Stria Vascularis Dysfunction in a Mouse Model of Mitochondrial Hearing Loss

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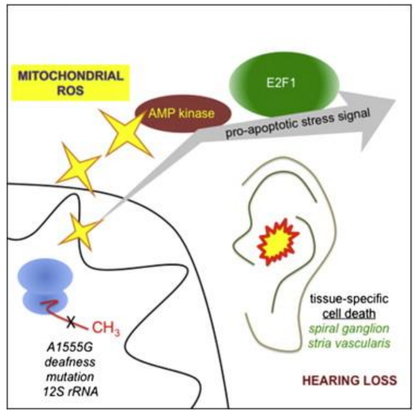
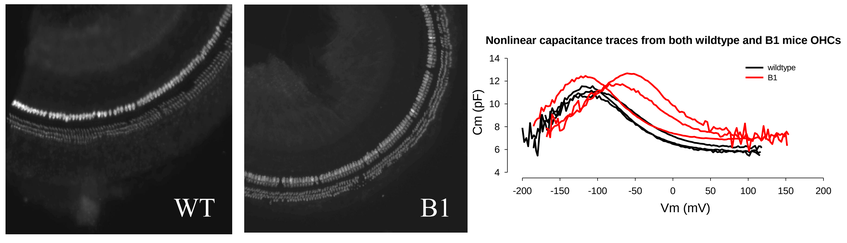
We recently described a transgenic mouse model of hearing loss induced by over-expression of the mitochondrial ribosomal RNA (rRNA) methyltransferase, TFB1M (Tg-TFB1M). These mice recapitulate maternally inherited deafness caused by the human A1555G mtDNA mutation, which results in increased methylation of the 12S rRNA in mitochondrial ribosomes and tissue-specific susceptibility to apoptosis. The present study aims to identify the specific cellular and tissue based pathologies underlying this form of deafness.



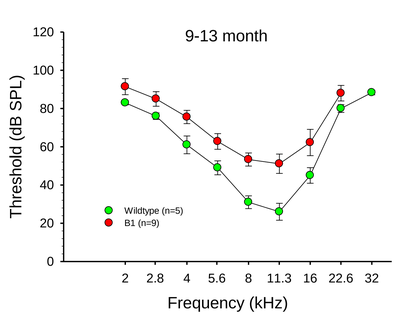
A Model of Mitochondrially Induced Deafness:
Overexpression of TFB1M or the presence of the A1555G mutation leads to the hypermethylation of the mitochondrial 12S ribosomal subunit. The result of hypermethylation is increased oxidative stress and hearing loss through an AMPK and E2F1 dependent pathway.

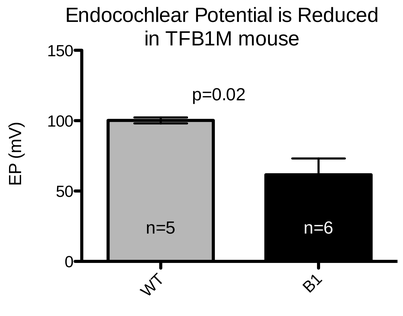
Hair Cell Loss Does not Correspond to Hearing Loss in TFB1M mice



TFB1M mice do not show significant loss of Myo7A labeled hair cells (left panels). The sporadic hair cell loss found in these animals does not correspond to the extent of ABR threshold increase. When studied in isolation, patch clamped outer hair cells show robust nonlinear capacitance and electromotility (right panel). The shift of NLC in TFB1 mice indicates some change of OHC function may have occurred. We intend to explore with OAE.

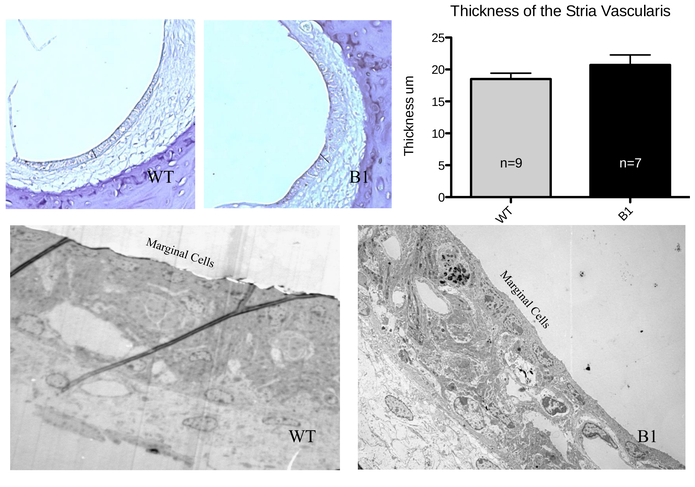
Stria vascularis dysfunction occurs in TFB1M mice





As reported earlier (see Raimundo et al 2012) TFB1M mice show progressive hearing loss at the age of 9-13 month. The across frequency threshold elevation measured by ABR (left panel) is accompanied with the loss of endocochlear potential (right panel).

Stria vascularis in the TFB1M mice is not atrophied.



The width of the stria was measured at the widest point on sections through the middle turn of the cochleae show no evidence of atrophy (upper panels). However, degenerative changes in the stria vascularis are evident under EM (lower panels).

Patterns of altered gene expression revealed by microarray analysis of the stria vascularis of TFB1M mice suggest several stress pathways that may mediate the hearing loss. Hybridization microarrays (Affymetrix) were performed on RNA collected from the stria vasculari of hearing and non-hearing (TFB1M) mice. The results were analyzed with the Ingenuity Pathway Analysis software (Qiagen) to identify groups of regulated genes associated with known signaling pathways or known to be regulated by specific transcription factors or drugs. The top ten signaling pathways implicated in the analysis include an immune response pathway (in yellow below.) What's more, one of the top implicated upstream regulators of genes with altered expression in the microarray is predinsone. Together these results support the idea that the stria is under inflammatory stress.

Top Ten Signaling Pathways Regulated in TFB1M Overexpressing Stria Vascularis:

Ingenuity Canonical Pathways	-log(p-value)
Sulfate Activation for Sulfonation	2.85
Role of BRCA1 in DNA Damage Response	2.81
Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I	1.85
Role of CHK Proteins in Cell Cycle Checkpoint Control	1.85
Hereditary Breast Cancer Signaling	1.69
Mismatch Repair in Eukaryotes	1.69
Mitochondrial Dysfunction	1.67
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	1.51

Upstream regulators with targets enriched in the stria of TFB1M Mice:

Upstream Regulator	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap
STAT4	transcription regulator		-1.366	4.44E-03
E2F4	transcription regulator			7.26E-03
KCNJ2	ion channel			9.25E-03
FHIT	enzyme		1.067	1.08E-02
CASP8	peptidase			1.38E-02
CD28	TM receptor		0.767	2.06E-02
RB1	transcription regulator		-1.178	2.37E-02
SLC22A1	transporter			2.99E-02
methylprednisolone	chemical drug		0.272	3.48E-02
E2F1	transcription regulator		-0.103	4.46E-02
HTT	transcription regulator			4.50E-02
CDK5R1	kinase			4.85E-02
Cd2+	chemical toxicant			4.85E-02
CXCL12	cytokine	Activated	2.425	4.71E-01
TNFSF11	cytokine		1.966	5.32E-01
IL17A	cytokine	Activated	2.174	1.00E00

Several recent studies have shown that mitochondrial reactive oxygen species activate cellular stress pathways, including DNA damage response and inflammatory pathways. The evidence provided here suggests that ROS induced deafness due to TFB1M over-expression may result in inflammatory changes in the stria vascularis that lead to hearing loss. The sparing of hair cells in our model may point to possible therapeutic options for patients affected by mitochondrially induced hearing loss.

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