Synergistic antitumor activity elicited by vaccination with the chemokine CCL21 in combination with IL-12-encoding plasmid DNA in vivo

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Abstract

Purpose: On the basis of the results of phase I clinical trial on patients with malignant melanoma (1), we are examining the possibility to improve the immune therapeutic treatment. Thus we asse different combination of therapies with Interleukin-12 (IL-12) and chemokines. The chemokine CCL21 plays an important role in attracting naive T cells and immature dendritic cells (DCs) from t periphery to secondary lymph organs associated with sites of inflammation or tumors. This feature, recruiting professional antigen-presenting DCs and T lymphocytes makes CCL21 a prir candidate for tumor therapy. Moreover, pronounced antitumor activity of IL-12 applied as plasmid DNA directing the expression of IL-12 was demonstrated in syngeneic primary and metasta mouse tumor models in gray horses that spontaneously develop melanoma and in a clinical phase I trial (1, 2). **Methods**: Groups of mice were injected intratumorally with recombinant CCL protein (recCCL21) or DNA encoding CCL21 (CCL21-DNA) in combination with DNA encoding IL-12. **Results and Conclusions**: Intratumoral application of plasmids encoding CCL21 or IL-12 exerted an antitumor effect against malignant melanoma and renal cell carcinoma in syngeneic mouse models. Furthermore, we observed a pronounced synergistic antitumor activity in the mou melanoma model when recCCL21 and CCL21-DNA was applied in combination with DNA encoding IL-12 (IL-12-DNA).
Keywords: chemokines, IL-12, melanoma

(1) Heinzerling L, Burg G, Dummer R, Maier T, Oberholzer PA, Schultz J, Elzaouk L, Pavlovic J, Moelling K, Hum Gene Ther, 16:35-48, 2005.
(2) Heinzerling LM, Feige K, Rieder S, Akens MK, Dummer R, Stranzinger G, Moelling K, J Mol Med, 78:692-702, 2001.

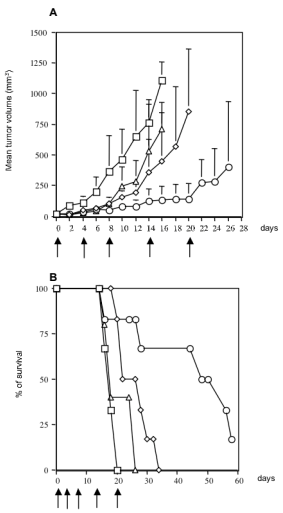

Fig.1. Time course of B16F10 melanoma tumor growth in C57BL/6 mice treated by administration of recCCL21. Tumors were established by subcutaneous inoculation of 1x10^5 B16F10 cells into the right hind flank. When a mean tumor volume of 15-20 mm^3 was reached (day 0), treatment was started. Groups of 6 mice were intratumorally injected with 0.4 ug recCCL21 (Δ) or 100 ug IL-12-DNA (◊) or combination of 0.4 ug recCCL21 and 100 ug IL-12-DNA (O) or 100ug control DNA (□) on days 0, 4, 8, 14, and 20. Mean tumor volumes of groups ± standard deviations are depicted. Tumor formation was significantly reduced in recCCL21/IL-12-DNA-treated mice compared to control mice. Mice were euthanized when the tumor volume was superior to 1400-1500 mm^3. **(A)** Growth kinetics and **(B)** percentage of mice survival treated with recCCL21 and IL-12-DNA.

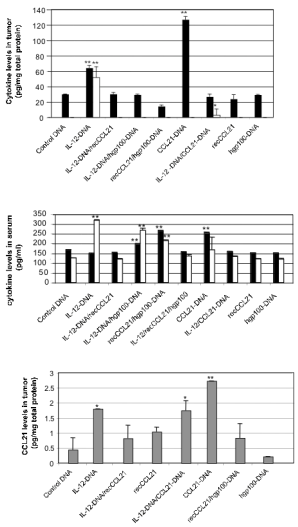

Fig.4. Levels of IP-10, IFN-γ, and IL-12 in mouse sera and tumors. Seven day-old established tumors were treated with intratumoral injection of either 100 ug IL-12-DNA or 0.4 ug recCCL21 or 100 ug hgp100-DNA or 100 ug CCL21-DNA alone or in combination every four days before tumors were harvested at day 16 resulting in a total of 4 injections for cytokine analysis. Tumors homogenates **(A)** and sera **(B)** taken at day 16 after the beginning of the therapy were evaluated for the presence of murine IP-10 (black columns), IFN-γ (white columns), and IL-12 (was not detectable). **(C)** Homogenates from tumors were evaluated also for the presence of CCL21 (gray columns). Results are expressed in pg/ml for sera and in pg/mg of total protein for tumor homogenates.

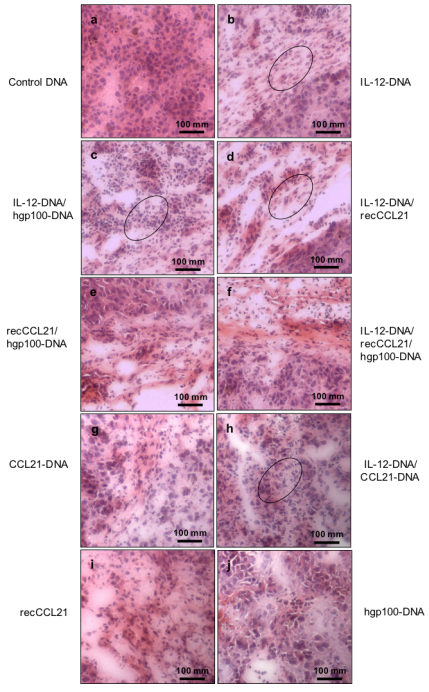

Fig.5. Intratumoral CCL21-DNA and IL-12-DNA therapies were accompanied by histological changes. Hematoxylin-Eosin staining of frozen-tumor sections (x100 magnification). **(a)** Tumors treated with control DNA consist of heavily pigmented tumor cells that cleared after treatment. **(b, c, d)** Tumors treated with IL-12-DNA or IL-12-DNA after a total of four injections showed signs of tumor cell apoptosis or formation of scarry connective tissue demonstrating fibrosis or T cells infiltration. **(e, f, g, h, i, j)** Tumors treated with different combinations of therapies recCCL21/hgp100-DNA, IL-12-DNA/recCCL21/hgp100-DNA, CCL21-DNA, IL-12-DNA/CCL21-DNA, recCCL21, and hgp100-DNA respectively, showed fibrosis formation. The circles indicate fibrosis formation in the tumor.

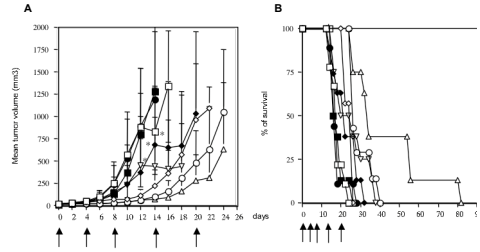

Fig.3. Time course of B16F10 melanoma tumor growth in C57BL/6 mice treated by administration of CCL21-DNA and hgp100-DNA. Tumors were established by subcutaneous inoculation of 1x10^5 B16F10 cells into the right hind flank. When a mean tumor volume of 15-20 mm^3 was reached (day 0), treatment was started. Groups of 7-9 mice were intratumorally injected with 100ug control DNA (□), 100 ug IL-12-DNA (◊), 0.4 u recCCL21 (●), 100 ug hgp100-DNA (▽), combination of 100 ug IL-12-DNA with either 0.4 u recCCL21 (O) or 100 ug hgp100-DNA (Δ), combination of 0.4 ug recCCL21 with 100 u hgp100-DNA (■), and combination of 0.4 ug recCCL21 with 100 ug IL-12-DNA and 100 u hgp100-DNA (♦) on days 0, 4, 8, 14, and 20. Mean tumor volumes of groups ± standar deviations are depicted. Tumor formation was significantly reduced in IL-12-DNA/hgp100 treated mice and in IL-12-DNA/recCCL21-treated mice compared to control mice. Asterisk indicated when one mouse was euthanized when the tumor volume was superior to 1400 1500 mm^3. (A) Growth kinetics and (B) percentage of mice survival.

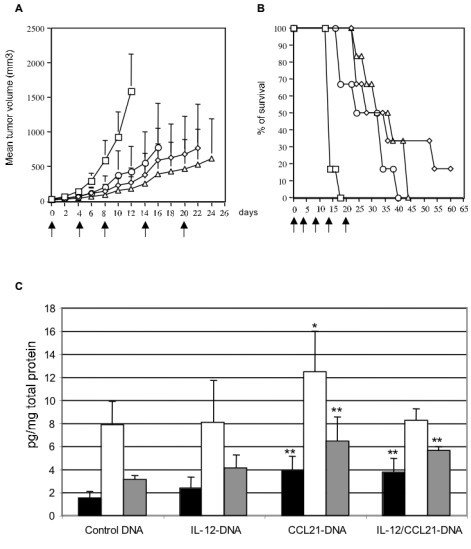

Fig.6. Time course of RENCA tumor growth in BALB/c mice treated by administration of CCL21-DNA. Tumors were established by subcutaneous inoculatior of 1x10^5 RENCA cells into the right hind flank. When a mean tumor volume of 15-20 mm was reached (day 0), treatment was started. Groups of 6 mice were intratumorally injected with 100 ug IL-12-DNA (◊) or 100 ug CCL21-DNA (O) or combination of 100 u CCL21-DNA and 100 ug IL-12-DNA (Δ) or 100 ug control DNA (□) on days 0, 4, 8, 1 and 20. Mean tumor volumes of groups ± standard deviations are depicted. Tumo formation was significantly reduced in CCL21-DNA/IL-12-DNA-treated mice compared tc control mice. Mice were euthanized when the tumor volume was superior to 1400-150 mm^3. **(A)** Growth kinetics and **(B)** percentage of mice survival treated with CCL21-DN and IL-12-DNA. **(C)** Homogenates from tumors at day 22 were evaluated also for the presence of murine IP-10 (black columns), IFN-γ (white columns), and CCL21 (gray columns). IL-12 was not detectable. Results are expressed in pg/mg of total protein.