1 The non-coding RNA MALAT1 is differentially expressed in the brains of patients with
2 schizophrenia.

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6 Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined
7 published and public microarray datasets^{2,3} to discover differentially expressed genes in
8 schizophrenia and schizoaffective disorder. We found significant differential expression of the
9 metastasis-associated lung adenocarcinoma transcript and long non-coding RNA MALAT1 in
10 neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and
11 schizoaffective disorder.
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27 Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems
28 biology of schizophrenia, MALAT1.

Psychiatric diseases schizophrenia and schizoaffective disorder have in common the existence of psychotic symptoms^{1,4,5}, like visual and auditory hallucinations, that can plague affected patients. Understanding the transcriptional profiles of cells and tissues from the brain of patients diagnosed with schizophrenia or schizoaffective disorder has the potential to provide insights into molecular mechanisms underlying disease initiation, progression and associated symptoms like psychosis. We mined published and public microarray data^{2,3} from layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder and from the layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex of patients with schizoaffective disorder to discover genes whose expression was most significantly different in the brains of patients with psychosis at the systems-level. MALAT1 was among the genes most differentially expressed in two cell types of the brain in patients with psychotic disorders.

Methods

We used microarray datasets GSE87610² and GSE93577³ and for this differential gene expression analysis in conjunction with GEO2R. GSE87610 was generated using Affymetrix Human Genome U219 with $n=72$ for control subjects and $n=65$ for patients with schizophrenia, using L3 and L5 pyramidal neurons from the dorsolateral prefrontal cortex. GSE93577 was generated using Affymetrix Human Genome U219 Array technology from layer 3 (L3) parvalbumin (PV) positive neurons of the dorsolateral prefrontal cortex, and we used $n=34$

control subjects each with paired replicates ($n=68$ total samples) and $n=35$ patients with schizophrenia or schizoaffective disorder with paired replicates ($n=70$ total samples) from this dataset. These patient diagnoses in this dataset included schizoaffective disorder, paranoid schizophrenia, undifferentiated schizophrenia, chronic paranoid schizophrenia, chronic disorganized schizophrenia, chronic undifferentiated schizophrenia, and disorganized schizophrenia.

The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether MALAT1 expression was significantly different when comparing neurons from the DLPFC from control subjects and or from patients with schizophrenia or schizophrenia and schizoaffective disorder, respectively, using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined public and published microarray data^{2,3} to identify in an unbiased fashion and at the global level genes most differentially expressed in the brains of patients with schizophrenia and schizoaffective disorder.

MALAT1 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

We observed differential expression of the $\alpha 1$ subunit of the γ -aminobutyric acid (GABA) receptor, encoded by the MALAT1 gene, in the layer 3 (L3) PV+ neurons of the dorsolateral prefrontal cortex (DLPFC) in patients with schizophrenia and schizoaffective disorder². When sorting each of the transcripts whose expression was measured by microarray based on significance of change in expression between diseased and control patients, in L3 PV+ DLPFC neurons from patients with schizophrenia. MALAT1 ranked 1 out of 49386 total transcripts. Differential expression of MALAT1 was statistically significant (Table 1; $p=1.01E-11$).

MALAT1 is differentially expressed in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

We also observed differential expression of MALAT1 in the layer 3 and layer 5 (L5) pyramidal neurons of the DLPFC in patients with schizophrenia. When sorting each of the transcripts whose expression was measured by microarray based on significance of change in expression between patients and control subjects, in L3 and L5 pyramidal neurons of the DLPFC from patients with schizophrenia, MALAT1 ranked 783 out of 49386 total transcripts (Table 3), equating to differential expression greater than 98.4% of all transcripts whose expression was detected and measured. Differential expression of MALAT1 in L3 and L5 pyramidal neurons of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=8.99E-04$).

MALAT1 expression is significantly higher in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

We also obtained exact mRNA expression levels for the differentially expressed MALAT1 transcript, from control subjects and from patients with schizophrenia, to directly compare MALAT1 expression levels in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex. We also performed a statistical test to determine if this difference in MALAT1 expression was statistically significant. MALAT1 was expressed at higher levels in the L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia, and this difference was statistically significant (Figure 1; $p < 0.0001$). We calculated a mean fold change of 1.102 ± 0.081 in MALAT1 expression when comparing L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia to that of control subjects (Table 1).

Thus, we found significant differential expression of MALAT1 in two types of neuronal cell types in patients with schizophrenia; MALAT1 expression was significantly higher in L3 and L5 pyramidal neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia.

Discussion

MALAT1 is a non-coding RNA greater than 8000 basepairs in length expressed from 11q13, and originally characterized as a transcript whose expression was significantly associated with metastasis in patients with non-small cell lung cancer⁶. MALAT1 can localize to nuclear

1 speckles, interact with serine/arginine SR splicing proteins including SRSF1, and regulate both
2 the localization of SR proteins in nuclear speckles and their phosphorylation status⁷. Depletion
3 of MALAT1 resulted in fragmentation of nuclei, disruption of cell cycle kinetics with
4 accumulation of cells at G2/M-phase, and increased cell death⁷. Though its control of SR
5 splicing protein phosphorylation status and localization, MALAT1 can regulate alternative
6 splicing of pre-mRNAs⁷. However, in mice deficient in MALAT1, nuclear speckles and
7 expression of nuclear speckle markers were intact; authors concluded that MALAT1 was not
8 important for formation of nuclear speckles in living mice⁸. The expression of another non-
9 coding RNA, Neat1, was however reduced in mouse embryonic fibroblasts from MALAT1-
10 knockout mice and in cells depleted of MALAT1 in culture⁸. However, the size and number of
11 another nuclear structure known as paraspeckles were significantly decreased in cells from
12 MALAT1-deficient mice and from cells depleted of MALAT1⁸.

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17 MALAT1 can localize to hundreds of sites on chromatin at active genes; in some of these
18 sites MALAT1 can co-localize with NEAT1⁹. Another study of MALAT1 function in living mice
19 demonstrated that MALAT1 was not required for pre- or post-natal development in mice, and
20 contrary to findings from other studies, was not required for alternative splicing or for
21 phosphorylation of splicing factors¹⁰. However, expression of neighboring genes was
22 dysregulated in adult MALAT1-null mice, suggesting that MALAT1 could influence gene
23 expression of neighboring loci in *cis*¹⁰. A role for MALAT1 in cell cycle control was supported
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1 by a study in human diploid fibroblasts that demonstrated levels of MALAT1 were regulated
2 based on cell cycle phase, and demonstrating activation of p53- and p53-target genes upon
3 depletion of MALAT, along with reduced expression of B-MYB (Mybl2), a transcription factor
4 regulating G2/M-phase progression¹¹.

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6 Multiple studies have described roles for MALAT1 in the brain, including in ischemic
7 stroke, traumatic brain injury, and in schizophrenia. In an *in vivo* middle cerebral artery
8 occlusion model, Malat1-knockout mice manifested worse neurological scores, increased size of
9 brain infarct, and decreased sensorimotor function as compared to wild-type mice¹².
10 Consistently, after focal cerebral ischemia, levels of E-selectin and inflammatory cytokines IL-6
11 and MCP-1 were significantly increased in Malat1-knockout mice¹². Significantly, Malat1 levels
12 increased following oxygen-glucose deprivation (OGD) in mouse brain microvascular
13 endothelial cells and in mouse cerebral microvessels and in the cerebral cortex following middle
14 cerebral artery inclusion (MCAO)¹². In an *in vitro* model of oxygen-glucose deprivation in
15 cerebral cortex neurons, MALAT1 and Beclin1, the mammalian homologue of Atg6, were up-
16 regulated coordinate with down-regulation of microRNA miR-30; depletion of MALAT1
17 resulted in decreased autophagic responses¹³. Importantly, depletion of MALAT1 in cortical
18 neurons significantly attenuated cell death caused by oxygen-glucose deprivation¹³. MALAT1
19 levels are significantly increased in the cerebellum as well as in the hippocampus and brain stem
20 in the brains of alcoholics¹⁴. In an investigation of the role of adipose-derived stem cell
21 exosomes on the regenerative response to traumatic brain injury (TBI), administration of
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1 exosomes depleted of MALAT1 resulted in decreased regenerative capacity in a Fisher 344 rat
2 model of mild controlled cortical impact as compared to administration of intact exosomes
3 containing MALAT1 in measures including elevated body swing test, forelimb akinesia and paw
4 grasp test, demonstrating effectiveness of exosome-derived MALAT1 in recovery from TBI
5 mediated by exosomes¹⁵. A study of long non-coding RNAs in schizophrenia found that while
6 MALAT1 levels were significantly different when comparing the peripheral blood of controls
7 and patients with schizophrenia, MALAT1 levels in the peripheral blood did correlate with
8 duration of disease and age of onset in patients as well as age in both controls and patients¹⁶.
9 Thus, MALAT1 has influence over the cell cycle, alternative splicing, and important association
10 with metastasis in cancer, and may have roles in the brain during regenerative responses.
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12 We found by mining independent microarray data, public and published, that the non-
13 coding RNA MALAT1 was among the transcripts whose levels was most significantly different
14 in two neuronal cell types of the dorsolateral pre-frontal cortex in patients with schizophrenia
15 when compared to the brains of healthy controls. MALAT1 may be relevant to the biology of
16 psychotic disorders including schizophrenia and schizoaffective disorder.
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Rank	ID	p-value	t	B	FC	Gene
1	11745723_a_at	1.01E-11	7.4391175	15.7392	1.102 ± 0.081	MALAT1

Table 1: MALAT1 is the most differentially expressed gene in the layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia.

The rank of differential expression, the probe ID, the *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene
783	11755605_s_at	8.99E-04	3.39	-0.753408	MALAT1

Table 2: MALAT1 is among the most differentially expressed genes in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

The rank of differential expression, the probe ID, the *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, the fold change of MALAT1 in L3 PV+ DLPFC neurons of patients with schizophrenia and schizoaffective disorder when compared to deep pyramidal neurons from control subjects, the gene and gene name are listed in this chart.

Figure 1: MALAT1 is expressed at significantly higher levels in the layer 3 and layer 5 pyramidal neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia.

The mRNA expression of MALAT1 in layer 3 and layer 5 pyramidal neurons of the DLPFC from control subjects (left) and from patients with schizophrenia (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in MALAT1 expression between control layer 3 and layer 5 pyramidal neurons of the DLPFC and layer 3 and layer 5 pyramidal neurons from patients with schizophrenia, a *p*-value, listed above.