Exploring BB_3 pharmacology to guide selective lung adenocarcinoma treatment

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Background and rationale

Lung cancer remains the leading cause of cancer-related deaths in Australia, responsible for ~17% of cancer fatalities in 2024[1]. Despite advances in treatment, prognosis is poor with an overall five-year survival of 26%[1]. Lung adenocarcinoma (LUAC) represents ~40% of lung cancers and is often diagnosed too late for significant intervention[2]. Even when LUAC harbours a targetable oncogenic driver, treatment resistance almost always develops[3].

G protein-coupled receptors (GPCRs) are the most successfully targeted protein family, accounting for ~30% of all FDA-approved drugs[4]. However, they remain underexploited in oncology because few GPCRs have been identified as oncogenes[5]. Our research has identified the bombesin 3 receptor (BB_3), an understudied GPCR owing to its unknown endogenous ligand, as a potential LUAC target. While BB_3 is typically absent in adult tissues, evidence suggests that related receptors are overexpressed in cancer[6]. If BB_3 is present in LUAC but not healthy tissue, the receptor could enable targeted treatment approaches with limited side effects.

Hypothesis/Aims

We hypothesised that BB_3 is a novel LUAC biomarker that can be targeted alongside current therapies to improve LUAC survival. Given that BB_3 is a peptide family receptor, we further hypothesised that AI-assisted peptide discovery could identify novel BB_3-targeting ligands with distinct pharmacological profiles. This study aimed to define BB_3 expression, activation and signal transduction, evaluate computationally predicted BB_3 peptide modulators, and determine possible interactions between BB_3 ligands and standard therapeutics.

Methodology

RNA-sequencing datasets were analysed to compare BB_3 expression across healthy and cancer tissues. Synthetic BB_3 ligands (MK5046[7], BAG-1[8] and BANTAG-1[9]) were tested across reporter gene assays measuring $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ signalling, NanoBiT β-arrestin recruitment, and BRET-based biosensors for ERK1/2 phosphorylation and cAMP accumulation.

To design BB_3-targeting peptides, BB_3 complexes with ~1000 hypothetical peptides were modelled by AlphaFold3[10], top-ranking predictions redesigned using RFdiffusion[11] and ProteinMPNN[12], and 7317 resulting sequences folded and evaluated with BB_3. 22 prioritised candidate peptides were synthesised and evaluated in Ca^{2+} mobilisation assays to identify agonists and antagonists. To assess the relevance of BB_3 activity in cancer, lung cancer cell lines were screened for BB_3 expression by qPCR, and the effect of ligand stimulation was measured using the CellTiter-Glo cell viability assay[13].

Results

RNA-sequencing analysis reveals that BB_3 is present in ~83% of LUAC, regardless of oncogenic driver, gender, ethnicity or disease stage, and is barely detected in other healthy or cancer tissue. BB_3 activates $G\alpha_{q/11}$, $G\alpha_{12/13}$ and $G\alpha_s$ in the absence of ligand (Emax = 439.8±31.2, 174.2±19.8, 118.7±27.7 RLU respectively), and agonism increased Ca^{2+} (MK5045 pEC_{50}=8.02, BAG-1 pEC_{50}=7.89). Of the 22 peptides prioritised by the AI-assisted discovery pipeline, two acted as partial agonists and two as antagonists in Ca^{2+} mobilisation assays, representing an experimental hit rate of 18%. BB_3-positive cell lines remained susceptible to standard cancer therapies, but BB_3 ligands alone had no effect on cell viability, nor did they alter the activity of standard therapies when administered together.

Conclusions

BB_3 is a highly selective cell-surface biomarker of LUAC, making it an ideal candidate for targeted approaches in a disease marked by late diagnosis and frequent treatment resistance. By characterising BB_3 expression, signalling and ligand pharmacology, we provide a strong foundation for the development of BB_3-targeted therapeutic strategies. Importantly, AI-assisted peptide discovery identified novel BB_3 modulators, supporting the feasibility of developing peptide-based tools for BB_3-positive LUAC.

Statement of the scientific innovation and significance of the study

To our knowledge, this study is the first to comprehensively characterise BB_3 in lung cancer, establishing it as a previously unrecognised biomarker. By resolving BB_3's pharmacology across multiple assay platforms, we have addressed a critical knowledge gap surrounding an orphan GPCR with high translational value. The identification of novel BB_3 modulators through AI-assisted peptide discovery demonstrates an innovative strategy for developing ligands capable of selectively engaging BB_3-positive LUAC.

Statement on the nominating student's specific role in the study

This project was conceived and designed by myself and my PhD supervisor A/Prof Nicola Smith. I performed all experiments, with replicates of the BB_3 peptide screen conducted by Honours student Olivia Clink under my supervision. Experimental advice was given by A/Prof Nicola Smith, Drs Chantal Donovan, Richard Kim and Madison

Coward-Smith. Drs Chantal Donovan and Richard Kim provided all lung cancer cell lines and qPCR reagents used in this study. Mr Joshua Andrew Nillama and A/Prof Luke Hunter synthesised the antagonist used in this study. Prof Irina Kufareva, Ms Lilian Spock and Mr Tyler Hurlimann designed the BB_3 -targeting peptides.

Declaration

We declare that this document was prepared primarily by the nominating student and has undergone minimal corrections by the supervisor or any other author on the accompanying abstract.

Student's name: Mariah Stavrou
Student's signature:

Date: 10/5/2026

Supervisor's name: Nicola J Smith
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