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Recombinant Fusion proteins for Antibody-Directed-Enzyme-Prodrug-Therapy (ADEPT) in colon cancer

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Inhalt

Rekombinante Fusionsproteine für antikörpervermittelte Enzym-Prodrug-Therapie (ADEPT) bei Kolonkarzinom

Die antikörpervermittelte Enzym-Prodrug-Therapie (englisch antibody-directed enzyme-prodrug therapy, ADEPT) ist ein Ansatz, die Spezifität onkologischer Chemotherapien durch künstliche auf Tumorzellen gerichtete Antikörperenzymkonstrukte zu verbessern. Dies ermöglicht die selektive Umwandlung einer ungiftigen Prodrug in eine im Tumorgewebe lokalisierte zytotoxische Substanz.

Wir wendeten diese Strategie bei Kolonkarzinom an, indem wir A33 als Antikörperkomponente verwendeten, welche das Differenzierungsantigen gpA33 erkennt. GpA33s gehört zur Immunglobulin-Superfamilie und wird von > 95 % aller Kolonkarzinome exprimiert. Da es in nicht gastrointestinalem Gewebe nicht vorkommt stellt es ein viel versprechendes Ziel der ADEPT dar.

Über die Merkmale des A33 Antigens bezüglich Zellwachstum und Zellzyklus sowie die jeweilige Wirkung auf die Antigenexpression ist wenig bekannt. Um die Funktion des gpA33 zu beschreiben haben wird die Zellwachstumskinetik in Abhängigkeit von der Anwesenheit von gpA33 untersucht und die Aufnahme in lebende Zellen visualisiert.

Neben der Untersuchung der Funktion des gpA33 ging es uns darum, bispezifische Fusionsproteine zu entwickeln, welche in der Lage sind, gpA33 zu erkennen, und gleichzeitig diagnostische oder therapeutische Funktion innehaben.

Hier werden die Struktur, die Klonierung, die Expression und die funktionelle Prüfung von zwei rekombinanten Fusionskonstrukten beschrieben, welche auf einem anti-gpA33 Einzelketten-Fragment, A33scFv genannt, basieren. Das erste Konstrukt, A33scFv::GFP, trägt das grün-fluoreszierende Eiweiß GFP (green fluorescent protein) und wurde als Musterkonstrukt für Biodistribution und zellbiologische Studien des gpA33 entworfen. Das andere, A33scFv::CDy, wurde für ADEPT entworfen und ist Träger des Enzyms Cytosindesaminase aus der Hefe S. cerevisiae (CDy). Es fungiert als Effektorprotein welches 5-Fluorocytosin (5-FC) in das als Chemotherapeutikum zugelassene 5-Fluorouracil (5-FU) umwandelt.

A33scFv::GFP ermöglichte eine quantitative Demonstration spezifischer Bindungen in vitro und in vivo. Indem wir das Reagens als Werkzeug verwendeten, konnten wir zeigen, dass die Expression des A33 auf der Membran kolorektaler Karzinomzellen unabhängig von der Expression von Haushaltsgenen durch die Zelldichte beeinflusst wurde und vom Zellzyklus abhängig war.

Das "therapeutische" Fusionsprotein A33scFv::CDy zeigte zweifache Funktion, d.h. Antikörperbindung und Enzymaktivität, und konnte in vitro erfolgreich im kompletten ADEPT System angewandt werden. Dies führt zu einer 300fachen Steigerung der Toxizität des Substrats 5-FC auf gpA33 positive Zelllinien, jedoch nicht auf gpA33 negative Kontrollen.

Zusammenfassung der Publikationspromotion

Zusammenfassung der Publikationspromotion zum Thema:

Recombinant fusion proteins for antibody-directed enzyme prodrug therapy (ADEPT) in colon cancer

Abstract

Antibody-directed enzyme prodrug therapy (ADEPT) is an approach to improve the specificity of anti-tumor chemotherapy by way of artificial antibody-enzyme constructs targeted at tumor cells. This allows for the selective conversion of a non-toxic prodrug into a cytotoxic agent localized to the tumor site.

Applying this strategy on colon cancer, we chose A33 as the antibody component, which recognizes the differentiation antigen gpA33. A novel member of the immunoglobulin family, gpA33 is expressed by > 95 % of human colon cancers, but is absent in all non-gastrointestinal tissues, making it a promising target for ADEPT. Little is known about the characteristics of the A33 antigen with regard to cell growth and cell cycle as well as the respective impact on antigen expression. In an attempt to elucidate the gpA33 function, here we have investigated the dependence of surface gpA33 presence on cell growth kinetics and visualized internalization in living cells. Along with the elucidation of the gpA33 function we also aimed to develop bispecific fusion proteins which at the same time are capable of recognizing the gpA33 and have either a diagnostic or a therapeutic effector function. The design, cloning, expression and functional assaying of two recombinant fusion constructs based on an anti-gpA33 single chain fragment, termed A33scFv, is described here. The first construct, A33scFv::GFP, carries the fluorophore green fluorescent protein and has been designed as a model construct for biodistribution and cell biology studies of gpA33. The other, A33scFv::CDy, has been designed for ADEPT and carries the enzyme cytosine deaminase from the yeast S. cerevisiae (CDy) as effector protein, which converts 5-fluorocytosine (5-FC) into the approved chemotherapeutic drug 5-fluorouracil (5-FU).

The A33scFv::GFP allowed the quantitative demonstration of specific binding *in vitro* and *in vivo.* Using this reagent as a tool, we showed that the expression of A33 on the membrane of colorectal cancer cells was influenced by cell density and is cell cycle dependent in a pattern distinct from that of housekeeping genes.

The “therapeutic” protein A33scFv::CDy demonstrated dual function, i.e. antibody binding and enzyme activity, and was successfully applied in the complete ADEPT system *in*

vitro, resulting in a 300-fold increase in the toxicity of the substrate, 5-FC, on gpA33-positive cell lines, but not on gpA33-negative controls.

Aims

The aim of this project was to design, clone, produce and characterize specific members of a novel class of recombinant fusion proteins, which consist of A33scFv with additional effector components, in this case CDy and GFP.

At the same time that we evaluated the feasibility and the potential of the generated molecules for use in an ADEPT approach for colon cancer, we intended to use them to characterize and elucidate the biological function of the gpA33 antigen.

Introduction

Recombinant technology allows for the design and expression of tailored genuine fusion proteins, providing defined molecules as to size, molar ratios of the functional components, and stability. The production of functional protein, however, is often limited or impossible due to refolding and solubility problems [1].

An appropriate expression system is very important. An approach to combine convenient culture conditions similar to those of bacteria with the posttranslational processing machinery of eukaryotes to maintain the original biological activity is offered by yeast species such as *Pichia pastoris* [2].

One of the more recent applications of antibody-enzyme conjugates is antibody-directed enzyme-prodrug therapy (ADEPT). This new cancer therapeutic approach is a promising strategy for tumor-specific drug targeting. It utilizes antibody – enzyme constructs for targeted enzyme delivery to tumors, and subsequent localized activation of a prodrug into an active chemotherapeutic agent.

Applying this strategy on colon cancer, the differentiation antigen gpA33 was selected as the molecular target of this therapy. The gpA33 antigen, expressed by > 95 % of human colon cancers, is a 43 KDa transmembrane glycoprotein of the immunoglobulin superfamily [3;4]. It is not secreted or shed which are obligatory characteristics for use in ADEPT. A number of clinical trials have been conducted with huA33 that have demonstrated an excellent uptake of this antibody [5-7]. However, the physiological function of gpA33 and its potential role in tumorigenesis are still unknown. In order to elucidate the role of the gpA33 in tumor biology, we have investigated the expression of this glycoprotein on the mRNA and cell surface protein levels by quantitative rt-PCR and flow cytometry respectively, in response to cell density in the culture and to cell cycle arrest in the G1,S, or G2/M phases.

Recombinant fusion proteins offer a promising strategy to overcome the low stability and large size of chemically linked conjugates and to provide defined reagents. We have designed fusion proteins of single-chain fragments (scFv) of the colon carcinoma targeting antibody huA33 with the yeast cytosine deaminase (CDy) and with the green fluorescent protein (GFP). CDy converts 5-fluorocytosine (5-FC), which is non-toxic to mammalian cells, into the approved chemotherapeutic drug for colon cancer 5-fluorouracil (5-FU). The A33scFv::CDy construct was generated to be used as a therapeutic agent. The complete ADEPT system was tested *in vitro* in cytotoxicity assays using the fluorescein diacetate method. Characterization of the protein was performed by FACS assays.

The A33scFv::GFP fusion protein was designed for two purposes: as an experimental tool and potential diagnostic reagent. It may be used to detect A33-positivity of a given tumor sample and hence aid in the histological diagnosis of gastrointestinal cancers and in therapy planning. At the same time it can also be used as a model molecule for the biodistribution and microlocalisation of this class of constructs without additional labeling. In this work the cloning and expression of A33scFv-GFP in *P. pastoris*, its purification and functional description by flow cytometry, surface plasmon resonance, fluorescence microscopy on sections of normal and cancerous colon tissue, and *in vivo* biodistribution studies were performed.

Methods

Vector design and host system. A33scFv [8], GFP cDNA and CDy were PCR-amplified. Primers were designed based on published sequences to remove start and stop codons and to add flanking restriction sites for vector cloning. Plasmid vectors were ampilified in *E. coli* and then transfected by electroporation into *Pichia pastoris* .

Screening and expression. Transformants were analyzed either by their ability to grow under a selective pressure, followed by western blot analysis, or analyzed by ELISA for the ability to bind to the A33 antigen. The clones suspected of having a strong expression of the recombinant protein were cultivated on a small scale. Samples were examined for protein production by SDS-PAGE and immunodetection, or protein staining.

Production of the fusion proteins. A well expressing clone of A33scFv::GFP and A33scFv::CDy recombinant constructs was chosen for protein production in 1L. *Pichia* cells were first inoculated for 24 hours in BMGY (buffered complex medium containing glycerol) to generate a seed culture. Cells were harvested and cultivated for 72 hours in

BMMY (buffered complex medium containing methanol) to induce production, following the production described [9].

Purification and detection of the A33scFv::GFP and A33scFv::CDy fusions. Briefly, the purification employed centrifugation followed by filtration and affinity chromatography with protein L. For detection and initial identification of A33scFv::GFP, the proteins were blotted onto a nitrocellulose membrane by electrophoresis and immunostained with a murine anti-GFP monoclonal antibody. A33scFv-CDy was identified by SDS-PAGE followed by protein staining.

Surface plasmon resonance. A direct assessment of the fusion protein affinity and specificity was achieved by surface plasmon resonance on immobilized recombinant A33 antigen. Here, off-rates of the recombinant scFv alone as well as the A33scFv-GFP fusion protein were compared.

Flow cytometry for antigen binding and detection. Antigen binding of both constructs (A33scFv::GFP and A33scFv::CDy) and fluorescence function of the A33scFv::GFP fusion protein were investigated simultaneously by flow cytometry. Cells from cultured colon cancer cell lines were harvested and incubated with the solutions containing the fusion proteins to be tested. Detection of the A33 antigen expression level on the surface of the cells was also performed by flow cytometry.

Flow cytometry for propidium iodide detection. Cells from colon cancer cell lines were incubated with cell-cycle blocking drugs. After incubation, their DNA content was measured based on propidium iodide staining.

Confocal Laser Scanning Microscopy. After reaching confluence in culture, cells were incubated with A33scFv::GFP using a microscope for viewing and imaging.

Fluorescence microscopy. Frozen microsections of normal and cancerous colon tissue were stained with A33scFv-GFP. Fluorescence-stained sections were examined.

Cytotoxicity. To test for the dual activity of the A33-CDy fusion protein and to determine its suitability for the prodrug therapy approach, a fluorescein diacetate assay was performed according to Nygren et al [10].

A33 positive tumor xenografts in mice. Nude mice carrying A33-positive xenografts from the pancreatic cancer cell line ASPC 1 or an A33-negative control were injected with

radiolabeled A33scFv-GFP. At defined time points, groups of three animals were sacrificed and blood, tissue and tumours analyzed for their relative radioactive doses

Results

Design and construction of the A33scFv::GFP and A33scFv::CDy expression cassette vector. The concept was to design and construct a vector system for the production of a scFv-effector enzyme comprising two functional cassettes, a single chain antibody Fv (huA33scFv) and the green fluorescent protein (GFP) or the yeast cytosine

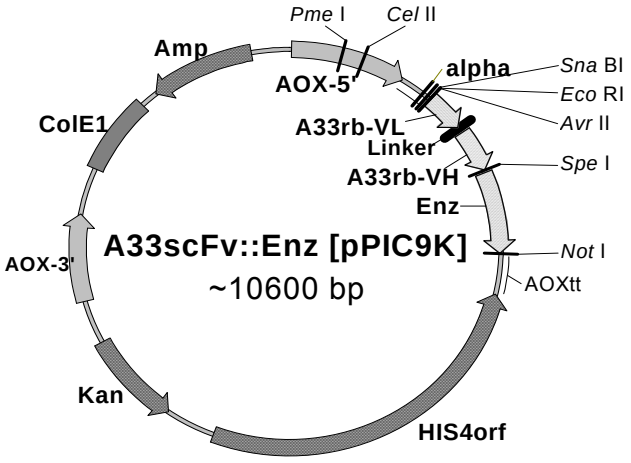


Figure 1: Plasmid design and overview of the A33scFv-GFP and A33scFv-CDy transfection vectors for yeast expression. Letters in italics designate restriction endonuclease recognition sites. A33rb-VL and A33rb-VH, variable fragment regions of the light and heavy variable chain, respectively; GFPuv – ultraviolet-enhanced green fluorescent protein; *Enz*: position of the enzyme; alpha, α-mating factor; AOX-5' and AOX-3', flanking regions of the AOX-I gene sequence; Amp, Kan, resistance genes against ampicillin and G418, respectively; ColE1, *E. coli* replication factor; HIS4orf, open reading frame of the histidine deaminase gene.

Deaminase (CDy) flanked by unique restriction sites (Fig. 1). Another vector system based on pPICZαA was also tested (data not shown here).

Expression of the generated proteins. The expression of the fusion proteins was carried out in the methylotrophic yeast *Pichia pastoris strains GS115* and *KM71*. Detection of protein production was performed by SDS-PAGE followed either by immunoblotting on a nitrocellulose membrane or by silver staining (Figs 2 and 3).

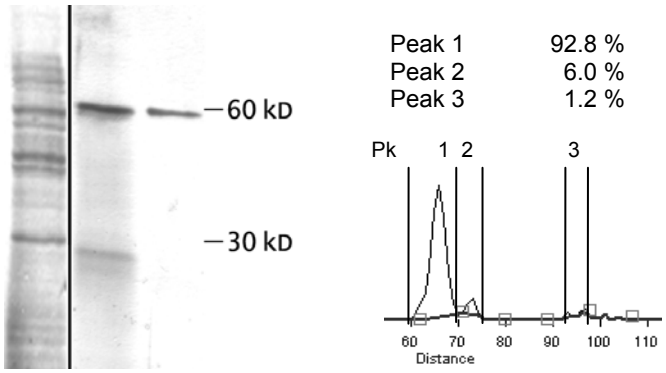


Figure 2: Purity of A33scFv-GFP following affinity chromatography. After dialysis against the loading buffer, the crude supernatant was passed through a sepharose gel carrying immobilized protein-L (details see text). Silver-stained SDS-PAGE gel with (1) crude culture supernatant, (2) eluate from protein L column, theoretical size of A33scFv-GFP, 53,4 kD; and (3) final eluate after protein L affinity and size exclusion chromatography. Bands of the final eluate have been electronically scanned and evaluated by densitometry as peaks 1, 2 and 3 as indicated.

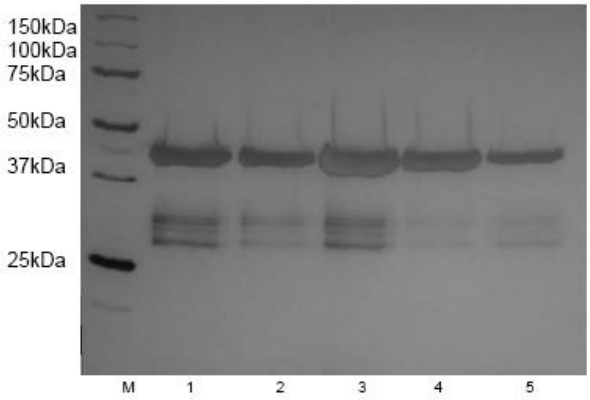
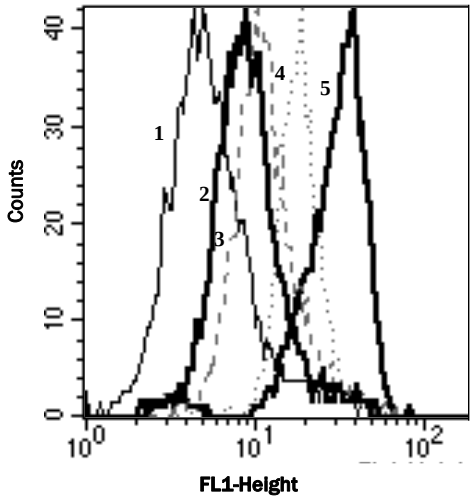


Figure 3 : Analysis of samples obtained on the large scale expression and purification of A33scFv::CDy
Lane M, molecular weight marker; Lane 1-5, different eluate fractions from the purification of 1L of *KM71* culture supernatant with proteinL 20µl of each sample was loaded into the gel after being boiled for 5min with 50µL of 1xSDS/β-mercaptoethanol buffer.

The protein constructs bind specifically to A33-positive cells. A direct assessment of the fusion protein affinity and specificity was achieved by surface plasmon resonance on immobilized recombinant A33 antigen (data not shown). Here, comparing off-rates, the recombinant scFv alone as well as the A33scFv-GFP fusion protein displayed about half the affinity of the complete IgG antibody, reflecting the predicted difference in avidity of monovalent vs. divalent antigen binding sites. By comparison, an unrelated antibody left the antigen almost immediately.

Antigen binding of both constructs (A33scFv::GFP and A33scFv::CDy) and fluorescence function of the A33scFv::GFP fusion protein were also investigated by various flow cytometry assays. In one of them the A33 antigen was first blocked with the A33scFv::CDy-containing culture supernatant and then incubated with A33scFv::GFP (Fig.4).



	Median Relative Fluorescence
1 – Native LIM 1215	5.05
2 – LIM 1215 + BMMY	8.28
3 – LIM 1215 + Pichia A33scFv::CDY supernatant	10.75
4 – LIM 1215 + Pichia A33scFvC::DY supernatant + Pichia A33scFv::GFP supernatant	20.54
5 – LIM 1215 + Pichia A33scFv::GFP supernatant	37.52

Figure 4 : **Blocking of the A33scFv::GFP ligation on the A33-positive LIM 1215 cells using the A33scFv::CDy- FACS Analyse**
Lim 1215 cells in suspension (1) were incubated with the green fluorescent A33scFv-GFP (5-positive control). Pre-incubation of A33scFvCDy in BMMY-Medium, with Lim 1215, reduced the ligation of the A33scFvGFP in the following incubation(4). As negative control, Lim 1215 cells were incubated just with A33scFvCDy in BMMY without A33scFv::GFP(3) or just with BMMY without adding either antibodies.

A33scFv-GFP allows morphologic staining of normal and cancerous colon tissue. To apply the fusion protein diagnostically as intended, snap-frozen microsections of healthy and cancerous colon tissue were stained with A33scFv-GFP (data not shown). The structure of crypts was clearly visualized by A33-staining of normal colon tissue, whereas the colon cancer slides displayed their complete destruction and an intense, irregular fluorescence pattern throughout the specimen. Staining identically treated slides from the same block with a control construct revealed no specific fluorescence pattern.

A33scFv-GFP localizes specifically to A33-positive xenografts in mice. Nude mice carrying A33-positive xenografts from the pancreatic cancer cell line ASPC 1 or an A33-negative control were injected with radiolabeled A33scFv-GFP. Overall, the activity retained in the animals was low with single digit total percentages of injected dose

ADEPT system in vitro. To test for the dual activity of the A33-CDy fusion protein and to determine its suitability for the prodrug therapy approach, a fluorescein cytotoxicity assay of the complete ADEPT system was established.

The colon cancer cell line Lim1215 was chosen as the antigen-positive control to perform this assay since this cell line is known for its high expression level of the A33 antigen.

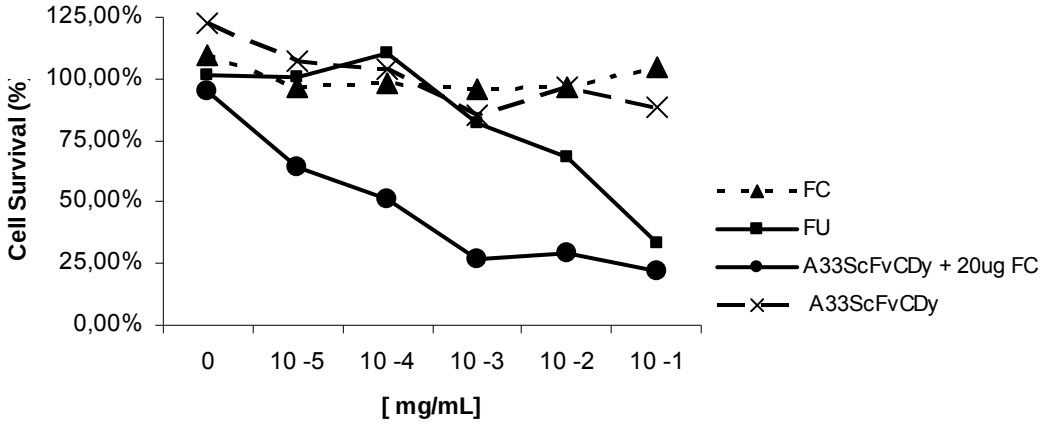


Figure 5 - A33scFvCdy mediated cytotoxicty on A33 antigen-positive cells: LIM 1215 cells were incubated with a dilution series of the A33scFv-CD fusion protein and, after washing, with a fix concentration of the 5-FC prodrug. As a control the A33scFvCDy was used without subsequent prodrug incubation. Survival was measured by the fluorescein diacetate method as described. Mean and s.d. of duplicate samples.

gpA33 surface expression is dependent on cell cycle phase with different patterns

Cell cycle–synchronized LIM1215 cells were incubated with A33scFv::GFP at the time of propidium iodide-staining in the previous experiment and analyzed by flow cytometry. While cells in G1 bind almost exactly as much A33scFv::GFP as unsynchronized cells, cells in S-phase show a marked reduction of about 20%, and those in G2/M an equal increase over unsynchronized cells. The differences among G2/M cells as well as those of S and G2/M cells compared to unsynchronized and G1 cells are statistically significant in the paired t-test (data not shown).

In order to quantify gpA33 expression at the mRNA level, the mRNA of LIM 1215 synchronized cells was extracted and quantified by PCR.

The difference in results between S-phase and G1 phase, as well as S phase and G2/M phase was statistically significant with p=0,045 and p=0,0490, respectively.

A33scFv::GFP binding and internalization

The intracellular uptake of gpA33 was analyzed by confocal laser scanning microscopy. For that purpose LIM1215 cells were incubated with A33scFv::GFP.

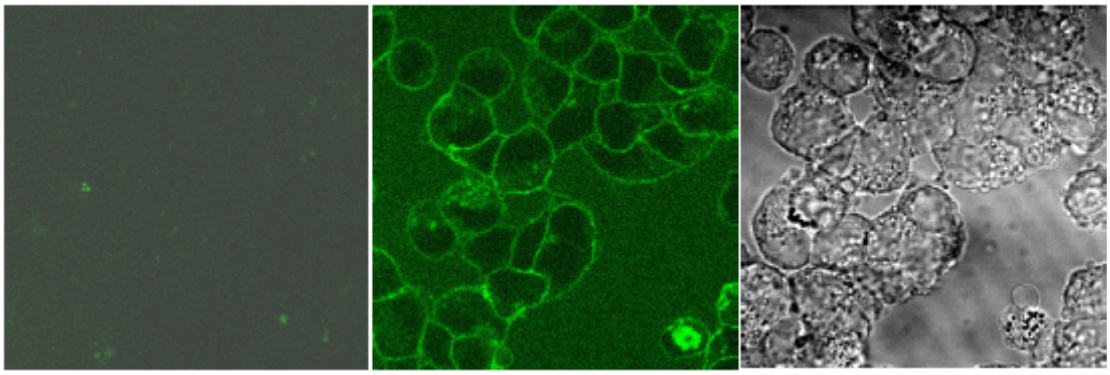


Figure 6: Binding of A33 antibody to colon cancer cells. LIM1215 human colon carcinoma cells were incubated with A33scFv::GFP. Confocal laser scanning microscopy was performed after washing. Left frame, background before A33scFv::GFP incubation; center, 10 min after A33scFv::GFP incubation; transmission image without fluorescence excitation. All frames show the same field of view.

Discussion

Recombinant fusion proteins could in principle overcome the limitations of chemical conjugates previously used in ADEPT systems, i.e. instability of the chemical linker and the presence of products with various molar ratios of antibody and enzyme. Applying this strategy on colon cancer, A33 has been selected for the antibody component.

In contrast to A33scFv::CDy, the A33scFv::GFP fusion protein allows easy detection of both its functions due to his own fluorescence. Using GFP as a reporter allowed the effective assessment of fusion protein expression in the yeast culture or of antigen

expression by transfected mammalian cells (not discussed here) and target binding of protein variants based on crude cell supernatant and purified material.

Binding specificity of the secreted fusion proteins was demonstrated by flow cytometry blocking experiments and by surface plasmon resonance on immobilized recombinant A33 antigen. The latter method also allowed for an estimation of actual affinity. With approximately half the retention of the divalent complete IgG antibody at a defined time point during the dissociation phase, both A33scFv and the A33scFv-fusion protein displayed the predicted avidity of the monovalent construct.

As one potential diagnostic application of the A33scFv::GFP fusion protein, histological fluorescence staining was investigated in specimens of normal and cancerous colon tissue, confirming specificity and demonstrating the feasibility of this application for routine use.

Biodistribution studies in tumour-xenografted mice were performed both to investigate potential diagnostic *in-vivo* applications and to gain preliminary data to guide the investigation in the development of future therapeutic fusion constructs based on the A33scFv. The overall percentage of injected activity detected in tumor tissue was considerably lower than that of bivalent A33 IgG. As in the surface plasmon resonance assay, this result reflects the smaller avidity of the monovalent compared to the divalent construct. However, both the fact that the tumor became the tissue with the highest radioactivity during the elimination phase, and the absence of any significant accumulation in the A33-negative control tumors, demonstrate that the accumulation in tumor tissue was due to immunologically specific binding rather than mere passive diffusion.

Thus, A33scFv::GFP can serve as a model for similarly designed A33-based fusion proteins with components such as prodrug-activating enzymes for antibody-targeted chemotherapy.

In this work we also report on the design, cloning, expression and in-vitro characterization of a new recombinant antibody-enzyme fusion protein for ADEPT, termed A33scFv::CDy. Several ADEPT systems have shown promising in vivo efficacy in a number of tumor models [11] and in several xenografts systems in nude mice [12-14]. There is clear evidence that ADEPT can deliver commonly used and novel cancer drugs reaching high concentrations and targeting the tumor tissue with high selectivity [15].

Radiolabeled huA33 had been shown to selectively target primary and metastatic colorectal tumors and penetrate to the center of large necrotic metastatic lesions. The

excellent targeting characteristics of this humanized antibody indicate clear potential for the targeted therapy for colorectal cancer [7;16].

In addiction, here we further characterized the gpA33 surface expression and its intracellular migration so that we could better explain the tumor specificity of anti-gpA33. We have seen that gpA33 presence on the cell surface increases with cell density until confluence has been reached, and declines after this point. We assumed that this fact may indicate a pathological mechanism not representative of the physiological function of gpA33.

The data obtained in our functional study of gpA33 lead us to the hypothesis that gpA33 surface density is regulated at least to a large part by internalization and resurfacing rather than gene expression in malignant cells. This may form a pathological mechanism blocking growth inhibitory signals.

The avidity of the A33scFv was also previously studied. Univalent A33scFv showed about half the binding activity of divalent huA33 IgG, and A33scFv-CD (with cytosine deaminase from E. coli) had slightly less binding activity than A33scFv [17].

Here we propose a new ADEPT system based on the A33scFv::CDy construct. Incubation of A33-positive tumor cells with this construct increased the 5-FC toxicity significantly. Neither A33scFv::CDy without 5-FC, nor the control construct with 5-FC inhibited cell growth, showing that specific enzymatic conversion was necessary for cytotoxicity. These results demonstrate dual (i.e. antibody and enzyme) specificity of this construct and functioning of this ADEPT system *in vitro*.

The evidence for specificity of antigen binding was enhanced by the blocking effect of the A33scFvCDy construct on A33scFv::GFP binding seen in the flow cytometry assay.

This study confirmed the feasibility of ADEPT strategies using scFv-based fusion proteins in general and of the A33 – cytosine-deaminase – 5-FC system in particular.

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Erklärung über den Anteil an den Publikationen

Publikation 1: Vânia Coelho, Jens Dernedde , Ulf Petrausch, Hossein Panjideh, Hendrik Fuchs, Christoph Menzel, Stefan Dübel, Ulrich Keilholz , Eckhard Thiel and P.Markus Deckert. **Design, construction, and *in vitro* analysis of A33scFv::CDy, a recombinant fusion protein for antibody-directed enzyme prodrug therapy in colon cancer**. International Journal of Oncology. 2007 October; 31: 951-957

Anteil: Konzeption der Arbeit, Leitung und Durchführung der wesentlichen Experimente, Verfassen des Manuskripts

Publikation 2: Ulf Petrausch, Jens Dernedde, Vânia Coelho, Dietmar Frey, Hendrik Fuchs, Eckard Tiel, P. Markus Deckert. **A33scFv-greeen Fluorescence Protein, a Recombinant Fusion Protein for Tumor Targeting: Cloning, Expression in P. pastoris , and Functional Analysis.** Protein Engineering, Design and Selection. 2007 Dec;20(12): 583-590. Epub 2007 Nov 22

Anteil: Maßgebliche Mitarbeit bei der Methodenentwicklung zur Expression und Aufreinigung des Fusionsproteins, Durchführung der Fluoreszenzzytometrie.

Publikation 3: Dietmar Frey, Vania Coelho, Ulf Petraush, Michael Schaefer, Ulrich Keilholz, Eckhard Thiel, P.Markus Deckert. **Surface expression of gpA33 is dependent on cell density and cell cycle phase and is modulated by intracellular migration rather than gene transcription.** Cancer Biotherapy & Radiopharmaceuticals.2008 Feb;23(1): 65-73

Anteil: Maßgebliche Mitarbeit bei Expression des Fusionsproteins und Zellkultur.

Due to copyright reasons publications could not be enclosed.
Please find the papers which refer to this dissertation in the journals listed bellow.

Publication 1: Vânia Coelho, Jens Dernedde , Ulf Petrausch, Hossein Panjideh, Hendrik Fuchs, Christoph Menzel, Stefan Dübel, Ulrich Keilholz , Eckhard Thiel and P.Markus Deckert. **Design, construction, and *in vitro* analysis of A33scFv::CDy, a recombinant fusion protein for antibody-directed enzyme prodrug therapy in colon cancer**. International Journal of Oncology. 2007 October; 31: 951-957

Publication 2: Ulf Petrausch, Jens Dernedde, Vânia Coelho, Dietmar Frey, Hendrik Fuchs, Eckard Tiel, P. Markus Deckert. **A33scFv-greeen Fluorescence Protein, a Recombinant Fusion Protein for Tumor Targeting: Cloning, Expression in P. pastoris , and Functional Analysis.** Protein Engineering, Design and Selection. 2007 Dec;20(12): 583-90. Epub 2007 Nov 22

Publication 3: Dietmar Frey, Vania Coelho, Ulf Petraush, Michael Schaefer, Ulrich Keilholz, Eckhard Thiel, P.Markus Deckert. **Surface expression of gpA33 is dependent on cell density and cell cycle phase and is modulated by intracellular migration rather than gene transcription.** Cancer Biotherapy & Radiopharmaceuticals.2008 Feb;23(1): 65-73

Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Erklärung über die Selbständigkeit

„Ich, Vânia Coelho, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: Recombinant fusion proteins for antibody-directed enzyme prodrug therapy (ADEPT) in colon cancer“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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