Bcl3 is differentially expressed in the brains of patients with schizophrenia.

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Visual and auditory hallucinations are a cardinal feature of psychotic disorders¹. We mined published and public microarray datasets^{2,3} to discover differentially expressed genes in schizophrenia and schizoaffective disorder. We found significant differential expression of Bcl3 in neurons of the dorsolateral pre-frontal cortex from patients with schizophrenia and schizoaffective disorder.

Keywords: schizophrenia, schizoaffective disorder, psychotic disorders, psychosis, systems biology of schizophrenia, Bcl3.

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 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. Bcl3 was among the most differentially expressed genes in patients with psychotic disorders in two cell types of the brain.

Methods

We used microarray datasets GSE93577² GSE87610³ and GSE145554⁴ for this differential gene expression analysis in conjunction with GEO2R. 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. 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. GSE145554 was generated using Affymetrix Human Gene 1.0 ST Array technology from deep pyramidal neurons isolated by laser capture microdissection. We utilized GSE145554 to compare the transcriptomes of deep pyramidal neurons from control subjects ($n=11$ samples utilized in this analysis) and from patients with schizophrenia that had not been treated with antipsychotic medications ($n=2$ samples utilized in this analysis). We also utilized GSE145554 to compare deep pyramidal neurons from control subjects ($n=11$ samples utilized in this analysis) and from patients with schizophrenia that had been treated with antipsychotic medications ($n=8$ samples utilized in this analysis).

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 Bcl3 expression was significantly different when comparing neurons of the DLPFC from control subjects and from patients with schizophrenia and schizoaffective disorder 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 published and public 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.

Bcl3 is differentially expressed in layer 3 parvalbumin-positive neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder.

We observed differential expression of Bcl3 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, Bcl3 ranked 175 out of 49386 total transcripts, equating to 99.6% differential expression. Differential expression of Bcl3 was statistically significant (Table 1; $p=1.14E-04$).

Bcl3 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 Bcl3 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

1 patients with schizophrenia, Bcl3 ranked 5958 out of 49386 total transcripts (Table 2), equating
2 to 87.9% differential expression. Differential expression of Bcl3 in L3 and L5 pyramidal neurons
3 of the DLPFC in patients with schizophrenia was statistically significant (Table 2; $p=3.9E-02$).
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6 Bcl3 is differentially expressed in the deep pyramidal neurons of two patients with schizophrenia
7 not treated with antipsychotics.
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9 We probed a third dataset to ask whether Bcl3 was differentially expressed in a third cell
10 type, the deep pyramidal neuron, in two patients not being treated with anti-psychotic
11 medications. By comparing the transcriptomes of deep pyramidal neurons of two patients who
12 were not being treated with antipsychotic medications to that of control subjects, we found that
13 Bcl3 was differentially expressed in the deep pyramidal neurons of patients with schizophrenia
14 off of antipsychotic medication. When sorting all of the genes expressed in deep pyramidal
15 neurons based on the significance of change between diseased patients who were not being
16 treated with antipsychotics and control subjects, Bcl3 ranked 98 out of 32321 total transcripts,
17 equating to 99.7% differential expression; this was statistically significant ($p=0.00564506$). Bcl3
18 was not significantly differentially expressed in the deep pyramidal neurons of eight patients
19 with schizophrenia who had been treated with anti-psychotic medications as compared to that of
20 the same eleven control subjects (Table 4; $p=0.400002$; Bcl3 ranked 9984 out of 32321 total
21 transcripts, equating to 69.1% differential expression).
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Bcl3 expression is significantly higher in layer 3 parvalbumin-positive neurons of the dorsolateral pre-frontal cortex in patients with schizophrenia and schizoaffective disorder.

We obtained exact mRNA expression levels for the differentially expressed Bcl3 transcript, from control subjects and from patients with schizophrenia and schizoaffective disorder, to directly compare Bcl3 expression levels in L3 PV+ DLPFC neurons. We also performed a statistical test to determine if this difference in Bcl3 expression was statistically significant. Bcl3 was expressed at higher levels in the L3 PV+ DLPFC neurons in patients with schizophrenia and schizoaffective disorder, and this difference was statistically significant (Figure 1; $p=0.0001$). We calculated a mean fold change of 1.1 ± 0.1 in Bcl3 expression when comparing L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder to that of control subjects (Table 1).

Bcl3 is expressed at significantly lower levels in the deep pyramidal neurons of patients with schizophrenia not treated with anti-psychotics when compared to deep pyramidal neurons from control subjects.

We also extracted exact mRNA levels for Bcl3 in the deep pyramidal neurons for both patient and control in order to directly compare Bcl3 transcript levels between patients with schizophrenia to non-affected controls. Bcl3 was expressed at lower levels in the deep pyramidal neurons from patients with schizophrenia not treated with anti-psychotics when compared to the same cells in the brains of non-affected controls; this difference in Bcl3 gene expression was also statistically significant (Figure 2; $p=0.0002$). We calculated a fold change of 0.910 ± 0.10 in Bcl3 mRNA levels when comparing patient deep pyramidal neurons to controls (Table 3).

Thus, we found significantly differential expression of Bcl3 in three types of neuronal cell types in patients with schizophrenia; Bcl3 expression was significantly higher in L3 PV+ neurons of the dorsolateral prefrontal cortex in patients with schizophrenia and schizoaffective disorder, while in the deep pyramidal neurons of two patients with schizophrenia not being treated with antipsychotic medications, Bcl3 was also differentially expressed but at lower levels as compared to controls.

Discussion

Between 1990 and 1994, at least six separate studies were published describing functions of a molecule with proto-oncogene function known as Bcl-3⁶⁻¹¹., originally identified as the gene of interest in genomic alterations observed in patients with B-cell chronic lymphocytic leukemias bearing t14:19 translocations^{12,13}. The transactivation and DNA-binding functions of the p50/p65 heterodimer of the nuclear factor of kappa light chain enhancer in B-cells, or NF-kB are inhibited by the Bcl-3 protein⁷. Nuclear translocation of NF-kB transcription factor subunits is controlled by cytoplasmic inhibitor molecules known as IκBs; Bcl-3 functioned similarly to an IκB but with specificity for the p50 NF-kB subunit⁸, and Bcl-3 contains an ankyrin repeat domain utilized by IκB-like molecules for association with NF-kB subunits like p50^{8,9} and thus Bcl-3 was deemed an IκB-like molecule of the NF-kB family^{7,8}. However, a subsequent study demonstrated that Bcl-3 had intrinsic transactivation functions, could translocate to the nucleus and be found in complex with p52, where p53/Bcl-3 heterodimers could transactivate expression

1 of NF- κ B target genes, and thus Bcl-3 was ascribed functions in the NF- κ B family unique from
2 that of an I κ B-like inhibitor of NF- κ B transcription factors⁹. While I κ B exerts its inhibitory
3 influence on nuclear translocation of p50/p65 and on NF- κ B target gene trans-activation through
4 interactions with p65 resulting in maintenance of cytoplasmic localization, Bcl-3 exerts its
5 inhibitory influence on NF- κ B transcriptional activation by interactions with p50¹⁰. Aside from
6 its properties related to direct transcriptional activation and inhibition of NF- κ B target gene
7 expression by cytoplasmic sequestration of NF- κ B proteins, Bcl-3 was initially described to
8 possess properties related to control of the cell cycle in response to mitogen stimulation⁶.

12 Transgenic mice over-expressing Bcl-3 under the control of E μ sequences (E μ -BCL3)
13 display splenomegaly and increases in mature B-lymphocyte populations in the bone marrow, the
14 spleen and the peritoneal cavity, as well as indications of an over-reactive humoral response such
15 as follicular hyperplasia and plasmacytosis in the lymph nodes, but with no frank neoplasia¹⁴.
17 Other signal transduction pathways influenced by Bcl-3 include the AP-1 transcription factor
18 pathway; Bcl-3 could be immunoprecipitated along with c-Jun in HeLa cells and microinjection
19 of Bcl-3 sequences into rat fibroblast cells induces expression of c-Jun¹⁵. The expression of
20 Bcl-3 can be induced by exposure to the interleukin-9 (IL-9) cytokine in T-helper and mast cell
21 lines¹⁶; in multiple myeloma cell lines, expression of Bcl-3 can also be induced by exposure to
22 IL-6¹⁷.

26 We found limited descriptions of Bcl-3 function in the brain. In patients with glioma,
27 high tumor expression of Bcl-3 was associated with resistance and inferior treatment responses
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1 to temozolomide¹⁸. In glioblastoma cells, Bcl-3 expression promoted mesenchymal
2 differentiation and associated gene expression¹⁸. In one analysis of whole exome data from 2545
3 Swedish schizophrenia cases, Bcl-3 was identified as an SLP, or “signed log p”, a measure of
4 whether rare, functional variants were present on average in excess in schizophrenia cases as
5 compared to controls¹⁹. Thus, Bcl-3 is a member of the NF- κ B transcription factor family, with
6 IkB-like inhibitory function as well as direct transactivation function; Bcl-3 expression in a
7 cancer of the brain is associated with poor response to chemotherapy and one analysis of whole
8 exome data from patients with schizophrenia found enrichment of a rare variant of Bcl-3 in
9 schizophrenia cases.

13 We found that Bcl-3 was among the genes most differentially expressed, transcriptome-
14 wide, in two neuronal cell types of the dorsolateral prefrontal cortex in patients with
15 schizophrenia: the layer 3 PV+ neurons and the layer 3 and layer 5 pyramidal neurons. Bcl-3 was
16 also differentially expressed in the deep pyramidal neurons of two patients not receiving anti-
17 psychotic medications, but not in the deep pyramidal neurons of eight patients who had been
18 receiving anti-psychotics. Differential expression of Bcl-3 should be validated in separate and
19 larger schizophrenia patient datasets. Bcl-3 may be of relevance to the cell biology of the brain
20 in schizophrenia and associated psychoses: visual and auditory hallucinations.

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Rank	ID	p-value	t	B	FC	Gene
175	11725445_x_at	1.14E-04	3.97	1.042493	1.1 ± 0.1	BCL3

Table 1: Bcl3 is differentially expressed in the layer 3 PV+ neurons of the dorsolateral prefrontal 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 Bcl3 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.

Rank	ID	p-value	t	B	Gene
5958	11725446_a_at	3.9E-02	2.0841553	-3.9737	BCL3

Table 2: Bcl3 is differentially expressed 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, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
98	8029465	0.00564506	-3.2522065	-3.76	0.910 ± 0.10	BCL3	B-cell CLL/ lymphoma 3

Table 3: Bcl3 is differentially expressed in the deep pyramidal neurons of two schizophrenic patients not treated with antipsychotic medications when compared to control subjects.

The rank of differential expression, the probe/transcript 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 gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
9984	8029465	0.400002	-0.8596946	-4.76	BCL3	B-cell CLL/ lymphoma 3

Table 4: Bcl3 is not differentially expressed in the deep pyramidal neurons of eight schizophrenic patients treated with antipsychotic medications when compared to control subjects.

The rank of differential expression, the probe/transcript 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 gene and gene name are listed in this chart.

Figure 1: Bcl3 is expressed at significantly higher levels in the layer 3 PV+ neurons of the dorsolateral pre-frontal cortex of patients with schizophrenia and schizoaffective disorder.

The mRNA expression of Bcl3 in L3 PV+ neurons of the DLPFC from control subjects (left) and from patients with schizophrenia and schizoaffective disorder (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in Bcl3 expression between control L3 PV+ DLPFC neurons and L3 PV+ DLPFC neurons from patients with schizophrenia and schizoaffective disorder, a *p*-value, listed above.

Figure 2: Bcl3 is expressed at significantly lower levels in the deep pyramidal neurons of the dorsolateral prefrontal cortex of patients with schizophrenia not treated with antipsychotic medications when compared to control subjects.

The mRNA expression of Bcl3 in the deep pyramidal neurons of control subjects (left) and in the deep pyramidal neurons of patients with schizophrenia not treated with antipsychotic medications (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in Bcl3 expression between the deep pyramidal neurons of control subjects and patients with schizophrenia, a *p*-value, listed above.