For each miRNA, three biological replicates were performed, and all reactions were run in triplicate. The cycle threshold was collected from each reaction, and the relative expression level of each miRNA to U6 snRNA was evaluated using the equation $2^{-(CTmiRNA-CTU6snRNA)}$, and the fold-change (log_2-ratio) was used to show the differential expression pattern of miR-184 in different samples (Saleh *et al*. 2011; Miles *et al*. 2012; Li *et al*. 2008).

MiR-184 target genes prediction and gene ontology enrichment analysis: We used the PicTar (Krek *et al*. 2005) and Target Scan (Lewis *et al*. 2003) softwares to identify potential miR-184 target genes. To improve the accuracy of the analysis, we chose all the genes in predicted results of two softwares to use for GO term enrichment (http://www.geneontology.org). If the *P* value less than 0.01 and at least 10 associated genes are in the GO term, it is considered these genes are enriched in the GO term.

RESULTS AND DISCUSSION

RNA quality and PCR melting curve: RNA samples were run on a 1% agarose gel at 100V for 30 min. All the samples show high-quality RNA because there is a clear appearance of the 28S and 18S rRNA bands (Figure 1). The results indicated that total RNA was isolated successfully and no degradation and could be used for further research.

From the melting curve of miR-184 in the pregnant goat ovaries (Figure 2) we could see there was no any other miscellaneous peaks except a main peak. The results suggested that there were no primer-dimer formation and unspecific amplification. However, in the non-pregnant goat ovaries, the successful melting curve of miR-184 was not detected and the amplification curve with a high cycle threshold (Ct value>35) was detected (Figures were not shown).

Expression of miR-184 in pregnant and non-pregnant goat ovaries: Real-time quantitative PCR analysis showed that miR-184 was expressed in the pregnant goat ovaries, but was not expressed in the non-pregnant goat ovaries (Figure 3).

There are many differently expressed miRNAs in the mammalian gonad development process. Some reproductive traits related miRNAs show certain differential expression pattern in the ovary during pregnancy and non-pregnancy because there are significant differences in the activity and endocrine characteristics during the two physiological stages (Judd *et al*. 1974; Richards 1994; Stephens and Moley 2009). Huang *et al*. found that miR-143 was the most highly expressed miRNA in the testis and ovaries of Holstein cows, and 10 putative miRNA target genes involved in the GnRH-signaling and insulin-signaling pathways, which are associated with endocrine system function, were identified (Huang *et al*. 2011). Carletti *et al*. reported that miR-21 exerted an anti-apoptotic effect during the transformation of ovarian granulosa cells into luteal cells, and knockdown of miR-21 induced granulosa cell apoptosis and significantly reduced the rate of ovulation, via a mechanism dependent on LH secretion (Carletti *et al*. 2010).

Our research group also had found many differently expressed miRNAs in the ovaries of pregnant and non-pregnant goats using Solexa sequencing. In this study, we found that miR-184 was expressed in the pregnant goat ovaries, but was not expressed in the non-pregnant goat ovaries. The result indicates that the maintenance of ovarian functions during goat pregnancy may require miR-184. MiRNAs usually regulate protein expression by binding to and repressing translation or promoting the degradation of their target mRNAs (Wienholds and Plasterk 2005; Carrington and Ambros 2003). In this study, miR-184 that upregulated in the ovaries of pregnant goats may inhibit the expression of target genes associated with follicular development, ovulation and estrogen secretion; thereby inhibiting ovulation and estrogen secretion and promoting luteinization and progesterone secretion.

MiR-184 is involved in regulation of development and cellular biosynthetic process: Total of common 38 genes were predicted with the two softwares and used for the GO term enrichment (Table 1). The results showed that 10 functional categories of GO terms were significantly enriched among the predicted miR-184 target genes (Table 2, Figure 4). These categories can be broadly defined as regulation of development and cellular biosynthetic process.

GO term enrichment analysis showed that the Hif1an, Inppl1, Runx1, Nr4a2, Creb3l1, Bin3, Fzd1,

Table 1. Target genes information predicted by TargetScan and PicTar softwares

Target gene	Representative transcript ID	Gene name	Target Scan	Pic Tar	Target Scan Aggregate P_{CT}	Pic Tar Score
ALDH4A1	NM_170726	aldehyde dehydrogenase 4 family, member A1	+	—	0.48	
BIN3	NM_018688	bridging integrator 3	+	+	0.34	2.18
C20orf112	NM_080616	chromosome 20 open reading frame 112	+	—	0.33	
C6orf68	NM_138459	chromosome 6 open reading frame 68	—	+		3.86
CARM1	NM_199141	coactivator-associated arginine methyltransferase 1	+	—	0.33	
CBX6	NM_014292	chromobox homolog 6	+	+	0.34	1.47
CREB3L1	NM_052854	cAMP responsive element binding protein 3-like 1	+	+	0.32	2.1
CRISPLD2	NM_031476	cysteine-rich secretory protein LCCL domain containing 2	+	—	0.33	
EIF2C2	NM_012154	eukaryotic translation initiation factor 2C, 2	+	+	0.33	3.23
ELN	NM_000501	elastin	+	—	0.49	
EPB41L5	NM_020909	erythrocyte membrane protein band 4.1 like 5	+	+	0.55	2.94
FBXO28	NM_015176	F-box protein 28	+	—	0.33	
FZD1	NM_003505	frizzled homolog 1	—	+		2.18
HIF1AN	NM_017902	hypoxia inducible factor 1, alpha subunit inhibitor	+	—	0.34	
HUWE1	NM_031407	HECT, UBA and WWE domain containing 1	+	—	0.33	
INPPL1	NM_001567	inositol polyphosphate phosphatase-like 1	+	+	0.33	3.37
LIMCH1	NM_001112717	LIM and calponin homology domains 1	+	—	0.33	
LOC283687	NM_175898	hypothetical protein LOC283687	—	+		1.44
MLEC	NM_014730	malectin	+	—	0.33	
NCOR2	NM_006312	nuclear receptor corepressor 2	+	—	0.33	
NR4A2	NM_006186	nuclear receptor subfamily 4, group A, member 2	+	—	0.33	
NUS1	NM_138459	nuclear undecaprenyl pyrophosphate synthase 1 homolog	+	—	0.33	
PODN	NM_153703	podocan	+	—	0.33	
PPAP2B	NM_003713	phosphatidic acid phosphatase type 2B	+	—	0.33	
PPP1CC	NM_002710	protein phosphatase 1, catalytic subunit, gamma isoform	—	+		4.94
PRKCB1	NM_002738	protein kinase C, beta 1, transcript variant 2	—	+		2.75
RAB1B	NM_030981	RAB1B, member RAS oncogene family	—	+		1.64
RASL10B	NM_033315	RAS-like, family 10, member B	+	—	0.32	
RUNX1	NM_001754	runt-related transcription factor 1	+	—	0.33	
SF1	NM_004630	splicing factor 1, transcript variant 1	—	+		3.48
SF1	NM_201995	splicing factor 1,transcript variant 2	+	+	0.33	4.42
SF1	NM_201998	splicing factor 1, transcript variant 3	—	+		4.17

SIDT2	NM_001040455	SID1 transmembrane family, member 2	+	—	0.33	
STC2	NM_003714	stanniocalcin 2	+	—	0.34	
TNRC6B	NM_015088	trinucleotide repeat containing 6B	+	—	0.33	
UBAP2	NM_148171	ubiquitin associated protein 2	—	+		1.01
ZIC4	NM_032153	Zic family member 4	—	+		2.05
ZNF865	NM_001195605	zinc finger protein 865	+	—	0.33	

+ represented the target gene was predicted successfully by this algorithm; — represented the target gene was not predicted successfully by this algorithm.

Table 2. GO terms that were significantly overrepresented among the predicted miR-184 target genes

Biological Process **GO Term**	**P-value**	**Sample frequency**	**Genes**
GO:2000112 regulation of cellular macromolecule biosynthetic process	0.00131	14/31 (45.2%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Cbx6/Eif2c2/Ncor2/Tnrc6b/Carm1/Sf1/Ppap2b
GO:0010556 regulation of macromolecule biosynthetic process	0.00176	14/31 (45.2%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Cbx6/Eif2c2/Ncor2/Tnrc6b/Carm1/Sf1/Ppap2b
GO:0031326 regulation of cellular biosynthetic process	0.00296	14/31 (45.2%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Cbx6/Eif2c2/Ncor2/Tnrc6b/Carm1/Sf1/Ppap2b
GO:0009889 regulation of biosynthetic process	0.00342	14/31 (45.2%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Cbx6/Eif2c2/Ncor2/Tnrc6b/Carm1/Sf1/Ppap2b
GO:0010468 regulation of gene expression	0.00479	14/31 (45.2%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Cbx6/Eif2c2/Ncor2/Tnrc6b/Carm1/Sf1/Ppap2b
GO:0032502 developmental process	0.000791	17/31 (54.8%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Bin3/Fzd1/Prkcb/Ppp1cc/Eif2c2/Huwe1/Ncor2/Nus1/Eln/Carm1/Sf1/Ppap2b
GO:0048731 system development	0.00482	14/31 (45.2%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Ppp1cc/Ncor2/Nus1/Eln/Carm1/Sf1/Ppap2b
GO:0048856 anatomical structure development	0.0057	15/31 (48.4%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Bin3/Fzd1/Prkcb/Ppp1cc/Ncor2/Nus1/Eln/Carm1/Sf1/Ppap2b
GO:0007275 multicellular organismal development	0.00602	15/31 (48.4%)	Hif1an/Inppl1/Runx1/Nr4a2/Creb3l1/Fzd1/Prkcb/Ppp1cc/Eif2c2/Ncor2/Nus1/Eln/Carm1/Sf1/Ppap2b
GO:0048869 cellular developmental process	0.00701	13/31 (41.9%)	Hif1an/Inppl1/Runx1/Nr4a2/Bin3/Fzd1/Ppp1cc/Eif2c2/Huwe1/Ncor2/Nus1/Carm1/Sf1

Prkcb, Ppp1cc, Eif2c2, Huwe1, Ncor2, Nus1, Eln, Carm1, Sf1 and Ppap2b in all of potential target genes of miR-184 were enriched in 5 GO terms related to development (P<0.01) such as developmental process, anatomical structure development, multicellular organismal development, cellular developmental process and system development (Table 2, Figure 4). In addition, the Hif1an, Inppl1, Runx1, Nr4a2, Creb3l1, Fzd1, Prkcb, Cbx6, Eif2c2, Ncor2, Tnrc6b, Carm1, Sf1 and Ppap2b in all of potential target genes of miR-184 were enriched in 5 GO terms related to cellular biosynthetic process (P<0.01) such as regulation of cellular macromolecule biosynthetic process, regulation of macromolecule biosynthetic process, regulation of cellular biosynthetic process, regulation of biosynthetic process and regulation of gene expression (Table 2, Figure 4).

Recent researches have indicated that miR-184 played an important role in cell proliferation, apoptosis and tumorigenesis. Yu *et al*. demonstrated not only that the lipid phosphatase SHIP2 was a target of miR-205 in epithelial cells, but, more importantly, that the corneal epithelial-specific miR-184 could interfere with the ability of miR-205 to suppress SHIP2 levels, which was associated with a marked increase in keratinocyte apoptosis and cell death (Yu *et al*. 2008).

Foley *et al*. demonstrate that the knockdown of endogenous miR-184 had the opposite effect of ectopic upregulation, leading to enhanced neuroblastoma cell numbers. MYCN (v-myc myelocytomatos is viral related oncogene, neuroblastoma derived) contributed to tumorigenesis, in part, by repressing miR-184, leading to increased levels of the serine/threonine kinase AKT2, a direct target of miR-184 (Foley *et al*. 2010). Nomura *et al*. reported that the expression of miR-184, a brain-specific microRNA repressed by the binding of MeCP2 (methyl CpG-binding protein 2) to its promoter, is upregulated by the release of MeCP2 after depolarization. The restricted release of MeCP2 from the paternal allele results in paternal allele-specific expression of miR-184. This finding provides a clue to the link between the miR-184 and DNA methylation pathways (Nomura *et al*. 2008). Liu *et al*. found that miR-184 regulated the expression of Numblike (Numbl), a known regulator of brain development, by binding to the 3'-UTR of Numbl mRNA and affecting its translation. Expression of exogenous Numbl could rescue the aNSC (adult neural stem/progenitor cell) defects that result from either miR-184 over expression or MBD1 (Methyl-CpG binding protein 1) deficiency. Therefore MBD1, miR-184, and Numbl form a regulatory network that helps control the balance between proliferation and differentiation of aNSCs (Liu *et al*. 2010).

In this study, we found that miR-184 was expressed in pregnant goat ovaries and targeted some specific genes associated with hormone secretion and reproduction. One interesting target gene among the 38 potential target genes is cAMP responsive element binding protein 3-like 1 (CREB3L1). CREB3L1 is an important element in the generation and transport processes of the cell second message (cAMP) (Vellanki *et al*. 2010). Another interesting target gene is frizzled homolog 1 (FZD1) that affects the classical Wnt/β-catenin signaling pathway, which play an important role in luteinization, progesterone secretion and the maintenance of pregnancy (Lapointe *et al*. 2010). Potential targets of miR-184 predicted in the 3' UTR of CREB3L1 and FZD1 were shown in Figure 5. Of course, the mechanisms regulating the ovarian endocrine and goat pregnancy effects of miR-184 still need to be investigated further. Future work to characterize the expression of miR-184 at different stages of reproduction and in different breeds of goat, or in specific cell lines derived from ovarian tissues, is necessary to fully elucidate the function of miR-184 in hircine follicular development and hormone secretion, which will help to understand the relationship between miR-184 and mammalian reproduction, while enhancing the development of artificial reproduction and marker assisted selection (MAS) techniques in goats.

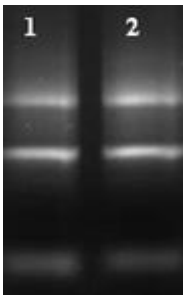

Figure 1. Result of the gel electrophoresis of total RNA isolated from goat ovaries samples. 1-pregnant goat, 2-non-pregnant goat.

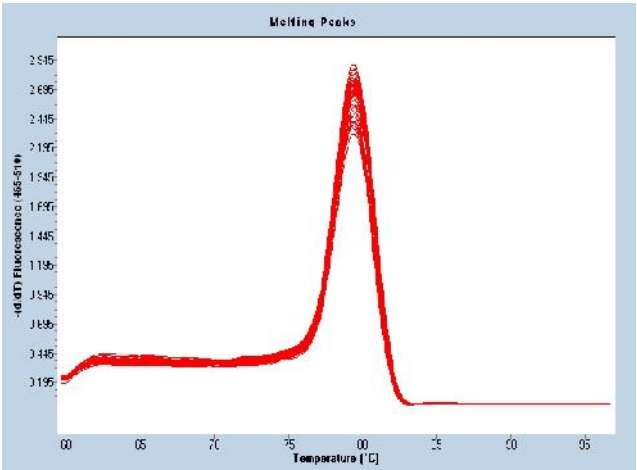

Figure 2. The melting curve of miR-184 in the pregnant goat ovaries.

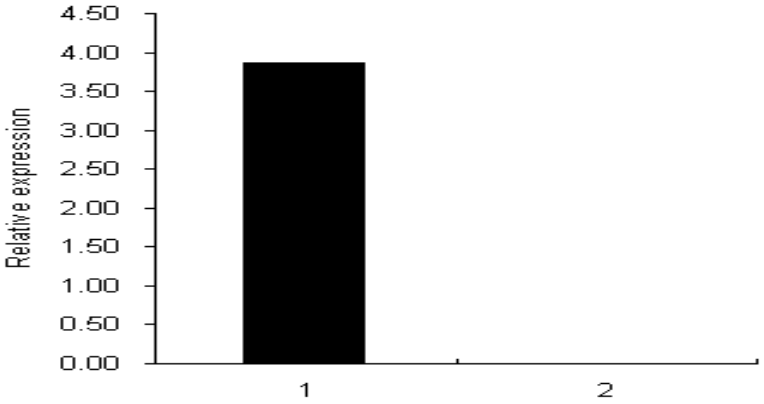

Figure 3. Relative expression of miR-184 in the ovaries of pregnant and non-pregnant goats. 1-pregnant goat, 2-non-pregnant goat. MiR-184 was not detected in non-pregnant goat ovaries (relative expression = 0).

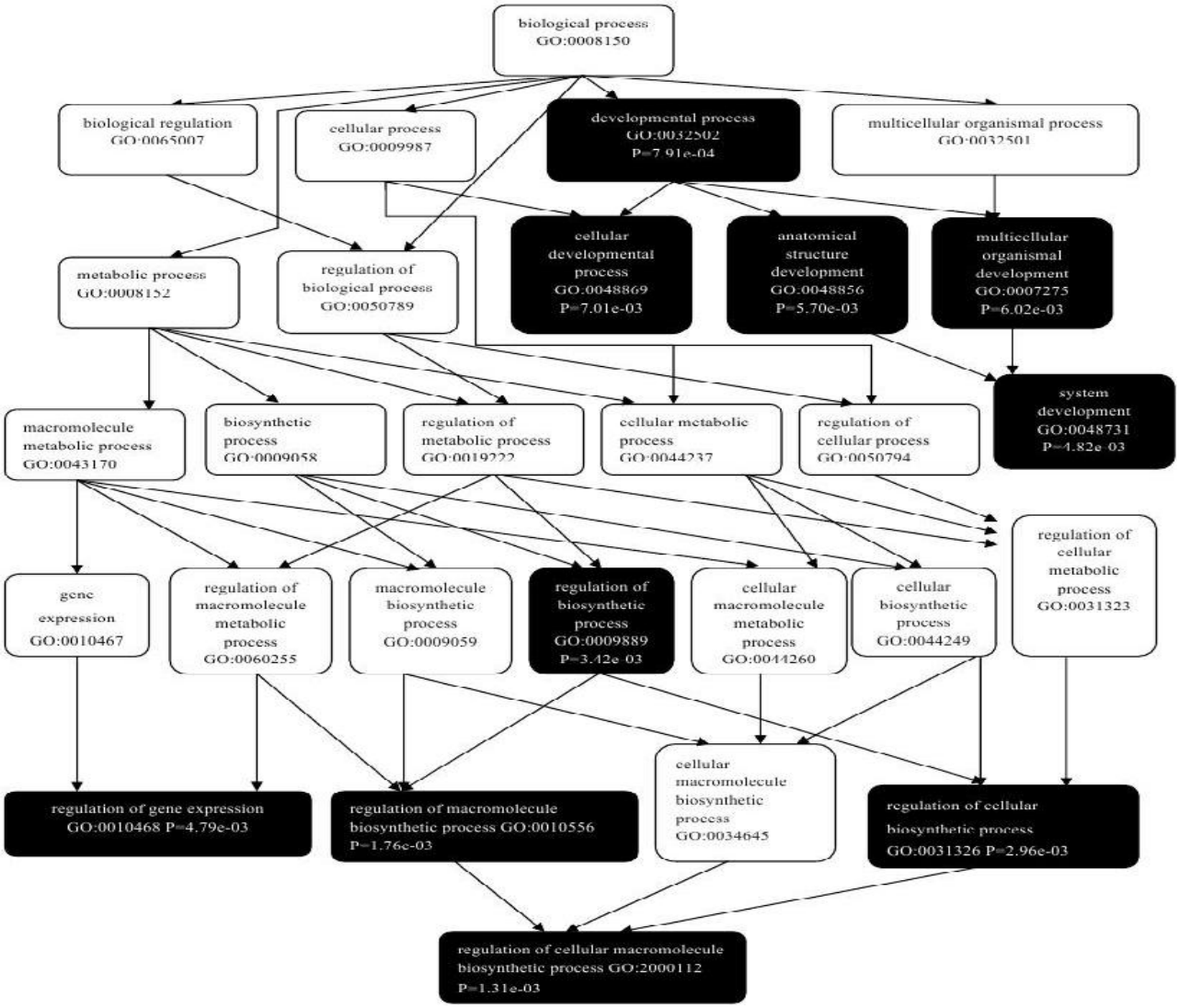

Figure 4. GO terms that were significantly enriched among the predicted miR-184 target genes. The diagram shows significantly overrepresented GO terms from the annotations of biological processes. Connections between broad high-hierarchy terms and more specific low-hierarchy terms are indicated by arrows. Black boxes represent significantly overrepresented terms that met cutoff criteria $P<0.01$. Open boxes represent terms that do not show any enrichment and are provided only as a guide to term connections and hierarchy.

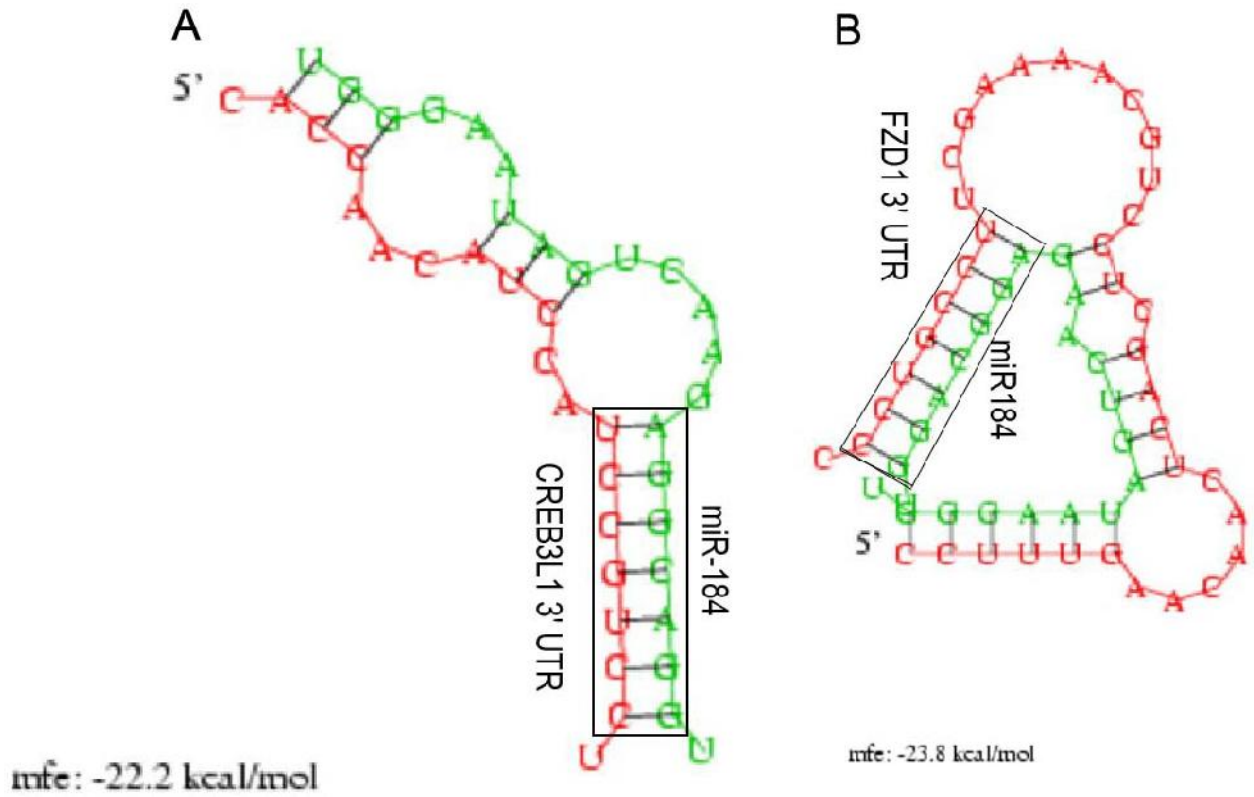

Figure 5. Potential targets of miR-184 were predicted in the 3' UTR of CREB3L1 and FZD1.
A: Presumable pairing between miR-184 and the CREB3L1 gene; B: Presumable pairing between miR-184 and the FZD1 gene. The black boxes indicate target sites of miR-184 in 3' UTR sequences of the two genes.

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REFERENCES

Ahn, H. W., R. D. Morin, H. Zhao, R. A. Harris, C. Coarfa, Z. J. Chen, A. Milosavljevic, M. A. Marra, and A. Rajkovic (2010). MicroRNA transcriptome in the newborn mouse ovaries determined by massive parallel sequencing. *Mol Hum Reprod* 16 (7):463-471.

Ambros, V. (2004). The functions of animal microRNAs. *Nature* 431 (7006):350-355.

Baley, J., and J. Li. (2012). MicroRNAs and ovarian function. *J. Ovarian Res* 5:8.

Carletti, M. Z., S. D. Fiedler, and L. K. Christenson. (2010). MicroRNA 21 Blocks Apoptosis in Mouse Periovulatory Granulosa Cells. *Biology of Reproduction* 83 (2):286-295.

Carrington, J. C., and V. Ambros. (2003). Role of microRNAs in plant and animal development. *Science* 301 (5631):336-338.

Chen, Y. X., and R. L. Stallings. (2007). Differential patterns of microRNA expression in neuroblastoma are correlated with prognosis, differentiation, and apoptosis. *Cancer Research* 67 (3):976-983.

Esau, C., X. Kang, E. Peralta, E. Hanson, E. G. Marcusson, L. V. Ravichandran, Y. Sun, S. Koo, R. J. Perera, R. Jain, N. M. Dean, S. M. Freier, C. F. Bennett, B. Lollo, and R. Griffey. (2004). MicroRNA-143 regulates adipocyte differentiation. *J Biol Chem* 279 (50): 52361-52365.

Foley, N. H., I. M. Bray, A. Tivnan, K. Bryan, D. M. Murphy, P. G. Buckley, J. Ryan, A. O'Meara, M. O'Sullivan, and R. L. Stallings. (2010). MicroRNA-184 inhibits neuroblastoma cell survival through targeting the serine/threonine kinase AKT2. *Molecular Cancer* 9.

Huang, J., Z. Ju, Q. Li, Q. Hou, C. Wang, J. Li, R. Li, L. Wang, T. Sun, S. Hang, Y. Gao, M. Hou, and J. Zhong. (2011). Solexa sequencing of novel and differentially expressed microRNAs in testicular and ovarian tissues in Holstein cattle. *Int J Biol*

Sci 7 (7):1016-1026.

Inoue, K. (2007). MicroRNA function in animal development. *Tanpakushitsu Kakusan Koso* 52 (3):197-204.

Iovino, N., Pane, A. and Gaul, U. (2009). miR-184 has multiple roles in Drosophila female germline development. *Dev. Cell* 17(1): 123-33.

Judd, H. L., G. E. Judd, W. E. Lucas, and S. S. Yen. (1974). Endocrine function of the postmenopausal ovary: concentration of androgens and estrogens in ovarian and peripheral vein blood. *J Clin Endocrinol Metab* 39 (6):1020-1024.

Krek, A., D. Grun, M. N. Poy, R. Wolf, L. Rosenberg, E. J. Epstein, P. MacMenamin, I. da Piedade, K. C. Gunsalus, M. Stoffel, and N. Rajewsky. (2005). Combinatorial microRNA target predictions. *Nat Genet* 37 (5):495-500.

Lapointe, E., M. Paquet, A. Boyer, J. S. Richards, and D. Boerboom. (2010). FZD1 May Transduce the WNT4/ RSPO1/ CTNNB1 Ovarian Developmental Signal. *Endocr Rev* 31 (3):S2460-S2460.

Lewis, B. P., I. H. Shih, M. W. Jones-Rhoades, D. P. Bartel, and C. B. Burge. (2003). Prediction of mammalian microRNA targets. *Cell* 115 (7):787-798.

Li, R., Y. Li, K. Kristiansen, and J. Wang. (2008). SOAP: short oligonucleotide alignment program. *Bioinformatics* 24 (5):713-714.

Liu, C. M., Z. Q. Teng, N. J. Santistevan, K. E. Szulwach, W. X. Guo, P. Jin, and X. Y. Zhao. (2010). Epigenetic Regulation of miR-184 by MBD1 Governs Neural Stem Cell Proliferation and Differentiation. *Cell Stem Cell* 6 (5):433-444.

Miles, J. R., T. G. McDaneld, R. T. Wiedmann, R. A. Cushman, S. E. Echternkamp, J. L. Vallet, and T. P. Smith. (2012). MicroRNA expression profile in bovine cumulus-oocyte complexes: possible role of let-7 and miR-106a in the development of bovine oocytes. *Anim Reprod Sci* 130 (1-2):16-26.

Nomura, T., M. Kimura, T. Horii, S. Morita, H. Soejima, S. Kudo, and I. Hatada. (2008). MeCP2-dependent repression of an imprinted miR-184 released by depolarization. *Human Molecular Genetics* 17 (8):1192-1199.

Richards, J. S. (1994). Hormonal control of gene expression in the ovary. *Endocr Rev* 15 (6):725-751.

Saleh, A. D., J. E. Savage, L. Cao, B. P. Soule, D. Ly, W. DeGraff, C. C. Harris, J. B. Mitchell, and N. L. Simone. (2011). Cellular stress induced alterations in microRNA let-7a and let-7b expression are dependent on p53. *PLoS One* 6 (10):e24429.

Shen, J. K., X. R. Yang, B. Xie, Y. J. Chen, M. Swaim, S. F. Hackett, and P. A. Campochiaro. (2008). MicroRNAs regulate ocular neovascularization. *Molecular Therapy* 16 (7):1208-1216.

Stephens, S. M., and K. H. Moley. (2009). Follicular origins of modern reproductive endocrinology. *Am J Physiol Endocrinol Metab* 297 (6):E1235-1236.

Vellanki, R. N., L. L. Zhang, M. A. Guney, J. V. Rocheleau, M. Gannon, and A. Volchuk. (2010). OASIS/CREB3L1 Induces Expression of Genes Involved in Extracellular Matrix Production But Not Classical Endoplasmic Reticulum Stress Response Genes in Pancreatic beta-Cells. *Endocrinology* 151 (9):4146-4157.

Wienholds, E., and R. H. Plasterk. (2005). MicroRNA function in animal development. *FEBS Lett* 579 (26):5911-5922.

Wong, T. S., X. B. Liu, B. Y. H. Wong, R. W. M. Ng, A. P. W. Yuen, and W. I. Wei. (2008). Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. *Clinical Cancer Research* 14 (9):2588-2592.

Yu, J., D. G. Ryan, S. Getsios, M. Oliveira-Fernandes, A. Fatima, and R. M. Lavker. (2008). MicroRNA-184 antagonizes microRNA-205 to maintain SHIP2 levels in epithelia. *Proc Natl Acad Sci U S A* 105 (49):19300-19305.