From Bench to Bed

New Gastrin-Releasing Peptide Receptor-Directed Radioligands and Their Use in Prostate Cancer

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KEYWORDS

• Gastrin-releasing peptide receptor targeting • Prostate cancer • Preclinical design
• Clinical translation • Theranostics

KEY POINTS

- Gastrin-releasing peptide receptors (GRPRs) are overexpressed in prostate cancer and may serve as molecular targets for diagnosis and therapy with GRPR-directed radiolabeled peptide probes.
- The amphibian bombesin and the mammalian gastrin-releasing peptide have served as motifs for the development of GRPR-directed diagnostic and therapeutic radiolabeled analogs.
- A shift of paradigm from internalizing radiolabeled GRPR-agonists to GRPR-radioantagonists has occurred owing to the higher biosafety and superior pharmacokinetics of the latter.
- Peptide chain, spacer, and chelator play a critical role in the final performance of GRPR antagonists labeled with medically relevant radiometals in mice and in humans.
- Translational studies have revealed the diagnostic value of GRPR-radioantagonists in prostate cancer; their role versus PSMA-based agents needs to be accurately evaluated.

INTRODUCTION

The gastrin-releasing peptide receptor (GRPR) has been widely regarded as an attractive molecular target for tumor diagnosis and therapy with radiolabeled peptide analogs, owing to its upregulation in major human cancers.[1–3] Thus, high-density expression of GRPR has been documented in primary prostate cancer as opposed to lack of expression in surrounding healthy or hyperplastic prostate tissue, thereby offering the opportunity for diagnosis of early neoplastic events in the prostate.[4,5] In most cases, disease infiltrated to adjacent lymph nodes still retains a high GRPR expression, allowing follow-up of metastatic spread. In advanced prostate cancer, GRPR expression seems to decline, especially in osseous metastases[6]; however, more and more thorough clinical studies are needed to fully understand the molecular background affecting GRPR expression in advanced states of the disease.

On the other hand, standard imaging modalities, such as MRI, computed tomography (CT), and ultrasound, as well as conventional nuclear medicine techniques (eg, PET with fluorodeoxyglucose F-18 [^{18}F]FDG/PET]) have shown low specificity that compromises their diagnostic value in prostate cancer.[7] Currently, reliable diagnosis and staging of the disease relies almost exclusively on biopsy; yet, the high number of inconclusive biopsies, which are associated with much patient discomfort and anxiety, as well as with an increase in health care costs, demonstrates the urgent

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clinical need for a noninvasive and accurate molecular diagnostic tool in prostate cancer.[8]

For molecular diagnosis and radionuclide therapy of GRPR-positive cancer, a wide array of suitably modified peptide analogs have been developed over the past 2 decades. Initial studies have involved tracers based on the amphibian tetradecapeptide bombesin (BBN) and its C-terminal octapeptide and nonapeptide fragments retaining full affinity for the GRPR.[9–11] Coupling of a variety of chelators on the N-terminus of such BBN-peptides, either directly or via different-length and hydrophilicity spacers, has allowed for labeling with a wide range of radiometals, suitable for SPECT (^{99m}Tc, ^{111}In, ^{67}Ga) or PET (^{68}Ga, ^{64}Cu) imaging, as well as for radionuclide therapy (^{177}Lu, ^{90}Y, ^{213}Bi). Preclinical evaluation in prostate cancer cells and animal models have demonstrated the impact of peptide chain, linker, chelator, and radiometal applied on the pharmacologic and pharmacokinetic profiles of resulting radioligands and has revealed analogs of interest for translation in humans.

Unlike BBN, which binds with high affinity to both the GRPR and the neuromedin B receptor (NMBR), the mammalian gastrin-releasing peptide (GRP) and neuromedin C (**Fig. 1**) are GRPR-preferring.[12] Therefore, more selective GRP-based radioligands have recently been introduced for diagnosis of GRPR-expressing cancer, with the aim to reduce background activity by evading NMBR-binding sites expressed in physiologic tissues in addition to GRPRs.[13] On the other hand, a search toward pan-BBN radioligands has been pursued as well, based on the rationale that NMBRs and/or BBN subtype 3 receptors (BB_3R) coexpressed in cancer together with GRPRs would enhance the clinical indications of GRPR-preferring radioligands.[14] Interestingly, research has recently shifted toward GRPR-antagonist–based radiopeptides,[15] following the successful shift of paradigm in the field of somatostatin receptor subtype 2 (sst_2)-radiotracers from agonists to antagonists.[16] Radioantagonists have often displayed faster background clearance and higher tumor localization in animal models and in humans.[17,18] In the case of GRPR-radioantagonists, an extra benefit in their use is associated to their inherent higher biosafety for human intravenous administration.

Exhaustive research in all these fronts has facilitated accumulation of crucial structure-activity relationships data that have synergistically fostered the design of improved radioligands and the selection of candidates for clinical translation. Clinical proof-of-principle studies have established the validity of this approach in patients with prostate cancer and have contributed in our better understanding of biological and biochemical GRPR-related processes during propagation of the disease. More extended clinical studies are now needed to evaluate the diagnostic and potentially also therapeutic value of radiolabeled GRPR-directed peptide probes in early and advanced stages of prostate cancer.

RADIOPEPTIDES BASED ON BOMBESIN

As already mentioned, frog BBN and its C-terminus fragments retaining full binding affinity for the GRPR have served as motifs for the development of peptide radioligands able to specifically localize in cancer-associated GRPR-sites. The first analogs were produced by replacing Arg^3 by Lys^3 in the full BBN sequence and subsequent coupling of the diaminedithiolate group in its lateral primary amine via different linkers to enable ^{99m}Tc labeling (**Table 1**).[19] Owing to the high lipophilicity of the ^{99m}Tc chelate, however, the resulting ^{99m}Tc radiotracers displayed high hepatobiliary excretion and hence unfavorably high accumulation in the abdomen. Subsequent introduction of a “built-in” diethylenetriaminepentaacetic acid (DTPA) hydrophilic modifier that replaced N-terminal Pyr^1 resulted in impressive reduction of abdominal accumulation and good targeting of prostate cancer xenografts in mouse models.[20,21]

Following a similar trend, coupling of DTPA or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) on either Lys^3 or Pro^1 of full-length BBN analogs (see **Table 1**) allowed for labeling with ^{111}In.[22–24] Formation of potent and hydrophilic radiotracers with excellent tumor-to-background ratios in the abdomen was achieved. These analogs exhibited clear agonistic activity at the GRPR

BBN pGlu-Gln-Arg-Leu-**Gly-Asn**-Gln-**Trp-Ala-Val-Gly-His-Leu-Met**-NH_2

GRP 27mer Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-**Gly-Asn**-His-**Trp-Ala-Val-Gly-His-Leu-Met**-NH_2

NMC: GRP(18–27) **Gly-Asn**-His-**Trp-Ala-Val-Gly-His-Leu-Met-**NH_2

Fig. 1. Amino acid sequences of amphibian tetradecapeptide BBN showing high affinity for GRPR and NMBR. The native human 27mer GRP and its C-terminal decapeptide fragment NMC, both GRPR-preferring, are also shown underneath with amino acids preserved across species highlighted in red.