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5. Research Theme Analysis on the bone metabolism regulation by osteopontin, a molecule involved in mechanical sensing

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7. Organization Tokyo Medical and Dental University  Medical Research Institute

8. Summary of Research

Space environment provides a gravity condition which induces rapid loss of bone. However, the mechanisms of such gravity-induced bone loss have not yet been fully understood. In order to let the bone sense the stress of physical forces, it is suspected that the cells in bone require certain gravitational sensing mechanisms, one of which could be the machinery of the cells to attach to the substrate, namely bone. Such attachment machineries of the cells include integrins and their ligands. Osteopontin is a molecule which contains a motif consisting of arginine, glycine, aspartic acid and serine (RGDS). This RGDS motif has been known to be recognized by a variety of integrins including $\alpha_v\beta_3$, an abundant integrin expressed in osteoclasts. In fact, osteopontin-deficient mice are resistant to bone loss upon ovariectomy which depletes estrogen to mimic a situation observed in post-menopausal osteoporosis. Tail suspension is a model used to mimic gravity-induced bone loss on the ground. By using tail suspension model, we have identified that osteopontin is a molecule which is required for the bone loss induced by tail suspension as well as increased in bone resorption due to tail suspension-dependent decrease in bone formation due to the lack of mechanical stress. It is known that even though the bone resorption could be prevented by the treatment with bisphosphonate, it is impossible to prevent another component of bone loss, which occurs due to the reduction in bone formation.

Since bone formation is also regulated by vascular function, we investigated whether osteopontin is involved in vascularization. Experiments using bone disc resorption in muscle environment indicated that osteopontin-knockout mice implanted with osteopontin-deficient bone revealed significant deficiency in bone resorption in the ectopic site. This was observed based on the X-ray pictures of the implanted bone disc examined at the end of the implantation experiments. We further examined whether local environment without vascularization could also be the place for osteopontin function by using organ culture systems where bone resorption by the osteoclasts residing within the bone explants was stimulated with PTH. PTH treatment resulted in resorption of bone in organ cultures, where release in calcium 45 used as a radio labeling tracer, into medium was increased. Osteopontin-deficiency in bones cultured in the presence of PTH suppressed bone resorption induced by this hormone. The osteoclasts’ morphology including the actin ring formation was not altered by the absence of osteopontin. These experiments indicated that osteoclast function by itself requires osteopontin to efficiently mediate the signals from PTH.

As osteopontin is a ligand for integrins, we tested whether such binding activities...
could be deficient in in vivo environment. For this purpose, we used melanoma metastasis model since melanoma cells such as B16 express integrin which can be recognized by osteopontin. In fact, in vitro experiment using osteopontin-coated plates indicated that melanoma cells efficiently attached to osteopontin in vitro. We then asked whether melanoma cells attachment to osteopontin-deficient animals would be similar or different from wild type. For this purpose, B16 melanoma cells were injected either through left ventricle by direct injection or through femoral vein. After the injection with B16 cells, organs were inspected to count the number of the metastasized B16 tumor foci. Intracardiac injection resulted in metastasis into bone, liver, adrenal gland and many other tissues. Melanoma metastasis levels into bone was significantly reduced in the absence of osteopontin. Intravenous route of B16 cell induced indicated metastasis predominantly in lung. In this case, the number of metastasized tumor was also less in osteopontin-deficient mice. These data suggest that the presence of osteopontin in the host environment facilitated the attachment of B16 cells, and thus indicated that osteopontin is involved in cell attachment in in vivo conditions.

Overall, our data suggested strongly that osteopontin is involved in cell attachment in vivo and this attachment in bone could be the site where the cells are sensing the mechanical stress in vivo. Since mechanical stress also affects vascularization as well as the flow stimuli in the microenvironment in bone, osteopontin may also play a role in this aspect. Experimental data accumulated within the period of 2000 and 2001 support the notion that osteopontin is one of the molecules required for the mediation of the mechanical environment signals to the cells in vivo. Thus, this in vivo observation should be subjected to further analysis to obtain molecular insights into the function of this molecule to link the mechanical environment to the function of osteoclasts and osteoblasts.

Publication List

Original articles