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授与した学位	博　士
専攻分野の名称	歯　学
学位授与番号	博甲第５９３４号
学位授与の日付	平成３１年３月２５日
学位授与の要件	医歯薬学総合研究科機能再生・再建科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Bone Marrow Cells Inhibit BMP-2-Induced Osteoblast Activity in the Marrow Environment. （骨髄細胞は BMP-2 誘導性骨芽細胞分化を抑制する）
論文審査委員	松本　卓也　教授　　窪木　拓男　教授　　上岡　寛　教授

学位論文内容の要旨

論文内容の要旨（2000字程度）

Objective: Bone morphogenetic protein 2 (BMP-2) is widely known as a potent growth factor that promotes bone and cartilage formation. However, an increasing number of reports have demonstrated the side effects of BMP-2 therapy. Therefore, a deeper understanding of the effect of BMP-2 on different cells other than those involved directly in bone remodeling, is of fundamental importance to promote a more effective delivery of BMP-2 to patients. In some clinical cases, it is necessary to induce bone formation in marrow area; however, the efficacy of BMP-2 in the marrow environment has not been thoroughly investigated. In this study, we aimed to investigate the effect of BMP-2 in the marrow environment.

Methods: At first, BMP-2 adsorbed onto dental titanium implants was delivered at the tooth extraction socket (less marrow site) or in a mandible marrow (rich marrow site) of beagle dogs. Next,

to investigate the effect of marrow on BMP-2 function, BMP-2 adsorbed in freeze-dried collagen pellets were transplanted into the calvarial bone with less marrow and inside the femoral cavity with rich marrow in C57BL/6 mice. In order to understand whether the marrow inhibits BMP-2-induced osteoblast differentiation, the appearance of osteoblasts was investigated using *Col1a1*-GFP transgenic mice and the ability of BMP-2 to induce bone formation was analyzed in marrow ablated femur. To analyze the effect of the marrow on the inhibition of BMP-2-induced the osteoblasts differentiation, *in vitro*, experiments analyzing luciferase activity of C2C12 cells with the BMP-responsive element (BRE) and ALP activity of MC3T3-E1 osteoblasts by co-culture cells technique directly or indirectly.

Results: BMP-2 could induce marked bone formation around the implant at the tooth extraction socket. Surprisingly, no bone formation was observed in the BMP-2-coated titanium implants inserted in the mandible marrow, hence, significantly inhibited osseo-integration in dental implant. In mice, BMP-2 could induce bone formation in marrow-absent calvarial bone. However, similar to the canine model, BMP-2 could not induce bone formation in the femur marrow. In fact, BMP-2 inhibited bone formation inside the marrow dose dependently. Analysis of osteoblast differentiation in *Col1a1*-GFP transgenic mice revealed a scarce number of osteoblasts in BMP-2-treated femurs, whereas in control group, osteoblasts were abundant. Further examination of BMP-2 function in ablated marrow femur showed that the ablation of femur marrow recovered the BMP-2 ability to induce bone formation. In co-culture cells experiment revealed that bone marrow cells inhibit BMP-2 induce osteoblast differentiation effect on osteoblasts by direct cell-cell contact.

Conclusion: Collectively, these results showed that the effect of BMP-2 in inducing bone formation is remarkably repressed by marrow cells via direct cell-cell contact with osteoblasts, and open new perspectives on the clarification of the side-effects associated with the BMP-2 application.

論文審査結果の要旨

Objective: Bone morphogenetic protein 2 (BMP-2) is a potent growth factor that promotes bone and cartilage formation. However, an increasing number of reports have demonstrated the side effects of BMP-2 therapy. Therefore, a deeper understanding of the effect of BMP-2 on different cells other than those involved directly in bone remodeling, is of fundamental importance to promote a more effective delivery of BMP-2 to patients. In this study, we aimed to investigate the effect of BMP-2 in the marrow environment.

Methods: At first, BMP-2 adsorbed onto dental titanium implants was delivered at the tooth extraction socket (less marrow site) or in a mandible marrow (rich marrow site) of beagle dogs. To investigate the effect of marrow on BMP-2 function, BMP-2 adsorbed in freeze-dried collagen was transplanted into the calvarial bone (less marrow) and inside the femoral cavity (rich marrow) in C57BL/6 mice. The appearance of osteoblasts was investigated using Col1a1-GFP transgenic mice and the ability of BMP-2 to induce bone formation was analyzed in ablated femur. *In vitro*, experiments analyzing luciferase activity of C2C12 cells with the BMP-responsive element (BRE) and ALP activity of MC3T3-E1 osteoblasts by co-culture cells technique directly or indirectly.

Results: BMP-2 induced marked bone formation around the implant at the tooth extraction socket but prohibited bone formation and osseointegration in the mandible marrow. In mice, BMP-2 could induce bone formation in marrow-absent calvarial bone or outside cortical bone, however, could not induce bone formation in the femur marrow. In fact, BMP-2 inhibited bone formation inside the marrow dose-dependently. In Col1a1-GFP transgenic mice, a scarce number of osteoblasts in BMP-2-treated femurs were observed, whereas in control group, osteoblasts were abundant. Further examination, the ablation of femur marrow recovered the BMP-2 ability to induce bone formation. In co-culture cells experiment revealed that bone marrow cells inhibit BMP-2 induce osteoblast differentiation effect on osteoblasts by direct cell-cell contact.

Conclusion: Collectively, these results showed that the effect of BMP-2 in inducing bone formation was remarkably repressed by marrow cells via direct cell-cell contact with osteoblasts, and open new perspectives on the clarification of the side-effects associated with the BMP-2 application.

Then, the committee determined that the thesis paper has sufficient scientific merit to fulfill the requirement for PhD (dentistry) degree.