Large Molecule Therapeutics

EpCAM-Selective Elimination of Carcinoma Cells by a Novel MAP-Based Cytolytic Fusion Protein

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Abstract

In normal epithelia, the epithelial cell adhesion molecule (EpCAM) expression is relatively low and only present at the basolateral cell surface. In contrast, EpCAM is aberrantly overexpressed in various human carcinomas. Therefore, EpCAM is considered to be a highly promising target for antibody-based cancer immunotherapy. Here, we present a new and fully human cytolytic fusion protein (CFP), designated “anti-EpCAM(scFv)-MAP,” that is comprised of an EpCAM-specific antibody fragment (scFv) genetically fused to the microtubule-associated protein tau (MAP). Anti–EpCAM(scFv)-MAP shows potent EpCAM-restricted proapoptotic activity toward rapidly proliferating carcinoma cells. In vitro assays confirmed that treatment with anti–EpCAM(scFv)-MAP resulted in the colocalization and stabilization of microtubules, suggesting that this could be the potential mode of action. Dose-finding experiments indicated that anti–EpCAM(scFv)-MAP is well tolerated in mice. Using noninvasive far-red in vivo imaging in a tumor xenograft mouse model, we further demonstrated that anti–EpCAM(scFv)-MAP inhibited tumor growth in vivo. In conclusion, our data suggest that anti–EpCAM(scFv)-MAP may be of therapeutic value for the targeted elimination of EpCAM+ carcinomas. Mol Cancer Ther; 13(9); 1–9. ©2014 AACR.

Introduction

Standard chemo- and radiotherapy remain routine cancer treatment regimens in daily clinical practice. Despite encouraging initial responses, most solid tumors eventually develop resistance, metastasize, and relapse as incurable disease in response to these treatments (1, 2). In addition, unacceptable side effects strongly limit the therapeutic applicability of conventional therapies. Thus, there is an urgent demand for novel therapeutic avenues that are both selective and have high cytotoxic efficacy against malignant tumor cells.

Among all cancers, carcinoma is the most diagnosed cancer type, originating in the skin, lungs, breasts, pancreas, glands, or various other organs. Although each type of carcinoma has its own specific properties, many types often aberrantly overexpress the epithelial cell adhesion molecule (EpCAM; CD326; ref. 3). In fact, the upregulation of this transmembrane glycoprotein has been associated with the development and progression of various carcinomas, including gastrointestinal carcinoma (gall bladder, pancreas; ref. 4, 5), lung carcinoma (squamous cell carcinoma, adenocarcinoma; refs. 6, 7), breast carcinoma (8), gynecological carcinoma (cervix; ref. 9), and urological carcinoma (urethral bladder, prostate; refs. 10, 11). The tumor-promoting nature of EpCAM has been accredited to its ability to modulate cell proliferation and cell–cell interactions, which consequently results in tissue invasion and metastasis (9, 12).

There are several characteristics of EpCAM+ carcinomas that make this protein an attractive target in cancer diagnosis and therapy. Although it is present in healthy epithelial tissue, the expression of EpCAM is low and the protein localizes solely to the basolateral membrane. In contrast, in carcinomas, EpCAM expression pattern is significantly altered and high levels of the protein can be found uniformly across the entire cell membrane (13). Moreover, EpCAM was shown to be hyperglycosylated in carcinomas versus in healthy epithelia (14). Overexpression of EpCAM has further been associated with poor prognosis and overall survival (15). Together, these differences would allow for the development of unique...
monoclonal antibodies (mAb) against EpCAM in carcinoma cells that are of high therapeutic value.

Several anti-EpCAM mAbs have been developed showing promising activity in preclinical studies. However, early-phase clinical evaluation of naked anti-EpCAM mAbs in cancer treatment has been disappointing (3, 16–18). The therapeutic effects of unmodified or “naked” mAbs are predominantly dependent on natural effector functions of a fully active human immune response and include complement-dependent cytolysis (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP). However, in patients with cancer, the immune system may be severely compromised. Moreover, cancer cells frequently develop resistance mechanisms that allow them to evade current mAb-based approaches.

Therefore, advanced antibody-based cancer therapeutics, such as antibody–drug conjugates (ADC) or immunotoxins, which deliver cytotoxic molecules to their specific target, were developed (19). Classic ADCs are conjugates in which a conventional antitumor mAb is chemically linked to a small synthetic cytotoxic drug molecule. The main goal of ADCs is not primarily the recruitment of immune effector cells via their Fc-domain, but rather the induction of cancer cell death after their internalization and the intracellular release of the coupled cytotoxic drug. However, because of their relative bulky size, ADCs have a reduced ability to efficiently penetrate solid tumors, limiting their application to early-stage disease and to nonsolid cancer types (20). In addition, a new generation of chimeric immunotoxins, which is composed of a small tumor-targeting ligand (e.g., growth factors and cytokines) or an antibody fragment [e.g., single-chain fragment variable (scFv)] and a cytotoxic protein, was developed (21). Although there have been encouraging results obtained in preclinical and clinical settings, the use of bacteria- or plant-derived protein toxins, such as _Pseudomonas aeruginosa_ exotoxin A (ETA) or ricin (22, 23), can result in the induction of neutralizing antibodies and dose-limiting side effects, including vascular leak syndrome and liver toxicity (24, 25).

During the last decade, various human proteins have been identified that seemed suitable to be exploited as antitumor effector proteins in immunotherapeutic approaches. Several human enzymes, including the protease granzyme B, RNase angiogenin, or death-associated protein kinase 2, have been demonstrated to induce apoptosis in target cells, when delivered by antigen-specific antibodies or antibody fragments (26–29). Similarly, death ligands or antibody fragments (26–29). Similarly, death ligands or antibody fragments (26–29). Similarly, death ligands or antibody fragments (26–29). Similarly, death ligands or antibody fragments (26–29).

In this study, we present a new human CFP, designated EpCAM(scFv)-MAP, consisting of a fully human anti-EpCAM scFv antibody fragment genetically fused to human MAP. Anti–EpCAM(scFv)-MAP was expressed in _Escherichia coli_ ( _E_. coli) and purified by standard chromatographic methods. Anti–EpCAM(scFv)-MAP potentially induced apoptosis in EpCAM + carcinoma cell lines, including L3.6pl (IC50, 43 nmol/L) and A431 (IC50, 67 nmol/L). The chimeric anti–EpCAM(scFv)-ETA' immunotoxin was used as a positive control. Colocalization and tubulin polymerization assays indicated that stabilization of microtubules could be a possible mode of action of anti–EpCAM(scFv)-MAP. Furthermore, anti–EpCAM(scFv)-MAP was well tolerated when injected in mice. L3.6pl cells transfected with the far-red fluorescent protein Katushka-2 were used in a mouse xenograft model _in vivo_ to confirm that anti–EpCAM(scFv)-MAP significantly inhibited tumor growth.

Here, we present a novel approach for the treatment of EpCAM + carcinomas combining the binding specificity of a human high-affinity anti-EpCAM scFv antibody fragment with the selective cytotoxicity of human MAP toward proliferating carcinoma cells.

**Materials and Methods**

**Generation of anti–EpCAM(scFv)-MAP**

The open reading frame (ORF) of MAP (Gene ID, 4137; modified as described in ref. 32) was modified with 5'-NotI and 3'-BlpI restriction sites by PCR followed by ligation into a NotI/BlpI-linearized pMT vector. The anti-EpCAM (scFv) sequence (33) was cloned 5' of MAP via XbaI/NotI. The sequence for anti–EpCAM(scFv)-ETA' was constructed using the same strategy. Expression, purification, and protein analysis was carried out as described previously (32).

**Cell culture**

EpCAM + human carcinoma cancer cell lines L3.6pl (34), A431 (ATCC: CRL-1555), C4-2 (kindly provided by Prof. Dr. Elsässer-Beile, University Hospital, Freiburg, Germany), SU86.86 (ATCC: CRL-1837), 22Rv1 (ATCC: CRL-2505), and the EpCAM + human embryonic kidney cell line HEK 293T (ATCC: CRL-11268) were grown in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mmol/L l-glutamine, 50 μg/mL penicillin, and 100 μg/mL streptomycin at 37°C, 100% humidity, and 5% CO2. Cell line authentication was carried out by short tandem repeat (STR) analysis. L3.6pl (6/2008), A431 (3/2005), HEK293 (12/2004), 22Rv1 (1/2013), and SU86.86 (10/2001) cells were authenticated at the Fraunhofer Institute IME in Aachen. The cell line C4-2 was not authenticated in-house.
Transfection of L3.6pl cells with Katushka-2

Transfection was carried out as described previously (35). Briefly, we seeded 1 × 10⁶ L3.6pl cells in a 12-well plate and transfected them with 1 μg DNA encoding the far-red fluorescent protein Katushka-2 (pTag-Katushka2-N; Evrogen) using Roti-Fect (Roth) according to the manufacturer’s instructions. Transfected cells were selected with 500 ng/mL G418 (Nalgene) and sorted using the FACSVantage Cell Sorter (Becton Dickinson).

Binding analysis

The cell-binding activity of purified anti–EpCAM(scFv)-MAP was analyzed by flow cytometry. We incubated 4 × 10⁵ cells with 1 μmol/L anti–EpCAM(scFv)-MAP in PBS (pH 7.4) containing 2 mmol/L EDTA and 0.5% (w/v) BSA for 30 minutes on ice followed by washing with PBS. Fluorescence staining was performed using an anti–penta-His-Alexa Fluor 488 antibody (1:100; Qiagen) for 30 minutes on ice in the dark. Finally, the cells were washed twice with PBS and analyzed on a FACS Calibur flow cytometer (Becton Dickinson).

In vitro cytotoxicity assay

The cytotoxic effect of anti–EpCAM(scFv)-MAP was assessed by measuring the conversion of XTT to a water-soluble orange formazan dye. In short, 1 × 10⁵ cells were seeded per well into a 96-well microtiter plate and were incubated with various dilutions of the recombinant protein for 72 hours at 37°C, 5% CO₂, and 100% humidity. The EpCAM⁺ cell line HEK293 and the chimeric immunotoxin anti–EpCAM(scFv)-ETA', and the SNAP-tagged anti–EpCAM(scFv) were used as a controls. Next, 50 μL XTT/phenazine methosulfate (100:1; SERVA and Sigma) were added to each well and incubated the plates as above for 3 to 4 hours before measuring the absorbance at 450 and 630 nm using an Epoch Microplate Spectrophotometer (BioTek). The concentration required to achieve 50% reduction of protein synthesis (IC₅₀) relative to untreated control cells was calculated using GraphPad Prism 5 (GraphPad Software). All experiments were carried out in triplicate.

Apoptosis assay

An AnnexinV/propidium iodide assay was used to determine the proapoptotic impact of anti–EpCAM(scFv)-MAP. We incubated 2.5 × 10⁵ cells/mL with 100 nmol/L anti–EpCAM(scFv)-MAP in a 12-well plate (Greiner Bio-One) for 48 hours at 37°C, 5% CO₂, and 100% humidity. EGFR⁺ HEK293 cells were used as a control. After incubation, the cells were washed twice with PBS (pH 7.4) and stained with AnnexinV-FITC (eBioscience) in AnnexinV binding buffer (10 mmol/L HEPES/NaOH (pH 7.4), 140 mmol/L NaCl, 2.5 mmol/L CaCl₂) for 30 minutes at room temperature. Finally, the cells were washed, resuspended in AnnexinV buffer containing 10 μg/mL propidium iodide, and analyzed by flow cytometry using a FACS Calibur flow cytometer (Becton Dickinson).

Tubulin polymerization assay

The stabilizing effect of anti–EpCAM(scFv)-MAP on tubulin polymerization was tested using a Tubulin Polymerization Assay Kit (tebu-bio) according to the manufacturer’s instructions. Paclitaxel (10 μmol/L) and general tubulin buffer were used as controls. All measurements were carried out in duplicate. Vₘₐₓ represents the slope of the linear phase in milli-extinction per minute.

Colocalization assay

To visualize colocalization of MAP with microtubules, EGFR⁺ A549 cells were transfected with a SNAP-TubB3 DNA construct (amplified by PCR from pCMV6-XL5-BIII-tubulin template [OriGene]) facilitating directed fluorescence labeling of tubulin via the SNAP-tag. Transfected A549SNAP-TubB3 cells were stained with 10 mmol/L SNAP-Cell TMR-Star (New England BioLabs) overnight. Afterward, cells were fixed by 4% (v/v) PFA/PBS (Polysciences Inc.) for 15 minutes at room temperature, permeabilized with 0.1% (v/v) Triton/PBS (Roth), and blocked with 3% (w/v) BSA/PBS. Of note, 1 μmol/L of EGF–MAP (32) or the truncated version EGF–MAP₁₅₁ diluted in 1% (w/v) BSA/PBS was added and incubated for 2 hours at room temperature. The primary antibody detecting MAP/MAP₁₅₁ was anti-human Tau [1:5,000 in 1% (w/v) BSA/PBS for 2 hours at room temperature (Thermo Fisher Scientific)]. The secondary antibody was goat-anti-mouse Alexa Fluor 647 [1:1,500 in 1% (w/v) BSA/PBS] and visualized with X-gal (Sigma) and DAPI (1:10,000 in PBS; Invitrogen) was used to counterstain the nucleus. Images were taken using an Opera Q EHS 2.0 confocal fluorescence microscope (PerkinElmer) at ×40 magnification.

In vivo efficacy

The local Animal Care and Use Review Committee officially approved the animal experiments. All animals received humane care in accordance with the requirements of the German Tierschutzgesetz, §8 Abs. 1 and with the guide for the care and use of laboratory animals published by the NIH in 2011. Animal experiments were carried out as described previously (35). Briefly, 1 × 10⁶ L3.6pl cells expressing Katushka-2 were suspended in approximately 20 μL of PBS (pH 7.4) and subcutaneously injected into the right hind limb of 3- to 8-week-old female Balb/C nu/nu mice (Charles River Laboratories). For imaging experiments, mice were placed under a purified, chlorophyll-free diet (AIN93G; SSNIFF GmbH) 11 days before the imaging experiments began. The animals were injected intravenously with anti–EpCAM(scFv)-MAP (4 mg/kg) or PBS (pH 7.4) on days 0, 1, 3, and 5. Readouts were taken on the same days as the treatment, using the Maestro CRI optical imaging system (Maestro CRI Inc.). Images were analyzed using the Maestro spectral imaging software as previously described (36). The Katushka-2 signal was detected using the yellow filter set (630–850 nm).
Statistical analysis
Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software). Data were expressed as mean ± SD or SEM as indicated. Statistical comparisons were made using a two-tailed unpaired Student t test or one-way ANOVA, where appropriate; *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001.

Results
Generation of anti–EpCAM(scFv)-MAP and characterization of binding activity
We previously reported the identification of a new cytolytic effector protein, called MAP, which proved effective in specifically killing proliferating cancer cells, when fused to EGF (32). Here, the complete ORF of MAP was cloned in frame with a 5′-ORF encoding the fully-human anti-EpCAM(scFv) into a bacterial expression vector. Anti–EpCAM(scFv)-MAP was successfully expressed under osmotic stress conditions in the presence of compatible solutes (37), yielding up to 1 mg of purified protein per liter of bacterial culture. After purification by affinity chromatography, specific binding activity to L3.6pl and A431 cells was confirmed by flow cytometry, which also revealed the absence of nonspecific binding to EpCAM+ HEK293 cells (Fig. 1).

Cytotoxicity of anti–EpCAM(scFv)-MAP in vitro
The EpCAM-specific scFv has been shown to be an attractive target for immunotherapy as it induces internalization of the receptor–ligand complex upon binding. Having taken this into consideration (33, 38), the in vitro cytotoxicity of anti–EpCAM(scFv)-MAP was determined using an XTT cell viability assay. Anti–EpCAM(scFv)-MAP showed specific toxicity toward L3.6pl and A431 cells reaching IC50 values of 43 nmol/L and 67 nmol/L, respectively (Fig. 2, top). Although the bacterial ETA is not comparable with MAP with respect to the mode of action, the cytotoxic efficacy of anti–EpCAM(scFv)-MAP was comparable with that obtained for anti–EpCAM(scFv)-ETA’ (409 nmol/L on L3.6pl and 91 nmol/L on A431 cells; Fig. 2, middle) that was used as a positive control.

The diagnostic tool molecule anti–EpCAM(scFv)-SNAP, which does not contain a cytotoxic load, showed no adverse effects on cell viability (Fig. 2, bottom). In addition, anti–EpCAM(scFv)-MAP bound and was cytotoxic to other cancer cell lines expressing different levels of EpCAM (Supplementary Fig. S1 and Table 1, respectively).

Anti–EpCAM(scFv)-MAP promotes apoptosis
The mode of action of anti–EpCAM(scFv)-MAP was investigated by incubating L3.6pl cells with anti–EpCAM (scFv)-MAP followed by staining with AnnexinV-FITC and propidium iodide. As this assay was performed to demonstrate qualitatively the proapoptotic effect of the anti–EpCAM(scFv)-MAP, the concentration (100 nmol/L ~ 2 × IC50) and the incubation time (48 hours) were appropriately chosen to capture the cells within the early and late apoptotic stage. Cells treated with equal volume of PBS were used as a control. The population of late apoptotic cells (upper right quadrant) increased strongly, demonstrating the cytotoxicity of anti–EpCAM(scFv)-MAP toward EpCAM+ target cells. In addition to apoptotic cells, the population in the upper left quadrant, representing necrotic cells, increased significantly. There was no induction of apoptosis in HEK293 cells (Fig. 3).

Anti–EpCAM(scFv)-MAP colocalizes with and stabilizes microtubules
To provide first indications for the mode of action of MAP-based CFPs, we established a cell-based in vitro fluorescence assay, in which colocalization of MAP can be visualized. Therefore, target cells had to be transfected with SNAP-tagged tubulin DNA constructs allowing the specific labeling of tubulin molecules with fluorescent dyes. Unfortunately, EpCAM+ L3.6 and A431 cells are difficult to transfect. Therefore, we used EGFR+ A549 cells in combination with previously described EGF–MAP and a truncated version EGF–MAPf151 to show the representative mode of action for MAP-based CFPs. Figure 4A exemplifies the successful demonstration of MAP/MAPf151-tubulin colocalization (hotspots indicated by arrows). Binding of MAP to tubulin was anticipated to interfere with mitosis by

![Figure 1. Cell-binding activity of anti–EpCAM(scFv)-MAP to L3.6pl and A431 cells. Purified anti–EpCAM(scFv)-MAP was tested for its ability to bind EpCAM+ target cells. EpCAM+ HEK293 cells were used as a negative control. Bound anti–EpCAM(scFv)-MAP was detected with anti–penta-His–Alexa Fluor 488 antibody. relMFI, relative mean fluorescence intensity = MFI stained cells/MFI background signal.](mct.aacrjournals.org)
stabilizing microtubules. To determine whether MAP could, in fact, stabilize microtubules and promote polymerization, we carried out a tubulin polymerization assay. Of note, 50 nmol/L (IC50) of anti–EpCAM(scFv)-MAP was able to stabilize microtubules effectively by promoting polymerization. Paclitaxel (10 μmol/L; concentration recommended by the manufacturer) was used as a control (Fig. 4B). Combining these two experimental outcomes indicates that MAP-based CFPs are able to bind to and to stabilize microtubules, thus affecting microtubule dynamics within the cell.

Antitumor activity of anti–EpCAM(scFv)-MAP in vivo
Before treatment experiments, anti–EpCAM(scFv)-MAP was tested in tumor-free mice to investigate its off-target toxicity. Three mice per treatment group received 0.5, 1.0, 2.0, or 4.0 mg/kg of anti–EpCAM(scFv)-MAP intravenously on days 0, 1, 3, and 5. The overall health status and body weight of the mice were monitored daily. All mice survived and showed normal behavior. No reduction of body weight was observed (Fig. 5A). Although a maximum tolerated dose could not

Table 1. Cytotoxicity of anti–EpCAM(scFv)-MAP to different EpCAM+ cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell type</th>
<th>IC50 (nmol/L)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>22Rv1</td>
<td>Prostate carcinoma</td>
<td>677</td>
<td>High</td>
</tr>
<tr>
<td>L3.6pl</td>
<td>Pancreas carcinoma</td>
<td>43</td>
<td>EpCAM expression</td>
</tr>
<tr>
<td>C4-2</td>
<td>Prostate carcinoma</td>
<td>161</td>
<td>Low</td>
</tr>
<tr>
<td>SU86.86</td>
<td>Pancreas carcinoma</td>
<td>333</td>
<td></td>
</tr>
<tr>
<td>A431</td>
<td>Epidermoid carcinoma</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>
be determined, our data suggest that anti–EpCAM(scFv)-MAP is well tolerated.

To test the efficacy of anti–EpCAM(scFv)-MAP in vivo, we transfected L3.6pl cells with the far-red fluorescent protein, Katushka-2, and injected \(1 \times 10^6\) transfected cells subcutaneously into the right hind leg of 10 Balb/C nude mice to induce tumors. We monitored tumor growth using the in vivo imaging system Maestro Cri, based on the premise that the total Katushka-2 signal correlates with cell number. Eleven days after inoculation, all mice showed palpable tumors (Fig. 5B). Mice were then randomly divided into two groups (\(n = 5\)) for one treatment cycle with anti–EpCAM(scFv)-MAP (4 mg/kg) or PBS (pH 7.4). Although the tumor volume of PBS-treated mice increased 2.9 fold on day 5 and 3.6 fold on day 7 (both compared with day 0), only 1.3 fold and 1.9-fold volume increase could be determined for mice treated with anti–EpCAM(scFv)-MAP, respectively. Hence, our results clearly demonstrate that anti–EpCAM(scFv)-MAP causes a significant reduction in tumor growth compared with the PBS control group.

Discussion

In this study, we present a novel MAP-based CFP anti–EpCAM(scFv)-MAP that has potent EpCAM-restricted proapoptotic activity toward carcinoma cells. Its cytotoxic mode of action is provided by the proapoptotic effector protein MAP that selectively affects proliferating cells (32). The effector protein MAP in anti–EpCAM(scFv)-MAP was cytotoxic toward cancer cells in a manner that mechanistically seems to be comparable with that of conventional microtubules modifying cytostatic agents.

![Figure 3](image-url)  
Figure 3. Cytotoxicity of anti–EpCAM(scFv)-MAP is based on induction of apoptosis. L3.6pl and HEK293 cells were incubated with 100 nmol/L of anti–EpCAM(scFv)-MAP for 48 hours. Detection of late apoptotic cells was carried out using recombinant AnnexinV-FITC and propidium iodide staining. Values are presented as mean ± SD of three independent measurements. Statistical analysis was carried out using one-way ANOVA; ***, \(P < 0.001\).

![Figure 4](image-url)  
Figure 4. Anti–EpCAM(scFv)-MAP colocalizes with and stabilizes microtubules. A, colocalization assays were performed using EGFR1 A549 cells transfected with a SNAP-tagged tubulin DNA construct. Expressed SNAP-tagged tubulin molecules were labeled with SNAP-Cell TMR-Star (green). EGF–MAP and the truncated version EGF–MAPf151 were used as representatives for MAP-based CFPs. MAP and MAPf151 were detected using mouse anti-human Tau and goat anti-mouse Alexa Fluor 647 antibodies (red). DAPI was used to counterstain the nucleus (blue). Images were taken using an Opera QEHS 2.0 confocal fluorescence microscope at \(x\) 40 magnification. Selected colocalization events of MAP/MAPf151 with tubulin are indicated by arrows. B, tubulin polymerization assay using 50 nmol/L (IC50) anti–EpCAM(scFv)-MAP (Ep-MAP), with general tubulin buffer and paclitaxel (10 \(\mu\)mol/L) as controls. \(V_{\text{max}}\) values were calculated using the slope of the linear phase (mE, milli-extinction). Statistical analysis was carried out using a two-tailed unpaired Student t test; ***, \(P < 0.001\) compared with the buffer control.
null
and/or signal sequences (45). However, the cytotoxic activity of human CFPs can be improved further by the insertion of adapter sequences that facilitate the translocation of the effector molecule from the endosome to the cytosol (29). Therefore, our future research will focus on improving the translocation efficiency of anti–EpCAM (scFv)-MAP, to further enhance its therapeutic potential. In regard to the cytotoxic mode of action, we have clearly demonstrated the proapoptotic effect of MAP, previously (32). Interestingly, but not totally unexpected, the surface expression levels of EpCAM did not correlate quantitatively (IC_{50} values) with the sensitivity of different cell lines. EpCAM is known to be a highly glycosylated protein. Differences in glycosylation patterns of EpCAM have been attributed to differences in protein stability and thus functionality (46). Furthermore, distinct EpCAM glycosylation has been suggested to be one possible discriminating criterion between healthy and malignant cells (14, 47). Incubation with anti–EpCAM(scFv)-MAP led to a significant increase of the necrotic cell population, which seems to be the result of prolonged treatment. In regard to immunogenicity, fully human CFPs seem superior to ADCs or chimeric immunotoxins due to the fact that they still did not reach the maximum tolerated dose, highlighting favorable tolerability of the new human CFP.

Given the fact that EpCAM is also expressed on healthy epithelia, a diagnostic preselection of patients with overexpression of EpCAM in cancerous lesions cells may provide a promising strategy to enhance the efficacy of EpCAM-directed cancer immunotherapy. Previously, it was demonstrated that postoperative intraperitoneal application of the anti–CD3/anti-EpCAM bispecific mAb catumaxomab proved to be beneficial in the treatment of patients with gastric cancer (48). It remains to be studied if perhaps anti–EpCAM(scFv)-MAP also shows enhanced efficacy when topically applied in a similar fashion.

In conclusion, the fully human fusion protein anti–EpCAM(scFv)-MAP shows promising carcinoma-selective cytotoxicity both in vitro and in an in vivo animal model and may be of potential in the treatment of EpCAM-overexpressing malignancies.

Disclosure of Potential Conflicts of Interest

D. Hristodorov, R. Mladenov, T. Thelen, and S. Barth have ownership interest (including patents) in MAP-based cytolytic fusion proteins. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

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Development of methodology: D. Hristodorov, M. Amoury, N. Berges, S. Di Fiore, T. Thelen, S. Barth

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc): D. Hristodorov, M. Amoury, R. Mladenov, J. Niesen, K. Arens, L. Hein, A.-T. Pham, W. Helfrich

Analysis and interpretation of data (e.g., statistical analysis, bios-statistics, computational analysis): D. Hristodorov, R. Mladenov, N. Berges, S. Di Fiore, M. Huhn, T. Thelen

Writing, review, and/or revision of the manuscript: D. Hristodorov, W. Helfrich, R. Fischer, T. Thelen, S. Barth

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.-T. Pham, M. Huhn

Study supervision: N. Berges, S. Di Fiore, T. Thelen

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References


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