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Ectodermal FGFs Induce Perinodular Inhibition of Limb Chondrogenesis *in Vitro* and *in Vivo* via FGF Receptor 2

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Abstract

The formation of cartilage elements in the developing vertebrate limb, where they serve as primordia for the appendicular skeleton, is preceded by the appearance of discrete cellular condensations. Control of the size and spacing of these condensations is a key aspect of skeletal pattern formation. Limb bud cell cultures grown in the absence of ectoderm formed continuous sheet-like masses of cartilage. With the inclusion of ectoderm, these cultures produced one or more cartilage nodules surrounded by zones of noncartilaginous mesenchyme. Ectodermal fibroblast growth factors (FGF2 and FGF8), but not a mesodermal FGF (FGF7), substituted for ectoderm in inhibiting chondrogenic gene expression, with some combinations of the two ectodermal factors leading to well-spaced cartilage nodules of relatively uniform size. Treatment of cultures with SU5402, an inhibitor FGF receptor tyrosine kinase activity, rendered FGFs ineffective in inducing perinodular inhibition. Inhibition of production of FGF receptor 2 (FGFR2) by transfection of wing and leg cell cultures with antisense oligodeoxynucleotides blocked appearance of ectoderm- or FGF-induced zones of perinodular inhibition of chondrogenesis and, when introduced into the limb buds of developing embryos, led to shorter, thicker, and fused cartilage elements. Because FGFR2 is expressed mainly at sites of precartilage condensation during limb development *in vivo* and *in vitro*, these results suggest that activation of FGFR2 by FGFs during development elicits a lateral inhibitor of chondrogenesis that limits the expansion of developing skeletal elements.

Keywords

limb development; pattern formation; fibroblast growth factor; FGF receptor; lateral inhibition

References

REFERENCES

1 E Akiba, S Yonei-Tamura, H Yajima, M Omi, M Tanaka, M Sato-Maeda, K Tamura, H Ide
Fibroblast growth factor-induced gene expression and cartilage pattern formation in chick limb bud recombinants
Dev. Growth Differ., 43 (2001), pp. 165-175

2 J Anderson, H.D Burns, P Enriquez-Harris, A.O.M Wilkie, J.K Heath

Apert syndrome mutations in fibroblast growth factor receptor 2 exhibit increased affinity for FGF ligand
Hum. Mol. Genet., 7 (1998), pp. 1475-1483

3 B Brand, B Christ, H.J Jacob
An experimental analysis of the developmental capacities of distal parts of avian leg buds
Am. J. Anat., 173 (1985), pp. 321-340

4 L Chandrasekaran, M.L Tanzer
Molecular cloning of chicken aggrecan
Biochem. J., 296 (1993), pp. 885-887

5 A.L Delezoide, C Benoist-Lasselin, L Legeai-Mallet, M Le Merrer, A Munnich, M Vekemans, J Bonaventure
Spatio-temporal expression of FGFR 1, 2 and 3 genes during human embryo–fetal ossification
Mech. Dev., 77 (1998), pp. 19-30
Article PDF (5MB)

6 C Deng, A Wynshaw-Boris, F Zhou, A Kuo, P Leder
Fibroblast growth factor receptor 3 is a negative regulator of bone growth
Cell, 84 (1996), pp. 911-921
Article PDF (919KB)

7 S.A Downie, S.A Newman
Morphogenetic differences between fore and hind limb precartilage mesenchyme: Relation to mechanisms of skeletal pattern formation
Dev. Biol., 162 (1994), pp. 195-208
Article PDF (1MB)

8 S.A Downie, S.A Newman
Different roles for fibronectin in the generation of fore and hind limb precartilage condensations
Dev. Biol., 172 (1995), pp. 519-530
Article PDF (460KB)

9 J.F Fallon, A Lopez, M.A Ros, M.P Savage, B.B Olwin, B.K Simandl
FGF-2: Apical ectodermal ridge growth signal for chick limb development
Science, 264 (1994), pp. 104-107

10 D.A Frenz, N.S Jaikaria, S.A Newman
The mechanism of precartilage mesenchymal condensation: A major role for interaction of the cell surface with the amino-terminal heparin-binding domain of fibronectin
Dev. Biol., 136 (1989), pp. 97-103
Article PDF (5MB)

11 Y Gañan, D Macias, J.M Hurle
Pattern regulation in the chick autopodium at advanced stages of embryonic development
Dev. Dyn., 199 (1994), pp. 64-72

12 D Givol, A Yayon
Complexity of FGF receptors: Genetic basis for structural diversity and functional specificity
FASEB J., 6 (1992), pp. 3362-3369

13 M.K Hajihosseini, C Dickson
A subset of fibroblast growth factors (Fgfs) promote survival, but Fgf-8b specifically promotes astroglial differentiation of rat cortical precursor cells
Mol. Cell. Neurosci., 14 (1999), pp. 468-485
Article PDF (2MB)

14 M.K Hajihosseini, S Wilson, L De Moerlooze, C Dickson
A splicing switch and gain-of-function mutation in FgfR2-IIIc hemizygotes causes Apert/Pfeiffer-syndrome-like phenotypes
Proc. Natl. Acad. Sci. USA, 98 (2001), pp. 3855-3860

15 B.K Hall, T Miyake
Divide, accumulate, differentiate: Cell condensation in skeletal development revisited
Int. J. Dev. Biol., 39 (1995), pp. 881-893

16 B.K Hall, T Miyake
All for one and one for all: Condensations and the initiation of skeletal development
Bioessays, 22 (2000), pp. 138-147

17 V Hamburger, H.L Hamilton
A series of normal stages in the development of the chick embryo
J. Morphol., 88 (1951), pp. 49-92

18 J.R Hassell, E.A Horigan
Chondrogenesis: A model developmental system for measuring teratogenic potential of compounds
Teratog. Carcinog. Mutagen., 2 (1982), pp. 325-331

19 J Heasman, M Kofron, C Wylie
Beta-catenin signaling activity dissected in the early Xenopus embryo: A novel antisense approach
Dev. Biol., 222 (2000), pp. 124-134
Article PDF (4MB)

20 J.M Hurle, Y Gañan
Interdigital tissue chondrogenesis induced by surgical removal of the ectoderm in the embryonic chick leg bud
J. Embryol. Exp. Morphol., 94 (1986), pp. 231-244

21 A Isacchi, M Statuto, R Chiesa, L Bergonzoni, M Rusnati, P Sarmientos, G Ragnotti, M Presta
A six-amino acid deletion in basic fibroblast growth factor dissociates its mitogenic activity from its plasminogen activator-inducing capacity
Proc. Natl. Acad. Sci. USA, 88 (1991), pp. 2628-2632

22 R.A Kosher, K.H Walker, P.W Ledger
Temporal and spatial distribution of fibronectin during development of the embryonic chick limb bud
Cell Differ., 11 (1982), pp. 217-228
Article PDF (1MB)

23 S Koshida, M Shinya, M Nikaido, N Ueno, S Schulte-Merker, A Kuroiwa, H Takeda
Inhibition of BMP activity by the FGF signal promotes posterior neural development in zebrafish
Dev. Biol., 244 (2002), pp. 9-20
Article PDF (300KB)

24 C Labarca, K Paigen
A simple, rapid, and sensitive DNA assay procedure
Anal. Biochem., 102 (1980), pp. 344-352
Article PDF (700KB)

25 K.K.H Lee, F.C.H Li, W.T Yung, J.L.S Kung, J.L Ng, K.S.E Cheah
Influence of digits, ectoderm, and retinoic acid on chondrogenesis by mouse interdigital mesoderm in culture
Dev. Dyn., 201 (1994), pp. 297-309

26 C.M Leonard, M Bergman, D.A Frenz, L.A Macreery, S.A Newman
Abnormal ambient glucose levels inhibit proteoglycan core protein gene expression and reduce proteoglycan accumulation during chondrogenesis: possible mechanism for teratogenic effects of maternal diabetes
Proc. Natl. Acad. Sci. USA, 86 (1989), pp. 10113-10117

27 C.M Leonard, H.M Fuld, D.A Frenz, S.A Downie, J Massagué, S.A Newman
Role of transforming growth factor-beta in chondrogenic pattern formation in the embryonic limb: stimulation of mesenchymal condensation and fibronectin gene expression by exogenous TGF-beta and evidence for endogenous TGF-beta-like activity
Dev. Biol., 145 (1991), pp. 99-109
Article PDF (7MB)

28 R Lev, S.S Spicer
Specific staining of sulfate groups with alcian blue at low pH
J. Histochem. Cytochem., 12 (1964), p. 309

29 E.J Mackie, I Thesleff, R Chiquet-Ehrismann
Tenascin is associated with chondrogenic and osteogenic differentiation in vivo and promotes chondrogenesis in vitro

J. Cell Biol., 105 (1987), pp. 2569-2579

30 G.R Martin
The roles of FGFs in the early development of vertebrate limbs
Genes Dev., 12 (1998), pp. 1571-1586

31 P Martin, J Lewis
Normal development of the skeleton in chick limb buds devoid of dorsal ectoderm
Dev. Biol., 118 (1986), pp. 233-246
Article PDF (19MB)

32 I McIntosh, G.A Bellus, E.W Jab
The pleiotropic effects of fibroblast growth factor receptors in mammalian development
Cell Struct. Funct., 25 (2000), pp. 85-96

33 R Merino, Y Gañan, D Macias, A.N Economides, K.T Sampath, J.M Hurle
Morphogenesis of digits in the avian limb is controlled by FGFs, TGFbetas, and noggin through BMP signaling
Dev. Biol., 200 (1998), pp. 35-45
Article PDF (764KB)

34 T Miura, K Shiota
TGFbeta2 acts as an "activator" molecule in reaction-diffusion model and is involved in cell sorting phenomenon in mouse limb micromass culture
Dev. Dyn., 217 (2000), pp. 241-249

35 M Mohammadi, G McMahon, L Sun, C Tang, P Hirth, B.K Yeh, S.R Hubbard, J Schlessinger
Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors
Science, 276 (1997), pp. 955-960

36 T Momose, A Tonegawa, J Takeuchi, H Ogawa, K Umesono, K Yasuda
Efficient targeting of gene expression in chick embryos by microelectroporation
Dev. Growth Differ., 41 (1999), pp. 335-344

37 S.A Newman
Sticky fingers: Hox genes and cell adhesion in vertebrate limb development
Bioessays, 18 (1996), pp. 171-174

38 S.A Newman, M.-P Pautou, M Kieny
The distal boundary of myogenic primordia in chimeric avian limb buds and its relation to an accessible population of cartilage progenitor cells
Dev. Biol., 84 (1981), pp. 440-448
Article PDF (8MB)

39 S.A Newman, J.J Tomasek
Morphogenesis of connective tissues
W.D Comper (Ed.), Extracellular Matrix, Harwood Academic Publishers, Amsterdam (1996), pp. 335-369

40 S.A Oberlender, R.S Tuan
Expression and functional involvement of N-cadherin in embryonic limb chondrogenesis
Development, 120 (1994), pp. 177-187

41 M Oldridge, E.H Zackai, D.M McDonald-McGinn, S Iseki, G.M Morriss-Kay, S.R Twigg, D Johnson, S.A Wall, W Jiang, C Theda, E.W Jabs, A.O Wilkie
De novo alu-element insertions in FGFR2 identify a distinct pathological basis for Apert syndrome
Am. J. Hum. Genet., 64 (1999), pp. 446-461
Article PDF (1MB)

42 E.B Pasquale
A distinctive family of embryonic protein-tyrosine kinase receptors
Proc. Natl. Acad. Sci. USA, 87 (1990), pp. 5812-5816

43 G Patstone, E.B Pasquale, P.A Maher
Different members of the fibroblast growth factor receptor family are specific to distinct cell types in the developing chicken embryo

embryo
Dev. Biol., 155 (1993), pp. 107-123
Article PDF (4MB)

44 D.F Paulsen, M Solursh
Microtiter micromass cultures of limb-bud mesenchymal cells
In Vitro Cell. Dev. Biol., 24 (1988), pp. 138-147

45 K Peters, D Ornitz, S Werner, L Williams
Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis
Dev. Biol., 155 (1993), pp. 423-430
Article PDF (1009KB)

46 K.G Peters, S Werner, G Chen, L.T Williams
Two FGF receptor genes are differentially expressed in epithelial and mesenchymal tissues during limb formation and organogenesis in the mouse
Development, 114 (1992), pp. 233-243

47 Saunders, J. W. Jr.1996. Operations on limb buds of avian embryos. In Methods in Avian Embryology (M. Bronner-Fraser, Ed.), pp. 125–145. Academic Press, San Diego.

48 M.P Savage, C.E Hart, B.B Riley, J Sasse, B.B Olwin, J.F Fallon
Distribution of FGF-2 suggests it has a role in chick limb bud growth
Dev. Dyn., 198 (1993), pp. 159-170

49 R Shiang, L.M Thompson, Y.Z Zhu, D.M Church, T.J Fielder, M Bocian, S.T Winokur, J.J Wasmuth
Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia
Cell, 78 (1994), pp. 335-342
Article PDF (1MB)

50 N.H Shubin
The implications of “the bauplan” for development and evolution of the tetrapod limb
J.R Hunchliffe, J.M Hurle, D Summerbell (Eds.), Developmental Patterning of the Vertebrate Limb, Plenum Press, New York (1991), pp. 411-421

51 M Solursh, R.S Reiter
Inhibitory and stimulatory effects of limb ectoderm on in vitro chondrogenesis
J. Exp. Zool., 248 (1988), pp. 147-154

52 M Solursh, C.T Singley, R.S Reiter
The influence of epithelia on cartilage and loose connective tissue formation by limb mesenchyme cultures
Dev. Biol., 86 (1981), pp. 471-482
Article PDF (10MB)

53 J Summerton
Morpholino antisense oligomers: The case for an RNase H-independent structural type
Biochim. Biophys. Acta, 1489 (1999), pp. 141-158
Article PDF (437KB)

54 G Szebenyi, M.P Savage, B.B Olwin, J.F Fallon
Changes in the expression of fibroblast growth factor receptors mark distinct stages of chondrogenesis in vitro and during chick limb skeletal patterning
Dev. Dyn., 204 (1995), pp. 446-456

55 J.J Tomasek, J.E Mazurkiewicz, S.A Newman
Nonuniform distribution of fibronectin during avian limb development
Dev. Biol., 90 (1982), pp. 118-126
Article PDF (10MB)

56 W.B Upholt, B.R Olsen
The active genes of cartilage
B.K Hall, S.A Newman (Eds.), Cartilage: Molecular Aspects, CRC Press, Boca Raton (1991), pp. 1-57

57 E Van Obberghen-Schilling, N.S Roche, K.C Flanders, M.B Sporn, A Roberts

Transforming growth factor beta-1 positively regulates its own expression in normal and transformed cells
J. Biol. Chem., 263 (1988), pp. 7741-7746

58 M.K Webster, D.J Donoghue
Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia
EMBO J., 15 (1996), pp. 520-527

59 R.B Widelitz, T.X Jiang, B.A Murray, C.M Chuong
Adhesion molecules in skeletogenesis. II. Neural cell adhesion molecules mediate precartilaginous mesenchymal condensations and enhance chondrogenesis
J. Cell. Physiol., 156 (1993), pp. 399-411

60 A.O Wilkie, S.F Slaney, M Oldridge, M.D Poole, G.J Ashworth, A.D Hockley, R.D Hayward, D.J David, L.J Pulleyn, P Rutland
Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome
Nat. Genet., 9 (1995), pp. 165-172

61 X Xu, M Weinstein, C Li, C Deng
Fibroblast growth factor receptors (FGFRs) and their roles in limb development
Cell Tissue Res., 296 (1999), pp. 33-43

62 S Yonei-Tamura, T Endo, H Yajima, H Ohuchi, H Ide, K Tamura
FGF7 and FGF10 directly induce the apical ectodermal ridge in chick embryos
Dev. Biol., 211 (1999), pp. 133-143
Article PDF (1MB)

63 K Yu, A.B Herr, G Waksman, D.M Ornitz
Loss of fibroblast growth factor receptor 2 ligand-binding specificity in Apert syndrome
Proc. Natl. Acad. Sci. USA, 97 (2000), pp. 14536-14541

64 K Yu, D.M Ornitz
Uncoupling fibroblast growth factor receptor 2 ligand binding specificity leads to Apert syndrome-like phenotypes
Proc. Natl. Acad. Sci. USA, 98 (2001), pp. 3641-3643

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