Comparative proteomics analysis reveals the difference during antler regeneration stage between red deer and sika deer

Hang Su[1], Xiaolei Tang[2], Xiaocui Zhang[2], Li Liu[2], Li Jing[1], Daian Pan[3], Weijie Sun[4], Huinan He[4], Chonghui Yang[4], Daqing Zhao[4], He Zhang[3] and Bin Qi[2]

[1] Practice Innovations Center, Changchun University of Chinese Medicine, Changchun, China
[2] College of Pharmacy, Changchun University of Chinese Medicine, Changchun, China
[3] School of Clinical Medicine, Changchun University of Chinese Medicine, Changchun, China
[4] Jilin Ginseng Academy, Changchun University of Chinese Medicine, Changchun, China

ABSTRACT

Deer antler, as the only mammalian regenerative appendage, provides an optimal model to study regenerative medicine. Antler harvested from red deer or sika deer were mainly study objects used to disclose the mechanism underlying antler regeneration over past decades. A previous study used proteomic technology to reveal the signaling pathways of antler stem cell derived from red deer. Moreover, transcriptome of antler tip from sika deer provide us with the essential genes, which regulated antler development and regeneration. However, antler comparison between red deer and sika deer has not been well studied. In our current study, proteomics were employed to analyze the biological difference of antler regeneration between sika deer and red deer. The proteomics profile was completed by searching the UniProt database, and differentially expressed proteins were identified by bioinformatic software. Thirty-six proteins were highly expressed in red deer antler, while 144 proteins were abundant in sika deer. GO and KEGG analysis revealed that differentially expressed proteins participated in the regulation of several pathways including oxidative phosphorylation, ribosome, extracellular matrix interaction, and PI3K-Akt pathway.

Subjects Agricultural Science, Biochemistry, Bioinformatics, Developmental Biology, Molecular Biology
Keywords Deer antler, Proteomics, Regeneration, Oxidative phosphorylation, Ribosome, Extracellular matrix interaction, PI3K-Akt

INTRODUCTION

Deer antlers are the mammalian regenerative organ, which is covered with abundantly vascular skin called velvet. The antler regeneration initiates in every spring (*Fennessy, 1984*). At early regeneration stage, the main beam of antler grows dramatically on posterior growth center (*Li et al., 2014*). Subsequently, at the fast regeneration stage, tines of antler will form on the anterior growth center. During the antler regeneration stage, the growth rate of red antler will reach to 100 g per day within 100 to 120 days (*Borsy et al., 2009*). The weight of the full size of red deer antler generally reached seven to nine kg (*Borsy et al., 2009*). However, the sika deer antler with three to six kg is less than red deer (*Hu et al., 2019*).

Submitted 10 January 2019
Accepted 14 June 2019
Published 17 July 2019

Corresponding authors
He Zhang, 13843148162@163.com
Bin Qi, qibin88@126.com

Academic editor
Ibolya Kiss

Additional Information and Declarations can be found on page 12

DOI **10.7717/peerj.7299**

Copyright
2019 Su et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

How to cite this article Su H, Tang X, Zhang X, Liu L, Jing L, Pan D, Sun W, He H, Yang C, Zhao D, Zhang H, Qi B. 2019. Comparative proteomics analysis reveals the difference during antler regeneration stage between red deer and sika deer. *PeerJ* **7**:e7299
http://doi.org/10.7717/peerj.7299

Following into autumn, the antler gradually reaches the full size and then has calcification accompanied with the loss of velvet and vessels (*Li et al., 2014*). Over the past decades, this special feature attracted researchers to study on the development mechanism underlying antler regeneration. The antler generation through modified endochondral ossification was first reported by *Banks & Newbrey (1983)*. Antler histogenesis and tissue depletion study provided sufficient evidence that pedicle periosteum drive antler to regeneration (*Li et al., 2007*; *Li & Suttie, 1994*; *Li, Suttie & Clark, 2004*). Besides the pedicle periosteum study, researches about the growing tip of antler with the fastest growth rate are also a hotspot (*Deb-Choudhury et al., 2015*; *Gyurjan et al., 2007*; *Yao et al., 2012*).

Molnar et al. (2007) identified two expression clusters for 36 genes that were mainly expressed in consecutive tissue zones of antler tip of red deer. The first gene expression cluster was found to involve with ribosome pathway and the second cluster could be related with tumor biology. Furthermore, the activated *runx2* pathway up-regulated the expression of mineralization proteins in cartilaginous tissue of red deer antler (*Steger et al., 2010*). *Steger et al. (2010)* demonstrated that antler regeneration model can be also used to study human osteoporosis. To further deeply study deer antler, the systematic study of sika deer antler tips based on transcriptome provided more information about the key regulated genes related to cartilage development (*Yao et al., 2012*). *Li et al. (2012a)* first used proteomic technology to disclose the molecular mechanism of antler stem cells from red deer. This study identified the key markers of antler stem cells, which were transcription factors POU5F1, SOX2, NANOG, and MYC. Moreover, distinctive pathways involved in sika deer antler development were found through the comparative proteomics between potentiated and dormant antler stem cells (*Dong et al., 2016*). Notably, PI3K-AKT, never identified in red deer antler, played an indispensable role in sika deer antler regeneration. We found that red deer or sika deer was selected as experimental animal to study on antler regeneration in last decade. Also, both of them have the potential to be developed as the model of regenerative medicine. However, the difference of antler regeneration between two deer species still remains unknown. Unfortunately, a previous study showed that the histogenetic aspects of these antlers were indistinguishable between red deer and sika deer (*Li, 2013*; *Li et al., 2014*). Except for the size of two antler groups, we never know any other differences between the two antler groups before. In some cases, the term ''antler'' means that the antler was collected from sika deer or red deer. The antler, as Chinese tradition medicine, is collected from sika deer or red deer according to Chinese Pharmacopeia (*Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China, 2015*). We realized that comparative study of antler regeneration between red deer and sika deer has been a ''scotoma'' all the time. Hence, it is necessary to make the comparative study of these two antler groups. In our current study, the proteomics technology was applied to reveal the differences of the protein level between them.

The aim of our study was to disclose the differentially expressed proteins between two antler groups. Furthermore, we want to remind other researchers that these two antler systems are different. The researchers should notice that whether these different pathways affect their study, when they choose antler to study on regenerative biomedicine. The proteomics based on mass spectrum can identify thousands of proteins, which were

processed by bioinformatic software to achieve the protein properties, consisting of protein abundance, protein subcellular distribution, protein functional classification, and correlative biological processes (*Larance & Lamond, 2015*). Thus, proteomics will accurately work out essentially changed molecular pathways of antler regeneration between red deer and sika deer. The differentially expressed proteins identified in comparative proteomic may facilitate the discovery of novel molecular pathways related with antler growth.

MATERIALS AND METHODS

Tissue sampling

We randomly chose three male red deer and three male sika deer as study subjects in Chinese local deer farm, which was located in Shuangyang district, Changchun city, Jilin province (N43°33′56.03″, E125°29′23.15″). All procedures of the sample collection were approved by the Animal Ethics Committee of Changchun University of Chinese Medicine. They are all 5-year-old deer with antler regeneration for 80 days after casting previous antlers. Antler tissues were harvested from anaesthetized deer. Briefly, the distal five cm of the antler tip, regarded as the growth center, was removed and sectioned sagittally by the electric saw to collect five mm to eight mm thick tissue section (*Li, Suttie & Clark, 2005*). Then, the tissue section was cut into small pieces, flash frozen and ground into powder in liquid nitrogen, combined and stored at −80 °C ready for proteomics study.

Protein extraction and FASP digestion

The antler powder samples were extracted with SDT-lysis buffer (4% (w/v) SDS, 100 mM Tris-HCl pH 7.6, 0.1 M DTT) in a sonic dismembrator for 20 min. And then the suspension was incubated at 4 °C for 60 min and subsequently heated at 95 °C for 5 min. The incubation solution was centrifuged at 16,000 g at 4 °C for 15 min. The clarified supernatant was collected to another tube, and then the pellet extracted again in lysis buffer according to the above method. The combination of supernatants was centrifuged at 30,000 g and 4 °C for 15 min. The concentration of proteins was quantified using the bicinchoninic acid assay (BCA assay). 200 μg of protein from each antler sample was digested with trypsin according to filter-aided sample preparation (FASP) protocol proposed by *Wisniewski et al. (2009)*. Subsequently, two μg of tryptic peptides were purified using C18 spin columns (Pierce^{TM} C18, Sigma), and then dried via lyophilization and rehydrated with 40 μl of 0.1% formic acid. The content of the peptide sample was quantified based on colorimetric protein concentration assays (BioRad).

LC-MS/MS analysis

Purified digested peptide fractions were analyzed by nano LC-MS/MS using EASY-nLC 1200 coupled to Q Exactive^{TM} mass spectrometer (Thermo Fisher Scientific). The peptide samples were separated with a 120 min linear gradient from 0% to 55% buffer B (84% acetonitrile, 0.1% Formic acid) on a reverse phase peptide column (Acclaim^{TM} PepMap^{TM} 100 C18, Thermo Fisher Scientific connected) to the EASY capillary C18 column (75 μm inner diameter, 10 cm long, three μm resin; Thermo Fisher Scientific) at a flow rate of 300 nl/min. The data-dependent top10 method was used to acquire MS data by choosing

the most abundant precursor ions for HCD fragmentation. The full MS survey scans were acquired at a resolution of 70,000, with automatic gain control target 3e6 and a range of m/z 300–1800. HCD spectra scan with a resolution of 17,500 at m/z 200 and the isolation window was two m/z. The dynamic exclusion was 40 s.

Database search and protein quantification

Mass spectra were processed by the peptide search engine Andromeda in MaxQuant software, which searched the data against uniprot_Cervus_20600_20180413.fasta retrieved from UniProt database. The correlated parameters were defined as follows: digest enzyme of trypsin, max missed cleavage of 2, fixed modifications of carbamidomethyl, variable modifications of methionine oxidation and N-terminal acetylation, the precursor mass window in the main search of six ppm and MS/MS tolerance of 20 ppm. The cutoff value of the false discovery rate was defined as 0.01 to identify peptides and proteins. The protein abundance was normalized and calculated according to the algorithms described in Cox *et al* (*Tyanova, Temu & Cox, 2016*). Data from the same antler group were merged into the same group, and the LFQ intensities of the proteins were log 2 transformed to obtain log 2 fold-change between two antler groups. To improve the accuracy of comparison, the data were retained with at least two of three valid values (greater than 0) in at least one group, and missing values were replaced by generating random numbers from a Gaussian distribution that well represents the distribution of low-abundance proteins (*Qian et al., 2018*). If a certain protein has two valid values in one group and only one in the other group, it will also be eliminated. Two-sample T-test is applied for determining if the means of the LFQ intensity values of two groups of deer antlers are significantly different from each other through the analysis of Perseus software (*Tyanova et al., 2016*). To define differentially expressed proteins (DEPs), fold change thresholds were set at 2.0 or 0.5 with p-value < 0.05. The mass spectrometry data in proteomics study have been submitted to the ProteomeXchange Consortium via PRIDE partner repository, with the dataset identifier PXD012090 (*Vizcaino et al., 2016*).

Bioinformatic analysis

To study on the biological function of the differentially expressed proteins, the identified proteins were used to run Gene Ontology (GO) analysis. Firstly, the homology search was performed for all identified sequences against the red deer database using localized NCBI BLAST+ software. And then GO annotation and enrichment analysis was completed using BLAST2GO software (*Gotz et al., 2008*). The result of GO annotation presented that the properties of the identified proteins in three functional categories, which are cellular component (CC), biological process (BP), and molecular function (MF). In addition, all differentially expressed proteins and significantly changed metabolites were searched against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and obtained the corresponding KEGG pathways (*Moriya et al., 2007*). To further disclose the mechanism underlying differentially expressed proteins and clarify the functional cluster of differentially expressed proteins, GO enrichment analysis was performed by Fisher's exact test. Notably, GO terms with p values < 0.05 were regarded as significant enrichment.

RESULTS

Protein identification and differentially expressed proteins (DEPs)

In total, 4,439 unique peptides and 1,060 proteins were identified through MaxQuant software against UniProt database. All identified protein from these two groups are listed in Table S1. An obvious difference in the number of proteins with changed abundance comparing red deer antler and sika deer antler make us to analyze the extent of shared and non-overlapping differences. A total of 578 proteins were identified in both the red deer antler proteome and the sika deer antler proteome, while 114 proteins were exclusively identified in sika deer antler (Fig. 1, Table S2). However, we found that only 19 exclusive proteins for red deer antler were observed (Fig. 1, Table S2). Among these antler proteins existed in both red deer and sika deer, 30 differentially expressed proteins (DEPs) were up-regulated and 17 down-regulated in the sika deer antler (Fig. 1, Table S2). Our current proteomics data indicated that more type of proteins were identified in sika deer antler than that in red deer.

Gene ontology annotation and enrichment analysis of differentially expressed proteins

To further study protein function in deer antler, Gene ontology (GO) annotation was processed to differentially expressed proteins by Blast2GO software. All terms were counted non-exclusively, when one protein possessed more than one term for biological process (BP), molecular function (MF), and cellular component (CC). The result of GO annotation was shown in Fig. 2 and Table S3. The most abundant category in molecular function was binding proteins (38%), especially ion bonding proteins (21%) and organic cyclic compound binding proteins (20%) (Fig. 2). The second highly abundant category in molecular function was catalytic activity proteins (23%), especially hydrolase activity proteins (10%). The cellular component analysis showed that the differentially expressed proteins were mainly located in the intracellular region, organelle and membrane (Fig. 2). 23 and 18% of proteins are in the organelle and membrane, respectively, whereas 9% of differentially expressed proteins are localized in the extracellular region. The major functional terms under biological process category were metabolic processes, cellular processes and biological regulation (18, 27, and 11%, respectively) (Fig. 2). The nitrogen compound metabolic process, which is a child term of metabolic process, possessed 22 differentially expressed proteins from two antler groups (Table S3).

To reveal the biological difference between sika deer and red deer antler, GO enrichment analysis was performed for differentially expressed proteins on three categories (Fig. 3). We found that differentially expressed proteins were mainly located in clathrin-coated pit/vesicles, organelle, specially mitochondrion and cytoskeleton. On the molecular function category, a high abundance of DEPs was enriched into transporter activity and ATPase activity. DEPs were mainly involved in the regulation of gene expression and extracellular structure organization under biological process.

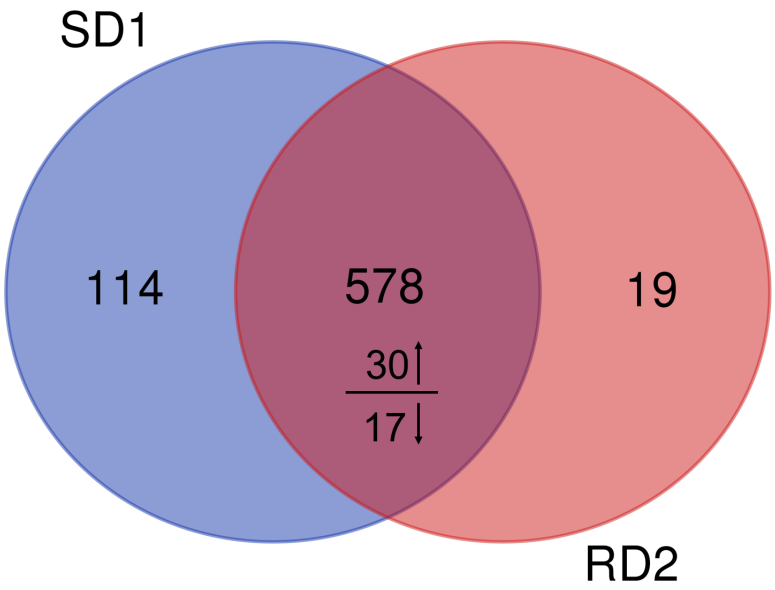

Figure 1 Venn diagram of identified proteins from sika deer and red deer groups. The identified antler proteins in at least two of three replicates were shown in the diagram. The overlapping regions indicate the number of shared proteins. The number above or below the horizontal line in each portion shows the number of up- and down-regulated shared proteins between sika deer group versus red deer group.
Full-size DOI: 10.7717/peerj.7299/fig-1

KEGG annotation of differentially expressed proteins

KEGG analysis was used to reveal the functional pathways about differentially expressed proteins. A total of 180 DEPs were processed to obtain 163 KEGG pathways (Table S4). The top 20 pathway terms were shown in Fig. 4. These differentially expressed proteins were mainly classified into PI3K-Akt signaling pathway, thermogenesis, oxidative phosphorylation, ECM-receptor interaction and focal adhesion, ribosome, synaptic vesicle cycle, complement and coagulation cascades, and other categories related with disease.

DISCUSSION

Proteins involved in oxidative phosphorylation

During antler regeneration stage, mesenchymal cells (MSCs) differentiate into chondrocytes participated in endochondral ossification, is one of the two key processes during bone development (*Rucklidge et al., 1997*). Previous study has shown that throughout the osteogenesis of MSCs, the copy number of mitochondrial DNA, oxygen consumption rate, OxPhos enzymes, mRNA associated with mitochondrial biogenesis, and intracellular ATP content were increased along with the significant decrease of endogenous reactive oxygen species (*Chen et al., 2008*; *Pattappa et al., 2011*). The production of ATP is mainly driven by OxPhos enzymes located in electron transport chain, where the proton gradient is built across the inner membrane by NADH-coenzyme Q oxidoreductase (complex I), Q-cytochrome c oxidoreductase (complex III), and cytochrome c oxidase (complex IV), which can drive ATP synthesis through ATP synthase (complex V) (*DiMauro & Schon, 2003*).

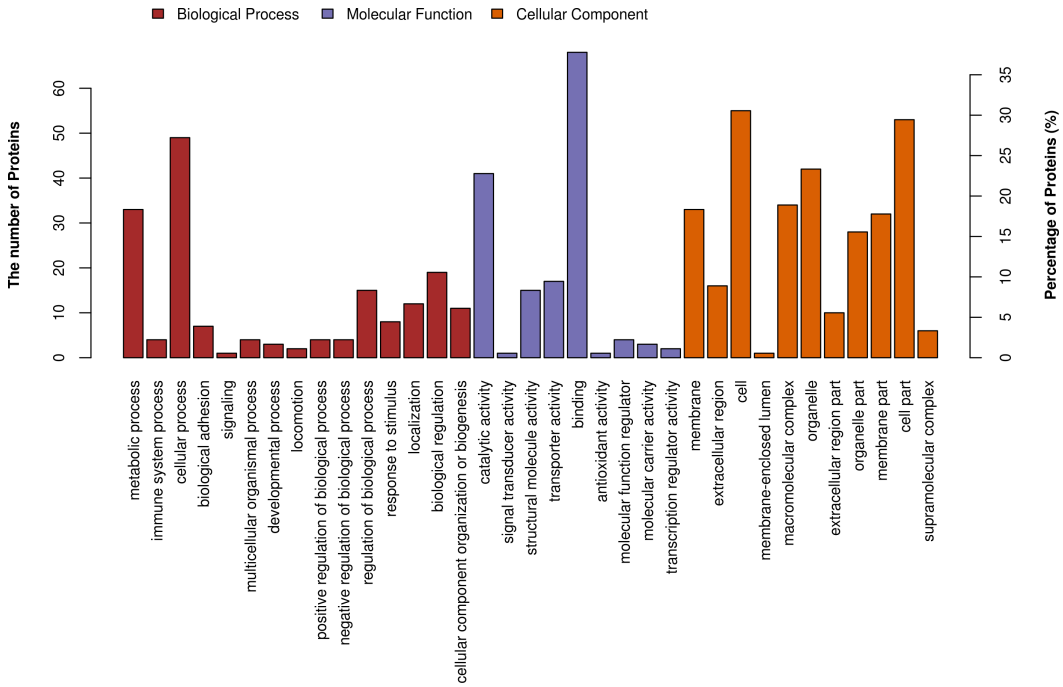

Figure 2 GO annotation for the differentially expressed antler proteins between sika deer and red deer. The GO annotation results are displayed under three main categories: biological process, molecular function, and cellular component.

Full-size DOI: 10.7717/peerj.7299/fig-2

According to KEGG annotation analysis, six up-regulated proteins in sika deer antler participated in oxidative phosphorylation (OxPhos) (Table 1). Of these six up-regulated proteins, vacuolar-type H-ATPase subunit E (ATP6E) belong to superfamily of related ATP synthases. Co-expression of the *ATP8* and *ATP6* genes provided stable protein expression for complex V assemble of the oxidative phosphorylation complexes (*Boominathan et al., 2016*). The up-regulation of the V-ATPase gene (*ATP6V0D2*) was found in the zone of hypertrophic chondrocytes, which was part of the process of endochondral ossification (*Ayodele et al., 2017*). The antlers were regenerated through a modified endochondral ossification process that involves the remodeling of cartilage (*Faucheux et al., 2001*). In this case, ATP6 could be the key enzyme involved in ATP production during antler development, whereas ATP8 may take over the roles of ATP6 in red deer. In addition, *ATP8*, which plays important roles in lipogenesis, was found during the stage of porcine adipose tissue development (*Zhang et al., 2016*). *Weiss et al. (2012)* reported that *ATP8* mutation led to mitochondrial ROS generation with dysregulation of mitochondrial OxPhos system. It indicated that the high abundance of ATP8 in red deer might maintain the stable of mitochondrial OxPhos system when antler regeneration faced an increasing demand for energy. Moreover, cytochrome c oxidase subunit 6a (COX6A) and cytochrome c oxidase subunit 6b (COX6B) were significantly up-regulated in sika deer (Table 1). The enzyme cytochrome c oxidase (COX, also called complex IV), that consist of 14 subunits, is part of mitochondrial respiration oxidase (*Balsa et al., 2012*; *Castresana et al., 1994*). The three

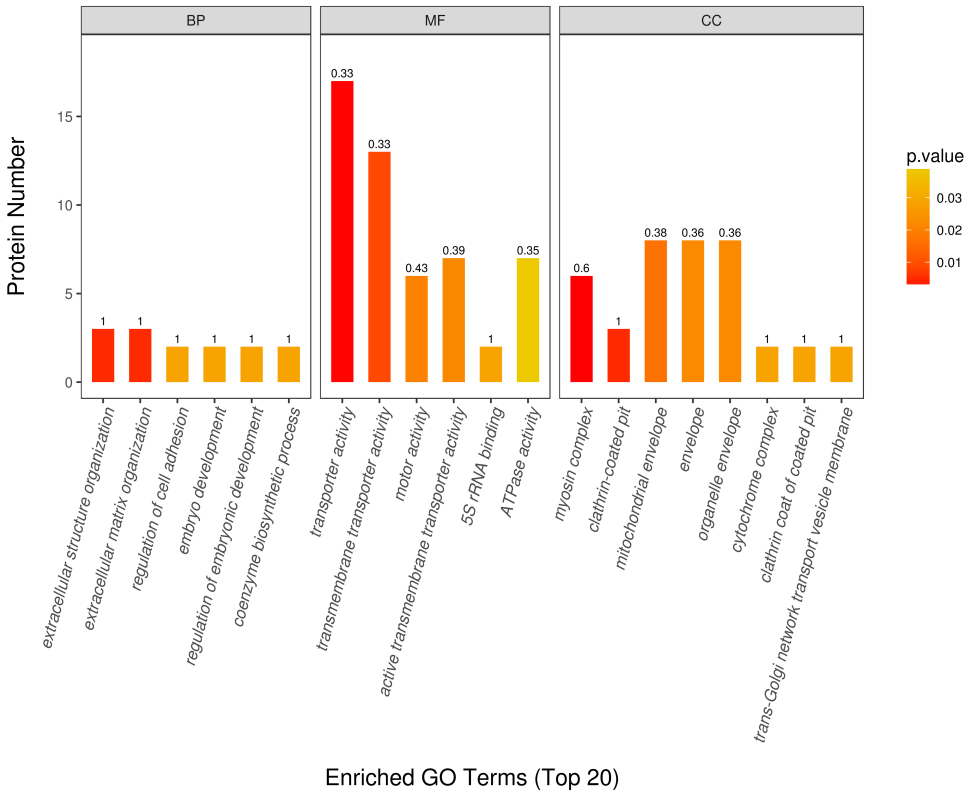

Figure 3 GO enrichment analysis for the differentially expressed antler proteins between sika deer and red deer. The color of the bar chart represents the significance of the GO Enrichment. The color gradient corresponds to the magnitude of *P* value . The gradient becomes red, which indicates a low *P* value. The value on the top of the bar chart is the rich factor (rich factor ≤ 1). The rich factor is the ratio of the number of differentially expressed proteins associated with a certain GO term to the number of all identified proteins associated with a certain GO term.

Full-size DOI: 10.7717/peerj.7299/fig-3

key subunits of COX are synthesized in mitochondrial to form the functional core of the enzyme, which is surrounded by 11 small nuclear-coded subunits (*Balsa et al., 2012*). The activity of cytochrome c oxidase, complex IV of the mitochondrial respiratory chain, has been shown to regulate the activity of osteoclasts necessary for adaptive bone remodeling (*Gandhi et al., 2017*; *Miyazaki et al., 2003*). The disruption of osteoclast COX activity could lead to the loss of bone-resorbing activity. The high expression of cytochrome c oxidase, accompanied with the up-regulation of ATP6 in sika deer, indicated that antler remodeling is more active than red deer. Accordingly, the higher ATP demand should be required for sika deer antler regeneration.

Previous studies have found that the overexpression of UQCRC1 enhanced complex III activity in mice, and its down-regulation was related with significant dysfunction in mitochondrion of epithelial cells (*Kriaucionis et al., 2006*; *Shibanuma et al., 2011*). This finding indicated that the up-regulation of UQCRC1 in sika deer (Table 1) contributes to maintain normal mitochondrial homeostasis, which is essential for osteoblast mediated bone formation (*Pan et al., 2018*). *Etzler et al. (2017)* have reported that the up-regulation

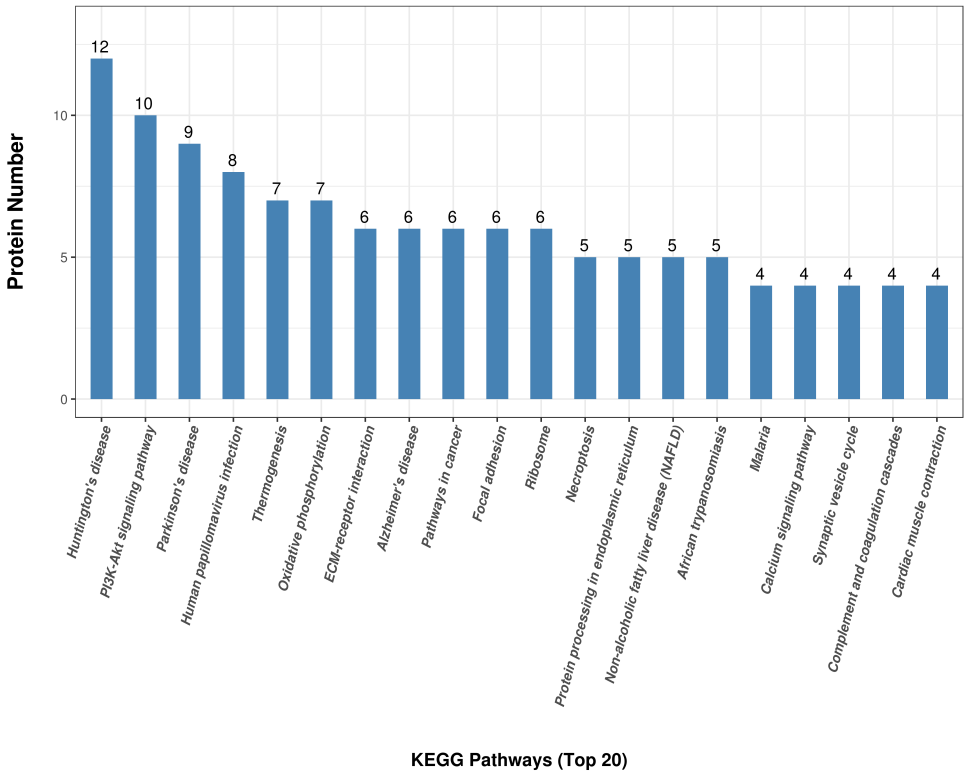

Figure 4 KEGG annotation for the differentially expressed antler proteins between sika deer and red deer. The top of the bar chart shows the number of identified proteins involved KEGG pathways.
Full-size DOI: 10.7717/peerj.7299/fig-4

of Complex III in HEK293 cells (human embryonic kidney cells) could enhance electron transport efficiency to survive from oxidative stress. Also, it was demonstrated that ROS inhibited the proliferation of chondrocytes and induced the chondrocyte hypertrophy (*Morita et al., 2007*). Therefore, the current proteomic study indicated that the antler development in sika deer require more complex III to regulate mitochondrial respiration in response to ROS, which was produced during endochondral ossification.

Recent work reported that the lower expression of NDUFS3 under oxidative stress impaired the Complex I assembly, which resulted in reduced Complex I content (*Sen et al., 2015*). Interestingly, the up-regulation of antioxidants, for example SOD2 and glutathione, could recover the complex I content (*Gopal et al., 2018*; *Sen et al., 2015*). According to the high abundance of NDUFS3, the content of antioxidants in sika deer antler should be higher that red deer (Table 1).

The high abundance of large subunit ribosomal proteins in sika deer

Another notable difference between red deer and sika deer antler was the higher abundance of large subunit ribosomal proteins (RPLs) in sika deer (Table 1). It is well known that ribosomes, as translational hub across the all living organisms, produce proteins based on mRNA template (*De la Cruz, Karbstein & Woolford Jr, 2015*; *Khatter et al., 2015*). The 80S ribosome in eukaryotes is comprised of a small (40S) and large (60S) subunit (*Khatter*

Table 1 Differentially expressed proteins involved in KEGG pathways.

UniProt ID	Protein Name	RD2/SD1	*P*-value
Oxidative phosphorylation pathway			
A0A172ZQ52	ATP8	2.232388	0.018918192
A0A212CA05	QCR1, UQCRC1	SD1[a]	–
A0A212CD21	ATPeV1E, ATP6E	SD1[a]	–
A0A212CE28	COX6C	SD1[a]	–
A0A212D8C1	COX6A	SD1[a]	–
A0A212D8Y9	QCR9, UCRC	SD1[a]	–
A0A212DI17	NDUFS3	SD1[a]	–
Ribosome pathway			
A0A212CGQ3	Large subunit ribosomal protein L14e	0.466	0.00337
A0A212CIR2	Large subunit ribosomal protein L35A	0.429	0.0408
A0A212CD44	Large subunit ribosomal protein L3e	SD1[a]	–
A0A212CH34	Large subunit ribosomal protein L5e	SD1[a]	–
A0A212CL29	Large subunit ribosomal protein L5e	SD1[a]	–
A0A212CVE3	Large subunit ribosomal protein L4e	SD1[a]	–
Extracellular matrix proteins			
A0A212C5S3	Laminin, alpha 4	0.400	0.0117
A0A212C632	Laminin, alpha 3/5	SD1[a]	–
A0A212C6B1	Collagen, type IX, alpha	SD1[a]	–
A0A212CFD7	Collagen, type IX, alpha	SD1[a]	–
A0A212D070	Cartilage oligomeric matrix protein	SD1[a]	–
E7D7Z2	Osteopontin	SD1[a]	–
PI3K/Akt pathway			
A0A212C5S3	Laminin, alpha 4	0.400	0.0117
A0A212D1I8	Immunoglobulin heavy chain	RD2[a]	–
A0A212CMX1	Serine/threonine-protein phosphatase 2A regulatory subunit B	RD2[a]	–
A0A212C632	Laminin, alpha 3/5	SD1[a]	–
A0A212C6B1	Collagen, type IX, alpha	SD1[a]	–
A0A212CF73	Guanine nucleotide-binding protein subunit gamma-12	SD1[a]	–
A0A212CFD7	Collagen, type IX, alpha	SD1[a]	
A0A212D070	Cartilage oligomeric matrix protein	SD1[a]	
A0A212DF15	HSP90A	SD1[a]	
E7D7Z2	Osteopontin	SD1[a]	

Notes.
[a]Proteins are expressed exclusively in a certain antler group.

et al., 2015). The up-regulation of ribosomal proteins was necessary to meet protein synthesis requirements in the rapid growth stage of coho salmon (*Causey et al., 2018*). It is consistent with our result, where the higher abundance of ribosomal proteins in sika deer as compared to red deer could lead to synthesize a greater variety of proteins in sika deer (Table S2). Therefore, ribosomal protein played a vital role in cell growth and proliferation by the regulation of protein biosynthesis. In bone marrow rRNAs, 60S subunit biogenesis associated with cycle circle (*Khincha et al., 2016*; *Ruggero & Shimamura, 2014*).

The mutation of RPL35A contributed to Diamond-Blackfan anemia, that is an inherited bone marrow failure syndrome (*Khincha et al., 2016*). Moreover, RPL4 was identified as a crucial regulator involved in MDM2-p53 pathway (*He et al., 2016*). RPL4 directly binds to MDM2 could significantly inhibit the ubiquitination and degradation of p53, resulting in the up-regulation of p53. Interestingly, RPL5 and RPL11 also could bind to MDM2 in the same way for p53 induction under the assistant of RPL4 (*Fumagalli et al., 2012*; *He et al., 2016*; *Sun, Dai & Lu, 2008*). Further study had reported that the knockdown of large ribosome subunit proteins, RPL7a, RPL11, RPL5, RPL14, RPL26 and RPL35 would impair the ribosomal biogenesis (*Bailly et al., 2016*). The activation of p53 may promote endochondral ossification through interaction with proteins related with matrix mineralization and chondrocyte maturation or apoptosis (*Li et al., 2012b*). However, rpl3 regulated the cell cycle progression and apoptosis induction through p53-independent manner (*Russo et al., 2016*; *Russo & Russo, 2017*). The combination of the data suggested that high abundance of ribosomal protein large subunits in sika deer improved antler development, involving the process of endochondral ossification. Intriguingly, the content of ribosomal proteins in red deer was at relatively low level in contrast to sika deer.

Difference of extracellular matrix proteins

Type IX collagen (COL9) and cartilage oligomeric matrix protein (COMP), which are specifically expressed in cartilage tissue, participate in the regulation of cartilage development (*Hecht et al., 2005*; *Myllyharju, 2014*). The skeletal deficiencies of mutations in COL9 or COMP strongly suggested that an adequate cartilage matrix was essential for extracellular matrix assembly and homeostasis (*Myllyharju, 2014*; *Shi et al., 2015*). Mutations in COL9 or COMP have been shown to cause multiple epiphyseal dysplasia (MED), which was a rare genetic disorder (*Briggs & Chapman, 2002*; *Briggs et al., 1995*). collagen IX knockout mouse model have implied that reduction of collagen IX changed the time course of callus differentiation during fracture repair (*Opolka et al., 2007*). The maturation of cartilage matrix was delayed in collagen IX knockout mice during the repair phase and the content of cartilage matrix was at relatively low concentration (*Opolka et al., 2007*). In addition, collagen IX will be gradually reduced as the mineralization of cartilage tissue. In the case of mutations in COMP, it reduced not only COMP secretion but type IX collagen in cartilage matrix (*Hecht et al., 2005*). In our antler proteomics study, the content of COL9 and COMP in sika deer was much higher than red deer (Table 1), which indicated that cartilage matrix assemble in sika deer was more active and red deer may possess more mineralized tissue zone. Consistently, laminin alpha 4 (LAMA4), that are the major constituent of hypertrophic chondrocytes, was highly expressed in sika deer (Table 1). It has reported that LAMA4 was implicated in chondrocyte mobility and chondrocyte hypertrophic zone (*Fuerst et al., 2011*; *Moazedi-Fuerst et al., 2016*). LAMA4 binding with integrins improved the chondrocyte mobility to promote the antler development, whilst the interaction between LAMA4 and integrins activated PI3K-AKT pathway to regulate the proliferation, differentiation and survival of chondrocytes. Osteopontin (OPN) is an extracellular matrix glycoprotein involved in bone remodeling (*Denhardt & Noda, 1998*). OPN was found to be secreted by different cell types as diverse as macrophages, epithelial

cells, and osteoclasts (*Rittling, 2011*; *Tardelli et al., 2016*). OPN, as migratory cytokine, stimulated the osteoclast migration to improve bone resorption. OPN is markedly expressed by MSCs and can be further up-regulated during the osteogenic differentiation (*Chen et al., 2014*; *Rickard et al., 1994*). OPN abundantly expressed in sika deer represented that the differentiation of MSCs putatively existed in the whole antler development stage. However, the abundance of OPN in red deer during late antler regeneration stage was significantly down-regulated, thereby retarded the mobility of osteoclast.

PI3K-Akt pathway

The Phosphoinositide 3-Kinase/Akt pathway is implicated in multiple cellular processes, including cell cycle entry, cell growth, cell survival and cell migration (*Cantley, 2002*; *Liu et al., 2009*). A set of hormones, cytokines, and extracellular matrix (ECM) proteins, as stimulators, can activate PI3K/Akt pathway (*Liu et al., 2009*). Furthermore, activation of Akt signaling in cartilage development enhanced the proliferation of chondrocytes and suppressed the hypertrophic differentiation of chondrocytes through the down-regulation of Runx2 expression, which participated in chondrocyte terminal differentiation (*Kita et al., 2008*). Compared with red deer, four ECM proteins in sika deer antler were significantly up-regulated (Table 1). Thereby, ECM proteins interacting with integrins can activate PI3K/Akt pathway through the action of focal adhesion kinase (FAK) in the sika deer. Meanwhile, the high abundance of guanine nucleotide-binding protein subunit gamma-12 (GNG12) in sika deer could can improve antler regeneration by activating PI3K (Table 1) (*Luo et al., 2018*). Another up-regulated protein belonging to PI3K/Akt pathway in sika deer, heat shock protein 90 (HSP90A), may contribute to mesenchymal stem cell migration to facilitate the antler development (Table 1) (*Gao et al., 2015*). Conversely, the up-regulation of serine/threonine protein phosphatase 2A (PP2A) in red deer will inhibit the dephosphorylate of Akt to inhibit PI3K/Akt pathway (Table 1) (*Eichhorn, Creyghton & Bernards, 2009*).

CONCLUSIONS

Our comparative proteomics study provided the comprehensive analysis of the differentially expressed antler proteins between sika deer and red deer based on label-free quantitative proteomics. The protein species and their abundance of sika deer antler were higher than in red deer. Differentially expressed proteins during the antler regeneration stage mainly participated in the regulation of several pathways including oxidative phosphorylation, ribosome, extracellular matrix interaction, and PI3K-Akt pathway.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This study was funded by the State Administration of Traditional Chinese Medicine of the People's Republic of China, Grant number ZYBZH-Y-JL-26. This work was also supported by the National Key Research and Development Program of China, Grant

number 2018YFC1706600. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
State Administration of Traditional Chinese Medicine of the People's Republic of China: ZYBZH-Y-JL-26.
National Key Research and Development Program of China: 2018YFC1706600.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Hang Su conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Xiaolei Tang performed the experiments, approved the final draft.
- Xiaocui Zhang contributed reagents/materials/analysis tools, approved the final draft.
- Li Liu contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.
- Li Jing analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.
- Daian Pan and Weijie Sun contributed reagents/materials/analysis tools, approved the final draft.
- Huinan He analyzed the data, contributed reagents/materials/analysis tools, approved the final draft.
- Chonghui Yang contributed reagents/materials/analysis tools, prepared figures and/or tables, approved the final draft.
- Daqing Zhao approved the final draft, technical support.
- He Zhang conceived and designed the experiments, prepared figures and/or tables, approved the final draft.
- Bin Qi conceived and designed the experiments, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The Animal Ethics Committee of Changchun University of Chinese Medicine provided full approval for this research.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Field experiments were approved by the Research Council of Changchun University of Chinese Medicine (project number: 17.03.001; Research Council Approval number:2017-120798).

Data Availability

The following information was supplied regarding data availability: PRIDE Archive: https://www.ebi.ac.uk/pride/archive/projects/PXD012090.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.7299#supplemental-information.

REFERENCES

Ayodele BA, Mirams M, Pagel CN, Mackie EJ. 2017. The vacuolar H(+) ATPase V0 subunit d2 is associated with chondrocyte hypertrophy and supports chondrocyte differentiation. *Bone Reports* **7**:98–107 DOI 10.1016/j.bonr.2017.08.002.

Bailly A, Perrin A, Bou Malhab LJ, Pion E, Larance M, Nagala M, Smith P, O'Donohue MF, Gleizes PE, Zomerdijk J, Lamond AI, Xirodimas DP. 2016. The NEDD8 inhibitor MLN4924 increases the size of the nucleolus and activates p53 through the ribosomal-Mdm2 pathway. *Oncogene* **35**:415–426 DOI 10.1038/onc.2015.104.

Balsa E, Marco R, Pereles-Clemente E, Szklarczyk R, Calvo E, Landazuri MO, Enriquez JA. 2012. NDUFA4 Is a subunit of complex IV of the mammalian electron transport Chain. *Cell Metabolism* **16**:378–386 DOI 10.1016/j.cmet.2012.07.015.

Banks WJ, Newbrey JW. 1983. In: Brown RD, ed. *Antlers development as unique modification of mammalian endochondral ossification*. Kingsville: Antler Development in Cervidae Cesar Kleburg Wildlife Research Institute, 231–260.

Boominathan A, Vanhoozer S, Basisty N, Powers K, Crampton AL, Wang X, Friedricks N, Schilling B, Brand MD, O'Connor MS. 2016. Stable nuclear expression of ATP8 and ATP6 genes rescues a mtDNA Complex V null mutant. *Nucleic Acids Research* **44**:9342–9357 DOI 10.1093/nar/gkw756.

Borsy A, Podani J, Steger V, Balla B, Horvath A, Kosa JP, Gyurjan I, Molnar A, Szabolcsi Z, Szabo L, Jako E, Zomborszky Z, Nagy J, Semsey S, Vellai T, Lakatos P, Orosz L. 2009. Identifying novel genes involved in both deer physiological and human pathological osteoporosis. *Molecular Genetics and Genomics* **281**:301–313 DOI 10.1007/s00438-008-0413-7.

Briggs MD, Chapman KL. 2002. Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations. *Human Mutation* **19**:465–478 DOI 10.1002/humu.10066.

Briggs MD, Hoffman SM, King LM, Olsen AS, Mohrenweiser H, Leroy JG, Mortier GR, Rimoin DL, Lachman RS, Gaines ES. 1995. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. *Nature Genetics* **10**:330–336 DOI 10.1038/ng0795-330.

Cantley LC. 2002. The phosphoinositide 3-kinase pathway. *Science* **296**:1655–1657 DOI 10.1126/science.296.5573.1655.

Castresana J, Lubben M, Saraste M, Higgins DG. 1994. Evolution of cytochrome oxidase, an enzyme older than atmospheric oxygen. *EMBO Journal* **13**:2516–2525 DOI 10.1002/j.1460-2075.1994.tb06541.x.

Causey DR, Kim JH, Stead DA, Martin SAM, Devlin RH, Macqueen DJ. 2018. Proteomic comparison of selective breeding and growth hormone transgenesis in fish: unique pathways to enhanced growth. *Journal of Proteomics* **192**:114–124 DOI 10.1016/j.jprot.2018.08.013.

Chen CT, Shih YR, Kuo TK, Lee OK, Wei YH. 2008. Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells. *Stem Cells* **26**:960–968 DOI 10.1634/stemcells.2007-0509.

Chen Q, Shou P, Zhang L, Xu C, Zheng C, Han Y, Li W, Huang Y, Zhang X, Shao C, Roberts AI, Rabson AB, Ren G, Zhang Y, Wang Y, Denhardt DT, Shi Y. 2014. An osteopontin-integrin interaction plays a critical role in directing adipogenesis and osteogenesis by mesenchymal stem cells. *Stem Cells* **32**:327–337 DOI 10.1002/stem.1567.

De la Cruz J, Karbstein K, Woolford Jr JL. 2015. Functions of ribosomal proteins in assembly of eukaryotic ribosomes *in vivo*. *Annual Review of Biochemistry* **84**:93–129 DOI 10.1146/annurev-biochem-060614-033917.

Deb-Choudhury S, Wang WY, Clerens S, McMahon C, Dyer JM, Li CY. 2015. Direct localisation of molecules in tissue sections of growing antler tips using MALDI imaging. *Molecular and Cellular Biochemistry* **409**:225–241 DOI 10.1007/s11010-015-2527-7.

Denhardt DT, Noda M. 1998. Osteopontin expression and function: role in bone remodeling. *Journal of Cellular Biochemistry* **72(Suppl 30-31)**:92–102 DOI 10.1002/(SICI)1097-4644(1998)72:30/31+<92::AID-JCB13>3.0.CO;2-A.

DiMauro S, Schon EA. 2003. Mitochondrial respiratory-chain diseases. *New England Journal of Medicine* **348**:2656–2668 DOI 10.1056/NEJMra022567.

Dong Z, Ba H, Zhang W, Coates D, Li C. 2016. iTRAQ-based quantitative proteomic analysis of the potentiated and dormant antler stem cells. *International Journal of Molecular Sciences* **17**:1778–1790 DOI 10.3390/ijms17111778.

Eichhorn PJ, Creyghton MP, Bernards R. 2009. Protein phosphatase 2A regulatory subunits and cancer. *Biochimica et Biophysica Acta/General Subjects* **1795**:1–15 DOI 10.1016/j.bbcan.2008.05.005.

Etzler JC, Bollo M, Holstein D, Deng JJ, Perez V, Lin DT, Richardson A, Bai Y, Lechleiter JD. 2017. Cyclophilin D over-expression increases mitochondrial complex III activity and accelerates supercomplex formation. *Archives of Biochemistry and Biophysics* **613**:61–68 DOI 10.1016/j.abb.2016.11.008.

Faucheux C, Nesbitt SA, Horton MA, Price JS. 2001. Cells in regenerating deer antler cartilage provide a microenvironment that supports osteoclast differentiation. *Journal of Experimental Biology* **204**:443–455.

Fennessy PF. 1984. Deer antlers: regeneration, function and evolution. *Journal of the Royal Society of New Zealand* **14**:290–291 DOI 10.1080/03036758.1984.10426948.

Fuerst FC, Gruber G, Stradner MH, Jones JC, Kremser ML, Angerer H, Setznagl D, Glehr M, Windhager R, Leithner A, Graninger WB. 2011. Regulation of MMP3 by laminin alpha 4 in human osteoarthritic cartilage. *Scandinavian Journal of Rheumatology* **40**:494–496 DOI 10.3109/03009742.2011.605392.

Fumagalli S, Ivanenkov VV, Teng T, Thomas G. 2012. Suprainduction of p53 by disruption of 40S and 60S ribosome biogenesis leads to the activation of a novel G2/M checkpoint. *Genes and Development* **26**:1028–1040 DOI 10.1101/gad.189951.112.

Gandhi SS, Muraresku C, McCormick EM, Falk MJ, McCormack SE. 2017. Risk factors for poor bone health in primary mitochondrial disease. *Journal of Inherited Metabolic Disease* **40**:673–683 DOI 10.1007/s10545-017-0046-2.

Gao F, Hu X, Xie X, Liu X, Wang J. 2015. Heat shock protein 90 stimulates rat mesenchymal stem cell migration via PI3K/Akt and ERK1/2 pathways. *Cell Biochemistry and Biophysics* **71**:481–489 DOI 10.1007/s12013-014-0228-6.

Gopal RK, Calvo SE, Shih AR, Chaves FL, McGuone D, Mick E, Pierce KA, Li Y, Garofalo A, Van Allen EM, Clish CB, Oliva E, Mootha VK. 2018. Early loss of mitochondrial complex I and rewiring of glutathione metabolism in renal oncocytoma. *Proceedings of the National Academy of Sciences of the United States of America* **115**:E6283–E6290 DOI 10.1073/pnas.1711888115.

Gotz S, Garcia-Gomez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talon M, Dopazo J, Conesa A. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research* **36**:3420–3435 DOI 10.1093/nar/gkn176.

Gyurjan I, Molnar A, Borsy A, Steger V, Hackler L, Zomborszky Z, Papp P, Duda E, Deak F, Lakatos P, Puskas LG, Orosz L. 2007. Gene expression dynamics in deer antler: mesenchymal differentiation toward chondrogenesis. *Molecular Genetics and Genomics* **277**:221–235 DOI 10.1007/s00438-006-0190-0.

He X, Li YH, Dai MS, Sun XX. 2016. Ribosomal protein L4 is a novel regulator of the MDM2-p53 loop. *Oncotarget* **7**:16217–16226 DOI 10.18632/oncotarget.7479.

Hecht JT, Hayes E, Haynes R, Cole WG. 2005. COMP mutations, chondrocyte function and cartilage matrix. *Matrix Biology* **23**:525–533 DOI 10.1016/j.matbio.2004.09.006.

Hu P, Wang T, Liu H, Xu J, Wang L, Zhao P, Xing X. 2019. Full-length transcriptome and microRNA sequencing reveal the specific gene-regulation network of velvet antler in sika deer with extremely different velvet antler weight. *Molecular Genetics and Genomics* **294**:431–443 DOI 10.1007/s00438-018-1520-8.

Khatter H, Myasnikov AG, Natchiar SK, Klaholz BP. 2015. Structure of the human 80S ribosome. *Nature* **520**:640–645 DOI 10.1038/nature14427.

Khincha P, Mirabello L, Ellis SR, Giri N, Brodie S, Chandrasekharappa S, Donovan F, Zhou WY, Hicks B, Boland J, Yeager M, Jones K, Zhu B, Wang MY, Alter BP, Savage SA. 2016. Novel and known ribosomal causes of diamond-blackfan anemia identified through comprehensive genomic characterization [Abstract 1495]. *Blood* **128**.

Kita K, Kimura T, Nakamura N, Yoshikawa H, Nakano T. 2008. PI3K/Akt signaling as a key regulatory pathway for chondrocyte terminal differentiation. *Genes to Cells* **13**:839–850 DOI 10.1111/j.1365-2443.2008.01209.x.

Kriaucionis S, Paterson A, Curtis J, Guy J, Macleod N, Bird A. 2006. Gene expression analysis exposes mitochondrial abnormalities in a mouse model of Rett syndrome. *Molecular and Cellular Biology* **26**:5033–5042 DOI 10.1128/MCB.01665-05.

Larance M, Lamond AI. 2015. Multidimensional proteomics for cell biology. *Nature Reviews Molecular Cell Biology* **16**:269–280 DOI 10.1038/nrm3970.

Li C. 2013. Histogenetic aspects of deer antler development. *Frontiers in Bioscience (Elite Ed)* **5**:479–489.

Li CY, Harper A, Puddick J, Wang WY, McMahon C. 2012a. Proteomes and signalling pathways of antler stem cells. *PLOS ONE* **7**:e30026 DOI 10.1371/journal.pone.0030026.

Li C, Mackintosh CG, Martin SK, Clark DE. 2007. Identification of key tissue type for antler regeneration through pedicle periosteum deletion. *Cell & Tissue Research* **328**:65–75 DOI 10.1007/s00441-006-0333-y.

Li C, Suttie JM. 1994. Light microscopic studies of pedicle and early first antler development in red deer (Cervus elaphus). *Anatomical Record-advances in Integrative Anatomy & Evolutionary Biology* **239**:198–215.

Li C, Suttie JM, Clark DE. 2004. Morphological observation of antler regeneration in red deer (Cervus elaphus). *Journal of Morphology* **262**:731–740 DOI 10.1002/jmor.10273.

Li C, Suttie JM, Clark DE. 2005. Histological examination of antler regeneration in red deer (Cervus elaphus). *The Anatomical Record Part A Discoveries in Molecular Cellular and Evolutionary Biology* **282**:163–174 DOI 10.1002/ar.a.20148.

Li C, Zhao H, Liu Z, McMahon C. 2014. Deer antler—a novel model for studying organ regeneration in mammals. *International Journal of Biochemistry and Cell Biology* **56**:111–122 DOI 10.1016/j.biocel.2014.07.007.

Li F, Lu Y, Ding M, Wu G, Sinha S, Wang S, Zheng Q. 2012b. Putative function of TAP63alpha during endochondral bone formation. *Gene* **495**:95–103 DOI 10.1016/j.gene.2011.12.057.

Liu P, Cheng H, Roberts TM, Zhao JJ. 2009. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nature Reviews Drug Discovery* **8**:627–644 DOI 10.1038/nrd2926.

Luo C, Zhao S, Dai W, Zheng N, Wang J. 2018. Proteomic analyses reveal GNG12 regulates cell growth and casein synthesis by activating the Leu-mediated mTORC1 signaling pathway. *Biochimica et Biophysica Acta - Proteins and Proteomics* **1866**:1092–1101 DOI 10.1016/j.bbapap.2018.08.013.

Miyazaki T, Neff L, Tanaka S, Horne WC, Baron R. 2003. Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. *Journal of Cell Biology* **160**:709–718 DOI 10.1083/jcb.200209098.

Moazedi-Fuerst FC, Gruber G, Stradner MH, Guidolin D, Jones JC, Bodo K, Wagner K, Peischler D, Krischan V, Weber J, Sadoghi P, Glehr M, Leithner A, Graninger WB. 2016. Effect of Laminin-A4 inhibition on cluster formation of human osteoarthritic chondrocytes. *Journal of Orthopaedic Research* **34**:419–426 DOI 10.1002/jor.23036.

Molnar A, Gyurjan I, Korpos E, Borsy A, Steger V, Buzas Z, Kiss I, Zomborszky Z, Papp P, Deak F, Orosz L. 2007. Identification of differentially expressed genes in the developing antler of red deer Cervus elaphus. *Molecular Genetics and Genomics* **277**:237–248 DOI 10.1007/s00438-006-0193-x.

Morita K, Miyamoto T, Fujita N, Kubota Y, Ito K, Takubo K, Miyamoto K, Ninomiya K, Suzuki T, Iwasaki R, Yagi M, Takaishi H, Toyama Y, Suda T. 2007. Reactive oxygen species induce chondrocyte hypertrophy in endochondral ossification. *Journal of Experimetnal Medicine* **204**:1613–1623 DOI 10.1084/jem.20062525.

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research* **35**:W182–W185 DOI 10.1093/nar/gkm321.

Myllyharju J. 2014. Extracellular matrix and developing growth plate. *Current Osteoporosis Reports* **12**:439–445 DOI 10.1007/s11914-014-0232-1.

Opolka A, Ratzinger S, Schubert T, Spiegel HU, Grifka J, Bruckner P, Probst A, Grassel S. 2007. Collagen IX is indispensable for timely maturation of cartilage during fracture repair in mice. *Matrix Biology* **26**:85–95 DOI 10.1016/j.matbio.2006.09.010.

Pan JX, Tang F, Xiong F, Xiong L, Zeng P, Wang B, Zhao K, Guo H, Shun C, Xia WF, Mei L, Xiong WC. 2018. APP promotes osteoblast survival and bone formation by regulating mitochondrial function and preventing oxidative stress. *Cell Death & Disease* **9**:1077–1094 DOI 10.1038/s41419-018-1123-7.

Pattappa G, Heywood HK, De Bruijn JD, Lee DA. 2011. The metabolism of human mesenchymal stem cells during proliferation and differentiation. *Journal of Cellular Physiology* **226**:2562–2570 DOI 10.1002/jcp.22605.

Pharmacopoeia Commission of the Ministry of Health of the People's Republic of China. 2015. *Pharmacopeia of the Peopole's Republic of China*. Beijing: China Medical Science Press.

Qian C, Chen HM, Johs A, Lu X, An J, Pierce EM, Parks JM, Elias DA, Hettich RL, Gu BH. 2018. Quantitative proteomic analysis of biological processes and responses of the Bacterium Desulfovibrio desulfuricans ND132 upon deletion of its mercury methylation genes. *Proteomics* **18**:ARTN 1700479 DOI 10.1002/pmic.201700479.

Rickard DJ, Sullivan TA, Shenker BJ, Leboy PS, Kazhdan I. 1994. Induction of rapid osteoblast differentiation in rat bone marrow stromal cell cultures by dexamethasone and BMP-2. *Developmental Biology* **161**:218–228 DOI 10.1006/dbio.1994.1022.

Rittling SR. 2011. Osteopontin in macrophage function. *Expert Reviews in Molecular Medicine* **13**:e15 DOI 10.1017/S1462399411001839.

Rucklidge GJ, Milne G, Bos KJ, Farquharson C, Robins SP. 1997. Deer antler does not represent a typical endochondral growth system: immunoidentification of collagen type X but little collagen type II in growing antler tissue. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **118**:303–308 DOI 10.1016/S0305-0491(97)00171-5.

Ruggero D, Shimamura A. 2014. Marrow failure: a window into ribosome biology. *Blood* **124**:2784–2792 DOI 10.1182/blood-2014-04-526301.

Russo A, Pagliara V, Albano F, Esposito D, Sagar V, Loreni F, Irace C, Santamaria R, Russo G. 2016. Regulatory role of rpL3 in cell response to nucleolar stress induced by Act D in tumor cells lacking functional p53. *Cell Cycle* **15**:41–51 DOI 10.1080/15384101.2015.1120926.

Russo A, Russo G. 2017. Ribosomal proteins control or bypass p53 during nucleolar stress. *International Journal of Molecular Sciences* **18**:ARTN 140 DOI 10.3390/ijms18010140.

Sen S, Domingues CC, Rouphael C, Chou C, Kim C, Yadava N. 2015. Genetic modification of human mesenchymal stem cells helps to reduce adiposity and improve glucose tolerance in an obese diabetic mouse model. *Stem Cell Research & Therapy* **6**:242–255 DOI 10.1186/s13287-015-0224-9.

Shi XW, Zhang F, Lv AL, Wen Y, Guo X. 2015. COL9A1 gene polymorphism is associated with Kashin-Beck disease in a Northwest Chinese Han population. *PLOS ONE* **10**:ARTN e0120365 DOI 10.1371/journal.pone.0120365.

Shibanuma M, Inoue A, Ushida K, Uchida T, Ishikawa F, Mori K, Nose K. 2011. Importance of mitochondrial dysfunction in oxidative stress response: a comparative study of gene expression profiles. *Free Radical Research* **45**:672–680 DOI 10.3109/10715762.2011.564169.

Steger V, Molnar A, Borsy A, Gyurjan I, Szabolcsi Z, Dancs G, Molnar J, Papp P, Nagy J, Puskas L, Barta E, Zomborszky Z, Horn P, Podani J, Semsey S, Lakatos P, Orosz L. 2010. Antler development and coupled osteoporosis in the skeleton of red deer Cervus elaphus: expression dynamics for regulatory and effector genes. *Molecular Genetics and Genomics* **284**:273–287 DOI 10.1007/s00438-010-0565-0.

Sun XX, Dai MS, Lu H. 2008. Mycophenolic acid activation of p53 requires ribosomal proteins L5 and L11. *Journal of Biological Chemistry* **283**:12387–12392 DOI 10.1074/jbc.M801387200.

Tardelli M, Zeyda K, Moreno-Viedma V, Wanko B, Grun NG, Staffler G, Zeyda M, Stulnig TM. 2016. Osteopontin is a key player for local adipose tissue macrophage proliferation in obesity. *Molecular Metabolism* **5**:1131–1137 DOI 10.1016/j.molmet.2016.09.003.

Tyanova S, Temu T, Cox J. 2016. The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. *Nature Protocols* **11**:2301–2319 DOI 10.1038/nprot.2016.136.

Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J. 2016. The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature Methods* **13**:731–740 DOI 10.1038/nmeth.3901.

Vizcaino JA, Csordas A, Del-Toro N, Dianes JA, Griss J, Lavidas I, Mayer G, Perez-Riverol Y, Reisinger F, Ternent T, Xu QW, Wang R, Hermjakob H. 2016. 2016 update of the PRIDE database and its related tools. *Nucleic Acids Research* **44(D1)**:D447–D456 DOI 10.1093/nar/gkv1145.

Weiss H, Wester-Rosenloef L, Koch C, Koch F, Baltrusch S, Tiedge M, Ibrahim S. 2012. The mitochondrial Atp8 mutation induces mitochondrial ROS generation,

secretory dysfunction, and beta-cell mass adaptation in conplastic B6-mtFVB mice. *Endocrinology* **153**:4666–4676 DOI 10.1210/en.2012-1296.

Wisniewski JR, Zougman A, Nagaraj N, Mann M. 2009. Universal sample preparation method for proteome analysis. *Nature Methods* **6**:359–362 DOI 10.1038/nmeth.1322.

Yao BJ, Zhao Y, Wang Q, Zhang M, Liu MC, Liu HL, Li J. 2012. De novo characterization of the antler tip of Chinese Sika deer transcriptome and analysis of gene expression related to rapid growth. *Molecular and Cellular Biochemistry* **364**:93–100 DOI 10.1007/s11010-011-1209-3.

Zhang J, Ma J, Long K, Jin L, Liu Y, Zhou C, Tian S, Chen L, Luo Z, Tang Q, Jiang A, Wang X, Wang D, Jiang Z, Wang J, Li X, Li M. 2016. Dynamic gene expression profiles during postnatal development of porcine subcutaneous adipose. *PeerJ* **4**:e1768 DOI 10.7717/peerj.1768.