International Journal of Biochemistry and Cell Biology 114 (2019) 105567

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Review article

Bombesin receptors as potential targets for anticancer drug delivery and imaging

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ARTICLE INFO

Keywords:
Bombesin receptors
Bombesin peptide
Gastrin releasing peptide
Cancer
Targeting
Drug delivery
Imaging

ABSTRACT

The biggest challenge in delivering anticancer agents is the ability to direct these molecules specifically to cancer cells. With this in mind, modern research is focussing on improving the precision of cancer drug delivery by incorporating a ligand that has the ability to specifically recognize cancer cells. Peptides are emerging as a new tool in drug and gene delivery. Peptide-drug conjugates, peptide-modified drug delivery systems, and peptide-coupled imaging agents have been shown to increase on-site delivery. This has allowed better tumor mass contouring in imaging and increased therapeutic efficacy of chemotherapies, reducing adverse effects. Benefits of peptide ligands include their small size, easy and affordable production, high specificity and remarkable flexibility regarding their sequence and conjugation possibilities. Bombesin (Bn) receptors have shown great promise for tumor targeting due to their increased expression in a variety of human cancers, including prostate, breast, small cell lung, and pancreatic cells. This review discusses the overexpression of Bn receptors in different cancers and various approaches to target these receptors for therapeutic and diagnostic interventions in human malignancies.

1. Introduction

Cancer was responsible for an estimated 9.6 million deaths globally in 2018 and approximately 18.1 million new cases are diagnosed every year (Bray et al., 2018). The number of new cases is expected to rise by approximately 70% in the next two decades, making cancer the second leading cause of death globally. Cancer is characterized by uncontrolled division of cells and the ability of these cells to invade other tissues/organs from the initial site *via* the circulatory or lymphatic systems (metastasis), leading to the formation of tumor mass and vascularization (Jiang et al., 2015). Although angiogenesis is vital in growth and development, it is also a fundamental step in the transition of tumors from a dormant to a malignant state (Folkman, 1995). Chemotherapy is one of the major approaches to treat cancer, delivering a cytotoxic agent to the cancer cells. The main problem with conventional chemotherapy is its inability to deliver the correct amount of drug directly to cancer cells without affecting healthy cells. Precise targeting (location, time, and concentration) is a major goal of any drug delivery system and this becomes more important in the case of anticancer drugs, where site-specific delivery is essential.

Targeted anticancer drug delivery has emerged as a potential way of enhancing therapeutic efficacy and cancer visualization. Targeted delivery vehicles not only maximize and maintain a relatively high concentration of therapeutic agents at the tumor site but also minimize their presence in normal tissues (Rosenblum et al., 2018; Shi et al., 2011). Development of an effective and targeted drug delivery system for anticancer chemotherapeutics depends on the overexpression of a molecular target, such as a receptor, in malignant cells rather than healthy cells. A targeted drug delivery system (TDDS) composed of three components: a drug carrier, chemotherapeutic agents (drugs/genes) and a targeting ligand. The success of a TDDS depends on its selective binding to malignant cells, which is contingent on the binding affinity of the targeting ligand at a specific target receptor (Sawyers, 2004). For a receptor to be considered as a target for drug delivery, it

Abbreviations: Bn, Bombesin; CME, clathrin-mediated endocytosis; DTX, Docetaxel; DOX, Doxorubicin; EGFR, Epidermal growth factor receptors; GRP, Gastrin-releasing peptide; MRI, Magnetic resonance imaging; NMB, neuromedin B; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic) acid; PTX, Paclitaxel; SPECT, single-photon emission computed tomography; SPION, Superparamagnetic iron oxide nanoparticles; TDDS, Targeted drug delivery system

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https://doi.org/10.1016/j.biocel.2019.105567
Received 1 February 2019; Received in revised form 2 July 2019; Accepted 5 July 2019
Available online 08 July 2019
1357-2725/ © 2019 Published by Elsevier Ltd.

Table 1
Different types of Bn receptors, their agonists, physiological role and overexpression in cancer.

Bombesin Receptor	Agonist	Physiological role	Overexpression in cancer	References
BB1 (NMB)	Neuromedin B	Urogenital and gastrointestinal smooth muscle contraction	Small cell lung, non-small cell lung, colon, pancreas, prostate, breast, and ovarian cancers	(Matusiak et al., 2005; Moody et al., 1995; Reubi et al., 2002)
BB2 (GRP)	Bombesin GRP	Stimulation of acid secretion, pancreatic exocrine and enteric peptide hormone secretion, gut motility, stimulation of immune response	Breast, prostate, lung, colon, glioblastoma, neuroblastoma, head, gastrointestinal, pancreatic, and neck squamous cell cancers	(Fleischmann et al., 2005; Gugger and Reubi, 1999; Jensen et al., 2008; Markwalder and Reubi, 1999; Moody et al., 2004; Reubi et al., 2004; Uri et al., 2018)
BB3	MK-50946 and Bantag-1	Regulation of blood glucose level, energy balance, weight control	Testis, lung, neuroendocrine, pancreas, pituitary, ovarian, and prostate cancers	(Moreno et al., 2013; Reubi et al., 2002)

should meet two basic criteria: first, it should be overexpressed on malignant rather than healthy cells, and second, it ideally needs to be specifically expressed on the target cells (Fernandes et al., 2019; Ziegler et al., 2014). Generally, threefold overexpression of a receptor on cancer cells compared with healthy cells is sufficient for significantly improved delivery of drugs to malignant cells (Srinivasarao et al., 2015). Peptide receptors act as important targets for imaging and targeted anticancer chemotherapy. Discovery of several peptide receptors and tumor-related peptides/proteins is expected to create a new wave of more effective and selective treatment for cancer (Aina et al., 2002; Enbäck and Laakkonen, 2007; Vlieghe et al., 2010).

Proteins and monoclonal antibodies are also used in cancer treatment; however, they are limited by poor delivery to tumors due to their large size and dose-limiting toxicity to the liver and bone marrow due to nonspecific uptake into the reticuloendothelial system. In contrast, peptides possess many advantages, such as small size, ease of synthesis and modification, tumor penetrating ability, and good biocompatibility. Furthermore, peptides play an important role in cancer, including early diagnosis, prognostic prediction, and the treatment of cancer patients. Commonly explored target receptors for anticancer drug delivery include folate, transferrin, sigma, epidermal growth factor and lectin. Recently, bombesin (Bn) receptors have been identified to be overexpressed in malignant cancer cells and their use as a targeted drug delivery strategy tested (Moody et al., 2018; Moreno et al., 2016). The focus of this review is Bn receptors and their role in cancer, and highlights the targeted approaches developed for imaging and delivery of anticancer drugs through Bn receptors.

2. Bombesin receptors and overexpression in cancer

Bn, an active tetradecapeptide, was initially isolated from the skin of two European aquatic toads of the family Bombinatoridae, *Bombina bombina,* and *Bombina variegata* (Anastasi et al., 1971). Bn is a small regulatory peptide with high cellular permeability and biocompatibility. As a result, Bn is more attractive as a targeting ligand than others such as folic acid, β-hydroxybutyric acid, biomolecules, or galactose. Due to its small size, Bn does not affect the size of the nanocarrier and can be incorporated at a high surface density (number of peptides per nanoparticle). However, peptides are usually hydrophilic in nature and cannot cross the blood-brain barrier (less than 0.1% of total injected peptide). Thus, the properties of Bn are beneficial when peripheral tumors are the desired targets (Reubi, 2003; Wang and Thanou, 2010).

Bn receptors are G protein-coupled receptors, consisting of three mammalian subtypes; BB1, BB2, and BB3 (Alexander et al., 2017; Ramos-Álvarez et al., 2015). The BB1 subtype is also known as the neuromedin B receptor (NMBR). The mammalian ligand neuromedin B (NMB) is a decapeptide involved in various physiological processes, including immune defence, thyroid and adrenocortical function, deglutition, weight regulation and cognition (Matusiak et al., 2005). NMB is also overexpressed in several neoplasms, such as lung, pancreas, colon, and carcinoids (bronchial, intestinal) (Reubi et al., 2002).

The BB2 subtype is also known as the gastrin-releasing peptide receptor (GRPR), because the C-terminal region of Bn is homologous to the mammalian gastrin-releasing peptide (GRP) (Jensen et al., 2008). GRP is a modulatory neurotransmitter that also acts as an endocrine cancer cell-growth factor for normal tissues and neoplastic cells of various origins; it stimulates several physiological processes including exocrine secretion and smooth muscle contraction and has trophic effects (Fleischmann et al., 2005; Varvarigou et al., 2004).

BB1 has more than 100-fold higher affinity towards NMB than GRP, whereas the BB2 receptor has approximately 50-fold higher affinity for GRP than NMB. Bombesin receptors, especially BB2, have been extensively studied and found to be overexpressed in several human cancers, including breast, colon, lung (NCI-H1299 human non-small cell lung cancer), central nervous system (gliomas, meningiomas),

Table 2
Expression levels of BB2 or gastrin-releasing peptide receptors in various cancers.

Cancer type	Expression of BB2 or GRP receptors	References
Lung	85–100% (Small cell lung cancer) 74–78% (Non-small cell lung cancer)	(Willey et al., 1984)
Prostate	60–100%	(Markwalder and Reubi, 1999)
Pancreatic	75%	(Montet et al., 2006)
Breast	40–70%	(Gugger and Reubi, 1999; Miyazaki et al., 1998)
Neuroblastoma	2%	(Bostwick and Bensch, 2018)

head/neck squamous cell, ovarian, pancreatic, and prostate cancers, and neuroblastomas (Moody et al., 2004; Moreno et al., 2016; Pu et al., 2015) (Tables 1 and 2). Bn receptor subtype 3 (BB3/BRS3) is an orphan G protein-coupled receptor that has 47–51% homology to BB1/BB2. Due to few selective ligands, the essential role of BB3 is largely unknown (Moreno et al., 2013). However, [D-Tyr^{6}, Ala^{11}, Phe^{13}, Nle^{14}] bombesin(6–14) is a potent ligand with high affinity for BB3 (Mantey et al., 1997; Pradhan et al., 1998). BB3 is overexpressed in neuroendocrine, lung, pancreas, pituitary, ovary, and prostate tumors (Reubi et al., 2002; Schulz et al., 2006).

3. Role of Bn receptors and peptides in cancer

Bn is structurally similar to human GRP, thus, several analogues of Bn have been synthesized to target Bn receptors in humans for imaging, diagnosis, and treatment of cancer (Fig. 1). These analogues have shown more metabolic stability than GRP/NMB (Uehara et al., 2011).

Bn peptides and their mRNAs are found in many tumors and act as autocrine growth factors that stimulate tumor growth through specific receptors (Bologna et al., 1989; Cuttitta et al., 1985; Yano et al., 1992). These peptides have also been implicated in neo-angiogenesis in several cancer models (Bajo et al., 2004; Heuser et al., 2005; Levine et al., 2003). This suggests that the corresponding receptor is systematically expressed in tumor cells as well as in tumor vascular beds and plays an important biological function.

Recent studies have demonstrated that activation of GRPR, NMBR or Bn-like receptor 3 (BRS-3) is frequently mediated by transactivation of tumor human epidermal growth factor receptors (EGFR) (Moody et al., 2016; Moreno et al., 2016). This signaling pathway frequently requires activation of the proto-oncogene tyrosine protein kinase (Src), the action of PKCs, stimulation of reactive oxygen species, and stimulation of matrix metalloproteinases, with the generation of EGFR ligands. This interaction could lead to the development of a novel therapeutic approach, using a combination of a Bn receptor antagonist and an EGFR inhibitor to combat tumor growth.

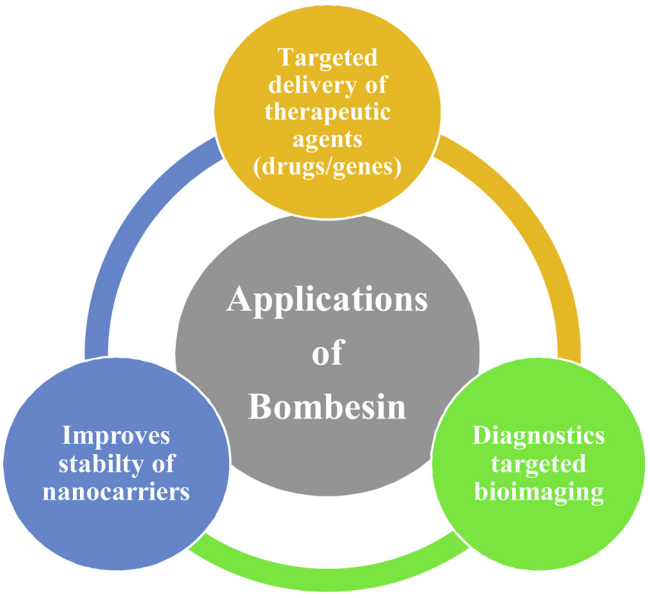

Fig. 1. Various applications of bombesin peptide.

4. Gastrin-releasing peptide receptor targeting for drug delivery

4.1. Drug-peptide conjugates

Peptide receptors have been used for targeted chemotherapy, whereby specific peptides are used as cognate ligands that are conjugated to cytotoxic drugs. These peptide-drug conjugates can target cancer cells that possess specific membrane receptors *via* ligand-receptor interactions (Fig. 2A).

Consequently, receptor selectivity enhances the efficacy of drugs against cancer cells. Similarly, paclitaxel (PTX), a taxane-derived anticancer drug, was conjugated to Bn peptide using a polyethylene glycol (PEG) cross-linker to improve drug solubility and targeted delivery. Safavy et al. (2006) proposed a multi-ligand approach and synthesized a model scorpion conjugate with two peptide claws and a PTX tail, to target Bn receptors. To enhance solubility, PEG derivatives of this conjugate were prepared by insertion into either the claw or tail regions. The conjugate showed superior cytotoxic activity in several GRP receptor-positive human cancer cell lines compared with free PTX (Safavy et al., 2006). These results demonstrated the potential of a multi-ligand approach in the design of receptor-targeting conjugates for tumor-specific drug delivery.

Nagy et al. (1997) synthesized a cytotoxic Bn-drug conjugate using highly active somatostatin hybrids containing doxorubicin (DOX). The cytotoxic Bn-drug conjugate AN-215 was prepared by linking the N-terminal of the Bn antagonist RC-23094 (Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2-NH)-Leu-NH_2) *via* a glutamic acid spacer to the 14 − OH group of DOX AN-201. This Bn-drug conjugate targeted Bn/GRP receptors and showed anti-tumor efficacy in various human cancer cells (Nagy et al., 1997). Subsequently, the efficacy of AN-215 was evaluated against human ovarian cancer cell (ES-2, SKOV-3, OV-1063, and UCI-107) xenografted nude mice (Engel et al., 2005). AN-215 significantly ($P < 0.05$) inhibited the growth of ES-2, OV-1063, and UCI-107 tumors. Furthermore, AN-215 did not downregulate the receptors, suggesting that it could also be useful for subsequent or repeated treatment.

The effect of the cytotoxic bombesin-drug AN-215 was evaluated in human ovarian cancer cells (UCI-107, ES-2, and OV-106), MX-1 human breast cancer cells, and HEC-1A, RL-95-2, and AN3CA human endometrial cancer cells. For all cancer cell lines, AN-215 significantly inhibited the growth of these tumors and prolonged the survival of nude mice bearing tumor xenografts (Engel et al., 2005). In another study, the actions of AN-215 were tested on U87MG human glioblastomas expressing BB1 and BB2 (Szereday et al., 2002). Treatment of nude mice bearing U87MG human glioblastomas with AN-215 significantly extended the tumor doubling time from 4.5 days to 8.2 days and significantly inhibited tumor growth (65% decrease in weight and 70% decrease in volume) compared with controls. Furthermore, AN-215 inhibited the growth of tumors from the renal cancer cell lines, ACHN, 786-0, and A-498 (Keller et al., 2005).

These drug conjugates may provide potential strategies for the

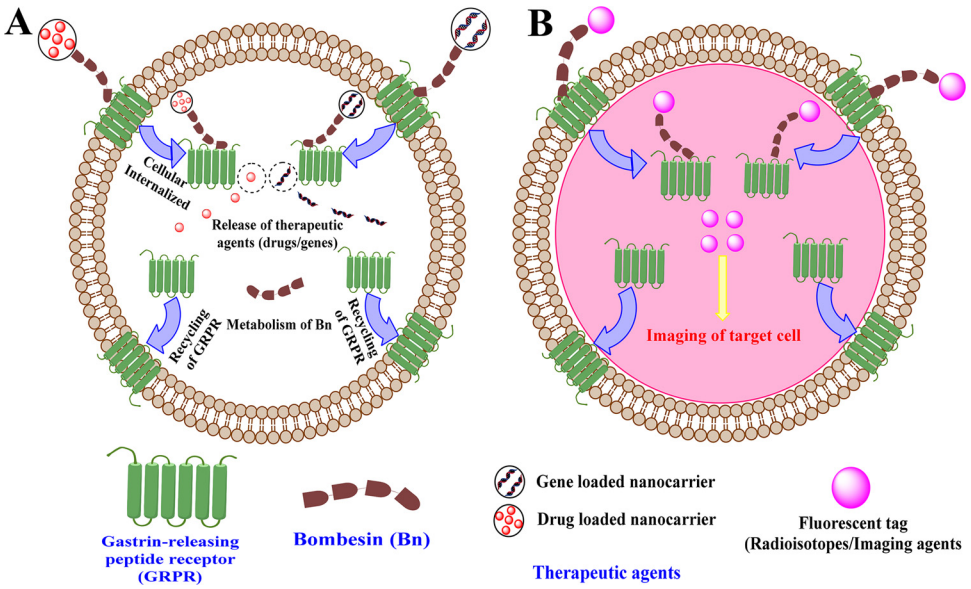

Fig. 2. Targeting of gastrin-releasing peptide receptor (GRPR): (A) Enhanced delivery of agents (drugs/genes) to improve their therapeutic effects (B) Diagnosis of tumors using fluorescently tagged (radioisotope/imaging agents) bombesin peptide.

treatment of tumors overexpressing Bn/GRP receptors. Yang et al. (2013) synthesized chimeric peptide B28Bn(6–14) by conjugating a Bn analogue, Bn(6–14), with the mitochondrial-disrupting peptide B28 to enhance the selectivity and toxicity of B28 against Bn receptor-overexpressing prostate cancer cells. Bn-conjugated B28 peptide was ten times more cytotoxic, with specific accumulation in mitochondria, than unconjugated B28 (Yang et al., 2013). Interestingly, Cescato et al. (2008) evaluated the *in vitro* and *in vivo* targeting potentials of the GRP receptor antagonist, Demobesin 4, in comparison with an agonist, Demobesin 1. The results demonstrated that GRP receptor antagonists could be better targeting agents than GRP receptor agonists (Cescato et al., 2008).

4.2. Bn-mediated targeting of drug-loaded nanoparticles

Active targeting of anticancer drugs to cancer cells and tissues is a promising field due to its potential to spare healthy cells and tissues, decrease toxicity, and improve the therapeutic effect by increasing intracellular drug concentrations in cancer cells. In order to enhance targeting, a high-affinity ligand that binds selectively at a receptor on cancer cells is attached to the surface of a nanocarrier. Many ligands have been used to modify the surface of nanoparticulate systems, including peptides such as bombesin, to target cancer cells. Accardo et al. (2012) prepared a liposome-based nanocarrier with an amphiphilic peptide derivative that contained the Bn(7–14) peptide and a chelating agent for high loading and targeted delivery of DOX to PC-3 human prostate cancer cells. Bn-conjugated liposomes showed specific binding to PC-3 cells compared to liposomes without the Bn peptide (Accardo et al., 2012).

To improve the binding properties and serum stability of liposomal nanocarriers, Bn-AA1 (a new sequence of Bn peptide) was developed. It demonstrated a significantly longer half-life compared with a Bn-conjugated liposome (Accardo et al., 2013). In a study carried out by Wang et al. (2016), DOX-loaded, Bn-conjugated solid lipid nanoparticles (Bn-DOX/SLN) showed excellent *in vitro* cytotoxicity and *in vivo* anti-tumor effects, in both multidrug-resistant MCF-7 human breast cancer cells and a breast cancer animal model. Bn-DOX/SLN even reversed the resistance to DOX, suggesting that chemotherapy using this example of a targeted nanocarrier may be of benefit in the treatment of multidrug-resistant human breast cancer (Wang et al., 2016).

Suresh et al. (2014) synthesized Bn-conjugated gold nanocages (AuNC-Bn) and validated the internalization of AuNC-Bn in GRP receptor-expressing human cancer cells using a radiolabelled competitive cell binding assay. The results showed that AuNC-Bn uptake in PC-3 cells was mediated by clathrin-mediated endocytosis (CME). Indeed, in the presence of CME inhibitors, AuNC-Bn uptake in cells was reduced up to 84%. Transmission electron microscope images further confirmed CME characteristics, *i.e.*, clathrin-coated pits and lysosomal release of AuNC. These results demonstrated that Bn conjugated to the surface of nanoparticles maintained target specificity. This strengthens the case for peptide robustness and their persisting functionality in intracellular vehicular delivery systems (Suresh et al., 2014).

To improve efficacy and selectivity towards cancer cells, drug-loaded nanoparticles can also be functionalized with two targeting ligands. Gold nanoparticles were conjugated with a Bn analogue (targeting peptide) and an RAF peptide analogue (a drug peptide ligand that inhibits Rb-Raf-1 binding *in vivo*) and then evaluated for anti-tumor activity against GRP-positive HeLa cells and GRP-negative neuroblastoma SH-SY5Y cells. The dual peptide functionalized nanoparticles had greater cytotoxicity and cellular selectivity than nanoparticles functionalized with either one of the peptides (see Fig. 3d, Hosta-Rigau et al., 2010).

Our research group has studied the potential of Bn-conjugated polymeric nanoparticles for the delivery of anticancer drugs to GRPR-overexpressing human cancer cells (Kulhari et al., 2014a). Docetaxel (DTX), an FDA-approved anticancer drug, was loaded in poly(lactic-co-glycolic) acid (PLGA) nanoparticles and Bn was conjugated to the surface of the drug-loaded nanoparticles. Bn-conjugated, DTX-loaded nanoparticles induced 12 times more cytotoxicity to Bn receptor-overexpressing MDA-MB-231 human breast cancer cells than DTX alone (Kulhari et al., 2014a). Similarly, these targeted nanoparticles induced 3.1 and 2.7 times more cytotoxicity to DU145 and PC-3 human prostate cancer cells, respectively (Kulhari et al., 2014a). Interestingly, we observed that Bn not only provided targeted delivery of the drug-loaded nanoparticles and increased the concentration of the drug in cancer cells but it also enhanced the colloidal stability of nanoparticles in various physiological media, such as phosphate buffered saline and serum, in comparison with unconjugated nanoparticles. Further, Bn-conjugated PLGA nanoparticles were stable in 0.7 M sodium sulphate salt medium (Kulhari et al., 2014b). Recently, we also demonstrated the ability of Bn-conjugated solid lipid nanoparticles to deliver natural drugs such as epigallocatechin gallate (EGCG). The Bn-conjugated

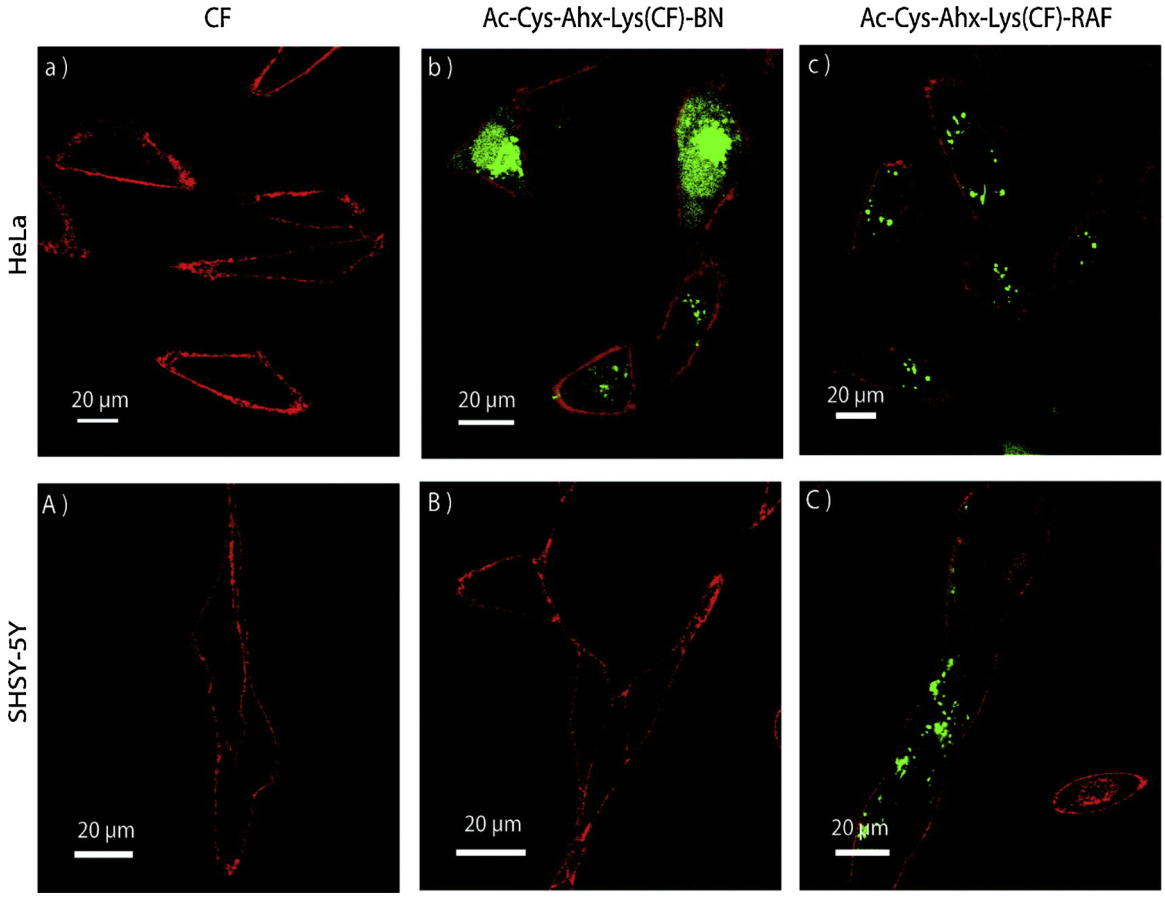

Fig. 3. Localization of carboxyfluoresceinated (CF) peptides (green) in HeLa cells and SH-SY5Y cells incubated for 24 h. Control CF (a, A), Ac-Cys-Ahx-Lys(CF)-BN (b, B), Ac-Cys-Ahx-Lys(CF)-RAF (c, C). Membranes (red) were stained with a fluorescent marker (wheat germ agglutinin). The peptides were added at a final concentration of 1×10^{-5} M. This figure is reprinted (adapted) with permission from Hosta-Rigau et al., 2010. Copyright (2010) American Chemical Society (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

nanoparticles showed targeting of EGCG to GRP receptor-overexpressing human breast cancer cells and B16F10 mouse melanoma cells (Radhakrishnan et al., 2019). Moreover, targeted nanoparticle-treated, tumor-bearing mice had greater survival than mice treated with non-targeted nanoparticles (Table 3).

5. Gastrin-releasing peptide receptor targeting for gene delivery

RNA interference (RNAi) has emerged as a potential strategy for treating human diseases (Kim and Rossi, 2007). This natural and potent mechanism down-regulates the expression of a specific gene in diseased cells (Fig. 2A). Most RNAi therapeutics are anionic macromolecules that have a short plasma half-life. Given the narrow therapeutic index and low cellular uptake, conventional delivery of RNAi therapeutics results in significant side- and off-target effects. Therefore, similar to anticancer drugs, RNAi needs to be delivered specifically and selectively to diseased cells. Wang et al. (2009) conjugated Bn to pH-sensitive, polymeric nanoparticles for the systemic and specific delivery of anti-hypoxia inducible factor-1R small interfering RNA (siRNA). Following intravenous administration, Bn-conjugated nanoparticles resulted in significant tumor growth inhibition in nude mice bearing human glioma U87 xenografts, with greater silencing of HIF-1R protein expression than free siRNA or non-targeted nanoparticles (Wang et al., 2009).

Hong et al. (1999) evaluated the efficiency of human adenovirus serotype 5 (Ad5) transgene delivery using a bimodular oligonucleotide (MH20) with a GRP receptor binding domain. They also evaluated the effect of the presence of a GRP-binding domain on the N-terminal or C-terminal side. One peptide (GRP-MH20) had the GRP domain on the N-terminal side of MH20, whereas the other (MH20-GRP) had the C-terminally amidified GRP on the C-terminal side of MH20. Only the GRP-MH20 peptide, not MH20-GRP, enhanced luciferase gene delivery to adenovirus-susceptible cells (Hong et al., 1999). Their results also showed that GRP-MH20-mediated enhancement of Ad5 gene transfer efficiency depends on the number of GRP receptors expressed on the cell surface and that the enhancing effect was inhibited in the presence of excess GRP peptide, indicating uptake of the delivery system through GRP receptors (Hong et al., 1999).

6. Gastrin-releasing peptide receptor targeting for diagnosis and imaging

Receptors for neuropeptides are overexpressed in different cancers (Moody et al., 2018). These receptors are not only implicated in carcinogenesis but have also been evaluated for developing novel diagnostic and therapeutic modalities for some cancers. *In vivo* receptor scintigraphy using radiolabelled somatostatin analogs has been established in the clinic as a diagnostic method to detect neuroendocrine tumors (Fig. 2B). Similar targeting methods are clinically needed for more common tumors. Therefore, evaluation of the overexpression of other peptide receptors in various cancers has gained increasing interest. Bn receptors are overexpressed in several types of frequently occurring cancers, qualifying these tumors as potential candidates for targeting approaches (Moreno et al., 2016). Due to the high frequency of overexpression of Bn receptors in many common tumors, reports using these receptors to image/target these tumors have markedly

Table 3
Bombesin-conjugated nanoparticles for drug delivery.

Nanocarrier	Drug	Cancer targeted	Comments	References
Solid lipid nanoparticles	Doxorubicin	Breast	*In vivo* tumor inhibition rate was increased to 81% from 14% after conjugation with Bn	(Wang et al., 2016)
PLGA nanoparticles	Docetaxel	Breast, Prostate	Bn-conjugated nanoparticles induced higher cytotoxicity, inhibited cell migration and colony formation compared with non-targeted nanoparticles or free DTX alone	(Kulhari et al., 2015, 2014a)
Lipid-polymer hybrid	Cabazitaxel	Prostate	The growth of LNCap cells (prostate cancer cells) *in vitro* was completely inhibited	(Chen et al., 2016)
Liposomes	Doxorubicin	Prostate	Conjugated liposomes showed increased binding, serum stability, and tumor growth inhibition	(Accardo et al., 2012, 2013)
Nanostructured lipid carriers (NLCs)	Doxorubicin	Lung	Bn-decorated NLCs promoted more stable and remarkably higher transfection efficiency and better anti-tumor efficacy than pre-bombesin decorated NLCs *in vivo* and *in vitro*	(Du and Li, 2016)
AuNPs	–	Prostate	Bn functionalized PEG-coated AuNPs showed high affinity binding to four subtypes of GPR	(Simpson et al., 2017)
AuNPs	–	HeLa cells (GRP positive) and neuroblastoma SH-SY5Y cells (GRP negative)	The dual peptide functionalized nanoparticles had increased cytotoxicity and cellular selectivity than nanoparticles functionalized with either one of the peptides	(Hosta-Rigau et al., 2010)
AuNC-Bn (gold nanocube Bn conjugate)	–	PC-3 cells	AuNC-Bn uptake in PC-3 cells was mediated by CME	(Suresh et al., 2014)
Solid lipid nanoparticles	Epigallocatechin gallate	Breast	Improved cytotoxicity of encapsulated drug	(Radhakrishnan et al., 2019)

increased in number.

Many synthetic Bn analogues have been developed by coupling with various radioisotopes such as ^{99m}Tc, ^{111}In, ^{125}I, $^{185/187}$Re, ^{18}F, ^{64}Cu, ^{67}Ga, ^{68}Ga, ^{90}Y and ^{177}Lu. ^{67}Ga is a radionuclide that emits γ radiation and is suitable for *in vivo* imaging by single-photon emission computed tomography (SPECT). Furthermore, its congener: ^{68}Ga, is an emerging positron emitter with increasing use in clinically relevant techniques such as positron emission tomography (PET) (Banerjee and Pomper, 2013). ^{99m}Tc-labelled Bn was shown to be taken up by Bn receptor-overexpressing cancer cell lines in *in vitro* studies, as well as by *in vivo* tumors, and is therefore applicable for imaging and scintigraphy detection of various cancers (Varvarigou et al., 2004). Similarly, ^{99m}Tc-labelled Bn was used as an imaging probe for detection/diagnosis of Capan-1 pancreatic adenocarcinoma in nude mice (Carlesso et al., 2015). The uptake of the conjugate was four-fold higher in pancreatic tumor cells compared to other tissues. Another study was carried out using Lys^3-bombesin-conjugated radiolabelled gold nanoparticles for radiotherapy and thermal ablation in prostate cancer (Jiménez-Mancilla et al., 2013). Two peptides, Lys^3-Bn peptide and Tat (a cell penetrating peptide), were conjugated to radiolabelled ^{99m}Tc/^{177}Lu-labelled gold nanoparticles. After laser irradiation, this multifunctional system caused an increase in the temperature up to 46.4 °C, which resulted in a significant decrease in viability of PC-3 cells. The nanoparticles exhibited properties suitable for plasmonic photothermal therapy and targeted radiotherapy for treatment of prostate cancer.

Accardo et al. (2010) prepared supramolecular aggregates by combining two different amphiphilic molecules. One was obtained from Bn peptide and the other was 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelating agent ((C18)$_2$DOTA) complexes with the radioactive ^{111}In(III) isotope. The Bn peptide was linked with a lipophilic moiety *via* a short linker containing five units of dioxoethylene or a long linker containing Peg3000. *In vitro* and *in vivo* studies showed that the short spacer-linked conjugate had higher affinity for the overexpressed Bn receptor binding site compared with the longer chain spacer (Accardo et al., 2010). ^{111}In-labelled Bn conjugates, comprising 2-nitroimidazole as a pharmacophore, was also used for imaging of PC-3 human prostate cancer cells (Wagh et al., 2012). Bn antagonist (PEG4-D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH$_2$)-based radioligands have also been constructed and evaluated for SPECT and PET nuclear imaging in PC-3 tumor-bearing nude mice (Abiraj et al., 2011). Zhang et al. (2017) first showed the clinical diagnostic efficacy of the ^{68}Ga-labelled heterodimeric peptide, Bn-RGD. This heterodimeric peptide was composed of two peptides, Bn and RGD (Arginylglycylaspartic acid), for targeting GRPR and integrin $\alpha_v\beta_3$ receptors, respectively (Zhang et al., 2017). RGD is a tripeptide that binds to the $\alpha_v\beta_3$ integrin receptor, which is also overexpressed in various cancer cells. To investigate the transport mechanism of Bn peptide-conjugated nanocarriers to overexpressed receptors, *in vivo* studies were carried out on human prostate tumor-bearing mice using ^{67}Ga-radiolabelled DOTA-coated AuNPs functionalized with the Bn peptide (Silva et al., 2016). *In vitro* studies using Bn-peptide-conjugated TDOTA (trimethyl 2,2′,2″-(10-2(3-(tritylthio)-propamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-trityl)-triacetate)-coated AuNPs showed higher cellular uptake of AuNPs in a GRPR overexpressing tumor cell line than unconjugated nanoparticles. However, these *in vitro* results could not be replicated in *in vivo* tumor uptake studies (Silva et al., 2016).

In addition to coupling with radioisotopes for tumor localization, Bn receptor ligands have also been coupled to other compounds for a wide range of applications. Examples include conjugation with fluorescent probes for optical imaging (Chen et al., 2014; Ma et al., 2007), gold nanoparticles coated with dendrimers for optical and nuclear imaging (Mendoza-Nava et al., 2016), gold nanorods coated with PEG for photoacoustical imaging (Heidari et al., 2014), and superparamagnetic iron oxide nanoparticles (SPIONs) for magnetic resonance imaging (MRI) (Moreno et al., 2016). Chanda et al. (2010) used a radiolabelled gold nanoparticle-Bn conjugate for the imaging of prostate cancer cells and

to study the Bn-mediated biodistribution of the nanocarrier. Gold nanoparticle-Bn conjugates accumulated to significantly higher levels in tumors than unconjugated gold nanoparticles. In addition, Mendoza-Sánchez et al. (2010) used ^{99m}Tc-labelled gold nanoparticles conjugated with Lys^3-bombesin for the imaging of GRP receptors *in vivo* in athymic mice with PC-3 tumors.

Recently, SPIONs have emerged as contrast agents for MRI. SPIONs can enhance the alteration of proton relaxation in tissue microenvironments, making them suitable for use as T_2 contrast agents in MRI. However, the clearance of SPIONs by macrophages or the reticuloendothelial system and their lack of specificity towards target cells or tissues are proving to be major disadvantages for TDDS. Therefore, targeted SPIONs could be used to overcome these problems. They have been studied *in vitro* or *in vivo* in several laboratories. Montet et al. (2006) first used SPIONs conjugated with an analogue of Bn to visualize pancreatic ductal adenocarcinoma in an inverse imaging strategy. Subsequently, Martin and co-workers demonstrated the potential of pan-bombesin conjugated with dye-functionalized SPIONs as an MRI probe for the detection of prostate cancer in *in vitro* conditions (Martin et al., 2010).

Jafari et al. (2015) conjugated SPIONs with a Bn analogue (KGG-CDFQWAV-βAla-HF-NIe) as a new targeted MRI contrast agent for breast cancer detection. This analogue has an affinity for all three subtypes of Bn receptors, overcoming the receptor heterogeneity in breast cancer cells. In addition, it is also hydrophilic and carries a negatively charged aspartic acid residue that enhances its renal clearance, thus solving the kidney retention problem of SPIONs (Jafari et al., 2015). The MRI study indicated that the SPION-Bn possessed good diagnostic ability as a GRP-specific contrast agent, with appropriate signal reduction in T_2-weighted color map MR images in mice with breast tumors.

Recently, Bn-conjugated and radioactive copper-64 (^{64}Cu)-labelled copper sulphide nanoparticles (Bn-PEG-[^{64}Cu] CuS NPs) were used for selective imaging of orthotopic prostate cancer. *In vitro* cellular binding/uptake assays clearly showed that Bn-PEG-[^{64}Cu] CuS NPs were better able to identify and rapidly accumulate into the prostate cancer cell line than unconjugated nanoparticles. In micro PET-computed tomography (PET-CT) imaging quantification, unconjugated nanoparticles were accumulated to only 1.20 ± 0.22 ID%/g (mean percent injected dose per gram; 1 h post-injection) and 1.41 ± 0.22 ID%/g (6 h post injection), whereas Bn-conjugated nanoparticles were accumulated to 3.64 ± 0.45 ID%/g ($P = 0.03$) and 4.82 ± 1.50 ID%/g ($P < 0.01$) at 1 h and 6 h post-injection, respectively (Cai et al., 2018).

Bn-conjugated gold nanoparticles are the most commonly explored nanocarrier system for imaging and delivery of drugs to GRP receptor-overexpressing cancer cells. Heidari et al. (2014) demonstrated the potential of Bn-conjugated PEG coated gold nanorods as a photoacoustic imaging agent with improved specificity and sensitivity for the detection of breast cancer. Gold nanorods can absorb about 1000 times more light than an equivalent volume of an organic dye, and when combined with their plasma resonance absorption and scatter in the near infrared region, they are suitable for *in vivo* imaging applications. Jokerst and co-workers reported that gold nanorods, under *in vivo* conditions, exhibit significant photoacoustic contrast and increase the diagnostic power of the photoacoustic imaging modality (Jokerst et al., 2014). The selective accumulation of Bn-conjugated gold nanorods was observed in a breast tumor, unlike unconjugated gold nanorods, following intravenous administration to breast tumor-bearing mice (Heidari et al., 2014). Bn-conjugated, PEG-coated gold nanoparticles (PEG-coated GNPs-Bn) were investigated as a new class of X-ray contrast agent for radiological imaging of breast cancer. A cytotoxicity study of PEG-coated GNPs-Bn on T47D breast cancer cells showed more than 80% cell viability up to a concentration of ~100 μg/mL after 24 h incubation. Additionally, Bn functionalized PEG-coated AuNPs were demonstrated to be great contrast agents for the detection of breast cancer in a mouse model (Salouti and Saghatchi, 2017).

Together with another targeting ligand, folic acid, Bn was conjugated to ^{177}Lu-labelled PAMAM dendrimers conjugated AuNPs to improve their cellular uptake and hence, imaging of cancer cells (Mendoza-Nava et al., 2016). The comparative potency of Bn and folate-targeted nanoparticles was evaluated against T47D breast cancer cells and compared with only folate-targeted or non-targeted dendrimer AuNPs. The dual receptor targeting nanoparticles demonstrated higher luminescence in T47D breast cancer cells compared to only folate-targeted or non-targeted dendrimer AuNPs. (Mendoza-Nava et al., 2016). Subsequently, the same group also demonstrated that a ^{177}Lu-DenAuNP-folate-bombesin conjugate was not only capable of enhancing the targeted luminescence (fluorescence-optical imaging) but also exhibited enhanced plasmonic–photothermal therapy and targeted radiotherapy in T47D breast cancer cells. The ^{177}Lu-DenAuNP-folate-bombesin conjugate killed ~90% of T47D breast cancer cells (Mendoza-Nava et al., 2017).

Bn-conjugated gold nanoparticles have also been investigated for prostate cancer detection *via* fluorescence imaging. Bn-conjugated gold nanoparticles were rapidly taken up by GRPR-overexpressing PC-3 human prostate cancer cells and more than 90% internalization of nanoparticles was observed after 4 h of incubation (Pretze et al., 2018). When the Bn receptors were blocked with Bn peptide, the uptake of nanoparticles was reduced by 54%, suggesting receptor-mediated uptake as well as the non-specific interaction of Bn-conjugated gold nanoparticles to the prostate cancer cells. A lower uptake of these nanoparticles by Bn receptor-negative A431 cells confirmed the Bn-receptor mediated uptake of Bn-conjugated gold nanoparticles. Simpson et al. (2017) demonstrated the targeting of all four subtypes of GRP receptor expressed on PC-3 human prostate cancer cells by using pan-bombesin peptide [D-Phe^6, β-Ala^{11}, Phe^{13}, Nle^{14}]Bn(6–14)-functionalized gold nanoparticles. Thus this conjugate can be used to target prostate cancer for diagnostic or therapeutic biomedical applications (Simpson et al., 2017).

Recently, Bn has been used in combination with c(RGDyK) peptide in the design of dual-receptor targeting diagnostic probes. A novel ^{64}Cu radiolabelled RGD_2-Bn(7–14) heterotrimer was synthesized for PET imaging of prostate cancer (Lucente et al., 2018). This conjugate successfully targeted two important receptors (integrin receptor and BB_2R) that are overexpressed in prostate cancer cells and it was stable in mouse serum for up to 1 h but it showed low tumor uptake *in vivo*. It was suggested that the low efficiency of the *in vivo* PET imaging was possibly due to poor pharmacokinetic properties of the imaging probe (Lucente et al., 2018).

7. Conclusions and future directions

Despite therapeutic advances, cancer remains one of the major causes of death. It is consequently imperative to discover new and effective treatment approaches for both early detection and advanced treatment of the disease. We have reviewed the importance of Bn receptors in imaging and targeting of tumor cells and shown how they are providing novel approaches to the treatment of cancer. We have discussed how the overexpression of Bn receptors in cancer cells helps to combat one of the major limitations of effective selective delivery of cytotoxic agents to tumor cells. This suggests that Bn receptor-targeting ligands, such as Bn agonists, antagonists, antibodies, *etc.* can be used to convert a non-specific drug molecule to a Bn receptor-specific drug molecule by direct covalent coupling or using a crosslinker. In addition, these ligands can also be conjugated to nanoparticles containing an imaging agent or anti-cancer agent for selective imaging or delivery of drugs to cancer cells, respectively. In both *in vitro* and preclinical studies in animal models, Bn-mediated targeted systems have shown promising results in controlling cancer cell growth. However, in comparison to other more established receptors that are overexpressed in cancer, such as folate or transferrin receptors, Bn receptor targeting is in its infancy, requiring further *in vivo* studies in tumor-bearing mice.

Declaration of Competing Interest

The authors report no conflict of interest.

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