IMPROVING BONE MORPHOGENETIC PROTEIN PRODUCTION IN CHO CELLS BY UNDERSTANDING ITS MATURATION, SIGNALING, AND ENDOCYTOSIS

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Bone morphogenetic proteins (BMPs) are a group of growth factors with the clinical potential to regulate cartilage and bone formation. Functionally active mature recombinant human BMPs (rhBMPs) are primarily expressed in Chinese hamster ovary (CHO) cells and have clinical applications. rhBMPs are considered difficult to express because they undergo maturation processes, signaling pathways, or endocytosis. Although BMPs are a family of proteins with similar mature domain sequence identities, their individual properties are diverse. Therefore, there is no general strategy to improve rhBMP production in CHO cells. The production of rhBMPs, such as rhBMP2, rhBMP4, rhBMP7, and GDF5, has been significantly improved in CHO cells by understanding the properties of individual rhBMPs with respect to their maturation processes, endocytosis, and signaling pathways. When the rhBMP gene is overexpressed as a large precursor in CHO cells, the content of proteolytic cleaving enzymes within the secretory pathway is likely insufficient to process the precursor. We observed that PC5/6DC overexpression in rhBMP7-producing recombinant rCHO (rCHO) cells resulted in complete processing of rhBMP7. No precursors were detected in the culture supernatants, thus resulting in a 1.5-fold increase in maximum rhBMP7 concentration. Some rhBMPs, such as rhBMP2, rhBMP4, and GDF5, secreted in the culture medium, are internalized into CHO cells via cell surface heparan sulfate proteoglycan (HSPG)-mediated endocytosis, which substantially lowers rhBMP titers in rCHO cell cultures. Secreted rhBMPs in the culture medium are internalized actively by binding to cell surface HSPGs in CHO cells. Therefore, secreted rhBMPs binding to HSPGs is inhibited by adding competitive inhibitors of HSPG, such as dextran sulfate (DS), to the culture medium, which effectively improves product yield by blocking undesirable endocytosis of secreted rhBMP into CHO cells. Adding 1.0 g/L of 15 kDa DS to the culture medium effectively blocked rhBMP4 internalization in CHO cells, resulting in 1.4-fold and 2.3-fold increase in the maximum rhBMP4 concentration in batch and fed-batch cultures, respectively. A more significant increase in the maximum concentration (22-fold increase) was obtained in batch cultures of rhBMP2-producing CHO cells by adding 15 kDa DS to the culture medium. In contrast, the addition of 15 kDa DS to the culture medium did not block rhGDF5 internalization in CHO cells, suggesting that a longer chain DS may be required. Indeed, the addition of 200 kDa DS to the culture medium at a concentration of 1.0 g/L significantly inhibited GDF5 internalization, resulting in a 10.3-fold increase in the maximum GDF5 concentration in batch culture. CHO cells express BMP receptors and Smad proteins, which are major mediators of canonical BMP signaling. Therefore, rhBMPs secreted into the conditioned medium may trigger autocrine signaling events that induce Smad signaling in rCHO cells. Exogenous BMP4 reduced the mRNA levels of endogenous BMP4 in CHO cells. rhBMP4-producing CHO cells, established using BMP receptor gene knockout host cells, lacked autocrine BMP signaling, did not experience growth inhibition induced by rhBMP4, and showed increased rhBMP4 mRNA expression. The mean maximum rhBMP4 concentration of knockout host cell-derived clones was approximately 2.4-fold higher than that of wild-type host cell-derived clones. In conclusion, as BMPs have very distinct characteristics, understanding the properties of individual rhBMPs with respect to their maturation processes, endocytosis, and signaling pathways is essential for improving rhBMP production in CHO cells.