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

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# Summary

G protein-coupled receptors (GPCRs) constitute the largest and most diverse family of membrane receptors in the human genome, encompassing over 800 members. With their unique transmembrane structure, GPCRs play pivotal roles in cellular communication by transducing extracellular signals into intracellular responses and mediating various biological and pathological processes. Indeed, nearly 34% of all marketed drugs act on over 120 GPCR family members, which accounts for 14% of all validated drug targets. However, despite a large body of evidence implicating GPCRs in cancer, there are only ten anti-cancer drugs targeting GPCRs approved up until 2025. GPCRs have been found to be involved in almost every essential step of cancer development, including cell proliferation and survival, inflammation, immune tolerance, angiogenesis, cell invasion and metastasis, and their potential in cancer therapy therefore remains largely underexploited. In the meantime, the advent of large-scale cancer genomics efforts has uncovered a surprising prevalence of somatic mutations within GPCR genes in cancer. In this thesis, we performed case studies for the adenosine $A_{2A}$ receptor ($A_{2A}$AR) and serotonin 5-$HT_{2C}$ (5-$HT_{2C}$R) receptor, for which we investigated the effects of cancer-associated mutations on ligand binding, receptor activation and inhibition. We also discussed the potential consequence of altered receptor pharmacology on cancer progress and treatment.

**Chapter 1** is a general introduction of the research background, aim and outline of this thesis. **Chapter 2** provides a comprehensive review of the current studies on GPCR-G protein mutations in cancer, covering important GPCR signaling pathways in cancer development and immune response, mutational landscape of GPCR and G proteins, and discussed the evidence of driver mutations that actively promote cancer progression and passenger mutations that accumulate as a consequence of genomic instability. This chapter suggests that a universal answer remains elusive due to the diversity of GPCRs and cancer contexts, calling for case-by-case investigation in the following chapters. In **Chapter 3**, the effects of 11 cancer-associated $A_{2A}$AR mutations on agonist/antagonist binding and receptor expression were investigated using radioligand displacement assay, and the effects of 8 mutations on receptor activation and inhibition were characterized using an impedance-based cell morphology assay, xCELLigence. We found that the binding affinity of agonist NECA was decreased by six mutations but increased by V275A. The binding affinity of antagonist ZM241385 was decreased by mutations A165V and A265V. The potency of NECA ($EC_{50}$) obtained from xCELLigence assay was decreased by I92M and A265V, which correlated with the decreased binding affinity. The increased affinity but decreased potency of NECA by V275A indicated a loss of coupling efficiency to downstream signaling pathways. Mutations S132L and H278N were found to decrease the potency while increase the efficacy ($E_{max}$) of NECA, and they also decreased the inverse agonism but increased the inhibitory potency ($IC_{50}$) of ZM241385. These together, these findings indicate that S132L and H278N may cause a conformation shift of $A_{2A}$AR towards the inactive state. Besides, the lost potency of ZM241385 carrying mutations V275A and P285L indicates that they could be problematic in cancer treatment and deserve follow-up studies. **Chapter 4** focuses on kinetic profiling of novel $A_{2A}$AR agonists and antagonists, where we found that the A265V and H278N mutations significantly increased the dissociation rate of ZM241385,

thus shortening its residence time (RT). However, the binding affinity of long-RT antagonist LUF6632 retained its high affinity at all mutant receptors, making it a promising candidate to include these mutants in its range of targets. The long-RT agonist UK432097 also retained its affinity at most mutants but was decreased by H278N. On the other hand, the apparent affinity of covalent antagonist LUF7445 was decreased by A165V, A265V and H278N to a similar extent as ZM241385. The affinity of non-ribose agonist LUF5833 was decreased by F70L and H278N but increased by A265S and A265V, displaying a unique profile. Followed by **Chapter 5**, we explored the role of $A_{2A}$AR and $A_{2B}$AR in growth regulation of breast cancer cells, providing further evidence of targeting GPCR in cancer treatment. We validated the inhibitory effects of $A_{2A}$AR and $A_{2B}$AR antagonists on three breast cancer cell lines with distinct receptor expression profiles. Results from cell morphology and cell growth assays indicated that the cellular responses to antagonists were predominantly mediated by $A_{2A}$AR in the $A_{2B}$AR low-expressing BT474 cells, and by $A_{2B}$AR in the $A_{2A}$AR low-expressing MCF-7 cells. In SKBR3 cells, which express high mRNA levels of both receptors, $A_{2A}$AR signaling was barely detectable in the cell morphology assay. Nonetheless, $A_{2A}$AR antagonists significantly inhibited cell growth, and these effects were further enhanced by knockdown of either $A_{2A}$AR or $A_{2B}$AR, suggesting that both receptors play a role in regulating cell growth. These findings highlight the importance of the cellular context determining the response to receptor antagonists and warrant further investigation to confirm whether the inhibitory effects of $A_{2A}$AR antagonists are target-specific, and to explore the potential consequences of mutations in cancer treatment. **Chapter 6** shifts focus to the 5-$HT_{2C}$R, another GPCR with oncogenic potential. In this chapter, the effects of 12 cancer-associated 5-$HT_{2C}$R mutations on ligand binding and receptor expression were investigated using radioligand displacement assay, and the effects of 5 mutations on receptor functioning were characterized using calcium-flux assay. We found that mutations in the orthosteric binding pocket, L209H and F328S, decreased the affinity of endogenous agonist 5-HT and antagonist mesulergine. F328S decreased the potency of 5-HT while increasing the efficacy. In contrast, 5-HT displayed higher affinity and potency at E306A and E306K, while a trend of decreased efficacy was observed. These two mutations may disrupt the conserved ionic interaction between E6.30 and R3.50 and thus increase the constitutive activity of the receptor. The inhibitory potency of mesulergine was increased for E306A but not E306K mutants. P365H decreased the expression level of the receptor by more than ten-fold. Such mutations may affect serotonin-mediated signaling in tumor cells as well as treatment strategies targeting this receptor. **Chapter 7** presents the overall conclusions of this thesis by integrating the findings from each chapter and offers future perspectives that have emerged from our research.