The good? The bad? The mutant! Characterization of cancer-related somatic mutations and identification of a selectivity hotspot in adenosine receptor

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Chapter 1

General Introduction

G protein-coupled receptors (GPCRs) are a family of membrane-bound proteins with approximately 800 members that have seven-transmembrane (7-TM) domains, an extracellular amino terminus and an intracellular carboxyl terminus[1,2]. According to sequence homology and phylogenetic analysis, human GPCRs can be classified into 5 main families, glutamate family (class C), rhodopsin family (class A), adhesion family, frizzled/taste2 and secretin family (class B), shorten as GRAFS[2,3]. The majority of GPCRs belongs to the class A subfamily resembling the visual pigment rhodopsin. GPCRs are responsive to a diverse set of physiological endogenous ligands including hormones and neurotransmitters. Upon activation, GPCRs induce a signal transduction cascade inside the cell via heterotrimeric G proteins, which consist of three subunits, α, β and γ[4,5]. Due to the various GPCR binding domains and their sensitivities to a diverse array of ligands, these proteins have shown to be very 'druggable' as they are the main target for an estimated 30% of approved drugs[6].

Mutations occurring in GPCRs can severely alter their normal function, including cell surface expression, basal activity, ligand binding and receptor – G protein interaction (Figure 1)[7], and may ultimately trigger their physiological roles to pathological ones. Mutations in 55 GPCR genes have been reported as causal link to 66 human monogenic diseases[8]. Different diseases can result from a single GPCR gene, due to the possibility of inactivating and activating mutations. Therapeutical approaches have been developed for the treatment of pathologies caused by GPCR malfunctions. For instance, symptomatic therapies with pharmacological and/or surgical intervention have been established to aim at the symptoms of the GPCR variants-linked diseases[9,10]. In addition, direct targeting of malfunctional GPCRs via genome editing approaches or small molecules could also be suitable strategies toward personalized therapeutics in GPCR pathologies[11,12]. Genetic variants in drug-targeted GPCRs, especially variants located in functional sites of GPCR structures, have been identified with altered drug responses[13]. Therefore, characterization of these GPCR variants is of great importance with respect to possibly impaired drug efficacy and undesired side effects.

In preclinical oncology the primary focus has mostly been on kinases due to their central role in the cell cycle[4,14]. However, a growing body of evidence shows a prominent role of GPCRs in all phases of cancer. Malignant cells can e.g., hijack GPCRs to increase proliferation or metastasis formation to distant tissue (Figure 2)[14]. In addition to an increased understanding of the role of GPCRs in cancer, recent investigations have shown that these proteins, present in patient isolates, are sensitive to mutation[15,16]. More specifically, it has been found that GPCRs are the second most mutated protein class with a mutations frequency of an estimated 20% of all cancers[17]. Higher mutations rates are often observed for certain conserved residues, and given the (evolutionary) importance of these residues the exact impact of these mutations in receptor pharmacology warrants considerable investigation[17,18].

One particular class of rhodopsin-like GPCRs included in this thesis are the adenosine receptors (ARs). This GPCR family consists of 4 subtypes, A_1AR, A_{2A}AR, A_{2B}AR, and A_3AR, which share the common local hormone adenosine as ligand[19]. Adenosine is a purine nucleoside that serves several important roles in a physiological context including DNA synthesis, a precursor in energy transfer (adenosine tri-phosphate, ATP) and secondary messenger (cyclic adenosine monophosphate, cAMP)[20]. Activation of A_1AR and A_3AR inhibits the activity of adenylyl cyclase via the $G_{\alpha i}$ protein, leading to decreased levels of intracellular cAMP[21,22]. The A_{2A}AR and A_{2B}AR are coupled to the $G_{\alpha s}$ protein upon activation, resulting in an increased production of intracellular cAMP[23,24]. The adenosine receptors are expressed throughout the body and are under investigation as drug targets in different disorders, including Parkinson's disease and Alzheimer's disease[25–27]. However, these GPCRs are also of interest in the context of cancer research due to the evidence of adenosine accumulation in the tumor microenvironment[28], which has been supported by several studies in immune cells[29]. In short, all the adenosine receptors might be targets for the development of novel approaches to the treatment of cancer, which will be further elaborated on in **Chapter 3** of this thesis.

Combining background knowledge on the role of GPCRs in cancer with the increased understanding of mutational patterns, it stands to reason that targeting GPCRs with drugs may have a beneficial effect on cancer in patients in general. More specifically, the group of adenosine receptors might form an interesting focus area for cancer treatment via GPCRs. For each of the four subtypes, a number of somatic mutations have been identified in patient isolates.

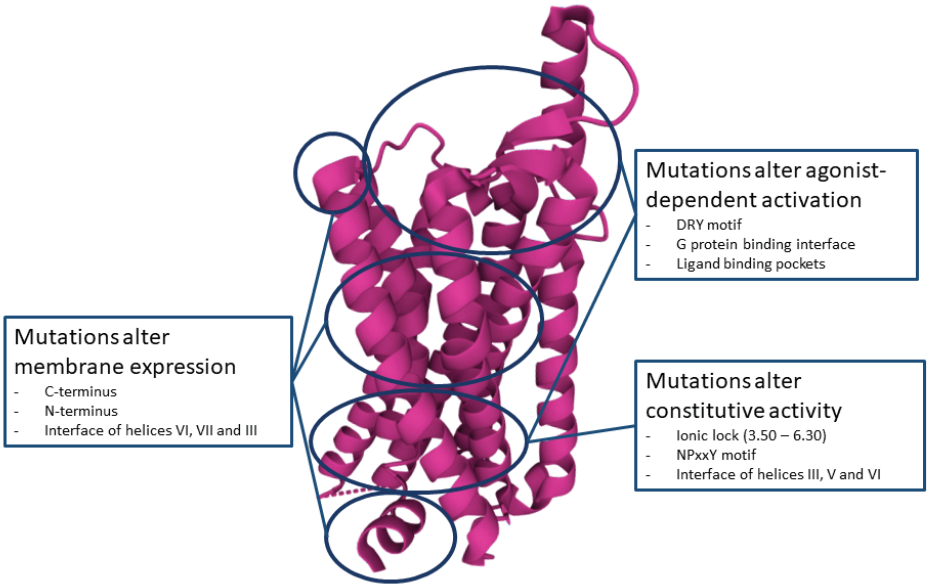

Figure 1. General consequences of class A GPCR mutations in receptor pharmacology. The 3D structure is adapted from A_1AR (PDB 7LD4).

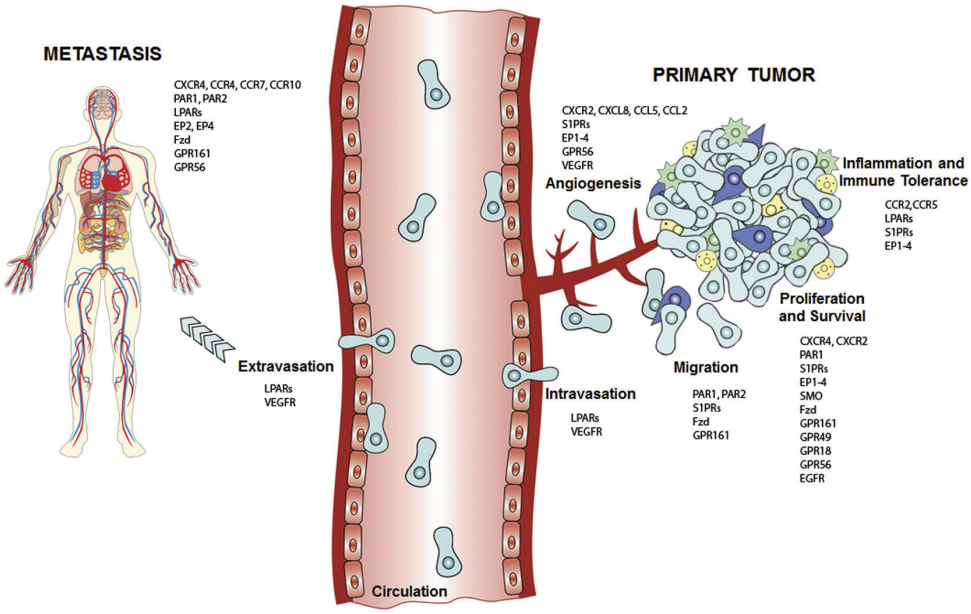

Figure 2. Examples of GPCRs and their roles in cancer progression[30]. Reproduced with permission.

Aim and outline of this thesis

To establish the role of cancer-related AR mutations at the molecular level in this thesis, we decided to express and study these in a yeast strain devoid of GPCRs and in some cases in a mammalian cell system. Here, we examined them using reference adenosine receptor ligands on receptor activation and ligand binding, and determined the impact mutations have on these pharmacological readouts.

Chapter 2 summarizes the strategies of using yeast systems in human GPCR studies with a focus on adenosine receptors. **Chapter 3** provides an overview of GPCRs and their mutations involved in cancer progression. Furthermore, evidence for adenosine receptors in cancer development is discussed in detail. As mutations of adenosine receptor have been identified from cancer patient isolates, **Chapter 4**, **5** and **6** provide information on the impact of these mutations in receptor functionality. **Chapter 4** focuses on receptor expression and activation of A_{2B} receptors using the engineered yeast system. The effects of cancer-related mutations in A_1 receptors on receptor activation and ligand binding are described in **Chapter 5** and **6**. **Chapter 5** describes mutations located at the loop regions, while **Chapter 6** focuses on mutations positioned in the 7-TM domains. **Chapter 7** reports the approach for the identification of a stereoselectivity hotspot in A_{2B} receptor antagonist recognition from both computational and experimental aspects. Finally, **Chapter 8** summarizes the results of the work described in this thesis, as well as future prospects and challenges that emerge from this thesis. Hopefully, this thesis will enrich the view of cancer-related mutations in GPCR pharmacology and ultimately contribute to novel strategies for the modulation of their activity using medicinal products.

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