Intratumoral expression of respiratory syncytial virus fusion protein in combination with cytokines encoded by adenoviral vectors as in situ tumor vaccine for colorectal cancer

Dennis Hoffmann, Wibke Bayer, Thomas Grunwald, and Oliver Wildner

Department of Molecular and Medical Virology, Institute of Microbiology and Hygiene, Ruhr-University Bochum, Bochum, Germany

Abstract
Although cancers can naturally elicit immune responses, immune ignorance is a common observation preventing immune-mediated elimination of tumor cells. We assessed whether intratumoral expression of respiratory syncytial virus fusion (RSV-F) protein, encoded by a replication-defective adenovirus vector (Ad.RSV-F), alone or in combination with local coexpression of cytokines can induce tumor-specific immune responses in a syngeneic murine colon cancer model. We confirmed in vitro by dye colocalization that transduction of murine cells with Ad.RSV-F induces cell-cell fusion. In vivo, we showed in a bilateral syngeneic s.c. colon cancer model in C57BL/6 and BALB/c mice that intratumoral injection of Ad.RSV-F leads to a significant volume reduction not only of the directly vector-treated tumor but also of the contralateral not directly vector-treated tumor. The intratumoral administration of Ad.RSV-F in combination with adenovirus vectors encoding interleukin (IL)-2, IL-12, IL-18, IL-21, or granulocyte macrophage colony-stimulating factor significantly enhanced the antitumor effect on the directly vector-treated tumor and also on the contralateral tumor. The antineoplastic efficacy of this combined treatment was significantly higher than that of the individual treatment components and was associated with the induction of a tumor-specific CTL response and increased infiltration of the tumors by natural killer cells and macrophages. Intratumoral coexpression of RSV-F and IL-21 resulted in the highest tumor growth inhibition and improved survival. Our experimental data indicate that intratumoral expression of RSV-F in combination with cytokines is a promising novel tool for the development of in situ tumor vaccination approaches. [Mol Cancer Ther 2007;6(7):1942–50]

Introduction
The destructive power of a host organism’s immunity is shown by the rapid rejection of organ grafts transplanted from an unrelated donor. However, tumors growing within the host are largely ignored by the immune system. CTLs recognize peptides derived from intracellular proteins that are loaded onto MHC class I molecules and presented on the cell surface (1). Immunotherapeutic applications using tumor-associated antigens as a vaccine component have shown promising results but are limited to patients with a defined cancer because only few antigens have been identified to date (2). Some studies circumvent this limitation by using tumor cell lysates, which probably include both known and unknown antigens (3, 4). Several studies showed that dendritic cells pulsed with tumor cell lysates could offer the potential advantage of inducing a broader T-cell immune response against uncharacterized tumors. However, this method is rather laborious and time consuming, as the dendritic cells have to be prepared from the patient’s blood for ex vivo pulsing with tumor cell lysates and are then reinfused (3–5). The possibility to elicit antitumor immunity by in situ vaccination by unmasking tumor antigens for appropriate presentation in a cytokine environment stimulating cell-mediated immunity would abrogate the need to obtain and culture a patient’s autologous tumor cells for manipulation in vitro, including transduction with cytokine genes, irradiation, and subsequent vaccination.

Using the fusogenic membrane protein G from vesicular stomatitis virus, which triggers cell fusion at pH 5.5, Linardakis et al. (5) showed recently in a syngeneic B16 murine melanoma model that expression of viral fusogenic membrane glycoproteins can enhance the efficacy of a weak allogeneic vaccine. Intratumoral expression of viral fusogenic glycoproteins leads to syncytia formation of infected cells with adjacent cells, thereby increasing the dispersion of viruses throughout the tumor, lateral spread of the transgene, virus release, and enhanced immunogenicity of tumor cells (6–9).

In this study, we assessed in two syngeneic bilateral s.c. colorectal cancer models in C57BL/6 and BALB/c mice...
whether the intratumoral expression of respiratory syncytial virus fusion protein (RSV-F) alone or in combination with local cytokine expression can serve as an in situ tumor vaccination strategy for colorectal cancer. We evaluated five cytokines encoded by replication-defective adenovirus vectors. Interleukin (IL)-2 acts as a growth factor for T, B, and natural killer (NK) cells and regulates T-cell survival by promoting activation-induced cell death (10). IL-12 stimulates proliferation of T as well as NK cells (11). IL-18 regulates Th1 and Th2 immune responses (12) and stimulates IFN-γ production from immune cells (13), whereas IL-21 has immunostimulatory effects on T and NK as well as dendritic cells (14) and promotes the proliferation of some B cells (14, 15). Granulocyte macrophage colony-stimulating factor (GM-CSF) acts mainly on CD4+ and CD8+ T cells and dendritic cells (16) but can also promote humoral immune responses (17, 18).

There have been several clinical studies evaluating the efficacy of cytokines, mostly IL-2, IL-12, and GM-CSF, given as recombinant proteins or expressed from DNA plasmid vectors in tumor vaccination trials, mostly in combination with chemotherapy, for cancer therapy (19, 20). High-dose cytokine therapy has proven to be effective in some cases, but there has been a considerable range of adverse side effects limiting the applicability (21, 22). In our study, the cytokines were expressed intratumorally, resulting in local but not systemic high cytokine concentration and therefore reduced systemic side effects (23).

Our data indicate that intratumoral expression of RSV-F particularly in combination with cytokine expression can serve as an in situ tumor vaccination strategy for colorectal cancer. To our knowledge, this is the first report evaluating RSV-F expression for in situ tumor vaccination.

Materials and Methods
Cell and Cell Culture
The human colon adenocarcinoma cell line HT-29 (ATCC HTB-38; ref. 24) was purchased from the American Type Culture Collection and the murine colon adenocarcinoma Colon26 cell line was purchased from CLS. The murine colon adenocarcinoma cell line MC38 was a gift from Steven A. Rosenberg (Surgery Branch, NCI, NIH, Bethesda, MD). The human embryonic kidney cell line 293 was purchased from Microbix Biosystems, Inc.

Viruses
To generate the vector Ad.RSV-F, the cDNA coding for the F protein of the RSV reference strain VR26 (American Type Culture Collection) was codon optimized for expression in human cells (NCBI protein sequence ABQ42594; GeneArt) and inserted into the adenovirus transfer vector pShuttle-TetO2 (25), to allow homologous recombination in Escherichia coli with the adenovirus AdEasy-1 backbone as described previously (26). Infectious Ad.RSV-F vector was rescued using T-REx-293 cells in the absence of tetracycline.

The adenovirus vectors Ad.II-12, Ad.II-18, Ad.II-21, and Ad.GM-CSF encoding the murine cytokines IL-12, IL-18, IL-21, and GM-CSF, respectively, were generated using the AdEasy-1 system (26). The cDNA for mIL-12 (pNGVL3-12; kindly obtained from Alexander Rakhmilevich, Department of Human Oncology, University of Wisconsin-Madison, Madison, WI; ref. 27), mIL-18 (pCR3.1::IL-18; kindly provided by Camille Locht, Laboratoire de Microbiologie Génétique et Moléculaire, Institut Pasteur de Lille, Lille, France; ref. 28), mIL-21 (pORF9-21, InvivoGen), and mGM-CSF (pGTF60mGM-CSF, InvivoGen) was cloned into the adenovirus transfer vector pAd.Track (26).

All adenovirus vectors used in this study were E1 and E3 deleted and produced in 293 cells. All viruses were purified with the Vivapure AdenoPACK 100 kit (Vivascience). The adenovirus particle concentration in purified preparations was determined by spectrophotometry as described previously (29) and expressed as viral particles per milliliter. With the used ion-exchange column purification kit, we obtained constant particle-to-plaque-forming unit ratios of ~30:1. The functionality of the cytokine-encoding adenovirus vectors was determined using cytokine-specific ELISA kits (Biosource International and R&D Systems). For this, 500,000 293 cells were transduced at a multiplicity of infection of 30 viral particles per cell in 1 mL with the vectors. Twenty-four hours after transduction with Ad.II-2, Ad.II-12, Ad.II-18, Ad.II-21, or Ad.GM-CSF, we detected in the supernatants 150, 500, 180, 130, and 500 pg of the respective cytokines.

Quantification of Syncytia Formation by Confocal Laser Scanning Microscopy and Flow Cytometric Analysis
To detect cell fusion, the opposing fusion partners where cytosolically stained with CellTracker Green CMFDA or CellTracker Orange CMTMR (Invitrogen Molecular Probes) according to the manufacturer’s instructions and seeded in an equal ratio onto culture slides (BD Biosciences Pharmingen). Next morning, 95% to 100% confluent cell monolayers were transduced with Ad.RSV-F at a multiplicity of infection of 1,000 viral particles per cell (MC38 and Colon26). The chosen multiplicity of infection for all cell lines resulted in ~100% transduction efficiency with the green fluorescent protein expressing Ad5.GFP.

For flow cytometric analysis, 24 h after vector transduction, cells were just detached by trypsin treatment, washed once with PBS, and analyzed (FACSCalibur flow cytometer, Becton Dickinson Immunocytometry Systems). For confocal laser scanning microscopy, 36 h after transduction, cells were washed and fixed with 2% paraformaldehyde. Slides were mounted and covered with thin coverslips before analyzing with the confocal laser scanning microscope TCS SP2 + DMIRE2 (Leica Instruments). Dual fluorescence, indicating membrane fusion, was quantified using the ImageJ (version 1.36b, NIH) software with the colocalization plug-in.

Animal Studies
This study was approved by the local Animal Care and Use Committee. Six- to 8-week-old female C57BL/6 and BALB/c mice were obtained from Janvier and maintained under specific pathogen-free conditions.

For the tumor growth study, C57BL/6 or BALB/c mice received s.c. 1 × 10^6 MC38 or Colon26 cells, respectively.
100 μL into the right hind flank and $1 \times 10^6$ cells in 100 μL into the left hind flank. Animals were randomly assigned to treatment groups ($n = 5$ for each tumor model) and treatment was initiated when the tumor on the right hind flank reached a volume of $\sim 200 \text{ mm}^3$ and the tumor on the left side was palpable. Animals were treated just with the Ad.RSV-F or the cytokine-encoding adenovirus vectors received on days 0 and 2 into the right tumor $6 \times 10^9$ viral particles in 100 μL PBS. When Ad.RSV-F was given in combination with the cytokine-encoding adenoviral vectors, $3 \times 10^9$ viral particles of each vector in 100 μL PBS were injected on days 0 and 2 into the right tumor. At least once a week, minimum and maximum perpendicular tumor axes were measured using Vernier calipers, and tumor volume was calculated using the simplified formula of a rotational ellipse ($l \times w^2 \times 0.5$). The skin thickness of 0.4 mm was subtracted from the measurements. To generate effector cells, mice were sacrificed and spleens were harvested and weighed 29 days after virus inoculation.

For tumor survival analysis, C57BL/6 mice received s.c. $1 \times 10^5$ MC38 cells in 100 μL into the right hind flank and $3 \times 10^4$ cells in 100 μL into the left hind flank. Animals were randomly assigned to treatment groups ($n = 6$) and treatment was initiated when the tumor on the right hind flank reached a volume of $\sim 100 \text{ mm}^3$ and the tumor on the left side was palpable. Treatment was conducted as described above. Survival of the animals was monitored. When animals seemed to be in distress, they were euthanized by CO$_2$ asphyxia.

### Lactate Dehydrogenase Release Assay

We analyzed the CTL response to tumor cells, using the lactate dehydrogenase–based CytoTox 96 (Promega) assay according to the manufacturer’s instructions. In brief, target cells (MC38 or Colon26) were plated at a density of 5,000 cells per well in round-bottomed 96-well plates. Target cells were then mixed with effector cells for 4 h at the indicated ratios. Lactate dehydrogenase release was determined measuring absorbance at 490 nm with a plate reader, and the specific lysis was calculated from triplicate samples as follows: specific lysis (%) = $\frac{\text{(experimental } A_{490} - \text{effector spontaneous } A_{490}) / (\text{target maximum } A_{490} - \text{target spontaneous } A_{490})}{\times 100}$.

### Immunohistochemistry

For sectioning, tumors were embedded in Jung tissue-freezing medium (Leica Instruments) as described previously (30). A Leica CM1900 (Leica Instruments) cryostat was used to prepare 10-micron cryosections, which were transferred to microscope slides, followed by acetone fixation at room temperature for 2 min. After washing with PBS three times, sections were immunostained with rat anti-mouse CD11b (M1/70.15.11.5) or rat anti-mouse CD49b (DX5) FITC-conjugated monoclonal antibodies (Miltenyi Biotec, Inc.). Digital images were taken with a high-resolution still camera (Olympus DP50) attached to a fluorescence microscope (Olympus BX51).

### Statistical Methods and Median Effect Analysis

The statistical software package SPSS 15 (SPSS, Inc.) was used for data analysis with indicated tests.

### Results

### RSV-F Expression Induces Cell-Cell Fusion of Murine Colorectal Cells

First, we analyzed by flow cytometry and confocal laser scanning microscopy whether transduction of confluent MC38 and Colon26 cell monolayers with the RSV-F protein...
encoding adenovirus Ad.RSV-F results in cell-cell fusion. For this, monolayers of two populations of cells stained with different dyes were transduced with Ad.RSV-F. When cell fusion occurs, colocalization of the dyes can be detected and quantified by flow cytometry or confocal laser scanning microscopy (31).

As shown in Fig. 1A, we analyzed cell-cell fusion by flow cytometry after 36 h. At this time point, we observed cell-cell fusion in the murine colon carcinoma cell lines. The flow cytometric data were confirmed qualitatively by confocal laser scanning microscopy 48 h after transduction with Ad.RSV-F (Fig. 1B).

Enhanced Growth Regression of the Directly Treated Tumor by Intratumoral Coexpression of RSV-F and Cytokines Encoded by Adenovirus Vectors

We evaluated in a syngeneic bilateral s.c. MC38 colorectal tumor model in C57BL/6 mice whether the coexpression of RSV-F and a cytokine (IL-2, IL-12, IL-18, IL-21, or GM-CSF) encoded by adenovirus vectors results in an enhanced in vivo treatment efficacy of the directly vector-treated tumor and the contralateral tumor when compared to the treatment with the individual components. As shown in Fig. 2, intratumoral inoculation of Ad.RSV-F alone resulted in a ~67% reduction of the treated tumor at day 28 [P < 0.001, ANOVA with Tukey’s honestly significant difference (HSD)]. Administration of vector encoding IL-12, IL-18, or IL-21 resulted in a ~10% to 47% reduction of the directly treated tumor (P < 0.01, ANOVA with Tukey’s HSD). Treatment with Ad.IL-2 and Ad.GM-CSF produced a ~10% reduction of the directly treated tumor (P = not significant, ANOVA with Tukey’s HSD). Intratumoral administration of Ad.RSV-F in combination with the vectors encoding IL-2, IL-12, IL-18, IL-21, or GM-CSF resulted in a ~92% to 98% reduction of directly treated tumor, respectively (P ≤ 0.05, ANOVA with Tukey’s HSD).

To assess whether our results are unique to MC38 cells and C57BL/6 mice (H-2b), we repeated the syngeneic bilateral tumor model with Colon26 cells in BALB/c mice (H-2d), which have contrasting susceptibilities to certain intracellular pathogens (32, 33). The experimental design was identical to that described above for MC38 cells in C57BL/6 mice. As shown in Fig. 2, the results are qualitatively similar to that obtained with MC38 cells.
The Efficacy of RSV-F Expression as Tumor Vaccine Is Enhanced by Intratumoral Cytokine Expression Encoded by Adenovirus

To determine whether intratumoral expression of RSV-F can serve as an *in situ* tumor vaccination, we monitored the tumor growth of the not directly vector-treated tumor on the left flank (Fig. 2). Intratumoral expression of RSV-F resulted in a ~41% reduction of the contralateral left tumor when compared with mock-treated animals (*P* < 0.001, ANOVA with Tukey’s HSD). Intratumoral treatment of animals with vectors encoding IL-2, IL-12, IL-18, IL-21, or GM-CSF resulted in a ~8%, 20%, 46%, 32%, or 21% reduction of the not directly vector-treated tumor, respectively (*P* < 0.05, ANOVA with Tukey’s HSD; *P* = not significant for Ad.IL-2). The combination of intratumoral Ad.RSV-F inoculation with the cytokine-encoding vectors resulted in a ~88% reduction of the not directly vector-treated tumor (*P* < 0.001, ANOVA with Tukey’s HSD). During the entire observation period, the levels of the vector-encoded cytokines in the serum were below the detection level of the respective cytokine ELISA (<10 pg/mL).

Intratumoral Coexpression of Adenovirus-Encoded Cytokine and RSV-F Induces Most Pronounced Splenomegaly

To analyze whether the observed growth regression of the not directly vector-treated tumor was immune mediated, we monitored on day 29 the spleen weight of the animals (Fig. 3A). We observed in both tumor models in animals treated with Ad.RSV-F a ~227% increased median spleen weight when compared with mock-infected animals. When compared with mock-infected animals, treatment with cytokine-encoding adenoviral vectors resulted in a ~30% increased spleen weight (*P* = not significant, ANOVA with Tukey’s HSD; *P* < 0.05 for Ad.IL-18). The combination of intratumoral Ad.RSV-F inoculation with the cytokine-encoding vectors resulted in a ~240% increased spleen weight. The treatment combination of intratumoral RSV-F and IL-18 expression resulted in the most pronounced splenomegaly. The spleens of representative mice of different treatment groups are shown in Fig. 3B.

Intratumoral Coexpression of Adenovirus-Encoded Cytokine and RSV-F Induces the Strongest Tumor-Specific CTL Response

To analyze whether the observed effects on tumor growth regression of the not directly vector-treated tumors were mediated by a tumor-specific lymphocyte response, we did a lactate dehydrogenase based cytotoxicity assay. As shown in Fig. 4, the effector splenocytes derived from untreated mice without tumor did not lyse target tumor cells (MC38 or Colon26 cells); in addition, splenocytes derived from mock-treated tumor-bearing animals did not lyse target tumor cells. A slight lysis of target cells was observed for splenocytes of animals treated with Ad.IL-12, Ad.IL-21, or Ad.GM-CSF, whereas Ad.IL-18–treated animals had the highest CTL activity resulting in ~25% cell lysis at an E:T ratio of 100:1. Splenocytes of animals treated with Ad.RSV-F showed a cytotoxicity of ~55% at a ratio of 100:1. The combination with the IL-encoding adenoviruses resulted in a median cytotoxicity of ~82% at an E:T of 100:1, whereas the highest cytotoxicity

![Figure 3](#) Effect of indicated treatments on the spleen weight. A, at day 29, animals were euthanized and spleen weight was determined. Columns, mean; bars, SD. B, spleens of representative mice of different treatment groups.
was observed with the splenocytes from Ad.RSV-F in combination with Ad.IL-21– or Ad.IL-12–treated animals.

**Local and Distant Antineoplastic Effects Are Associated with the Tumor Infiltration of NK Cells and Macrophages**

As shown in Fig. 5A by immunohistochemistry, the combination therapy consisting of Ad.RSV-F and Ad.IL-18 or Ad.IL-21 resulted in a strongly enhanced infiltration of macrophages and NK cells into the not directly vector-treated tumors when compared with mock-treated or single vector-treated animals.

**Intratumoral RSV-F and Cytokine Expression Results in Enhanced Survival**

The Kaplan-Meier survival analysis revealed a median survival of the untreated animals of 29 days (Fig. 5B). Animals that received intratumoral injections of Ad.IL-18 or Ad.IL-21 had a median survival of 36 days, respectively \((P < 0.001\), log-rank test). The median survival of mice treated with Ad.RSV-F alone was 48 days \((P < 0.001\), log-rank test). The combination of Ad.RSV-F with Ad.IL-18 or Ad.IL-21 resulted in a median survival of 71 or 87 days, with three and five long-term survivors, respectively. We showed in the tumor model that the combination of RSV-F expression in combination with IL-18 or IL-21 expression resulted in a significantly higher treatment efficacy when compared with the single treatment therapy \((P < 0.01\), log-rank test).

**Discussion**

Clinical trials using engineered adenovirus or herpes simplex virus-1–based vectors for the treatment of patients with advanced cancer revealed not only that these viruses are safe but also that their oncolytic efficacy as a single agent is limited (34–36). In a controlled clinical trial, the use of an oncolytic adenovirus \((dl1520;\) ref. 37) in combination with clinically relevant chemotherapy in patients with advanced head and neck cancer resulted in promising treatment efficacy of directly treated tumors, but no significant immune-mediated reduction of distant tumors was observed (34).

The intratumoral expression of viral fusogenic membrane proteins is a promising approach for cancer gene therapy because their expression in tumor cells is directly cytotoxic and associated with a local bystander effect (6) but can also stimulate an antitumor immunity (38, 39). In this study, we

---

![Figure 4. T-cell–mediated tumor regression by expression of RSV-F alone or in combination with cytokines. To elucidate the mechanism for the growth reduction of the second, not directly treated tumor, we determined the cytotoxic activity of spleen lymphocytes from mice that received indicated treatment against target cells (MC38 or Colon26) using a lactate dehydrogenase release assay. Data of all animals are expressed as the percentage of specific release of three independent experiments. Points, mean; bars, SD.](image-url)
showed dye colocalization by flow cytometry and confocal laser scanning microscopy in murine cells on transduction with Ad.RSV-F, indicating cell-cell fusion (31) and thus confirming previous results using a vaccinia virus encoding RSV-F (40).

The key findings of the colorectal cancer models in C57BL/6 and BALB/c mice were the following: first, intratumoral expression of RSV-F by the adenovirus vector Ad.RSV-F resulted, despite the limited intratumoral spread and transduction efficiency of the replication-defective adenovirus vector, in tumor regression of the directly vector-treated tumors, confirming previous studies (6). Due to the host specificity of adenovirus, human adenovirus will in general not productively infect murine cells (41). Thus, a trans-complementation of the replication-defective vectors for replication (42) to improve tumor transduction efficiency (43) is not possible in this model. Second, we confirmed that expression of viral fusogenic membrane proteins can serve as a tumor vaccination platform (5, 38) because we observed regression of the not directly vector-treated tumor. Third, intratumoral expression of IL-12, IL-18, and IL-21 resulted in reduction of both the directly vector-treated and the contralateral untreated tumor. However, in both models, the intratumoral expression of IL-2 did not result in a regression of the contralateral tumor. This finding is in accordance with other publications, reporting no enhanced outcome in colorectal cancer treatment when IL-2 was given in combination with other therapeutics (44). Fourth, intratumoral expression of RSV-F in combination with the cytokines IL-2, IL-12, IL-18, IL-21, or GM-CSF encoded by adenovirus vectors resulted in a significantly improved treatment efficacy not only of the directly vector inoculated tumors but also of the contralateral not vector-treated tumors when compared with single-agent therapy. Fifth, treatment of animals with the combination of Ad.RSV-F and/or cytokine expression induced tumor-specific CTL responses and a massively increased spleen weight. Sixth, we observed by immunohistochemistry a pronounced tumor infiltration with macrophages of animals that received intratumoral injections of adenovirus vector encoding RSV-F in combination with intratumoral IL-18 or IL-21 expression. Previously

Figure 5. Fluorescent immunohistochemical staining for tumor-infiltrating NK cells and macrophages (Mφ) and survival analysis. A, in a syngeneic bilateral s.c. MC38 tumor model, the right tumor of naive C57BL/6 mice was directly mock- or vector-treated. Treatment was carried out as described in Fig. 2. Fourteen days after initiation of therapy, continuous serial sections of the tumors were prepared and individually immunostained for macrophages and NK cells. Each treatment group consisted of three animals. Representative slides. Original magnification, ×400. Similar data were obtained with the Colon26 tumor model (data not shown). B, the MC38 tumor model as shown in Fig. 2 was repeated with six animals each group and survival time was monitored up to day 90.
Shimura et al. (45) showed that tumor-associated macrophages are inversely correlated with tumor progression in human prostate cancer because macrophages provide important antigen-presenting functions (46).

A conceivable mechanism for the induction of tumor-specific immunity by expression of RSV-F in combination with cytokines might be the xenogenization of tumor cells by presentation of viral antigens on the cell surface in conjunction with MHC-I molecules leading to CTL-mediated tumor cell destruction (47, 48).

Because it has been shown that adenovirus vector vaccines induce strong cytotoxic T-cell responses (49), the immunologic properties of adenovirus might have contributed to the observed vaccination effects. Furthermore, the expression of viral fusogenic membrane proteins has been postulated to result in an efficient presentation of tumor antigens on antigen-presenting cells having taken up debris of apoptotic cells or exosomes of fused cells (50, 51). The effects of RSV-F expression seen in our study confirms the findings published thus far, showing that dendritic cell maturation and naive T-cell activation are effectively primed on contact with vesicular stomatitis virus-G–expressing, syncytia-forming tumor cells (39). We assume that the immune stimulatory properties of RSV-F are similar to those of other viral fusogenic membrane glycoproteins (e.g., vesicular stomatitis virus-G or measles virus H and F). The efficacy of the cytokines IL-12, IL-18, IL-21, and GM-CSF in enhancing the antitumor immune response might be due to their ability to stimulate T-cell and NK cell activation and survival (11–14, 16).

In summary, our data show that the intratumoral expression of RSV-F in combination with intratumoral cytokine expression gives the best results not only with regard to the antineoplastic effects on the directly vector-treated tumor but also with regard to the antitumor immunity and thus the effect on an untreated tumor. To further improve the treatment efficacy, it should be advantageous to use an oncolytic vector expressing both RSV-F and cytokines, most likely resulting in a more efficient liberation of potential tumor-associated antigens (52).

Acknowledgments

We thank Malcolm Brenner (St. Jude Children’s Research Hospital, Memphis, TN) for providing via Jay Ramsey the Ad-IL-2 vector; Alexander Rakhmilevich for providing the mll-12–encoding plasmid; Camille Locht for giving the mll-18–encoding plasmid; Steven A. Rosenberg for the MC38 cells; Klaus Uberla for providing support; and Cathrin Walter (West German Cancer Center, University of Duisburg-Essen, Essen, Germany) for critical review of this manuscript.

References


Expression of RSV-F and Cytokines for Tumor Vaccination


# Intratumoral expression of respiratory syncytial virus fusion protein in combination with cytokines encoded by adenoviral vectors as *in situ* tumor vaccine for colorectal cancer

Dennis Hoffmann, Wibke Bayer, Thomas Grunwald, et al.


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at: <a href="http://mct.aacrjournals.org/content/6/7/1942">http://mct.aacrjournals.org/content/6/7/1942</a></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Cited articles</th>
<th>This article cites 52 articles, 23 of which you can access for free at: <a href="http://mct.aacrjournals.org/content/6/7/1942.full#ref-list-1">http://mct.aacrjournals.org/content/6/7/1942.full#ref-list-1</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citing articles</td>
<td>This article has been cited by 1 HighWire-hosted articles. Access the articles at: <a href="http://mct.aacrjournals.org/content/6/7/1942.full#related-urls">http://mct.aacrjournals.org/content/6/7/1942.full#related-urls</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E-mail alerts</th>
<th>Sign up to receive free email-alerts related to this article or journal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, contact the AACR Publications Department at <a href="mailto:permissions@aacr.org">permissions@aacr.org</a>.</td>
</tr>
</tbody>
</table>