Loss of Glutaredoxin-2 contributes to tumor oncocytic transformation via differential protein S-glutathionylation and hampering of oxidative phosphorylation

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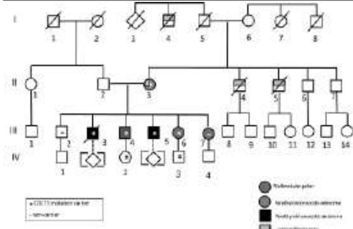
Introduction

A family affected by autosomal dominant Hyperparathyroidism-jaw tumor syndrome (HPT-JT), carrying a unique large deletion of tumor suppressor *CDC73*, showed an unusual predisposition to develop oncocytic tumors of the parathyroid characterized by the accumulation of somatic mtDNA mutations. Hallmarks of oncocytomas, the latter hamper mitochondrial function leading to the acquisition of an indolent clinical behavior. We hypothesize that the *CDC73* deletion might be causative of the oncocytic phenotype occurrence in the tumors of these patients.

Aim&Results

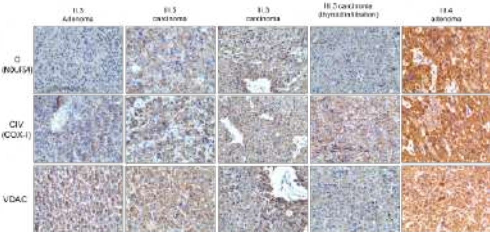
Pedigree of the family with HPT-JT



Pathogenic mutations load correlates with phenotype severity in HPT-JT family

Family ID	Base change	AA change	Gene (coding region)	Allele frequencies in total patients	disease
II.3	**m.2356A>G**	-	**MT-RNR2 (16S)**	**0.000220**	**ND**
	m.2635G>A	-	MT-RNR2 (16S)	ND	ND
	m.14973G>A	G76D	MT-CYB (CIII)	0.00000	91%
III.5	**m.2356A>G**	-	**MT-RNR2 (16S)**	**0.000220**	**ND**
	m.5147G>A	syn	MT-ND2 (CI)	0.040615	ND
	m.3380G>A	R25Q	MT-ND1	0.000439	88%
III.3	m.14387A>G*	L96S	MT-ND6	0.00000	74%
III.4	**m.2356A>G**	-	**MT-RNR2 (16S)**	**0.000220**	**ND**
	m.10371G>A	E105K	MT-ND3	0.000220	89%

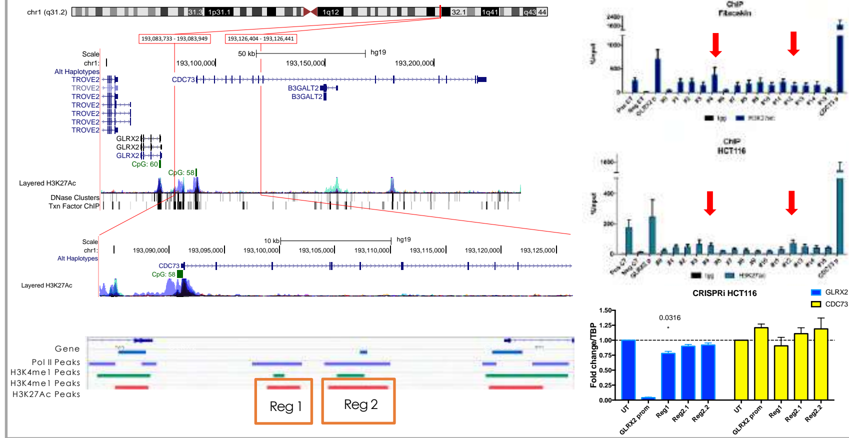
Prioritization criteria: HmtVar Prediction for pathogenic variants: disease score ≥ 0.43, allele frequency < 0.003264.
*****The m.14387A>G was detected only within the thyroid infiltration of the parathyroid carcinoma, but not in the parathyroid.



IHC showed Complex I disassembly and reduced mitochondrial – encoded COX-I only in II.3 adenoma, suggesting that the unique combination of both the germline m.2356A>G and the somatic m.2635G>A rRNA mutations, may be so pathogenic as to hamper mitochondrial protein synthesis.

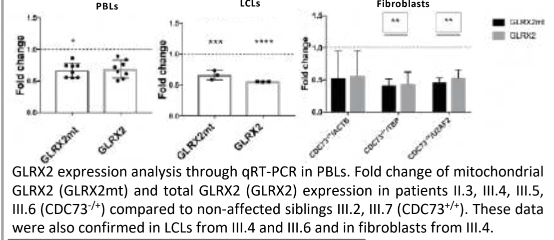
MtDNA sequencing and immunohistochemistry analysis confirmed the acquisition of an oncocytic phenotype in HPT-JT patients. ChIP-seq, CRISPR interference (CRISPRi) and qRT-PCR analysis were used to reveal a candidate gene for the triggering of oncocytic transformation. The deletion inherited by HPT-JT patients spans a region with putative regulatory elements of GLRX2, a mitochondrial enzyme that catalyzes the interplay between the glutathione (GSH) pool and protein thiols in response to altered redox states. Loss of GLRX2 might be causative for a dysfunctional mitochondrial redox system, which may set the environment for the onset and accumulation of mtDNA mutations in the patients' tumors. To investigate the effects of GLRX2 loss on tumor phenotype we generated TPC1$^{GLRX2-/-}$ and HCT116$^{GLRX2-/-}$.

ChIP- H3K27Ac and CRISPR interference analyses confirmed the presence of *GLRX2* active regulatory regions



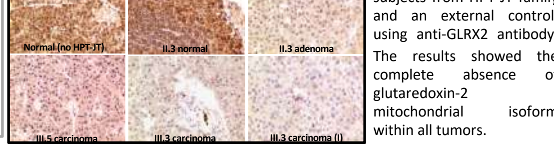
CDC73 locus on chromosome 1 and breakpoints of the germline deletion carried by HPT-JT patients. ChIP-seq confirmed the presence of active regulatory regions in HPT-JT fibroblasts$^{CDC73+/+}$ and in HCT116 colorectal cancer line (N=3). CRISPRi was used to repress the transcription of region 1 (Reg1) and 2 (Reg2) . The reduction of *GLRX2* expression observed in HCT116 confirmed that the regulatory elements near *CDC73* deletion induce *GLRX2* expression.

GLRX2 mRNA expression is reduced in PBLs, LCLs and fibroblasts



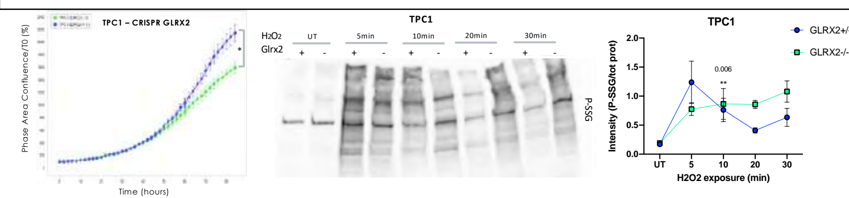
GLRX2 expression analysis through qRT-PCR in PBLs. Fold change of mitochondrial GLRX2 (GLRX2mt) and total GLRX2 (GLRX2) expression in patients II.3, III.4, III.5, III.6 (CDC73$^{-/+}$) compared to non-affected siblings III.2, III.7 (CDC73$^{+/+}$). These data were also confirmed in LCLs from III.4 and III.6 and in fibroblasts from III.4.

GLRX2 is not expressed in HPT-JT family tumors



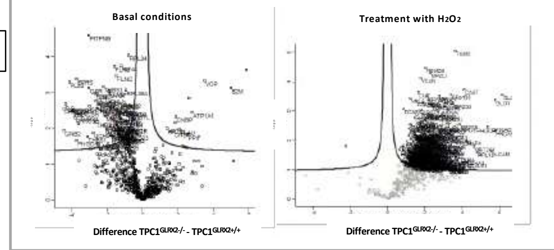
IHC analysis of FFPE tissue from parathyroid glands of subjects from HPT-JT family and an external control, using anti-GLRX2 antibody. The results showed the complete absence of glutaredoxin-2 mitochondrial isoform within all tumors.

GLRX2-/- cell lines shows reduced growth and a slowdown in proteome S-glutathionylation under H_2O_2 treatment



TPC1$^{GLRX2-/-}$, TPC1$^{GLRX2+/+}$ proliferation was monitored by analyzing the % of confluence over time with IncuCyte® live imaging system. Western blot showed an impaired capacity to restore basal levels of total protein glutathionylation (P-SSG) over time in GLRX2-/- after treatment with H_2O_2. Data are normalized on total proteins; densitometric analysis of n=3 experiments is shown. Unpaired T-test was used for statistical analysis. Comparable results were obtained with HCT116$^{GLRX2-/-}$ (data not shown).

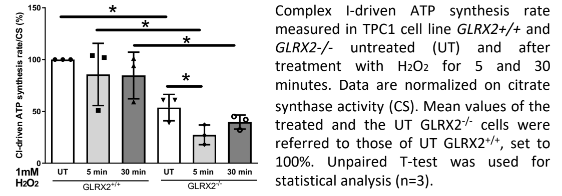
S-glutathionylated proteome is altered in *GLRX2-/-*



Identification and quantification of S-glutathionylated proteins in TPC1 (*GLRX2+/+* and GLRX2-/-) cell lines, through liquid chromatography-mass spectrometry. Volcano plots show a significant enrichment of glutathionylated proteins in TPC1$^{GLRX2-/-}$ in basal conditions, while following H_2O_2 treatment TPC1$^{GLRX2+/+}$ resulted in more glutathionylated proteins.

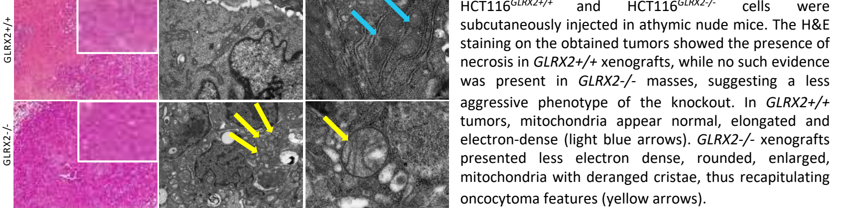
These results confirmed a basal alteration of the antioxidant system and revealed a reduced ability to protect the proteome under oxidative stress in these models. Loss of GLRX2 in tumors may lead to a dysfunctional detoxification system that, by altering mitochondrial protein S-glutathionylation, may favor oncocytic transformation. Moreover, CI-driven ATP synthesis is significantly reduced in GLRX2-/- in basal conditions and is further compromised following treatment with H_2O_2, suggesting a greater impairment of the mitochondrial respiration under oxidative stress.

Complex I-driven ATP synthesis is impaired in *GLRX2-/-*



Complex I-driven ATP synthesis rate measured in TPC1 cell line *GLRX2+/+* and *GLRX2-/-* untreated (UT) and after treatment with H_2O_2 for 5 and 30 minutes. Data are normalized on citrate synthase activity (CS). Mean values of the treated and the UT GLRX2$^{-/-}$ cells were referred to those of UT GLRX2$^{+/+}$, set to 100%. Unpaired T-test was used for statistical analysis (n=3).

GLRX2-/- xenografts are enriched in dysfunctional mitochondria



HCT116$^{GLRX2+/+}$ and HCT116$^{GLRX2-/-}$ cells were subcutaneously injected in athymic nude mice. The H&E staining on the obtained tumors showed the presence of necrosis in *GLRX2+/+* xenografts, while no such evidence was present in *GLRX2-/-* masses, suggesting a less aggressive phenotype of the knockout. In *GLRX2+/+* tumors, mitochondria appear normal, elongated and electron-dense (light blue arrows). *GLRX2-/-* xenografts presented less electron dense, rounded, enlarged, mitochondria with deranged cristae, thus recapitulating oncocytoma features (yellow arrows).

Conclusions

Our findings shed light on the role of *GLRX2* in mitochondrial response to oxidative stress in a tumorigenic context and offer new perspectives on the possible mechanisms which may confer a more indolent phenotype.