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Human tissue kallikreins and testicular cancer

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Human tissue kallikreins are fifteen homologous genes encoding for secreted serine proteases and residing tandemly on chromosome 19q13.4. These enzymes are highly expressed in a variety of tissues and participate in diverse physiological processes. Human tissue kallikreins were found to be associated with several malignancies, especially endocrine-related cancers, including prostate, ovarian, breast and testicular cancer. In testicular germ cell tumors, some tissue kallikrein genes, including KLK5, KLK10, KLK13 and KLK14, were found to be significantly down-regulated. Tissue-specific splice variant forms of some kallikreins have been identified in the testis. In this paper, the expression of KLK5, KLK10, KLK13 and KLK14 in testicular cancer and their possible roles during testicular cancer development, as well as their clinical applications are briefly reviewed.

Key words: Human tissue kallikreins; testicular cancer; tumor markers; splice variants; tissue-specific expression; prognostic markers.

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OVERVIEW OF THE HUMAN TISSUE KALLIKREIN GENE FAMILY

Historically, kallikreins referred to a group of proteases that could release vasoactive peptides from high molecular weight precursors (1). There are two categories of kallikrein enzymes: plasma kallikrein and tissue kallikrein. Plasma kallikrein is encoded by a single gene (KLKB1) residing on chromosome 4 (2). This enzyme is produced solely by liver cells and cleaves high molecular weight kininogen to release bradykinin (3). Tissue kallikrein is now a generic term describing all serine proteases that tandemly locate on human chromosome 19q13.4 and share extensive homology at both the DNA and protein level (4). This definition is not based on the enzymatic activities, but more on sequence homology and physical linkage. Initially, the human tissue kallikrein gene family was thought to include only three members: KLK1 (renal/pancreatic kallikrein), KLK2 (glandular kallikrein), and KLK3 (prostate specific antigen) (1). During the past few years, another 12 new members were identified in the same locus (4). These new kallikrein genes were given the names as KLK4 to KLK15. The encoded proteins were designated as hK4 to hK15 (5).

LOCUS ORGANIZATION

The human tissue kallikrein gene locus spans about 340 kb of genomic sequence. The organization of the kallikrein genes in the locus is shown in Fig. 1. The kallikreins reside tandemly in the locus without any other intervening genes. The testicular acid phosphatase (ACPT) gene and the SIGLEC-9 gene define to the 5′ and 3′ end of the locus, respectively. KLK2 and KLK3 are transcribed in the same direction, from centromere to telomere, whereas the rest

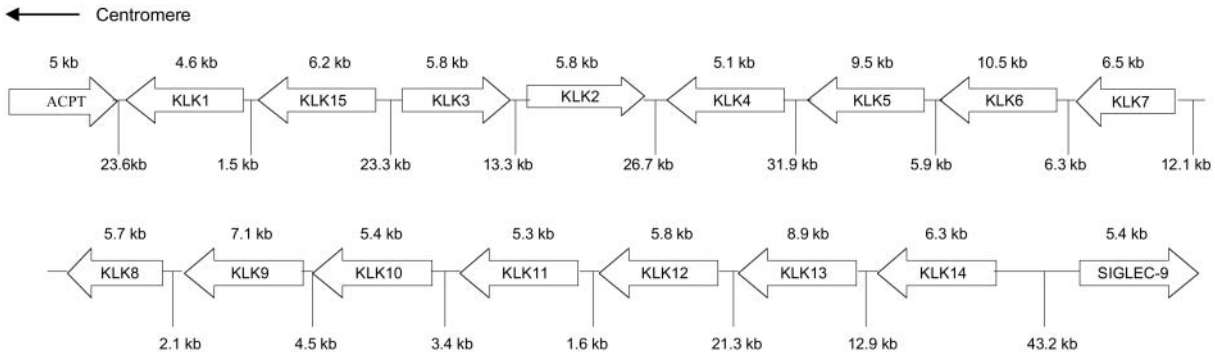

Fig. 1. Schematic diagram showing the organization of the human tissue kallikrein locus on chromosome 19q13.4. The kallikrein genes (KLK1 to KLK15) are represented by arrows, pointing to direction of transcription. Lengths of genes and between genes are indicated in kilobases. The genes adjacent to the kallikrein locus, testicular acid phosphatase (ACPT) and SIGLEC-9, are also shown.

of the kallikreins transcribe in the opposite direction.

TISSUE EXPRESSION AND HORMONAL REGULATION OF THE HUMAN TISSUE KALLIKREINS

Human tissue kallikreins were found to be expressed in a variety of tissues, such as the salivary glands, esophagus, tonsil and skin, determined by both RT-PCR (mRNA) and immunoassays (proteins). They are also highly expressed in endocrine-related tissues, including breast, ovary, testis and prostate (4). Interestingly, the expression of most kallikreins is regulated by steroid hormones. KLK1 has been reported to be up-regulated by estrogen as demonstrated by its differential expression during the menstrual cycle (6). KLK2 and KLK3 are well-known to be up-regulated by androgens and progestins in both cancer cell lines and animal models (7, 8). Androgen response elements (AREs) within the KLK2 and KLK3 promoters that mediate the hormonal regulation have been identified (9–12). Although an estrogen response element (ERE) was also predicted to be present in the KLK1 gene promoter, it has not been functionally tested (13). For the rest of the tissue kallikreins, to some extend, their expression is regulated by estrogen, androgen and progestin, based on studies performed in cancer cell lines (4). Considering the high expression level of the tissue kallikreins in sex organs, and their regulation by sex hormones, it is possible that the tissue kallikreins are down-stream mediators of sex hormone action.

ENZYMATIC ACTIVITIES AND PHYSIOLOGICAL FUNCTIONS OF THE HUMAN TISSUE KALLIKREINS

Among all the tissue kallikreins, hK1 is the only one that has true kininogenase activity. It can cleave low molecular weight kininogen to release Lys-bradykinin, which binds to the G-protein coupled kinin receptor B to modulate a variety of physiological processes, such as regulation of blood flow, vascular permeability and Na^+/water homeostasis (14). hK2 and hK3 (also known as prostate specific antigen) are two tissue kallikreins that are predominantly expressed in the prostate. hK2 has been shown to have trypsin-like enzymatic activity and be able to activate hK3 (15–18). However, its exact physiological functions remain unknown. hK3 has chymotrypsin-like enzymatic activity and its major physiological function is to cleave seminogelin to liquefy semen clots (19, 20). For the rest of the tissue kallikreins, their enzymatic activities have not been studied in detail so far. Preliminary experiments have shown that hK6, hK8 and hK11 have trypsin-like enzymatic activity (21–24), whereas, hK7's enzymatic activity is chymotrypsin-like (25, 26). hK6 (also known as zyme/neurosin) is highly expressed in the brain. It is thought to be involved in the cleavage of amyloid precursor protein and thus, it could be implicated with Alzheimer's disease

(27, 28). hK7 (also known as human stratum corneum chymotryptic enzyme, HSCCE) was initially identified from a keratinocyte derived-library (25). In some studies, it has been shown that abundant expression of hK7 is restricted to the skin and hK7 appears to catalyze the degradation of intercellular cohesive structures in outermost cornified layer of the skin and contributes to the cell shedding process at the skin surface (25, 26). The physiological substrates of hK8 (also known as neuropsin) remain unclear although some experiments have indicated that it can cleave fibronectin (23). Through protein sequence homology comparison, hK4, hK5, hK10, hK12, hK13 and hK14 are predicted to have trypsin-like activity and hK9 and hK15 could possess chymotrypsin-like activity (4). However, these predicted enzymatic activities have not been experimentally tested and hence, their physiological substrates and functions are still not yet known.

HUMAN TISSUE KALLIKREINS AND CANCER

Human tissue kallikreins are secreted serine proteases, which can be detected in various biological fluids. During malignant transformation, the expression of tissue kallikreins could change. Therefore, they may have potential as biomarkers of various malignancies. hK2 and hK3 have applications mainly in prostate cancer. During prostate cancer development, the serum levels of both kallikreins are elevated (29). Serum hK2, in combination with hK3, has value in the differential diagnosis between prostate cancer and benign prostatic hyperplasia (30). Serum hK3 is the best prostate cancer biomarker discovered so far and has been widely used as a screening test and for prostate cancer monitoring. The expression of hK3 is actually down-regulated in aggressive prostate tumors (31, 32). hK3 also has potential clinical utility in breast cancer. It has been reported that high levels of PSA in breast tumors predict favorable prognosis of breast cancer (33). For the newly discovered tissue kallikreins, the malignancy that they are more closely associated with, is ovarian cancer (34, 35). KLK4, KLK5, and KLK15 are found to be overexpressed in ovarian cancer, as demonstrated by RT-PCR (36–38). Similarly, hK6 and hK10 proteins are increased in ovarian cancer tissues, as determined by immunoassays (39, 40). All these overexpressions are associated with more aggressive forms of ovarian cancer and poor patient survival. In contrast, high levels of KLK8, KLK9 and KLK14 expression are indicators of good prognosis in ovarian cancer (41–43). Overexpression of these tissue kallikreins in ovarian tumor tissues suggests that their serum levels may also be elevated. Indeed, serum hK6 and hK10 have already been found to be elevated in ovarian cancer patients and have value in improving ovarian cancer diagnosis when combined with CA125 (44, 45). In addition, serum hK11 is elevated in 70% of ovarian cancer and 60% of prostate cancer patients (46). For more detailed descriptions of tissue kallikreins and ovarian cancer, please refer to reference (34). Besides ovarian cancer, breast cancer is another malignancy that is often associated with tissue kallikreins. KLK6 and KLK10 have been reported to be down regulated in breast cancer, whereas, high KLK13 expression correlates with favorable prognosis in subgroups of breast cancer patients (47–49).

HUMAN TISSUE KALLIKREINS AND TESTICULAR CANCER

Many tissue kallikreins are highly expressed in normal testicular tissue, including KLK5, KLK9, KLK10, KLK12, KLK13 and KLK14. Therefore, it is reasonable to speculate that their expression may be affected during testicular malignancy initiation and progression. Thus, these kallikreins may represent candidate biomarkers for testicular cancer. The association between KLK5, KLK10, KLK13 and KLK14 expression and testicular cancer has been studied preliminarily. In contrast to their overexpression in ovarian cancer, the expression of these genes appears to be universally down-regulated in testicular cancer.

The expression level of the KLK5 gene was determined with quantitative RT-PCR technique. It was found that KLK5 is expressed by both normal and cancerous testicular tissues. However, in 93% of testicular cancer patients, KLK5 expression in the cancerous region was significantly (50%) lower than in the adjacent, histologically

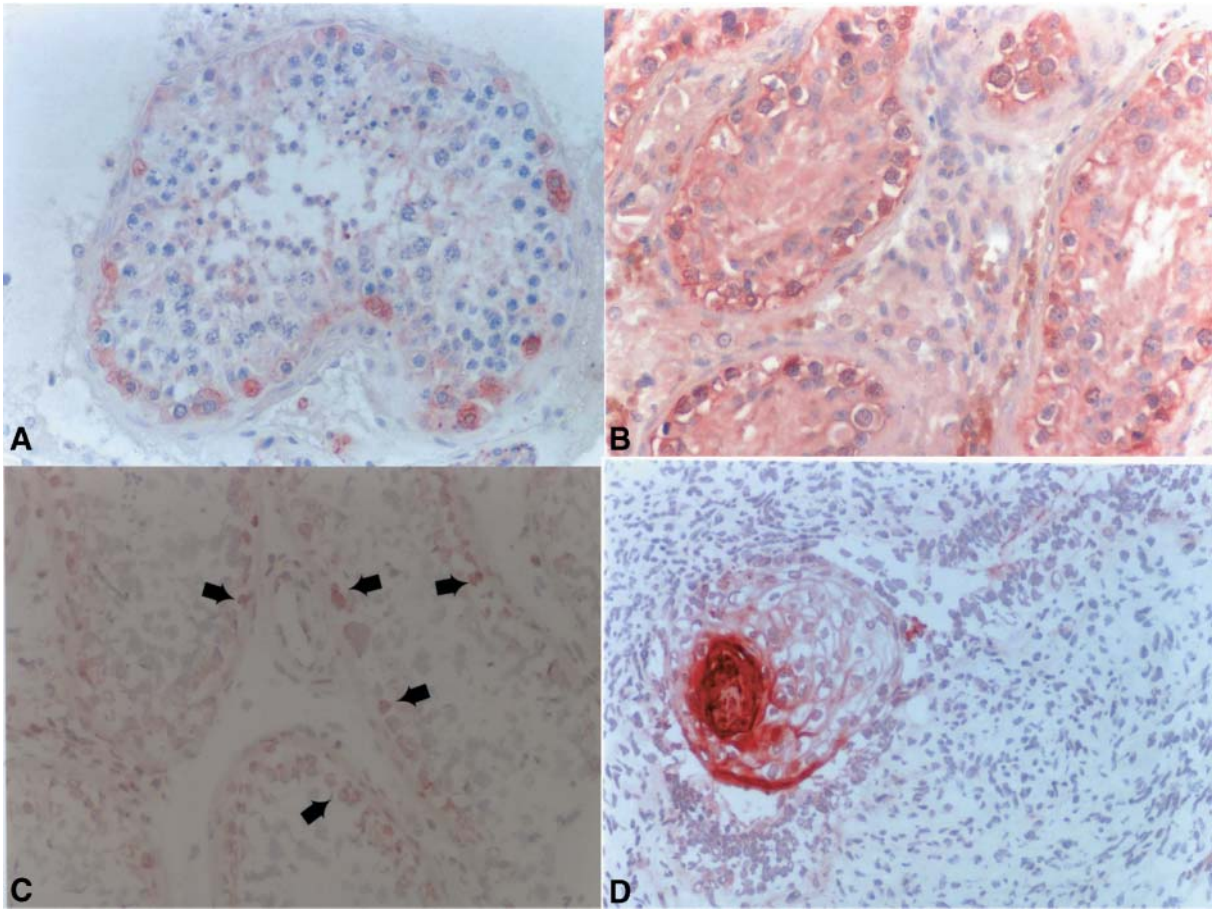

Fig. 2. Cellular localization of hK10 protein in normal and malignant testicular tissues. A. A paraffin-fixed tubule from the normal testis. Note a strong staining of hK10 in spermatogonia. B. A frozen section of the normal testis demonstrating the strongest staining in spermatogonia (indicated by arrows). C, A section with carcinoma in situ. There is a strong staining of CIS cells and diffuse staining of Sertoli cells. D. An example of hK10 staining in teratoma. Note the high hK10 expression in a well-differentiated epithelial component, whereas, the less differentiated parts of the tumor are negative. Reprinted by permission from Ref. 51.

normal testicular tissues. Lower KLK5 expression was also more frequently observed in seminomas than in nonseminomas. Furthermore, low KLK5 expression seemed to be associated with aggressiveness of testicular tumors. In late stage (II/III) tumors and tumors with vascular/lymphatic invasion, KLK5 expression was lower than those with early stage (stage I) or tumors limited to the testis, respectively (50).

The expression of KLK10 in normal testicular tissue has been investigated with immunohistochemistry. As Fig. 2 shows, germ cells, mainly spermatogonia, stain strongly for hK10 protein. A diffuse staining was also observed in Sertoli cells, especially in areas adjacent to spermatogonia, and in the basement membrane of seminiferous tubles. A weak reaction was seen in Leydig cells. Also, with RT-PCR, the expression of KLK10 was investigated in normal and cancerous testicular tissues. CIS (carcinoma in situ) and Leydig cell tumors have nearly as high level of KLK10 expression as normal parachyma, whereas, seminoma and nonseminoma have very low or no expression of KLK10, compared to their adjacent normal counterparts. This observation was further confirmed with randomly selected normal and cancerous testicular tissues (51). Immunohistochemical staining of hK10 protein in germ cell tumors verified that the malignant germ cells lacked hK10 staining, whereas, some well-differentiated epithelial components still retained strong hK10 staining (51). These results indicate that normal germ cells express high levels of KLK10 but

during germ cell malignant transformation, the expression of the KLK10 gene is down-regulated, which leads to low or no expression of hK10 protein in germ cell tumors.

The expression of KLK13 in testicular cancer was also investigated. KLK13 has seven splice variants, which originate by alternative splicing of exon 3. Among these splice variants, one (classic form) gives rise to the full-length pre-pro-hK13 protein. For the rest of the six splice variants, they are predicted to encode for truncated forms of hK13. The classic form and all the variant forms are expressed at the mRNA level, in normal testicular tissues. Interestingly, the expression of the variant forms is restricted to normal testicular tissue, while the classic form is widely expressed in many other normal tissues. In germ cell tumors, the classic form has similar expression level as in normal testicular tissues, however, all the variant forms become undetectable in tumors (52).

Similarly to KLK5 and KLK10, KLK14 expression was also found to be lower in 70% of testicular tumors (53).

The physiological functions of kallikreins in testicular tissue are still not clear. KLK5, KLK10, KLK13 and KLK14 are all serine proteases with trypsin-like enzymatic activity (4). However, their physiological substrates in testicular tissues remain undetermined. In the testis, the differentiation of diploid spermatogonia into haploid spermatozoa involves successive rounds of mitotic and meiotic cell divisions and extensive morphological reconstruction. Proteolytic reactions leading to cell proliferation, apoptosis, differentiation and migration are crucial steps in the process of normal germ cell development. The kallikreins may participate in one or more of these proteolytic events. Alternatively, during spermatogenesis, communication between germ cells and Sertoli cells through specific soluble factors are essential (54). It is possible that tissue kallikreins may be involved in the cleavage of these specific factors. During germ cell tumor development, the abnormal germ cell proliferation and differentiation may lead to down-regulation of the tissue kallikrein genes. In addition, given that multiple tissue kallikrein genes are expressed in the testicular tissue, it is possible that they are components of proteolytic cascade reactions as we proposed for ovarian cancer (34).

Some experimental evidence has suggested that KLK10 may function as a tumor suppressor gene. Overexpression of KLK10 can reduce tumor formation in nude mice and inhibit anchorage independent growth of breast cancer cell lines (55). Furthermore, KLK10 expression is down-regulated in breast and prostate cancer cell lines as well as in breast tumors (48, 55). It has been shown that this down-regulation is due to hypermethylation of the KLK10 gene itself (56). The down-regulation of KLK10 in testicular cancer is in accord with the hypothetical tumor suppressor function of KLK10. Whether KLK10 and the other kallikrein genes are down-regulated in testicular cancer through the same mechanisms needs to be experimentally determined.

The down-regulation of kallikrein genes in testicular cancer is in sharp contrast to their overexpression in ovarian cancer (34). This differential expression of kallikrein genes in different malignancies may be due to their tissue-specific functions. Tissue kallikrein genes are widely expressed in a variety of tissues, such as testis, breast, prostate, skin, tonsil, and the gastrointestinal tract. Their diverse tissue expression indicates that the kallikreins may have different physiological substrates and hence, different physiological functions, in different physiological environments.

Testicular cancer is a common malignancy in young males. Usually, tumors cannot be diagnosed until symptoms develop. Biological factors that are involved in tumor development may facilitate early diagnosis. One or more tissue kallikreins may be potential biomarkers for testicular cancer. Tissue kallikreins are secreted proteins and can be detected in many biological fluids, such as serum and seminal plasma (57–59). For the KLK13 splice variants, their absence of expression could aid in prognosis or diagnosis of germ cell tumors. Similar applications of KLK2 splice variants have been reported in prostate cancer (60).

CONCLUSION

Tissue kallikreins are a group of serine proteases that are expressed in diverse tissues and participate in many physiological processes. They are differentially expressed in various ma-

lignancies. In testicular cancer, some of the tissue kallikrein genes, including KLK5, KLK10, KLK13 and KLK14, are found to be universally down-regulated. These findings warrant further studies to investigate their physiological functions during testicular cancer development and their clinical applications in testicular cancer diagnosis.

REFERENCES

1. Clements J. The molecular biology of the kallikreins and their roles in inflammation. In: Farmer S, editor. The Kinin System. San Diego, CA: Academic Press, 1997;5:71–97.
2. Yu H, Bowden DW, Spray BJ, Rich SS, Freedman BI. Identification of human plasma kallikrein gene polymorphisms and evaluation of their role in end-stage renal disease. Hypertension 1998;31:906–11.
3. Bhoola K, Figueroa C, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 1992;44:1–80.
4. Yousef GM, Diamandis EP. The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr Rev 2001;22: 184–204.
5. Diamandis EP, Yousef GM, Clements J, Ashworth LK, Yoshida S, Egelrud T, Nelson PS, Shiosaka S, Little S, Lilja H, Stenman UH, Rittenhouse HG, Wain H. New nomenclature for the human tissue kallikrein gene family. Clin Chem 2000;46:1855–8.
6. Clements JA, Mukhtar A, Ehrlich A, Yap B. Kallikrein gene expression in the human uterus. Braz J Med Biol Res 1994;27:1855–63.
7. Riegman PH, Vlietstra RJ, van der Korput HA, Romijn JC, Trapman J. Identification and androgen-regulated expression of two major human glandular kallikrein-1 (hGK-1) mRNA species. Mol Cell Endocrinol 1991;76:181–90.
8. Cleutjens KB, van der Korput HA, Ehren-van Eekelen CC, Sikes RA, Fasciana C, Chung LW, Trapman J. A 6-kb promoter fragment mimics in transgenic mice the prostate- specific and androgen-regulated expression of the endogenous prostate- specific antigen gene in humans. Mol Endocrinol 1997;11:1256–65.
9. Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 1991;5:1921–30.
10. Cleutjens KB, van der Korput HA, van Eekelen CC, van Rooij HC, Faber PW, Trapman J. An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate- specific antigen promoter. Mol Endocrinol 1997;11:148–61.
11. Schuur ER, Henderson GA, Kmetec LA, Miller JD, Lamparski HG, Henderson DR. Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 1996;271:7043–51.
12. Huang W, Shostak Y, Tarr P, Sawyers C, Carey M. Cooperative assembly of androgen receptor into a nucleoprotein complex that regulates the prostate-specific antigen enhancer. J Biol Chem 1999;274:25756–68.
13. Murray SR, Chao J, Lin FK, Chao L. Kallikrein multigene families and the regulation of their expression. J Cardiovasc Pharmacol 1990;15:S7–16.
14. Maier M, Austen KF, Spragg J. Kinetic analysis of the interaction of human tissue kallikrein with single-chain human high and low molecular weight kininogens. Proc Natl Acad Sci U S A 1983;80:3928–32.
15. Schedlich LJ, Bennetts BH, Morris BJ. Primary structure of a human glandular kallikrein gene. DNA 1987;6:429–37.
16. Kumar A, Mikolajczyk SD, Goel AS, Millar LS, Saedi MS. Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. Cancer Res 1997;57:3111–4.
17. Takayama TK, Fujikawa K, Davie EW. Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein. J Biol Chem 1997;272: 21582–8.
18. Lovgren J, Rajakoski K, Karp M, Lundwall A, Lilja H. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. Biochem Biophys Res Commun 1997; 238:549–55.
19. Akiyama K, Nakamura T, Iwanaga S, Hara M. The chymotrypsin-like activity of human prostate-specific antigen, gamma-seminoprotein. FEBS Lett 1987;225:168–72.
20. Lilja H. A kallikrein -like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein. J Clin Invest 1985;76:1899–903.
21. Gomis-Ruth FX, Bayes A, Sotiropoulou G, Pampalakis G, Tsetsenis T, Villegas V, Aviles FX, Coll M. The structure of human kallikrein 6 reveals a novel activation mechanism for the kallikrein family. J Biol Chem 2002;16:16.
22. Bernett MJ, Blaber SI, Scarisbrick IA, Dhanarajan P, Thompson SM, Blaber M. Crystal structure and biochemical characterization of human kallikrein 6 reveals that a trypsin-like kallikrein is expressed in the central nervous system. J Biol Chem 2002;277:24562–70.
23. Shimizu C, Yoshida S, Shibata M, Kato K, Momota Y, Matsumoto K, Shiosaka T, Midorikawa R, Kamachi T, Kawabe A, Shiosaka S. Char-

acterization of recombinant and brain neuropsin, a plasticity- related serine protease. J Biol Chem 1998;273:11189–96.
24. Yoshida S, Taniguchi M, Suemoto T, Oka T, He X, Shiosaka S. cDNA cloning and expression of a novel serine protease, TLSP. Biochim Biophys Acta 1998;1399:225–8.
25. Hansson L, Stromqvist M, Backman A, Wallbrandt P, Carlstein A, Egelrud T. Cloning, expression, and characterization of stratum corneum chymotryptic enzyme. A skin-specific human serine proteinase. J Biol Chem 1994;269: 19420–6.
26. Tanimoto H, Underwood LJ, Shigemasa K, Yan Yan MS, Clarke J, Parmley TH, O'Brien TJ. The stratum corneum chymotryptic enzyme that mediates shedding and desquamation of skin cells is highly overexpressed in ovarian tumor cells. Cancer 1999;86:2074–82.
27. Yamashiro K, Tsuruoka N, Kodama S, Tsujimoto M, Yamamura Y, Tanaka T, Nakazato H, Yamaguchi N. Molecular cloning of a novel trypsin-like serine protease (neurosin) preferentially expressed in brain. Biochimica et Biophysica Acta 1997;1350:11–4.
28. Little SP, Dixon EP, Norris F, Buckley GW, Johnson M, Dobbins JR, Wyrick T, Miller JR, Mackellar W, Hepburn D, Corvalan J, McClure D, Liu X, Stephenson D, Clements J, John-stone EM. Zyme, a novel and potentially amyloidogenic enzyme cDNA isolated from Alzeimer's disease brain. J biol Chem 1997;272:25135–42.
29. Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. Crit Rev Clin Lab Sci 1998;35:275–368.
30. Magklara A, Scorilas A, Catalona WJ, Diamandis EP. The combination of human glandular kallikrein and free prostate-specific antigen (PSA) enhances discrimination between prostate cancer and benign prostatic hyperplasia in patients with moderately increased total PSA. Clin Chem 1999;45:1960–6.
31. Pretlow TG, Pretlow TP, Yang B, Kaetzel CS, Delmoro CM, Kamis SM, Bodner, DR, Kursh E, Resnick MI, Bradley EL Jr. Tissue concentrations of prostate-specific antigen in prostatic carcinoma and benign prostatic hyperplasia. Int J Cancer 1991;49:645–9.
32. Magklara A, Scorilas A, Stephan C, Kristiansen GO, Hauptmann S, Jung K, Diamandis EP. Decreased concentrations of prostate-specific antigen and human glandular kallikrein 2 in malignant versus nonmalignant prostatic tissue. Urology 2000;56:527–32.
33. Yu H, Giai M, Diamandis EP, Katsaros D, Sutherland DJ, Levesque MA, Roagna R, Ponzone R, Sismondi P. Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 1995c;55:2104–10.
34. Yousef GM, Diamandis EP. Kallikreins, steroid hormones and ovarian cancer: is there a link? Minerva Endocrinol 2002;27:157–66.
35. Diamandis EP, Yousef GM. Human tissue kallikreins: a family of new cancer biomarkers. Clin Chem 2002;48:1198–205.
36. Obiezu CV, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, Rigault de la Longrais IA, Arisio R, Diamandis EP. Higher human kallikrein gene 4 (KLK4) expression indicates poor prognosis of ovarian cancer patients. Clin Cancer Res 2001;7:2380–6.
37. Dong Y, Kaushal A, Bui L, Chu S, Fuller PJ, Nicklin J, Samaratunga H, Clements JA. Human kallikrein 4 (KLK4) is highly expressed in serous ovarian carcinomas. Clin Cancer Res 2001;7: 2363–71.
38. Kim H, Scorilas A, Katsaros D, Yousef GM, Massobrio M, Fracchioli S, Piccinno R, Gordini G, Diamandis EP. Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br J Cancer 2001;84: 643–50.
39. Hoffman B, Katsaros D, Scorilas A, Diamandis P, Fracchioli S, Rigamlt de la Longrais IA, Colgan T, Pnopolo M, Giardina G, Massobrio M, Diamandis EP. Immunofluorometric quantitation and histochemical localization of kallikrein 6 protein in ovarain cancer tissue: a new independent unfavorable marker. Br J Cancer 2002;87(7): 763–71.
40. Luo LY, Katsaros D, Scorilas A, Fracchioli S, Piccinno R, Rigault de la Longrais IA, Howarth DJ, Diamandis EP. Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. Clin Cancer Res 2001;7:2372–9.
41. Magklara A, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, Danese S, Diamandis EP. The human KLK8 (neuropsin/ovasin) gene: identification of two novel splice variants and its prognostic value in ovarian cancer. Clin Cancer Res 2001;7:806–11.
42. Yousef GM, Kyriakopoulou LG, Scorilas A, Fracchioli S, Ghiringhello B, Zarghooni M, Chang A, Diamandis M, Giardina G, Hartwick WJ, Richiardi G, Massobrio M, Diamandis EP, Katsaros D. Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: A new independent and favorable prognostic marker. Cancer Res 2001;61:7811–8.
43. Yousef GM, Scorilas A, Fracchioli S, Borgono CA, Iskander L, Richiardi G, Massobrio M, Katsaros D, Diamandis EP. Steroid hormone regulation and prognostic value of human kallikrein gene 14 (KLK14) in ovarian cancer. Amer J Clin Pathol 2003;in press.
44. Luo LY, Bunting P, Scorilas A, Diamandis EP.

Human kallikrein 10: a novel tumor marker for ovarian carcinoma? Clin Chim Acta 2001;306: 111–8.
45. Diamandis EP, Yousef GM, Soosaipillai AR, Bunting P. Human kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma. Clin Biochem 2000;33:579–83.
46. Diamandis EP, Okui A, Mitsui S, Luo LY, Soosaipillai A, Grass L, Nakamura T, Howarth DJC, Yamaguchi N. Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. Cancer Res 2002;62(1):295–300.
47. Anisowicz A, Sotiropoulou G, Stenman G, Mok SC, Sager R. A novel protease homolog differentially expressed in breast and ovarian cancer. Mol Med 1996;2:624–36.
48. Dhar S, Bhargava R, Yunes M, Li B, Goyal J, Naber SP, Wazer DE, Band V. Analysis of normal epithelial cell specific-1 (NES1)/kallikrein 10 mRNA expression by in situ hybridization, a novel marker for breast cancer. Clin Cancer Res 2001;7:3393–8.
49. Chang A, Yousef GM, Scorilas A, Grass L, Sismondi P, Ponzone R, Diamandis E P. Human kallikrein gene 13 (KLK13) expression by quantitative RT-PCR: an independent indicator of favourable prognosis in breast cancer. Br J Cancer 2002;86:1457–64.
50. Yousef GM, Obiezu C, Jung K, Stephan C, Scorilas A, Diamandis EP. Differential expression of kallikrein gene 5 (KLK5) in cancerous and normal testicular tissues. Urology 2002;60(4):714–8.
51. Luo LY, Rajpert-De Meyts ER, Jung K, Diamandis EP. Expression of the normal epithelial cell-specific 1 (NES1; KLK10) candidate tumour suppressor gene in normal and malignant testicular tissue. Br J Cancer 2001;85:220–4.
52. Chang A, Yousef GM, Jung K, Rajpert-De Meyts E, Diamandis EP. Identification and molecular characterization of five novel kallikrein gene 13 (KLK13; KLK-L4) splice variants: differential expression in the human testis and testicular cancer. Anticancer Res 2001;21:3147–52.
53. Yousef GM, Magklara A, Chang A, Jung K, Katsaros D, Diamandis EP. Cloning of a new member of the human kallikrein gene family, KLK14, which is down-regulated in different malignancies. Cancer Res 2001;61:3425–31.
54. Kierszenbaum AL. Mammalian spermatogenesis in vivo and in vitro: a partnership of spermatogenic and somatic cell lineages. Endocr Rev 1994; 15:116–34.
55. Goyal J, Smith KM, Cowan JM, Wazer DE, Lee SW, Band V. The role for NES1 serine protease as a novel tumor suppressor. Cancer Res 1998;58: 4782–6.
56. Li B, Goyal J, Dhar S, Dimri G, Evron E, Sukumar S, Wazer DE, Band V. CpG methylation as a basis for breast tumor-specific loss of NES1/ kallikrein 10 expression. Cancer Res 2001;61: 8014–21.
57. Yousef GM, Grass L, Polymeris M-E, Soosaipillai A, Chan P-C, Scorilas A, Borgono C, Harbeck N, Schmalfeldt B, Dorn J, Schmitt M, Diamandis EP. Human kallikrein 5 (hK5): a novel serum biomarker for breast and ovarian cancer. Cancer Res 2002;in press.
58. Luo LY, Grass L, Howarth DJ, Thibault P, Ong H, Diamandis EP. Immunofluorometric assay of human kallikrein 10 and its identification in biological fluids and tissues. Clin Chem 2001;47: 237–46.
59. Kapadia C, Chang A, Sotiropoulou G, Yousef GM, Grass L, Soosaipillai A, Xing X, Howarth DHC, Diamandis EP. Human kallikrein 13: production and purification of recombinant protein, monoclonal and polyclonal antibodies and development of a sensitive and specific immunofluorometric assay. Clin Chem 2003;49:77–86.
60. Slawin KM, Shariat SF, Nguyen C, Leventis AK, Song W, Kattan MW, Young CY, Tindall DJ, Wheeler TM. Detection of metastatic prostate cancer using a splice variant-specific reverse transcriptase-polymerase chain reaction assay for human glandular kallikrein. Cancer Res 2000;60: 7142–8.

COMMENTS

Leendert Looijenga (Rotterdam, The Netherlands): I conclude from your presentation that the only subtype of kallikrein genes downregulated in the early stage of development of testicular cancer is KLK 10. You used immunocytochemistry (ICH) to compare CIS with invasive tumours, but in all your other studies you used RT-PCR to compare invasive tumours with normal testicular parenchyma. You did not use ICH on gonocytes or prenatal germ cells.

Eleftherios Diamandis (Toronto, Canada): A major limitation of our studies is that we have only looked at a single case of CIS with ICH.

We are also limited by our methodology and the number of kallikreins which we have studied. We have antibodies for many of the kallikreins, and it would be interesting to detect KLK expression at the protein level by ICH comparing normal tissue, CIS and overt cancer.

Leendert Looijenga: Interestingly, you suggest that KLK 10 is a tumour suppressor gene.

Eleftherios Diamandis: We must look at KLK 10 but also other genes. We have excellent monoclonal antibodies which react with numerous different kallikreins and these can be used on paraffin sections.

Anders Bjartell (Malmö, Sweden): Are some of the kallikreins rapidly degraded in tissue, because they may be involved in a cascade reaction? This would cause difficulty in detecting them by IHC.

Eleftherios Diamandis: Kallikreins are involved in cascade reactions: many are trypsin-like proteases and act together activating different steps in the pathway, and many are degraded in the process. It is not always easy to demonstrate the tissue expression of these proteins, and sometimes we have to predict their presence. Some kallikreins are, therefore, silencing other members by their proteolytic enzymatic action. We need further experiments in order to build the pathways and to localise the genes. The mode of regulation, and more importantly the mode of expression, suggest that these genes are acting together as a group rather than single enzymes acting one at a time.