Guest Editorial

The 3rd International Symposium on Kallikreins and Kallikrein-Related Peptidases

The 3rd International Symposium on Kallikreins and Kallikrein-Related Peptidases (IKS 2009) took place from August 30 to September 2, 2009, in Munich, Germany. Pioneering work of H. Kraut, E.-K. Frey, and E. Werle conducted here in Munich in the first half of the last century led to the identification of tissue kallikrein as the proteolytic enzyme liberating the vasoactive peptide kallidin from its substrate low-molecular-weight kininogen. As we know today, tissue kallikrein (KLK1) is just one member of the human kallikrein-related peptidase family (for reviews see, e.g., Lundwall et al., 2006; Lundwall and Brattsand, 2008; Sotiropoulou et al., 2009). This family encompasses 15 *KLK* genes located on chromosome 19q13.4 and constitutes the largest cluster of serine proteases in the human genome. *KLKB1*, the gene encoding plasma kallikrein, which is more closely related to blood coagulation factor XI than to KLK1, is located on a different chromosome, 4q35.

In contrast to KLK1, the other members of the KLK family display weak or no kininogenase activity. KLK1 thus is the only 'true' tissue kallikrein, whereas the kallikrein-related peptidases target other substrates and fulfill other functions. The highly homologous members KLK1, KLK2 (glandular kallikrein), and KLK3 (prostate-specific antigen, PSA) form a subgroup within the KLK family (named 'classical' KLKs) sharing the so-called kallikrein-loop, a unique 11-residue insertion in the 99-loop of serine proteases that lies just above the active-site cleft and strongly impacts enzyme/substrate interactions. Whereas KLK1, -2, and -3 have been known for a long time, the other members of the KLK family were identified rather late in the 1990s. All KLKs are produced as single-chain pre-pro-proteases containing a single chymotrypsin- or trypsin-like catalytic domain. After secretion as inactive zymogens, the KLKs are extracellularly activated by removal of their short propeptides. The majority of these kallikrein-related peptidases prefer, like trypsin, basic P1 residues, whereas few members cleave similar to chymotrypsin or with a mixed P1 specificity.

The identification of the KLK gene cluster has boosted intensive research focusing on the analysis of the (patho)physiological functions of the KLK proteases. More than 150 participants from 20 different countries (14 European countries, USA, Canada, Australia, Japan, Mexico, and Brazil) attended the symposium in Munich that was supported in particular by the German Research Association (DFG), the International Union for Biochemistry and Molecular Biology, and the E.K. Frey-E. Werle Foundation.

The research progress in the KLK field presented and discussed at the symposium is particularly exemplified by the work of the recipients of the three awards presented by the E.K. Frey-E. Werle Foundation. The 'Commemorative Gold Medal' was given to Michael Blaber, mainly for his fundamental findings on the involvement of KLK6 in neurological disorders and the elucidation of activation relationships within the KLK family and with the thrombostasis system. In 2002, Blaber and colleagues solved the crystal structure of the first mature, active human kallikrein-related peptidase, KLK6, which is abundantly expressed in the central nervous system. At the same time, the structure of pro-KLK6 was reported displaying a completely closed specificity pocket and other striking structural differences. Comparison of both structures allowed a detailed analysis of rearrangements occurring upon proteolytic activation. Meanwhile, the structures of several other human KLK proteases, i.e., KLK1, -3, -4, -5, and -7, have been published.

In normal and malignant tissues, members of the kallikrein family and various other endopeptidases are coexpressed, raising the possibility that KLKs can participate in proteolytic cascades. To analyze the activation of the human pro-KLKs, Blaber and coworkers established a high-throughput system allowing characterization of KLK propeptide cleavage. For this, all propeptides of the KLK protease family were individually fused to a soluble, protease-resistant carrier protein and these fusion proteins incubated with mature KLKs or other active proteases revealing a series of potential activating pairs (for a review see Blaber et al., 2010). Other findings emphasize that KLKs are integrated within proteolytic networks. Several trypsin-like KLKs have, e.g., been shown to be potent activators of the zymogens of the serine protease urokinase-type plasminogen activator (uPA) as well as the metalloproteases meprin α and β. Interestingly, in a complementary setting, meprin β cleaves pro-KLK7 two amino acids upstream its activation cleavage site. Such processing, although leaving the zymogen inactive, can prime pro-KLK7 towards further activation by endoproteases and, potentially, exoproteases as well (Ohler et al., 2010).

Many members of the KLK family represent biomarkers, particularly in prostate, ovarian, colon, and breast cancer, either regarding prediction of the course of the disease (prognosis) or response to therapy. Andreas Scorilas received the 'E.K. Frey-E. Werle Promotion Prize' for his innovative investigations on the development of highly sensitive quantitative assays for biomarkers, particularly KLKs, and the evaluation of their clinical relevance. In the present highlight

issue, effects on KLK5 gene expression in prostate cancer cells upon treatment with chemotherapeutic agents are described (Mavridis et al., 2010). Analysis of mRNA expression of the KLK gene family is complex, as alternative splicing is frequent. Increased expression of an alternatively spliced KLK3 variant was observed in prostate cancer (Whitbread et al., 2010). In addition to mRNA quantification and determination of protein levels by ELISA, expression of KLK proteases within normal and malignant tissues has been extensively analyzed by immunohistochemical methods (see, e.g., Gabril et al., 2010, and Seiz et al., 2010). To investigate the levels of kallikrein-related peptidase activity in ascitic fluids of ovarian cancer patients, an irreversible inhibitor of trypsin-like proteases, hooked to biotin via a chemical linker, was used to precipitate proteases with streptavidin-coated magnetic beads (Oikonomopoulou et al., 2010a). Interestingly, only low levels of active KLK enzymes relative to the total amounts determined by ELISA were present in these ascitic fluids. One possible explanation is that active KLK proteases rapidly form complexes with natural inhibitors also present in ascitic fluids. Nevertheless, as over- or *de novo* expression of KLKs is often associated with poor patient outcome, these proteases certainly represent attractive targets for tumor therapy. Although still a matter of debate, even KLK3 (prostate-specific antigen, PSA), which at present is clinically used as a diagnostic and monitoring marker, could be a candidate for cancer treatment by inhibiting its proteolytic activity (LeBeau et al., 2010). The use of engineered sunflower trypsin inhibitors to target KLK4 or KLK14 (Swedberg et al., 2010) or other scaffolds such as ecotin (Stoop et al., 2010) could allow a tight control of proteolytic activity in cancer patients. Furthermore, cyclic peptides were developed to inhibit the proteolytic activity of KLK2 (Hekim et al., 2010). A novel approach to regulate KLK overproduction is the use of microRNAs, small RNAs that do not code for proteins but function by controlling the expression of other genes (White et al., 2010).

In addition to their contribution to the degradation of extracellular matrix proteins and their supposable involvement in inflammatory pathways (Sotiropoulou and Pampalakis, 2010), it is now clear that certain KLKs serve as signaling molecules controlling cell functions through specific membrane receptors, the protease-activated receptors (PARs). The signals, induced by proteolytic cleavage of the receptors, modulate processes including platelet aggregation, vascular relaxation, cell proliferation, cytokine release, and inflammation. Katerina Oikonomopoulou, recipient of the 'E.K. Frey–E. Werle Young Investigator Award 2009', demonstrated in cell model systems that KLK5, -6, and -14 can activate PAR1, -2, and -4 to different extents (reviewed in Oikonomopoulou et al., 2010b). Together with independent work by other groups, these results suggest that some KLK proteases could be major modulators of PAR-mediated signaling in inflammatory diseases and/or cancer.

Although the emphasis of the IKS 2009 symposium in Munich was on the analysis of the (patho)physiological functions of the 'new' kallikreins KLK4–KLK15, it offered a series of presentations on the progress in research concerning the 'classical' kallikreins KLK1–KLK3 as well. This is reflected by several articles in the present highlight issue, including a review on the role of KLK1 in various types of diseases and wound healing (Chao et al., 2010), the presentation of a protein network linking the kallikrein-kinin and renin-angiotensin systems developed by employing three-dimensional protein-protein interaction databases (Stoka and Turk, 2010), and the analysis of transgenic mice expressing rat tonin, the homolog of KLK2, in the brain (Cardoso et al., 2010). In the case of KLK1 and KLK8, knockout mouse models have also been utilized to elucidate their physiological functions. Interestingly, analyses of $KLK8^{-/-}$ mice indicate that this protease can also have functions in the brain as well as in the skin (see Yoshida, 2010).

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