ABSTRACT

Growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15) are receptor-dependent growth factors that are well known for their important roles in regulating folliculogenesis and female fertility. However, recent publications implicating their involvement in human cancers raise questions about whether GDF-9 and BMP-15 might exert diverse biological functions in non-ovarian tissues. We have purified recombinant mouse GDF-9 and recombinant human BMP-15 from Chinese Hamster Ovary (CHO) cells and evaluated their bioactivities using a range of bioassays. In a functional ELISA, mouse GDF-9 and human BMP-15 bind to the recombinant human/mouse BMP-RII extracellular domain with Kd values of 1.4 nM and 2 nM, respectively. In addition, the two proteins activate Smad 2/3 in P19 cells. Similar to other members of the transforming growth factor-β family, recombinant mouse GDF-9 is able to induce apoptosis in Mv1Lu and DU145 cells. In addition, recombinant human BMP-15, like other BMP family members, is osteogenic and promotes differentiation of MC3T3-E1 cells to osteoblasts. We also found that human BMP-15 is very potent in supporting the survival and proliferation of NIH3T3 cells under nutrient deprived conditions. The differential in vitro functions of GDF-9 and BMP-15 provide a basis for new research initiatives and also open a new avenue to explore their functions in cancer biology and reproductive physiology. These studies in turn may lead to an expanded interest into their potential use as therapeutic targets.

INTRODUCTION

GDF-9 and BMP-15 are members of the transforming growth factor-β (TGF-β) superfamily and possess similar structural and functional properties. Both molecules form non-covalently linked homodimers or heterodimers due to the lack of the fourth of seven conserved cysteines present in the majority of the TGF-β family members. GDF-9 and BMP-15 are predominately expressed in the ovary and play important roles in regulating folliculogenesis through several paracrine mechanisms. In GDF-9-deficient mice, expression levels of several ovarian marker genes are reduced and follicular development is arrested at the preantral stage. GDF-9 and BMP-15 promote the growth and differentiation of granulosa cells, theca cells, and oocytes. GDF-9 in concert with BMP-15 modulates the cumulus expansion, which is essential for ovulation, fertilization, and implantation. However, the biological significance of GDF-9 and BMP-15 outside the ovary is largely unknown. Recent publications implicating their biological functions in human cancers raised questions of whether GDF-9 and BMP-15 might exert diverse functions in non-ovarian tissues. We have purified the recombinant mouse GDF-9 and human BMP-15 from Chinese Hamster Ovary (CHO) cells and evaluated bioactivity using several bioassays. Members of the TGF-β superfamily transmit signals to the nucleus by signaling through type II and type I serine-threonine kinase receptors, and intracellular effectors known as Smads. Ligands bind to a type II receptor on the surface of the cell and then activate their receptor, activating it via phosphorylation. This activated complex phosphorylates Smads, which translocate to the nucleus to transduce the signal. Studies indicate GDF-9 and BMP-15 signaling utilizes the TGF-β/BMP pathway, acting through BMP-RII and Smad2/3, and eliciting cell proliferation, differentiation, and many other cellular processes during folliculogenesis.

SUMMARY

• Both rmGDF-9 and rhBMP-15 signal through the BMP-RII receptors and activate Smad2/3.
• rhBMP-15 displays TGF-β-like activity in vitro, inducing apoptosis of Mv1Lu embryonic mouse cells with various concentrations of BMP-15 as indicated, for 24 hours. The effect of rhBMP-15 on cell viability was measured using MTT assay. The effect of rhBMP-15 on NIH3T3 cells was measured by the amount of H2-Ras bioassay incorporated into the DNA.

REFERENCES