Summary

Galectins as targets for angiostatic cancer therapy
Summar

Galectins are a family of proteins that are characterized by the presence of a conserved carbohydrate recognition domain (CRD). Via this CRD, galectins bind to certain carbohydrates present on glycoproteins and glycolipids. In line with the numerous diverging cellular functions of galectins, the deregulation of these proteins is shown to contribute to various pathologies, including cancer. Indeed, multiple studies confirmed the prognostic and therapeutic value of certain galectins in several types of cancer. It has been shown that galectins facilitate several key steps during tumor progression, such as tumor cell transformation, metastasis and tumor immune escape. In addition, several galectins have been implicated in the sprouting of novel blood vessels from pre-existing capillaries, i.e. angiogenesis. Tumors induce angiogenesis to be able to grow beyond a few cubic millimeters in size. This process provides the tumor with a continuous blood supply, which facilitates the transport of nutrients, cells and gasses. Consequently, the tumor mass can be expanded and a route for metastasis of tumor cells is formed. Therefore, the inhibition of angiogenesis is considered as a promising anti-cancer therapy. Endothelial cells (EC), which form the inner lining of blood vessels, are genetically stable cells and are easily accessible for therapeutics via the blood stream. These characteristics make them attractive targets for angiostatic therapy. Recently, evidence has accumulated that galectins in the tumor endothelium might provide opportunities for the inhibition of angiogenesis. Of the fifteen mammalian galectins, only galectin-1, -3, -8 and -9 are found to be expressed in the endothelium. This expression appears to be differentially regulated upon endothelial cell activation, suggesting a role for galectins in tumor angiogenesis. The knowledge with regard to the expression and function of galectins in endothelial cell biology and angiogenesis as well as the opportunities to target galectins are summarized in chapter 1.

In this thesis, we aimed to assess the prognostic value of galectins in cancer patients. In addition, we set out to further unravel the expression and function of galectins in endothelial cells. Finally, we explored methods to therapeutically target galectins.

To address these aims, several known angiogenesis methods were adapted to study the role of galectins as well as of galectin inhibitors in endothelial cell biology in vitro (Chapter 3) and angiogenesis in vivo (Chapter 4).

In chapter 2, we evaluated the prognostic value of all human galectins in patients with stage I/II non-small cell lung cancer (NSCLC). It is important to identify early stage NSCLC patients with poor survival, since 30-40% of the early stage patients will present tumor recurrence within two years after surgical resection and these patients are likely to benefit from adjuvant chemotherapy. Extensive galectin mRNA expression profiling confirmed the prognostic value of galectin-1 in these patients, where high galectin-1 expression significantly correlated with shorter overall survival (OS) and disease free survival (DFS). This corroborates with other studies which described elevated galectin-1 expression levels to be associated with poor patient outcome. In addition, we assessed the expression of the three main galectin-9...
splice variants, and identified galectin-9Δ5 as a novel potential prognostic marker in early stage NSCLC. The observation that different galectin-9 splice variants appear to have different prognostic value suggests diverging functions of the isoforms. Together with the observation that galectin-9 was abundantly expressed in the tumor endothelium, this prompted us to assess the expression of all possible galectin-9 isoforms in endothelial cells, as described in chapter 5. The galectin-9 transcript is subject to extensive post-transcriptional splicing varying in the exclusion of exons 5 and 6, which encode for the linker region between the two CRDs, and exon 10, which encodes the C-terminal CRD. Apart from the three main galectin-9 splice variants (gal-9 full length, gal-9Δ5 and gal-9Δ5/6), we found two additional splice variants, i.e. gal-9Δ5/10 and gal-9Δ5/6/10, lacking also exon 10, which results in a truncated C-terminal CRD. These splice variants were shown to be differentially regulated during endothelial cell activation. The function of the most abundant isoform, i.e. gal-9Δ5, in endothelial cell biology and angiogenesis appeared to depend on cellular localization, local concentration and the context in which the protein was presented to the cells. Overall, gal-9Δ5 appeared to have only a minor inhibitory effect on angiogenesis.

In chapter 6, we further examined the prognostic value of all known galectin-9 splice variants, including the two novel isoforms that were described in chapter 5, in renal carcinoma patients. Here we identified increased gal-9Δ5 and gal-9Δ5/6/10 as novel markers for better patient survival, again stressing the importance of distinguishing between different splice variants. The latter isoform contains a truncated C-terminal CRD, which potentially affects protein function. Therefore, we compared the effects of recombinant gal-9N and gal-9Δ5 on endothelial cell function. As compared to gal-9Δ5, gal-9N appeared to be a more potent inhibitor of endothelial migration in vitro and angiogenesis in vivo, whereas endothelial cell sprouting and transwell migration were more potently stimulated. As was mentioned before, the environmental context appears to be an important determinant of galectin-9 function. Further research into the regulation and functional diversity of different splice variants is warranted for the development of effective galectin-targeted cancer therapies.

Apart from being valuable as prognostic or diagnostic markers, galectins are increasingly appreciated as potential targets for angiostatic cancer therapy. Previously, we identified galectin-1 as the receptor for the angiostatic peptide anginex. The structural and functional similarities between anginex and the endogenous angiostatic chemokine platelet factor 4 (CXCL4), prompted us to hypothesize that galectin-1 could also serve as a functional binding partner for CXCL4. This hypothesis was addressed in chapter 7, where we indeed proved binding between CXCL4 and galectin-1. As a consequence of the interaction, the carbohydrate binding affinity of galectin-1 is altered. However, the functional relevance of this modulation remains elusive. We showed that CXCL4 neutralizes the stimulatory effects of galectin-1 on proliferation and migration of endothelial cells. Whether this is a result of e.g. decreased H-Ras signaling or increased uptake of galectin-1-binding receptors needs further investigation. Interestingly, CXCL4 also affected galectin-1-induced effects in blood platelets. Both platelet activation and
aggregation induced by galectin-1 were potentiated by CXCL4. Possibly, the observed effects on endothelial cell and platelet function can be explained by the altered carbohydrate binding affinity of galectin-1 upon interaction. Alternatively, by binding to galectin-1 the transport of CXCL4 to e.g. distant sites of inflammation might be facilitated, or galectin-1 might interfere with CXCL4 function. Evidently, further research on CXCL4 as a potential galectin-targeting agent should be encouraged.

In general, research on galectin-targeting therapies focuses on interfering with galectin function by using blocking antibodies, carbohydrates or peptides. However, the importance of reducing environments for the correct protein folding and functioning of galectins opens alternative avenues to interfere with protein activity. In chapter 8 we initiated research into a new approach to possibly interfere with galectin function by modulation of the cellular redox state. Metallothioneins (MTs) are small cysteine-rich proteins which are involved in e.g. metal homeostasis, metal detoxification and regulation of the redox balance. In addition, evidence suggests MTs to be involved in angiogenesis, both in vitro as in vivo. Since little is known regarding the expression and function of MTs in EC, we performed extensive expression profiling of all human MTs in endothelial cells. We detected a broad repertoire of endothelial MTs, the expression of which was influenced by different triggers, including hypoxia. In addition, we revealed a role for the most prevalent MT-isoform, i.e. MT2A, in endothelial cell function in vitro. Future research should reveal whether MT2A could be a valuable target for angiostatic therapy and whether interfering with MT2A indeed affects galectin-1 activity.

The research described in this thesis further increased the knowledge on the prognostic value of galectins in cancer and emphasized the importance of distinguishing between different splice variants. Furthermore, we identified novel galectin-9 isoforms with potentially diverging functions in angiogenesis. Moreover, we propose several galectin-interfering strategies which might provide leads for the development of galectin-targeting drugs as angiostatic cancer therapy.