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Short Communication

Expression of the fibroblast activation protein during mouse embryo development

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ABSTRACT Human Fibroblast Activation Protein (FAP), a member of the serine prolyl oligopeptidase family, is a type II cell surface glycoprotein that acts as a dual-specificity dipeptidyl-peptidase (DPP) and collagenase *in vitro*. Its restricted expression pattern in embryonic mesenchyme, in wound healing and in reactive stromal fibroblasts of epithelial cancers, has suggested a role for the FAP protease in extracellular matrix degradation or growth factor activation in sites of tissue remodeling. The FAP homologue in *Xenopus laevis* has been reported to be induced in the thyroid hormone–induced tail resorption program during tadpole metamorphosis supporting a role for FAP in tissue remodeling processes during embryonic development. However, *Fap*-deficient mice show no overt developmental defects and are viable. To study the expression of FAP during mouse embryogenesis, a second *Fap*-deficient mouse strain expressing β-Galactosidase under the control of the *Fap* promoter was generated by homologous recombination (*Fap*$^{-/-}$ *lacZ* mice). FAP deficiency was confirmed by the absence of FAP-specific dipeptidyl-peptidase activity in detergent-soluble extracts isolated from 17.5 d.p.c. *Fap*$^{-/-}$ *lacZ* embryos. We report that *Fap*$^{-/-}$ *lacZ* mice express β-Galactosidase at regions of active tissue remodeling during embryogenesis including somites and perichondrial mesenchyme from cartilage primordia.

KEY WORDS: *Mouse Fibroblast Activation Protein, Serine prolyl oligopeptidase, Fap*$^{-/-}$ *LacZ mice, expression pattern, X-gal staining.*

In this study we have generated *Fap* knockout mice expressing β-Galactosidase under the control of the endogenous *Fap* promoter to analyze FAP expression and function at various stages of embryonic development. A targeting construct was generated in which a *lacZ-PGK-1-neo*-cassette was fused in frame into exon 4 of the *Fap* gene such that part of the exon 4 and exon 5 were replaced (Fig. 1A). Consequently, X-gal staining should indicate areas of *Fap*-expression. The targeting vector was electroporated into E14.1 ES-cells followed by positive/negative selection with G418 and gancyclovir. One cell clone, identified by Southern blot analysis, was expanded and injected into *C57Bl/6* recipient blastocysts. Male founder animals exhibiting extensive coat-color chimerism were crossed with *C57Bl/6* females, off-springs were screened for agouti coat-color and germ-line transmission was assessed by Southern blotting of tail DNA (data not shown). To confirm the absence of FAP at the functional level, FAP-specific activity assays were performed. The α-mFAP monoclonal antibody 3D11 (Niedermeyer *et al.*, 2000) was used to immunoprecipitate FAP protein present in detergent-soluble extracts isolated from 17.5 d.p.c. *Fap*$^{+/+}$ and *Fap*$^{-/-}$ embryos. Subsequently, the immunopurified material was analyzed in a dipeptidyl-peptidase (DPP) activity assay. FAP mediated DPP activity was present in *Fap*$^{+/+}$, but not in *Fap*$^{-/-}$ *lacZ* embryos (Fig. 1B).

To analyze FAP expression at various stages of embryonic development, whole-mount X-gal stainings of 8.5 to 17.5 d.p.c. Fap$^{+/+}$, Fap$^{+/-}$, and Fap$^{-/-}$ *lacZ* embryos were performed (Fig. 2 A-D). Both in 8.5 d.p.c. and in 9.5 d.p.c. *Fap*$^{-/-}$ *lacZ* embryos no FAP expression could be detected (data no shown). β-Galactosidase activity was first observed in 10.5 d.p.c. *Fap*$^{-/-}$ *lacZ* embryos within the somites (Fig. 2B). 11.5 and 12.5 d.p.c. *Fap*$^{-/-}$ *lacZ* embryos showed a similar expression pattern. Beginning 12.5 d.p.c., *Fap*$^{-/-}$ *lacZ* embryos also demonstrated β Galactosidase activity in paravertebral regions (Fig. 2 A,C). Analysis of *Fap*$^{+/-}$ *lacZ* embryos revealed the same expression pattern (data not shown). In contrast, *lacZ* staining was absent in *Fap*$^{+/+}$ wildtype embryos at any stage analyzed (Fig. 2D and data not shown).

Abbreviations used in this paper: DPP, dipeptidyl-peptidase; FAP, fibroblast activation protein.

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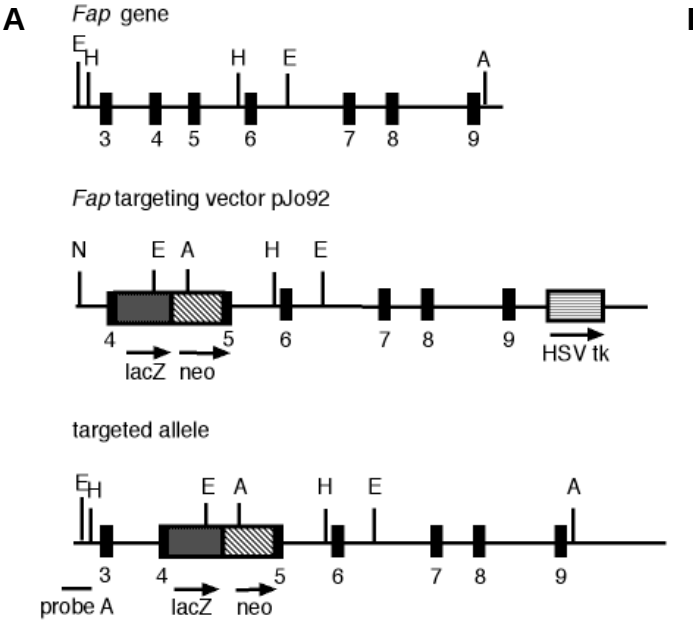

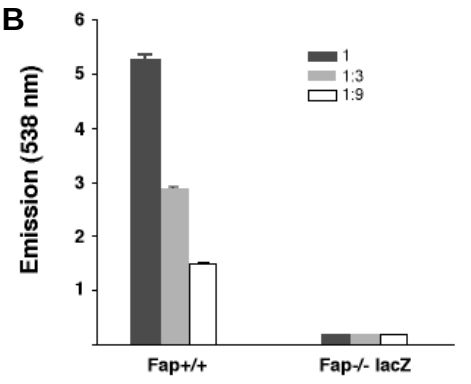

Fig. 1. Targeted disruption of the *Fap* gene. **(A)** *The targeting vector containing a β-Galactosidase-cassette (lacZ), its relationship to the wild-type allele, and the organization of the targeted allele are depicted. The relative positions of exons 3 to 9 (closed boxes) are indicated. Relevant restriction sites (A, Apa I; E, EcoR I; and H, Hind III; N, Not I) and the location of the probe (probe A) used for Southern blot analysis are shown.* **(B)** *FAP-specific dipeptidyl-peptidase activity assay. Detergent-soluble membrane fractions obtained from 17.5 d.p.c. Fap$^{+/+}$ and Fap$^{-/-}$ lacZ embryos were immunopreciptated with the monoclonal antibody 3D11 and different dilutions (1:3; 1:9) of the immunoprecipitates were used in the dipeptidyl-peptidase-assay.*

To determine the localization of FAP expression observed in the whole-mounts at the cellular level, X-gal stained *Fap$^{-/-}$ lacZ* embryos from 7.5 d.p.c. to 16.5 d.p.c. were serially sectioned and counterstained with azocarmine (Fig. 3 A-F). From all organs and tissues analyzed, LacZ expression was restricted to somites, myotubes and perichondral mesenchyme from cartilage primordia. Within the somites LacZ is expressed in the dermomyotome component of the rostral portion but is absent in the sclerotome component. An example for a 12.5 d.p.c. *Fap$^{-/-}$ lacZ* embryo is shown in Fig. 2B. Wildtype *Fap$^{+/+}$* embryos did not show β-Galactosidase activity in the somites (Fig. 2C). Furthermore, specific X-gal staining is detected in myotubes at 11.5 d.p.c and older stages, whereas earlier stages show no expression (Fig. 3A and data not shown). At 16.5 d.p.c. the scattered developing intercostal muscle fibres express FAP (Fig. 3E and data not shown). Finally, in support to our previous findings with a monoclonal antibody against FAP (Niedermeyer *et al.*, 2000), at all stages present, primitive mesenchymal cells surrounding the cartilaginous primordia of the bones exhibited specific β-Galactosidase activity. Examples of perichondrial mesenchymal cell around the cartilage primordium of the ribs and around the lower thoracic vertebral bodies are shown in Fig. 3 D and F, respectively.

Despite the very restricted expression pattern of FAP in certain primitive mesenchymal cells of the developing embryo, no obvious phenotypes were observed in the underlying organs and tissues. This finding suggests that the proteolytic activity of FAP is not sufficient to trigger processes like tissue remodeling. It is tempting to speculate that the loss of FAP expression may result in the upregulation of other compensatory collagenolytic enzymes, such as serine-proteases or metallo-proteases (MMPs), or that a functional redundancy of other collagenolytic proteins may mark the loss of FAP. The generation of double- or multiple knockouts with animals deficient for other serine-proteases or MMPs will address this issues.

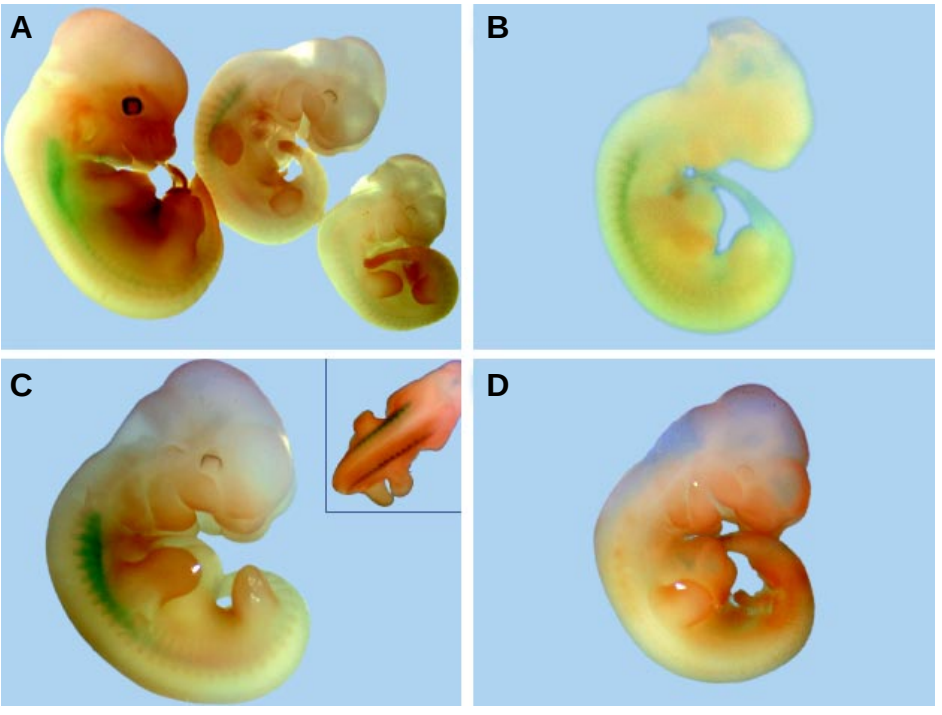

Fig. 2. **β-Galactosidase activity in Fap$^{-/-}$ *lacZ* embryos at different stages of development**. **(A)** *Lateral view of 12.5, 11.5, and 11 d.p.c. whole-mount stained embryos (from left to right).* **(B)** *Lateral view of a 10.5 d.p.c. whole mount stained embryo.* **(C)** *Lateral and dorsal view (insert) of an 11.5 d.p.c. whole-mount stained embryo.* **(D)** *Lateral view of a whole-mount stage-matched 11.5 d.p.c. Fap$^{+/+}$ wildtype embryo.*

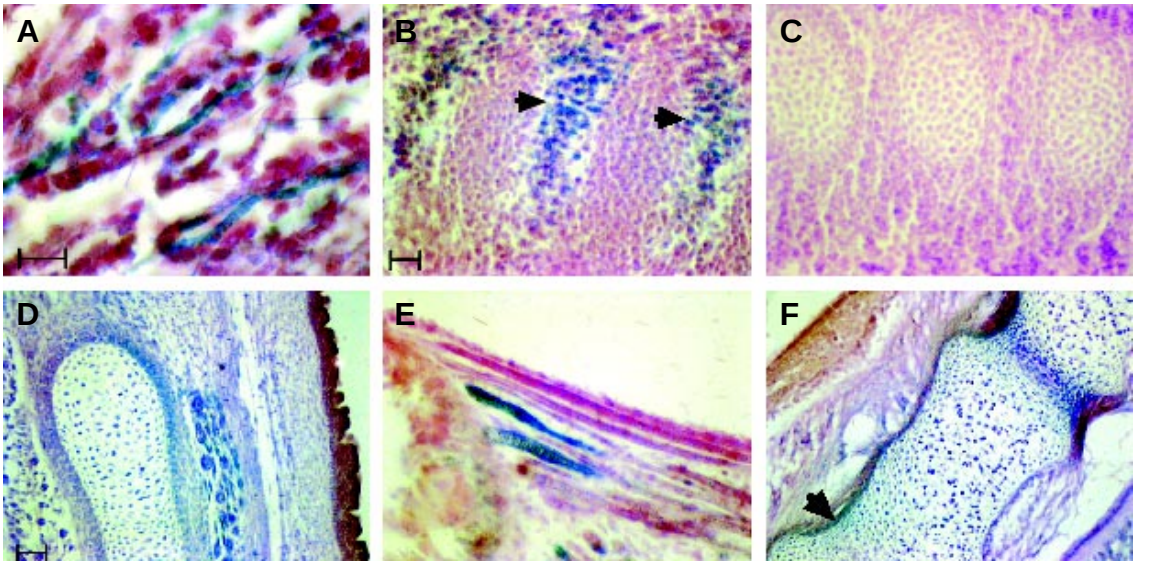

Fig. 3. **Microscopic sections of the *Fap*$^{-/-}$ *lacZ* embryos.** *Serial sections of embryos were stained with the X-gal substrate (blue staining) and counterstained with azocarmine (red staining).* **(A)** *β-Galactosidase activity is restricted to scattered developing myotubes (pre-muscle mass of subscapularis) from an 11.5 d.p.c. embryo.* **(B)** *Negative staining in the sclerotome component of the somites and specific blue staining of the dermomyotome component of the somites from the rostral portion of a 12.5 d.p.c. embryo.* **(C)** *A wildtype Fap*$^{+/+}$ *12.5 d.p.c. embryo stained as in B showing no β-Galactosidase activity in the somites.* **(D,E)** *A 16.5 d.p.c. embryo showing specific activity in the perichondrial mesenchymal cells from the cartilage primordium of the ribs* **(D)**, *and in scattered developing intercostal muscle fibres* **(E)**. **(F)** *β-Galactosidase activity in perichondrial mesenchymal cells around the cartilage primordia of lower thoracic vertebral bodies from a 17.5 d.p.c. embryo. Scale bars in* **(A**,**B**,**C**,**E)**, *20 μm, and in* **(D,F)**, *50 μm.*

Materials and Methods

Generation and characterization of Fap$^{-/-}$ lacZ *mice*

A 7.5 kb *Xba I*-fragment of the m*Fap* gene spanning parts of exons 5 and exons 6 to 9 was isolated from a mouse genomic libray (Stratagene) and cloned into pPNT (Tybulewicz *et al.*, 1991), resulting in plasmid pJo44. Subsequently, a 1.2 kb genomic PCR-fragment containing intron 3 and part of exon 4 was cleaved by *Not I* and *Kpn I* and fused in-frame to an *E.coli* lacZ-cassette, obtained by *Xho I/Kpn I* cleavage from pGNA (Le Mouellic *et al.*, 1990). The resulting *Xho I/Not I* fragment was cloned into pJo44 to yield the final targeting construct pJo92.

Embryonic stem (ES)-cell culture and generation of *Fap*$^{-/-}$lacZ-mice followed protocols described recently (Niedermeyer *et al.*, 2000). Wholemount *lacZ* stainings for β-gal activity were performed as described (Pfeffer *et al.*, 2000)

Immunological reagents

Protein A-Sepharose was obtained from Pharmacia Fine Chemicals (Stockholm, Sweden). Antisera used were: 3D11, a mouse monoclonal antibody (IgG2b) recognizing an extracellular epitope of mouse and human FAP; rabbit anti-mouse IgG (Dianova, Hamburg, Germany); H194-112, a rat IgG2a antibody recognizing mouse DPP IV/CD26 (PharMingen; San Diego CA).

Immunopurification of FAP and dipeptidyl-peptidase (DPP) assays

Preparation of membrane extracts and dipeptidyl-peptidase assays have been performed as previously described (Niedermeyer *et al.*, 2000).

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