RESEARCH ARTICLE

OAZ1 knockdown enhances viability and inhibits ER and LHR transcriptions of granulosa cells in geese

Bo Kang1,2*, Dongmei Jiang1,2*, Rong Ma1, Hui He1, Zhixin Yi1, Ziyu Chen1

1 College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan Province, People’s Republic of China, 2 Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan Province, People’s Republic of China

☯ These authors contributed equally to this work.
* bokang@sicau.edu.cn (BK); jiangdm@sicau.edu.cn (DMJ)

Abstract

An increasing number of studies suggest that ornithine decarboxylase antizyme 1 (OAZ1), which is regarded as a tumor suppressor gene, regulates follicular development, ovulation, and steroidogenesis. The granulosa cells in the ovary play a critical role in these ovarian functions. However, the action of OAZ1 mediating physiological functions of granulosa cells is obscure. OAZ1 knockdown in granulosa cells of geese was carried out in the current study. The effect of OAZ1 knockdown on polyamine metabolism, cell proliferation, apoptosis, and hormone receptor transcription of primary granulosa cells in geese was measured. The viability of granulosa cells transfected with the shRNA OAZ1 at 48 h was significantly higher than the control (p<0.05). The level of putrescine and spermidine in granulosa cells down-regulating OAZ1 was 7.04- and 2.11- fold higher compared with the control, respectively (p<0.05). The CCND1, SMAD1, and BCL-2 mRNA expression levels in granulosa cells down-regulating OAZ1 were each significantly higher than the control, respectively (p<0.05), whereas the PCNA and CASPASE 3 expression levels were significantly lower than the control (p<0.05). The estradiol concentration, ER and LHR mRNA expression levels were significantly lower in granulosa cells down-regulating OAZ1 compared with the control (p<0.05). Taken together, our results indicated that OAZ1 knockdown elevated the putrescine and spermidine contents and enhanced granulosa cell viability and inhibited ER and LHR transcriptions of granulosa cells in geese.

Introduction

Ornithine decarboxylase antizymes (OAZs) bind to ornithine decarboxylase (ODC), which in turn enhance the degradation of the enzyme protein by the 26S proteasome and also inhibit intracellular polyamine influx [1–3]. Thus, OAZs are considered as negative regulators of intracellular polyamines. Polyamines are essential for cellular processes such as cell growth and proliferation, and hence play important roles in reproduction [4, 5]. Among four types of OAZs described to date [6], the prototype and the most highly investigated form is OAZ1,
which is expressed ubiquitously at significantly higher levels and is believed to be the predomi-
nant factor in the regulation of ODC [7, 8].

The essential role of OAZ1 in inhibiting ODC and polyamine uptake suggests that OAZ1 is a negative regulator of cell proliferation and tumor development [9–11]. Recent studies have indicated that OAZ1 also prevents centrosome abnormalities and facilitates DNA double-
strand break repairs [12, 13]. Fong et al. reported that OAZ1 overexpression reduced the for-
estomach cell proliferation and increased apoptosis in mice with forestomach carcinogenesis
[14]. Further, OAZ1 overexpression has been shown to have a tumor-suppressive effect in
C57BL/6 and DBA/2 mice [15]. In addition to accelerate the degradation of ODC, an increas-
ing number of studies indicate that OAZ1 also binds to and accelerates the degradation of
other proteins shown to regulate cell proliferation, such as AURKA, CCND1, and SMAD1
[16–18].

The goose is one of the most important waterfowl species and is also a vital component in
the fast-growing poultry industry of China [19, 20]. The study to improve goose laying perfor-
ance is very important for poultry industry development. Our previous study suggested that
increased OAZ1 expression might disrupt polyamine homeostasis by inhibiting ODC activity
and suppress follicular development in geese [21]. Recently, studies from our and others labo-
ratories indicate that OAZ1 regulates the ovarian and follicular development and ovulation by
mediating intracellular polyamine homeostasis in the ovary [21–23]. Granulosa cells play a
critical role in ovarian functions such as follicular development, ovulation, and steroidogenesis
in both mammals and birds. To date, and to our knowledge, studies on OAZ1 regulating ovar-
ian functions, particularly granulosa cells, are scarce. The action of OAZ1 mediating physio-
logical functions of granulosa cells is obscure. We therefore undertook studies using short
hairpin RNA (shRNA) targeted to OAZ1, with the aim of determining the importance of
OAZ1 in polyamine metabolism, cell proliferation, apoptosis, and hormone responsiveness of
primary granulosa cells in geese. The results indicated that OAZ1 knockdown elevated the
putrescine and spermidine contents and enhanced granulosa cell viability and inhibited estro-
gen receptor (ER) and luteinizing hormone receptor (LHR) transcriptions of granulosa cells in
geese.

Materials and methods
Ethics statement
All animal experiments of this study were approved by the Animal Care and Use Committee
of the Sichuan Agricultural University (Chengdu, China), in order to ensure compliance with
international guidelines for animal welfare.

Animals, primary granulosa cell collection and culture
The Sichuan white goose (Anser cygnoides) used in this study were from the Experimental
Farm of Waterfowl Breeding in Sichuan Agricultural University (Ya’an, China). The animals
were kept under same environmental conditions and provided ad libitum water and locally
available commercial feed. The geese were exposed to natural lighting and temperature. Adult
female geese during the egg-laying stage were killed by exsanguination to obtain the whole
ovary under anesthesia with 3% isoflurane (35 mg/kg body weight). The primary granulosa
cells were collected as the method described by Gilbert et al. [24]. Granulosa cells were cul-
tured in a humidified incubator at 37°C and 5% CO₂ in a Dulbecco’s Modified Eagle Medium/
Nutrient Mixture F-12 (DMEM/F-12, Thermo Fisher scientific, Shanghai, China) medium
supplemented with 3% fetal bovine serum (FBS) and 100 U/ml penicillin/streptomycin to a
concentration of 1 × 10⁵ cells/ml.
Granulosa cell viability assay

Granulosa cell viability was determined using a 3-(4, 5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Granulosa cells were plated at a density of 3–5×10³ cells per well in a 96-well plate. Viable cells were stained with MTT solution (0.1 mg/ml, 4 h). Formazan crystals were dissolved by 100 µl dimethylsulfoxide (DMSO). Absorbance was measured at 570 nm using a spectrophotometer (Thermo Fisher scientific, Vantaa, Finland). Each analysis was performed using three replicate wells.

shRNA OAZ1 construction and transfection

Plasmid DNA encoding green fluorescent protein (GFP) and shRNA OAZ1 vector was constructed from Pentr/U6/shRNA/GFP vector (BGI, Shenzhen, China). The targeted OAZ1 sequence (GenBank accession number KC845302) was 5′-GCGGATACTCAACAGTCAGTCACTGCGAACAGTGAC TGTTGAGTATCCG-3′ using the specific primers (forward: 5′-CACCCGGTACTCAACAGTCAGTCACTGCGAACAGTGAC TGTTGAGTATCCG-3′ and reverse: 5′-AAAAGCGGATACTCAACAGTCAGTCACTGCGAACAGTGAC TGTTGAGTATCCG-3′). Ligation products with shRNA OAZ1 were transformed into Escherichia coli DH5α competent cells, and verified by sequencing. For transfection, 1.0 µg of shRNA OAZ1 plasmid, 3.0 µl X-tremeGENE™ HP DNA Transfection Reagent (Roche, Shanghai, China), and up to 100 µl DMEM/F-12 medium were applied per well of 12-well culture plates and co-incubated overnight. The level of OAZ1 mRNA expression was detected using quantitative real-time PCR (qPCR) as detailed below. Granulosa cells and culture medium were harvested at 24, 48, and 72 h after transfection for the analyses. Each group had three replicates, and the same treatment was repeated in triplicate.

Total RNA extraction and qPCR

Total RNA was extracted from granulosa cells using the RNAiso Plus kit (Takara, Dalian, China) following the manufacturer’s protocol. Reverse transcription to obtain cDNA was performed using a PrimeScript™ RT reagent kit with a gDNA Eraser (Takara). Primers used in this experiment were synthesized in BGI Company (Shenzhen, China) (Table 1). qPCR detection and expression analysis of genes was then carried out using the iQ SYBR Green Supermix kit (Bio-Rad Laboratories, CA, USA). Threshold and threshold cycle (Ct) values were determined automatically by the CFX Manager™ Software (Bio-Rad Laboratories) using default parameters. The comparative cycle threshold (2^{-\Delta\Delta Ct} method) was used to analyze the expression levels of genes examined in this study. The abundance of each gene transcripts was normalized by GAPDH gene expression levels and expressed as arbitrary units (AU). The relative quantization of gene expression was performed in three replicates for each sample.

Polyamine contents measurement

Putrescine, spermidine, and spermine contents in granulosa cells were determined by high performance liquid chromatography (HPLC) analysis with an Agilent 1100 Series system (Agilent Technologies, CA, USA) following a benzylation procedure. The separation was achieved on a chromatographic column Chromstar™ C18 (5 µm, 4.6 × 250 mm, Agilent Technologies) in an HPLC system using a fluorescence detector set excitation wavelength at 229 nm. The proportion of mobile phase A (methyl alcohol) and B (water) was 62:38 (v/v). The isocratic elution was performed as follows: 17 min, 62% mobile phase A. The column temperature was maintained at 25°C, the flow rate was at 1 ml/min, and the injection volume was 20 µl. Results were compared to the internal standard (1, 6-hexanediamine) and the standard curves for
Table 1. Oligonucleotide PCR primer sets used in this study.

| Primers | Sequence (5’ - 3’) | Size | Tm  |
|---------|--------------------|------|-----|
| OAZ1 F: CAGGTGGCAGGGGAATAGT | 142 bp | 65˚C |
| OAZ1 R: GACATCTAAGCCCTGACTGGAC | | | |
| OAZ2 F: AAGCCTATGTTGTCACACTTC | 146 bp | 65˚C |
| OAZ2 R: GTGCTGATAACCCTTCCTTG | | | |
| AZIN F: GCTCTTACCTCCACATGGCACA | 180 bp | 58˚C |
| AZIN R: TGAATGTACGTTTGCAGTTCTTG | | | |
| ODC F: TGATCTGTGTTGGAAGTGGTG | 146 bp | 60˚C |
| ODC R: CAGGAGACTATCTGTCCGGAAC | | | |
| SAMDC F: GCTTGACCAGTAGTTAGGGACA | 180 bp | 58˚C |
| SAMDC R: TGAATAGTCCAGTAAACCCTACGG | | | |
| SPDS F: TCTGCTGACAAGGTGATGC | 111 bp | 55˚C |
| SPDS R: AGGGATGGTGCAATAGGGGTGTA | | | |
| SPMS F: GTCTGATCCCTTGGAGGTTGGT | 110 bp | 55˚C |
| SPMS R: TTCACGCCGAGATCACCATTT | | | |
| SMO F: GSCATCAATACACCAAGACAA | 116 bp | 55˚C |
| SMO R: TAAAGTCAGCTCTCGCTCCGG | | | |
| SSAT F: CACCCCTTCCTCACAAGTCCTG | 173 bp | 58˚C |
| SSAT R: CCAATGCCAGTCCTCCTG | | | |
| APAO F: GAGTTTAGAGCCACACCTTCTGG | 143 bp | 58˚C |
| APAO R: TGGCTGAGGACCAACCAAA | | | |
| CCND1 F: CGTCTGTCCTCCACATGCCTTG | 163 bp | 55˚C |
| CCND1 R: TTCTTGAGAGCTGAGAAAACA | | | |
| AURKA F: ATCATACTGTCATCAAGAAGGCTG | 169 bp | 55˚C |
| AURKA R: CATTTCAAGGCAAGTATGCAAG | | | |
| PCNA F: AGAATTGATGAGGGAGAGCTGAC | 178 bp | 55˚C |
| PCNA R: TTCAATCTTTGGAGCAAGATTGT | | | |
| BCL-2 F: GATGCCTGCTGGGATGTCATG | 98 bp | 60˚C |
| BCL-2 R: GCTCCCACAGAACAAAC | | | |
| SMAD1 F: CCGCTGTAGGTGTTAGAGATGA | 146 bp | 55˚C |
| SMAD1 R: AATTACATGCGGCGCCTTTT | | | |
| BAX F: CCGAAGCAAGCAAGGAACGC | 162 bp | 58˚C |
| BAX R: CAGTAAAGGGCACTTGAAATGCTTC | | | |
| CASPASE 8 F: GGTGTCGAGTTTCAGGTA | 127 bp | 57˚C |
| CASPASE 8 R: CATTGTAGTTTCCAGGCTTT | | | |
| CASPASE 9 F: TTCAGCTCTGCTGCGGGTA | 150 bp | 64˚C |
| CASPASE 9 R: GTCCAGCTTCCACATACCA | | | |
| CASPASE 3 F: CTGTGATCGAGGAGCACTTGG | 158 bp | 60˚C |
| CASPASE 3 R: CAGCCACCCCTACACAGGACTGAA | | | |
| ER F: ACCCAACAGCACCCTTCAACGAA | 187 bp | 56˚C |
| ER R: CGCCAGACTAGGCCCACACCATCAG | | | |
| FSHR F: TCTCGTGTCTACCCCTTTCTCT | 207 bp | 59˚C |
| FSHR R: AACCAGTGATATAATAGTCCCATC | | | |
| LHR F: GTACACTGUGAATAAGGGAAAT | 191 bp | 53˚C |
| LHR R: GAAGGGCTTGATGTTGATA | | | |
| GAPDH F: GTGGTGCAGGAGCATGCTGAC | 86 bp | 55˚C |
| GAPDH R: GTGATGCTCCTGATGCTGAT | | | |

https://doi.org/10.1371/journal.pone.0175016.t001
putrescine, spermidine and spermine standards (Sigma-Aldrich, Shanghai, China). The inter-assay coefficients of variation were <10%.

Hormone assays

Levels of follicle-stimulating hormone (FSH), LH and estradiol in the culture medium for granulosa cells were determined by using a Goose hormone ELISA kit (Bangyi, Shanghai, China). Briefly, the 50 µl standard was added to the standard wells, and 10 µl of the measuring sample and 40 µl of the sample diluent were added to the measuring sample wells. Then, 100 µl of horseradish peroxidase-conjugate reagent was added, and the wells covered with microplate sealers and incubated for 60 min at 37˚C. Each well was aspirated and washed five times with washing buffer. Next, 50 µl of chromogen solution A and 50 µl of chromogen solution B were added to each well. Wells were gently mixed and incubated for 15 min at 37˚C, and protected from light. Finally, 50 µl stopping solution was added, and hormone levels were determined by absorbance at 450 nm within 15 min using a spectrophotometer (Thermo Fisher Scientific). The standard curve indicated a direct relationship between optical density and hormone content.

Statistical analyses

The data were analyzed by a one-way analysis of variance using SAS 9.1 statistical software (SAS Institute Inc., NC, USA). Statistically significant results were further analyzed by Duncan’s multiple range test. The data were presented as the mean ±SEM. A value of $p<0.05$ was considered significant.

Results

**OAZ1 knockdown enhanced the viability of granulosa cells**

Geese primary granulosa cells were either transfected with shRNA targeting *OAZ1* or control shRNA. As shown in Fig 1A, the level of *OAZ1* mRNA expression in granulosa cells transfected with shRNA *OAZ1* at 48 and 72 h was significantly lower than the control ($p<0.05$). The viability of granulosa cells transfected shRNA *OAZ1* at 48 h was significantly higher than the control ($p<0.05$) (Fig 1B). Thus, granulosa cells at 48 h after shRNA *OAZ1* transfection were employed to conduct the subsequent research.

**OAZ1 knockdown elevated putrescine and spermidine contents by mediating the expression of polyamine metabolic enzyme genes**

To determine the action of *OAZ1* regulating polyamine homeostasis in primary granulosa cells from geese, the expression levels of polyamine metabolic enzyme [3, 25, 26] genes and polyamine levels (Figs 2 and 3, and Table 2) were measured. The *OAZ2, SAMDC, SPDS, and APAO* mRNA expression levels in granulosa cells silencing *OAZ1* were significantly higher than the scramble ($p<0.05$). The *ODC* and *SSAT* mRNA expression levels in granulosa cells down-regulating *OAZ1* were significantly lower than the control ($p<0.05$). The putrescine and spermidine contents in granulosa cells transfected shRNA *OAZ1* were 7.04- and 2.11-fold higher compared with the control, respectively ($p<0.05$). However, spermine concentration was not significantly different compared with the control ($p>0.05$).
Fig 1. Characteristics of granulosa cells in geese 24 h, 48 h and 72 h after shRNA OAZ1 transfection. A: The OAZ1 mRNA expression level in granulosa cells. B: The activity of granulosa cells.

https://doi.org/10.1371/journal.pone.0175016.g001

Fig 2. Expression levels of genes related to polyamine metabolism in granulosa cells. The data were presented as the mean ± SEM. Bars with a star were significantly different (p<0.05).

https://doi.org/10.1371/journal.pone.0175016.g002
altered expression abundance of cell proliferation and apoptosis genes in granulosa cells

Further, we performed qPCR to elucidate the effect of OAZ1 knockdown on the transcriptions of genes related to granulosa cell proliferation and apoptosis. As shown in Fig 4, the amount of CCND1 (encoding the Cyclin D1 protein which is crucial for the transition of cells from G1 to S phase), SMAD1 (encoding the SMAD1 protein which is an intracellular protein that transduce extracellular signals from TGF beta ligands to the nucleus), and BCL-2 (encoding the BCL-2 protein which is specifically considered an important anti-apoptotic protein) mRNA expression

\[ \text{Table 2. Polyamines concentration (nmol/10}^6\text{ cell) in granulosa cells.} \]

|                 | shRNA Scramble | shRNA OAZ1 |
|-----------------|----------------|------------|
| Putrescine      | 0.49 ± 0.03    | 3.45±0.09* |
| Spermidine      | 3.65 ± 0.05    | 7.69±0.07* |
| Spermine        | 1.55 ± 0.24    | 2.24±0.47  |

The data were presented as the mean ± SEM. *denotes a significantly different (p<0.05).
in granulosa cells down-regulating OAZ1 was significantly higher than the control ($p<0.05$), and were 1.41-, 2.13- and 1.35-fold higher compared with the control, respectively. Whereas the PCNA (a cell proliferation marker) and CASPASE 3 transcripts in granulosa cells down-regulating OAZ1 were 0.61- and 0.75-fold higher, respectively, compared with the control ($p<0.05$). However, the expression levels of AURKA (encoding the AURKA protein which plays an essential role in mitotic events, peaks during the G2 phase to M phase transition), BAX (encoding the BAX protein which forms a heterodimer with BCL-2 and functions as an apoptotic activator), CASPASE 8 and 9 were not significantly different than the vehicle group ($p>0.05$).

**Fig 4.** Expression levels of genes related to granulosa cell proliferation and apoptosis. The data were presented as the mean ± SEM. Bars with a star were significantly different ($p<0.05$).

[https://doi.org/10.1371/journal.pone.0175016.g004](https://doi.org/10.1371/journal.pone.0175016.g004)

**Discussion**

Numerous studies have found that polyamines play key roles in both normal and abnormal functions of cells [27]. OAZ1 plays a central role in the regulation of polyamine homeostasis.
Increased OAZ1 levels in response to polyamines led to inhibition of growth in IEC-6 cells, which was restored by inhibiting OAZ synthesis by asparagine in the presence of spermidine or spermine [29]. In agreement with that study, our result corroborated further that down-regulated OAZ1 enhanced the growth of primary granulosa cells. Enhanced levels of polyamines, including putrescine and spermidine, are associated with cell proliferation and hyper-proliferation [30]. Furthermore, OAZ1 affects cell proliferation and viability solely by modulating cellular polyamine metabolism in NIH3T3 and HEK-293 cells [31]. However, Ray et al. suggested that the effect of OAZ1 on IEC-6 cell growth was independent of polyamines [32]. In the present study, OAZ1 silencing resulted in an increase of the putrescine and spermidine concentrations, and an unchanged spermine concentration. This finding was in keeping with the result in HEK-CMV-Luc2-Hygro cells reported by Xiao et al. [33]. Thus, in consideration of the mechanism of polyamine action varying among cell types, whether increased viability of granulosa cells silenced OAZ1 resulted from suppressed OAZ1 expression, or decreased the putrescine and spermidine contents, remains to be determined.

OAZ2 has structure and tissue distribution similar to that of OAZ1, but it is expressed at significantly lower levels [8]. OAZ1 knockdown in granulosa cells induced OAZ2 expression, suggesting that OAZ2 played an important role in suppressing ODC by compensating for decreased OAZ1 [8, 34]. Increased OAZ2 and decreased ODC transcription might contribute to counteract sharp increase of the putrescine content in granulosa cells silencing OAZ1. It is possible that augmented spermidine content in granulosa cells down-regulating OAZ1 resulted from enhanced levels of SAMDC and SPDS mRNA expression. These results suggested that OAZ1 knockdown disturbed intracellular polyamine homeostasis with elevated putrescine and spermidine contents, and accelerated the growth of granulosa cells of geese in vitro.

OAZ1 has been shown to function by inhibiting cell proliferation and antitumor activity [14]. In the current study, CCND1, SMAD1, and BCL-2 mRNA expression in granulosa cells down-regulating OAZ1 with decreased CASPASE 3 expression level were significantly higher.
than the scramble group. Polyamine biosynthesis has been shown to peak at the G1/S transition regulated by CCND1 [35]. OAZ1 overexpression in zinc-deficient mice restrained CCND1 expression in the forestomach [14]. SMAD1 is known to serve as a signaling intermediate for bone morphogenetic proteins and anti-mullerian hormone, which are critical in regulating granulosa cell growth and differentiation [36]. OAZ1 overexpression induced a small decrease in the concentration of BCL-2 protein [37]. It corroborated the hypothesis that OAZ1 functions as inhibiting cell proliferation and promoting apoptosis. These results, including our data, indicate that OAZ1 knockdown enhanced granulosa cell growth by up-regulating the transcriptions of proliferation-promoting and anti-apoptosis genes, and suppressing the activation of apoptosomal CASPASE 3 cascade. As mentioned above, OAZ1 is a negative regulator of cell proliferation [9, 10], and PCNA is a cell proliferation marker. Our present data showed that the viability of granulosa cells down-regulating OAZ1 increased, while the level of PCNA mRNA expression decreased. However, the reason remains to be elucidated. Additionally, though OAZ1 can mediate degradation of AURKA [17], our study suggested that silencing of OAZ1 did not alter AURKA transcription.

Numerous studies have suggested that FSH, LH, and estradiol induce ODC activity and hence polyamines [4, 38]. Bastida et al. reported that ODC/polyamines played important roles in mediating the effect of LH on follicular development and luteinization [39]. Polyamines are also important in directly regulating the ligand–membrane receptor interaction and gene-activating functions of ER in human breast cancer cells [40, 41]. Our results showed that knockdown of OAZ1 decreased estradiol content in culture media and suppressed ER and LHR mRNA expression in granulosa cells of geese. On the contrary, OAZ1 overexpression suppressed ER mRNA expression in human breast cancer cells [42]. One possible explanation was that the function of OAZ1 mediating ER transcription might vary between primary normal and cancer cells. Additionally, oligoamines (a specific polyamine analogues) suppressed expression of ER in human breast cancer cells [43]. Whether increased putrescine and spermidine concentrations decreased ER mRNA expression level remains to be determined. Taken together, data from our study and others indicate that OAZ1 and/or polyamines play important roles in the responsiveness of granulosa cells toward estradiol and LH in geese, though a possible mechanism remains to be elucidated.

Conclusions

In conclusion, OAZ1 knockdown, with elevated putrescine and spermidine contents enhanced granulosa cell viability by affecting the transcriptions of genes related with granulosa cell proliferation and apoptosis, as well as inhibited ER and LHR transcriptions of granulosa cells in geese. Our current study provides the first evidence that OAZ1 and/or polyamines play an important role in regulating primary granulosa cell viability and hormone receptor expression.

Author Contributions

Conceptualization: BK DMJ.

Data curation: BK DMJ RM.

Formal analysis: BK RM.

Funding acquisition: BK.

Investigation: BK RM HH ZXY ZYC.

Methodology: BK DMJ RM.
Project administration: BK DMJ.
Resources: BK.
Supervision: BK DMJ.
Validation: BK DMJ RM HH.
Writing – original draft: BK DMJ RM HH ZXY ZYC.
Writing – review & editing: BK DMJ RM HH ZXY ZYC.

References
1. Murakami Y, Matsufuji S, Kameji T, Hayashi S, Igarashi K, Tamura T, et al. Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. Nature. 1992; 360(6404):597–9. https://doi.org/10.1038/360597a0 PMID: 1334232
2. Suzuki T, He Y, Kashiwagi K, Murakami Y, Hayashi S, Igarashi K. Antizyme protects against abnormal accumulation and toxicity of polyamines in ornithine decarboxylase-overproducing cells. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91(19):8930–4. PubMed Central PMCID: PMCPMC44720. PMID: 8090747
3. Pegg AE. Functions of Polyamines in Mammals. The Journal of Biological Chemistry. 2016; 291(29):14904–12. PubMed Central PMCID: PMCPMC4946908. https://doi.org/10.1074/jbc.R116.731661 PMID: 27268251
4. Lefevre PL, Palin MF, Murphy BD. Polyamines on the reproductive landscape. Endocrine reviews. 2011; 32(5):694–712. Epub 2011/07/28. https://doi.org/10.1210/er.2011-0012 PMID: 21791588
5. Ma R, Jiang D, Kang B, Bai L, He H, Chen Z, et al. Molecular cloning and mRNA expression analysis of antizyme inhibitor 1 in the ovarian follicles of the Sichuan white goose. Gene. 2015; 568(1):55–60. https://doi.org/10.1016/j.gene.2015.05.014 PMID: 25959024
6. Coffino P. Regulation of cellular polyamines by antizyme. Nature Reviews Molecular Cell Biology. 2001; 2(3):188–94. https://doi.org/10.1038/3506500 PMID: 1165248
7. Kahana C. Antizyme and antizyme inhibitor, a regulatory tango. Cellular and molecular life sciences: CMLS. 2009; 66(15):2479–88. Epub 2009/04/29. https://doi.org/10.1007/s00018-009-0033-3 PMID: 19399584
8. He H, Kang B, Jiang D, Ma R, Bai L. Molecular cloning and mRNA expression analysis of ornithine decarboxylase antizyme 2 in ovarian follicles of the Sichuan white goose (Anser cygnoides). Gene. 2014; 545(2):247–52. https://doi.org/10.1016/j.gene.2014.05.022 PMID: 24831833
9. Feith DJ, Origanti S, Shoop PL, Sass-Kuhn S, Shantz LM. Tumor suppressor activity of ODC antizyme in MEK-driven skin tumorigenesis. Carcinogenesis. 2006; 27(5):1090–8. Epub 2006/01/10. https://doi.org/10.1093/carcin/bgi343 PMID: 16400186
10. Feith DJ, Shantz LM, Pegg AE. Targeted antizyme expression in the skin of transgenic mice reduces tumor promoter induction of ornithine decarboxylase and decreases sensitivity to chemical carcinogenesis. Cancer research. 2001; 61(16):6073–81. Epub 2001/08/17. PMID: 11507056
11. Iwata S, Sato Y, Asada M, Takagi M, Tsujimoto A, Inaba T, et al. Anti-tumor activity of antizyme which targets the ornithine decarboxylase (ODC) required for cell growth and transformation. Oncogene. 1999; 18(1):165–72. https://doi.org/10.1038/sj.ong.1202275 PMID: 9926931
12. Mangold U, Hayakawa H, Coughlin M, Munger K, Zetter BR. Antizyme, a mediator of ubiquitin-independent proteasomal degradation and its inhibitor localize to centrosomes and modulate centriole amplification. Oncogene. 2008; 27(5):604–13. Epub 2007/08/02. https://doi.org/10.1038/sj.ong.1210685 PMID: 17669742
13. Tsuji T, Katsurano M, Ibaragi S, Shima K, Sasaki A, Hu GF. Ornithine decarboxylase antizyme upregulates DNA-dependent protein kinase and enhances the nonhomologous end-joining repair of DNA double-strand breaks in human oral cancer cells. Biochemistry. 2007; 46(31):8920–32. Epub 2007/07/17. https://doi.org/10.1021/bi0703328 PMID: 17630775
14. Fong LY, Feith DJ, Pegg AE. Antizyme overexpression in transgenic mice reduces cell proliferation, increases apoptosis, and reduces N-nitrosomethylbenzylamine-induced forestomach carcinogenesis. Cancer research. 2003; 63(14):3945–54. Epub 2003/07/23. PMID: 12873989
15. Feith DJ, Shantz LM, Shoop PL, Keefee KA, Prakashagowda C, Pegg AE. Mouse skin chemical carcinogenesis is inhibited by antizyme in promotion-sensitive and promotion-resistant genetic backgrounds.
15. Bercovitch Z, Snapir Z, Keren-Paz A, Kahana C. Antizyme affects cell proliferation and viability solely through regulating cellular polyamines. The Journal of Biological Chemistry. 2011; 286(39):33778–83. Epub 2011/06/01. PubMed Central PMCID: PMC3190834. https://doi.org/10.1074/jbc.M111.270637 PMID: 21832095

16. Ray RM, Bhattacharya S, Bavaria MN, Viar MJ, Johnson LR. Antizyme (AZ) regulates intestinal cell growth independent of polyamines. Amino acids. 2014; 46(9):2231–9. PubMed Central PMCID: PMC4134479. https://doi.org/10.1007/s00726-014-1777-9 PMID: 24930035

17. Xiao S, Chen YC, Buehler E, Mandal S, Mandal A, Betenbaugh M, et al. Genome-scale RNA interference screen identifies antizyme 1 (OAZ1) as a target for improvement of recombinant protein production in mammalian cells. Biotechnology and Bioengineering. 2016; 113(11):2403–15. https://doi.org/10.1002/bit.26017 PMID: 27215166
34. Fraser AV, Goodwin AC, Hacker-Prietz A, Sugar E, Woster PM, Casero RA Jr. Knockdown of ornithine decarboxylase antizyme 1 causes loss of uptake regulation leading to increased N1, N11-bis(ethyl)nor-spermine (BENSpm) accumulation and toxicity in NCI H157 lung cancer cells. Amino acids. 2012; 42 (2–3):529–38. Epub 2011/08/05. PubMed Central PMCID: PMC3240712. https://doi.org/10.1007/s00726-011-1030-z PMID: 21814790

35. Aim K, Oredsson S. Cells and polyamines do it cyclically. Essays in biochemistry. 2009; 46:63–76. Epub 2010/01/26. https://doi.org/10.1042/bse0460005 PMID: 20095970

36. Ocon-Grove OM, Poole DH, Johnson AL. Bone morphogenetic protein 6 promotes FSH receptor and anti-Mullerian hormone mRNA expression in granulosa cells from hen prehierarchical follicles. Reproduction. 2012; 143(6):825–33. https://doi.org/10.1530/REP-11-0271 PMID: 22495888

37. Liu GY, Liao YF, Hsu PC, Chang WH, Hsieh MC, Lin CY, et al. Antizyme, a natural ornithine decarboxylase inhibitor, induces apoptosis of haematopoietic cells through mitochondrial membrane depolarization and caspases’ cascade. Apoptosis: an international journal on programmed cell death. 2006; 11 (10):1773–88. Epub 2006/08/24.

38. Thomas TJ, Thomas T, John S, Hsu HC, Yang P, Keinanen TA, et al. Tamoxifen metabolite endoxifen interferes with the polyamine pathway in breast cancer. Amino acids. 2016; 48(10):2293–302. https://doi.org/10.1007/s00726-016-2300-6 PMID: 27438264

39. Bastida CM, Cremades A, Castells MT, Lopez-Contreras AJ, Lopez-Garcia C, Tejada F, et al. Influence of ovarian ornithine decarboxylase in folliculogenesis and luteinization. Endocrinology. 2005; 146 (2):666–74. Epub 2004/10/30. https://doi.org/10.1210/en.2004-1004 PMID: 15514084

40. Huber M, Poulin R. Permissive role of polyamines in the cooperative action of estrogens and insulin or insulin-like growth factor I on human breast cancer cell growth. The Journal of Clinical Endocrinology & Metabolism. 1996; 81(1):113–23.

41. Swift TA, Dias JA. Effects of the polyamine spermine on binding of follicle-stimulating hormone to membrane-bound immature bovine testis receptors. Biochimica et Biophysica Acta. 1986; 885(2):221–30. PMID: 3004602

42. Zhu Q, Jin L, Casero RA, Davidson NE, Huang Y. Role of ornithine decarboxylase in regulation of estrogen receptor alpha expression and growth in human breast cancer cells. Breast cancer research and treatment. 2012; 136(1):57–66. Epub 2012/09/15. PubMed Central PMCID: PMC3715085. https://doi.org/10.1007/s10549-012-2235-x PMID: 22978807

43. Huang Y, Keen JC, Pledgie A, Marton LJ, Zhu T, Sukumar S, et al. Polyamine analogues down-regulate estrogen receptor alpha expression in human breast cancer cells. The Journal of Biological Chemistry. 2006; 281(28):19055–63. PubMed Central PMCID: PMC1829667. https://doi.org/10.1074/jbc.M600910200 PMID: 16679312