ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 10338–10340

www.rsc.org/chemcomm

COMMUNICATION

Using boronolectin in MALDI-MS imaging for the histological analysis of cancer tissue expressing the sialyl Lewis X antigen†

Chaofeng Dai,[a] Lisa H. Cazares,[b] Lifang Wang,[a] Yong Chu,[ac] Siming L. Wang,[a] Dean A. Troyer,[b] O. John Semmes,[b] Richard R. Drake*[b] and Binghe Wang*[a]

Received 30th March 2011, Accepted 4th August 2011
DOI: 10.1039/c1cc11814e

Certain carbohydrate-based biomarkers are known to correlate with cancer formation and progression. By targeting sialyl Lewis X, we have developed the first boronolectin–MS tag conjugate, which allows for MALDI-based imaging of cancer based on its cell surface carbohydrate.

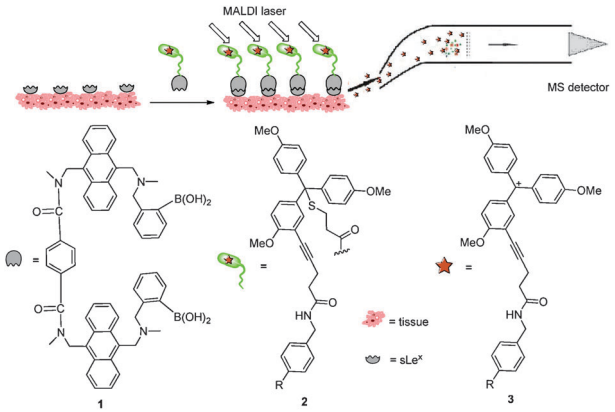
Biomarker-based histological work is known to save lives, help the formulation of therapeutic intervention strategies, and allow for improved prognosis.[1,2] Among all the known biomarkers, cancer cell surface carbohydrate antigens play a very important role, and most clinically measured cancer biomarkers are glycoproteins.[3] Cell surface carbohydrate structures as part of glycosylated proteins, peptides, and lipids are characteristic signatures of different cell types[4–8] and are associated with many forms of cancer.[9,10] For example, the sialyl Lewis X (sLe^x) antigen is being assessed in many cancers; serum sLe^x and the cytokeratin 19 fragment are said to be predictive factors for recurrence in patients with stage I non-small cell lung cancer;[11] and sLe^x plus CA 15.3 levels in serum of breast cancer patients were reported to be more effective than CA 15.3 plus CEA in diagnosing cancer.[12] Furthermore, the combination of sLe^x and sLe^a expression is known to be correlated with the adhesion of urothelial cancer cells to activated endothelium.[13] Detection of changes in the expression of these cell surface carbohydrates is clearly very important in cancer histological work.

In histological work, fluorescent and/or color staining agents are most commonly used. However, such approaches suffer from difficulties in multiplexing due to spectral resolution/overlap issues and in quantitation. A novel but maturing technology, MALDI imaging mass spectrometry (MALDI-IMS)[14,15] allows for direct examination of tissue biopsies without the need for micro-dissection and solubilization of tissue biomarkers prior to analysis, and ion desorption can be targeted to specific "points" in a grid pattern and the data rasterized. The resulting spectra can then be used to generate two-dimensional molecular maps of hundreds of biomolecules directly from the surface of a tissue section. These molecular maps display the relative abundance and spatial distribution of these molecules. MALDI tissue profiling has the power to link the molecular detail of mass spectrometry with molecular histology, generating mass spectra correlated to known locations within a thin tissue section.[15] We and others have recently demonstrated the potential of MALDI-IMS to clinical histopathology applications.[15–18]

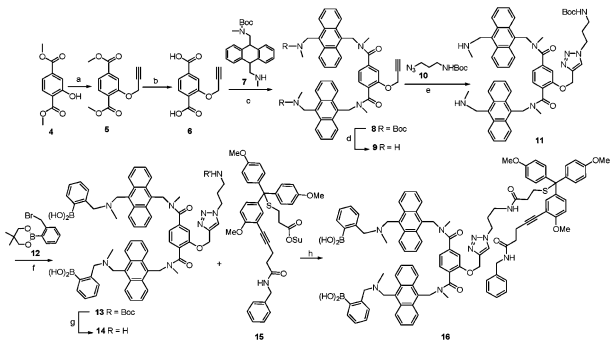
A recently developed variant of MALDI-IMS, termed Targeted Imaging Mass Spectrometry (TIMS) or TAMSIM for Targeted multiplex Mass Spectrometry Imaging, first described by Thiery *et al.*,[19] allows for the targeted analysis and spatial visualization of a molecule of interest directly from tissue sections by the use of laser-reactive photo-cleavable molecular tags attached to affinity molecules.[20,21] The bond conjugating the mass tag to the affinity molecule is photo-cleavable so that exposure to the UV laser in a MALDI mass spectrometer releases the tag without the need for matrix assistance. The released tag is readily detected. Changing the mass of the tags also allows for multiplexed detection of different molecules simultaneously within the same tissue, and sections prepared by standard methods (fixed or frozen) can be used for TIMS so that existing pathology workflows are the same.

We have had a long-standing interest in the development of boronic acid-based "receptors" (named boronolectins)[10] that can recognize carbohydrate biomarkers. In one study, a bisboronic acid sensor for sLe^x was developed.[22,23] We envisioned that conjugation of this boronolectin **1** with a trityl-based tag[24–29] would allow matrix-free MALDI-IMS analysis of cancer tissues with a high level of sLe^x (Scheme 1). Because the trityl cation is very stable, MALDI laser can ionize compounds similar to **2** by removing the thiol linkage to give carbocation **3**. Therefore, this boronolectin was conjugated to a trityl-based MS tag **15** for imaging applications (Scheme 2). Herein we describe the first example of conjugating a carbohydrate biomarker-targeting small molecule with a matrix-free MALDI mass spectrometric tag for MALDI-IMS work by tracking cancer cell surface sLe^x expression.

[a] *Department of Chemistry and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, Georgia 30302-4098, USA. E-mail: wang@gsu.edu; Tel: +1 404-413-5544; +1 404-413-5888*
[b] *Department of Microbiology and Molecular Cell Biology and The Leroy T. Canoles Jr. Cancer Research Center, Eastern Virginia Medical School, Norfolk, Virginia, 23507, USA. E-mail: drakerr@evms.edu*
[c] *Department of Medicinal Chemistry, School of Pharmacy, Fudan University, No.826, Zhangheng Rd, Shanghai, 201203, China*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc11814e



Scheme 1 The general concept of boronolectin–MS tag conjugation for imaging applications.



Scheme 2 (a) Propargyl bromide, K_2CO_3, CH_3CN, reflux 24 h, quantitative; (b) 2 N NaOH, CH_3OH, reflux 3 h, 84%; (c) CH_2Cl_2, EDCI, HOBT, DMAP, 88%; (d) TFA, CH_2Cl_2, 82%; (e) CuI, DIPEA, DMSO, 80 °C, microwave, 55%; (f) K_2CO_3, CH_3CN, 79%; (g) TFA, CH_2Cl_2, 73%; (h) Et_3N, DMF, CH_2Cl_2, 36%.

Scheme 2 shows a general flowchart for the synthesis of the boronolectin–MS tag conjugate. Specifically, compound **9** can be synthesized following procedures similar to the synthesis of the parent boronolectin without the side arm.[23] Subsequent Cu-mediated Huisgen cycloaddition[30–33] allowed for the installation of a side chain functional group for conjugation. We use click chemistry for side chain modification in order to allow for future diversity generation and conjugation to various molecules. The final compound (**16**) was obtained following alkylation and amidation reactions. The trityl tag was synthesized following similar literature procedures (see ESI† for details) and its MALDI MS in the absence of any matrix showed formation of an intense peak corresponding to the trityl tag (Fig. 1a, top panel).[24–29]

The boronolectin–trityl reporter conjugate (**16**) was then tested with fresh-frozen renal tissues containing both tumor and normal regions. The same peak for the trityl tag (Fig. 1a, bottom panel) was observed and monitored for imaging studies. In Fig. 1b, a picture of the renal tissue slide is shown, with immunostaining of the sLe^x (mouse monoclonal antibody, GenWay Biotech San Diego, CA) expressing regions coincident with the tumor area (dark stained bottom tip area; confirmed by a pathologist).

An adjacent tissue slice (7 μm) was placed on a conductive slide and incubated with the sLe^x–trityl probe in 100% methanol (2 ng μl^{-1}) in a humid chamber overnight at 4 °C. Slides were washed for 5 min in PBS followed by brief water wash to remove any unbound material. The slides were placed in a desiccator for 20 min and analyzed directly by MALDI-TOF (no matrix is added) in the reflectron mode using a laser raster width of 200 μm.

Only in the region of tumor with sLe^x expression was there binding of the sLe^x–trityl conjugate, as shown in red pixel intensities (Fig. 1b). These red pixels correlate to the MALDI peaks in the spectra shown in the Fig. 1a panel and the color intensity is related to peak abundance as shown in the expression scale. Peaks were obtained in the absence of any chemical matrix. Addition of external sLe^x clearly attenuated the ability of the conjugate to bind to the tissue (Fig. S1, ESI† section). This same probe also has shown the ability to bind to alcohol fixed renal tissues, as shown in Fig. 1b. This opens the possibility for probing tumor tissue microarrays prepared from alcohol or formalin fixed tissues. Thus, it is clear that MALDI-IMS results tracked with the immunostaining results and tumor tissue location, showing feasibility of using a boronolectin–MS tag conjugate for MALDI-IMS work. This represents the first

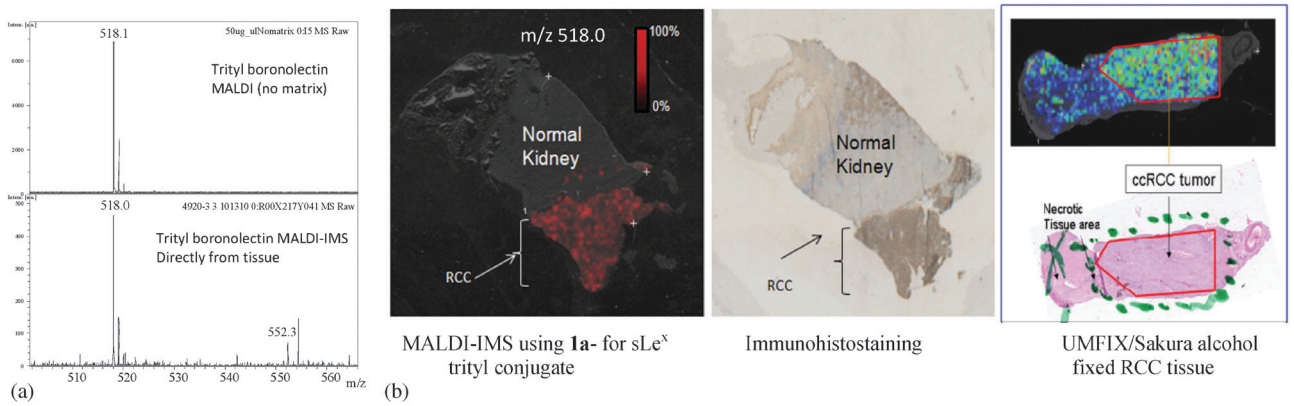


Fig. 1 (a) MALDI-IMS trityl peak obtained using frozen kidney tissue cut and stored at −80 °C. Other peaks track to the RCC (renal cell carcinomas). (b) MALDI-IMS (left) and immunostaining (middle) images of kidney tissue described in (a). A pathologist confirmed that immunostaining and MALDI-IMS-boronolectin signal results overlap in the tumor region, and not in normal cell areas. The third panel (right) shows boronolectin staining of a Sakura/UMFix alcohol fixed renal tumor tissue (top).

example of using small molecules for targeting carbohydrate-based cancer biomarkers in MALDI-IMS work.

In conclusion, using a sLe^x-recognizing boronolectin, we were able to develop a mass spectrometric probe for histological work on cancer tissues expressing the target carbohydrate using MALDI-IMS. Compared with protein-based targeting molecules, small molecule boronolectins have the advantage of excellent stability, easy storage, well-defined conjugation chemistry, and compatibility with organic solvents in sample preparation and tissue handling. The availability of additional boronolectins will allow for the development of a ''toolbox'' for MALDI-IMS of cancer based on carbohydrate biomarkers.

We gratefully acknowledge the financial support of this work by the National Institutes of Health (GM084933 (BW) and GM086925 (BW); CA137704/CA135087 (RRD); CA085067 (OJS)) and Department of Defense W81XWH-10-1-0136 (RRD).

Notes and references

1 B. N. Nathwani, S. J. Sasu, A. N. Ahsanuddin, A. M. Hernandez and M. R. Drachenberg, *Adv. Anat. Pathol.*, 2007, **14**, 375–400.
2 J. Teruya-Feldstein, *Arch. Pathol. Lab. Med.*, 2010, **134**, 1659–1665.
3 J. A. Ludwig and J. N. Weinstein, *Nat. Rev. Cancer*, 2005, **5**, 845–856.
4 *Lectins and Cancer*, ed. H.-J. Gabius and S. Gabius, Springer-Verlag, New York, 1991.
5 A. Danguy, I. Camby and R. Kiss, *Biochim. Biophys. Acta, Gen. Subj.*, 2002, **1572**, 285–293.
6 *Molecular Glycobiology*, ed. M. Fukuda and O. Hindsgaul, Oxford University Press, New York, 1994.
7 *Chemical Glycobiology*, ed. X. Chen, R. Halcomb and P. G. Wang, American Chemical Society, Washington, DC, 2008.
8 *Molecular and Cellular Glycobiology*, ed. M. Fukuda and O. Hindsgaul, Oxford University Press, 2000.
9 Y. Cheng, M. Li, S. Wang, H. Peng, S. Reid, N. Ni, H. Fang, W. Xu and B. Wang, *Sci. China Chem.*, 2010, **53**, 3–20.
10 S. Jin, Y. F. Cheng, S. Reid, M. Li and B. Wang, *Med. Res. Rev.*, 2010, **30**, 171–257, and references cited therein.
11 S. Mizuquchi, N. Nishiyama, T. Iwata, T. Nishida, N. Izumi, T. Tsukioka, K. Inoue and S. Suehiro, *Eur. J. Cancer Suppl.*, 2007, **5**, 374.
12 J. Kurebayashi, T. Nomura, M. Hirono, S. Okubo, K. Udagawa, S. Shiiki, M. Ikeda, K. Nakashima, K. Tanaka and H. Sonoo, *Jpn. J. Clin. Oncol.*, 2006, **36**, 150–153.
13 Y. Fujii, M. Yoshida, L. J. Chien, K. Kihara, Y. Kageyama, Y. Yasukochi and H. Oshima, *Urol. Int.*, 2000, **64**, 129–133.
14 P. Chaurand, M. Stoeckli and R. M. Caprioli, *Anal. Chem.*, 1999, **71**, 5263–5270.
15 K. Schwamborn and R. M. Caprioli, *Nat. Rev. Cancer*, 2010, **10**, 639–646.
16 J. A. Bauer, A. B. Chakravarthy, J. M. Rosenbluth, D. M. Mi, E. H. Seeley, N. D. Granja-Ingram, M. G. Olivares, M. C. Kelley, I. A. Mayer, I. M. Meszoely, J. A. Means-Powell, K. N. Johnson, C. J. Tsai, G. D. Ayers, M. E. Sanders, R. J. Schneider, S. C. Formenti, R. M. Caprioli and J. A. Pietenpol, *Clin. Cancer Res.*, 2010, **16**, 681–690.
17 L. H. Cazares, D. Troyer, S. Mendrinos, R. A. Lance, J. O. Nyalwidhe, H. A. Beydoun, M. A. Clements, R. R. Drake and O. J. Semmes, *Clin. Cancer Res.*, 2009, **15**, 5541–5551.
18 S. Rauser, C. Marquardt, B. Balluff, S. O. Deininger, C. Albers, E. Belau, R. Hartmer, D. Suckau, K. Specht, M. P. Ebert, M. Schmitt, M. Aubele, H. Hofler and A. Walch, *J. Proteome Res.*, 2010, **9**, 1854–1863.
19 G. Thiery, M. S. Shchepinov, E. M. Southern, A. Audebourg, V. Audard, B. Terris and I. G. Gut, *Rapid Commun. Mass Spectrom.*, 2007, **21**, 823–829.
20 G. Thiery, E. Anselmi, A. Audebourg, E. Darii, M. Abarbri, B. Terris, J. C. Tabet and I. G. Gut, *Proteomics*, 2008, **8**, 3725–3734.
21 R. Lemaire, J. Stauber, M. Wisztorski, C. Van Camp, A. Desmons, M. Deschamps, G. Proess, I. Rudlof, A. S. Woods, R. Day, M. Salzet and I. Fournier, *J. Proteome Res.*, 2007, **6**, 2057–2067.
22 W. Yang, S. Gao, X. Gao, V. R. Karnati, W. Ni, B. Wang, W. B. Hooks, J. Carson and B. Weston, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2175–2177.
23 W. Yang, H. Fan, S. Gao, X. Gao, W. Ni, V. Karnati, W. B. Hooks, J. Carson, B. Weston and B. Wang, *Chem. Biol.*, 2004, **11**, 439–448.
24 K. R. Birikh, P. L. Bernad, V. V. Shmanai, A. D. Malakhov, M. S. Shchepinov and V. A. Korshun, *Single Nucleotide Polymorphisms: Methods and Protocols*, in *Methods in Molecular Biology*, ed. J. Walker, Humana Press, New York, 2008, pp. 345–361.
25 K. R. Birikh, P. L. Bernad, V. V. Shmanai, A. D. Malakhov, M. S. Shchepinov and V. A. Korshun, *Methods Mol. Biol.*, 2009, **578**, 345–361.
26 K. R. Birikh, V. A. Korshun, P. L. Bernad, A. D. Malakhov, N. Milner, S. Khan, E. M. Southern and M. S. Shchepinov, *Anal. Chem.*, 2008, **80**, 2342–2350.
27 M. S. Shchepinov, R. Chalk and E. M. Southern, *Nucleic Acids Symp. Ser.*, 1999, 107–108.
28 A. V. Ustinov, V. V. Shmanai, K. Patel, I. A. Stepanova, I. A. Prokhorenko, I. V. Astakhova, A. D. Malakhov, M. V. Skorobogatyi, P. L. Bernad, S. Khan, M. Shahgholi, E. M. Southern, V. A. Korshun and M. S. Shchepinov, *Org. Biomol. Chem.*, 2008, **6**, 4593–4608.
29 I. G. Gut, *EP2163900*, 2010.
30 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
31 Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless and M. G. Finn, *J. Am. Chem. Soc.*, 2003, **125**, 3192–3193.
32 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
33 M. Meldal and C. W. Tornøe, *Chem. Rev.*, 2008, **108**, 2952–3015.

