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A Role for Retinoic Acid in Regulating the Regeneration of Deer Antlers

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Abstract

Deer antlers are the only mammalian organs that can be repeatedly regenerated; each year, these complex structures are shed and then regrow to be used for display and fighting. To date, the molecular mechanisms controlling antler regeneration are not well understood. Vitamin A and its derivatives, retinoic acids, play important roles in embryonic skeletal development. Here, we provide several lines of evidence consistent with retinoids playing a functional role in controlling cellular differentiation during bone formation in the regenerating antler. Three receptors (α, β, γ) for both the retinoic acid receptor (RAR) and retinoid X receptor (RXR) families show distinct patterns of expression in the growing antler tip, the site of endochondral ossification. RARα and RXRβ are expressed in skin ("velvet") and the underlying perichondrium. In cartilage, which is vascularised, RXRβ is specifically expressed in chondrocytes, which express type II collagen, and RARα in perivascular cells, which also express type I collagen, a marker of the osteoblast phenotype. High-performance liquid chromatography analysis shows significant amounts of Vitamin A (retinol) in antler tissues at all stages of differentiation. The metabolites all-*trans*-RA and 4-*oxo*-RA are found in skin, perichondrium, cartilage, bone, and periosteum. The RXR ligand, 9-*cis*-RA, is found in perichondrium, mineralised cartilage, and bone. To further define sites of RA synthesis in antler, we immunolocalised retinaldehyde dehydrogenase type 2 (RALDH-2), a major retinoic acid-generating enzyme. RALDH-2 is expressed in the skin and perichondrium and in perivascular cells in cartilage, although chondroprogenitors and chondrocytes express very low levels. At sites of bone formation, differentiated osteoblasts which express the bone-specific protein osteocalcin express high levels of RALDH2. The effect of RA on antler cell differentiation was studied *in vitro*; all-*trans*-RA inhibits expression of the chondrocyte phenotype, an effect that is blocked by addition of the RAR antagonist Ro41-5253. In monolayer cultures of mesenchymal progenitor cells, all-*trans*-RA increases the expression of alkaline phosphatase, a marker of the osteoblastic phenotype. In summary, this study has shown that antler tissues contain endogenous retinoids, including 9-cis RA, and the enzyme RALDH2 that generates RA. Sites of RA synthesis in antler correspond closely with the localisation of cells which express receptors for these ligands and which respond to the effects of RA.

Keywords

retinoic acid; deer antler; cartilage; bone; retinoic acid receptor; retinoid X receptor; chondrocyte; osteoblast; RALDH2

REFERENCES

1 R.T. Ballock, A. Haydemann, L.M. Wakefield, K.C. Flanders, A.B. Roberts, M.B. Sporn
Inhibition of the chondrocyte phenotype by retinoic acid involves the upregulation of metalloprotease genes independent of TGF-β
J. Cell. Physiol., 159 (1994), pp. 340-346

2 J.W. Banks, W.J. Newbry
Antler development as a unique modification of mammalian endochondral ossification
R.D. Banks (Ed.), "Antler Development in Cervidae", Cesar Kleburg Wildlife Research Institute, Kingsville, TX (1983), pp. 279-306

3 K. Berggren, P. McCaffery, U. Drager, C.J. Forehand
Differential distribution of retinoic acid synthesis in the chicken embryo as determined by immunolocalization of the retinoic acid synthetic enzyme, RALDH2
Dev. Biol., 210 (1999), pp. 288-304
Article PDF (11MB)

4 K. Berggren, E.B. Ezerman, P. McCaffery, C.J. Forehand
Expression and regulation of the retinoic acid synthetic enzyme RALDH-2 in the embryonic chick wing
Dev. Dyn., 222 (2001), pp. 1-16

5 J.P. Brockes
Amphibian limb regeneration: Rebuilding a complex structure
Science, 276 (1997), pp. 81-87

6 D.E. Cash, C.B. Bock, K. Schugart, E. Linney, T.M. Underhill
Retinoic acid receptor α function in vertebrate limb skeletogenesis: A modulator of chondrogenesis
J. Cell Biol., 136 (1997), pp. 445-457

7 P. Chambon
A decade of molecular biology of retinoic acid receptors
FASEB J., 10 (1996), pp. 940-954

8 P.F.M. Choong, T.J. Martin, K.W. Ng
Effects of ascorbic acid, calcitrol, and retinoic acid on the differentiation of preosteoblasts
J. Orthop. Res., 11 (1993), pp. 638-647

9 R.A. Conlon, J. Rossant
Exogenous retinoic acid rapidly induces anterior ectopic expression of murine Hox-2 genes in vivo
Development, 116 (1992), pp. 357-368

10 F. De Luca, J.A. Uyeda, V. Mericq, E.E. Mancilla, J.A. Yanovski, K.M. Barnes, M.H. Zile, J. Baron
Retinoic acid is a potent regulator of growth plate chondrogenesis
Endocrinology, 141 (2000), pp. 346-353

11 H. de The, P. del Mar Vivanco-Ruiz, P. Tiollais, H. Stunnenberg, A. Dejean
Identification of a retinoic acid responsive element in the retinoic acid receptor β gene
Nature, 343 (1990), pp. 177-180

12 P. Dollé, E. Ruberte, P. Kastner, M. Petkovich, C.M. Stoner, L.J. Gudas, P. Chambon
Differential expression of genes encoding α, β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse
Nature, 342 (1989), pp. 702-704

13 P. Dollé, V. Fraulob, P. Kastner, P. Chambon
Developmental expression of murine retinoid X receptor (RXR) genes
Mech. Dev., 45 (1994), pp. 91-104
Article PDF (4MB)

14 C. Faucheux, J.P. Price
Parathyroid hormone-related peptide may play a role in deer antler regeneration
J. Danks, C. Dacke, G. Flik, C. Gay (Eds.), "Calcium Metabolism: Comparative Endocrinology", Bioscientifica Ltd, Bristol (1999), pp. 131-138

15 C. Faucheux, S.A. Nesbitt, M.A. Horton, J.S. Price
Cells in regenerating antler cartilage provide a microenvironment that supports osteoclast differentiation

J. Exp. Biol., 204 (2001), pp. 443-455

16 R.J. Goss
"Principles of Regeneration.", Academic Press, New York (1969)

17 R.J. Goss
"Deer Antlers: Regeneration, Function and Evolution.", Academic Press, New York (1983)

18 A.E. Grigoriadis, P.M. Petkovich, E.E. Rosenthal, J.N. Heersche
Modulation by retinoic acid of 1, 25-dihydroxy vitamin-D3 effects on alkaline phosphatase activity and parathyroid hormone responsiveness in an osteoblast like osteosarcoma cell line
Endocrinology, 119 (1986), pp. 932-939

19 R. Hein, T. Krieg, P.K. Mueller, O. Braun-Falco
Effect of retinoids on collagen production by chondrocytes in culture
Biochem. Pharmacol., 33 (1984), pp. 3263-3267
Article PDF (726KB)

20 J.A. Helms, C.H. Kim, G. Eichele, C. Thaller
Retinoic acid signaling is required during early chick limb development
Development, 122 (1996), pp. 1385-1394

21 D. Henrique, J. Adam, A. Myat, A. Chitnis, J. Lewis, D. Ish-Horowicz
Expression of a Delta homologue in prospective neurons in the chick
Nature, 375 (1995), pp. 787-790

22 D.S. Hill, C.W. Ragsdale Jr., J.P. Brockes
Isoform-specific immunological detection of newt retinoic acid receptor δ1 in normal and regenerating limbs
Development, 117 (1993), pp. 937-945

23 C. Horton, M. Maden
Endogenous distribution of retinoids during normal development and teratogenesis in the mouse embryo
Dev. Dyn., 202 (1995), pp. 312-323

24 H. Ide, H. Aono
Retinoic acid promotes proliferation and chondrogenesis in the distal mesodermal cells of the chick limb bud
Dev. Biol., 130 (1988), pp. 767-778

25 M. Iwamoto, E.B. Golden, S.L. Adams, S. Noji, M. Pacifici
Responsiveness to retinoic acid changes during chondrocyte maturation
Exp. Cell Res., 205 (1993), pp. 213-224
Article PDF (1MB)

26 U. Kierdorf, L. Bartos
Treatment of the growing pedicle with retinoic acid increased the size of first antlers in fallow deer (*Dama dama* L.)
Comp. Biochem. Physiol., 124 (1999), pp. 7-9
Article PDF (63KB)

27 U. Kierdorf, H. Kierdorf
Effects of retinoic acid on pedicle and first antler growth in a fallow buck (*Dama dama* L.)
Ann. Anat., 180 (1998), pp. 373-375
Article PDF (2MB)

28 D.M. Kochhar
Limb development in mouse embryos. 1. Analysis of teratogenic effects of retinoic acid
Teratology, 7 (1973), pp. 289-298

29 E. Koyama, E.B. Golden, T. Kirsch, S.L. Adams, R.A.S. Chandraratna, J-J. Michaille, M. Pacifici
Retinoid signaling is required for chondrocyte maturation and endochondral bone formation during limb skeletogenesis
Dev. Biol., 208 (1999), pp. 375-391
Article PDF (910KB)

30 R.M. Langille, D.F. Paulsen, M. Solursh

Differential effects of physiological concentrations of retinoic acid in vitro on chondrogenesis and myogenesis in chick craniofacial mesenchyme
Differentiation, 40 (1989), pp. 84-92
Article PDF (1MB)

31 W.F. Lau, I. Tertinegg, J.N.M. Heersche
Effects of retinoic acid on cartilage differentiation in a chondrogenic cell line
Teratology, 47 (1993), pp. 555-563

32 M. Leid, P. Kastner, P. Chambon
Multiplicity generates diversity in the retinoic acid signalling pathways
Trends Biochem. Sci., 17 (1992), pp. 427-433
Article PDF (901KB)

33 D. Lohnes, P. Kastner, A. Dierich, M. Mark, M. LeMeur, P. Chambon
Function of retinoic acid receptor γ in the mouse
Cell, 73 (1993), pp. 643-658
Article PDF (17MB)

34 D. Lohnes, M. Mark, C. Mendelsohn, P. Dollé, A. Dierich, P. Gorry, A. Gansmuller, P. Chambon
Function of the retinoic acid receptors (RARs) during development (1) Craniofacial and skeletal abnormalities in RAR double mutants
Development, 120 (1994), pp. 2723-2748

35 H-C. Lu, J-P. Revelli, L. Goering, C. Thaller, G. Eichele
Retinoid signaling is required for the establishment of a ZPA and for the expression of Hoxb-8, a mediator of ZPA formation
Development, 124 (1997), pp. 1643-1651

36 T. Lufkin, D. Lohnes, M. Mark, A. Dierich, P. Gorry, M.P. Gaub, M. LeMeur, P. Chambon
High postnatal lethality and testis degeneration in retinoic acid receptor α (RARα) mutant mice
Proc. Natl. Acad. Sci. USA, 90 (1993), pp. 7225-7229

37 M. Maden
Vitamin A and pattern formation in the regenerating limb
Nature, 295 (1982), pp. 672-675

38 M. Maden, E. Sonneveld, P.T. van der Saag, E. Gale
The distribution of endogenous retinoic acid in the chick embryo: Implications for developmental mechanisms
Development, 125 (1998), pp. 4133-4144

39 P. McCaffery, K.C. Posch, J.L. Napoli, L. Gudas, U.C. Drager
Changing patterns of the retinoic acid system in the developing retina
Dev. Biol., 158 (1993), pp. 390-399
Article PDF (659KB)

40 P. McCaffery, U.C. Drager
Hot spots of retinoic acid synthesis in the developing spinal cord
Proc. Natl. Acad. Sci. USA, 91 (1994), pp. 7194-7197

41 C. Mendelsohn, E. Ruberte, M. LeMeur, G. Morriss-Kay, P. Chambon
Developmental analysis of the retinoic acid-inducible RAR-beta 2 promoter in transgenic animals
Development, 113 (1991), pp. 723-734

42 C. Mendelsohn, E. Ruberte, P. Chambon
Retinoid receptors in vertebrate limb development
Dev. Biol., 152 (1992), pp. 50-61
Article PDF (8MB)

43 J.B. Moss, J. Xavier-Neto, M.D. Shapiro, S.M. Nayeem, P. McCaffery, U.C. Drager, N. Rosenthal
Dynamic patterns of retinoic acid synthesis and response in the developing mammalian heart
Dev. Biol., 199 (1998), pp. 55-71
Article PDF (7MB)

44 K. Niederreither, P. McCaffery, U.C. Drager, P. Chambon, P. Dollé

44 K. Niederreither, P. McCaffery, U.C. Dräger, P. Chambon, P. Dollé
Restricted expression and retinoic acid-induced downregulation of the retinaldehyde dehydrogenase type 2 (RALDH2) gene during mouse development
Mech. Dev., 62 (1997), pp. 67-78
Article PDF (12MB)

45 K. Niederreither, S. Abu-Abed, B. Schuhbaur, M. Petkovich, P. Chambon, P. Dolle
Genetic evidence that oxidative derivatives of retinoic acid are not involved in retinoid signaling during mouse development
Nat. Genet., 31 (2002), pp. 84-88

46 A. Oliva, F. Della Ragione, M. Fratta, G. Marrone, R. Palumbo, V. Zappia
Effect of retinoic acid on osteocalcin gene expression in human osteoblasts
Biochem. Biophys. Res. Commun., 191 (1993), pp. 908-914
Article PDF (326KB)

47 C-K. Park, Y. Ishinmi, M. Ohmura, M. Yamaguchi, S. Ikegami
Vitamin A and carotenoids stimulate differentiation of mouse osteoblastic cells
J. Nutr. Sci. Vit., 43 (1997), pp. 281-296

48 L.T. Pecorino, C.D. Lo, J.P. Brockes
Isoform-specific induction of a retinoid response antigen after biolistic transfection of chimaeric retinoic acid/thyroid hormone receptors into a regenerating limb
Development, 120 (1994), pp. 325-333

49 L.T. Pecorino, A. Entwistle, J.P. Brockes
Activation of a single retinoic acid receptor isoform mediates proximodistal respecification
Curr. Biol., 6 (1996), pp. 556-563

50 J.S. Price, B.O. Oyajobi, R.O.C. Oreffo, R.G.G. Russell
Cells cultured from the growing tip of red deer antler express alkaline phosphatase and proliferate in response to insulin-like growth factor-I
J. Endocrinol., 143 (1994), pp. R9-R16

51 J.S. Price, B.O. Oyajobi, A.M. Nalin, A. Frazer, R.G.G. Russell, L.J. Sandell
Chondrogenesis in the regenerating antler tip in red deer: Expression of collagen types I, IIA, IIB, and X demonstrated by in situ nucleic acid hybridization and immunocytochemistry
Dev. Dyn., 205 (1996), pp. 332-347

52 R.D. Riddle, R.L. Johnson, E. Laufer, C. Tabin
Sonic hedgehog mediates the polarizing activity of the ZPA
Cell, 75 (1993), pp. 1401-1416
Article PDF (15MB)

53 S.R. Scadding, M. Maden
Retinoic acid gradients during limb regeneration
Dev. Biol., 162 (1994), pp. 608-617
Article PDF (619KB)

54 J.G. Schilthuis, A.A.F. Gann, J.P. Brockes
Chimeric retinoic acid/thyroid hormone receptors implicate RAR-alpha1 as mediating growth inhibition by retinoic acid
EMBO J., 12 (1993), pp. 3459-3466

55 S.K. Sessions, N. Wanek, S.V. Bryant
Effects of localized application of retinoic acid on pattern in developing and regenerating salamander limbs
Am. Zool., 29 (1989)

56 J.M.W. Slack
A serial threshold theory of regeneration
J. Theor. Biol., 82 (1980), pp. 105-140
Article PDF (2MB)

57 M.C. Slootweg, J.P. Salles, C. Ohlsson, C.P. De Vries, C.P. Engelbregt, J.C. Netelenbos
Growth hormone binds to a single high affinity receptor site on mouse osteoblasts: Modulation by retinoic acid and cell differentiation

differentiation
J. Endocrinol., 150 (1996), pp. 465-472

58 S.M. Smith, I.J. Kirstein, Z-S. Wang, J.F. Fallon, J. Kelley, J. Bradshaw-Rouse
Differential expression of retinoic acid receptor-beta isoforms during chick limb ontogeny
Dev. Dyn., 202 (1995), pp. 54-66

59 H. Sucov, K.K. Murakami, R.M. Evans
Characterization of an autoregulated response element in the mouse retinoic acid receptor type β gene
Proc. Natl. Acad. Sci. USA, 87 (1990), pp. 5392-5396

60 M. Takigawa, H. Ishida, T. Takano, F. Suzuki
Polyamine and differentiation: induction of ornithine decarboxylase by parathyroid hormone is a good marker of differentiated chondrocytes
Proc. Natl. Acad. Sci. USA, 77 (1980), pp. 1481-1485

61 R.A. Tassava
Retinoic acid enhances monoclonal antibody WE3 reactivity in the regenerate epithelium of the adult newt
J. Morphol., 213 (1992), pp. 159-169

62 C. Thaller, G. Eichele
Identification and spatial distribution of retinoids in the developing chick limb bud
Nature, 327 (1987), pp. 625-628

63 C.M. Viviano, J.P. Brockes
Is retinoic acid an endogenous ligand during urodele limb development
Int. J. Dev. Biol., 40 (1996), pp. 817-822

64 C.M. Viviano, C.E. Horton, M. Maden, J.P. Brockes
Synthesis and release of 9-cis retinoic acid by the urodele wound epidermis
Development, 121 (1995), pp. 3753-3762

65 A.D. Weston, V. Rosen, R.A. Chandraratna, T.M. Underhill
Regulation of skeletal progenitor differentiation by the BMP and retinoid signaling pathways
J. Cell Biol., 148 (2000), pp. 679-690

66 H.B. Wood, S.J. Ward, G.M. Morriss-Kay
Effects of all-trans-retinoic acid on skeletal pattern, 5′HoxD gene expression, and RAR beta 2/beta 4 promoter activity in embryonic mouse limbs
Dev. Genet., 19 (1996), pp. 74-84

67 L.N.Y. Wu, Y. Ishikawa, D. Nie, B.R. Genge, R.E. Wuthier
Retinoic acid stimulates matrix calcification and initiates type I collagen synthesis in primary culture of avian weight-bearing growth plate
J. Cell. Biochem., 65 (1997), pp. 209-230

68 M. Yamaguchi, M. Nakamoto, H. Honda, T. Nakagawa, H. Fujita, T. Nakamura, H. Hirai, S. Narumiya, A. Kakizuka
Retardation of skeletal development and cervical abnormalities in transgenic mice expressing a dominant-negative retinoic acid receptor in chondrogenic cells
Proc. Natl. Acad. Sci. USA, 95 (1998), pp. 7491-7496

69 J-X. Zhu, Y. Sasano, I. Takahashi, I. Mizoguchi, M. Kagayama
Temporal and spatial gene expression of major bone extracellular matrix molecules during embryonic mandibular osteogenesis in rats
Histochem. J., 33 (2001), pp. 25-35

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