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Tumor-suppressive activities by chemokines introduced into OV-HM cells using fiber-mutant adenovirus vectors

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In this study, fiber-mutant adenovirus vectors encoding chemokines, Ad-RGD-mCCL17, Ad-RGD-mCCL21 and Ad-RGD-mCCL22 were constructed. The insertion of integrin-targeting RGD sequence into fiber knob of adenovirus vectors notably enhanced the infection efficiency into tumor cells. Among three chemokine-encoding vectors evaluated, Ad-RGD-mCCL22 showed significant tumor-suppressive activity via transduction into OV-HM cells.

Cytokine or chemokine encoded by a viral vector is currently regarded as a promising way of cancer gene immunotherapy. Chemokines consist of a superfamily of small secreted proteins that attract their target cells by interacting with G protein-coupled receptors expressed on these cells. Researchers have paid attention to chemotactic activity of chemokines for immune cells, and have expected that they may be able to play a pivotal role in cancer treatment, because the basis and premise of immunotherapy is the accumulation of immune cells in tumor tissues. More than 40 chemokines have been identified so far (Yoshie et al. 2001), but only a few have been demonstrated as candidates for cancer therapy by using as sole agents or with adjuvant (Gao et al. 2003; Maric and Liu 1999).

In the present report, three CC family chemokines, thymus and activation-regulated chemokine/CCL17, secondary lymphoid-tissue chemokine/CCL21 and macrophage-derived chemokine/CCL22 have been studied. CCL17 and CCL22 are chemotactic for memory CD4 + T cells via CCR4 while CCL21 induces migration of T cells, B cells, dendritic cells and NK cells via CCR7 (Campbell et al. 1999, Nagira et al. 1997). CCL22 was also shown to have a chemoattractant activity for dendritic cells, NK cells and T cells (Godiska et al. 1997). We hypothesized that if tumor cells could be genetically modified *in vitro* to produce chemokines *in vivo*, the chemokines would accumulate immune cells in the tumor. The facilitated interaction of immune cells with the tumor cells *in vivo* might induce anti-tumor activity. To test this hypothesis, we developed a recombinant adenovirus vector with a fiber mutation containing the integrin-targeting Arg-Gly-Asp (RGD) sequence in the fiber knob (Mizuguchi et al. 2001b). As shown in the Table, This vector has been demonstrated to possess higher transduction efficacy to OV-HM cells, a mouse ovary carcinoma line (Hashimoto et al. 1989), compared to that of conventional adenovirus vector.

Table: EGFP expression in OV-HM cells infected with Ad-EGFP and Ad-RGD-EGFP

	250 PT % gated	500 PT % gated
Non-infection	0.94%	0.94%
Ad-EGFP	47.1%	76.3%
Ad-RGD-EGFP	98.7%	99.6%

OV-HM cells were infected with 250 or 500 particles/cell of Ad-EGFP or Ad-RGD-EGFP for 48 h and EGFP expression was measured by flow cytometric analysis

In this study, we infected OV-HM with fiber-mutant adenovirus vectors encoding mCCL17, mCCL21 or mCCL22 and examined their expression by RT-PCR. The migration assay of chemokine-encoding vectors was also conducted *in vitro*. The results demonstrated that the efficient production of biologically active mCCL17, mCCL21 and mCCL22 could be detected in the culture supernatants of cells infected with these vectors, and the vectors could efficiently migrate the specific receptor-expressing cells (data not shown). Then OV-HM cells infected with Ad-RGD-mCCL17, Ad-RGD-mCCL21, Ad-RGD-mCCL22 or Ad-RGD-NULL (the control vector only) were intradermally inoculated into B6C3F1 mice to evaluate their effects on tumor growth *in vivo*. As shown in the Fig., OV-HM infected with Ad-RGD-mCCL22 showed significant suppression in tumor growth. On the other hand, OV-HM infected with either Ad-RGD-mCCL17 or Ad-RGD-mCCL21 did not show any difference in tumor growth from that infected with Ad-RGD-NULL. In rechallenge experiment, mice that had complete regression were intradermally injected with OV-HM or B16/BL6 cells 90 days after the initial challenge. Results demonstrated that 100% of mice rechallenged with OV-HM remained tumor-free. In contrast, all of the mice rechallenged with B16/BL6 developed palpable tumors within 2 weeks (data not shown). These results indicated the generation of specific immunity against OV-HM in mice that rejected OV-HM expressing mCCL22. To exclude the possibility that the growth suppression of the tumor cells by Ad-RGD-mCCL22 was due to the cytotoxicity of adenovirus or

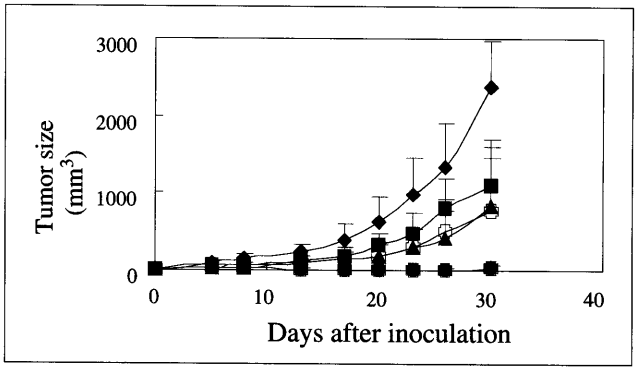

Fig.: Tumor-suppressive activity of Ad-RGD-mCCL22
—◆— No-treat, —■—Ad-RGD-NULL, —▲— Ad-RGD-mCCL17, —●— Ad-RGD-mCCL22, —○— Ad-RGD-mCCL21

chemokine, OV-HM cells transfected with Ad-RGD-mCCL17, Ad-RGD-mCCL21, Ad-RGD-mCCL22 or Ad-RGD-NULL were cultured for 48 h, and the cell viability was measured by the MTT assay. The *in vitro* growth of the cells infected with these vectors was essentially identical to that of control cells (data not shown).

In summary, our study suggests that CCL22, a CC family chemokine, may be a good candidate for cancer gene immunotherapy.

Experimental

1. Cell lines and animals

OV-HM ovarian carcinoma cells were kindly provided by Dr. Hiromi Fujiwara (School of Medicine, Osaka University, Japan) and were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS. Human embryonic kidney (HEK) 293 cells were cultured in DMEM supplemented with 10% FBS. All the cell lines were cultured at 37 °C in a humidified atmosphere with 5% CO_2. Female B6C3F1 mice (6–8 week of age) were purchased from SLC Inc. (Hamamatsu, Japan). All of the experimental procedures were in accordance with the Osaka University guidelines for the welfare of animals in experimental neoplasia.

2. Procedures

2.1. Infection of chemokines into OV-HM cells using fiber-mutant adenovirus vectors

Replication-deficient adenovirus vectors used in this study were based on the adenovirus serotype 5 backbone with deletions of E1 and E3 and the expression cassette in the E1 region (Mizuguchi et al. 2001a). The integrin-targeting RGD sequence was inserted into the HI loop of the fiber knob using a two-step method (Mizuguchi et al. 2001b). Fiber-mutant adenovirus vectors, Ad-RGD-mCCL17, Ad-RGD-mCCL21 and Ad-RGD-mCCL22 carrying the murine chemokine cDNA under the control of the cytomegalovirus promoter, were constructed by an improved *in vitro* ligation method as described (Mizuguchi and Kay 1998). The Ad-RGD-NULL vector, serving as a negative control, is identical to the Ad-RGD-chemokine vectors without the chemokine gene in the expression cassette. The adenovirus vectors were propagated in HEK 293 cells and purified by cesium chloride gradient ultracentrifugation, and their titer was determined by plaque-forming assay.

2.2. Tumor rejection in mice and subsequent rechallenge by tumor reinoculation

1×10^6 OV-HM cells that had been infected with Ad-RGD-mCCL17, Ad-RGD-mCCL21 or Ad-RGD-mCCL22 at a MOI (Multiplicity of Infection) of 10 for 24 h were inoculated intradermally into the flank of mice. The length and width of the tumor were measured twice a week. Animals were euthanized when one of the two measurements were greater than 15 mm. Three months after complete regression of primary tumors, mice were rechallenged with freshly isolated OV-HM tumor cells or B16/BL6 melanoma cells by intradermal injection of 1×10^6 cells into the flank.

References

Campbell JJ, Haraldsen G, Pan J et al. (1999) The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature 400: 776–780.

Gao JQ, Tsuda Y, Katayama K et al. (2003) Anti-tumor effect by interleukin-11 receptor alpha-locus chemokine/CCL27, introduced into tumor cells through a recombinant adenovirus vector. Cancer Res 63: 4420–4425.

Godiska R, Chantry D, Raport CJ et al. (1997) Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J Exp Med 185: 1595–1604.

Hashimoto M, Niwa O, Nitta Y et al. (1989) Unstable expression of E-Cadherin adhesion molecules in metastatic ovarian tumor cells. Jpn J Cancer Res 80: 459–463.

Maric M, Liu Y (1999) Strong cytotoxic T lymphocyte responses to a macrophage inflammatory protein 1 alpha-expressing tumor: linkage between inflammation and specific immunity. Cancer Res 59: 5549–5553.

Mizuguchi H, Kay MA, Hayakawa T (2001a) In vitro ligation-based cloning of foreign DNAs into the E3 and E1 deletion regions for generation of recombinant adenovirus vectors. Biotechniques 30: 1112–1114.

Mizuguchi H, Koizumi N, Hosono T et al. (2001b) A simplified system for constructing recombinant adenoviral vectors containing heterologous peptides in the HI loop of their fiber knob. Gene Ther 8: 730–735.

Mizuguchi H, Kay MA (1998) Efficient construction of a recombinant adenovirus vector by an improved *in vitro* ligation method. Hum Gene Ther 9: 2577–2583.

Nagira M, Imai T, Hieshima K et al. (1997) Molecular cloning of a novel human CC chemokine secondary lymphoid-tissue chemokine that is a potent chemoattractant for lymphocytes and mapped to chromosome 9p 13. J Biol Chem 272: 19518–19524.

Yoshie O, Imai T, Nomiyama H (2001) Chemokines in immunity. Adv Immunol 78: 57–110.