The kallikreins: old proteases with new clinical potentials

Manfred Schmitt1; Thomas Renné2,3; Andreas Scorilas4

1Clinical Research Unit, Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technische Universität Muenchen, Munich, Germany; 2Department of Molecular Medicine and Surgery, Karolinska Institutet and University Hospital, Stockholm, Sweden; 3Division of Clinical Chemistry, University Hospital Eppendorf, Hamburg, Germany; 4Department of Biochemistry and Molecular Biology, University of Athens, Athens, Greece

Tissue kallikrein (KLK1), kallikrein-related peptidases (KLK2-15), and plasma kallikrein (KLKB1) are secreted serine proteases with broad expression and physiological roles (1-4). KLK1–15 genes are tightly clustered in a tandem array on chromosome 19q13.3–q13.4 spanning ~300 kb. This locus represents the largest contiguous cluster of protease genes within the entire human genome. The KLKB1 gene is located apart from the kallikreins (KLKs) within the genetic locus 4q35.

The 10 articles in this Theme Issue comprise a broad spectrum of areas of investigation and give an up-to-date summary on structure and function correlations of KLKB1 and KLKs including their clinical relevance in human disease states such as cancer, cardiovascular disorders, diabetes and skin diseases. Cumulatively, this collection highlights important roles of KLKB1 and KLKs in a broad array of pathophysiological processes.

Lundwall (5), in this Theme Issue, gives a concise overview of the evolution of the KLK locus, which is subdivided into two distinct regions. The genetic sublocus, in that KLK1–4 are localised, has undergone several alterations during its evolution, which gives rise to novel genes, closely related to KLK1, such as KLK3, which encodes the prostate-specific antigen (PSA), the most broadly used tumour biomarker in routine clinical prostate cancer management (6). The same sublocus gave birth to 13 unique genes in the mouse and nine unique ones in the rat.

The other sublocus, encompassing KLK5–15, has been more strictly conserved through evolution. Interestingly, the KLK gene family may have arisen as part of the process that generated features that are unique to mammals, such as skin with hair and sweat glands, and specialised anatomical sites of the brain and the reproductive system. Upon secretion, KLKs flow into bodily fluids such as sweat, milk, saliva, seminal plasma, cerebrospinal fluid, or remain in the pericellular space (7).

Despite similarities in name, tissue kallikreins (KLK1–15) and plasma kallikrein (KLKB1) exert different functions. KLKB1 and KLK1 serine proteases are involved in the production of kinin-peptides, bradykinin and Lys-bradykinin (kallidin), respectively. The article by Pathak et al. (8) reviews the protein structural data available for plasma kallikrein (KLKB1) and tissue-associated KLKs and examines their molecular mechanisms of zymogen activation and substrate recognition, focusing on KLKB1 and KLK1-mediated cleavage of the high-molecular-weight kinin precursors, the kininogens. The authors claim that the structural information has proved highly informative in understanding the molecular mechanisms of kinin production and they envision that these structures eventually will serve as templates to design inhibitors for treatment of various kinin-mediated diseases.

The 15 tissue-associated KLKs are mainly secreted by epithelial cells and are found within the glandular epithelia of many organs, such as the colon, stomach, pancreas, breast, ovary, and prostate. Some KLKs are present in the skin as well, which is one of the most extensively studied organs in terms of KLK enzymatic activity and its regulation. Yet, the exact mechanism underlying the tight transcriptional regulation of KLK genes in skin is still matter of ongoing research. In the skin, KLKs are expressed as enzymatically inactive preforms; they require proteolytic cleavage to become enzymatically active.

KLKs may activate each other or get activated by other proteases such as the matrix metalloproteinases or proteases of the thrombostasis axis, e.g. the serine proteases plasmin and urokinase-type plasminogen activator (uPA). Egelrud et al. have originally identified two KLK family members, namely KLK5 and KLK7, in the uppermost part of the skin (stratum corneum) (9). These serine proteases are profoundly involved in skin desquamation and inflammatory processes. Since KLKs are tightly regulated to achieve physiological skin functions, their dysregulation usually results in critical pathological conditions, as reviewed by Fischer et al. in this Theme Issue (10). KLK5 and KLK7 proteolytic activity is balanced by specific endogenous protease inhibitors, either irreversibly (serpin peptidase inhibitors) or reversibly (serine peptidase inhibitors, Kazal-type; SPINKs). This implies that novel synthetic candidate drugs to KLKs could constitute an innovative way of targeting skin diseases (11).

Three of the state-of-the-art review articles selected for this Theme Issue summarise KLK functions in cancer, with focus on tumour growth and metastasis of patients with cancer of the gastrointestinal or the genitourinary tract. Kontos et al. looked at the functional role of KLKs in the digestive system and describe their current status as prognostic and/or predictive biomarkers in gastrointestinal cancer (12).

The tissue-based expression of KLK family members has been associated with various clinicopathological parameters of patients suffering from gastric, colorectal, pancreatic, hepatic or esophageal cancer. In gastric cancer, several KLKs are involved in...
cancer initiation and progression, thus facilitating tumour invasion and metastasis. In this disease, KLKs are also regulators of proteinase-activated receptors (PARs). Cleavage of these G-protein-coupled receptors by KLKs triggers the activation of the MEK/ERK pathway and, subsequently, cancer cell survival and proliferation. Pericellular proteolysis of e.g. the extracellular matrix proteins E-cadherin, fibronectin, vitronectin and collagens, but also other extracellular matrix components, may modify the tumour microenvironment; thus affecting tumour cell growth and invasion, apoptosis, angiogenesis and metastasis.

Focal point of attention of the article by Dorn et al. (13) is the clinical impact of a group of KLKs in female and male urogenital tract malignancies. Remarkably, all of the 15 KLKs are expressed in the normal prostate, testis, and kidney. The uterus, ovary and urinary bladder, however, express a limited number of KLKs only. Most of the information regarding KLK expression in tumour-affected urogenital organs is available for ovarian cancer. All of the 12 KLKs tested so far were found to be elevated in ovarian cancer depicting them as valuable prognostic/predictive biomarkers and targets of cancer therapy to affect tumour invasion and metastasis.

The tumour-tissue-associated KLK2 and KLK3 (PSA) proteins, which are released into the blood, are among the most well-studied and clinically relevant KLKs in prostate cancer. KLK2 and KLK3 expression is regulated by the androgen receptor, whose activity has a fundamental role in prostate tissue development and progression of the disease. The review by Thorék et al. (14) discusses the biological roles of KLK2 and KLK3 as well as the historical and advanced use of their detection to accurately detect and guide treatment of prostate cancer.

Four of the articles of this Theme Issue focus on the clinical impact of tissue kallikrein (KLK1) or plasma kallikrein (KLKB1) in cardiovascular diseases or diabetes. KLK1, the initial member of the KLK family, constitutes the most important constitutive kallidin-forming enzyme in arteries, heart and kidney of mammals and hence plays an important role in both homeostasis and pathophysiology. Translation of KLK1 mRNA results in a pre-proenzyme containing a short signal peptide, followed by a propeptide and a catalytic domain, which remains in the mature, enzymatically active, protein. Murine and human genetic models of KLK1 deficiency, including its enzymatic inactivation in the former and a major loss-of-function polymorphism in the respective gene of the latter, have provided a useful tool for the investigation of the physiological role of this enzyme, as reviewed by Wæckel et al. (15).

Impaired KLK1 activity induces arterial dysfunction and renal tubular defects. On the other hand, data implicating KLK1 in blood pressure regulation and in protection against hypertension seem to be contradictory. Causality between KLK1 and kinin deficiency, and severity of cardiovascular diseases, has been well-documented so far; cardiac as well as peripheral ischaemic heart diseases constitute the most prominent examples. Diabetic renal disease can also worsen due to KLK1 deficiency. The authors summarise substantiating studies in support of this notion, concluding that KLK1 and kinins should be viewed as putative therapeutic targets.

KLKB1 is a serine protease with well-characterised roles in the intrinsic coagulation cascade, in inflammation, vascular permeability control, the fibrinolytic system, the renin-angiotensin system, the alternative complement pathway and in the innate immune system. The KLKB1 precursor plasma prekallikrein (pre-KLKB1) is produced by the KLKB1 gene, mostly expressed in the liver, pancreas and kidney but low KLKB1 levels are detectable in various other organs as well, including the brain, heart, spleen, thymus, testis and intestine (16).

Most of the physiological actions of KLKB1 have been attributed to cleavage of its two major substrates, coagulation factor XII and high-molecular-weight kininogen (17). KLKB1 circulates in plasma as a complex with high-molecular-weight-kininogen. The review of Björkqvist et al. (18) gives a comprehensive overview of the biochemistry of this enzyme. Focus of their article is on recent in vivo studies that have established important functions of KLKB1 in thrombotic and proinflammatory disease states.

Feener et al. (19) highlight important roles of KLKB1 in diabetes mellitus, and present potential mechanisms that mediate activation of the KLKB1-kinin system in this disease. The authors summarise their studies elucidating the effects of KLKB1 inhibition. For instance, KLKB1 inhibitors have been demonstrated to ameliorate impairments in cerebral haemostasis and to decrease retinal vascular permeability induced by hypertension and diabetes. As suggested by the authors of this review, pharmacological approaches inhibiting KLKB1 could contribute to the control of proinflammatory effects of bradykinin peptides with concurrent preservation of the physiological functions of KLKB1, mediated by bradykinin receptors. Therefore, the blockade of the KLKB1-kinin system appears as a promising therapeutic strategy in diabetes mellitus (20).

Finally, de Maat et al. (21) provide information about the physiological role of the plasma protein factor XII (FXII), a member of the contact system, that is involved in KLKB1 activation. FXII has a crucial role in thrombotic diseases and contributes to inflammatory conditions as well. The article describes the potential application of novel-type FXII-blocking nanobodies to investigate the role of activated FXII under normal physiology and in the disease-state.

Molecular analysis of proteases remains one of the most promising areas of translational research since there is strong evidence for an association between protease levels and pathophysiology. We emphasise that further understanding of the clinical impact of one of the most fascinating groups of proteases, the kallikreins, will lead to an increase in scientific knowledge for the well-being of the patients. The task now is to conduct that kind of clinical research that would determine whether the use of plasma kallikrein (KLKB1) or the tissue-associated KLKs as molecular biomarkers would provide perceptible benefits to those who are at increased risk of developing cancer, cardiovascular disease or diabetes.

Conflicts of interest
None declared.
References