ProCA1.GRPR: a new imaging agent in cancer detection

“Development of protein MRI contrast agent such as ProCA1.GRPR is expected to make a major leap toward preclinical and clinical applications...”

First draft submitted: 10 February 2016; **Accepted for publication:** 18 February 2016;
Published online: 13 April 2016

Keywords: bombesin • breast cancer • gastrin releasing peptide • MRI • molecular imaging
• prostate cancer

Precision molecular imaging addresses major medical gaps including the early detection of small lesion and biomarker expression, especially for high-risk patients, and monitoring the dynamic changes of biomarkers during disease progression and upon therapeutic treatment. One of the major challenges in achieving precision medicine is developing our capabilities to select patients with genetic and phenotypic heterogeneity while monitoring treatment responses. Current golden standard of histological analysis coupled with biopsy has many limitations, such as invasiveness, significant sampling errors especially for small lesions and risks of tumor peritoneal seeding [1]. Important avenues to achieve these goals include identifying biomarkers whose expression are capable of accurately reflect disease stages and treatment effects, and developing sensitive and noninvasive precision molecular imaging methodology for early responsive, predictive and prognostic biomarkers with increased levels of spatial and temporal resolution [2]. Here, we will first discuss GRPR as an attractive biomarker for molecular imaging of various types of cancers. We will then report our development of protein-based MRI contrast agent against GRPR with significantly improved sensitivity and spatial resolution.

GRPR is a G-protein coupled receptor belonging to the bombesin receptor family [3]. The nature ligand for GRPR is gastrin releasing peptide (GRP). The C-terminal

peptide sequence of GRP is highly similar to its amphibian counterpart, bombesin, a 14 amino acid peptide with high affinity to both GRPR and neuromedin B receptor. GRP and bombesin have strong affinity to GRPR at nanomolar range. The binding of these peptides to GRPR triggers downstream signaling cascades and activates a series of physiological and biological events, such as hormone release in endocrine organs [4].

GRPR is an attractive biomarker expressed on a series of human malignancies including prostate cancer, lung cancer, breast cancer, colon cancer, ovarian cancer, pancreatic cancer, gastrointestinal carcinoid tumor, head and neck cancer, various CNS/neural tumors, renal cell cancers and uterine cancer. As investigated by PCR, immunohistochemistry and radionuclide binding assay of clinic samples, GRPR is expressed in almost 100% on the prostate tumors. The expression level of GRPR in prostate cancer is significantly higher than that of other normal tissues. In addition, 33–72% of breast cancer, 40–50% of gastric cancer, 85% of carcinoids cancer and 29–85% of small cell lung cancer also have high GRPR expression [5].

GRPR is highly expressed in prostatic intraepithelial neoplasias, primary prostate cancer and invasive prostate carcinomas, whereas the expression level in normal prostate tissue and benign prostate hyperplasia are relatively low [6,7]. The distribution of GRPR in prostate cancer is heteroge-

Fan Pu[†]

Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA
and
Inlight Biosciences LLC, Marietta, GA 30068, USA

Shenghui Xue[†]

Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA

Jenny J Yang

Author for correspondence:
Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA
Tel.: +1 404 413 5520
Fax: +1 404 413 5551
jenny@gsu.edu

[†]Authors contributed equally

Future
Medicine part of

neous [8]. The expression level of GRPR is higher in well-differentiated carcinomas than that of poorly differentiated prostate carcinomas, suggesting that GRPR may be a biomarker for the early prostate carcinogenesis events, such as neoplastic transformation. GRPR is a biomarker to differentiate prostate neoplasia and prostate hyperplasia. Additionally, 57% of bone metastases from androgen-independent prostate cancers are GRPR-positive [7]. GRPR is also expressed in 85.7% of lymph node metastases of prostate cancer [9].

Lung cancer is the leading cause of cancer related death in both women and men in USA. A recent study shows that GRPR is expressed in 62.5% of non-small-cell lung carcinoma and 52.6% small cell lung carcinoma of patients. GRPR has low expression in clinical stage I and II and high expression in clinical stage III. There is a good correlation between the stronger GRPR staining and higher clinical stages, and such correlation is independent of tumor cell types [10].

High GRPR expression is found in the majority of the breast cancer cases. GRPR is heterogeneously expressed in neoplastic epithelial mammary cells, invasive carcinoma and ductal carcinomas *in situ*. GRPR is also expressed in lymph node metastases from breast cancer, whereas the GRPR expression level in normal lymph node tissue is very low. In addition, GRPR mRNA levels are correlated with smaller pathologic tumor size, positive estrogen receptor 1, progesterone receptor status and negative HER2 status [11,12].

Great efforts have been made toward the development of bombesin/GRP-based agents for the image and treatment of these diseases. Cytotoxic compounds were fused to bombesin analogues for the treatment of cancers in animal models. Additionally, several nonradioactive GRPR antagonists have been developed for the antiproliferation treatment of cancers. Moreover, radioactive compounds were conjugated to bombesin analogues for the imaging and treatment of cancers. Several of these compounds such as AMBA, [Leu12]-bombesin, RP527, BHZ3 and CB-TH2A-AR have been tested for the imaging of different types of cancers in patients using SPECT, PET and/or planar scintigraphy. However, these imaging methods have limitations for repeated applications to monitor disease progression and treatment effects due to their radiation. Achieving precision molecular imaging also requires higher resolution for histological correlation. Nevertheless, these studies and clinical trials clearly demonstrated that GRPR is a promising target for imaging and treatment of prostate cancers and other cancers expressing GRPR [8].

With high spatial and temporal resolution, no depth limitation, superior soft tissue penetration and relatively low costs, MRI became the leading imag-

ing technique for disease diagnosis. Beside the above advantages of MRI over SPECT, PET and planar scintigraphy, the MRI image collection process does not require the use of any radioactive isotopes. In addition, MRI is also capable of evaluating several physiological conditions of diseases, such as water perfusion, tissue temperature, blood vessel density and permeability, the concentration and distribution of various metabolites and tissue oxygen level. The combination of the information collected by MRI provides formative guidance for the disease diagnosis as well as image-guided interventions.

Because of these unique features, MRI combined with biomarker-targeted contrast agents is a promising approach for the noninvasive evaluation of the expression and distribution of disease biomarkers during clinical diagnosis and prognosis for the same patient. However, the reported literature on the molecular imaging of disease biomarkers using MRI is very limited despite its advantages over molecular imaging using PET, SPECT and planar scintigraphy. There are two major factors restricting the development of biomarker-targeted MRI contrast agents. First, the sensitivity of clinical MRI contrast agents is much lower than the theoretically predicted value. For example, the relaxivity of Gd-DTPA is around $4 \text{ mM}^{-1}\text{s}^{-1}$ at 1.4 T. In order to induce significant signal increase in MRI, a local gadolinium concentration of approximately 0.1 mM is requested for most clinical gadolinium-based MRI contrast agents [13–15]. Based on the fact that the expression level of biomarkers in diseases is in the nanomolar or picomolar level, more sensitive MRI contrast agents with high affinity biomarker binding moiety are needed to visualize biomarkers in MRI. Second, the imaging agents must have good blood vessel penetration in order to image disease biomarkers located outside of the blood vessel. Because of the high molecular weight and low blood vessel penetration, antibody is not the ideal targeting moiety for such application. On the other hand, the use of natural peptides as targeting moieties is limited by the intrinsic properties of the peptides, such as undefined structure, low solubility, short half-life and prone to protease cleavage [16–18].

To overcome these limitations in the development of GRPR-targeted MRI contrast agents, we fused full length of bombesin peptide in the position 52 of protein-based MRI contrast agents using grafting approach. Unlike clinical MRI contrast agents, the protein contrast agent was developed by creating a Gd^{3+} binding site into a scaffold protein. Flexible peptide linkers were introduced between targeting peptide and contrast agent allowing the peptide to maintain its native structure. This engineered protein is further

PEGylated to improve its biocompatibility [19]. This newly developed MRI contrast agent, named ProCA1.GRPR [20], maintains strong GRPR affinity with $K_d = 2.7$ nM. In addition, ProCA1.GRPR has about 10-times higher r_1 at 1.5 T than that of Gd-DTPA. Due to its small size compared with antibodies and nanoparticles, ProCA1.GRPR has strong blood vessel penetration in the xenografted tumors. Because of these unique features, ProCA1.GRPR can semiquantitatively evaluate GRPR expression levels in prostate and lung cancers in mice model. Consistent with histological staining of GRPR in mice tumor and reported clinical samples [8], ProCA1.GRPR is able to visualize the heterogeneous distribution of GRPR in tumor by MRI [20]. In addition, ProCA1.GRPR is capable of detecting GRPR in H441 tumors, which express 2×10^4 GRPR per cell. Development of protein MRI contrast agent such as ProCA1.GRPR is expected to make a major leap toward preclinical and clinical applications with the capability to quantify expression level and identify spatial locations of molecular biomarker GRPR of various types of cancer in the whole animal and human body by noninvasive MRI. It has strong potential to extend MRI application to early detection

of cancers, monitoring disease progression, evaluating treatment effects and facilitating image-guided local treatment with significantly improved sensitivity and accuracy.

Acknowledgements

The authors thank M Liu for her critical reviews of the manuscript.

Financial & competing interests disclosure

JJ Yang is the Principal Investigator for the biomarker-targeted ProCAs projects funded by NIH research grants (EB007268, GM62999, CA118113 and 1R41CA183376) and the Georgia Research Alliance VentureLab grant. F Pu and S Xue are participants of the biomarker-targeted ProCAs project founded by NIH research grants (EB007268, GM62999, CA118113 and 1R41CA183376) and the Georgia Research Alliance VentureLab grant. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- 1 Stigliano R, Burroughs AK. Should we biopsy each liver mass suspicious for HCC before liver transplantation? – no, please don't. *J. Hepatol.* 43(4), 563–568 (2005).
- 2 Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science* 339(6127), 1546–1558 (2013).
- 3 Spindel ER, Giladi E, Brehm P, Goodman RH, Segerson TP. Cloning and functional characterization of a complementary DNA encoding the murine fibroblast bombesin/gastrin-releasing peptide receptor. *Mol. Endocrinol.* 4(12), 1956–1963 (1990).
- 4 Gonzalez N, Moody TW, Igarashi H, Ito T, Jensen RT. Bombesin-related peptides and their receptors: recent advances in their role in physiology and disease states. *Curr. Opin. Endocrinol. Diabetes Obes.* 15(1), 58–64 (2008).
- 5 Patel O, Shulkes A, Baldwin GS. Gastrin-releasing peptide and cancer. *Biochim. Biophys. Acta* 1766(1), 23–41 (2006).
- 6 De Visser M, Van Weerden WM, De Ridder CM *et al.* Androgen-dependent expression of the gastrin-releasing peptide receptor in human prostate tumor xenografts. *J. Nucl. Med.* 48(1), 88–93 (2007).
- 7 Markwalder R, Reubi JC. Gastrin-releasing peptide receptors in the human prostate: relation to neoplastic transformation. *Cancer Res.* 59(5), 1152–1159 (1999).
- 8 Mansi R, Fleischmann A, Macke HR, Reubi JC. Targeting GRPR in urological cancers – from basic research to clinical application. *Nat. Rev. Urol.* 10(4), 235–244 (2013).
- 9 Ananias HJ, Van Den Heuvel MC, Helfrich W, De Jong IJ. Expression of the gastrin-releasing peptide receptor, the prostate stem cell antigen and the prostate-specific membrane antigen in lymph node and bone metastases of prostate cancer. *Prostate* 69(10), 1101–1108 (2009).
- 10 Mattei J, Achcar RD, Cano CH *et al.* Gastrin-releasing peptide receptor expression in lung cancer. *Arch. Pathol. Lab. Med.* 138(1), 98–104 (2014).
- 11 Gugger M, Reubi JC. Gastrin-releasing peptide receptors in non-neoplastic and neoplastic human breast. *Am. J. Pathol.* 155(6), 2067–2076 (1999).
- 12 Dalm SU, Sieuwerts AM, Look MP *et al.* Clinical relevance of targeting the gastrin-releasing peptide receptor, somatostatin receptor 2, or chemokine C-X-C motif receptor 4 in breast cancer for imaging and therapy. *J. Nucl. Med.* 56(10), 1487–1493 (2015).
- 13 Caravan P. Strategies for increasing the sensitivity of gadolinium based MRI contrast agents. *Chem. Soc. Rev.* 35(6), 512–523 (2006).
- 14 Xue S, Qiao J, Jiang J *et al.* Design of ProCAs (protein-based Gd(3+) MRI contrast agents) with high dose efficiency and capability for molecular imaging of cancer biomarkers. *Med. Res. Rev.* 34(5), 1070–1099 (2014).
- 15 Xue S, Yang H, Qiao J *et al.* Protein MRI contrast agent with unprecedented metal selectivity and sensitivity for liver cancer imaging. *Proc. Natl Acad. Sci. USA* 112(21), 6607–6612 (2015).
- 16 Xue S, Qiao J, Pu F, Cameron M, Yang JJ. Design of a novel class of protein-based magnetic resonance imaging contrast agents for the molecular imaging of cancer biomarkers. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 5(2), 163–179 (2013).

- 17 Qiao J, Xue S, Pu F *et al.* Molecular imaging of EGFR/HER2 cancer biomarkers by protein MRI contrast agents. *J. Biol. Inorg. Chem.* 19(2), 259–270 (2014).
- 18 Pu F, Xue S, Qiao J, Patel A, Yang JJ. Towards the molecular imaging of prostate cancer biomarkers using protein-based MRI contrast agents. *Curr. Protein Pept. Sci.* 17,1 (2016) (Epub ahead of print).
- 19 Li S, Jiang J, Zou J *et al.* PEGylation of protein-based MRI contrast agents improves relaxivities and biocompatibilities. *J. Inorg. Biochem.* 107(1), 111–118 (2012).
- 20 Pu F, Qiao J, Xue S *et al.* GRPR-targeted protein contrast agents for molecular imaging of receptor expression in cancers by MRI. *Sci. Rep.* 5, 16214 (2015).