Pathogenic mitochondrial DNA mutations are associated with ROS signature and are prognostic markers in hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the most frequent form of primary liver cancer, with a high mortality rate even when detected and treated at its early stage.
Mitochondrial DNA (mtDNA) mutations have been shown to be involved in tumor progression by altering the cancer cells' bioenergetics and thus contributing to the metabolic rewiring and plasticity of tumors.

Aim: to investigate the contribution of mtDNA mutations to the pathogenesis and prognosis of HCC patients.

Results

A total of 328 germline variants were found. With the exception of two variants, namely the m.8505A>G mapping in MT-ATP8 and the m.12965T>C in MT-ND5 for which the clinical relevance could not be estimated, all of the events were classified as non-pathogenic, thus ruling out a strong predisposing role for mtDNA germline variants in HCC.
We detected 148 somatic variants in 75/104 (72.1%) tumor samples: 18 (12%) synonymous variants in 19 patients, 49 (33%) missense variants in 45 patients and three (2%) nonsense mutations in as many patients, while most of the somatic events were in the non-protein coding part, i.e. 78 (53%) variants in 72 cases. Out of the variants falling in protein-coding genes, the largest set observed in HCC tumors was constituted by missense variants, most of which were classified as pathogenic, suggesting these to be tolerated by, or even advantageous for cancer cells (**Fig.2**).

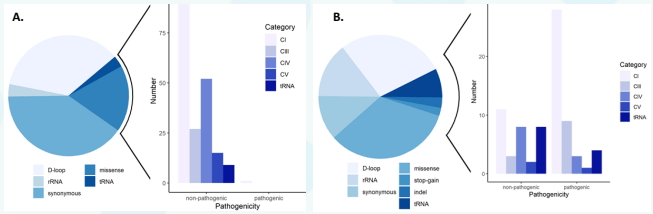
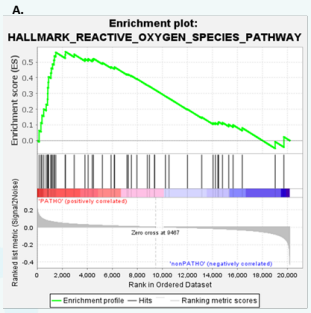


Fig.2. Distribution of germline (**A**) and somatic (**B**) variants in the TCGA-LIHC cohort based on their pathogenicity classification. While the major part of germline variants were benign, most of the somatic mtDNA variants were pathogenic, with a high number of mutations falling in genes coding for respiratory complex I.

Missense mtDNA mutations, especially those affecting respiratory complex I (CI) and III (CIII), may cause a severe to mild OXPHOS defect and are more frequently associated with an increased tumorigenic potential than disassembling mutations. To investigate the impact of such mutations, we further exploited the transcriptomics data available for the same TCGA patients and performed a gene set enrichment analysis (GSEA) which revealed a significant enrichment of the HALLMARK REACTIVE OXYGEN SPECIES PATHWAY (ROS) gene set in patients harbouring pathogenic mtDNA mutations (**Fig.3.A**). The core enrichment of this pathway was represented by genes part of ROS scavengers pathways (glutaredoxins, thioredoxins, peroxiredoxins and mitochondrial superoxide dismutase 2), indirectly suggesting an increased ROS production in these tumors. This compensatory increase in antioxidant proteins can contribute to the survival, proliferation and metastatization capacity of cancer cells by maintaining pro-tumorigenic ROS levels.
MtDNA mutations are often appointed as being the cause for higher ROS production. The ROS-induced mutation pattern (A>C and C>A transversions), was found only in a small fraction of the somatic nucleotide changes suggesting the main mutational signature being due to mtDNA replication errors (**Fig.3.B**), like in most tumors. These findings suggested that somatic mtDNA mutations in HCC may be the cause, rather than the consequences of a ROS overproduction.



	N	%
ROS mutational signature	9	5.4
POLG errors signature	144	86.7

Fig.3. A. GSEA showing an enrichment of the hallmark gene set for Reactive oxygen species in the group of patients presenting tumors with pathogenic mtDNA mutations (p-value=0.049). **B.** Mutational signatures of somatic mtDNA variants in TCGA-LIHC tumors.

Material and methods

mtDNA variants extraction was done using WXS data (paired tumoral and non-tumoral tissues) from the TCGA Liver Hepatocellular Carcinoma (LIHC) project, using the MToolBox pipeline. Variant annotation and classification was done according to the HmtVar pathogenicity scoring system and to the ACMG/AMP standard guidelines for clinical evidence and interpretations of mtDNA variants (**Fig.1**). Whole transcriptome data was used to perform a gene set enrichment analysis with the Bioconductor GSEA v4.1 software. MtDNA mutations were mapped on the 3D structures of their corresponding proteins using Chimera 1.14 (UCSF).

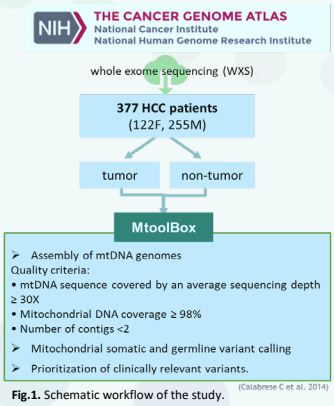
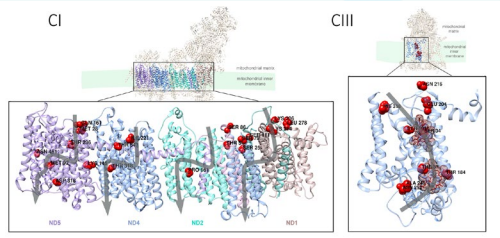


Fig.1. Schematic workflow of the study.

Mapping of the amino acids changes on the 3D structures of their respective subunits (**Fig.4**) showed that most of the mutations fell in the alpha helices forming the proton channels with a possible impact on CI function. For variants located in the CIII cytochrome *b*, some pathogenic mutations involved amino acids located in close proximity of the heme group, likely interfering with protein–heme interaction, thus increasing the probability of electrons escaping the mitochondrial complexes.

Fig.4. Overview of the pathogenic aminoacid changes (red) by mtDNA variants in CI and CIII in the TCGA-LIHC cohort. MtDNA encoded subunits are showed in colors while the rest of the nuclear encoded subunits are colored in tan. Gray arrows are a schematic representation of the proton translocation channels.



We further analyzed whether the presence of pathogenic mtDNA mutations may impact on patients overall survival (OS). Kaplan-Meier survival analysis revealed a significant decrease in the OS of patients harboring pathogenic mtDNA variants (**Table 1; Fig.5**), even when other factors such as disease stage, age, gender or AFP levels were taken into account.

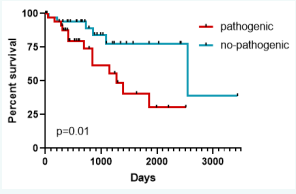


Fig.5. Survival curves of TCGA-LIHC patients with somatic mt-DNA variants. The presence of a pathogenic mtDNA mutation is associated with a poorer overall survival.

mtDNA variants	Median Estimate (days)
No pathogenic	2542
pathogenic	1271

Table 1. Estimated survival time of the TCGA- LIHC cohort (Log-rank test).

Conclusion

We report a high number of somatic mtDNA variants found in the analyzed HCC patients among which more than one third were classified as pathogenic variants. Most of these mutations were located in CI and CIII subunits, the main ROS generators in cells.

Tumors harboring pathogenic mtDNA mutations showed a significant enrichment for genes present in the ROS pathway, which may contribute to a more aggressive phenotype, thus leading to a decreased survival of HCC patients.

Selected references

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7-34. doi:10.3322/caac.21551

Giannini EG, Farinati F, Ciccarese F, et al. Prognosis of untreated hepatocellular carcinoma. Hepatology. 2015;61(1):184-190. doi:10.1002/hep.27443

Ju YS, Alexandrov LB, Gerstung M, et al. Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. Elife. 2014;3. doi:10.7554/eLife.02935