Manipulation of neuronal cell line NG108-15, as an *in vitro* model for investigating the role of schizophrenia candidate gene *ERBB4*

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INTRODUCTION

- Numerous genes and environmental factors play an important role in the susceptibility to schizophrenia, a psychiatric disorder that affects approximately 1% of the population worldwide.
- It is poorly understood what these factors are, how they function and interact in convergent signalling pathways resulting in such a polygenic disorder.
- *ERBB4* is a strong candidate susceptibility gene for schizophrenia for several reasons including; positive genetic association in several populations[1, 2], evidence of brain region specific differential expression in *post mortem* tissue from schizophrenia patients[3, 4] and its important roles in neurodevelopment[5].
- Converging evidence is beginning to emerge; nonetheless, the pathophysiological role of *ERBB4* in schizophrenia is not yet fully established.
- Manipulation of neuronal cell lines allows the investigation of signalling pathways that may be affected in schizophrenia, such as the NRG1-ERBB4 pathway.

AIM

- To investigate the mouse neuroblastoma x rat glioma neuronal cell line NG108-15, as an appropriate *in vitro* model for the exploration of the Nrg1-Erbb4 signalling pathway by manipulating *Erbb4* expression and analysing the downstream effects.

METHODS

WESTERN BLOTTING- Total protein lysate, isolated form NG108-15 cells, was electrophoresed through Tris-HCL gel then blotted onto PVDF membrane which was subsequently probed with a primary antibody against Erbb4 and an anti- rabbit-HRP labelled secondary antibody (both Santa Cruz Biotechnology).

IMMUNOFLUORESCENCE- NG108-15 cells were plated onto poly-L-lysine coated chamber slides, fixed in 100% methanol, then labelled with antibody against Erbb4 and an anti-rabbit-alexa488 labelled secondary antibody (both Santa Cruz Biotechnology).

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)- Total RNA was isolated from NG108-15 cells and first stand cDNA was synthesised (VILO™ Superscript®III, Invitrogen). *Erbb4* transcripts were amplified by PCR using forward and reverse PCR primers, designed specifically to the mouse or rat transcripts of *Erbb4* (Sigma Genosys), using Platinum® Taq-hiFidelity polymerase (Invitrogen). PCR products were then electrophoresed through 2% (w/v) agarose gel and visualised by UV light.

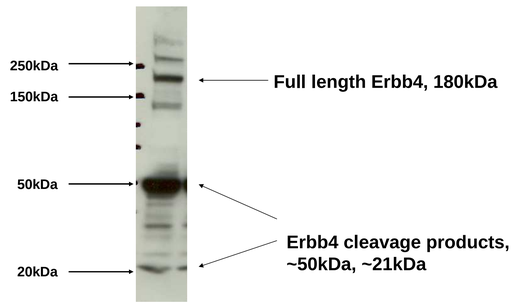
$RNA_{interference}$- NG108-15 cells were transfected with varying final concentrations of small interfering RNAs (siRNAs) for mouse *Erbb4, Glyceraldehyde-3-phosphate dehydrogenase* (*Gapd*, positive control) and non-targeting sequence (NT, negative control) (Ambion) using varying volumes of Lipofectamine™-RNA_{iMAX} transfection reagent (Invitrogen) for 24hours at 37℃/5% $C0_2$ before total RNA was isolated.

REAL-TIME QAUNTITATIVE POLYMERASE CHAIN REACTION (RT-qPCR)- NG108-15 cell cDNA was synthesised as described for RT-PCR. The cDNA was amplified by PCR and levels of *Erbb4* and *Gapd* were measured in real-time.

RESULTS

- Western blotting (Figure 1) and immunofluorescence (Figure 2) were used to show that NG108-15 cells express Erbb4.

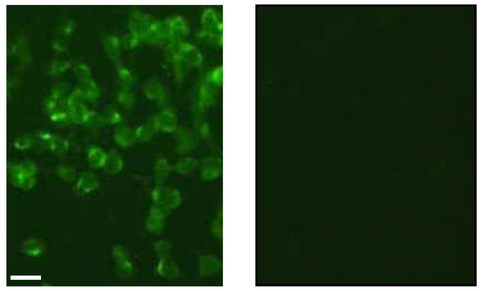
Figure 1



65µg NG108-15 total protein lysate with 1:2000 α-Erbb4 Ab+ 1:2000 α-rabbit-HRP Ab

- Western blotting of NG108-15 cell protein lysate showed multiple bands, representing the full length Erbb4 protein at 180kDa and multiple cleaved forms of Erbb4. These results are consistent with previous findings of Erbb4 expression in other tissues[4,7].

Figure 2

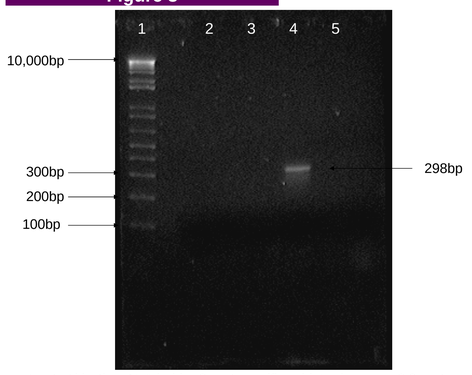


NG108-15 cells with 1:500 α-Erbb4 Ab+1:400 α-rabbit-488. Scale bar represents 20µM

NG108-15 cells with 1:400 α-rabbit-488 (secondary Ab only)

- Expression of *Erbb4* mRNA was detected by RT-PCR using PCR primers specific for either rat or mouse *Erbb4* (Figure 3). The PCR product was sequenced (Figure 4) and bioinformatics performed to confirm species specificity of the product (Figure 5).

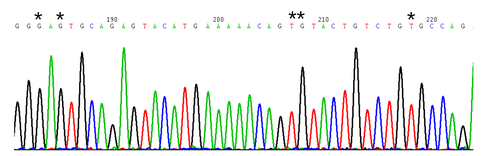
Figure 3



Key:1=1kb Plus DNA ladder 2= rat specific Erbb4 primers+H_20 3= rat specific Erbb4 primers+NG108-15 cDNA 4= mouse specific Erbb4 primers+H_20 5=mouse specific Erbb4 primers+NG108-15 cDNA

- RT-PCR showed *Erbb4* expression with mouse primers but not in negative controls or when using rat primers. These primers amplify *Erbb4* in cDNA made from rat brain and sequencing confirmed rat transcript.

Figure 4



Chromatogram showing sequence of mouse PCR product. Sequence is mouse *Erbb4* specific. Bases which are heterogeneous between species are marked with an asterisk (*).

Figure 5

```
F1 Product        1                                                  ATC     3
                                                                     |||
Amplicon Seq      1 AGGCTACATGACTCCAATGCATGACAAGCCCAAACAAGAATATCTGAATC    50
F1 Product        4 CTGTGGAAGAGAACCCTTTTGTGTCCCGAAGGAAGAATGGAGATCTTCAA    53
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
Amplicon Seq     51 CTGTGGAAGAGAACCCTTTTGTGTCCCGAAGGAAGAATGGAGATCTTCAA   100
F1 Product       54 GCTTTAGATAATCCGGAGTATATCACAGTGCTTCCAGCGGTCCACCCAAGGC  103
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
Amplicon Seq    101 GCTTTAGATAATCCGGAGTATATCACAGTGCTTCCAGCGGTCCACCCAAGGC  150
F1 Product      104 GGAGGATGAAATACGTGAATGAGCCTCTATACCTCAACACCTTCGCCAATG  153
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
Amplicon Seq    151 GGAGGATGAAATACGTGAATGAGCCTCTATACCTCAACACCTTCGCCAATG  200
F1 Product      154 CCTTGGGGAGTGCAGAGTACATGAAAAACAGTGTACTGTCTGTGCCAGAG  203
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
Amplicon Seq    201 CCTTGGGGAGTGCAGAGTACATGAAAAACAGTGTACTGTCTGTGCCAGAG  250
F1 Product      204 AAAGCCAAGAAAGCATTTGACAACCCCGACTACTGGAAA              242
                    ||||||||||||||||||||||||||||||||||||||
Amplicon Seq    251 AAAGCCAAGAAAGCATTTGACAACCCCGACTACTGGAA
```

Alignment of sequence from PCR product to mouse *Erbb4* transcript shows 100% sequence identity. Bases which are heterogeneous between species are highlighted in red.

- Specific knockdown of *Gapd* was optimised (Figure 6) for use as a positive control. Specific knockdown of *Erbb4* was optimised (Figure 7) for investigating Erbb4 signalling pathways. *Erbb4* expression was reduced in all samples transfected with the *Erbb4* siRNA. These samples did not show decreased expression of other genes tested (Figure 8) suggesting that there are no off-target effects.

Figure 6

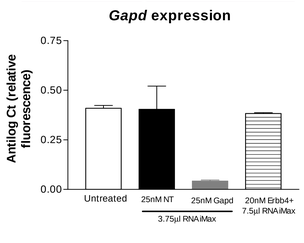


Figure 7

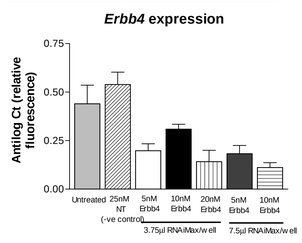
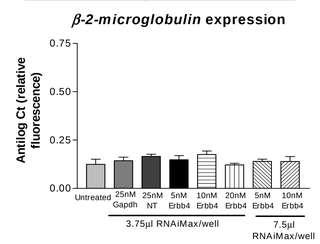


Figure 8



CONCLUSIONS

- The mouse neuroblastoma x rat glioma cell line NG108-15, expresses schizophrenia candidate gene *Erbb4* which is evident both at the mRNA and protein level.
- NG108-15 cells solely express the mouse transcript of the *Erbb4* gene.
- We have shown specific knock-down of *Erbb4* with no off-target effects in NG108-15 cells.
- NG108-15 cells are therefore, a promising model cell line for the investigation of signalling pathways that are possibly aberrant in schizophrenia.

REFERENCES

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ACKNOWLEDGEMENTS

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