I. GENETICS

STERNUM MALFORMATIONS AS A RESULT OF BMP4 DEFICIENCY

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Abstract

Recent data shows that the Bmp4 plays significant roles in a large number of developmental processes, including branching morphogenesis of the lung, tooth development, lens development, neuroepithelial cell diferentiation, primordial germ cell formation, and not leats most importantly bone formation and development.

The purpose of this study was to identify the Bmp4 expression and its role in the development of ribs and sternum and analysis of the skeletal phenotypes caused by the genetic inactivation of Bmp4. along with the study of it's expression patterns.

This conclusion of this study is based in part on the finding that some heterozygous mutants, which presumably produce half the amount of active BMP protein as wild type appear to have some skeletal defects with regards to the sternum and ribs.

Key words : bone morphogenetic protein, bone, development, transgenic mice, sternum, ribs

Introduction

BMPs are multifunctional cytokines that are members of the TGF-beta superfamily proteins, which consist of 43 members. [10]

The role of the TGF- beta superfamily involves cell growth, differentiation, and embryonic pattern formation. Currently, approximately 20 BMPs are known, including the addition of various growth/differentiation factors (GDFs) based on sequence homology, that are responsible for inducing ectopic bone formation, chondroblast formation, and visceral development. So BMPs are involved in cell proliferation, differentiation, apoptosis, and morphogenesis. [10]

They are broadly divided into three subclasses based on derived amino acid sequences.

The first subclass is the BMP-2 and BMP-4. They differ mainly in the amino terminal group.

The second subgroup is BMP-5, 6, 7 and 8.

These molecules are larger than the first group. The third subgroup is BMP-3 (osteogenin), which is more distantly related. [10]

Bone morphogenic proteins (BMPs) are known to promote osteogenesis, and clinical trials are currently underway to evaluate the ability of certain BMPs to promote fracture-healing. The optimal BMPs to be used in different clinical applications have not been elucidated, and a comprehensive evaluation of the relative osteogenic activity of different BMPs is lacking. [10]

The function of BMPs is multifaceted. Besides involvement in bone and cartilage formation, BMPs create an environment for red bone marrow formation and contribute to systemic hematopoietic production. [10]

Recent data shows that the Bmp4 gene plays significant roles in a large number of developmental processes, including branching morphogenesis of the lung, tooth development, lens development, neuroepithelial cell differentiation, primordial germ cell formation, and not least most importantly bone formation and development. [12]

Bmp4 is actively expressed during early sternum and rib morphogenesis and plays an important role in the development of the bonny thorax.. [12]

Despite increasing experimental insight into the Bmp4 gene regulation in vitro, the in vivo mechanisms controlling Bmp4 expression during development are unknown. [12]

The sternum originates from two distinct mesenchymal condensations (sternal bands) that arise dorsally, extend caudally while migrating toward the ventral midline, and eventually fuse. Rib anlagen emerge independently from the sternal bands. Upon cell proliferation they grow toward the ventral midline and fuse to the sternal bands [Chen, 1952 a; Storm and Kingsley, 1996]. It is generally considered that the ribs and the sternum arise from distinct mesenchymal condensations. [24]

Anterior body wall defects in the thoracic region may be severe, leading to ectopia cordis, or mild, as in skin-covered sternal clefts. The embryologic basis for other sternal abnormalities, such as pectus excavatum and pectus carniatum, is not clear; however, abnormalities of rib morphogenesis and growth are the most likely causes.

Ethiopathogenesis of pectus excavatum and carinatum remains unsettled.

Disturbances in endochondral ossification and growth of costal cartilage seem to be more probable cause of the deformities than diaphragm underdevelopment.

The etiology of sternal cleft deformity is unknown. Afamilial predisposition has not been described.

Sternal clefts vary from minor ‘‘V’’- shaped defects associated with an orthotopic heart to complete separation of sternal halves to the xiphisternum associated with thoracic or thoracoabdominal ectopia cordis, the ‘‘pentalogy of Cantrell“. The latter conditions are associated with intrinsic cardiac anomalies and a far higher mortality. [9]

Isolated sternal clefts are rarely associated with significant intrinsic cardiac defects.

Several anomalies of somatic fusion have been reported, including fibrous bands extending from the defect to the umbilicus and diastasis recti. [9]

Material and Methods

The purpose of this study was to identify the Bmp4 expression and its role in the development of ribs and sternum and analysis of the skeletal phenotypes caused by the genetic inactivation of Bmp4. along with the study of it’s expression patterns. [24]

Bmp4 lacZ mice, where expression of the inserted lacZ is controlled by the entire endogenous Bmp4 gene, were used for mapping all Bmp4 expression domains in the bones that form the thorax.

Although Bmp4 is widely expressed in different tissues during development, we chose to examine its expression in the ribs and sternum.

Mouse Bmp4 promoter lacZ constructs:

Three fragments of the Bmp4 1A promoter and part of 5`-exon 1 ()2372/+258,) 1140/+212, and)260/+212) were linked to pUC19/AUG b-gal containing the lacZ gene. [12]

Expression of lacZ in mice harboring this construct is a sensitive reflection of expression of the entire endogenous Bmp4 gene. [12]

B-Galactosidase expression assay and immunostaining:

To examine the onset of endogenous Bmp4 expression in ribs, heterozygous Bmp4 lacZ newborn mice (PN1) were utilized for analysis of ß-gal activity.

B-Galactosidase staining was assessed in newborn mice using the method described by Lawson et al. Briefly newborn mice were fixed with ice-cold 4% paraformaldehyde for 30 min to 1 h, and then washed three times with PBS for 5 min each. The specimens were then stained overnight in freshly made X-Gal solution (1 mg/ml) at 32^0 C. [12]

Bone and cartilage staining:

The ribs and sternum from newborn mice were dissected and the stained with alcian blue and alizarin red for bone and cartilage.

The chemicals used in the fetal skeletal processing— ethanol, potassium hydroxide (KOH) and glycerin. [2]

The specific stains used in the study were Alizarin Red S and Alcian Blue (Sigma Chemical Co.). [2]

Before preparation of the staining solution, stock solutions of Alcian Blue and Alizarin Red S were prepared as follows: Alcian Blue 0. 15% (w/v) in 70% ethanol and Alizarin Red S 0. 1% (w/v) in 95% ethanol. [2]

The clearing solution was 70% ethanol: glycerin: 100% benzyl alcohol solution (2:2:1) and the holding solution was glycerin 50% (v/v) in 70% alcohol. [2]

The double-staining procedure described in the methods section was effective at staining both ossified and cartilaginous skeletal structures in the newborns. The ossified structures are stained red and the cartilage is stained blue.

Results

External analysis of the newborn pups did not reveal any significant abnormality for the Bmp4, heterozygotes. In particular, no differences in size were observed, indicating the absence of major patterning and growth defects.

At this stage Bmp4 lacZ signals are present in the perichondrium along the entire course of the ribs from the vertebrae to the sternum sometimes being dispersed but often being more intense towards the outerlayers.

As shown in figure 1, initial Bmp4 signals are evident in the ribs and sternum at post natal day 1.

Asynchronous ossification of inferior sternal segment 5 was recorded. This ossification segment differed from all the other four centers and had a modified shape. We have noticed that in some cases this center was reduced in size and in others fused with segment 4 giving it an unusual shape as seen in the figure 2.

Ectopic calcification of the site of rib attachment to the sternum was not detected in any of the single heterozygote analyzed.

Even when patterning defects were observed, each rib attached to a cartilaginous region of the sternum and these cartilaginous regions were clearly separated by ossified areas.

It has been proposed that the process of rib attachment to the sternum inhibits ossification and/or promotes chondrocyte proliferation at the site of fusion, and that this process is controlled at least partly by Bmps [Storm and Kingsley, 1996; Solloway et al., 1998].

This is consistent with the hypothesis that rib extensions inhibit ossification at the site of fusion [Chen, 1953]. The number of ribs attached to the sternum was always normal in the Bmp4 heterozygous mice. [24]

The sternum is formed by the progressive anterior to posterior fusion of two parallel sternal bands, a process that is normally completed by 15. 5 dpc [Chen, 1952b].

The presence of an unfused distal part of the sternum neonatally indicates a severe delay in the completion of this process. [24]

Analysis of the xiphoid process revealed some defects in Bmp4 heterozygotes.

Both the cartilaginous and ossified part of this element was split medially, while wild-type animals have a completely fused process.

As seen in figure 2 the sternum malformation was associated in all cases with the defect of ossification at the level of the inferior sternal segment 5.

Very mild modifications of the ossification center 4 were noted in correlation with the malformed 5th center.

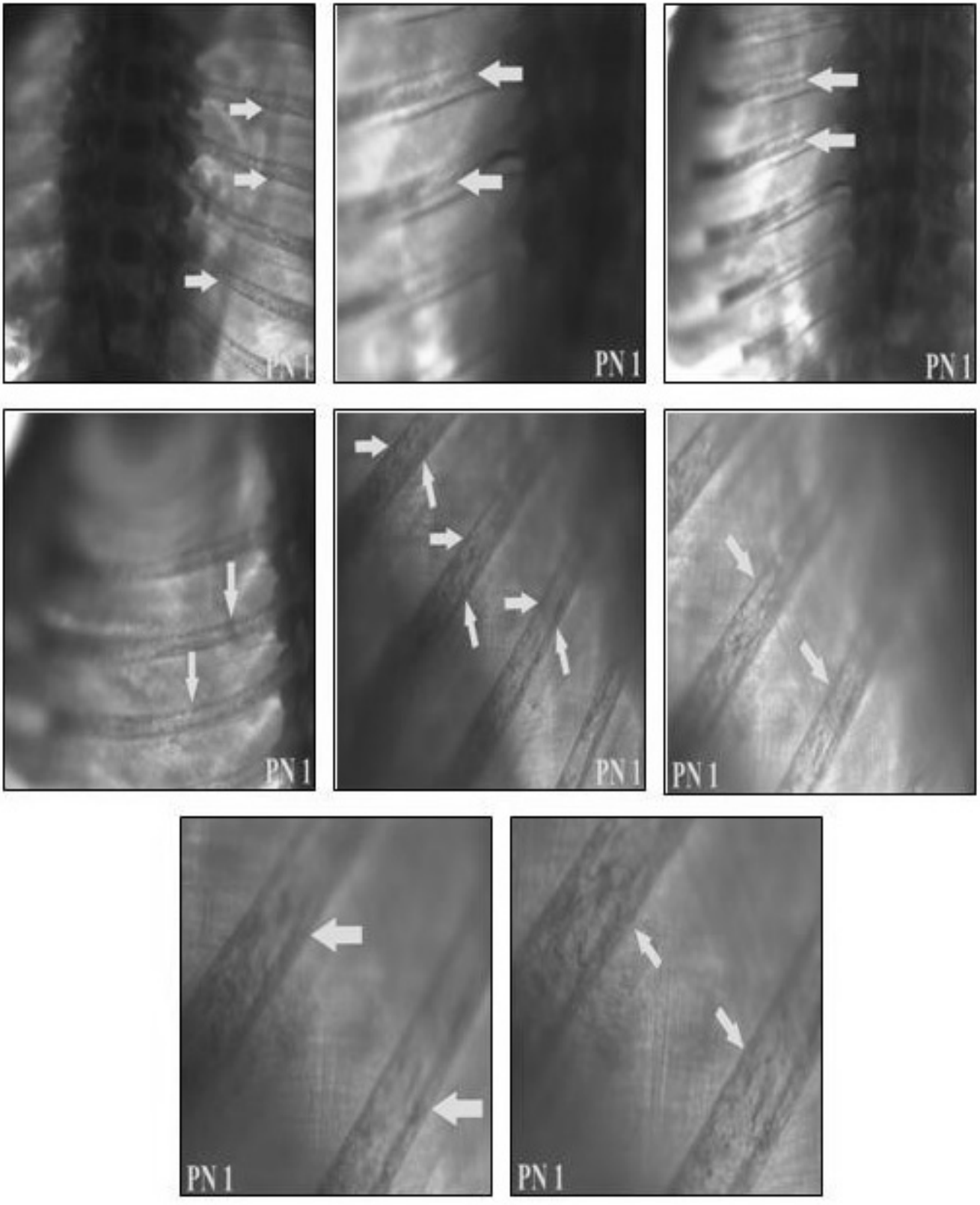

Fig. 1. Bmp4 lacZ signals are present in the perichondrium along the entire course of the ribs from the vertebrae to the sternum sometimes being dispersed but often being more intense towards the outerlayers. The arrows show the blue lacZ staining.

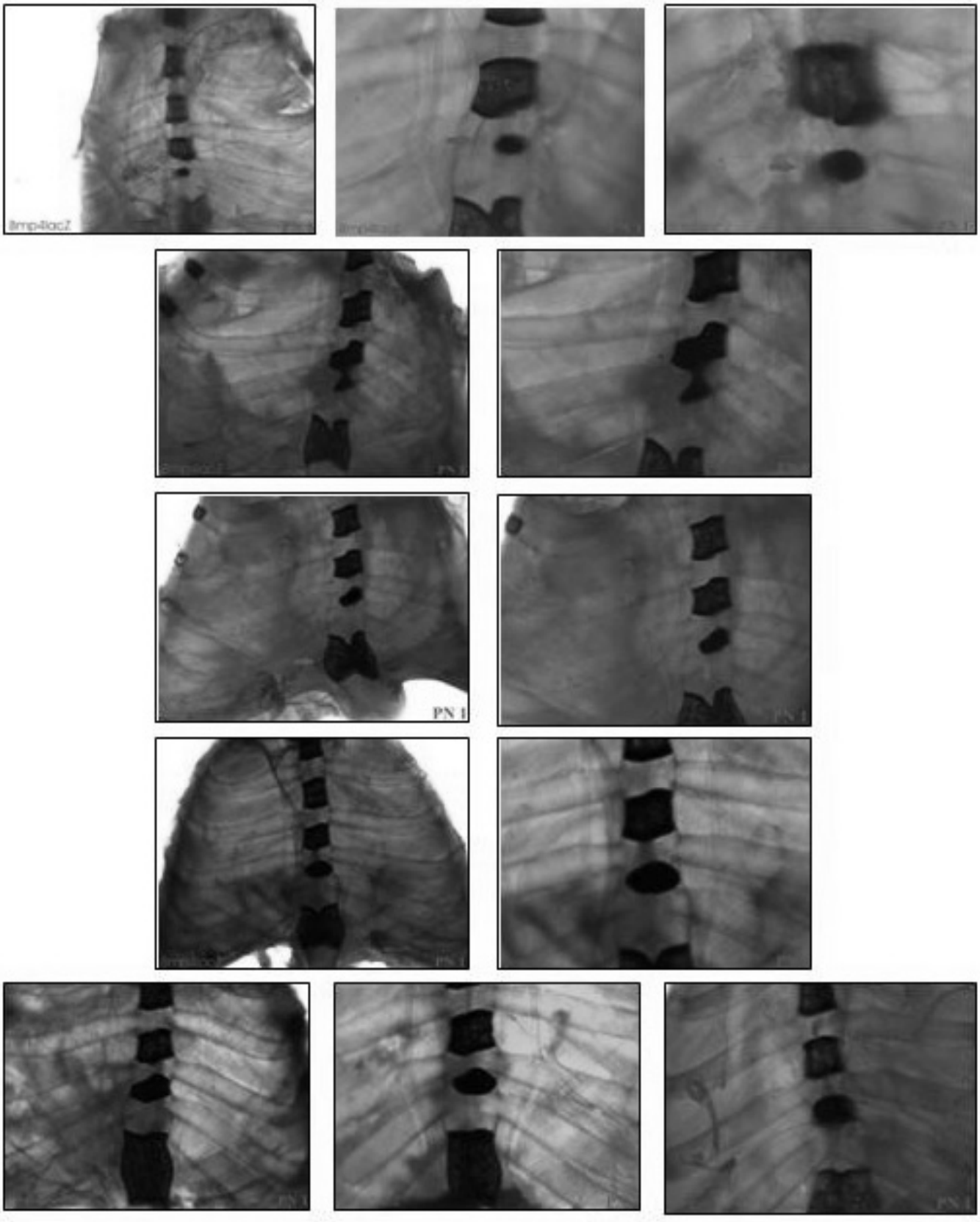

Fig. 2 Asynchronous ossification of inferior sternal segment 5 was recorded at the BMP4 +/- mice as shown by the pointing arrows. (Alcian blue/ alizarin red staining).

Conclusions

These results indicate that Bmp4 gene dosage is essential for the normal development of the sternal bone and ribs. [17]

Whatever the precise mechanism, the amount of BMP4 secreted by a signaling source appears to be important for achieving the appropriate response in a target tissue. [17]

This conclusion is based in part on the finding that some heterozygous mutants, which presumably produce half the amount of active BMP protein as wild type appear to have some skeletal defects with regards to the sternum and ribs.

Discussions:

These noted malformations in the mouse model might not have a major effects on the future development of the chest cavity largely because of the fact that the mouse does not have a vertical position of the body so that even if

Findings suggest that a nonossified sternal segment is abnormal and is an indication for further imaging in humans. Few investigators in recent articles have addressed the issue of timing of ossification of the sternal segments. [25]

In many anatomic regions in young children, bone structures ossify in a predictable fashion. A lack of knowledge of the order of ossification can lead to misinterpretation of these findings. [25]

These sternal ossification abnormalities have been reported in human subjects to occur in infants with there are any tendencies of the sternum to sink in the excavatum shape the chest internal organs will act as a force that will keep the sternum in the right position.

With regards to the human body witch has a vertical position thus any changes in the consistency of the anterior chest wall will not be contrabalanced by an internal force to prevent the sternum from sinking.

If the ossification of the sternum occurs with delay the possibility that the anterior chest wall will modify its shape increases.

A more detailed analyses of these modifications will determine the efficacy of osteogenesis that we know may depend not only on the type of BMP or the combination of BMPs that is present but also on the cell types that are present.

The timing of ossification of the xiphoid, or sternal segment 5, varies and the xiphoid may remain nonossified for years in humans. Some researchers think that asynchronous ossification of one of the five ossification sites may be suggestive of a number of disease processes that involve the anterior chest wall. [25]
congenital heart disease and in patients with several of the bone dysplasias. [25]

Dysplasias associated with delayed sternal ossification include camptomelic dysplasia, Noonan syndrome, and trisomy 17–18 in humans. [25]

It can be suggested that there is a possibility of a normal variation in sternal ossification centers with regards to there presence or absence, shape and size, not investigated.

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