Volume 50(9): 1247–1261, 2002
The Journal of Histochemistry & Cytochemistry
http://www.jhc.org

ARTICLE

Human Kallikrein 10 Expression in Normal Tissues by Immunohistochemistry

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SUMMARY The normal epithelial cell-specific 1 (NES1) gene (official name kallikrein gene 10, KLK10) was recently cloned and encodes for a putative secreted serine protease (human kallikrein 10, hK10). Several studies have confirmed that hK10 shares many similarities with the other kallikrein members at the DNA, mRNA, and protein levels. The enzyme was found in biological fluids, tissue extracts, and serum. Here we report the first detailed immunohistochemical (IHC) localization of hK10 in normal human tissues. We used the streptavidin–biotin method with two hK10-specific antibodies, a polyclonal rabbit and a monoclonal mouse antibody, developed in house. We analyzed 184 paraffin blocks from archival, current, and autopsy material, prepared from almost every normal human tissue. The staining pattern, the distribution of the immunostaining, and its intensity were studied in detail. Previously, we reported the expression of another novel human kallikrein, hK6, by using similar techniques. The IHC expression of hK10 was generally cytoplasmic and not organ-specific. A variety of normal human tissues expressed the protein. Glandular epithelia constituted the main immunoexpression sites, with representative organs being the breast, prostate, kidney, epididymis, endometrium, fallopian tubes, gastrointestinal tract, bronchus, salivary glands, bile ducts, and gallbladder. The choroid plexus epithelium, the peripheral nerves, and some neuroendocrine organs (including the islets of Langerhans, cells of the adenohypophysis, the adrenal medulla, and Leydig cells) expressed the protein strongly and diffusely. The spermatic epithelium of the testis expressed the protein moderately. A characteristic immunostaining was observed in Hassall's corpuscles of the thymus, oxyphilic cells of the thyroid and parathyroid glands, and chondrocytes. Comparing these results with those of hK6, we observed that both kallikreins had a similar IHC expression pattern. **(J Histochem Cytochem 50:1247–1261, 2002)**

KEY WORDS
human kallikrein 10
immunohistochemical expression
normal human tissues
secreted proteins
serine proteases
cancer biomarkers

THE BASIC COMPONENTS of the kallikrein–kinin system were discovered in the 1930s (Kraut et al. 1930; Clements 1997). The human tissue kallikrein gene family was identified in the 1980s (reviewed in Diamandis et al. 2000b; Yousef and Diamandis 2001). Until recently it was believed that this gene family consisted of only three genes: pancreatic/renal kallikrein (KLK1, encoding for hK1 protein), human glandular kallikrein 2 (KLK2, encoding for hK2 protein), and human kallikrein 3 (KLK3, encoding for hK3 protein or prostate-specific antigen, PSA). KLK1 gene expression is highest in the pancreas, kidney, and salivary glands. The other two kallikreins, PSA and hK2, are relatively prostatic-specific and have already found important applications as biomarkers for diagnosis and monitoring of prostate cancer (McCormack et al. 1995; Chu 1997; Rittenhouse et al. 1998; Stenman 1999). hK3 and hK2 proteins and mRNA have been found at significant amounts in the female breast

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Received for publication November 16, 2001; accepted April 10, 2002 (1A5684).

0022-1554/02/$3.30

and at lower levels in many other tissues (Diamandis and Yu 1997; Black and Diamandis 2000).

In the past 3 years, new members of the human kallikrein gene family have been discovered (Diamandis et al. 2000b; Yousef and Diamandis 2001). This gene family now consists of 15 genes, all of which encode for trypsin-like or chymotrypsin-like serine proteases, show significant homology at both the DNA and amino acid levels, and are all localized at the chromosomal locus 19q13.4 (Diamandis et al. 2000b; Gan et al. 2000; Yousef and Diamandis 2001). Several empirical names were given to these new genes, but recently official names have been adopted. The genes are designated as KLK1–KLK15 and their encoded proteins as hK1–hK15 (Diamandis et al. 2000a). KLK4 seems to be prostatic-specific by Northern blotting analysis, but by RT-PCR it was demonstrated that it is also expressed in breast and other tissues (Yousef et al. 1999a). The remaining kallikreins are not tissue-specific, although certain genes are preferentially expressed in specific organs (Diamandis et al. 2000b).

The normal epithelial cell-specific 1 (NES1) gene (official name kallikrein gene 10, KLK10) was cloned by subtractive hybridization techniques based on its downregulation in radiation-transformed breast epithelial cells (Liu et al. 1996). KLK10 resides on chromosome 19q13.4, spans ~5.5 kb of genomic DNA sequence, and contains six exons (one untranslated) and five introns (Luo et al. 1998). It encodes for a putative secreted serine protease (human kallikrein 10, hK10) that has 35–40% identity and 50–60% similarity with PSA and other members of the human kallikrein gene family. The gene is expressed in various human tissues, including breast, ovary, prostate, and testis. Its physiological function is still not clear but experimental data suggest that KLK10 controls normal cell growth, may function as a tumor suppressor, and is downregulated during breast, prostate, and testicular cancer progression (Goyal et al. 1998; Dhar et al. 2001; Luo et al. 2001d). Until recently, no suitable method for measuring hK10 with high sensitivity and specificity was available. We reported the first immunofluorometric assay for hK10 protein, which is suitable for measuring hK10 with high sensitivity and specificity (Luo et al. 2001b). With this method, we quantified hK10 in serum, breast milk, seminal plasma, cerebrospinal fluid, and amniotic fluid, as well as in various tissue extracts (Luo et al. 2001b).

There are very few reports describing the expression of kallikreins by IHC in human normal or diseased tissues (Diamandis et al. 2000b). Recently we reported the first extensive IHC study of hK6 expression in normal tissues (Petraki et al. 2001). The protein is not tissue-specific and is mainly expressed by glandular epithelia, the choroid plexus, and several neuroendocrine tissues, such as the pancreatic islets and cells of the anterior lobe of the pituitary gland.

Here we describe the IHC localization of hK10 in diverse human tissues.

Materials and Methods

This is an IHC study on almost all normal human tissues, aiming to establish the expression pattern of hK10. Parts of an organ with different histology (e.g., stomach, fundus, body, antrum) were examined separately. A paraffin block of three different cases for every tissue (organ, all parts with different histology) was selected. Tissues that exist in several organs (e.g., fat, muscle, vessels, peripheral nerves, ganglia, and neuroendocrine cells) were not studied separately. A total of 184 paraffin blocks were examined. Of these, 150 concerned archival or current material from 117 cases and 34 were autopsy material from two cases. The archival and the current material were biopsy or surgical specimens. We used autopsy material to study the different parts of the brain, including the pituitary gland, because the routine material is usually from neoplastic cases. We did not study any specimens from the pineal gland and the spinal cord.

The IHC staining was performed on 4-μm-thick paraffin sections of tissues fixed in buffered formalin according to a streptavidin–biotin–peroxidase protocol using the DAKO LSAB+Kit Peroxidase (Carpinteria, CA). An hK10-specific rabbit polyclonal antibody (1:400) and an hK10 mouse monoclonal antibody (Code 5D3, 1:100) were raised by immunizing with full-length recombinant hK10 produced in yeast, as described elsewhere (Luo et al. 2001b). The specificity of the antibodies was evaluated during development of the immunofluorometric assay (Luo et al. 2001b). We found no detectable crossreactivity from other closely related antigens, including kallikreins hK2–hK15 (Luo et al. 2001b; and our unpublished data). In addition, we tested the crossreactivity of the polyclonal antibodies for hK6 and hK10 against each other by using Western blots. Staining procedures included deparaffinization in warm xylene for 5 min with two changes of xylene at room temperature (RT), followed by rehydration by transfer through graded alcohols. Endogenous peroxidase activity was blocked with 0.5% H_2O_2 in methanol for 10 min. The sections were pretreated with 10 mmol/liter citrate buffer (pH 6.1) in a microwave for 5 min and were incubated overnight at 4C with the hK10 primary rabbit polyclonal and mouse monoclonal antibodies in 3% BSA. After two washes of the sections in 50 mM Tris buffer (pH 7.6), the biotinylated link (DAKO) was applied for 15 min and a streptavidin–peroxidase conjugate was added for another 15 min. The enzymatic reaction was developed in a freshly prepared solution of 3,3′-diaminobenzidine tetrahydrochloride using DAKO liquid DAB substrate–chromogen solution for 10 min (brown color). The sections were then counterstained with hemalum, dehydrated, cleared in xylene, and mounted.

The staining pattern, the distribution of the immunostaining in each tissue, and the intensity of the staining were studied in detail.

In selected tissues the primary antibody was replaced by a non-immune rabbit serum (1:500) in 3% BSA to assess nonspecific binding. For the same reason, an immunoabsorption test was also performed in which the primary polyclonal hK10 antibody was incubated for 1 hr at RT with recombinant hK10 antigen (10 μg/liter) before immunostaining.

Results

The hK10 immunoreactivity using the polyclonal and the monoclonal antibody was generally localized in the cytoplasm. Both antibodies revealed the same distribution of the antigen in all tissues. Replacement of the primary polyclonal antibody with non-immune rabbit serum and immunoabsorption of the antibody with hK10 antigen abolished the immunostaining in the tissues, supporting the specificity of staining. The protein was found in a variety of tissues, indicating that it is not tissue-specific. hK10 was mainly expressed by glandular tissues, but we obtained evidence that it could also serve as a neuroendocrine marker. The distribution and the quantitative expression of hK10 in various tissues are described below and are further summarized in Table 1. It is noteworthy that there were no major differences between the immunolocalizations of hK10 and hK6 in the examined tissues. Western blots confirmed the specificity of the two polyclonal antibodies for hK6 and hK10. As shown in Figure 1, no crossreactivity was detected and the two antibodies recognize only their cognate antigens.

Central and Peripheral Nervous Systems

Our most striking finding in the CNS was the strong and diffuse positivity in the epithelium of the choroid plexus, the specialized organ that lines the ventricular system and is responsible for the production of the cerebrospinal fluid (Figure 2A). In the cerebellar cortex the antigen was expressed weakly to moderately in the Purkinje cells (neural cells), whereas the granular cells were negative (Figure 2B). In the gray matter of the cerebral cortex, neurons and glial cells were weakly to moderately immunoreactive (Figures 2C and 2D). It is worth mentioning that although the majority of neurons were positive, the immunoreactivity in the glial cells was focal and concerned both protoplasmic astrocytes and oligodendrocytes. Glial cells were morphologically easily distinguishable from neurons. We verified the distinction of small neurons from protoplasmic astrocytes using synaptophysin, neurofilaments, and GFAP (glial fibrillary acidic protein) antibodies. The former cells react with synaptophysin and neurofilaments and the latter cells with GFAP. Oligodendrocytes were easily distinguished by their smaller size, their characteristic location against the cell membrane of neurons, and their CD57/synaptophysin positivity. Microglia were not easily recognized in our sections, and we could not comment about its reactivity with hK10 antibodies. In the white matter of the cerebral cortex, both fibrillary astrocytes and oligodendrocytes expressed hK10 focally. The distinction between these cell types was difficult. The nuclei of oligodendrocytes are somewhat smaller and more hyperchromatic, but usually these two cell types

Table 1 Tissues positive for hK10 immunoexpression[a]

Tissue	Immunostaining characteristics
Choroid plexus	D, strong
Glial cells and neurons	D
Peripheral nerves, ganglia	D, strong
Breast	DC, LS, focal, strong
Endometrium	Focal, DC (droplets)
Decidua	D, weak
Endocervix	PN
Fallopian tube	SpN
Ovary	Primordial follicles, granulosa lutein cells, surface epithelium
Prostate	DC
Epididymis, seminal vesicles, vas deferens, ejaculatory ducts, rete testis	C, BB
Testis, Leydig cells	D, strong
Testis, spermatic epithelium	D, weak
Kidney (urinary tubuli)	C
Colon, appendix	IfN (colon), SpN (appendix)
Small intestine	C, BB
Stomach	C, IfN
Bile ducts and gallbladder	C
Pancreas (islets of Langerhans, ducts)	D, strong
Bronchus, larynx, trachea, rhinopharynx, paranasal sinuses	C
Salivary glands, submucosal laryngeal, tracheal, bronchial glands, sweat glands	C (ductal epithelium)
Thymus	Hassall's corpuscles
Adrenal gland (medulla)	C
Thyroid gland	Focal, mainly in hyperplasia
Parathyroid glands	Oxyphilic cells

[a]D, diffuse; C, cytoplasmic; BB, brush border; LS, luminal secretions; PN, paranuclear; SpN, supranuclear; IfN, infranuclear.

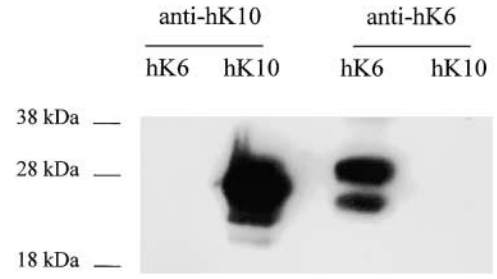

Figure 1 Western blotting analysis of hK6 and hK10 with anti-hK6 or anti-hK10 antibodies. Note the specific reaction of the two antibodies with their cognate antigens (no crossreactivity). Bands with molecular weight <28 kD represent fragmented recombinant proteins [hK6; made in a mammalian expression system (Little et al. 1997) and hK10 in yeast (Luo et al. 2001b)].

do not fall into two clearly defined groups. The CD57 positivity in the oligodendrocytes was helpful. No staining was identified in the meninges.

We studied hK10 immunostaining in the peripheral nervous system, examining nerves and ganglia, which were contained in different specimens. All nerves, motor, sensory, and autonomic, showed the same strong positivity for hK10 (Figure 3A). We suggested that the immunoexpression concerned Schwann cells because the staining pattern was similar to that of S100 protein. Strong positivity for hK10 was also observed in the ganglia of the peripheral nervous system. Both the neurons and their satellite cells that have an origin similar to that of Schwann cells were immunoreactive.

Female Reproductive System

We examined non-malignant breast specimens from women with mild fibrocystic disease. We tried to focus on almost "normal" structures. Cytoplasmic immunoexpression was identified in the columnar cells of ductal and lobuloalveolar structures. Myoepithelial cells remained unstained. Luminal secretions were also positive (Figures 3B and 3C). Foci of apocrine metaplasia and apocrine cysts showed strong staining (Figure 3D). The staining in breast tissues was in most cases focal. Not every structure expressed the protein, and there were differences in the staining intensity.

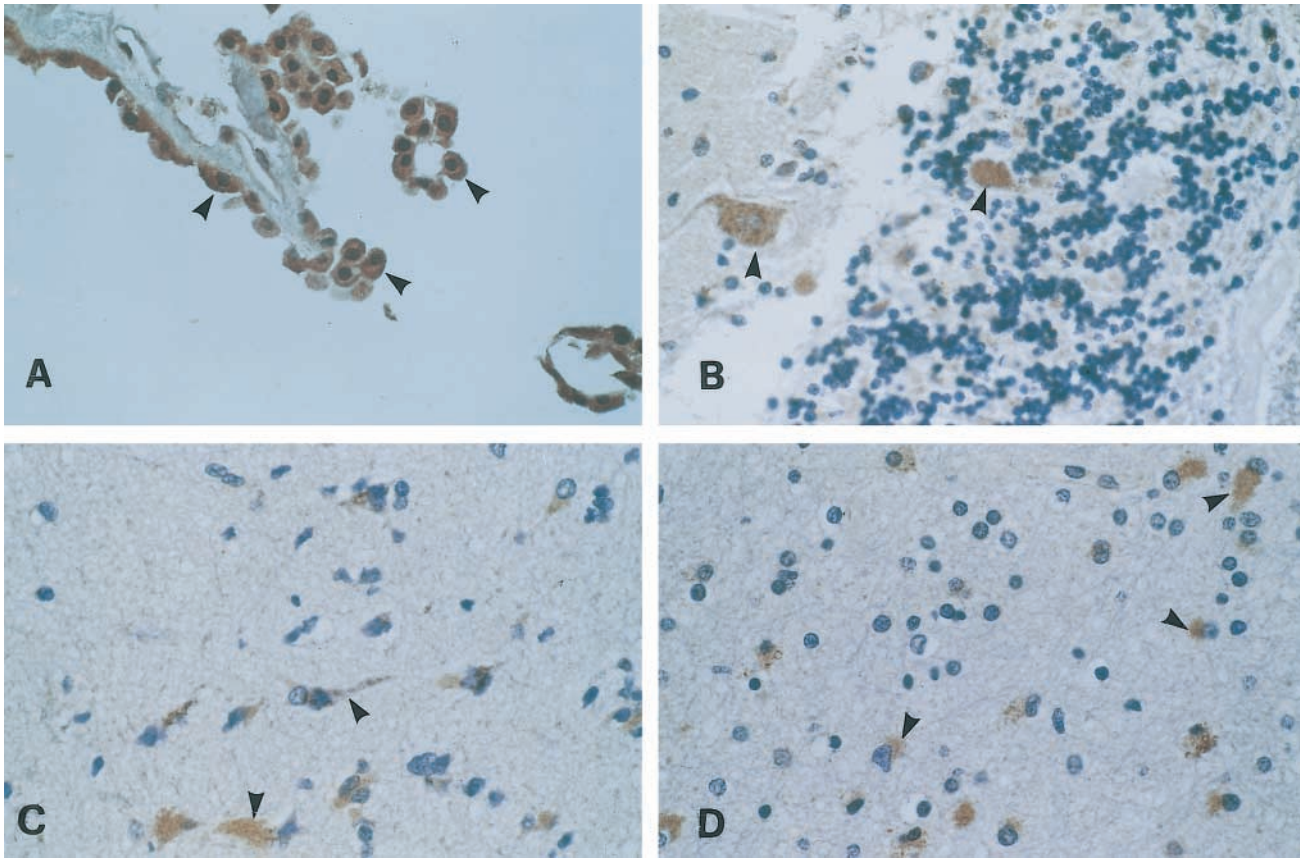

Figure 2 (**A**) Strong hK10 positivity in the choroid plexus epithelium. Polyclonal antibody. (**B**) hK10 immunostaining in the neurons in the cerebellum (Purkinje cells). Monoclonal antibody. (**C**) hK10 immunoexpression mainly in the neurons of the gray matter of the cerebral cortex. Monoclonal antibody. (**D**) hk10 immunoexpression in protoplasmic astrocytes of the gray matter of the cerebral cortex. Monoclonal antibody. Original magnification ×200.

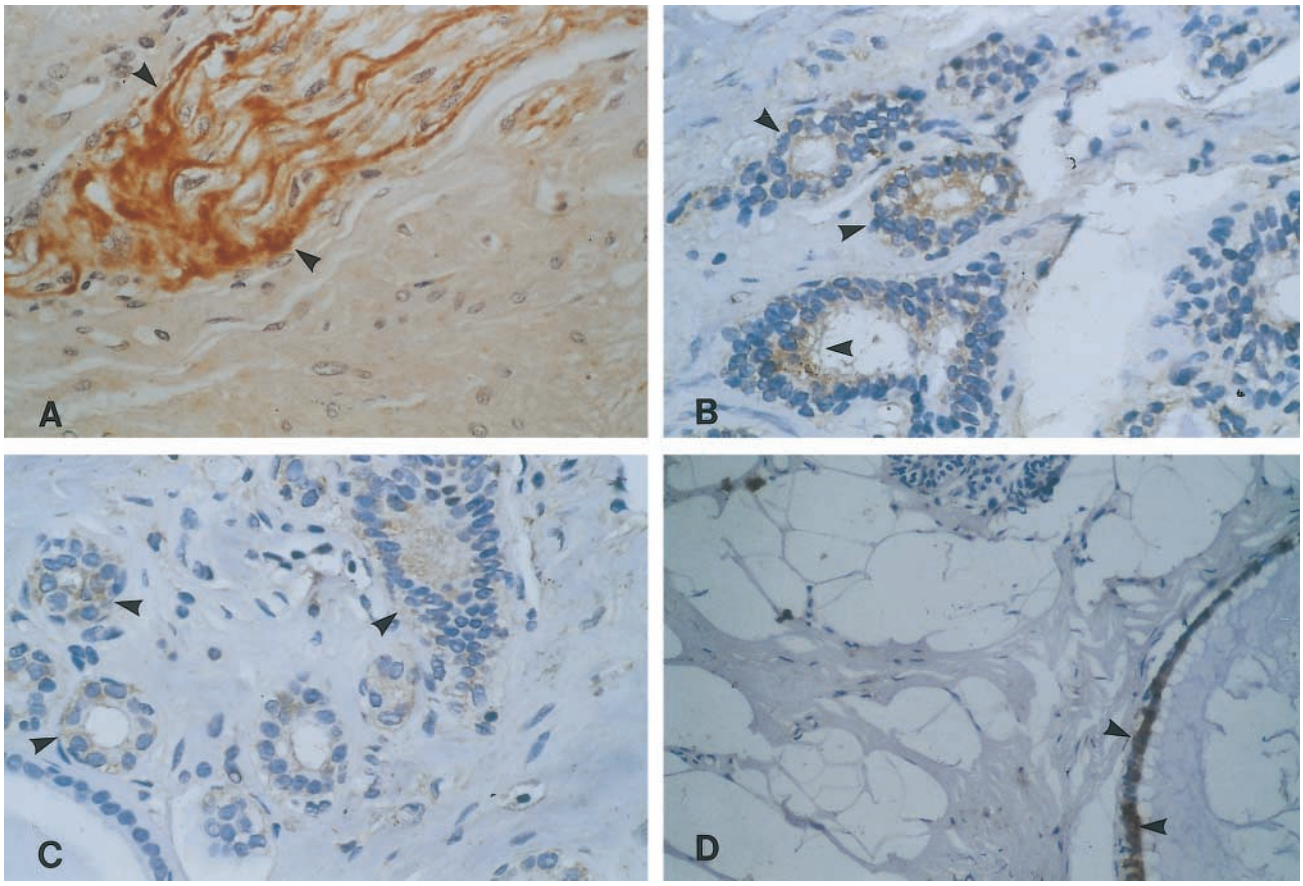

Figure 3 (**A**) Immunoexpression of hK10 in an autonomic nerve of the neurovascular bundles close to the prostate gland. Polyclonal antibody. (**B**) Cytoplasmic hK10 immunostaining in columnar cells in the breast; no staining in the myoepithelial cells. Polyclonal antibody. (**C**) Cytoplasmic immunoexpression of hK10 in the columnar cells of ductal and lobuloalveolar structures in the breast and positivity in luminal secretions. Polyclonal antibody. Original magnification ×200. (**D**) Immunoexpression of hK10 in an apocrine cyst in the breast. Monoclonal antibody. Original magnification ×100.

We studied both layers of the endometrium, the basalis and the functionalis, during the different phases of the menstrual cycle. Strong but focal hK10 immunostaining was seen in the epithelium of the endometrium in both the proliferative and secretory phase, with cytoplasmic localization. A characteristic infranuclear droplet-like, in some areas widely distributed, expression was noted in the secretory phase (Figures 4A and 4B). The staining in the functionalis layer was more diffuse and intense than in the basalis layer. Characteristic was the moderate hK10 immunoexpression in areas of decidual change of the endometrial stroma due to hormonal changes during the menstrual cycle, in cases of hormone intake for therapeutic reasons, and in pregnancy. No staining was identified in the myometrium.

Cytoplasmic immunoexpression in the mucin-secreting epithelium of the endocervix and the tubular cervical glands was observed. A ciliar staining pattern was observed in ciliated cells. Foci of squamous metaplasia and the squamous epithelium of the exocervix were negative (data not shown), as was the squamous epithelium of the vagina.

Ovarian specimens from pre- and post-menopausal women were examined. hK10 immunostaining was observed in the surface epithelium. A clear immunoreaction was also observed in the primordial follicles, the granulosa lutein cells in the corpus luteum, and sparse luteinized cells in the stroma of the ovary (data not shown). No other cells expressed the protein. Strong, diffuse cytoplasmic and ciliar staining was revealed in the epithelium of the fallopian tubes (Figure 4C).

Genitourinary Tract

We studied specimens from the renal cortex and the renal medulla. The epithelium of all types of the urinary tubuli showed a cytoplasmic IHC expression of hK10. Stronger staining was observed in the epithelium of the proximal and distal convoluted tubuli (Fig-

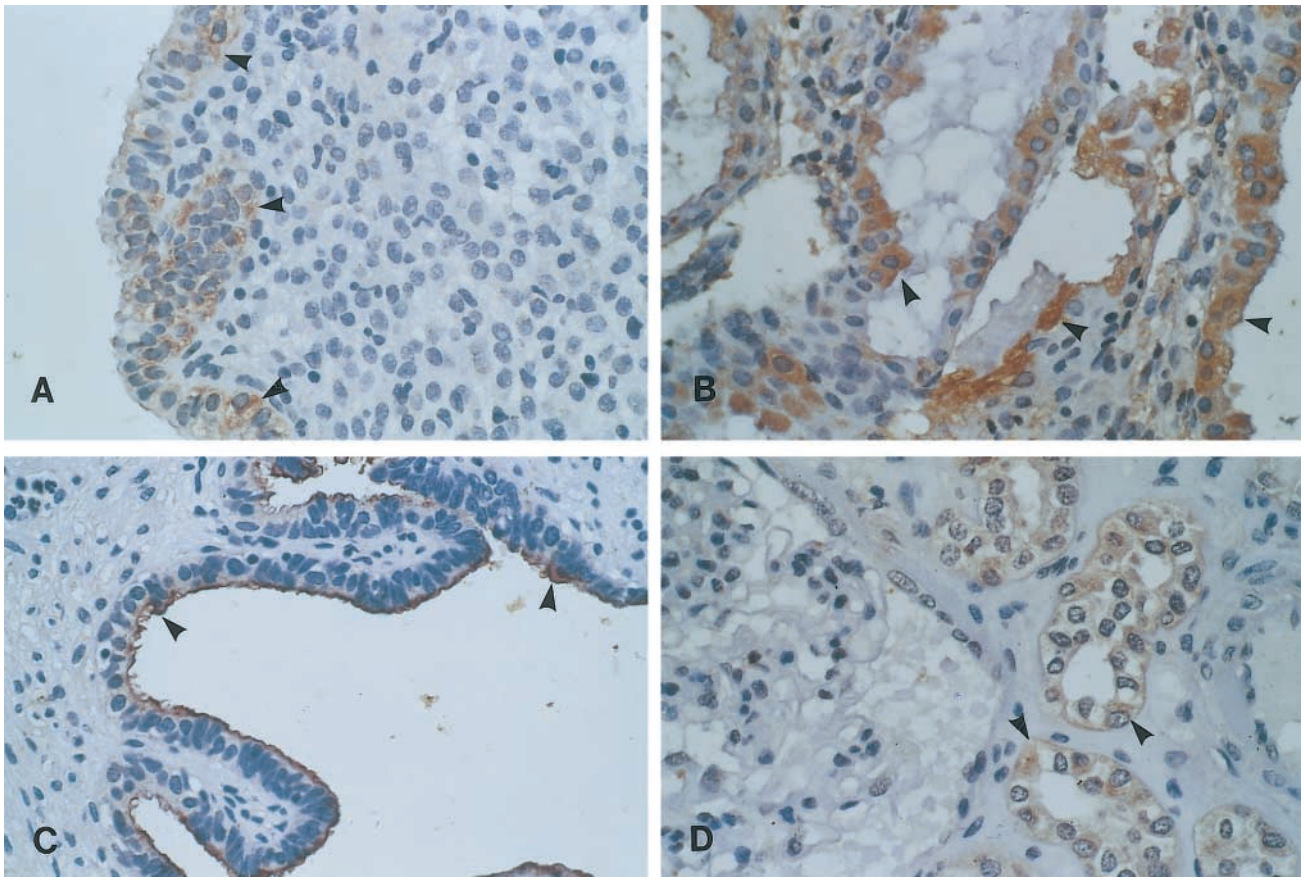

Figure 4 **(A)** Cytoplasmic hK10 immunoexpression in the surface epithelium of the endometrium in the proliferative phase of the menstrual cycle. Polyclonal antibody. **(B)** Cytoplasmic hK10 immunoexpression in the glandular epithelium of the endometrium in the secretory phase of the menstrual cycle. Polyclonal antibody. **(C)** Cytoplasmic and cilia hK10 immunostaining in the epithelium of the fallopian tube. Monoclonal antibody. **(D)** Cytoplasmic hK10 immunoexpression in the epithelium of distal convoluted tubules in the cortical area of the kidney. Polyclonal antibody. Original magnification ×200.

ure 4D). In the proximal tubuli a brush border staining was obvious. No cell type of the renal glomeruli expressed hK10.

The urothelium of the urinary bladder, as well as the other parts of the urinary tract, showed a characteristic immunoexpression of hK10. The superficial umbrella cells and only some scattered intermediate urothelial cells expressed the protein (Figure 5A).

Male Reproductive System

We studied hK10 immunoexpression in all zones of the prostate gland. Variable but mostly strong and diffuse hK10 immunostaining in the prostate secretory cells was observed in all parts. Although the basal cells remained unstained, hK10 was often expressed in basal cell hyperplasia. In these areas the columnar cells expressed the protein weakly or remained unstained. Another interesting finding was the most intense staining in scattered cells among the columnar epithelium. Chromogranin positivity in these cells supported their neuroendocrine nature (Figures 5B–5D). The epithelium of the ejaculatory ducts revealed hK10 positivity as well.

Strong cytoplasmic, brush border, and ciliar staining was revealed in the epithelium of the efferent ductules, the epididymis, the ductus deferens, the ampulla of ductus deferens, the seminal vesicle, and the ejaculatory duct (Figure 6A).

Strong hK10 immunostaining in the Leydig cells of the testis was observed. The spermatic epithelium showed variable expression from case to case. Weak to moderate staining throughout the epithelium was mostly observed. In cases with atrophy of the spermatic epithelium, spermatogonia and Sertoli cells expressed the protein more intensely (Figures 6B and 6C). The rete testis showed cytoplasmic hK10 expression (Figure 6D).

The columnar epithelium of the penile urethra, as well as the Littre's and Cowper's glands, showed mod-

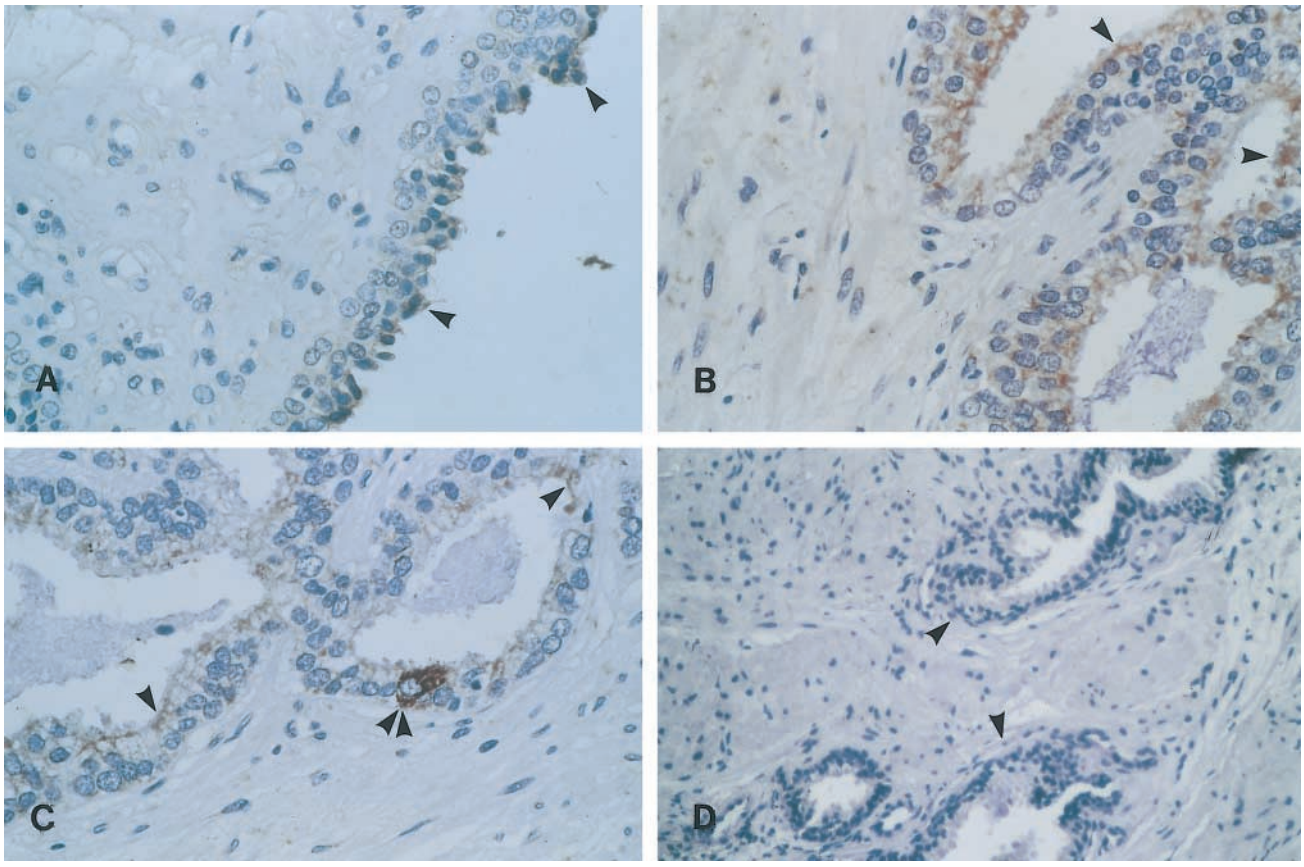

Figure 5 (**A**) hK10 immunoexpression in the umbrella cells of the urothelium of the urinary bladder. Monoclonal antibody. (**B**) Positive staining of hK10 in secretory cells in the peripheral zone of the prostate gland. Monoclonal antibody. (**C**) Positive staining of hK10 in secretory cells in the peripheral zone of the prostate gland (single arrowheads). More intense staining in a neuroendocrine cell (double arrowheads). Monoclonal antibody. Original magnification ×200. (**D**) Unstained prostate epithelium. Non-immune rabbit serum (using hK10 polyclonal antibody). Original magnification ×100.

erate hK10 expression (data not shown). The smooth muscle and the connective tissue of the corpus cavernosum and the corpus spongiosum, as well as other structures in the penis, were negative.

Gastrointestinal Tract

The non-keratinizing squamous epithelium of the esophageal mucosa was negative. The mucus-secreting esophageal glands located in the lamina propria expressed hK10, mainly in the ductal epithelium (data not shown).

Specimens from cardiac, antral, and fundic mucosa of the stomach were examined. The columnar mucus-secreting surface epithelium in all parts of the gastric mucosa showed focal hK10 cytoplasmic, mainly infranuclear staining. The cardiac and antral gastric glands, as well as the mucous neck cells in the fundus, expressed the kallikrein in the same manner. Immunoexpression in the fundic mucosa was more extensive and diffuse. Both parietal and chief cells expressed hK10, but the immunoexpression in the former cells was constant and more intense (Figure 7A). Strong positivity in foci of intestinal metaplasia in the gastric mucosa was the rule. Stronger expression of some cells in some glands could concern neuroendocrine cells, but this suggestion was not further investigated in the present study.

We studied specimens from all parts of the small intestine. No apparent differences in hK10 expression were noted in the mucosa of the duodenum, the jejunum, and the ileum. Both the villous and the crypt epithelium expressed the kallikrein with a focal distribution. Intense cytoplasmic, mainly supranuclear, and brush border immunoexpression was observed in the absorptive cells. Goblet cells were positive to a lesser degree, expressing the protein in their cytoplasm and only rarely in the mucin droplets (Figure 7B). As in the gastric mucosa, there was a strong suggestion that endocrine cells in the crypts were positive. Staining of Paneth cells was not always obvious. Therefore, these cells require further investigation. The submucosal Brunner's glands in the bulb of the duodenum showed moderate focal immunostaining.

Specimens from all segments of the large intestine

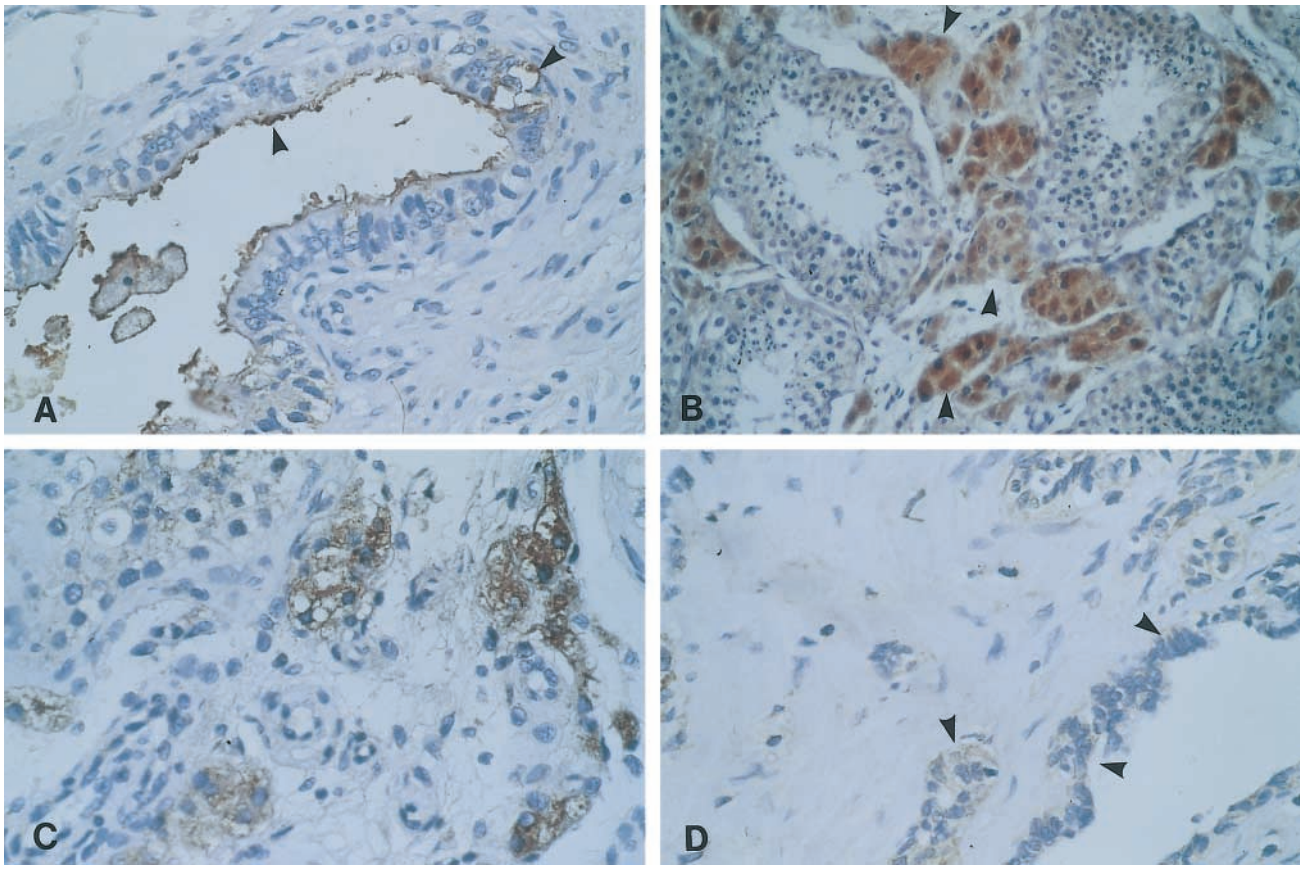

Figure 6 (**A**) Brush border and ciliar hK10 immunostaining in the epithelium of the ductus deferens near the junction with the epididymis. Monoclonal antibody. (**B,C**) Strong hK10 staining in the Leydig cells and weak staining in the spermatic epithelium of the testis. Monoclonal antibody. (**D**) Cytoplasmic hK10 immunostaining in the epithelium of the rete testis. Monoclonal antibody. Original magnification ×200.

were examined. No clear differences in hK10 expression were noted in the mucosa of the different regions of the colon. Both the surface and the crypt epithelium expressed the kallikrein strongly and mostly diffusely. Cytoplasmic, mainly infranuclear immunoexpression dominated in the absorptive cells. Goblet cells also showed cytoplasmic, mainly supranuclear staining, whereas most mucin droplets remained unstained (Figure 7C).

A mainly supranuclear cytoplasmic immunostaining was expressed in the absorptive cells of the appendix (Figure 7D). A brush border pattern in the surface epithelium, covering lymphoid aggregates, concerned membranous cells. A cytoplasmic, also supranuclear, immunoexpression was revealed in goblet cells, whereas the apical mucin droplets usually remained unstained.

Pancreas

In the exocrine pancreas, cytoplasmic hK10 immunoexpression was observed in the medium and small pancreatic ducts, whereas the acinar cells were negative. We found strong positivity in the cells of the islets of Langerhans. Scattered hK10-positive cells were localized in relationship to pancreatic acinar cells (Figures 8A–8D). Using a double immunostaining method, we demonstrated that all cell types in the islets revealed a co-localization of each hormone and hK10 in the same cell population. It is well known that scattered cells in the exocrine pancreas express the different hormones that are produced in the different cell types in the islets. These cells probably express hK10 as well. This finding was documented by double immunostaining techniques (our unpublished data).

Liver, Gallbladder, and Extrahepatic Bile Ducts

Hepatocytes were negative. Cytoplasmic immunostaining was observed in the bile ducts of the portal tracts (data not shown).

Cytoplasmic immunostaining was observed in the columnar epithelium of the gallbladder, the cystic duct, and the extrahepatic bile ducts (data not shown).

Respiratory Tract

Specimens from most regions of the upper and lower respiratory system were examined. The pseudostrati-

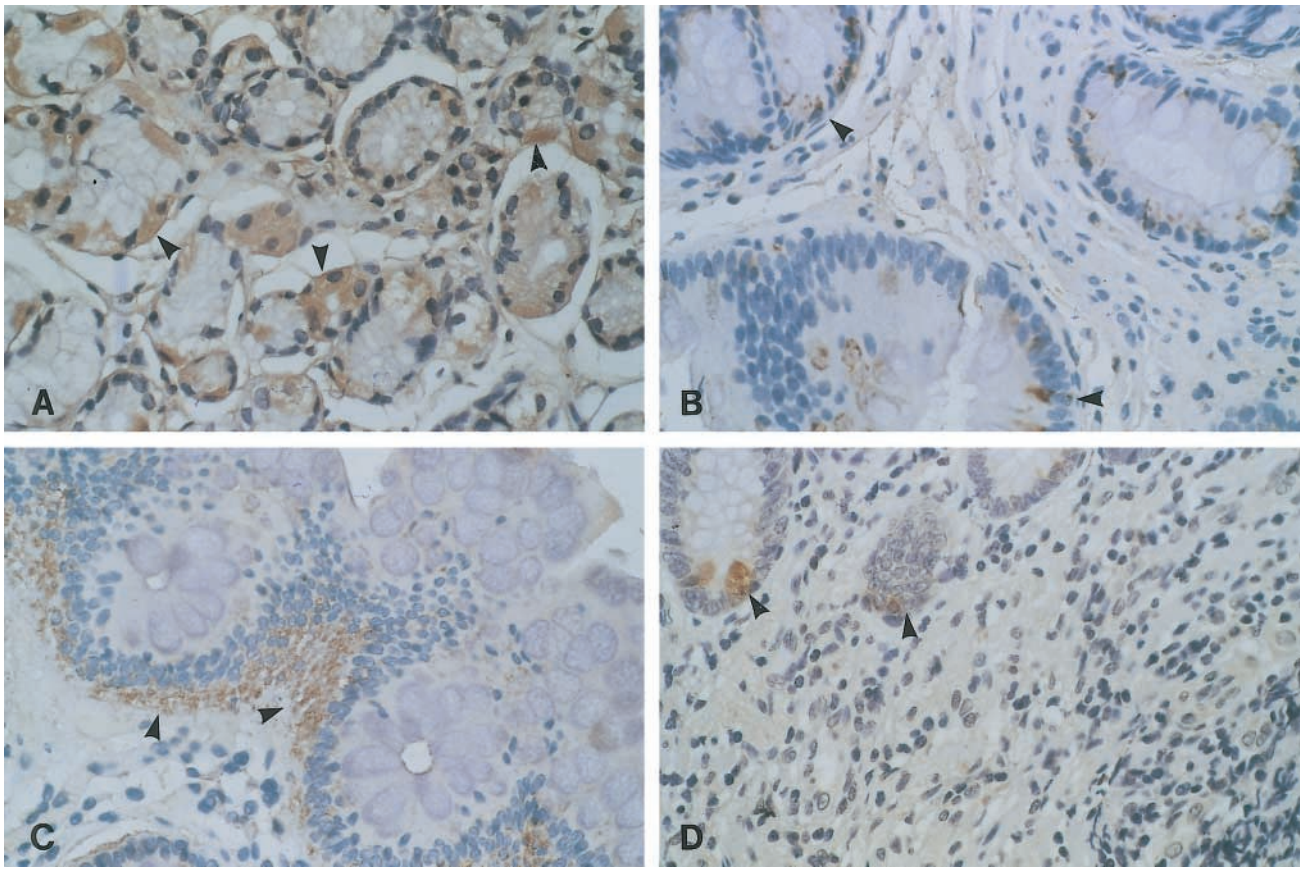

Figure 7 (**A**) hK10 immunoexpression in all cell types in the neck portion of the fundic gastric mucosa. Monoclonal antibody. (**B**) hK10 immunoexpression in absorptive and goblet cells in the villous epithelium of the duodenum. Monoclonal antibody. (**C**) hK10 immunoexpression in absorptive and goblet cells in the surface epithelium of the colon. Monoclonal antibody. (**D**) hK10 immunoexpression in absorptive and goblet cells in the crypt epithelium of the appendix. Polyclonal antibody. Original magnification ×200.

fied ciliated columnar epithelium that covers the upper and lower respiratory tract (nose, paranasal sinuses, larynx, trachea, bronchial tree) showed cytoplasmic and ciliar hK10 staining. Intermingled goblet cells were also positive (data not shown). A characteristic immunostaining was observed in the submucosal glands in the larynx, trachea, and larger bronchi. The epithelium of the excretory ducts and the serous alveoli expressed hK10 strongly and diffusely, and mucous alveoli stained to a lesser degree (Figure 9A). The alveolar epithelium of the lung parenchyma was negative.

Salivary Glands and Skin Appendages

We examined specimens from the major and minor salivary ducts. hK10 immunoexpression in the different cells in serous, mucous, and mixed glands was evaluated. Strong cytoplasmic positivity in the epithelium of the excretory ducts was constant in all cases (Figure 9B). Most mucous and serous alveoli were negative. In mixed glands, crescent-shaped formations of serous cells on the periphery of mucous alveoli expressed the kallikrein often.

The ductal epithelium of skin appendages expressed hK10.

Spleen, Tonsils, Lymph Nodes, and Bone Marrow

The lymphatic organs, such as the spleen and the lymph nodes, generally did not express hK10. Only characteristic positivity in the Hassall's corpuscles of the thymus was only observed (Figure 9C). In inflammatory lesions in different tissues, hK10 was expressed by leukcocytes, mainly neutrophils.

Adrenal Glands

Moderate positivity was observed in the cytoplasm of the cells in the adrenal medulla (Figure 9D).

Thyroid Gland

Focal protein immunoexpression was revealed in the follicular cells in the thyroid gland, mainly in hyperplastic conditions and in oxyphilic cell metaplasia (Figure 10A).

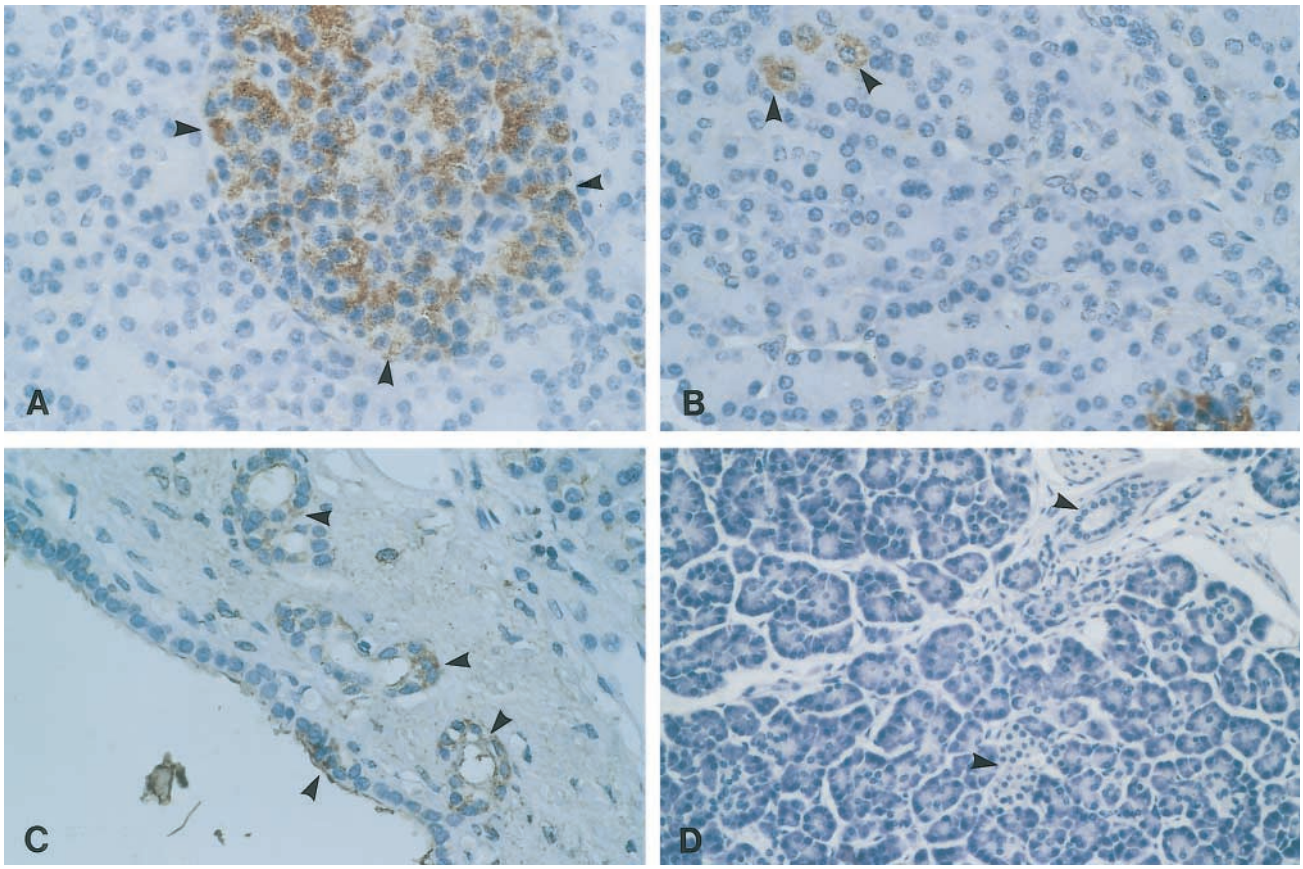

Figure 8 (**A**) Strong and diffuse hK10 immunostaining in an islet of Langerhans in the pancreas. Monoclonal antibody. (**B**) Scattered hK10 positive cells between acinar cells in the pancreas. Monoclonal antibody. (**C**) hK10 positivity in the epithelium of medium sized ducts in the pancreas. Monoclonal antibody. Original magnification ×200. (**D**) Unstained islets and duct epithelium in the pancreas. Immunoabsorption (using hK10 polyclonal antibody). Original magnification ×100.

Parathyroid Glands

hK10 immunoexpression by the oxyphilic cells was noted in the parathyroid glands. Chief cells remained mostly unstained (data not shown).

Pituitary Gland

In the anterior lobe of the adenohypophysis, many cells expressed the protein strongly (Figure 10B). These cells had the morphology of lactotrophs and corticotrophs. Lactotrophs usually wrap around adjacent cells and sometimes show paranuclear staining, a pattern corresponding to prolactin in the Golgi apparatus. Corticotrophs are larger, ovoid to polyhedral, have a tendency to cluster, and many have an unstained region near the nucleus. Furthermore, they reacted with prolactin and ACTH antibodies, respectively. Characteristic strong positivity was also shown in epithelium-lined follicular and ductal formations in the poorly developed (in humans) pars intermedia. (Figure 10C). It is well known that these cystic formations are lined by a single layer of cuboidal to columnar epithelium which may be non-ciliated, ciliated, mucin-producing, or granulated (endocrine). These endocrine cells show variable immunoreactivity for pituitary hormones. In our case, they were prolactin-immunoreactive. The pituicytes of the pars nervosa were negative.

Mesothelium

Specimens including pleural, pericardial, and peritoneal mesothelium were studied. The positivity for hK10 was variable, from weak to strong. In reactive processes, where the mesothelial cells undergo marked proliferative and hyperplastic changes, the staining was more prominent (data not shown).

Squamous Epithelia

Squamous epithelia (skin, uterine cervix, mouth mucosa) were generally negative. In some cases weak focal expression by keratinocytes was observed but could not be considered safely as positive.

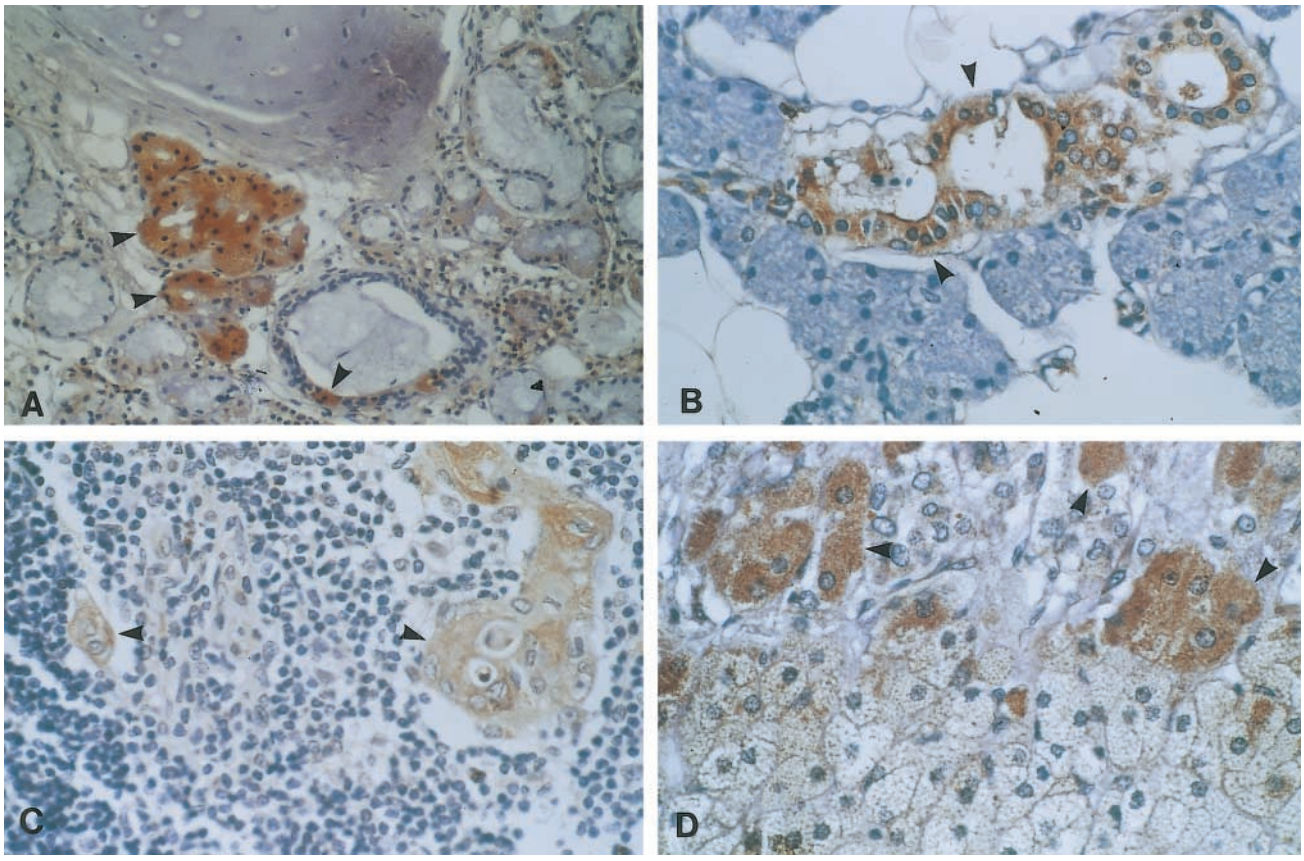

Figure 9 (**A**) Intense cytoplasmic hK10 staining in serum alveoli and in an excretory duct in submucosal bronchial glands; weaker staining in mucous alveoli. Polyclonal antibody. Original magnification ×100. (**B**) Cytoplasmic hK10 immunostaining in the ductal epithelium of the parotid gland. Polyclonal antibody. (**C**) hK10 immunoexpression in the Hassall's corpuscles of the thymus. Polyclonal antibody. (**D**) hK10 immunoexpression in the cells of the adrenal medulla. Monoclonal antibody. Original magnification ×200.

Mesenchymal Tissues

In general, no expression was observed in mesenchymal tissues. Positivity was expressed in chondrocytes (Figure 10D) and in the wall of small vessels in some cases.

In short, hK10 is expressed by many normal human tissues. Glandular epithelia constitute the main hK10 immunoexpression sites, with representative organs being the prostate, epididymis, spermatic duct, kidney, breast, endometrium, endocervix, fallopian tube, colon, appendix, small intestine, stomach, salivary ducts, bile ducts, gallbladder, bronchus, and the upper respiratory tract. Both the Leydig cells and the spermatic epithelium in the testis also expressed hK10. Focal immunostaining in glial cells in the CNS was also observed. Diffuse and strong expression was noted in the choroid plexus epithelium, the peripheral nerves, the ganglia, cells of the anterior lobe of the pituitary, and the islets of Langerhans in the pancreas. A characteristic immunostaining was observed in the Hassall's corpuscles of the thymus, the oxyphilic cells in the thyroid and parathyroid glands, and in chondrocytes.

Discussion

The KLK10 gene, initially named NES1, was cloned by subtractive hybridization from radiation-transformed breast epithelial cells (Liu et al. 1996). KLK10 resides on the same chromosomal locus as other human kallikreins and shares considerable similarities at the gene and protein levels (Diamandis et al. 2000b; Yousef and Diamandis 2001). The recent development of monoclonal and polyclonal antibodies against hK10 and of the first immunofluorometric assay for quantifying the protein has contributed in defining the distribution of hK10 in biological fluids and tissue extracts (Luo et al. 2001b). The gene is expressed in many human tissues (e.g., skin, colon, salivary glands, prostate, fallopian tubes, testis, endometrium, pituitary, and lung) and is also secreted in many biological fluids, including amniotic fluid, seminal plasma, breast milk, serum, and cerebrospinal fluid (Luo et al. 2001b). However, hK10 has not previously been studied by IHC.

This is the first report describing the IHC localization of hK10 in diverse normal human tissues. Similar work has been reported recently by our group for the

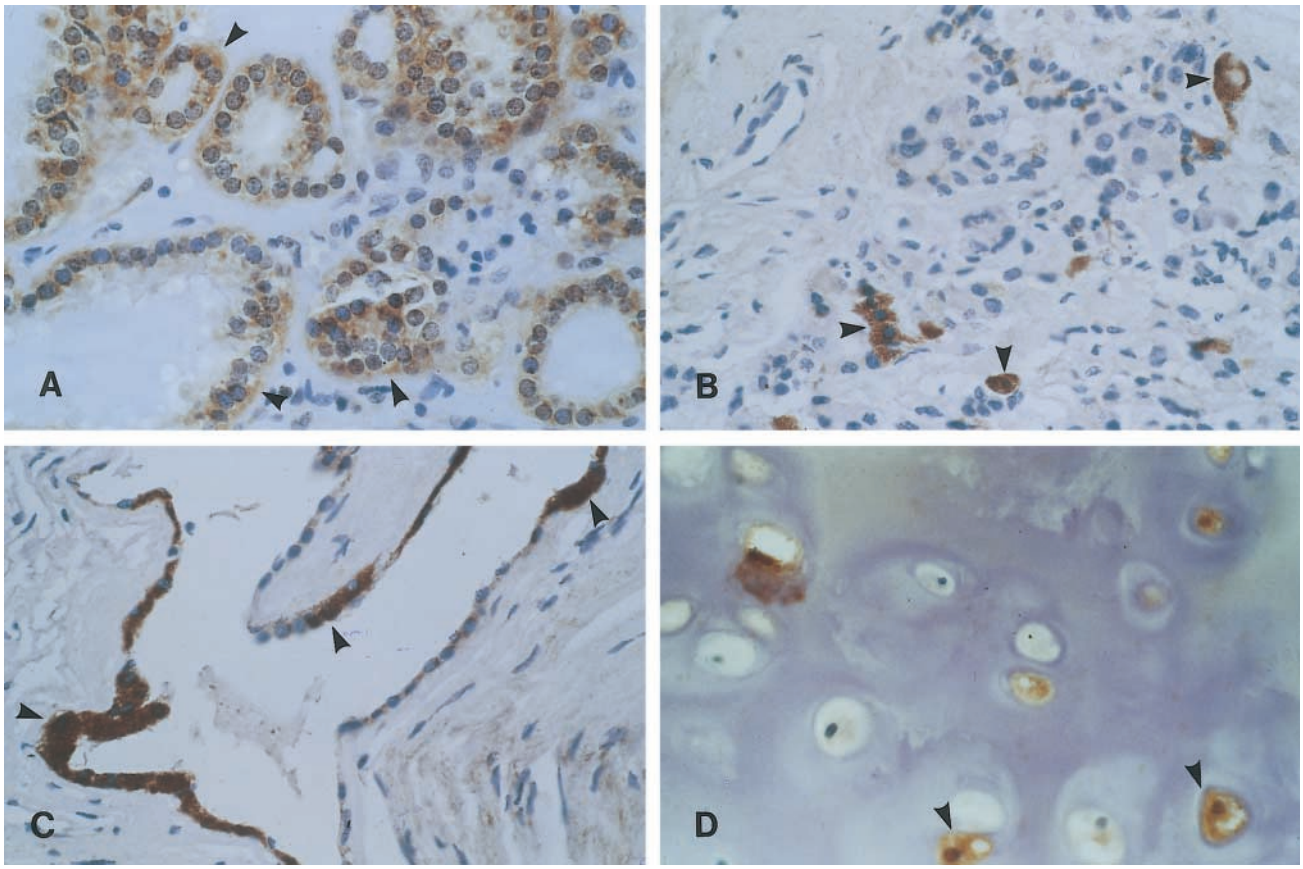

Figure 10 (**A**) Intense cytoplasmic hK10 immunoexpression in hyperplastic follicles of the thyroid gland. Polyclonal antibody. (**B**) Pituitary gland: hK10-positive endocrine cells (corticotrophs) in the adenohypophysis. Monoclonal antibody. (**C**) Pituitary gland: hK10-immunoreactive endocrine cells in epithelium-lined spaces of the pars intermedia. Monoclonal antibody. (**D**) Intense cytoplasmic hK10 immunoexpression in chondrocytes of hyaline cartilage. Polyclonal antibody. Original magnification ×200.

homologous kallikrein hK6 (Petraki et al. 2001). Comparison of the expression patterns of the two kallikreins reveals no major differences, suggesting that they share a common mode of regulation. Indeed, both kallikreins are regulated by steroid hormones and, most profoundly, by estrogens (Diamandis et al. 2000b; Yousef and Diamandis 2001; Yousef et al. 1999a; Luo et al. 2000). Importantly, in any case of simultaneous quantification of hK6 and hK10 in biological fluids, including serum of ovarian cancer patients and breast cancer cytosols, there is a significant correlation between hK6 and hK10 concentrations (data not shown), further supporting our suggestion of coordinated expression. Both the polyclonal and the monoclonal hK10 antibodies revealed the same immunostaining pattern in all tissues. Replacement of the primary polyclonal antibody by non-immune rabbit serum and the immunoabsorption tests resulted in abolition of the immunostaining, suggesting good specificity.

The hK10 protein was localized in a large number of normal human tissues and therefore, like hK6, it cannot be considered a specific tissue marker, in contrast to the homologous proteins hK2 and PSA (hK3), which have prostate-restricted expression (McCormack et al. 1995; Chu 1997; Rittenhouse et al. 1998; Stenman 1999). With the availability of highly sensitive techniques, it has become apparent that both hK2 and hK3 are also expressed at lower levels in many other tissues, including breast, thyroid, and salivary glands (Yu and Diamandis 1995; Black and Diamandis 2000; Black et al. 2000; Magklara et al. 2000). The expression of hK10 mainly by glandular epithelia suggests that it is secreted. This is further supported by the finding of hK10 in many biological fluids, including milk, seminal plasma, serum, cerebrospinal fluid (CSF), and amniotic fluid (Luo et al. 2001b). In this study, luminal secretions in the breast stained positive for hK10 (Figure 3).

Our IHC findings correspond well with the data of hK10 quantification in tissue extracts by immunoassay (Luo et al. 2001b). The IHC study is certainly a superior tool because it defines the protein distribution in different cell types. The high concentration in

skin extracts (Luo et al. 2001b) probably corresponds to the IHC expression of hK10 by the skin appendages, as reported in the present study. In the prostate gland the IHC staining was localized in the columnar cells. Basal cells remained unstained. An interesting finding was the strong hK10 immunoexpression by hyperplastic basal cells. A possible explanation is that both cell types derive from the same stem cell and can have similar phenotypes in several pathological benign or malignant conditions. A similar observation was made with hK6 staining. Some scattered strongly positive cells likely correspond to neuroendocrine cells because chromogranin staining revealed positive cells in these areas as well. In general, hK10 and hK6 had the same immunostaining pattern in the benign prostate gland as hK2 and hK3. The pattern of hK10 and hK6 expression in the urothelium is similar to that of keratin 20, with staining of the superficial cells and of scattered intermediate cells. We suggest that both kallikreins could be, like keratin 20, markers for urothelial differentiation (Harnden et al. 1996). Further studies will clarify this finding. In the testis, both Leydig cells and spermatic epithelium showed positive staining. The immunoexpression in the Leydig cells was strong, as in other examined neuroendocrine organs. A detailed study including cases with spermatic epithelium atrophy and testicular germ cell and sex cord stromal tumors will yield further information. Our present findings in the testis are in accordance with previous work investigating the expression level of the KLK10 gene in normal and malignant testicular tissues using RT-PCR and IHC. The expression was markedly reduced in the germ cell tumors compared to adjacent normal tissues, suggesting that KLK10 may play a role in the pathogenesis and progression of this malignancy (Luo et al. 2001a). Another interesting finding was the presence of hK10 and hK6 in cells of the diffuse neuroendocrine system and in nerves. The staining was relatively strong, and we believe that it could be representative of neuroendocrine differentiation. The expression of hK6 and hK10 is reminiscent of CD56 (natural killer cell-associated antigen, neural cell adhesion molecule) and CD57 (Leu-7, T-cell surface marker) which are sensitive but not specific for cells and neoplasms with neuroendocrine differentiation (Kaufmann et al. 1997). These suggestions need verification with a study of a variety of neuroendocrine tumors. The IHC analysis of the pituitary tissue in this study localized strong staining in some cell types, probably lactotrophs or corticotrophs of the anterior lobe of the adenohypophysis, and in epithelium-lined spaces of the intermediate lobe remnant, which are also prolactin-immunoreactive. The same distribution was observed with the hK6 expression in our previous work (Petraki et al. 2001). It is noteworthy that the pancreatic kallikrein (hK1) has been found in lactotrophs of the rat and in human anterior pituitary (Jones et al. 1990; Vio et al. 1990). Interesting is the strong expression of hK10 and hK6 by the islet cells in the human pancreas. Furthermore, both kallikreins were expressed in the epithelium of the pancreatic ducts but not in the acinar cells of the exocrine pancreas. It is possible that hK6 and hK10 are involved in islet hormone processing, a property already attributed to many other proteolytic enzymes (Seidah and Chretien 1999). Pancreatic kallikrein has been also evaluated in the β-cells of the pancreatic islets (Ole–Moi Yoi et al. 1979; Pinkus et al. 1983). The expression of kallikreins in the anterior lobe of the pituitary and in the endocrine pancreas supports the suggestion that kallikreins are involved in the processing of precursors to polypeptide hormones (Mason et al. 1983). Interestingly, KLK10 mRNA in the pancreas appears to be shorter (1.1 kb) than mRNA from other tissues (1.4 kb) (Liu et al. 1996). The strong immunoexpression of hK10 by the ducts of the salivary gland is in accordance with the report that almost all kallikreins are expressed by these glands (Diamandis et al. 2000b). The discovery of hK1 in the pancreas, salivary glands, and kidney was reported by Werle and his colleagues (Kraut et al. 1930; Frey et al. 1950). It is well known that there are not only morphological but also functional similarities between the salivary glands, and the pancreas and, except for kallikrein, amylase, glucagon, and peptide P-C are present in both tissues (Ito et al. 1983). Other neuroendocrine organs that expressed both hK10 and hK6 in our studies were the adrenal medulla and scattered neuroendocrine cells in the gastrointestinal tract (Petraki et al. 2001). Positive hK10 immunoexpression in glial cells and in the choroid plexus epithelium was observed. Like hK6, hK10 may have some connection to Alzheimer's disease (Little et al. 1997; Diamandis et al. 2000c). An interesting finding was the expression of hK10 and hK6 in oxyphilic cells in the thyroid and parathyroid glands. These findings accord with the demonstration of hK2 and hK3 in oxyphilic cells of the thyroid (Magklara et al. 2000), suggesting another similarity among these four kallikreins.

Kallikreins are regulated by steroid hormones (Diamandis et al. 2000b; Yousef and Diamandis 2001). The KLK10 gene is primarily upregulated by estrogens but also by androgens and progestins in the breast cancer line BT-474, indicating that it may be involved in a pathway that counterbalances the action of estrogens and androgens in steroid hormone-responsive tissues (Yousef et al. 1999a; Luo et al. 2000). KLK6 is also regulated by steroid hormones (Yousef et al. 1999a). It will be interesting to study steroid hormone responsive organs for hK10 and hK6 expression and to investigate their possible involvement in tumor development and progression (Diamandis et al. 2000b; Yousef and Diamandis 2001).

The contribution of hK3 (PSA) and hK2 in the diagnosis and monitoring of prostate cancer suggests that other kallikreins may also have value as candidate biomarkers. We have already shown that serum hK6 and hK10 concentration is increased in ovarian carcinoma (Diamandis et al. 2000d; Luo et al. 2001a). We also reported downregulation of KLK10 in malignant breast, prostate, and testicular tissues (Liu et al. 1996; Goyal et al. 1998; Luo et al. 2001d). Many other kallikrein genes are either underexpressed or overexpressed in certain carcinomas. Upregulation of the kallikreins KLK4, KLK5, KLK6, KLK7, KLK8, KLK9, and KLK10 in ovarian carcinomas has been reported (Tanimoto et al. 1999; Underwood et al. 1999; Yousef et al. 1999b,2001; Dong et al. 2001; Kim et al. 2001; Luo et al. 2001c; Magklara et al. 2001; Obiezu et al. 2001). In general, kallikreins may serve to positively or negatively regulate cell growth or differentiation by cleavage of cell surface receptors or cell growth regulatory and angiogenic proteins and by activation of other proteases for invasion and metastasis. Because they are secreted into the extracellular space, kallikreins could have potential as circulating tumor markers (Diamandis et al. 2000b; Yousef and Diamandis 2001).

In conclusion, we report here the expression of hK10 protein in various human tissues by immunohistochemistry. We believe that this study further illumines the role of this protein in human tissues and will help to generate hypotheses of its function and pathophysiology.

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