Immunohistochemistry

Tissues were stored in formalin and paraffin-embedded sections were cut using a microtome. Paraffin was removed by placing slides first in two changes of xylene, followed by 100% alcohol, 90% alcohol and then in distilled water. Antigen retrieval of sections was achieved using a pressure cooker. Following antigen retrieval, slides were blocked for endogenous peroxidase activity with hydrogen peroxide in PBS (1.5%) (1 H_2O_2 : 1 PBS) for 20 minutes and then washed with TBS-T. Sections were incubated in serum for 20 minutes. The slide was washed with TBS-T between and after primary and secondary antibody incubations. Sections stained with Vectastain Universal Elite ABC (Avidin and Biotinylated horseradish peroxidase macromolecular Complex solution) kit (Catalog No. PK-6200; Vector Laboratories, CA, USA). They were then incubated with DAB solution, rinsed with distilled water and washed with TBS-T before counterstaining with hematoxylin solution.

SDS-PAGE Gel Electrophoresis and Western Blot

Sample media was mixed with 4X loading (sample) buffer containing 5% β-mercaptoethanol (Sigma, MO, USA) and Radio-Immuno Precipitation Assay (RIPA) buffer, pH 7.4 (Catalog No. BP-115, Boston BioProducts, MA, USA). The samples were then heated for 5 minutes at 95°C. 10-30μg of sample was loaded onto SDS-PAGE, NuPAGE Bis-Tris pre-cast polyacrylamide gels using the mini-cell system (Invitrogen, CA, USA). NuPAGE MOPS SDS running buffer (Invitrogen, CA, USA) was used. 500μl of antioxidant was added to the running buffer. Electrophoresis was performed at 140V-

200V until adequate spread of the protein molecular marker was achieved. Following SDS-PAGE gel electrophoresis, proteins were then transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA). Transfer was achieved using a wet-blot (Bio-Rad) transfer system. Standard Towbin transfer buffer was used containing 25mM Tris, pH 8.3, 192 mM glycine, 20% (v/v) methanol. Proteins were then visualized with an enhanced chemiluminescence detection system.

Supplemental Table 1

Peptide	Full length only	Q-Value	m/z
AHALAWHVYNEK	Yes	7.82E-09	719.8652
CMASELVR	Yes	0.007687 0.000167	483.23 [a] 491.2282 [a,b]
ELGVTHYR	No	0.043227	487.76
GASIWDTFTHHPLAPPGDSR	No	0.003633	1081.526
GLFYVDFLSQDK	No	1.76E-09	716.3616
ISIALQADWIEPACPFSQK	Yes	1.32E-07	1087.552 [a]
KIIDSNGFPGPETLER	Yes	0.001707	591.6443 [c]
LCFQELGHHVK	Yes	0.00599	684.3484 [c]
LIQGTFDFLALSHYTTILVDSEK	Yes	1.09E-07	871.1276
LWITMNEPYTR	Yes	1.46E-07	712.3559
NNFLLPYFTEDEK	Yes	1.92E-07	815.3926
QGAWENPYTALAFAEYAR	Yes	1.49E-11	1029.49
SSALFYQK	No	5.35E-05	472.2479
VNITPVVALWQPMAPNQGLPR	Yes	2.77E-03	1151.13
VYYMQNYINEALK	Yes	2.30E-05	824.9063

		1.49E-06	832.9036 [b]
YAADQFEPK	Yes	1.36E-05	534.7531

Supplemental Table 1: Candidate peptide signatures identified from recombinant human αKlotho.

[a] Alkylated Cysteine
[b] Oxidised Methionine
[c] Triply charged peptide

Supplemental table 2

PEPTIDE		**GLFYVDFLSQDK** *Isoforms 1 and 2* **716.36 m/z**	**QGAWENPYTALAFAEYAR** *Isoform 1 only* **1029.49 m/z**	**NNFLLPYFTEDEK** *Isoform 1 only* **815.39 m/z, 2+**	**LWITMNEPYTR** *Isoform 1 only* **712.35, 2+**	**VYYMQNYINEALK** *Isoform 1 only* **824.91, 2+**
SITE	rh-αKlotho	+	+	+	+	+
	Kidney	+	+	+	+	-
	Kidney cortex	+	+	-	+	-
	Kidney medulla	+	+	+	+	+
	Kidney proximal tubular epithelial cells	+	-	+	-	+
	Keratinocyte	+	-	+	-	-
	Parathyroid	+	-	+	-	-
	Mammary epithelial Cells	+	-	-	-	-
	Prostate epithelial cells	+	-	-	-	-

	Pancreas	+	-	-	-	+
	Cerebral Cortex	+	+	-	-	-
	Cerebellum	+	-	-	-	+
	Neuron cells	+	+	+	+	+
	Aorta	+	-	+	-	-
	Artery	+	+	-	+	-

Supplemental Table 2: rh-αKlotho – Recombinant human αKlotho. Peptide sequences shown represent the 5 peptide signatures used to identify αKlotho. Cells shaded in green indicate that the peptide was identified in the corresponding sample.