SUMMARY

GENERAL DISCUSSION and FUTURE PERSPECTIVES
Summary

Since the discovery of the first ADAM family members about two decades ago, ADAM-related research has focused on the genetic, developmental and biochemical properties of ADAMs. Although it has become clear that ADAMs are key players in various cellular processes, the number of studies that address the role of ADAMs in human disease is still limited. Particularly little effort has been devoted to the evaluation of the tissue distribution and functional activities of ADAMs in the human kidney and vasculature. ADAM family member ADAM17 and to lesser extent also ADAM19 have been identified as crucial sheddases of EGFR ligands, which positions these proteins as regulators of EGFR signalling. EGFR signalling can direct cellular behaviour towards cellular growth, migration and proliferation, which has important pathophysiological consequences. The aim of this thesis was to explore the potential implications of ADAM17 and ADAM19 in the physiology of the normal kidney and in the pathophysiology of renal disease and atherosclerosis.

The best-characterized and most appealing function of catalytically active ADAM family members is protein ectodomain shedding, which is also referred to as cleavage or proteolysis (pacman activity). Protein ectodomain shedding is the process of the proteolytic release of growth factors, cytokines and receptors from their membrane-anchored precursors. In most events, protein ectodomain shedding implicates the transformation of an inactive membrane-anchored growth factor into its active alter ego: the released form is able to bind autocrine, juxtacrine and paracrine receptors, whereas often its membrane-anchored precursor is not. As such, ADAM family members have been recognized as central modulators of receptor-driven signalling pathways by mediating the availability of ligands to their respective receptors (chapter 2). Recently, ADAMs, and in particular ADAM17, have been identified as the sheddases responsible for the release of membrane-anchored EGF receptor (EGRR) ligands such as TGF-α and HB-EGF. ADAM-dependent shedding of EGFR ligands can be induced by factors that bind G-protein coupled receptors (GPCRs), such as angiotensin II binding the AT1 receptor. This mechanism of GPCR-induced, ADAM-assisted EGFR activation has been termed EGFR transactivation. In the kidney, EGFR signalling is indispensable for normal renal organogenesis. Experimental targeting of ADAM17 and the EGFR using pharmacological inhibitors has shown therapeutic potential in the treatment of renal fibrotic disorders, which positions the ADAM17 - EGFR signalling axis as potential target for intervention in human renal disease (chapter 3).

The lack of qualitative data on the distribution in the kidney prompted us to examine ADAM17 expression in various stages of human nephrogenesis, in healthy adult human kidneys and in a variety of human renal diseases. In human nephrogenesis, ADAM17 was strongly expressed throughout all gestational ages. In normal adult kidneys, ADAM17 expression was weak except for high distal tubular expression. In human renal diseases, including diabetic nephropathy, hypertensive nephropathy, and membranous glomerulopathy, ADAM17 was upregulated or de novo expressed in almost all renal structures. Rather than being connected to one specific renal disease, the amount of renal ADAM17 expression was associated with markers of renal
histological damage and renal function throughout renal disease. Since TGF-α, one of the ligands for the EGFR and substrate for ADAM17, had been previously linked with fibrogenesis, we examined the expression of TGF-α and ADAM17 within the same renal biopsy and identified colocalization of ADAM17 and TGF-α in interstitial fibrotic lesions, suggesting functional interaction. In vitro, TGF-α shedding could be inhibited with a pharmacological ADAM17 inhibitor. Moreover, ADAM17 inhibition significantly reduced cellular proliferation (chapter 4). In a subsequent study, we extended our knowledge of ADAM17 distribution in the human kidney by examining ADAM17 expression in various stages of renal allograft disease, including histological normal renal allografts, acute allograft rejection and chronic allograft injury. ADAM17 was significantly upregulated in chronic allograft injury when compared to normal kidneys. We did not observe exclusive ADAM17 expression in one of the allograft disease states; rather, similar to our analysis of native renal diseases, we detected strong ADAM17 expression in tissue areas with severe histological damage. Furthermore, we observed colocalization of ADAM17 with EGFR ligand HB-EGF in interstitial fibrotic lesions and demonstrated that ADAM17 inhibition reduced HB-EGF shedding from cultured human mesangial cells. Subsequently, we tested the significance of pharmacological ADAM17 inhibition as method of intervention in a rat model of unilateral renal ischemia-reperfusion injury. Daily pharmacological inhibition of ADAM17 did not effectuate a major beneficial effect on renal tissue outcome. Although the number of tissue macrophages was decreased after 4 days of reperfusion in rats undergoing ADAM17 inhibition, this did not attenuate renal fibrosis, the principle outcome parameter reflecting renal damage, after 14 days of reperfusion (chapter 5).

In the same series of human developing kidneys, normal adult human kidneys and renal disease groups as described in chapter 4, we investigated the renal expression profile of ADAM19. Widespread ADAM19 expression was detected in the nephrogenic zone of human fetal kidneys, suggesting developmental regulation. Normal human kidneys showed weak ADAM19 expression, whereas ADAM19 was de novo expressed or upregulated throughout human renal disease. Independent of the renal disease diagnosis group, ADAM19 expression was associated with glomerular and interstitial damage and with decreased renal function. Based on ADAM19’s postulated sheddase activity with regard to EGF-like growth factor neuregulin and EGFR ligand HB-EGF, we performed a tissue colocalization staining and identified ADAM19 to be coexpressed with tubular and interstitial neuregulin but not with HB-EGF (chapter 6). In human renal allograft disease, we demonstrated a significant upregulation of ADAM19 when compared to healthy human kidneys. To determine whether ADAM19 was specifically related to chronic allograft disease, we studied renal transplant biopsies with and without allograft nephropathy, acute rejection and non-transplant-related interstitial fibrosis. In most renal structures, ADAM19 was significantly higher in chronic allograft disease when compared with histological normal renal allografts or acute rejection. When compared to chronic renal allograft disease, ADAM19 was expressed to a similar extent in non-transplant-related interstitial and glomerular fibrosis, interstitial atrophy and inflammation, indicating that ADAM19 is not specifically linked to chronic renal allograft pathology (chapter 7). In a following study, we assessed the role of syndecan-1 in
renal allograft disease. Syndecan-1 is a heparan sulphate proteoglycan that can bind several growth factors. As such, it is involved in the presentation of growth factors to their respective receptors. Its functioning has been considered to be important for wound healing and inflammation. We identified increased syndecan-1 expression on tubular epithelial cells in renal allografts when compared to normal kidneys and non-transplant renal interstitial fibrosis. The increased tubular syndecan-1 expression in renal allografts correlated with increased renal function and prolonged allograft survival. Knockdown of tubular syndecan-1 in vitro reduced cellular proliferation and positive staining for the proliferation marker KI-67 in renal allografts correlated with syndecan-1 expression. Increased tubular syndecan-1 was associated with local binding of HB-EGF, suggesting that syndecan-1 affects tubular proliferation by binding and presentation of HB-EGF. These results suggest that syndecan-1 plays a role in regeneration of the tubular epithelium upon renal allograft transplantation, which, potentially via mediating HB-EGF availability to the EGFR, may help to shift the balance from non-functional tissue fibrosis towards functional repair (chapter 8).

Besides the postulated involvement of ADAM17 in renal pathophysiology, ADAM17 has also been proposed as a promising target to reduce atherosclerotic cardiovascular disease. To test this concept, we investigated ADAM17 expression in the normal human vasculature and in early human atherosclerotic lesions. Upregulated ADAM17 expression was identified in early human atherosclerotic lesions when compared to non-atherosclerotic vasculature, suggesting a role in disease initiation. To investigate this further, we analyzed the effects of pharmacological ADAM17 inhibition on the development of early atherosclerosis in mice. ADAM17 inhibition for 4 weeks in apoE/- mice fed a Western-type diet resulted in a significant increase in plasma total cholesterol and triglyceride levels. However, despite this proatherogenic lipid profile, atherosclerotic lesion size did not differ between treated animals and controls, indicating that systemic ADAM17 inhibition may not represent a viable treatment strategy for early atherosclerotic cardiovascular disease (chapter 9).
General discussion and future perspectives

The studies described in this thesis provide insight into the expression profile and functional activities of ADAM17 and ADAM19 in the physiology of the normal human kidney and the pathophysiology of renal disease. From this thesis it appears that both ADAM17 and ADAM19 are abundantly expressed in key morphological structures of the developing human kidney, which makes a role in renal development likely. As was postulated in animal studies, ADAM17, and also ADAM19, may contribute to tissue remodelling via the proteolytic release of growth factors, in particular those from the epidermal growth factor (EGF) family. One way to identify genes that play a role in development is to study animal genetic knockout models. It was previously demonstrated that ADAM17 knockout mice display developmental disorders that relate to defects in epithelial cell maturation and organization, among which failing eye lid fusion, severe cardiac and pulmonic abnormalities, and hair and skin defects. ADAM19 knockout mice exhibit cardiac abnormalities, including ventricular septal defects, aberrant formation of cardiac and pulmonic valves and abnormal cardiac vasculature. Due to these developmental defects, all ADAM17 deficient mice and almost all ADAM19 deficient mice died before or shortly after birth. Although no macroscopic and microscopic abnormalities were observed in the kidneys of ADAM17 or ADAM19 deficient mice, reliable assessment of renal development and function was precluded due to this high perinatal mortality. Further clues towards the roles of ADAM17 and ADAM19 in renal development may be obtained from studying long-living heterozygous ADAM17 and ADAM19 knockout mice. The upregulation and de novo expression of ADAM17 and ADAM19 in human renal disease as described in this thesis is closely linked with renal histological damage and decline in renal function. High ADAM expression was identified particularly in areas of renal fibrosis. Moreover, ADAM expression was observed in co-expression with the EGFR ligands TGF-α and HB-EGF. Additionally, in vitro studies in human renal cells showed that EGFR ligands could be shed by ADAM17. The biological implications of ADAM17 in renal fibrotic disease are considered significant as based on a report by Lautrette et al, who showed that ADAM17 inhibition reduced renal fibrosis and improved renal function in an animal model of angiotensin II-induced renal fibrotic disease. This report is convincingly linking ADAM17 directly to renal fibrosis, and one recent paper has been published to support this pathophysiological concept. Kassiri et al. demonstrated that lack of TIMP3, a natural inhibitor of ADAM17, resulted in enhanced ADAM17 expression with a concomitant increase in interstitial nephritis and fibrosis of the kidney. As described in this thesis, pharmacological ADAM17 inhibition in a rat model of ischemia-reperfusion injury did not have a beneficial treatment effect on the principle outcome parameter renal fibrosis. Further studies are needed to corroborate the theory that ADAM17-mediated EGFR signalling is a critical pathway in the development of renal fibrosis. Other animal models with renal fibrosis as principle tissue outcome, such as, for example, unilateral ureteral obstruction, need to be explored to define the potential value of ADAM17 inhibition in renal fibrotic disease. The technology of Cre-Lox recombination, in which a specific sequence of DNA can be targeted and
spliced with the help of the enzyme cre recombinase, would allow for knockout of individual genes in specific tissue types or cells.

A crucial role in renal physiology is related to the abundant expression of ADAM17 and ADAM19 in distal tubular cells of healthy human kidneys. A central function of distal tubules is to fine-tune the amount of electrolytes in the definitive urine by regulating the activity of reabsorption channels. Evidence from recent studies demonstrates a mechanism by means of which ADAM17 and ADAM19 may influence tubular reabsorption. Given the role of ADAM17 (and to lesser extent also ADAM19) in EGFR ligand shedding, it was interesting to note that EGFR ligands seem to play central functions in tubular physiology. EGFR ligand HB-EGF could regulate tubular transepithelial resistance by adjusting the configuration of tight junction proteins in tubules, which is a general mechanism to manage the settings for paracellular ion conductance. EGFR ligand EGF could specifically control sodium reabsorption across distal nephron epithelia by adjusting epithelial sodium channel (ENaC) activity. Moreover, EGF-mediated EGFR signalling was critically involved in renal magnesium homeostasis in a pathway of EGFR-induced activation of Transient Receptor Potential Melastatin 6 (TRPM6), a Mg²⁺ permeable channel in distal tubules that determines fine-tuning of urine magnesium excretion. These preclinical data were corroborated in a clinical cohort study of 98 colorectal cancer patients who, after treatment with anti-EGFR monoclonal antibodies as anticancer treatment, developed hypomagnesaemia resulting from renal Mg²⁺ wasting. These studies illustrate the contributions of EGFR signalling to renal physiology. Further characterization of the involved ADAMs and substrates, particularly in the cascade of EGFR signalling-related tubular processes, needs to be pursued. A first step in this direction can be taken by mimicking tubular physiology in vitro. One approach would be to culture a monolayer of aligned tubular cells with flow of respectively blood and urine on the basolateral and apical side of tubular cells. In this flow system, selective inactivation of individual ADAMs, substrates, receptors and/or electrolyte channels by means of pharmacological inhibition or RNA silencing will be helpful in identifying candidate cascades with implications in tubular physiology.

In certain fields of ADAM research, substantial preclinical data have been gathered to support the start of clinical trials with ADAMs inhibitors in human disease. The most appealing target for investigators has been ADAM17 because it was this ADAM that was discovered to be the sheddase responsible for cleaving the pro-inflammatory cytokine TNF-α. TNF-α is a central player in the pathophysiology of inflammatory conditions, such as rheumatoid arthritis, psoriatic disease, and septic shock. The value of TNF-α in inflammation is already clinically appreciated, as treatment with pharmacological TNF-α receptor blockers forms part of clinical practice in rheumatoid arthritis and psoriatic disease. Similarly, anti-TNF-α treatment has promising potential to treat Crohn’s disease and ulcerative colitis. Therefore, the discovery of TNF-α’s sheddase, ADAM17, was expected to lead to the identification of new treatment strategies for patients suffering from TNF-α-driven inflammatory disorders. Indeed, ADAM17 inhibition in animal models reduced joint arthritis and pneumococcal meningitis, and further animal interventional studies in inflammatory disorders are underway. Human clinical studies are needed to confirm the
promising potential of ADAM17 inhibition as treatment in inflammatory disease. At the same time, it should be realized that also undesirable effects might occur when ADAM-mediated shedding is inhibited in a systemic approach. A clinical example that may carry relevance is the observation that patients who undergo TNF-α receptor blockade for rheumatoid arthritis seem to be more susceptible to viral infections due to insufficient immune reactivity. Cancer is another interesting field in which ADAM17 has shown potential as target of intervention. Because EGFR signalling is critically engaged in some types of human cancer, the discovery that ADAMs can mediate EGFR signalling by shedding EGFR ligands has led to the design of various in vitro and in vivo studies to address the involvement of ADAMs within this pathway. Animal interventional studies have revealed therapeutic success from ADAM17 inhibition, with or without simultaneous EGFR inhibition, to reduce breast cancer. These experimental studies have led to a currently ongoing phase II clinical trial assessing the effect of EGFR blockade, with or without ADAM17 inhibition, on treating human breast cancer. The outcome of this trial is expected in the second half 2009. In the near future, ADAM17 inhibition may emerge as a valuable addition to EGFR blockade when intervening in EGFR signalling as target of intervention in human cancer treatment.

In ADAM research, some general questions illustrate that many more studies need to be performed to completely understand ADAM biology. One issue relates to the similarities in expression profiles of ADAM17 and ADAM19 in the kidney as described in this thesis, which raises the question as to whether ADAM17 and ADAM19 possess redundant functions. Redundancy has been described for the orthologs of ADAM family members ADAM10 and ADAM17 in Caenorhabditis elegans. Moreover, although ADAM17 was identified as the principle sheddase of TNF-α, also ADAM19 has to capacity to shed TNF-α from its membrane-anchored precursor, indicating an overlapping functional profile. Redundancy is a trait that would enhance the complexity of ADAM biology. Moreover, redundancy would render difficulties in the interpretation of studies that rely on the inhibition of an individual ADAM, as other ADAMs may come into action to compensate for the selective inhibitory effect. Another fairly unexplored area is how ADAMs are activated to execute their proteolytic functioning. Moreover, which other factors contribute to ADAM-mediated signalling? What is the significance of heparan sulphates in binding and presenting ADAM-shed growth factors? How do heparan sulphates as syndecan-1 promote ADAM-mediated EGFR activation? Based on these considerations and given the ubiquitous expression of ADAMs and the wide repertoire of substrates for one individual ADAM, it remains a challenging task to generate a complete overview of the involved players in ADAM biology.

In conclusion, ADAM biology forms an exciting area of research and recent scientific work has already revealed many pathways in which ADAMs are involved. This generates great enthusiasm to further understand ADAM biology, particularly in the search for new therapeutic strategies to treat human disease. Future studies that intervene in ADAM biology in renal physiology and disease are eagerly awaited.
References


