Eur J Nucl Med Mol Imaging (2010) 37:1559–1565
DOI 10.1007/s00259-010-1446-9

SHORT COMMUNICATION

# Tumour-targeting properties of antibodies specific to MMP-1A, MMP-2 and MMP-3

**Stefanie Pfaffen · Katharina Frey · Irène Stutz · Christoph Roesli · Dario Neri**

Received: 8 January 2010 / Accepted: 8 March 2010 / Published online: 20 April 2010


**Abstract**
*Purpose* Matrix metalloproteinases (MMPs), a group of more than 20 zinc-containing endopeptidases, are upregulated in many diseases, but several attempts to use radiolabelled MMP inhibitors for imaging tumours have proved unsuccessful in mouse models, possibly due to the limited specificity of these agents or their unfavourable pharmacokinetic profiles. In principle, radiolabelled monoclonal antibodies could be considered for the selective targeting and imaging of individual MMPs.
*Methods* We cloned, produced and characterized high-affinity monoclonal antibodies specific to murine MMP-1A, MMP-2 and MMP-3 in SIP (small immunoprotein) miniantibody format using biochemical and immunochemical methods. We also performed comparative biodistribution analysis of their tumour-targeting properties at three time points (3 h, 24 h, 48 h) in mice bearing subcutaneous F9 tumours using radioiodinated protein preparations. The clinical stage L19 antibody, specific to the alternatively spliced EDB domain of fibronectin, was used as reference tumour-targeting agent for in vivo studies.
*Results* All anti-MMP antibodies and SIP(L19) strongly stained sections of F9 tumours when assessed by immunofluorescence methods. In biodistribution experiments, SIP (SP3), specific to MMP-3, selectively accumulated at the tumour site 24 and 48 h after intravenous injection, but was rapidly cleared from other organs. By contrast, SIP(SP1) and SIP(SP2), specific to MMP-1A and MMP-2, showed no preferential accumulation at the tumour site.
*Conclusion* Antibodies specific to MMP-3 may serve as vehicles for the efficient and selective delivery of imaging agents or therapeutic molecules to sites of disease.

**Keywords** MMP-1A · MMP-2 · MMP-3 · Antibody · Tumour targeting

## Introduction

Monoclonal antibodies are increasingly being used for the selective delivery of bioactive molecules (e.g. radionuclides, drugs, cytokines) to the site of disease, thus sparing normal organs [1]. Although antibodies specific to membrane antigens on cancer cells have mainly been used so far for tumour-targeting applications, there is growing interest in the use of stromal antigens and of markers of angiogenesis as antibody targets [2], which have the potential to offer broad tumour coverage and low expression in normal tissues.

Matrix metalloproteinases (MMPs) are a group of more than 20 zinc-containing extracellular proteinases that are capable of degrading multiple components of the extracellular matrix. Abnormal expression of MMPs contributes to a variety of pathological conditions. In the tumour environment, for example, host- and tumour-derived MMPs are often upregulated, while being expressed at low levels in normal organs [3, 4]. Several studies of the use of MMPs as targets for imaging purposes have been reported [5]. These approaches include the use of radiolabelled MMP inhibitors as targeting agents, or the use of fluorogenic

**Electronic supplementary material** The online version of this article (doi:10.1007/s00259-010-1446-9) contains supplementary material, which is available to authorized users.

S. Pfaffen · K. Frey · I. Stutz · C. Roesli · D. Neri (✉)
Department of Chemistry and Applied Biosciences, ETH Zürich,
Institute of Pharmaceutical Sciences,
Wolfgang-Pauli-Strasse 10,
8093 Zürich, Switzerland
e-mail: dario.neri@pharma.ethz.ch

MMP-specific substrates combined with optical imaging modalities. Since attempts to use radiolabelled MMP inhibitors for in vivo tumour targeting applications have often proved unsuccessful, possibly due to the limited specificity of these agents or their unsuitable pharmacokinetic properties [5], radiolabelled monoclonal antibodies could in principle be considered as an alternative.

We have previously reported on the isolation of three high-affinity human monoclonal antibodies (termed SP1, SP2 and SP3) specific to murine MMP-1A, MMP-2 and MMP-3, respectively, using phage display technology. These antibodies have been thoroughly characterized in vitro and tested by immunofluorescence in a wide panel of healthy and diseased tissues [4]. Here we describe the cloning, production and characterization of these antibodies in small immunoprotein (SIP) miniantibody format [6] (Fig. 1a) and the comparative analysis of their tumour-targeting properties in an allograft mouse model of cancer. SIP(SP1) and SIP(SP2) showed no preferential accumulation in the tumour. By contrast, both SIP(SP3) and SIP (L19), a clinical stage recombinant antibody specific to the alternatively spliced EDB domain of fibronectin (a marker of tumour angiogenesis [2, 6]), exhibited selective accumulation at the tumour site, as assessed by quantitative biodistribution studies with radioiodinated antibody preparations. These results indicated that recombinant monoclonal antibodies specific to MMP-3 may be used for imaging purposes or for the selective delivery of bioactive moieties to tumours or to disease areas where MMP-3 is known to be overexpressed (e.g. in rheumatoid arthritis).

## Material and methods

### Cloning, expression, and purification of antibodies in the SIP format

Single chain variable fragments (scFv) were converted into the SIP format by cloning VH and VL into pcDNA3.1 (Invitrogen) using a previously described strategy [7, 8] (Fig. 1a). The plasmids were transfected into CHO-S cells (Invitrogen) using a Cell Line Nucleofector Kit V (Lonza), following the manufacturer's protocol. Transfectants were grown in RPMI medium supplemented with 10% fetal calf serum (FCS) and selected by addition of 0.5 mg/ml Geneticin (G418) (Merck Chemicals). Monoclonal cultures were obtained by fluorescent-activated cell sorting or by limited dilution, as described previously [8]. After 10 to 14 days of selection, cells were suspended and cultured in PowerCHO-2 CD medium (Lonza). SIP antibodies were purified from culture medium by affinity chromatography using a Protein A Sepharose Fast Flow resin (GE Healthcare), as described previously [8].

### Characterization of the SIP antibodies

Purified SIP antibodies were analyzed by SDS-PAGE, size exclusion chromatography on Superdex 200 HR10/300 columns (Amersham Biosciences) and by surface plasmon resonance measurements on a low-density coated antigen chip as described previously [7] to determine the apparent $K_D$.

### Deglycosylation

To deglycosylate purified SIP(SP1), 15 μg protein samples were incubated with 500 U PNGase F (New England Biolabs) for 3 h at 37°C. The resulting product was analysed directly by SDS-PAGE.

### Immunofluorescence analysis/confocal laser scanning microscopy of frozen tissue sections

F9 teratocarcinoma tissues were embedded in freezing medium (Microm) and stored at −80°C until sectioned. Tissue sections (10 μm) were fixed for 10 min with ice-cold acetone, rehydrated with phosphate-buffered saline (PBS) and blocked with 20% FCS in PBS. Biotinylated SIP(L19) (2 μg/ml; specific to the alternatively spliced EDB domain of fibronectin, used as positive control antibody of proven tumour-targeting properties [6]), purified SIP(HyHEL-10) (2 μg/ml; specific to hen egg lysozyme, used as negative control antibody) or the purified antibodies scFv(SP1), scFv (SP2) and scFv(SP3) (10–20 μg/ml) containing a *myc*-tag were added onto the sections. As secondary reagents for the scFvs a rat anti-*myc* antibody (Genetex) and for the SIP (HyHEL-10) a rabbit anti-human-IgE (Dako) were applied. The scFvs were detected using streptavidin Alexa Fluor 488 for SIP(L19), goat anti-rabbit IgG Alexa Fluor 488 (Invitrogen) for SIP(HyHEL-10) or donkey anti-rat IgG Alexa Fluor 488 antibody (Invitrogen). Nuclei were counterstained with DAPI (Invitrogen). All commercial binding reagents were diluted according to the manufacturer's recommendation. Sections were rinsed with PBS between all incubation steps. Slides were mounted with fluorescent mounting medium (Dako) and analysed using Zeiss AxioVision 4.7 image analysis software (Carl Zeiss).

For laser scanning microscopy analysis all steps were performed as described above. In addition, the cytoskeleton was counterstained with Phalloidin-Alexa 647 (Invitrogen). Slides were analysed using a LSM 510 META microscope from Zeiss (Carl Zeiss).

### Radioiodination of the SIP antibodies

Antibody radioiodination was performed as previously described [7]. Briefly, 300 μg of protein was mixed with

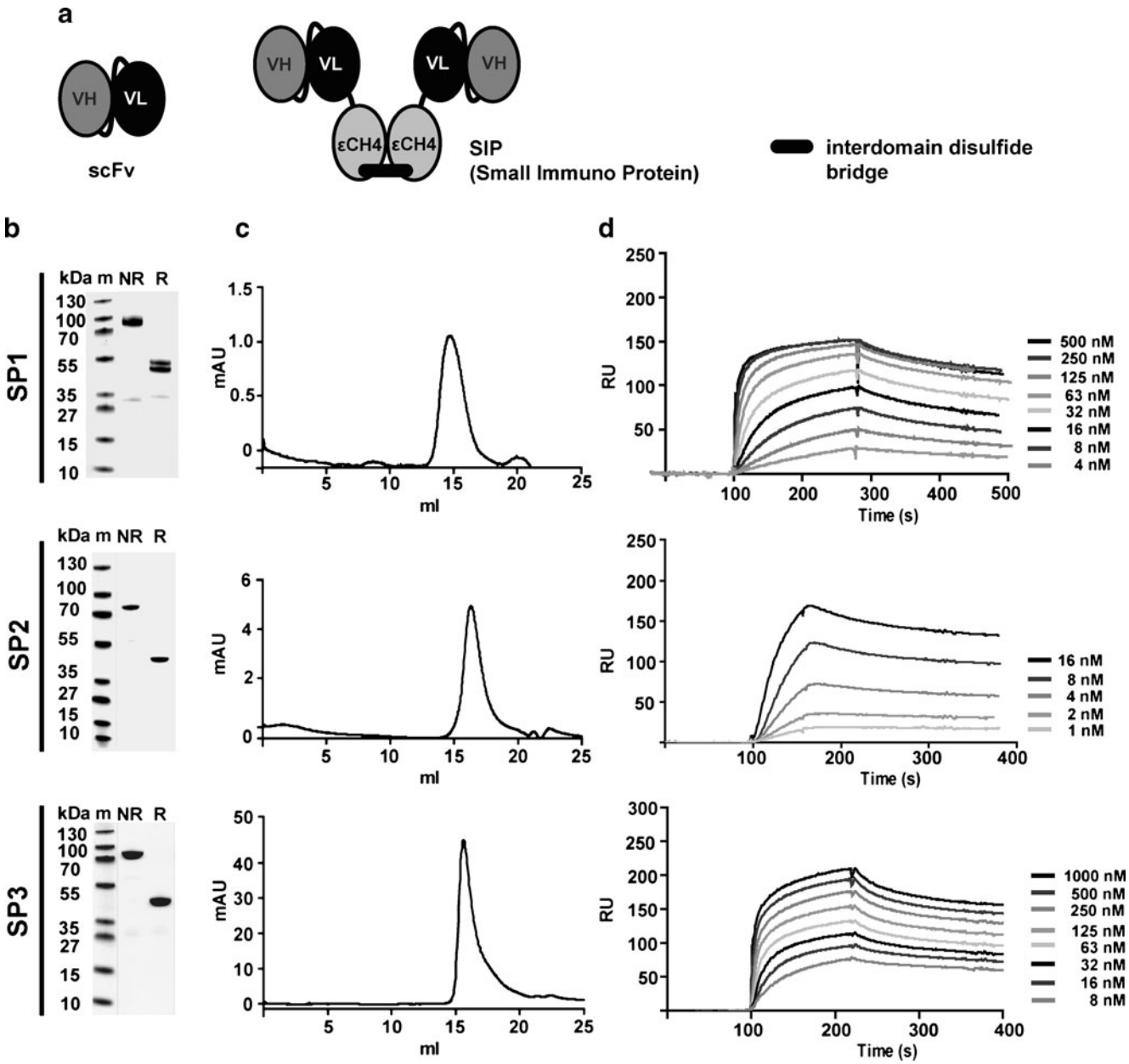


**Fig. 1** Antibodies against the catalytic domain of murine MMP-1A, MMP-2 and MMP-3. **a** Schematic illustration of the scFv format consisting of a heavy chain (*VH*) and a light chain (*VL*) linked by a peptide linker, and a schematic illustration of the SIP format consisting of a disulphide-linked homodimer. **b** SDS-PAGE analysis of affinity-purified anti-MMP antibodies SIP(SP1), SIP(SP2) and SIP (SP3) (*m* molecular weight marker, *R* SIP antibodies under reducing conditions, *NR* SIP antibodies under nonreducing conditions). **c** Size exclusion chromatogram of SIP(SP1), SIP(SP2) and SIP(SP3) on a Superdex 200 HR10/300 column. The major peaks eluting at about 15 ml for SIP(SP1) and at about 16 ml for SIP(SP2) and SIP(SP3) correspond to the molecular weight of the covalent homodimer. **d** BIAcore sensograms of the antibodies SIP(SP1), SIP(SP2) and SIP (SP3). Kinetic constants were calculated with the BIA evaluation 4.1 software

200 μCi of $^{125}$I (Perkin Elmer) and filtered aqueous solution of chloramine T (Sigma) (5 mg/ml; 0.25 μg of chloramine T per microgram of protein) for 1 min 45 s followed by separation from unincorporated iodine using a PD-10 disposable gel filtration column (GE Healthcare). Antibody immunoreactivity after labelling was evaluated by affinity chromatography as previously described [6]. Binding reactivity, defined as the ratio between the counts of the eluted antibody and the sum of the counts (flow-through, wash and eluate), was 79% for SIP(L19), 62% for SIP(SP1), 67% for SIP(SP2), 84% for SIP(SP3) and 73% for SIP(HyHEL-10).

### Biodistribution of radiolabelled SIP antibodies in tumour-bearing mice

F9 murine teratocarcinoma cells ($2\times10^7$) were implanted subcutaneously into the left flank of 10- to 12-week-old 129/SvEv mice (Taconic) and tumours were allowed to grow for 8 days. The $^{125}$I-labelled antibody fragment (15–20 μg; 3–6 μCi) in 100 μl saline solution, radiolabelled immediately prior to use, was injected intravenously. Mice were sacrificed 3 h, 24 h and 48 h after injection. Organs were weighed and radioactivity was counted. Five to six animals were used for each time point and each construct. The radioactivity contents of representative organs are expressed as the percentage of the injected dose per gram of tissue (%ID/g) ±SE.

### Biodistribution of radiolabelled SIP antibodies in healthy mice

$^{125}$I-labelled antibody fragments (30–40 μg; 3–6 μCi) in 200 μl saline solution, radiolabelled immediately prior to use, were injected intravenously. Mice were sacrificed 30 min after injection. Urine was collected and was applied to a disposable gel filtration column (GE Healthcare). Fractions of 500 μl were collected and radioactivity was measured. Organs were excised, weighed and the radioactivity was counted. Two animals were used for each construct. The radioactivity content of urine fractions is expressed as counts per minute (cpm).

### Murine blood serum analysis by surface plasmon resonance

Murine blood serum was analysed by surface plasmon resonance on a BIAcore 3000 instrument (GE Healthcare), using a high-density coated SIP antibody chip as described previously [7]. A rabbit anti-human IgE antibody (Dako) (final concentration 83 μg/ml) and buffered saline solution were used as a positive control and as a negative control, respectively.

The radioactivity content of representative organs is expressed as the percentage of the injected dose per gram of tissue (%ID/g).

## Results

### Production and characterization of antibodies SIP(SP1), SIP (SP2) and SIP(SP3)

The human monoclonal antibody fragments scFv(SP1), scFv(SP2) and scFv(SP3) were cloned in SIP format by genetically fusing the scFv moiety at the N-terminal extremity of a human εCH4 domain of the secretory isoform S2 of human IgE [6] (Fig. 1a). This domain promotes the formation of homodimers that are further stabilized by disulphide bonds between the COOH terminal cysteine residues, resulting in a 75-kDa bivalent miniantibody (Fig. 1a). This format has previously been shown to offer a good pharmacokinetic compromise between the rapidly cleared scFv antibody fragments and the antibodies in full IgG format, which display long circulatory half-lives in vivo. Tumour-targeting SIPs typically show high tumour-to-organ ratios at 24 h and at later time points after injection [7].

SIP(SP1), SIP(SP2) and SIP(SP3) were expressed in Chinese hamster ovary cells (CHO-S) from a pcDNA3.1-based expression vector and purified by protein A affinity chromatography of the cell culture supernatant. All three recombinant antibodies quantitatively formed cysteine-linked covalent homodimers, as shown by SDS-PAGE under reducing and nonreducing conditions (Fig. 1b) and by size-exclusion chromatography (Fig. 1c). SDS-PAGE of SIP(SP1) revealed the presence of an N-glycosylation site, which was confirmed by N-glycanase treatment (Supplementary Fig. S1). The purified SIP(SP1), SIP(SP2) and SIP (SP3) antibodies were also analysed by real-time interaction analysis on a BIAcore instrument on a low-density microsensor chip, which confirmed their high affinity to the cognate antigens (Fig. 1d). The apparent $K_D$ values were 1.8 n*M* for SIP(SP1), 2.4 n*M* for SIP(SP2) and 1.9 n*M* for SIP(SP3).

### Biodistribution studies of SIP(SP1), SIP(SP2) and SIP(SP3) in F9 tumour-bearing mice

The in vivo targeting performance of radioiodinated SIP (SP1), SIP(SP2) and SIP(SP3) antibody preparations was evaluated by biodistribution studies in mice bearing F9 teratocarcinoma. $^{125}$I-labelled SIP(L19), a clinical stage recombinant antibody of proven tumour-targeting performance [2, 6], was used as positive control. SIP(HyHEL-10), an antibody specific to hen egg lysozyme, was used as negative control. All three anti-MMP antibodies, as well as L19, strongly stained F9 tumour sections as revealed by immunofluorescence analysis, while HyHEL-10 did not exhibit any detectable staining, as expected (Fig. 2a). Confocal laser microscopy analysis revealed that MMP-1A and MMP-3 were strongly expressed in the cytoplasm and on the membrane of the F9 tumour cells, while MMP-2 was predominantly found in intracellular structures (Supplementary Fig. S2). The antibodies were injected intravenously. At different time points (3 h, 24 h, 48 h) animals were sacrificed, organs were excised and weighed, and the radioactivity was counted. The biodistribution results are expressed as percent injected dose per gram of tissue or body fluid (Table 1, Fig. 2b).

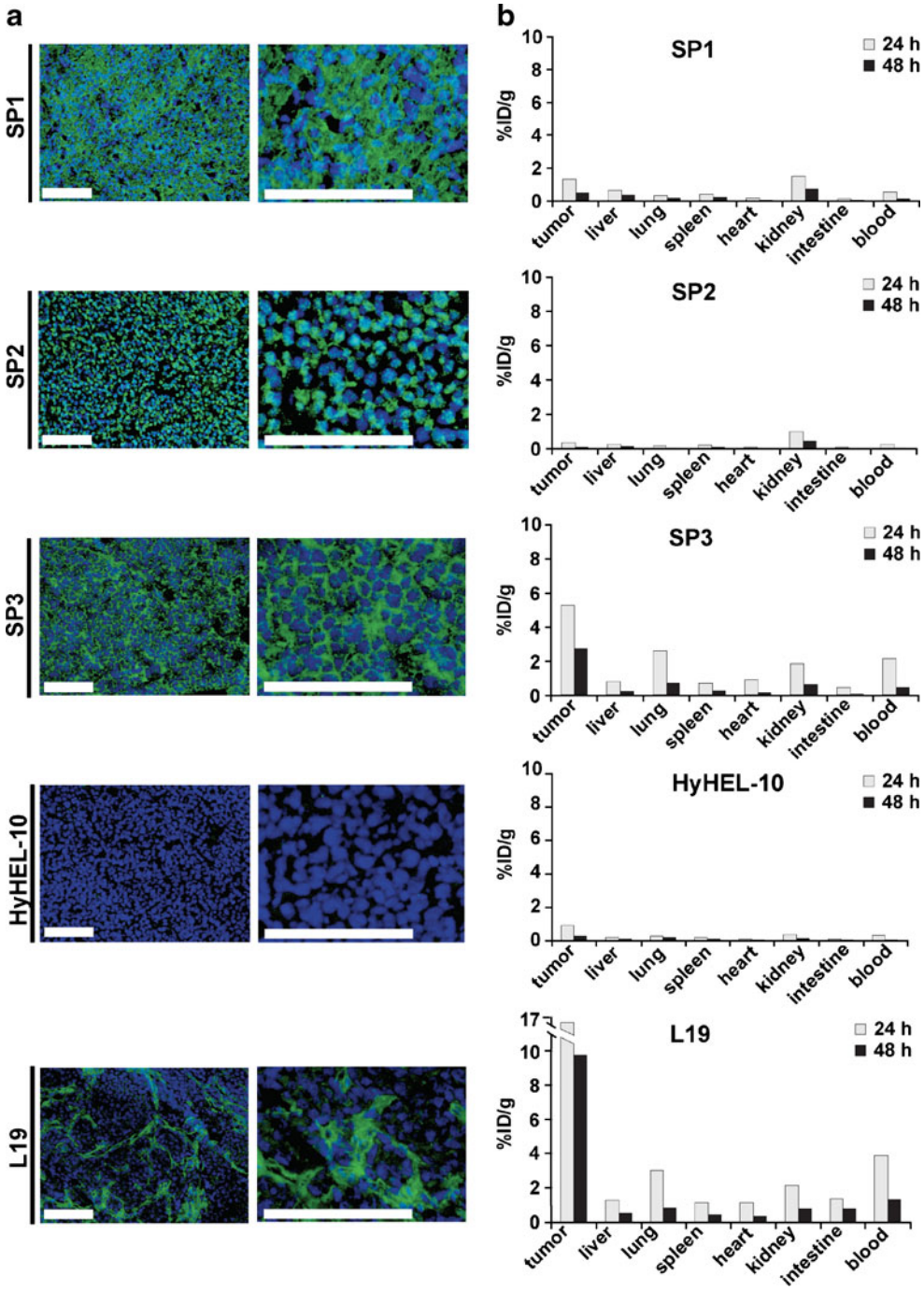


**Fig. 2** Immunofluorescence analysis and biodistribution studies after intravenous injection of antibodies in F9 tumour-bearing mice. **a** Immunofluorescence analysis was performed on tumour cryosections. MMP-1A, MMP-2, MMP-3 and extradomain B of fibronectin are stained in *green*, whereas cell nuclei were stained in *blue* using DAPI (*scale bar* 100 μm). **b** Biodistribution of $^{125}$I-labelled SIP(SP1), SIP (SP2), SIP(SP3) and of two control antibodies SIP(L19) and SIP(HyHEL-10) at 24 and 48 h. Each value is the average from five or six animals

The negative control antibody SIP(HyHEL-10) and the antibodies SIP(SP1) and SIP(SP2), specific to MMP-1A and MMP-2, respectively, were cleared very rapidly from the bloodstream and from most normal organs and did not show selective accumulation at the site of tumour (Table 1, Fig. 2b). By contrast, both SIP(SP3), specific to MMP-3, and SIP(L19) preferentially localized to the tumour (Table 1, Fig. 2b).

The blood clearance profile of SIP(SP1) and SIP(SP2), which both carry a VL domain consisting of the DPL-16 germline gene [4], was more rapid than that of SIP(L19) and SIP(SP3), which carry a VL domain based on the DPK-22 germline segment [4, 6]. All four antibodies contain a VH domain based on the DP-47 germline gene [7] and are identical in other portions of the SIP molecule. Comparison of the biodistribution of three SIP antibodies based on DPK-22 VL domains and five SIP antibodies based on DPL-16 light chains revealed a general trend to faster blood clearance for DPL-16-based antibodies (Supplementary Fig. S3). The excretion of the SIP(SP2) antibody 30 min after intravenous injection was more rapid than that of SIP (SP3) and SIP(L19) (Supplementary Fig. S4a). While

**Table 1** Biodistribution of radiolabelled antibody preparations in F9 tumour-bearing mice. Values are %ID/g ±SE

| | SIP(SP1) | | | SIP(SP2) | | | SIP(SP3) | | | SIP(HyHEL-10) | | | SIP(L19) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 hours | 24 hours | 48 hours | 3 hours | 24 hours | 48 hours | 3 hours | 24 hours | 48 hours | 3 hours | 24 hours | 48 hours | 3 hours | 24 hours | 48 hours |
| Tumour | 4.15±0.60 | 1.34±0.18 | 0.51±0.16 | 2.19±0.11 | 0.36±0.06 | 0.09±0.01 | 14.42±0.74 | 5.28±0.72 | 2.71±0.85 | 5.54±0.60 | 0.91±0.36 | 0.28±0.10 | 20.21±1.21 | 16.44±1.49 | 9.73±0.48 |
| Liver | 4.72±0.90 | 0.62 ±0.05 | 0.35±0.02 | 3.72±0.21 | 0.27±0.01 | 0.11±0.01 | 12.72±0.33 | 0.82±0.05 | 0.24±0.03 | 2.70±0.29 | 0.20±0.03 | 0.10±0.03 | 4.81±0.27 | 1.24±0.10 | 0.53±0.03 |
| Lung | 3.55±0.35 | 0.33±0.03 | 0.17±0.03 | 1.68±0.18 | 0.18±0.03 | 0.06±0.02 | 11.91±0.41 | 2.59±0.10 | 0.73±0.07 | 3.93±0.51 | 0.27±0.11 | 0.16±0.09 | 8.96±0.85 | 3.00±0.36 | 0.83±0.07 |
| Spleen | 3.23±0.48 | 0.40±0.01 | 0.23±0.01 | 2.22±0.17 | 0.21±0.04 | 0.07±0.01 | 9.51±0.69 | 0.73±0.11 | 0.29±0.01 | 2.42±0.28 | 0.19±0.06 | 0.08±0.02 | 4.94±0.43 | 1.12±0.11 | 0.43±0.05 |
| Heart | 2.49±0.40 | 0.20±0.01 | 0.06±0.01 | 1.03±0.04 | 0.08±0.01 | 0.03±0.01 | 10.11±0.30 | 0.90±0.03 | 0.19±0.01 | 2.28±0.22 | 0.10±0.03 | 0.03±0.01 | 6.32±0.60 | 1.13±0.10 | 0.36±0.04 |
| Kidney | 7.39±1.91 | 1.49±0.09 | 0.75±0.06 | 6.75±0.30 | 1.00±0.08 | 0.43±0.04 | 19.31±0.31 | 1.86±0.14 | 0.66±0.06 | 4.72±0.52 | 0.36±0.10 | 0.15±0.04 | 10.73±0.85 | 2.12±0.24 | 0.80±0.03 |
| Blood | 7.76±1.25 | 0.53±0.04 | 0.12±0.02 | 2.78±0.23 | 0.24±0.05 | 0.03±0.01 | 33.36±1.39 | 2.17±0.08 | 0.49±0.07 | 7.89±0.80 | 0.30±0.10 | 0.07±0.02 | 19.64±1.38 | 3.89±0.37 | 1.33±0.07 |
| Intestine | 1.69±0.32 | 0.15±0.02 | 0.05±0.004 | 0.98±0.06 | 0.07±0.02 | 0.01±0.004 | 6.34±1.09 | 0.50±0.03 | 0.11±0.02 | 2.02±0.26 | 0.10±0.03 | 0.02±0.01 | 6.41±1.61 | 1.35±0.27 | 0.78±0.09 |

clearance via the hepatobiliary route predominates, antibody dehalogenation in the liver leads to the excretion of free radioiodine through the kidneys, as revealed by a gel filtration analysis of urine (Supplementary Fig. S4b). We hypothesized that the longer retention of SIP(SP3) and SIP (L19) in the blood could have been due to a noncovalent association with serum components. Supplementary Fig. S5 reveals that indeed SIP(SP3), but not SIP(SP1) or SIP(SP2), formed a complex with serum proteins, as revealed by real-time interaction analysis on a BIAcore 3000 instrument.

## Discussion

We have reported the first biodistribution analysis of the tumour-targeting performance of three radiolabelled high-affinity monoclonal antibodies, specific to three different murine MMPs. While all three antibodies strongly stained various tumour sections [4], including F9 tumours (Fig. 2a), only the SIP(SP3) antibody, specific to murine MMP-3, exhibited a preferential localization at the tumour site.

The biodistributions of the SP1, SP2, SP3 and L19 antibodies in SIP format were compared in mice bearing murine tumours facilitated by the fact that the EDB antigen has an identical sequence in mice and humans [2], thus allowing characterization of tumour-targeting properties in a syngeneic setting. To our knowledge, only one biodistribution study with a monoclonal antibody specific to membrane-bound human MMP-1 has previously been reported [9]. In that study, which involved a mouse and a rat xenograft model, the antibody exhibited no preferential localization to the tumour, which is in line with our results.

Our recent immunohistochemical analysis of the patterns of murine MMP-1A, MMP-2 and MMP-3 expression [4] has shown that these antigens are undetectable in most normal mouse tissues (except in the liver for MMP-2 and MMP-3, and in the lung, brain and kidney for MMP-2), but are strongly expressed in the majority of tumours tested, making them suitable candidates for biomolecular anticancer pharmacodelivery strategies. In order to qualify for antibody-based tumour-targeting applications, an antigen needs to be accessible in vivo and also needs to mediate an "immobilization" of the antibody on the neoplastic lesion. The biodistribution results presented here indicate that MMP-3 is sufficiently abundant and accessible in F9 tumours to permit a preferential antibody localization at the neoplastic site. This protease is probably anchored to extracellular matrix components via, for example, the NC1 domain of type IV collagen or the laminin-binding domain of agrin, which are structurally similar to tissue inhibitors of metalloproteinases (reviewed in reference [10]). MMP-1A and MMP-3 showed a similar cellular localization in F9

tumour cells, but only MMP-3 could be efficiently targeted in vivo. By contrast, in this tumour model, the majority of MMP-2 was intracellular and thus not accessible to antibodies in vivo (Supplementary Fig. S2).

Antibodies were used in SIP format for biodistribution studies. Miniantibody formats have been extensively studied by our group and in other laboratories, and appear to provide an ideal compromise between the fast-clearing scFv fragments and the long-lived IgGs [6]. Unlike scFv and Fab fragments, antibodies in SIP format are mainly cleared via the hepatobiliary route [6, 7], so that the kidneys would be spared in radioimmunotherapy applications. Indeed, $^{131}$I-labelled SIP(L19) and SIP(F16) are currently being investigated in phase II radioimmunotherapy trials, with a special focus on haematological malignancies [2], and these two SIPs labelled with $^{124}$I are being studied in immuno-PET clinical trials.

In line with previous observations in biodistribution studies with antibodies in IgG, Fab and scFv formats, the results presented in Table 1 show differences in blood clearance properties among different SIPs, with the L19 and SP3 antibodies being the longest-lived recombinant antibodies in circulation. The reasons for these differences are not obvious, since the cognate antigens are present at negligibly low concentrations (nanograms per millilitre levels or lower) in blood and since all antibodies have a VH domain based on the same (DP-47) germline V segment [4]. Interestingly, both SP3 and L19 carry a Vκ domain based on the DPK-22 germline V segment, while SP1 and SP2 have a Vλ domain, based on the DPL-16 germline segment. Biodistribution studies revealed that DPL-16-based antibodies in SIP format clear more rapidly from the blood than antibodies containing DPK-22 light chains (Supplementary Fig. S3). A previously unsuspected noncovalent binding interaction of SP3 with serum, clearly revealed by BIAcore analysis (Supplementary Fig. S5), may account for the longer residence time of this antibody in the bloodstream (Fig. 2b, Table 1). Both L19 and SP3 exhibited a long residence time on the tumour in vivo, a favourable property for the use of these antibodies as building blocks for the development of targeted anticancer biopharmaceuticals.

The selective tumour localization properties of SIP(SP3) suggest that anti-MMP-3 antibodies may be useful for the immuno-PET visualization of this antigen in patients with cancer, but also in other pathologies where MMP-3 is strongly overexpressed (e.g. rheumatoid arthritis). In addition to nuclear medicine applications, it would be conceivable to use anti-MMP-3 antibodies for pharmaco-delivery applications, in full analogy with antibodies specific to splice isoforms of fibronectin and tenascin-C [2, 6, 7], which have been fused to cytokines, photosensitizers, procoagulant factors, enzymes and drugs. Some of these derivatives (most notably L19-IL2, L19-TNF, F16-IL2, F8-IL10) are currently being investigated in clinical trials. However, since the catalytic domain of MMP-3 displays only an 82% homology between mouse and man, human-specific monoclonal antibodies will be needed for clinical applications.

**Acknowledgments** The SIP(L19) antibody used in this study was a kind gift from Alessandro Palumbo (ETH Zurich, Switzerland).

Financial support from ETH Zürich (ETHIRA grant), the Swiss National Science Foundation (grant 3100A0-105919/1), the Swiss Cancer League (Robert-Wenner Award), the SWISSBRIDGE-Stammbach Foundation and European Union Projects IMMUNO-PDT (grant LSHC-CT-2006-037489), DIANA (grant LSHB-CT-2006-037681) and ADAMANT (HEALT-F2-2008-201342) is gratefully acknowledged.

## References

1. Carter PJ. Potent antibody therapeutics by design. Nat Rev Immunol 2006;6:343–57.
2. Neri D, Bicknell R. Tumour vascular targeting. Nat Rev Cancer 2005;5:436–46.
3. Overall CM, Kleifeld O. Tumour microenvironment – opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer 2006;6:227–39.
4. Pfaffen S, Hemmerle T, Weber M, Neri D. Isolation and characterization of human monoclonal antibodies specific to MMP-1A, MMP-2 and MMP-3. Exp Cell Res 2010;316:836–47.
5. Scherer RL, McIntyre JO, Matrisian LM. Imaging matrix metalloproteinases in cancer. Cancer Metastasis Rev 2008;27:679–90.
6. Borsi L, Balza E, Bestagno M, Castellani P, Carnemolla B, Biro A, et al. Selective targeting of tumoral vasculature: comparison of different formats of an antibody (L19) to the ED-B domain of fibronectin. Int J Cancer 2002;102:75–85.
7. Villa A, Trachsel E, Kaspar M, Schliemann C, Sommavilla R, Rybak JN, et al. A high-affinity human monoclonal antibody specific to the alternatively spliced EDA domain of fibronectin efficiently targets tumor neo-vasculature in vivo. Int J Cancer 2008;122:2405–13.
8. Zuberbühler K, Palumbo A, Bacci C, Giovannoni L, Sommavilla R, Kaspar M, et al. A general method for the selection of high-level scFv and IgG antibody expression by stably transfected mammalian cells. Protein Eng Des Sel 2009;22:169–74.
9. Temma T, Sano K, Kuge Y, Kamihashi J, Takai N, Ogawa Y, et al. Development of a radiolabeled probe for detecting membrane type-1 matrix metalloproteinase on malignant tumors. Biol Pharm Bull 2009;32:1272–77.
10. Overall CM, Lopez-Otin C. Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat Rev Cancer 2002;2:657–72.