A Bone Morphogenetic Protein Subfamily: Chromosomal Localization of Human Genes for BMP5, BMP6, and BMP7

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Bone morphogenetic proteins (BMPs) were originally identified by the ability of a demineralized bone extract to induce endochondral osteogenesis in vivo. Seven BMP cDNAs (BMP1 through BMP7) have been recovered through molecular cloning. Recombinant protein products from six of these clones (BMP2 through BMP7) are members of the transforming growth factor β (TGF-β) superfamily of regulatory molecules. Based upon a high degree of amino acid sequence homology, BMP5, BMP6, and BMP7 constitute a subfamily within the BMPs. Using human–rodent somatic cell hybrid lines and cDNA probes, we mapped the three members of this subfamily of genes to the human chromosomes. BMP5 and BMP6 are syntenic on human chromosome 6, while BMP7 is syntenic with previously localized BMP2 on human chromosome 20. This analysis reveals that BMP6 maps to a conserved region between the mouse and human genomes. Sequence analysis suggests that the Drosophila 60A gene is the dipteran homolog of this BMP subfamily and may provide clues to the physiologic functions of the products of these genes in human biology.

INTRODUCTION

Bone possesses the remarkable ability to regenerate completely following injury. This pathway of endochondral osteogenesis begins with proliferation of mesenchymal tissue and leads to the formation of calcified cartilage and bone (Campbell and Kaplan, 1992). While many factors influence bone cell growth and differentiation (Canalis, 1985; Centrella et al., 1991), the molecular mechanisms responsible for the induction and regulation of osteogenesis remain unknown.

In 1965, Urist first described bone morphogenetic protein (BMP), an extract derived from demineralized bone matrix that could independently induce endochondral osteogenesis in an in vivo extraskeletal site (Urist, 1965). Recently, the protein-coding regions for seven distinct BMPs (BMP1–BMP7) have been cloned and sequenced (Wozney et al., 1988; Celeste et al., 1990; Ozkaynak et al., 1990). Six of these genes (BMP2–BMP7) encode proteins belonging to the transforming growth factor β (TGF-β) superfamily of polypeptides (Wozney et al., 1988; Celeste et al., 1990; Ozkaynak et al., 1990). All TGF-β superfamily members, including BMP2–BMP7, are secreted as large precursor proteins and undergo proteolytic cleavage to an active form (Centrella et al., 1991). The mature carboxy-terminal region of all TGF-β-related peptides is characterized by the stringent conservation of seven cysteine residues (Centrella et al., 1991).

The BMPs have been categorized into subfamilies based on comparative amino acid sequence analysis. BMP5–BMP7 constitute one subfamily that share 71–80% sequence identity to one another in the mature protein region and 52–64% sequence similarity in the propeptide region (Celeste et al., 1990). Although the precise chondrogenic and osteogenic properties of this subfamily are unknown, recombinant human BMP5 can independently induce endochondral osteogenesis in vivo (D’Alessandro et al., 1991), and preliminary data on BMP7 suggest a role for this molecule in bone induction (Ozkaynak et al., 1990).

The BMPs have been implicated in embryonic development and tissue differentiation (Jones et al., 1991; Kawamura and Urist, 1988; Kaplan et al., 1990; Lyons et al., 1989, 1990; Wang et al., 1988, 1990). Further support for the developmental importance of the BMPs comes from the evolutionary conservation of these genes. The BMP2 and BMP4 gene products exhibit a striking 75% homology with the Drosophila dpp protein, which regulates development of the dorsal ectoderm, visceral mesoderm, and imaginal discs (Gelbart, 1989; Kaplan et al.,...
Recently, the dipteran homolog to the BMP5–BMP7 subfamily, the *Drosophila* 60A gene, has been identified and cloned (Wharton et al., 1991). The *Drosophila* 60A gene product shares 68–73% amino acid sequence homology with the mature polypeptide regions of BMP5–BMP7 (Wharton et al., 1991; Doctor et al., 1992).

BMP1–BMP4 have been localized and their chromosomal assignments suggest possible associations with several disorders of bone and cartilage formation (Tabas et al., 1991, 1993). In the present study, we used human–rodent somatic cell hybrid lines and cDNA probes to map the BMP5–BMP7 subfamily of genes to the human chromosomes.

**MATERIALS AND METHODS**

**Somatic cell hybrids.** Chromosomal assignments for BMP5, BMP6, and BMP7 were performed using a human chromosome mapping panel consisting of 17 human × mouse and 1 human × Chinese hamster somatic cell hybrid lines. All hybrids were characterized by both cytogenetic analysis and in situ hybridization to determine retention of human chromosomes (NIGMS, Human Genetic Mutant Cell Repository, Coriell Institute, Camden, N.J). A hybrid cell line was considered to contain the human chromosome if the human chromosome was present in >25% of the cells and to be negative if the human chromosome was present in <5% of the cells (data not shown).

**Hybridization probes.** The 0.2-kb BamHI/XbaI fragment of a partial-length cDNA for human BMP5, the 0.24-kb BamHI/XbaI fragment of a partial-length cDNA for human BMP6, and the 0.26-kb BamHI/XbaI fragment of a partial-length cDNA for human BMP7 were gel purified (Wozney et al., 1988; Celeste et al., 1990). All partial-length probes contain the 3' region of the full-length cDNA clone and were labeled by random priming using [α-32P]dCTP (Sambrook et al., 1989).

**Hybridization.** Prehybridization and hybridization were carried out at 42°C in hybridization buffer [6× SSC, 5× Denhardt's reagent, 0.5% (w/v) SDS, 1 mg/ml sonicated salmon sperm DNA, 50% (v/v) formamide]. Filters were washed in 2× SSC, 0.1% (w/v) SDS at room temperature, then 0.5× SSC, 0.1% (w/v) SDS at 65°C. Filters were exposed to Kodak XAR-2 film using intensifying screens at −70°C (Sambrook et al., 1989).

**RESULTS**

BMP5 was assigned to human chromosome 6 by analysis of a panel of *Pst*I-digested DNA from 18 human–rodent somatic cell hybrid lines. A single 9.4-kb fragment was detected in control human DNA and was easily distinguishable from the mouse and hamster patterns (Fig. 1). The 9.4-kb fragment was detected in all hybrid cell lines that retained human chromosome 6. There were zero discordancies for localization to this chromosome, and there were at least two discordancies for localization to any other chromosome (Table 1).

BMP6 was also assigned to human chromosome 6 by analysis of the same panel of *Pst*I-digested DNA that was used for the localization of BMP5. A single 1.2-kb fragment was detected in control human DNA and was easily distinguishable from the mouse and hamster patterns (Fig. 1). The 1.2-kb fragment was detected in all hybrid cell lines that retained human chromosome 6. There were zero discordancies for localization to this chromosome, and there were at least two discordancies for localization to any other chromosome (Table 1).

BMP7 was assigned to human chromosome 20 by analysis of a panel of *Hind*III-digested DNA from 18 human–rodent somatic cell hybrid lines. A single 11.2-kb fragment was detected in control human DNA and was easily distinguishable from the mouse and hamster patterns (Fig. 1). The 11.2-kb fragment was detected in all hybrid cell lines that retained human chromosome 20. There were zero discordancies for localization to this chromosome, and there were at least three discordancies for localization to any other chromosome (Table 1).

**DISCUSSION**

**Chromosomal Assignments**

We have localized the genes for three bone morphogenetic proteins: BMP5 and BMP6 to human chromosome 6, and BMP7 to human chromosome 20. Previous localizations of members of the TGF-β superfamily have revealed that these genes are widely dispersed throughout the human genome (Barton et al., 1988, 1989; Tabas et al., 1991, 1993). Chromosomal dispersion may have facilitated the development of tissue-specific functions for the various TGF-β superfamily members (Barton et al., 1989). Although the BMP family members are dispersed in the genome, our study reveals that several of the genes are syntenic (Table 2). Further sublocalization by in situ hybridization or somatic cell hybrid deletion panels will be helpful in determining whether these genes are clus-
TABLE 1

Discordancy Data for the Localization of BMP5, BMP6, and BMP7 to Human Chromosomes in Somatic Cell Hybrids

<table>
<thead>
<tr>
<th>Human chromosome</th>
<th>Number of discordant hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1    2    3    4    5    6    7    8    9    10    11    12    13    14    15    16    17    18    19    20    21    22    X    Y</td>
<td></td>
</tr>
<tr>
<td>BMP5 8     8     7     2     6     0     8     3     13    10    7     5     9     4     6     12    3     6     6     4     8     7     13    10</td>
<td></td>
</tr>
<tr>
<td>BMP6 8     8     7     2     6     0     8     3     13    10    7     5     9     4     6     12    3     6     6     4     8     7     13    10</td>
<td></td>
</tr>
<tr>
<td>BMP7 7     7     8     5     6     5     6     8     12    7     8     5     6     3     8     11    4     5     7     0     7     6     12    10</td>
<td></td>
</tr>
</tbody>
</table>

tered (BMP2 and BMP7 on chromosome 20, BMP5 and BMP6 on chromosome 6) or whether they occupy distinct nonlinked loci on those chromosomes.

BMP6 and Vgr-1 Map to Conserved Regions between Human and Murine Genomes

Vgr-1 is the murine homolog of human BMP6 based on amino acid sequence comparison (Celeste et al., 1990). The two peptides are >91% similar (Celeste et al., 1990). The Vgr-1 (Vg-related) gene was initially identified by cross-hybridization with a Vg1 cDNA, a Xenopus gene also belonging to the TGF-β superfamily (Lyons et al., 1989). The Vgr-1 gene has been previously mapped to murine chromosome 13, 2.2 ± 1.5 cM distal to the Friend MuLV integration site 1 (FIM-1), 14.3 ± 3.7 cM proximal to the hepatitis B virus transgenic integration site (4/12), and 1.6 ± 1.6 cM proximal to the satin (sa) mutation site (Dickinson et al., 1990). The FIM-1 gene maps to the p arm of human chromosome 6 and the close linkage with Vgr-1 suggested that BMP6 may also map to chromosome 6 (Dickinson et al., 1990). Our report confirms that BMP6 is localized to chromosome 6 and thus suggests yet additional regions of homology between the murine and human genomes. Linkage data utilizing additional markers are needed for further confirmation.

Possible Disease Associations with BMP5, BMP6, and BMP7

Localizations of the BMP genes have suggested possible associations with disorders of bone and cartilage formation (Table 2). BMP5 and BMP6 localize to chromosome 6, which has not yet been associated with any musculoskeletal disorders (Ziegler et al., 1991). However, Vgr-1, the murine homolog of BMP6, is a candidate gene for the congenital hydrocephalus (ch) mutation in the mouse based upon linkage analysis and mRNA expression data. This mutation is characterized by hydrocephalus and abnormalities in skeletal and renal embryogenesis (Green, 1970). Vgr-1 mRNA transcripts are found at high levels in several tissues including cartilage, epidermis, esophagus, kidney, and the meninges (Lyons et al., 1989). These data also suggest that BMP6 may play an important developmental role in a wide variety of tissues.

BMP7 maps to chromosome 20 which has recently been proposed as a possible locus for the Holt–Oram syndrome (HOS) (Yang et al., 1990), a disorder of cardiac and skeletal development characterized by atrioventricular septal defects and upper limb abnormalities (McKusick, 1990). This syndrome has been tentatively assigned to the q23–q24.2 region of human chromosome 14 based on a de novo deletion in a boy with skeletal and cardiac abnormalities consistent with HOS (McKusick, 1990). Yang et al. (1990) recently reported a child with HOS exhibiting a pericentric inversion of chromosome 20 with breakpoints at p13 and q13.2. They suggested that the mutation may occur at one of these breakpoints. BMP genes have been suggested as possible candidate genes for Holt–Oram syndrome based upon mRNA expression in developing tissues such as the atrioventricular cushion and the truncus arteriosus, as well as the api-

TABLE 2

BMP Gene Family: Localizations and Possible Disease Associations

<table>
<thead>
<tr>
<th>Human gene</th>
<th>Chromosomal location</th>
<th>Possible disease association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP1</td>
<td>8</td>
<td>Multiple hereditary osteochondromatosis</td>
<td>Tabas et al., 1991</td>
</tr>
<tr>
<td>BMP2</td>
<td>20p12</td>
<td>Holt–Oram syndrome; FOP</td>
<td>Tabas et al., 1991; Rao et al., 1992; Kaplan et al., 1990</td>
</tr>
<tr>
<td>BMP3</td>
<td>4p14.8–q21</td>
<td>Dentinogenesis imperfecta Type II</td>
<td>Tabas et al., 1991</td>
</tr>
<tr>
<td>BMP4</td>
<td>14</td>
<td>Holt–Oram syndrome; FOP</td>
<td>Kaplan et al., 1990; Tabas et al., 1993</td>
</tr>
<tr>
<td>BMP5</td>
<td>6</td>
<td>?</td>
<td>This report</td>
</tr>
<tr>
<td>BMP6</td>
<td>6</td>
<td>?</td>
<td>This report</td>
</tr>
<tr>
<td>BMP7</td>
<td>20</td>
<td>Holt–Oram syndrome</td>
<td>This report</td>
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Polycystic renal disease (Jones et al., 1991). Linkage analysis studies in large families with HOS will determine if BMP2, BMP4, or BMP7 is related to the HOS phenotype.

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REFERENCES


