Ablation of long noncoding RNA MALAT1 decreases reactive oxygen species and sensitizes the insulin responses in mice

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Background

The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long noncoding RNA and its overexpression is associated with the development of many types of malignancy. MALAT1 null mice have no reported overt phenotype, however, the expression of antioxidant genes was highly enriched in hepatocytes with a significant reduction in reactive oxygen species (ROS) in hepatocytes and isolated pancreatic islets.

Result

Figure 1

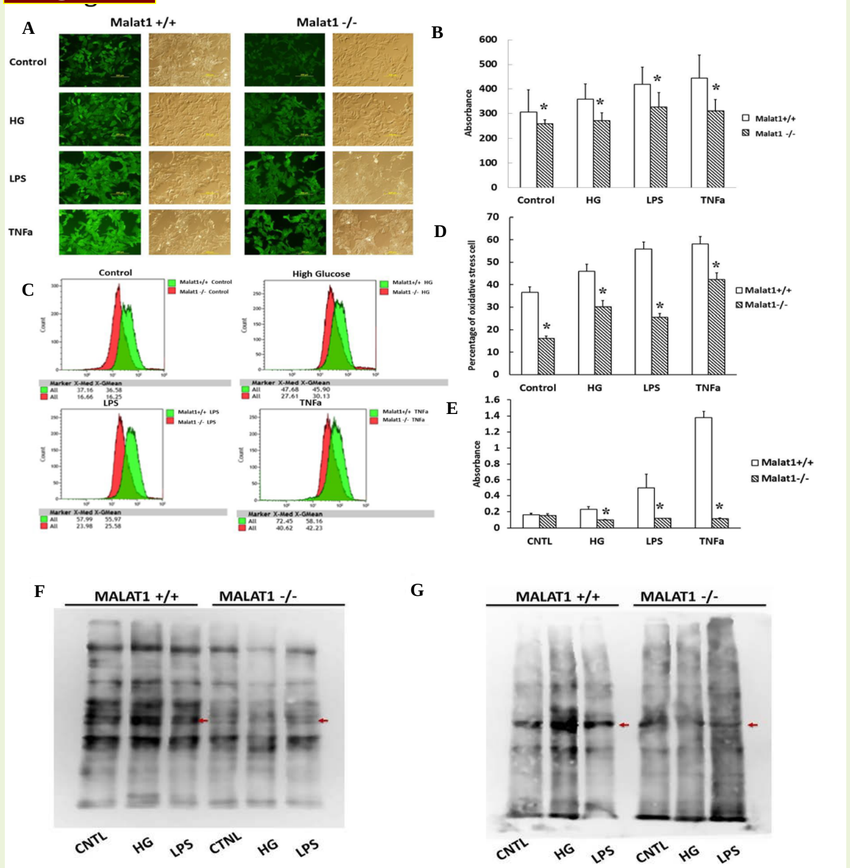


Figure 1. MALAT1 ablation inhibits ROS generation and total protein carbonylation. A, The ROS generation in mouse hepatocytes determined by immunofluorescence microscopy using DCFH-DA. **B,** ROS levels in MALAT1 and WT hepatocytes challenged with high glucose, LPS and TNF-α determined by fluorescence quantification with the plate reader. **C and D**, Flow cytometric analysis of ROS generation in MALAT1 -/- and MALAT1 +/+ hepatocytes**. E,** ROS levels in isolated pancreatic islets from wild type and MALAT1 null mice challenged with high glucose, LPS and TNF-α determined by fluorescence quantification with the plate reader (BioTek). **F and G**, Detection of protein carbonylation by Western blotting. Equal amount of protein from hepatocytes (F) and pancreas (G) from MALAT1 and wild type control treated with high glucose (HG, 2g/kg, 3 times in 24h) or LPS (30 mg/kg,2h).

Figure 2

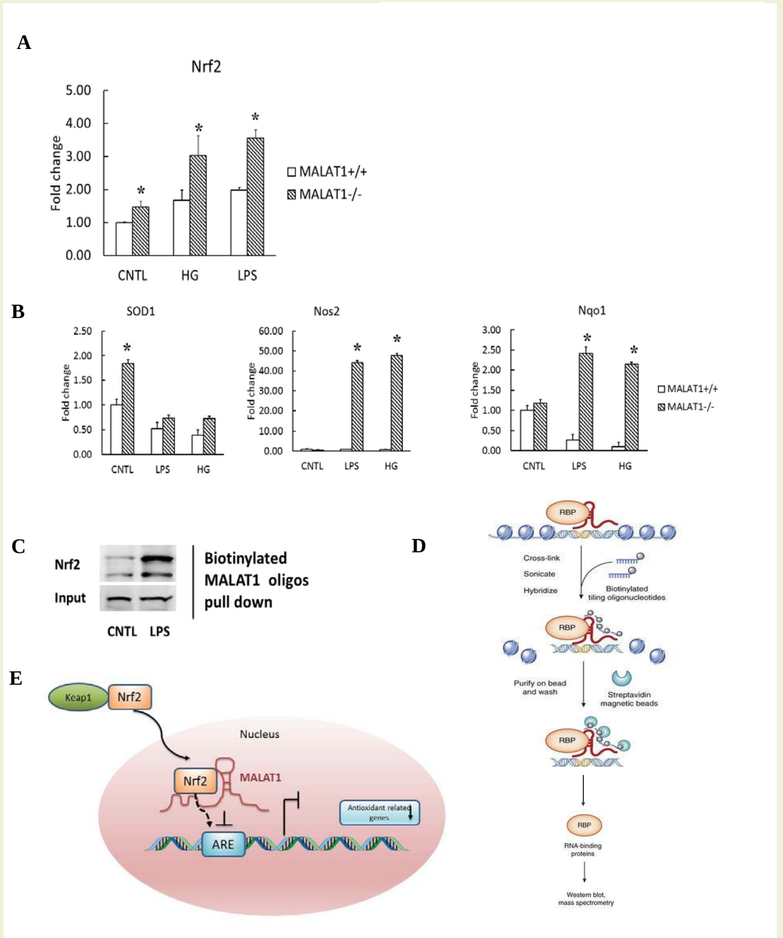


Figure 2. MALAT1 interact with Nrf2 and inhibit the Nrf2/ARE-driven pathways. A, Nrf2 expression in primary hepatocytes from MALAT1 ablated mice and wild type mice treated with oxidative stress inducer. **B,** Antioxidant genes expression in primary hepatocytes treated with oxidative stress inducer including LPS and High glucose. **C**, Western blot of Nrf2 expression pulled down by biotinylated MALAT1 oligos from nuclear extracts from wild type mice treated with LPS. Error bars show standard deviations. *, P <0.05.

Figure 3

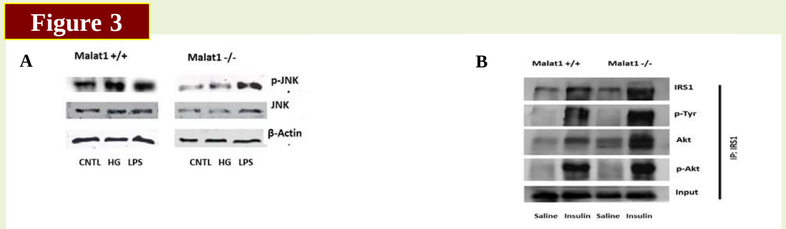


Figure 3. MALAT1 ablation inhibits JNK activation and enhances the insulin signaling capacity. A, Western analysis of the JNK phosphorylation as well as proteins levels of IRS1, p-Tyr of IRS1, Akt and p-Akt in primary hepatocytes from MALAT1 null mice and wild type mice. Hepatocytes were treated with control medium, high glucose medium (HG), LPS and TNF-a. **B**, Protein extracted from liver was immunoprecipitated for IRS-1 and immunoblotted (IB) for phosphorylated, total IRS-1, phosphorylated and total AKT.

Figure 4

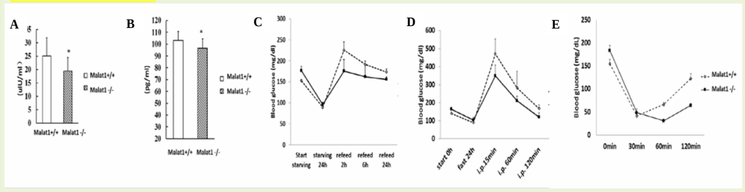


Figure 4. MALAT1 ablation improves insulin signaling responses in mice. A and B, Plasma insulin and glucagon levels in random-fed 6-week-old wild type (N=18) and MALAT1 null mice (N=20). **C**, Blood glucose levels under fast-refeed challenge. **D,** Blood glucose levels of MALAT1 in glucose tolerance test. **E,** Blood glucose levels in insulin tolerance test (n=6). *P < 0.01.

Figure 5

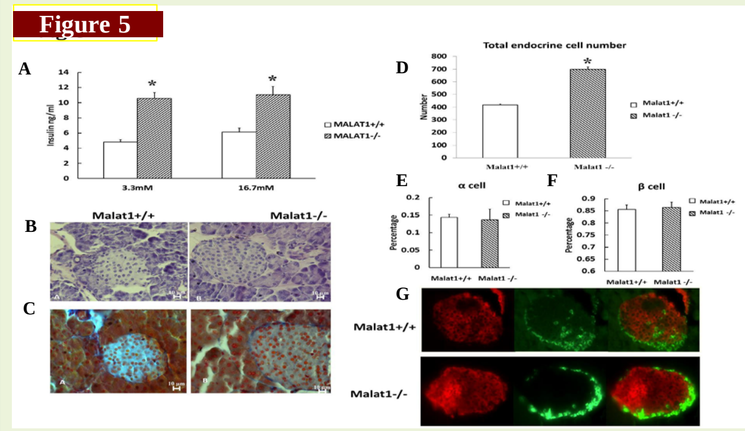
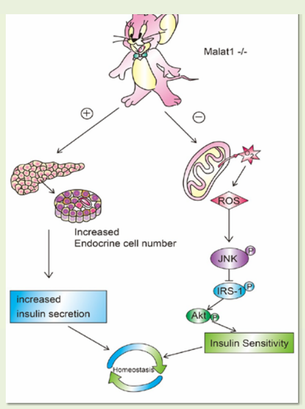


Figure 5. Pancreatic islets morphology and cellularity of MALAT1 null mice. A, Glucose-stimulate insulin secretion was measured by mouse insulin ELISA kit in isolated islets from MALAT1 null and wild type control mice. **B and C,** Representative section of pancreas from 6 week-old MALAT1 null and wild type mice using HE staining and Heidenhain's AZAN trichrome staining**. D,** Quantification of total endocrine cell number per total pancreatic area. **E and F**, Percentage of α and β cells in total endocrine cells per pancreatic area. **G,** Representative sections of pancreas visualized by double immunofluorescence after staining with anti-insulin (red) and anti-glucagon (green) antibodies.

Conclusion



Proposed mechanisms for the improved insulin signaling capacity in the MALAT1 ablated mice: 1) MALAT1 ablation improves insulin responses by upregulating the antioxidant gene expression thereby quenching ROS, and improving the insulin signal capacity through decreasing the JNK activation and increasing the functions of IRS1, and AKT pathways. 2) MALAT1 ablation leads to enhanced pancreatic endocrine functions by increasing the islet endocrine cellularity.