

Editorial Focus

Using kallikrein-related peptidases (KLK) as novel cancer biomarkers

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The scientist Emil-Karl Frey, a scholar of the famous surgeon 'Geheimrat' Ferdinand Sauerbruch, observed in 1925 a considerable reduction in arterial blood pressure when he injected human urine into dogs, an until then unknown cardioactive and vasoactive effect which he attributed to an unspecified substance with potential biological functions (1, 2). This then uncharacterized kininogenase was named kallikrein (Greek synonym for pancreas: kallikreas) by the three German scientists H. Kraut, E.-K. Frey and E. Werle, who in 1930 reported that the pancreas is a rich source of this endogenous hypotensive substance (3). A few years later, E.-K. Werle identified kallikrein as a proteolytic enzyme (KLK1 [EC 3.4.21.35] initially named tissue kallikrein, glandular kallikrein, kallikrein 1 or hK1) that liberates the biologically highly active, basic polypeptide 'DK' or kallidin (i.e. lys-bradykinin) from the blood plasma protein kallidinogen (now termed high- and low-molecular-weight kininogen, HMWK- and LMWK-kininogen) (4). The serine protease KLK1 is different from the later discovered plasma serine protease kallikrein (KLKB1 or Fletcher factor [EC 3.4.21.34], located on chromosome 4q34-q35) which is part of the plasma contact activation system and structurally related to coagulation factor XI (5), whereas KLK1 located on chromosome 19q13.4 is structurally related to the serine protease trypsin (5–7). Later on, two more glandular kallikreins were identified in humans: KLK2 (initially named human glandular kallikrein 1, hGK1, and later human kallikrein 2, hK2) (8) and KLK3 (also known as prostate-specific antigen [PSA]) (9, 10), both located on chromosome 19q13.4 as well (6). Some time later, before the turn of the millennium, 12 additional serine protease genes were independently discovered by three major research groups to be tandemly located on chromosome 19q13.4 and therefore assigned to the glandular kallikrein family as well, based on their localization to chromosome locus 19q13.4 and sequence/structural similarities with the three traditional glandular kallikrein genes (11–13), thus forming a new, "extended" kallikrein gene family (6, 7). Hence, a new comprehensive nomenclature was needed, and since 2006 of the 15 tissue-related kallikreins 14 are denoted kallikrein-related peptidases (KLK2 to KLK15) (14), except for kallikrein 1, since this is the only serine protease in the KLK-family with significant kininogenase activity (15).

Now, with the comprehensive description of the human KLK gene locus available, one of the main bio-medical research endeavors is focused on the clarification of the (patho)physiological functions of the KLK (16). KLK are expressed in a wide range of tissues, although individual KLK do have quite restricted expression patterns suggesting a functional role in diverse (patho)physiological processes (17), e.g. skin desquamation and other skin diseases, tooth development and enamel defects, Alzheimer's disease, Parkinson's disease, and cancer (6, 15). Besides KLK3 (PSA), a well known biomarker for prostate cancer, other members of the KLK family are now considered as potential biomarkers in malignant disease states as well. Recently, several of the kallikrein-related peptidases have shown promise as prognostic and predictive cancer biomarkers, e.g. for cancer of the prostate, testis, kidney, breast, ovary, lung, colon, pancreas, and brain (6, 18). Interestingly, some of the KLK can auto-activate, while others activate each other, suggesting that the KLK may be part of an enzymatic cascade similar to the urokinase/plasminogen system or the matrix metalloprotease family, which are associated with the malignant potential of multiple malignant diseases (19, 20).

Currently, three well-established technologies are in use to quantify kallikrein-related peptidase expression: RT-PCR (mRNA), ELISA (protein) and DNA-methylation (epigenetic modification of CpG islands in genomic DNA) (6, 21). From these assays, it can be assumed that certain members of the kallikrein-related peptidase gene family are differentially expressed in a wide variety of carcinomas. Although still under investigation, it has been shown that these factors can serve as new biomarkers for diagnosis, prognosis, and monitoring of cancer (6, 7, 15, 16). In Figure 1A the number of citations per year are displayed with reference to KLK3 (PSA) and cancer, encompassing investigations in human and animal tissues and cell lines, respectively. Between 1982 and 2008, 12,135 articles were published in MedLine including 1,631 reviews. Most of the experiments were conducted with human tumor tissues, tumor cell lines or blood components (12,047); 1,523 considering clinical trials. Only a small portion of the published articles dealing with the clinical impact of kallikrein-related peptidases (556, includ-

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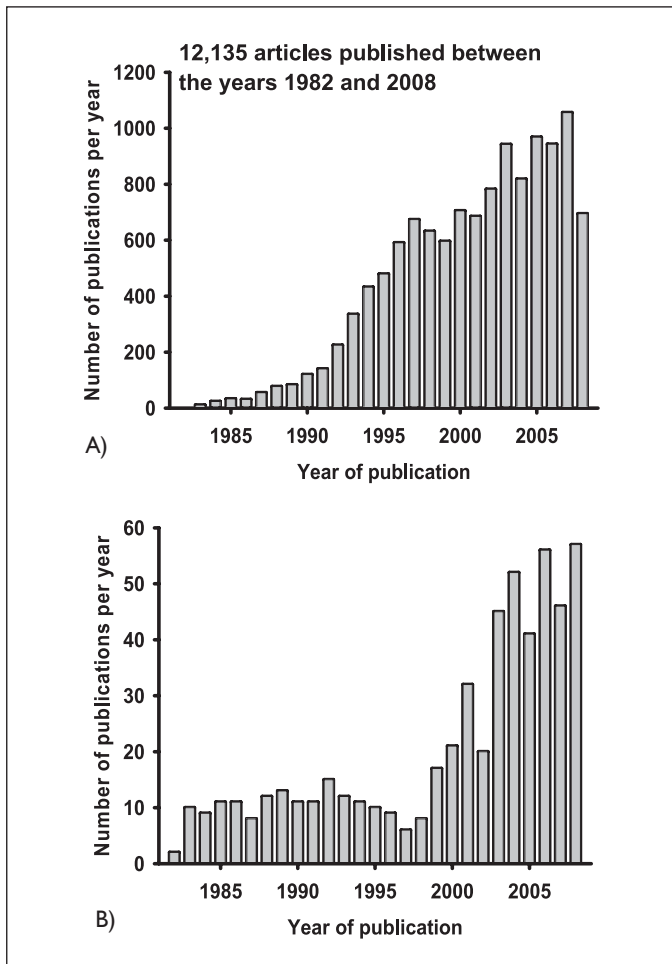


Figure 1: Articles published between 1982 and 2008 relevant to KLK and cancer. A) Articles relevant to KLK3 (PSA) and cancer. B) Articles relevant to cancer and KLKs, except KLK3.

ing 79 reviews) did not involve KLK3 but any combination of the other fourteen KLK; this is displayed in Figure 1B.

Interestingly, from 1982 to 2008, most of the articles published regarding the (pre)clinical relevance of kallikrein-related peptidases in cancer (n=12,123) centered on the investigation of prostate cancer tumor tissue specimens or blood components, followed by rather low numbers of articles focusing on other types of solid malignant cancers (Fig. 2).

KLK are primarily known for their biomarker value in prostate, ovarian, and breast cancer, but regarding prediction of the course of the disease (prognosis) and/or response to therapy more recent data point to an important role of certain KLK in several other malignancies, including those of the gastrointestinal tract, lung, brain, head and neck (22). Nonetheless, for the other cancer types, current efforts lie in establishing kallikrein-related peptides as cancer biomarkers to increase the accuracy of cancer diagnosis, prognosis and prediction of therapy to allow individualized therapy and to improve clinical management (23).

Pioneering work in this direction has been conducted by E. P. Diamandis (University of Toronto, Canada) (6), and his former research associate, A. Scorilas (University of Athens, Greece). Scorilas and colleagues have published innovative work regard-

Target organ of KLK research	Number of published articles	Number of reviews included
Prostate	12,123	1,637
Bone	939	128
Breast	475	71
Head and neck	260	18
Ovary	218	35
Lung	210	34
Liver	177	17
Colon	148	25
Muscle, soft tissue	147	16
Skin	109	11
Pancreas	90	8
Brain	75	13
Stomach	43	4
Kidney	30	7

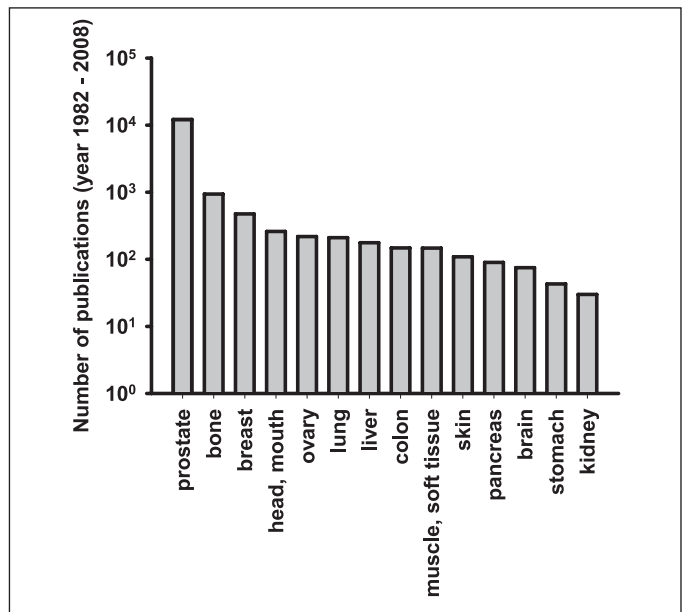


Figure 2: Articles published between 1982 and 2008 with reference to KLK expression and cancer, itemized according to organ specificity.

ing the biochemistry and clinical impact of various kallikrein-related peptidases, other than KLK3, for different types of cancer such as cancer of the ovary (23), breast (24), testis (25), head and neck (26) and prostate (27). Now, new results by Scorilas and co-workers with reference to four different cancer entities are published in this and the upcoming issue of *Thrombosis and Haemostasis*, describing clinical impact of either KLK4, -5, -7, -8, or -11 in prostate, breast, ovarian, or colon cancers (28–31).

In the paper by Thomadaki et al. (28), the objective was to investigate possible alterations in mRNA expression of the KLK5 and KLK11 genes in prostate cancer cells in response to treatment with various chemotherapeutics. Indeed, for the first time, a drug-dependent prostate cancer cell response, especially in modulating KLK5 expression, was demonstrated by the authors,

pointing to the fact that variation in KLK5 mRNA expression may be used as a potential cancer biomarker to predict treatment outcome in prostate cancer patients. The study by Pachristopoulou et al. (29), also for the first time, reveals that expression analysis of the KLK4 gene may serve as a novel biomarker in breast cancer since KLK4 mRNA is elevated in breast cancer tissue compared to benign tumor specimens. Elevated expression is correlated with advanced tumor grade, tumor aggressivity and negative progesterone receptor status, features pointing to poor clinical outcome of a breast cancer patient.

KLK8 expression in ovarian cancer has been investigated before by Scorilas and colleagues and was described as a favorable biomarker associated with early stages of ovarian cancer disease, employing immunoenzymometric (ELISA) determination of KLK8 protein expression in tumor tissue extracts (32). Now, Scorilas and colleagues present a novel way of quantitative KLK8 protein expression analysis, by making use of a modified, fluorescence-based immunohistochemical technique, AQUA (automated in situ quantitative protein analysis) (30). AQUA allowed to quantify KLK8 protein expression in a tumor tissue microarray mainly consisting of tumor specimens obtained from advanced stage ovarian cancer patients. AQUA, or similar technologies, will help to improve quantitative protein analysis of KLK8 protein expression, determined as single analytes or in combination with other KLK or different cancer biomarkers such as CA-125, HER2, urokinase-type plasminogen activator, to name a few. Advances by Scorilas and colleagues have oc-

curred in other aspects of oncology as well, for instance with respect to first-time description of KLK7 mRNA expression in colon cancer tumor tissue samples and clinical outcome of the cancer patients (31). The data indicate that upregulation of KLK7 mRNA expression in tumor tissues is correlated with poor prognosis of patients afflicted with colon cancer.

The initial claim to fame of kallikrein-related peptidases was mainly attributed to the clinical impact of KLK3 (PSA) as a biomarker for screening and monitoring of prostate cancer (6, 9, 15), but recent reports, including the four articles by Scorilas and coworkers (28–31), have demonstrated that several other KLK are promising cancer biomarkers as well, with specific prognostic and predictive (therapy response) value. Furthermore, KLK may not only be targets for cancer prognosis and therapy response but also molecular targets for therapeutic intervention, as highly specific inhibitors of KLK activity have been developed and thus may represent promising agents for cancer treatment (33). Structural, functional, and regulatory studies may help to develop such new strategies to prevent and treat disorders to which individual members of the KLK protease family contribute significantly (34). The biological and clinical implications of these important findings for cancer and other pathophysiological processes will also be a topic of the upcoming 3rd International Symposium on Kallikreins and Kallikrein-related Peptidases, in Munich, Germany, August 30 – September 2, 2009 (<http://www.IKS2009.de>).

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