

Supplementary data

Supplementary Figure 1:

Sanger sequencing traces from matched normal, primary tumour and PDCL for TKCC-10, showing the presence of a somatic heteroplasmic insertion in primary tumour, which progresses to homoplasmy in the PDCL.

Supplementary Table 1: Nuclear mitochondrial and metabolic genes analysed for somatic mutations.

Supplementary Table 2: Detailed description of function in nuclear mitochondrial and metabolic genes with non-synonymous mutations in PDCLs.

Supplementary Table 3: Intracellular metabolites (n=72) targeted in metabolomics analysis.

Supplementary Methods

1. Culture Medium for TKCC-02, 03, 04.

RPMI 1640 (Gibco®)	500 ml
Fetal Bovine Serum (FBS) (HyClone)	10%
hEGF (Invitrogen) (1 mg/ml)	20 ng/ml
Penicillin Streptomycin (Gibco®)	5 ml
(+5,000 U/ml Pen, +5,000 mg/ml Strep)	
Gentamicin (Pfizer) (40 mg/ml)	0.02 mg/ml

2. Culture Medium for TKCC-07, 08, 09, 10, 15-LO, 16-LO, 17-LO, 26-LO.

M199/F12 (HAM) 1:1 (Gibco®)	500ml
HEPES (Gibco®) 1 M	15 mM
L-glutamine (Gibco®) 200mM	2 mM
hEGF (Invitrogen) 1 mg/ml	20 ng/ml
Hydrocortisone 1 mg/ml	40 ng/ml
Transferrin (Sigma) 2.5 mg/ml	5 ug/ml
Insulin (ActRapid) 100 IU/ml	0.2IU/ml
Glucose (Sigma) 10%	0.06%
FCS (HyClone)	7.5%
Tri-iodothyronine (Sigma) 0.1 mg/ml	0.5 pg/ml
MEM vitamins (Gibco®) (100x)	5 ml
O-phosphoryl ethanolamine (Sigma) (20 mg/ml)	2 ug/ml
Penicillin Streptomycin (Gibco®)	5 ml
Gentamicin (Pfizer) (40 mg/ml)	0.02 mg/ml

3. Culture Medium for TKCC-05, 06 (HPAC Modified Medium).

DMEM/F12 (HAM) (Gibco®)	500ml
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HEPES (Gibco®)	15 mM
Transferrin (Sigma) 2.5 mg/ml	5 ug/ml
FCS (HyClone)	7%
Insulin (ActRapid) (100 IU/ml)	0.1 IU/ml
hEGF (Invitrogen) 1 mg/ml	10 ng/ml
Hydrocortisone 1 mg/ml	40 ng/ml
Glucose Sigma (10%)	0.12%
Penicillin Streptomycin (Gibco®)	5 ml
Gentamicin (Pfizer) (40 mg/ml) mg/ml	0.02

4. Culture Medium for TKCC-18-LO, 19-LO (IMDM Modified Medium).

IMDM (Gibco®)	
FCS (HyClone)	20%
hEGF (Invitrogen) 1 mg/ml	20 ng/ml
Transferrin (Sigma) 2.5 mg/ml	2.5 ug/ml
Insulin (ActRapid) 100 IU/ml	0.2IU/ml
MEM vitamins (Gibco®) (100x)	2.5 ml
Penicillin Streptomycin (Gibco®)	5 ml
Gentamicin (Pfizer) (40 mg/ml)	0.02 mg/ml

5. Primers used for mtDNA sequencing

Amplicon 1

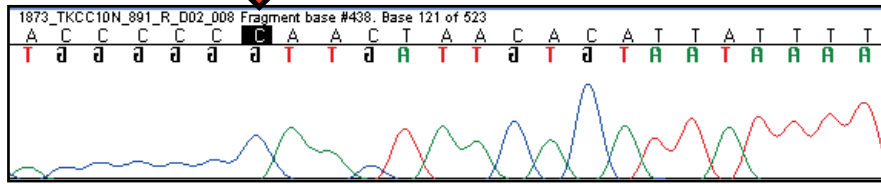
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12899-R	5'-CATGCTAAGGCGAGGATGAA-3'
12834-F	5'-AGCAGCCATTCAAGCAATCC-3'
13306-F	5'-CTAGCATTCTGCACATCTG-3'
13588-F	5'-CTGACAAGCGCCTATAGCAC-3'
14447-R	5'-AGGAGTATCCTGAGGCATGG-3'
14371-F	5'-TCCTACCTCCATCGCTAACC-3'
15087-F	5'-ACATCGGCATTATCCTCCTG-3'
15759-F	5'-TCGGAGGACAACCAGTAAGC-3'
16492-F	5'-ATCCGACATCTGGTTCCTAC-3'
42-R	5'-AGAGCTCCCGTGAGTGGTTA-3'
891-R	5'-GGCACGAAATTGACCAACC-3'
751-F	5'-ACAAGCATCAAGCACGCAGC-3'
1721-R	5'-GGCTAAGGTTGTCTGGTAGT-3'
1551-F	5'-GGAGACAAGTCGTAACATGG-3'
2324-F	5'-TTCTCCTCCGCATAAGCCTG-3'

Amplicon 2

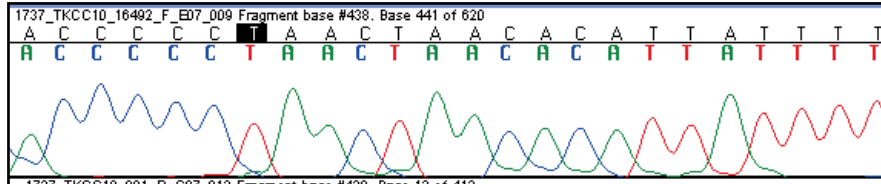
2909-F 5'-GACCAACGGAACAAGTTACC-3'
3106-R 5'-GTAGATAGAAACCGACCTGG-3'
3322-F 5'CTCCTACTCCTCATTGTACC-3'
3340-R 5'-GTACAATGAGGAGTAGGAGG-3'
3559-R 5'-GTAGAAGAGCGATGGTGAGA-3'
4162-R 5'-TGAGTTGGTCGTAGCGGAAT-3'
4040-F 5'-CAACATATGACGCACTCTCC-3'
4344-F 5'-TCGAACCCATCCCTGAGAAT-3'
4838-F 5'-TCTGACATCCGGCCTGCTTC-3'
5603-F 5'-CCACTCTGCATCAACTGAAC-3'
5911-F 5'-CCGACCGTTGACTATTCTCT-3'
5995-R 5'-GTGCCTAGGACTCCAGCTCA-3'
6325-F 5'-CCTCCGTAGACCTAACCATC-3'
6413-R 5'ATTGATAATTGTTGTGATGA-3'
6946-F 5'-TCACCGTAGGTGGCCTGACT-3'
7400-F 5'-CCCACCCTACCACACATTTCG-3'
7743-F 5'-CTAACATCTCAGACGCTCAG-3'
8254-F 5'-CGTATTTACCCTATAGCACC-3'
8782-R 5'-CGAGGAGGTTAGTTGTGGCA-3'
8642-F 5'-ACAACCGACTAATCACCACC-3'
9001-F 5'-CGCCTAACCGCTAACATTAC-3'
9378-F 5'-TGGCGCGATGTAACACGAGA-3'
10380-R 5'-GTAGTCACTCATAGGCCAGA-3'
10131-F 5'-CCACAACCTAACGGCTACAT-3'
10534-F 5'-ATCGCTCACACCTCATATCC-3'
10961-F 5'-CTAACTACCTGACTCCTACC-3'
11680-F 5'-AAGCTTCACCGGCGCAGTCA-3'

Supplementary Figure 1 - Hardie et al

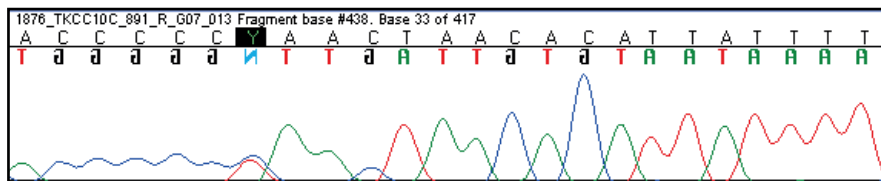
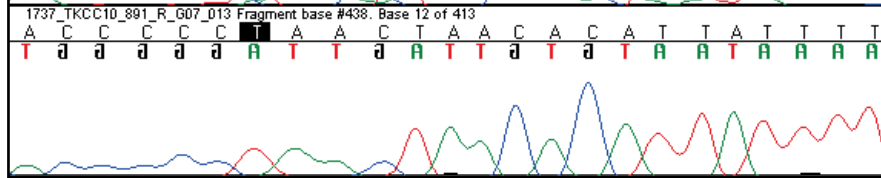
bp 438 L strand promoter



normal



PDCL



primary tumour

Supplementary Table 2: Detailed description of function in nuclear mitochondrial and metabolic genes with non-synonymous mutations in PDCLs.

Gene mutated	Name and function of protein encoded	Cell line
TRIT1	tRNA isopentenyl transferase, lung cancer tumour suppressor (Spinola et al. 2005).	TKCC-02
TP53	Tumour protein 53, regulates cell cycle and acts as tumour suppressor (Cho et al. 1994).	TKCC-04, TKCC-17-LO (different site)
ACACA	Acetyl Co-A carboxylase, involved in rate limiting step of fatty acid synthesis.	TKCC-04
CPT1C	Carnitine palmitoyltransferase I, an acetyltransferase involved in transport of long chain fatty acids across outer mitochondrial membrane by binding them to carnitine. Important in fatty acid metabolism.	TKCC-04
PIK3CA	Phosphatidylinositol 3-kinase, signalling important in many cancers, eg. Mutation associated with improved colorectal cancer survival with adjuvant aspirin therapy (Liao et al. 2012).	TKCC-04
PDHA2	Pyruvate dehydrogenase (lipoamide) alpha 2, a testis-specific mitochondrial matrix enzyme that catalyses the oxidative decarboxylation of pyruvate, producing acetyl-CoA and CO ₂ . Key enzyme in controlling the balance between lipid and glucose oxidation depending on substrate availability.	TKCC-04
AKT3	RAC-gamma serine/threonine-protein kinase, enzyme, AKT kinases are regulators of cell signalling in response to insulin and growth factors. This kinase has been shown to be stimulated by platelet-derived growth factor (PDGF), insulin, and insulin-like growth factor 1 (IGF1).	TKCC-09
YME1L1	ATP-dependent metalloprotease YME1L1, gene proposed to plays a role in mitochondrial protein metabolism and could be involved in mitochondrial pathologies (Coppola et al. 2000).	TKCC-09
ADCK4	aaRF domain containing kinase 4, This gene encodes a protein with two copies of a domain found in protein kinases. The encoded protein has a complete protein kinase catalytic domain, and a truncated domain that contains only the active and binding sites of the protein kinase domain, however, it is not known whether the protein has any kinase activity.	TKCC-09, TKCC-16-LO (different site)
MTCP1	Protein p13 MTCP-1, oncoprotein (Yang et al. 1998).	TKCC-15-LO
HMGCL	3-Hydroxymethyl-3-Methylglutaryl-CoA Lyase, mitochondrial enzyme that catalyzes the final step of leucine degradation and plays a key role in ketone body formation. Mutations in this gene are associated with HMG-CoA lyase deficiency.	TKCC-16-LO
TARS2	Threonyl-TRNA Synthetase 2, Mitochondrial (Putative), mitochondrial aminoacyl-tRNA synthetase.	TKCC-18-LO
LDHB	Lactate dehydrogenase B, enzyme which catalyzes the reversible conversion of lactate and pyruvate, and NAD and NADH, in the glycolytic pathway. Mutations in this gene are associated with lactate dehydrogenase B deficiency. Implicated in cancers (McClelland et al. 2012; Brown et al. 2013)	TKCC-26-LO