Prevention of postoperative progression of pulmonary metastases in osteosarcoma by anti-angiogenic therapy using endostatin

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Introduction: We have recently demonstrated the animal experimental and clinical data suggesting the positive linkage of postopeartive upregulation of systemic angiogenic activity and disease relapse (1-4). The finding that the significant downregulation of endostatin was critical in the angiogenic elevation after primary tumor removal suggests that endostatin is a candidate for the anti-angiogenic therapy for osteosarcoma. In the current study, we investigated whether endostatin therapy after primary tumor removal can prevent the progreesion of pulmonary metastasis.

Materials and Methods: To examine whether postoperative anti-angiogenic therapy could prevent the postoperative progression of pulmonary metastasis, we administrated Ad5CMV-mEnd (Q-BIO gene, Carlsland, CA) after primary tumor removal. Mice were inoculated subcutaneously with mouse osteosarcoma cell line LM8 cells. Two weeks after tumor inoculation, Two weeks after the s.c. inoculation of mouse osteosarcoma cell line LM8 cells, the animals were anesthetized and the primary tumor was removed surgically. Then, Ad5CMV-mEnd (1×106, 1×107, 1×108 particles) or Ad5.CMV-LacZ (1×108 particles) was injected into the tail vein. Two weeks after the viral injection, mice were sacrificed and the number of macroscopic pulmonary metastases was counted. Systemic angiogenic activity was evaluated by Matrigel plug assay.

Results: The number of pulmonary metastasis was smaller in the mice injected with 1×107 and 1×108 particles of Ad5CMV-mEnd than in controls (LacZ v.s. 7 PFU; p=0.002, La Z v.s. 8 PFU; p=0.003, Figure 1), accompanied by significant suppression of systemic angiogenic activity (LacZ v.s. 7 PFU; p=0.0005, LacZ v.s. 8 PFU; p=0.0003, Figure 2). In addition, the sizes of the pulmonary metastases of the mice injected with 1×107 and 1×108 particles of Ad5CMV-mEnd were smaller than in control group (Figure 3) suggesting that postoperative anti-angiogenic therapy had the potential to keep the smaller metastatic lesions as a dormant state. In contrast, no significant reduction in the number of pulmonary metastases have not been observed in the mice which injected with 1×106 particles of Ad5CMV-mEnd (intact-tumor group v.s. 6 PFU; p=0.0001, Figure 1) and the sizes of metastatic lesions were larger than those of intact-tumor group (Figure 3). The serum level of endostatin in the injected mice was lower than in intact-tumor group (1 week after the infection, intact-tumor group, 420±36 ng/ml and 6 PFU, 395±21 ng/ml; 2 weeks after the infection, intact-tumor group, 866±56 ng/ml and 6 PFU, 673±56 ng/ml, Figure 1) and the hemoglobin concentration of the serum obtained from these injected mice was higher than in the intact-tumor group (p=0.005, Figure 2).

Discussion: In the current study, we demonstrated that postoperative endostatin therapy could suppress the postoperative enhancement of systemic angiogenic activity and led to the prevention of postoperative progression of pulmonary metastasis. These results suggest that postoperative treatment of endostatin can be a new therapeutic tool for osteosarcoma. We conclude that anti-angiogenic therapy using endostatin has the potential to prevent postoperative progression of pulmonary metastasis and enable patients to coexist with dormant pulmonary metastasis. Although this therapeutic strategy cannot provide a cure for osteosarcoma, improvement of the prognosis for patients with osteosarcoma is highly expected.

References: 1 Kaya M, Wada T, Akatsuka T, et al. Clin Cancer Res 2000; 6: 572-577. 2 Kaya M, Wada T, Kawaguchi S et al. Br J Cancer. 2002; 86: 864-869. 3 Tsunemi T, Nagoya S, Kaya M, et al. Clin Orthop 2003; 407: 159-166. 4 Kaya M, Wada T, Nagoya S et al. J Bone Joint Surg (B)2004; 86: 143-147.

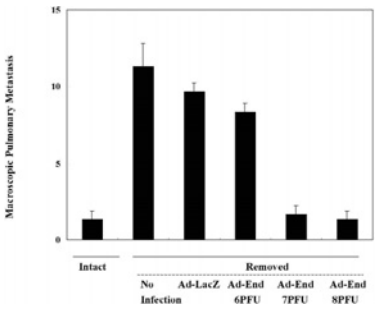

Figure 1 Prevention of the progression of pulmonary metastasis by Ad5CMV-mEnd

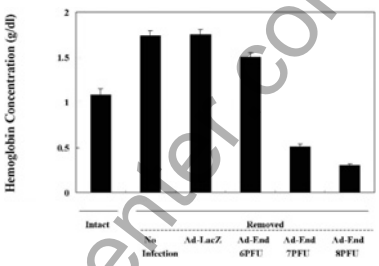

Figure 2 Angiogenesis-inducing ability of the serum after injection of Ad5CMV-mEnd

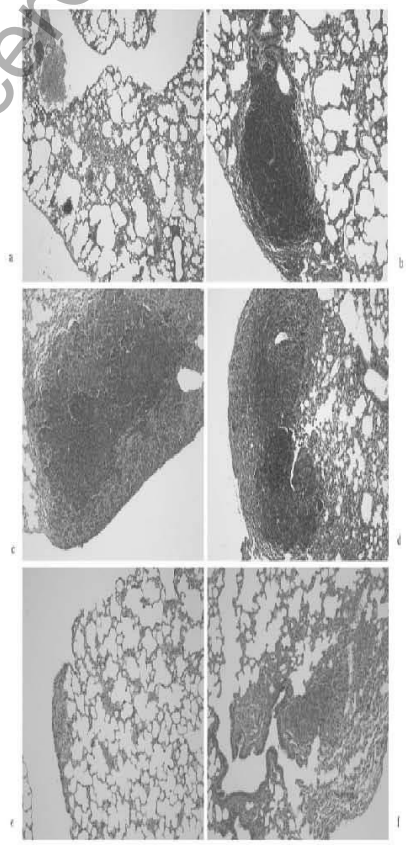

Figure 3 Microscopic pulmonary metastasis after injection of Ad5CMV-mEnd a; tumor intact, b; tumor removed, c; Ad-LacZ, d; Ad-End 6PFU, e; Ad-End 7PFU and f; Ad-End 8PFU