The biology of ADAMs in renal disease
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
INTRODUCTION
**Chronic kidney disease**

Chronic kidney disease (CKD), characterized by renal structural deterioration and progressive renal function loss, is an important cause of morbidity and mortality worldwide\(^1\). Patients who suffer from CKD may ultimately develop end stage renal disease (ESRD), leading to the need for renal replacement therapy, i.e. haemodialysis or kidney transplantation. Despite quality improvements in haemodialysis, patients with ESRD still have an increased risk for morbidity and mortality and suffer from a reduced quality of life when compared to the general population\(^2,3\).

With regard to kidney transplantation, only \(~ 50\%\) of renal allografts survive for 10 years due to the development of chronic transplant dysfunction (CTD), which, for the majority of patients, implicates a return to the state of ESRD and the renewed need for haemodialysis\(^4\). The incidence and prevalence of ESRD have increased dramatically over the last decades due to ageing of the population, reduced cardiovascular mortality of patients with CKD, and the increasing incidence of obesity and type 2 diabetes\(^5\). These demographic observations have led to the expectation that the number of patients that reaches ESRD will increase even further in the near future, requiring more financial and human resources to care for these patients\(^6\). Taken together, the consequences of ESRD for patient and society are tremendous. In order to develop novel strategies to deal with the emerging problem of ESRD, better understanding of the pathophysiology of renal disease - and associated disorders such as atherosclerosis - is needed.

In the pathophysiology of renal disease, the cellular processes that form the basis for kidney tissue destruction are diverse and complex. A great variety of signalling events can direct the transcriptional behaviour of kidney cells and, as such, influence renal tissue outcome. Many of the involved signalling and transcriptional processes depend on growth factor-mediated activation of cellular receptors. An example of such a receptor is the epidermal growth factor receptor (EGFR), which has been recognized as a central modulator of tissue remodelling. The study of the regulation of growth factors and -related cellular signalling pathways is a valuable area to explore, since it may lead to a deeper understanding of the pathophysiology of renal disease and may result in the exploration of novel treatment modalities.

**ADAM family members: proteases with Pacman-like activity**

ADAM (A Disintegrin And Metalloproteinase) proteins (Figure 1) belong to the adamalysin subfamily of the metzincin superfamily of Zn-dependent metalloproteinases\(^7\) (see chapter 2). The best characterized function of catalytically active ADAMs is *protein ectodomain shedding*, also referred to as *proteolysis* or *cleavage* or *shedding*, which involves the process of release of the ectodomain of a membrane-anchored protein\(^8\) (Figure 2). A large diversity of molecules undergoes protein ectodomain shedding, among which growth factors, cytokines, adhesion molecules and receptors.

Protein ectodomain shedding may serve different signalling purposes (reviewed in\(^8\)). 1: Shedding of growth factors may enable their binding to (and activation of) specific receptors on the same (autocrine), adjacent (juxtacrine) or distant (paracrine) cells. For example, ADAMs are key sheddases of EGFR ligands. As such, ADAMs are central regulators of EGFR signalling (see
Ectodomain shedding of receptors into the extracellular environment may result in their inactivation. Ectodomain shedding of receptors into the extracellular environment may lead to sequestration of soluble ligands, which prevents the binding of these ligands to cellular receptors, impeding the initiation of signalling pathways. Ectodomain shedding of a receptor may lead to its activation: cleavage within the transmembrane domain releases the extracellular part, but can also release the cytoplasmic domain from its membrane anchor, which allows it to travel through the cytoplasm and enter the nucleus to participate in the transcriptional regulation of specific target genes. This process is named regulated intramembrane proteolysis (RIP).

**Figure 1.** ADAM domain structures. This schematic figure demonstrates the domain structures of a typical cell membrane-anchored ADAM protein, including a metalloprotease domain, disintegrin domain, cysteine-rich region, EGF-like domain, transmembrane domain and cytoplasmic tail (see chapter 2). With permission from: Blobel CP (Nat Rev Mol Cell Biol. 2005 Jan;6(1):32-43).

**Figure 2.** Protein ectodomain shedding. Upon their activation, ADAMs can shed the ectodomain of cell membrane-bound factors into the extracellular environment, leaving a residual membrane-anchored part. With permission from: Blobel CP (Nat Rev Mol Cell Biol. 2005 Jan;6(1):32-43).
The different outcomes of cellular shedding events illustrate the variety of processes to which ADAM-functioning may contribute. The result of shedding varies to a large extent, and depends on the cell type and tissue, the extracellular environment and stimulatory agent, the involved ADAM and substrate, and potential co-working colleague ADAMs. These multi-directed and complex interactions make it a challenging task to understand the implications of individual ADAM-dependent signalling, as well as to understand the interacting roles of ADAM family members in complex circumstances, such as development or disease.

Thus far, the majority of ADAM-related scientific efforts have been devoted to the genetic, developmental and biochemical properties of ADAMs. From these studies, it has become clear that ADAMs are key players in various cellular processes. Moreover, ADAM biology has been linked to disease-related processes, such as the development of Alzheimer's disease, thrombotic thrombocytopenic purpura, endotoxic shock, asthma and cancer (see chapter 3). Nevertheless, the number of studies that address the role of ADAMs in human disease is still limited, and hardly any data are available on the tissue distribution and roles of ADAMs in the human kidney and vasculature.

**Aim and outline of thesis**

This thesis explores the potential implications of ADAM17 and ADAM19 in the development of renal disease and atherosclerosis.

In order to provide background information on the ADAM family, we first discuss the biochemical, genetic, physiologic and pathophysiologic aspects of ADAMs, with specific focus on their involvement in human disease (**chapter 2**). The best-characterized function of ADAMs involves the release of growth factors from their cell membrane precursors, allowing for binding of these factors to their receptors. In recent years, particular interest was raised to unravel the role of ADAMs in mediating EGFR signalling. The mechanism of ADAM-mediated activation of the EGFR, and the consequences of EGFR signalling in the kidney, are described in **chapter 3**.

Given the limited number of reports on ADAM17 and ADAM19 expression in human tissue, especially in the kidney, it was our aim to investigate their tissue expression in the developing human kidney, in normal human kidneys, and in a variety of human renal diseases. Making use of a large series of biopsies from patients with renal disease, we studied potential associations between ADAM17 and ADAM19 tissue expression on one hand and parameters of renal structural integrity and renal function on the other hand. In order to identify potential mechanisms through which ADAM17 and ADAM19 are involved in renal (patho)physiology, we studied colocalization between ADAMs and EGFR ligands in diseased renal tissue. Moreover, we analyzed the shedding activity of ADAM17 in cultured human tubular epithelial cells and glomerular visceral epithelial cells (**chapter 4 and 5**). In addition to our study of native human renal diseases, we analyzed the expression pattern of ADAM17 and ADAM19 in various forms of human renal allograft disease. Additionally, to elaborate on the in vivo implications of ADAM17 activity, we applied an experimental model of renal ischemia-reperfusion injury in the rat to assess
the value of pharmacological ADAM17 inhibition as therapeutic regimen (chapter 6 and 7). As an additional approach to identify mediators of ADAM-mediated EGFR signalling, we investigated the role of the heparan sulphate proteoglycan syndecan-1 in human allograft disease. Syndecan-1, a growth factor-binding protein, is thought to facilitate cellular signalling by presenting growth factors to their receptors. In chapter 8, we analyzed whether the expression of syndecan-1 is correlated with human renal allograft survival. Moreover, we addressed syndecan-1’s potential to bind growth factors such as the EGFR ligand HB-EGF, which may provide further insight into the processes that contribute to EGFR signaling. In a separate study, we investigated the expression of ADAM17 in the normal human vasculature and in different stages of human atherosclerosis (chapter 9). Furthermore, we studied the potential of ADAM17 inhibition as treatment for atherosclerotic vessel disease in an atherosclerotic animal model of Western-type diet-fed apolipoprotein deficient mice. The results from the studies as delineated above, as well as the implications and directions for future research efforts, are summarized and discussed in chapter 10.
References


