Supplemental Methods:

Quantitative PCR

Total RNA was isolated using the Total RNA purification Kit (Norgen Biotek Corp) with on-column DNase-I treatment. RNA concentrations were determined by measuring the spectrophotometric absorption at 260 nm using the GeneQuant (Amersham). cDNA synthesis was performed using 500 ng total RNA and 8 µl 5X iScript reaction mix (Bio-Rad) containing oligo dT primers, with a total volume of 40 µl. cDNA synthesis was performed using a heating block. Samples were denatured for 10 min at 70 ^{0}C and cDNA synthesis was performed for 60 min at 42 ^{0}C, followed by an enzyme inactivation step for 5 min at 99 ^{0}C. Quantitative PCR (qPCR) was performed to determine the expression levels of HK-2, GLUT-3 and β2 Microglobulin (β2M) mRNAs in 22 PPGL tumor tissues. The primers used for the reactions are as follows: HK-2 Forward Primer 5' TCAGATTGAGAGTGACTGCC 3' and Reverse Primer 5' TTTCTCGTATCCTGTCCACC 3' GLUT-3 Forward Primer 5' GTGGCCCAGATCTTTGGTC 3' and Reverse Primer 5' AAGGGCTGCACTTTGTAGG 3' β2M Forward Primer 5'ATGAGTATGCCTGCCGTGTG 3' Reverse Primer 5'CCAAATGCGGCATCTTCAAAC3'. Reactions were performed using SYBR green PCR master mix (Applied Biosystems). cDNA equivalent of 10 ng total RNA was used for each reaction and the reactions were performed in a total volume of 10 µl in ABI Prism 7500 sequence detector (Applied Biosystems). The amplification reactions were carried out with denaturation at 95 ^{0}C for 10 min, 40 cycles of 15 s at 95 ^{0}C (melting), and 60 s at 60 ^{0}C (annealing and elongation). The expression of the mRNAs was calculated relative to the levels of the internal control gene β2M. The relative expression levels were determined by the $2^{-\Delta Ct}$ method.

Immunohistochemical staining and quantification

Tumor tissue sections were deparaffinized, rehydrated and washed with 50 mM phosphate buffered saline (PBS). Antigen retrieval was performed by boiling the sections in 10 mM citrate, pH 6.0 in a microwave for 3 minutes at 850W followed by 10 minutes at 150 W. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide $_{(H2O2)}$ in PBS for 30 minutes. For HK-1, 2 and 3 staining,

endogenous peroxidase activity was blocked by incubation with 0.6% $_{H2O2}$ in 40% methanol and 60% 50 mM PBS for 30 minutes. Subsequently, the sections were washed with PBS. Non-specific interactions were blocked using normal human serum (5% in PBS for 30 minutes) for HK-1, 2 and 3, normal goat serum (20% in PBS) for VEGF, MCT-4, (30 minutes) GLUT-1 and -3 (10 minutes) respectively. For GLUT-1 prior to incubation with the primary antibody, avidin and biotin block (Vector Laboratories, SP-2001) was performed for 15 minutes each. Primary antibody diluted in 0.1% BSA/PBS was applied (Supplemental Table 2) for 60 minutes for HK-1, HK-2, HK-3, CD34 and GLUT-3, 90 minutes for MCT-4 and overnight for GLUT-1 and VEGF at 4 °C. The sections were then washed with 50 mM PBS, followed by incubation with the secondary antibody for 30 minutes (Supplemental Table 2). Slides stained for HK-1, HK-2, HK-3 and GLUT-1 were incubated with avidin biotin reagent (Vectastain Elite ABC Kit, Vector Laboratories, PK6100) for 45 minutes. The sections were washed with PBS and incubated with 3,3-diaminobenzidine (DAB) (Immunologic, BS04-110) for 10 minutes. After washing DAB with running tap water for 5 minutes, the sections were counterstained with hematoxylin for 10 seconds, washed and dehydrated in ethanol series (50%, 70%, 100%) followed by xylene and mounted with permount.

Expression of various markers was quantified by evaluating the amount of DAB staining by an experienced pathologist (BK) who was unaware of the patient's clinical data while CD34 was scored automatically. The percentage of the positive stained area of the sections was graded in a scale ranging from 0-100%. The intensity of the DAB color was graded as non-staining (0), faint staining (I), medium staining (II) and intense staining (III), ignoring the background color of the staining. Areas containing adrenal cortex, brown fat and capillaries were used as controls for comparison between different sections. The intensity of each marker was evaluated separately. Cytoplasmic staining was evaluated for HK-1, HK-2, HK-3 and VEGF. For GLUT-1, GLUT-3 and MCT-4 cytoplasmic and/or membrane staining was graded as they were expressed both in the cytoplasm and membrane. The score of the staining represents the expression of the markers and is based on the multiplication of the intensity with the percentage of the positive stained area.

For CD34 scoring, image acquisition was performed using a CCD color camera (AxioCam MRc; Carl Zeiss AG, Jena, Germany) mounted on a conventional light microscope (AxioPhot; Carl Zeiss AG, Jena, Germany) and attached to a personal computer. Images were acquired using a x20 objective (Plan Neofluar, NA = 0.5, resulting in specimen level pixel size of 0.53x0.53 μm^2). Prior to analysis of the immunohistochemical staining, an image of an empty microscopic field was acquired, which was used for correction for unequal illumination. Image acquisition and analysis were performed using a custom written macro in KS400 image analysis software (Carl Zeiss), as described previously (*29*). Thresholds for recognition of CD34-positivity were determined from a set of training slides and were found adequate for almost all slides analyzed in this study. When the initial thresholds led to unrealistic patterns, interactive adjustment was performed by the operator. For each patient, the percentage of anti-CD34 stained area, the microvessel density and the vessel perimeter were calculated per surface area tumor in ten randomly selected images.

SUPPLEMENTAL TABLE 1. Patients characteristics and results for immunohistochemical markers of glucose uptake and metabolism and [^{18}]F-fluorodeoxyglucose uptake

Genotype	Sex	Age (y)	Tumor dimensions (mm)	Tumor location	Tumor stage	HK-2	HK-3	GLUT-1	GLUT-3	VEGF	MCT-4	SUV max	SUV mean
Sporadic[1]	F	70	12 x 8 x 16	EA	metastasis	200	100	120	100	200	90	0.79	0.70
Sporadic	F	66	30 x 27 x 32	RA	primary	80	80	90	90	90	5	2.78	1.27
Sporadic	F	56	21 x 17 x 22	RA	primary	40	80	110	130	160	100	1.67	0.78
Sporadic	M	66	27 x 23 x 23	RA	rest tumor	0	60	0	80	90	90	1.93	1.14
Sporadic	F	75	35 x 26 x 37	RA	primary	270	80	25	180	270	100	5.82	2.81
Sporadic	M	44	23 x 26 x 32	RA	primary	90	60	40	150	80	60	2.32	1.08
Sporadic	M	70	52 x 40 x 50	RA	primary	0	40	20	0	5	20	1.28	0.62
Sporadic	M	41	22 x 22 x 21	RA	primary	190	120	66	210	180	30	0.83	0.60
Sporadic	M	36	70 x 63 x 60	RA	primary	20	60	100	10	150	40	2.15	0.90
Sporadic	M	49	75 x 56 x 73	RA	primary	0	10	165	110	90	160	2.28	0.97
Sporadic	F	53	35 x 32 x 33	LA	primary	50	120	120	150	160	160	1.87	1.10
Sporadic	M	20	78 x 52 x 70	RA	primary	160	20	15	180	180	270	4.58	1.63
Sporadic	M	62	31 x 35 x 38	LA	primary	180	30	160	90	180	160	1.64	0.95
Sporadic	F	50	50 x 52 x 53	LA	primary	90	160	60	150	150	270	3.06	1.62
Sporadic	F	55	43 x 40 x 50	LA	primary	110	160	200	90	140	160	6.47	3.26
RET	M	36	17 x 13 x 20	LA	local relapse 2	35	160	30	160	90	80	1.61	1.01
RET	M	36	23 x 19 x 30	LA	primary	150	40	85	150	180	40	1.57	0.83
RET	M	44	27 x 18 x 38	RA	primary	20	180	100	160	130	200	2.42	1.25
RET	M	71	22 x 17 x 17	RA	primary	50	160	50	160	90	160	1.83	0.99
RET	F	47	49 x 45 x 45	RA	primary	30	70	10	50	150	70	1.90	1.11
MAX	M	63	38 x 25 x 41	LA	local relapse 1	100	90	30	70	90	30	2.30	0.99
NF1	F	70	46 x 33 x 65	RA	primary	70	70	90	70	10	70	1.97	0.94
SDHD	M	32	21 x 14 x 21	EA	primary	120	180	30	200	200	160	10.2	3.99
SDHD	F	60	55 x 45 x 50	EA	primary	210	140	110	200	270	60	9.28	5.42
SDHD	M	46	30 x 30 x 21	RA	primary	280	160	60	90	180	80	1.64	0.95
SDHB	M	41	22 x 22 x 20	EA	local relapse 2	230	200	200	90	180	160	11.8	4.93
SDHB	M	21	13 x 14 x 17	EA	primary	200	90	20	150	270	270	8.83	4.76

Abbreviatons: M=male; F=female; y = years; EA = extra-adrenal; LA = left adrenal; RA = right adrenal; HK-2 = hexokinase-2; HK-3 = hexokinase-3; GLUT-1 = glucose transporter type 1; GLUT-3 = glucose transporter type 3; VEGF = vascular endothelial growth factor; MCT-4 = monocarboxylate transporter type 4; SUV = standard uptake value (max = maximum).

[1] Patients were tested for the presence of germline mutations and large deletions in *SDHB/C/D*, *RET*, *VHL* and -since 2011- in *SHDA*, *SDHAF2*, *TMEM127* and *MAX*.

SUPPLEMENTAL TABLE 2. Details of antibodies used for immunohistochemistry

Primary antibody	Host	Concentration (µg/ml)	Manufacturer / Catalog number	Secondary antibody	Concentration (µg/ml)	Manufacturer / Catalog number
HK-1	Goat	10	Santa Cruz Biotechnology SC 6518	Rabbit anti goat biotin	4	DAKO, EO466
HK-2	Goat	3	Santa Cruz Biotechnology SC 6521			
HK-3	Goat	10	Santa Cruz Biotechnology SC 6523			
GLUT-1	Rabbit	1	Thermo Scientific RB-9052-PO	Goat anti rabbit biotin	7.5	Vector Laboratories, BA-1000
GLUT-3	Mouse	Neat	Thermo Scientific PA1-28204	Goat polyclonal anti-mouse/rat/ rabbit peroxidase	1:1 of neat	Immunologic, DPVO 110HRP
MCT-4	Rabbit	5	Millipore AB3316P			
VEGF	Rabbit	32	Gifted by dept. of Laboratory Medicine, RUNMC			
CD-34	Mouse	1:200	Immunologic ILM 1343			Immunologic, DVPO 999HRP

SUPPLEMENTAL FIGURE 1. Expression of (A) Hexokinase 2 and (B) glucose transporter type 3 mRNA in PGL tumors. Expression of mRNAs in PPGL tumors was investigated using qPCR and their relative expression levels across different genotypes are plotted. Groups with different letters as superscripts are significantly different.