Mutation-driven modulation of GPCR pharmacology in cancer: insights from adenosine and serotonin receptors

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Chapter 1

General introduction

G protein-coupled receptors (GPCRs) constitute the largest and most diverse family of membrane receptors in the human genome, encompassing over 800 members. These receptors play pivotal roles in cellular communication by transducing extracellular signals into intracellular responses through coupling with heterotrimeric G proteins [1]. Given their central role in physiology, it is not surprising that GPCRs are implicated in numerous pathological conditions, including neurological disorders [2], cardiovascular diseases [3], metabolic syndromes [4], and notably, cancer [5]. As a result, GPCRs are among the most targeted protein families in pharmacotherapy, with approximately one-third of all marketed drugs acting on these receptors [6].

Despite their established therapeutic relevance, GPCRs have not been the primary focus in oncology like classical oncogenes or tumor suppressors, and their involvement in cancer biology has remained relatively underexplored until recent years. The advent of large-scale cancer genomics efforts such as The Cancer Genome Atlas (TCGA), International Cancer Genome Consortium (ICGC), and Genomic Data Commons (GDC) have uncovered a surprising prevalence of somatic mutations within GPCR genes across diverse cancer types [7, 8]. Although individually rare and dispersed across multiple members of the GPCR family, these mutations raise interests about their functional significance and potential roles in tumorigenesis. Despite their low frequency and distribution across various receptor domains, the structural complexity and conformational flexibility of GPCRs suggest that even single amino acid substitutions can substantially alter receptor pharmacology. Indeed, several studies have demonstrated that certain GPCR mutations can influence ligand binding, receptor expression, and downstream signal transduction [9-11]. Understanding the mutation-driven modulation of GPCR function is not only critical for dissecting cancer signaling networks, but also for refining therapeutic strategies that leverage GPCRs as drug targets. These findings raise important questions: Do GPCR mutations in cancer function as oncogenic drivers that actively promote cancer progression, or are they merely passenger mutations that accumulate as a consequence of genomic instability? Most crucially, can such mutations be exploited for therapeutic intervention? While a universal answer remains elusive due to the diversity of GPCRs and cancer contexts, focused case studies can offer valuable insights.

Among GPCR subfamilies, adenosine and serotonin (5-hydroxytryptamine, 5-HT) receptors have drawn increasing attention due to their established roles in cell proliferation, immunomodulation, and angiogenesis [12, 13]. In particular, the adenosine A_{2A} receptor (A_{2A}AR) and A_{2B} receptor (A_{2B}AR) are known for their immunosuppressive roles in the tumor microenvironment (TME), where elevated extracellular adenosine levels suppress T-cell function and facilitate tumor immune evasion [14]. On the other hand, serotonin receptors such as the 5-HT_{2C} receptor (5-HT_{2C}R) have also been implicated in cancer cell proliferation and immune response [15, 16]. Despite these associations, the specific impact of cancer-associated mutations on the pharmacology and function of these receptors remains poorly understood. Functional characterization of such mutations may uncover previously unrecognized mechanisms of drug resistance, help predict patient responses to GPCR-targeting agents, and inspire the development of next-generation precision therapies.

Aim and outline of this thesis

This thesis seeks to investigate how cancer-associated mutations affect the pharmacological properties of selected GPCRs—primarily A_{2A}AR and 5-HT_{2C}R—through a combination of ligand binding assays, signaling readouts, kinetic profiling, and cellular functional assays. By characterizing the functional consequences of naturally occurring missense mutations, this work aims to shed light on the broader question of whether such mutations could serve as biomarkers for treatment stratification or as targets for novel therapeutic strategies.

In **Chapter 2**, a comprehensive review outlines the current understanding of GPCR-G protein signaling in the context of cancer and summarizes the mutational landscape of GPCRs identified from large-scale cancer datasets. The chapter also discusses whether GPCR mutations identified in cancer act as drivers or passengers, and the methodological and conceptual challenges in making this distinction.

Chapters 3 and **4** focus on the adenosine A_{2A} receptor, analyzing how specific cancer-associated mutations influence ligand-receptor interactions and receptor function. Using both classical and newly developed agonists and antagonists, these chapters delve into how pharmacological parameters such as binding affinity and residence time, potency and efficacy are affected by structural alterations in the receptor. The implications of these findings for cancer cell behavior and therapeutic strategies are also discussed.

In **Chapter 5**, the functional role of A_{2A}AR and A_{2B}AR in cancer cell growth is investigated using breast cancer cell lines. The study evaluates the impact of pharmacological activation and inhibition, and discusses how receptor signaling contributes to cancer progression in a cell type dependent manner. This chapter provides an important link between *in vitro* pharmacology and oncogenic phenotypes.

Chapter 6 shifts focus to the 5-HT_{2C} receptor, another GPCR with oncogenic potential. Using a similar approach as with A_{2A}AR, this chapter investigates how cancer-associated 5-HT_{2C}R mutations affect receptor pharmacology, including ligand binding and signal transduction. Despite the dispersed nature of these mutations, the study identifies several variants with altered functional profiles, suggesting potential relevance to cancer biology.

Chapter 7 summarizes the findings presented in this thesis, offering a thorough discussion of how mutations can rewire GPCR pharmacology in a context-dependent manner. These insights underscore the need for case-by-case characterization of GPCR variants, especially in cancer where receptor function and therapeutic response are tightly linked to the cellular environment. This chapter also highlights underexplored opportunities, such as covalent ligands, biased signaling, and allosteric modulation as promising strategies to overcome drug resistance associated with GPCR mutations. Taken together, by combining molecular pharmacology, cancer biology, and translational perspectives, this thesis contributes to a growing recognition of GPCRs as dynamic and druggable nodes in cancer signaling networks.

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