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RESEARCH ARTICLE SUMMARY

RUMINANT GENOMICS

Genetic basis of ruminant headgear and rapid antler regeneration

Yu Wang*, Chenzhou Zhang*, Nini Wang*, Zhipeng Li*, Rasmus Heller*, Rong Liu*, Yue Zhao*, Jiangang Han*, Xiangyu Pan, Zhuqing Zheng, Xueqin Dai, Ceshi Chen, Mingle Dou, Shujun Peng, Xianqing Chen, Jing Liu, Ming Li, Kun Wang, Chang Liu, Zeshan Lin, Lei Chen, Fei Hao, Wenbo Zhu, Chengchuang Song, Chen Zhao, Chengli Zheng, Jianming Wang, Shengwei Hu, Cunyuan Li, Hui Yang, Lin Jiang, Guangyu Li, Mingjun Liu, Tad S. Sonstegard, Guojie Zhang, Yu Jiang†, Wen Wang†, Qiang Qiu†

INTRODUCTION: All pecoran families, except the Moschidae, have cranial appendages or headgear, a unique structure among mammals that has a different morphology in each family (ossicones in giraffids, pronghorns in pronghorn, antlers in cervids, and horns in bovids). Moreover, the deer antler is the only completely regenerable organ found in mammals, thus providing a unique model for regenerative biology. Antlers also have extremely rapid growth rates (~1.7 cm/day in red deer),

with rates of cell proliferation that surpass even cancerous tissue growth. Cervids also have low cancer rates. The relation between a tight regulation of antler growth and inhibition of oncogenesis in deer may provide insights for cancer prevention and therapy in humans and other organisms.

RATIONALE: We obtained 221 transcriptomes from bovids and cervids and sequenced three genomes representing the two pecoran lin-

eages that convergently lack headgear. Comparing the data with a large set of ruminant genomes, including nine cervids, we detect genetic changes (lineage-specific positively selected genes and conserved elements) in pecorans with headgear (PWH), particularly cervids. Using the observed genetic changes and gene expression in headgear, we explore the genomic basis of ruminant headgear origin and antler regeneration.
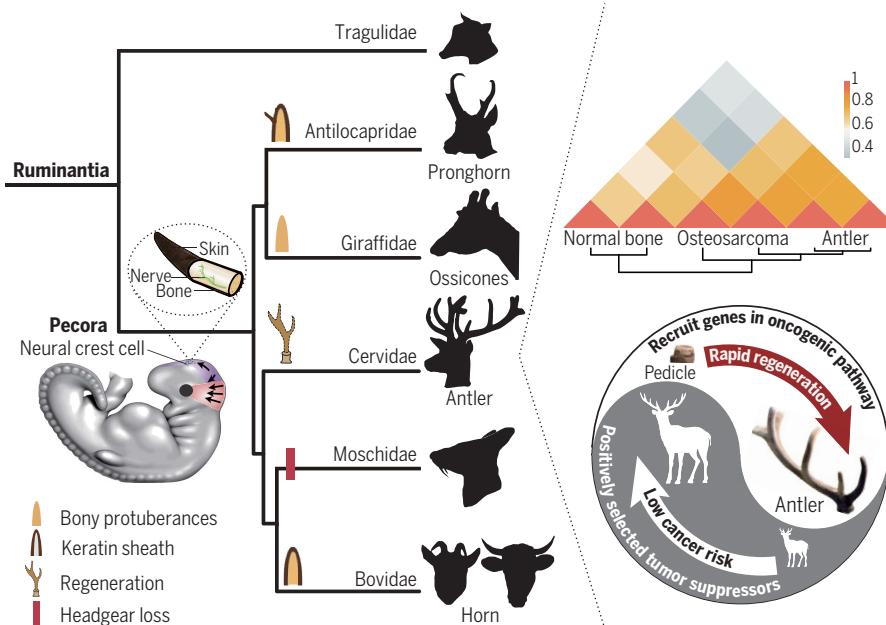
RESULTS: We find that highly or specifically expressed genes in horns and antlers are most

frequently coexpressed in bone, skin, nerve tissues, and testis. Many genes under positive selection in PWH (e.g., *OLIG1*, *OTOP3*), PWH-specific genes associated with highly conserved elements (e.g., *HOXD* gene cluster, *SNAI2*, *TWIST1*, *SOX9*), and genes highly or specifically expressed in headgear (*RXFP2*, *SOX10*, *NGFR*) are involved in neural functions. In addition, *RXFP2*, which is specifically expressed in headgear and testis, was convergently pseudogenized in the headgearless lineages of Moschidae and Hydropotinae. The expression profile of antler is more correlated with osteocarcinoma than with normal bone tissue expression profiles. A number of proto-oncogenes (*FOS*, *REL*, *FAM83A*) and tumor suppression genes have been positively selected in cervids, especially several cofactor genes (*PML*, *NMT2*, and *CD2AP)* and regulator genes (*ELOVL6*, *S100A8*, *ISG15*, *CNOT3*, and *CCDC69*) of the p53 tumor suppressor, suggesting that these adaptive changes may enhance cancer resistance in deer.

CONCLUSION: Together, the phylogeny, gene expression profiles, and convergent headgear losses support a single evolutionary origin of the ruminant headgear. Pecoran headgear likely share a common cellular origin from neural crest stem cells, and the determination of the chondrogenic and neural lineages is important for headgear development. In addition, cervid-specific genetic changes in tumor suppressor and proto-oncogenes imply that the regenerative properties of antler tissue exploit oncogenesis pathways. Our study reveals genetic mechanisms underlying the evolutionary, developmental, and histological origin of ruminant headgear, as well as antler regeneration. The identified genes and their unique mutations provide guidelines for future functional studies of headgear development, regeneration of mammalian organs, and oncogenesis. ∎

Neural crest cellular origin of ruminant headgear and the tight control of rapid antler regeneration and low cancer risk in cervids. (Left) Phylogenomic relationships of the six ruminant families. Anatomic features of family-specific headgear are depicted, showing that headgear of ruminants share tissue and cellular origins. (Upper right) The gene expression profile of antler correlates more strongly with osteocarcinoma than with normal bone tissue. (Lower right) The balance between rapid antler regeneration, which depends on genes in the oncogenic pathway, and reduced cancer risk, which may involve adaptive evolution of tumor suppressor genes.

RESEARCH ARTICLE

Genetic basis of ruminant headgear and rapid antler regeneration

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Ruminants are the only extant mammalian group possessing bony (osseous) headgear. We obtained 221 transcriptomes from bovids and cervids and sequenced three genomes representing the only two pecoran lineages that convergently lack headgear. Comparative analyses reveal that bovid horns and cervid antlers share similar gene expression profiles and a common cellular basis developed from neural crest stem cells. The rapid regenerative properties of antler tissue involve exploitation of oncogenetic pathways, and at the same time some tumor suppressor genes are under strong selection in deer. These results provide insights into the evolutionary origin of ruminant headgear as well as mammalian organ regeneration and oncogenesis.

Ruminants are the only group of extant mammals with osseous cranial appendages, which are collectively termed headgear [1]. Osseous headgear are exclusively found in the pecorans (all ruminants, excluding Tragulidae), a group that radiated ~23.3 million to 20.8 million years (Ma) ago [2] (Fig. 1). Each family in the pecoran group exhibits a distinct headgear morphology [3]. The ossicones of Giraffidae consist of bony protuberances covered only by skin and hair. The pronghorns of Antilocapridae are composed of bone covered by skin, hair, and an annually deciduous forked keratinous sheath. The horns of Bovidae also have a bony core but are covered by a nondeciduous, nonforked keratinous sheath. The antlers of Cervidae are wholly deciduous, regenerating annually as an outgrowth of bone from the frontal skull. Despite this variation, all these types of pecoran headgear (including those of extinct species) share characteristic features, such as their frontal cranial position and a bony core covered by integument (fig. S1). The evolutionary origin of headgear, specifically whether headgear evolved only once or multiple times, has been a matter of considerable scientific discussion [1]. Resolving this has proved challenging because of the lack of consensus regarding the family-level phylogeny of the ruminants.

Results

A comprehensive phylogenetic analysis of the ruminants [2] resolved the family-level topology and shows that the most phylogenetically parsimonious hypothesis is a single origin of headgear in pecorans followed by two independent losses (Fig. 1). Multiple independent origins would be at odds with the rapid radiation of the five Pecora families, which took place during an ~2.5-million-year interval (23.3 to 20.8 Ma ago), and furthermore it is difficult to explain why headgear would have evolved multiple times in pecorans yet be absent in all other mammalian

taxa. The Ruminant Genome Project [2] provides an opportunity to investigate the genomic background of ruminant headgear evolution and address its implications in organ regeneration.

Shared gene recruitment in horns and antlers

The evolutionary origin of any new organ typically depends on the recruitment of genes that were originally expressed in other tissues [4]. To identify genes recruited in bovid horns, we compared 181 transcriptomes obtained in this study—representing 16 tissues and including 7 transcriptomes of goat horn sprouts, 61 of other goat tissues, 3 of sheep horn sprouts, and 110 of other sheep tissues—and added 49 published sheep transcriptomes [5] (table S1). In addition, transcriptomes from two fetal sheep horn buds and adjacent frontal skin tissues were sequenced to identify differentially expressed genes (DEGs) between these two tissue types at this important developmental stage. For cervid antlers, we sequenced 20 roe deer (Capreolus capreolus) and 20 sika deer (Cervus nippon) samples representing 16 tissues, including neonatal antlers (table S1). Genes specifically expressed in headgear tissues (hereafter headgear-specific genes) were defined as those that have a τ index exceeding 0.8 and are expressed most strongly or second most strongly in headgear tissues [5]. We identified 624 horn-specific genes (table S2), and these were most highly coexpressed in bone, skin, testis, and brain tissues (Fig. 2A and fig. S2A). We also identified 761 antler-specific genes that were most highly coexpressed in the same four tissues (Fig. 2A, fig. S2B, and table S3). In addition, 201 headgear-specific expression genes were shared by both antler and horn tissues (fig. S3A), and these genes were enriched in bone development, skin development, and neurogenesis pathways (Fisher's exact test, adjusted P value < 1×10^{-5}) (fig. S3B and table S4). The DEGs (table S5) between fetal sheep horn bud and adjacent frontal skin tissues were enriched in nerve development pathways (fig. S4 and table S6). Histological analysis of cattle fetal horn buds suggested that, in contrast to the frontal skin of polled fetuses, neural tissue exists only in the horn buds [6]. Overall, the gene expression results suggest that the development of both kinds of headgear considered here (horns and antlers) depends on similar gene expression profiles, largely recruited from nerve, bone, and skin tissues.

A common genetic and cellular basis of headgear

To identify the genetic basis of headgear evolution, we used the branch-site likelihood ratio test [5] to detect positively selected genes shared only by pecorans with headgear (PWH). A total of 240 genes were identified as positively selected in PWH (table S7). Enriched functional Gene Ontology (GO) categories of these genes included biomineral tissue development (Fisher's exact test, adjusted P value = 4.9×10^{-2}) (table S8), providing further evidence that the evolution of osseous headgear depends on bone development.

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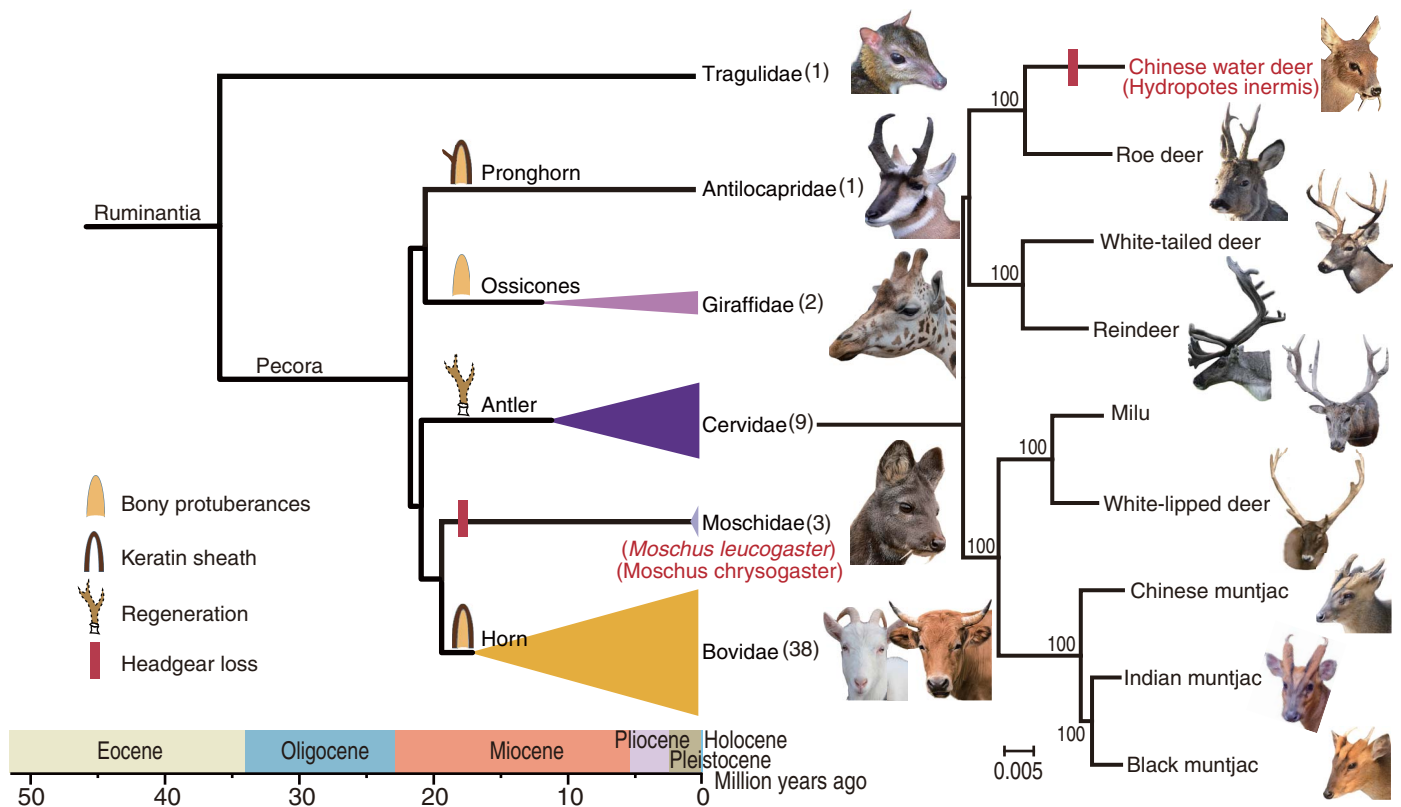
Fig. 1. Phylogenomic placement of species without headgear in the Ruminantia and Cervidae. (**Left**) The phylogenomic relationships of the six ruminant families from (*2*). (**Right**) Maximum-likelihood tree for the nine studied cervid species obtained using 3,316,385 four-fold degenerate sites. The anatomic structure of headgear in each family is depicted, including the keratin sheath of Antilocapridae and Bovidae and the regenerable antler of Cervidae. The red bar indicates secondary headgear loss and the red text highlights the de novo assembled species in this study. The number of species in each family used in this study is indicated in parentheses. Sources and credits for species photos are listed in table S26.

Among these genes, we found that *OTOP3* was under positive selection in PWH along with a headgear-specific gene (fig. S5 and tables S2, S3, and S7). *OTOP3* is a member of the otopetrin gene family, which regulates biomineralization processes (*7*). Of the eight Pecora-specific amino acid mutations in the OTOP3 protein, four are located in the otopetrin functional domain (PF03189) (fig. S5). *OTOP3* is expressed in the neural crest of *Xenopus* embryos, suggesting a role in neural crest function (*8*). *OLIG1* (fig. S6), another positively selected gene in PWH, is also related to neural crest differentiation pathways (*9*). A previous study showed that a 212–base pair (bp) duplication (~65 kbp) flanking the *OLIG1* gene region is the causal mutation of polled cattle, i.e., cattle that completely lack horns (*10*). We detected nine distinct amino acid changes in *OLIG1* in the inferred common ancestor of Pecora, resulting in domain structure changes as revealed by protein structure homology modeling (fig. S6). Given that the frontal cranial bones are derived from cells of the cranial neural crest (*11*), changes in the *OTOP3* and *OLIG1* genes likely played crucial roles in the evolution and development of pecoran headgear (Fig. 2B).

We also identified 8732 lineage-specific highly conserved elements (HCEs) (≥20 bp) (table S9) in PWH using the phylogenetic hidden Markov model approach (*5*). The PWH-specific HCE-associated genes are enriched in the signaling pathway regulating the pluripotency of stem cells (Fisher's exact test, adjusted *P* value = 2.5×10^{-9}) and transforming growth factor–β (TGF-β) (Fisher's exact test, adjusted *P* value = 3.5×10^{-7}) (table S10). The TGF-β signaling pathway plays an important role in bone formation and the regulation of cranial neural crest cell proliferation during frontal bone development (*12*). The PWH-specific HCE-associated genes *SNAI2*, *TWIST1*, *SOX9*, and the *HOXD* gene cluster are involved in neural crest cell migration (*9*) (Fig. 2B and table S9). Specifically, we identified a PWH-specific 25-bp HCE 15 kbp downstream from the *HOXD* gene cluster (fig. S7A) that serves as a master regulator in neural crest patterning, particularly in the cranial region (*13*). Further analysis indicates that this element resulted from a 3.6-kbp Pecora-specific transposable element insertion (fig. S7, B and C) located in the candidate region causing the four-horned phenotype in sheep (*14*). These results suggest that the evolution of PWH-specific regulatory elements may also play a role in reprogramming neural crest cells to develop into headgear.

In addition, we found that six neural crest cell migration-related genes (*SOX10*, *SNAI1*, *SNAI2*, *TFAP2A*, *NGFR*, and *COL11A2*) are specifically expressed in headgear (tables S2 and S3). *SOX10*, *SNAI1*, and *TFAP2A* are highly expressed in fetal sheep horn buds but not in adjacent skin tissue (fig. S4 and table S5). Notably, *SOX10* and *NGFR* are used as marker genes of neural crest cells (*15*), and our immunohistochemical analysis confirmed that *SOX10*- and *NGFR*-positive cells are present in the embryonic horn bud of sheep (fig. S8). In addition, two headgear-specific genes, *FOXL2* and *TWIST1*, are related to horn abnormalities (*16*, *17*) (tables S2 and S3) and both interact with *SOX9*, a marker gene for the determination of the chondrogenic lineage in the cranial neural crest (*18*). From the results of our comparative genomic analysis and previous findings regarding horn-related genes (*10*, *14*, *16*, *17*), we conclude that pecoran headgear likely share a common cellular origin in neural crest stem cells (Fig. 2B). This supports a single evolutionary origin for pecoran headgear despite their morphological diversity.

Convergent pseudogenization led to secondary loss of headgear

We supplemented the 51 ruminant genomes in the Ruminant Genome Project (*2*) with a high-quality reference genome from a secondarily antlerless species, the Chinese water deer (*Hydropotes inermis*) of the cervid subfamily Hydropotinae
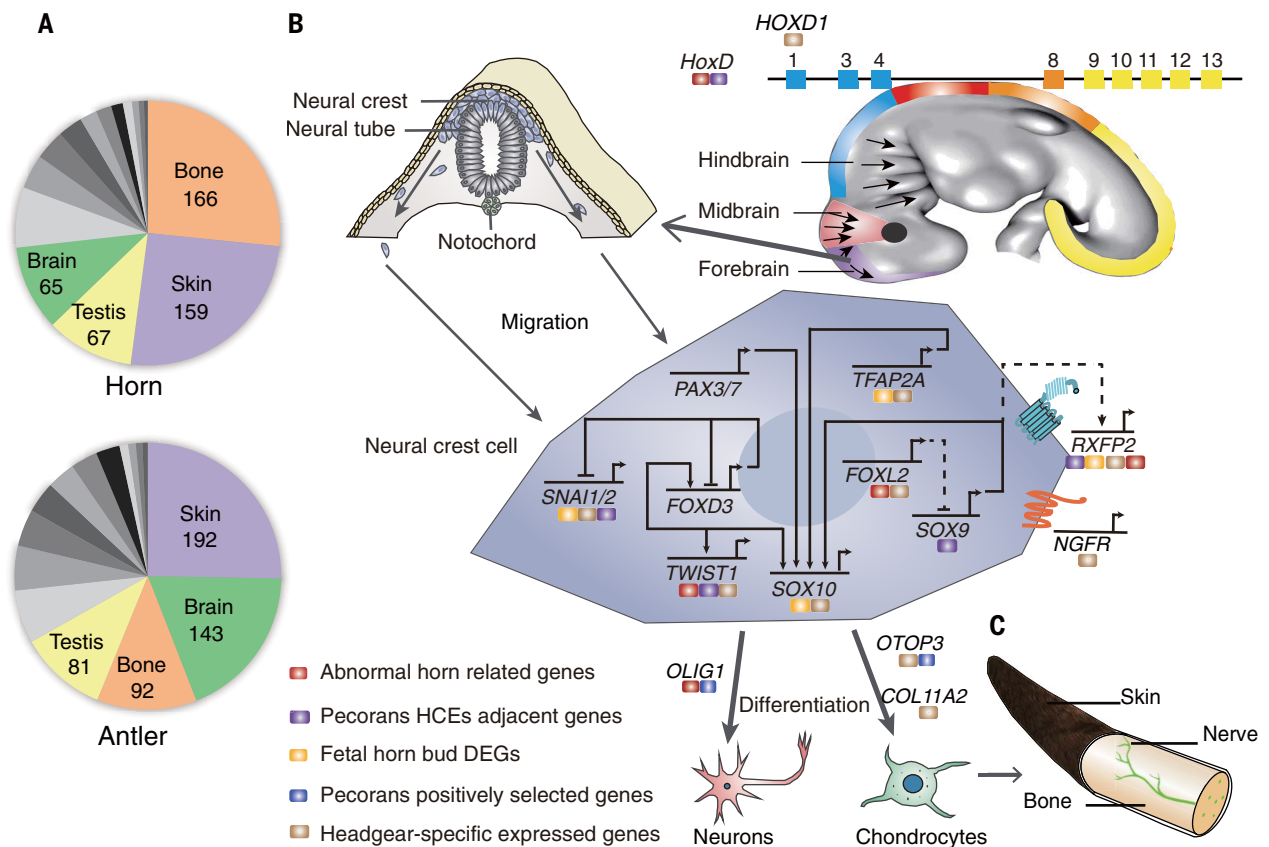
Fig. 2. Gene recruitment and cellular origin of ruminant headgear.
(**A**) Genes recruited to headgear from different organs. Bone, skin, testis, brain, and others are marked as orange, purple, yellow, green, and various shades of gray, respectively. (**B**) Diagram of neural crest cell genes involved in headgear development. The *HOXD* gene cluster is depicted as the hypothesized master regulator of headgear. Genes annotated in the neural crest cell migration and differentiation pathway are labeled with different colors to indicate positively selected genes (PSGs), HCEs, fetal horn bud DEGs, and genes related to abnormal horn development. The solid arrows represent known neural crest cell pathways. The dashed arrows indicate the pathway known to be related to other cell types that has not been recorded in neural crest cells. (**C**) Diagram of the headgear of the ruminant ancestor, mainly containing bone, skin, and nerve tissues.

(fig. S9 and tables S11 to S13), and two contig-level genomes of Moschidae species (*Moschus chrysogaster* and *M. leucogaster)* (table S14). From the high-confidence phylogenetic tree obtained with whole-genome data (Fig. 1), it is clear that Moschidae and Hydropotinae have independently lost their headgear. To explain this convergent feature and learn more about genes controlling headgear, we investigated pseudogenization in the shape of premature stop codons and frameshifts in both of these lineages (Fig. 1 and tables S15 and S16). Of 289 pseudogenes identified in these two distant lineages, *RXFP2* was the only headgear-specific gene that was convergently pseudogenized, although the mutations occur at different sites of the *RXFP2* gene in these lineages (fig. S10). *RXFP2*, which our transcriptomic data indicate was recruited from testis tissue into horn and antler, is highly expressed in the fetal sheep horn bud (fig. S10) and has two PWH-specific HCEs in its intron region, one of them with a binding motif for the horn-specific gene *ARNT* (fig. S11). Previous studies identified *RXFP2* as a sexually selected gene associated with horn morphology in bighorn sheep

(*Ovis canadensis*) (*19, 20*). Additionally, a 1.8-kbp insertion in the 3′ untranslated region (3′UTR) of *RXFP2* is associated with lack of horns in sheep (*21*). Collectively, these results indicate that pseudogenization of *RXFP2* is the most likely functional mechanism behind the convergent secondary loss of headgear in the Moschidae and Hydropotinae lineages.

Neural processes involved in antler regeneration

The deer antler is the only completely regenerable organ found in mammals and thus provides a unique model for regenerative biology. Antler regeneration is a stem cell–based process (*22*), and we demonstrated that antler stem cells may originate from cranial neural crest cells, which have the potential to rapidly proliferate and differentiate into cartilage and neural cells (Fig. 2B). Notably, growing antlers are richly innervated by sensory fibers, and resection of the antler pedicle sensory nerve markedly reduces antler regeneration and stunts antler size (*23*). Both antler-specific expression genes (table S3) and cervid-specific HCE-associated genes (table S17)

are enriched in annotation terms associated with the axon guidance pathway, particularly the genes coding for key guidance molecules for axon growth: slits, ephrins, and semaphorins (Fig. 3A and tables S18 and S19). In addition, the top eight rapidly evolving genes in cervids with adjacent HCEs are all related to neural functions (fig. S12 and table S20). We also found that the nerve growth factor receptor (*NGFR*) gene is strongly and specifically expressed in antlers (table S3). This is consistent with findings that neural growth factors promote the growth of antler nerves (*24*), and these lines of evidence corroborate the involvement of neural processes in annual antler regeneration.

Similarities between antler growth and cancer cell growth programs

Antlers grow extremely quickly, as exemplified by red deer antlers, which have average growth rates of 1.7 cm/day and can reach a weight of up to 30 kg (*25*). These antlers regrow annually (from spring to summer) owing to very fast cell proliferation (Fig. 3B) that surpasses even cancerous tissue growth (*25*). Antler growth mainly

Fig. 3. Oncogenesis and neurogenesis pathways involved in rapid antler regeneration. (**A** and **D**) Positively selected genes in cervids (blue), cervid-specific HCE-associated genes (yellow), and antler-specific expression genes (green) in axon guidance (A) and oncogenesis (D) pathways. (**B**) Annual antler regeneration cycle. Antlers are shed in winter and regenerate in spring, growing most rapidly in summer. Antlers then calcify and shed their velvet in autumn. (**C**) The anatomy of the antler. The source and credit for the red deer photo are listed in table S26.
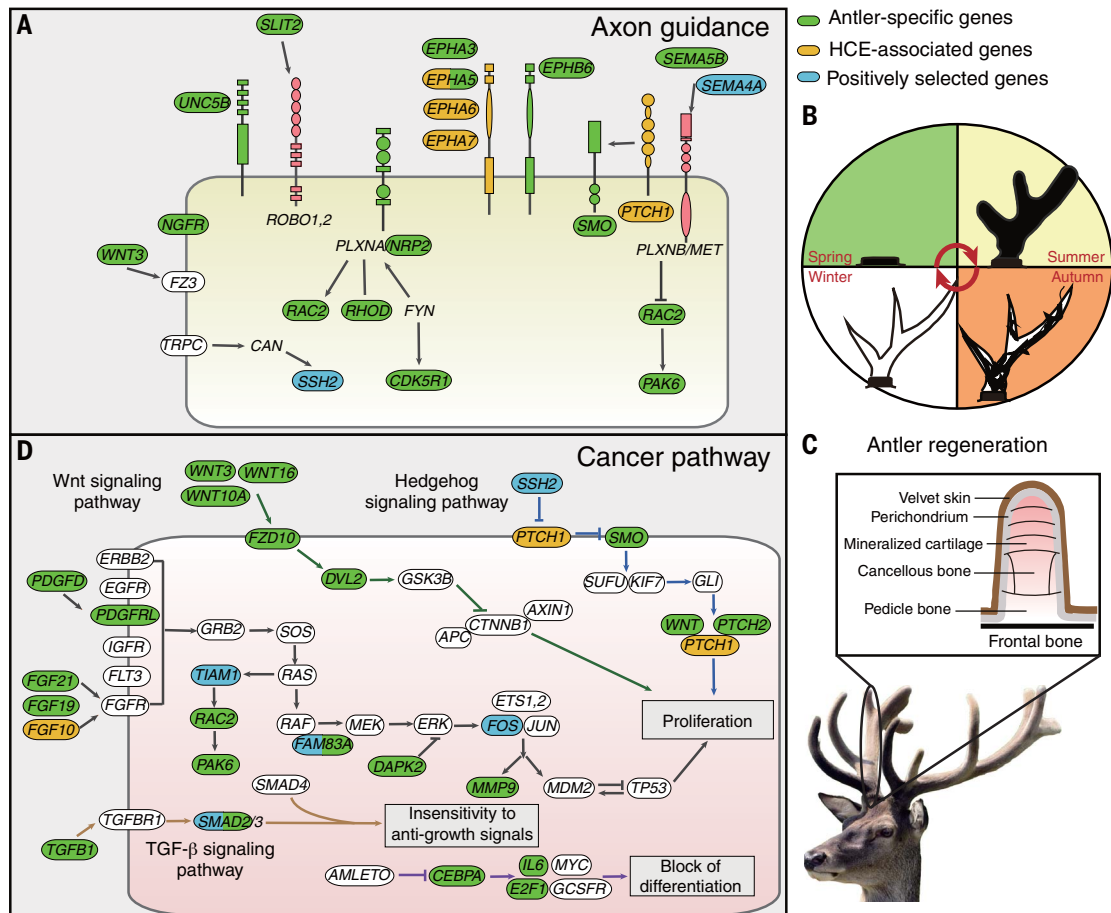
proceeds by chondrocyte proliferation and ossification (Fig. 3C) and thus provides a research model for osteosarcoma. We found a higher correlation between the gene expression profiles of antler and osteosarcoma ($r = 0.67$ to 0.78) than between those of antler and normal bone tissues ($r = 0.33$ to 0.47) (fig. S13), showing similar patterns of developmental programs in antler growth and oncogenesis.

We found evidence that three proto-oncogenes (*FOS*, *FAM83A*, and *REL*) were under positive selection in the cervid ancestor (fig. S14 and table S21). Of these, *FOS* acts as a downstream growth factor signaling pathway that regulates cell proliferation and differentiation. Overexpression of *FOS* induces osteosarcoma formation in mice via the transformation of chondroblasts and osteoblasts (*26*). Additionally, *FAM83A* has been identified as an oncogene involved in the epidermal growth factor receptor (EGFR) signaling pathway (*27*). We also observed antler-specific expression of five growth factor and receptor genes (*FGF19*, *FGF21*, *FGFBP3*, *PDGFD*, and *PDGFRL*) that play important roles in driving cancer cell proliferation and survival (*28*) (table S3). In addition, a cervid-specific HCE is located in the 3′UTR of *NOVA1*, which is believed to activate telomerase and promote tumor growth in vivo (*29*) (figs. S15 and S16 and table S17). Taken together, these cell growth–associated cervid-specific changes and

the expression of tumor promoters in antlers (Fig. 3D) indicate that the rapid proliferation of cells required for rapid antler growth has similarities with cancer cell growth programs.

Regulation of antler growth may confer cancer resistance

Cancer frequency records from both the Philadelphia and San Diego zoos indicate that cancer incidence rates are ~5 times lower in cervids than in other mammals (0.4 to 0.8% and 2.1 to 4.6%, respectively) (*30*, *31*). This tentatively suggests that the precisely regulated cell growth regulators required for controlled rapid antler regeneration may confer protection against the development of cancers in cervids because of specific genetic changes relevant to cancer avoidance. Accordingly, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of DEGs between antler and osteosarcoma shows enrichment for cancer- and metabolism-related pathways (fig. S13B and table S22).

We also observed that many tumor suppressor genes are under positive selection (table S21) and strongly expressed in antlers. Among these, the tumor suppressor gene *PML* has 11 cervid-specific nonsynonymous changes and carries the strongest signal of positive selection detected in cervids (Fig. 4A and table S21). PML is a transcriptional coactivator of p53, and its overexpression enhances p53 transcriptional activation and leads

to cell growth arrest (*32*). The *TP53* (encoding the protein p53) signaling pathway plays a central role in regulating cell division and preventing tumor formation (*33*). We observed that three p53 cofactor genes (*PML*, *NMT2*, and *CD2AP*) and five p53 regulator genes (*ELOVL6*, *S100A8*, *ISG15*, *CNOT3*, and *CCDC69*) were under positive selection in the cervid lineage and expressed in antlers (Fig. 4B and table S21). We also noticed that *TP53* itself was identified as a rapidly evolving gene in the cervid lineage from the evolutionary analysis of 51 ruminant genomes (*2*).

In addition to the *TP53* pathway–related genes, we also observed several other tumor suppressor genes that were under selection in the cervid lineage and expressed in antlers. One such gene, *ADAMTS18* (Fig. 4A), belongs to the *ADAMTS* family, which encompasses disintegrin and metalloproteinase-like proteinases that inhibit growth of carcinoma cells by controlling the structure and function of the extracellular matrix (ECM) and regulating the tumor microenvironment (*34*). The components and function of the ECM are known to play an important role in cancer resistance in the naked mole-rat (*35*). In addition, the *ADAMTS* family members *ADAMTS2*, *ADAMTS4*, *ADAMTS12*, *ADAMTS14*, *ADAMTS17*, and *ADAMTS18* are not only all antler-specific genes (fig. S17) but are also more highly expressed in antler than in osteosarcoma (fig. S13C).
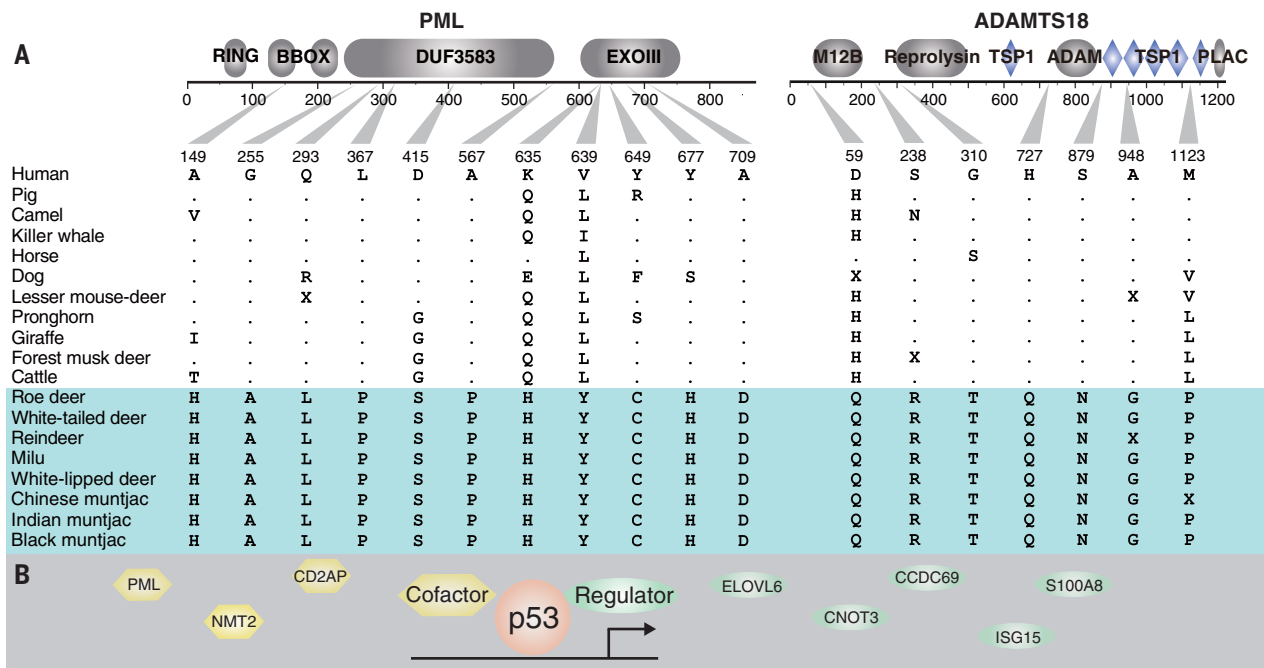
Fig. 4. Examples of positively selected tumor suppressor genes in cervids. (**A**) Gene models showing cervid-specific mutations of two positively selected tumor suppressor genes, *PML* and *ADAMTS18*. *PML* has the strongest selection signals detected in cervids (likelihood ratio test, *P* value = 1.63 × 10⁻⁶) (table S21). (**B**) Genes positively selected in the p53 signaling pathway of cervids. Selected p53 cofactors are highlighted with yellow hexagons, and selected regulators are marked with green ovals.

Finally, several genes involved in DNA damage response pathways showed signatures of cervid-specific evolution in our transcriptomic and comparative genomic analyses. *TP73* and *TP53I13* suppress tumors through their roles in the p53-mediated DNA damage response pathway (*36*) and are specifically expressed in antlers (table S3). Moreover, we found that three more DNA damage response genes (*SLF1*, *RHNO1*, and *DDB2*) were under positive selection in the cervid lineage (table S21).

The cervid-specific expression and genetic changes in these tumor suppressor and DNA repair genes may play important roles in the fine-tuned regulation of rapid antler regeneration, while at the same time preventing the onset of cancers. Further detailed functional studies may therefore be of great scientific significance in demonstrating the mechanisms underlying rapid but controlled cell growth and exploring the potential of cervids as a cancer model.

Discussion

Headgear, or cranial appendages (antlers in cervids, horns in bovids, pronghorns in pronghorn, and ossicones in giraffids), are conspicuous and diverse features of the Pecora lineage within Ruminantia and are unique among all mammals. Headgear evolution is still debated, partly because of differences in the evolutionary scenarios suggested by different phylogenic trees, coupled with the notable differences in headgear anatomy and development (*1*). Therefore, it has been proposed that pecoran headgear could have multiple independent origins. We show that the most parsi-

monious explanation is a single headgear origin (*2*). Our comparative genomic and transcriptomic analyses indicate that horns and antlers are very similar in their gene expression profiles and that pecoran headgear share cellular origins from neural crest stem cells. Moreover, the headgear-specific gene *RXFP2* was convergently pseudogenized in secondarily headgearless lineages, corroborating the single evolutionary origin hypothesis by providing a simple genetic mechanism for the otherwise puzzling convergent loss of headgear. Notably, the extremely rapid growth and peculiar regeneration of cervid antlers are biological features of interest. For instance, an incision in an antler tine results in a slight scar that in the following year leads to a small tine, whereas injury to the pedicle leads to permanent inhibition of either pedicle or antler growth (*37*, *38*). Furthermore, it has been shown that electrical stimulation of antler nerves increases antler length and weight (*39*), suggesting that neural tissue plays an important role in antler regeneration. This link was corroborated by the neural associations of several antler-specific genes and cervid-specific HCE-associated genes identified in this study. Further integrated functional, physiological, and transcriptomic analyses of a wider range of samples during various antler growth stages are warranted to clarify the roles of these antler-specific neural genes in development and their potential utility in regenerative medicine and in vitro organ regeneration.

The antler is an exclusively male trait in cervids (except reindeer), which has been strongly selected by sexual selection, and some species most

likely became extinct as a result of exaggerated antler size (*40*). Our data suggest that fast-growing cervid antlers have expression profiles that are more similar to those of osteosarcoma than to those of normal bone tissues (fig. S13). On the other hand, cervids have much lower cancer incidence than other mammals (*30*, *31*). It is conceivable that natural selection might have selected for efficient cancer-defense mechanisms in deer. It has previously been shown that elephants have a reduced cancer risk because of functional duplicates of the master tumor suppressor *TP53* (*41*). In contrast, cervids have a single copy of *TP53*, but other genes (*PML*, *NMT2*, *CD2AP*, *ELOVL6*, *S100A8*, *ISG15*, *CNOT3*, and *CCDC69*) functioning in the p53 pathway are under positive selection, suggesting that cervids may have evolved an enhanced *TP53* signaling pathway to constrain tumor growth. Elephants and deer may therefore have independently evolved different strategies to avoid cancer by targeting the same central tumor-controlling p53 regulatory pathway. We also found evidence that other tumor suppressor genes and proto-oncogenes have been under strong positive selection in cervids and/or are strongly and specifically expressed in the antler (e.g., *ADAMTS18*, *FOS*, *REL*, and *FAM83A*). Our study reveals the genetic mechanisms underlying the evolutionary, developmental, and histological origins of pecoran headgear and provides insights into the molecular mechanisms of regeneration of deer antler and its relevance to cancer resistance. The identified genes and their specific mutations provide a starting point for future functional studies of headgear

development, regeneration of mammalian organs, and oncogenesis.

Materials and methods

We sequenced and collected 221 and 49 transcriptomes, respectively, including 20 of roe deer, 20 of sika deer, 68 of goat, and 162 of sheep. RNA-sequencing reads were aligned with HISAT2 v2.0.3 (*42*), and the gene expression levels were quantified with StingTie v1.2.2 (*43*). Tissue-specific expressed genes were calculated with the τ index method (*44*). The DEGs in the embryonic horn bud were identified with DESeq2 v1.20.0 (*45*). Immunohistochemical analysis was performed to detect the expression of neural crest marker genes *NGFR* and *SOX10* in the embryonic horn bud. The genome of the Chinese water deer (*H. inermis*) was sequenced using 10X genomic platform and assembled with Supernova software v1.2.1 (*46*). Two contig-level assemblies of Moschidae species were obtained by Illumina HiSeq 2000 sequencing and assembled using the SOAPdenovo software v2.04 (*47*). Pseudogenes of the three headgearless genomes were identified with GeneWise v4.0 (*48*). Whole-genome alignments of 54 ruminant species were carried out with LAST vlast867 (*49*) and multiz v11.2 (*50*) using goat as the reference genome. Positively selected genes were identified by using the branch-site model in PAML v4.9e (*51*). PWH-specific HCEs were identified with phastCons v1.4 (*52*). Elements showing accelerated evolution in Cervidae were detected using phyloP v1.4 with the CONACC mode and LRT test method (*52*).

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S18
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Science

Genetic basis of ruminant headgear and rapid antler regeneration

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Phylogeny and characteristics of ruminants

Ruminants are a diverse group of mammals that includes families containing well-known taxa such as deer, cows, and goats. However, their evolutionary relationships have been contentious, as have the origins of their distinctive digestive systems and headgear, including antlers and horns (see the Perspective by Ker and Yang). To understand the relationships among ruminants, L. Chen *et al.* sequenced 44 species representing 6 families and performed a phylogenetic analysis. From this analysis, they were able to resolve the phylogeny of many genera and document incomplete lineage sorting among major clades. Interestingly, they found evidence for large population reductions among many taxa starting at approximately 100,000 years ago, coinciding with the migration of humans out of Africa. Examining the bony appendages on the head —the so-called headgear—Wang *et al.* describe specific evolutionary changes in the ruminants and identify selection on cancer-related genes that may function in antler development in deer. Finally, Lin *et al.* take a close look at the reindeer genome and identify the genetic basis of adaptations that allow reindeer to survive in the harsh conditions of the Arctic.

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