## *Summary*

The glucocorticoid receptor (GR) signaling pathway is essential for the survival and wellbeing of most vertebrate organisms and in addition, it significantly contributes in combating inflammation (reviewed in **Chapter 1)**. Hence, the scope of the present thesis was to elucidate the biological significance and action of the glucocorticoid receptor by means of genetic manipulation and stimulation with synthetic glucocorticoids (GCs). As a model organism, we employed the zebrafish, that allows fine genetic, molecular and cellular experimental approaches and as our main readout we used transcriptome analysis, since the GR is a transcription factor. Our aim was also to further characterize the function of this versatile signaling cascade in zebrafish, in order to establish this animal model as a valid system for detailed as well as high throughput research on GR.

In **chapter 2**, we explored the role of the zebrafish GR α-isoform (zGRα) with respect to modulating the inflammatory response to a wound injury. For that reason, a tail fin amputation assay was employed in 3-day-old zebrafish larvae which were subsequently treated with the synthetic GC beclomethasone. Amputation elicited a migratory behavior towards the wound site for both macrophages and neutrophils as well as induction of several immune-related signaling routes. Using cell imaging as well as whole transriptome analysis, we studied the GC effect on the cellular trafficking of leukocytes as well as on the transcriptional rate of genes involved in molecular networks altered due to amputation. Our results show that beclomethasone treatment of amputated larvae attenuated the migratory behavior of neutrophils, but not of macrophages. Additionally, GC treatment had a very general dampening effect on the induction of gene expression upon amputation, without any apparent specificity for particular pathways. These results show that the zebrafish larva model of tail fin amputation and beclomethasone treatment recapitulates the anti-inflammatory GC effects, thus providing a reliable model system to further elucidate the molecular mechanisms of GC signaling.

In **chapter 3**, we investigated the specificity and function of both zebrafish GR α- and β-isoforms (zGRα and zGRβ, respectively). Zebrafish embryos were injected with two splice-blocking morpholinos (one leading to knockdown of both zGRα and zGRβ, and another targeting the alternative splicing of the zGR pre-mRNA in favor of the zGR β-isoform) and with zGRβ mRNA (resulting in specific zGRβ overexpression). Embryos were treated with the synthetic GC dexamethasone and transcriptome analysis was performed using microarray technology. This experimental design allowed us to answer 3 questions. First, which specific genes are affected by zGRα under different physiological conditions. Second, whether zGRβ exhibits a dominant-negative activity on zGRα's transcriptional properties. Third, which genes are specifically altered due to a possible intrinsic zGRβ transcriptional activity (independent of zGRα). Our transcriptome analysis showed two distinct gene clusters regulated by zGRα. One cluster that was affected by zGRα under basal conditions, and one that was regulated upon activation of zGRα by dexamethasone treatment. In general, the first cluster mainly contains cell-cycle related genes whereas the second cluster mainly involves genes related to metabolism. Furthermore, our data did not support a significant role of zGRβ as a dominant-negative inhibitor of zGRα. Nevertheless, zGRβ could have an intrinsic transcriptional activity that would require high expression levels of this splice variant.

In **chapter 4**, we embarked on a series of experimental approaches allowing us to elucidate the biological significance of the zGR β-isoform. In particular, we were interested in answering two questions. First, whether zGRβ plays a role as a dominant-negative inhibitor on zGRα's transcriptional properties. Second, whether zGRβ exhibits an intrinsic transcriptional activity, meaning independent of zGRα. We overexpressed zGRβ transiently by cell transfections using a zGRβ expression plasmid. Luciferase reporter assays in transiently transfected COS-1 cells demonstrated a dominant-negative effect of zGRβ on the transactivation properties of zGRα. However, no such effect was observed in zebrafish PAC2 cells using the induction of the *fkbp5* gene as readout. In addition, upon zGRβ mRNA injections in zebrafish embryos, no dominant-negative effect was observed on the zGRα-induced transactivation of the *fkbp5*, *pepck* and *nfkbiaα* genes. Subsequently, we generated a transgenic fish line with inducible zGRβ overexpression. Transcriptome analysis by microarray and subsequent qPCR validation using this line did not reveal any regulatory effect of zGRβ (either as a dominant-negative inhibitor of zGRα or as a transcription factor independent of zGRα). Based on these results, we suggest that the zebrafish GR β-isoform does not have a regulatory role in transcription and that splicing of the GR pre-mRNA into a messenger encoding an alternative splice variant could instead represent a physiological mechanism to downregulate the levels of the canonical receptor variant.

In **chapter 5**, results from all three experimental chapters were discussed collectively in order to draw conclusions about the validity of zebrafish as a model system for GC research and, most importantly, the role and function of both zGR splice variants. Our work showed that the zebrafish is a reliable system to study the GR signaling pathway since this model organism recapitulates the classical effects of GRα activation such as transactivation of genes and immunosuppressive activity. Furthermore, our whole organism transcriptome analysis provided us with a wealth of information on GRα-dependent gene expression regulation under different conditions. These data can further be investigated in more detail, in order to study the biological significance and molecular properties and interactions of this receptor with respect to specific physiological settings. For instance, different inflammatory responses could easily be modeled in zebrafish, and comparative analysis of zGRα-mediated regulation of the immune response could be undertaken. As for the biological role of zGRβ, our data do not support any zGRβ dominant-negative activity on zGRα's transcriptional properties, whereas some specific zGRβ intrinsic transcriptional activity (meaning independent of zGRα) cannot be ruled out.