Investigating the patterning of the cerebral cortex; role of Eph/ephrin family members

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INTRODUCTION

The neocortex, the outer-most structure of the brain, processes and integrates the sensory information perceived by sensory organs, to build a representation of our environment and initiates the appropriate response. To fulfill these complex higher functions, the neocortex is organised into functional units, or cortical areas, each characterised by a specific cell architecture, connectivity and gene expression profile. Cortical area identity is specified during embryonic development, when the cortical neurons migrate into the forming neocortex. Molecules involved in regulating cell migration, including members of the Eph/ephrin family, participate in patterning cortical identity by steering newborn neurons to a particular area. Ephrins are membrane-bound ligands which activate Eph receptors, eliciting a bi-directional attractive or repulsive response in both ligand- and receptor bearing cell. In order to understand the role of ephrin-A2 in the patterning the developing mouse brain, we propose to alter its expression by gain and loss of function approach. **My project was to validate 4 siRNA targeting *efnA2* and analyse cortical sections overexpressing *efnA2.***

AIMS

- Transfect CHO cells with pCAGS-efnA2-IRES-GFP
- Test in vitro for **transient knockdown of *efnA2* expression**
- Validate the plasmid delivery method using *in utero* electroporation

METHODS

CHO cells were transfected with the plasmid pCAGS-efnA2-IRES-GFP by **lipofection** using Lipofectamine in Optimem medium over 48 hours.

3 conditions

- Negative control: no plasmid/no siRNA
- Positive control: pCAGS-efnA2-IRES-GFP
- Experimental: pCAGS-efnA2-IRES-GFP + siRNA mix

TRIzol LS reagent used to isolate RNA and proteins

- Total RNA isolated and expression measured using RT-PCR
  - PCR carried out in a total volume of 25uL with Go Taq ready mix.
  - Quantity of cDNA normalised using housekeeping gene m_GAPDH
  - Percentage of knockdown quantified using qPCR, 17uL reaction volume
- Total proteins isolated and expression measured using Western Blot
  - Bradford assay to calculate protein concentration of samples
  - Western blot carried out in a total volume of 60uL
  - Antibodies: **mouse anti ephrin-A2** and **mouse anti β-actin** (internal control)*
  - Membrane stripping buffer: Glycine 100mM, NaCl 0.2M, 1% β-mercaptoethanol, 0.1% Tween in H20

RESULTS

Lanes 1-2: CHO
Lanes 3-4: CHO, pCAGS-efnA2-IRES-GFP 500ng
Lanes 5-6: CHO, pCAGS-efnA2-IRES-GFP 500ng, siRNA09-12, 25uM each

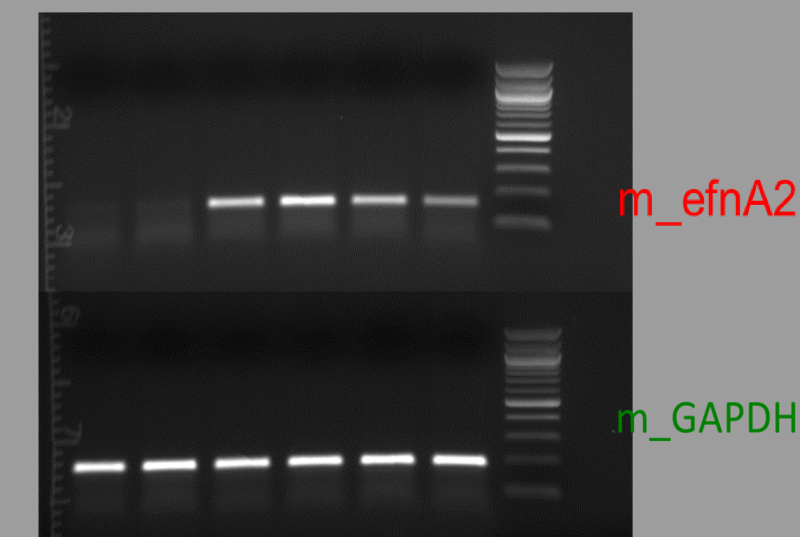

Figure 1. RT-PCR gel image Specific knockdown of efnA2 RNA expression

	GAPDH	m_efna2	ΔCt	ΔΔCt	$2^{-ΔΔCt}$	% fraction difference
GAPDH	16	19.7433	3.74333			
GAPDH	16.4533	19.3967	2.94333			
EfnA2 RNA	16.2033	21.1033	4.9	1.15667	0.44855	44.85%
EfnA2 RNA	15.62	24.5833	8.96333	6.02	0.01541	1.54%

Figure 2. Quantification of changes in gene expression caused by siRNA using qPCR. Positive ΔΔCt value indicates lower number of efnA2 transcripts in the presence of siRNA targeting *efnA2* compared to control.

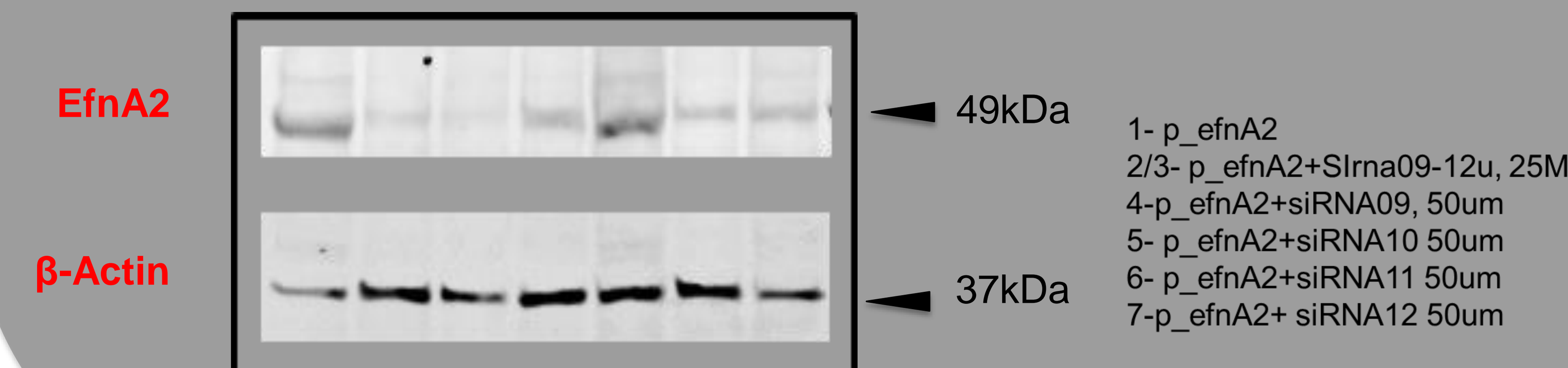

Figure 3. Western Blot membrane images Level of protein expression in control and siRNA conditions

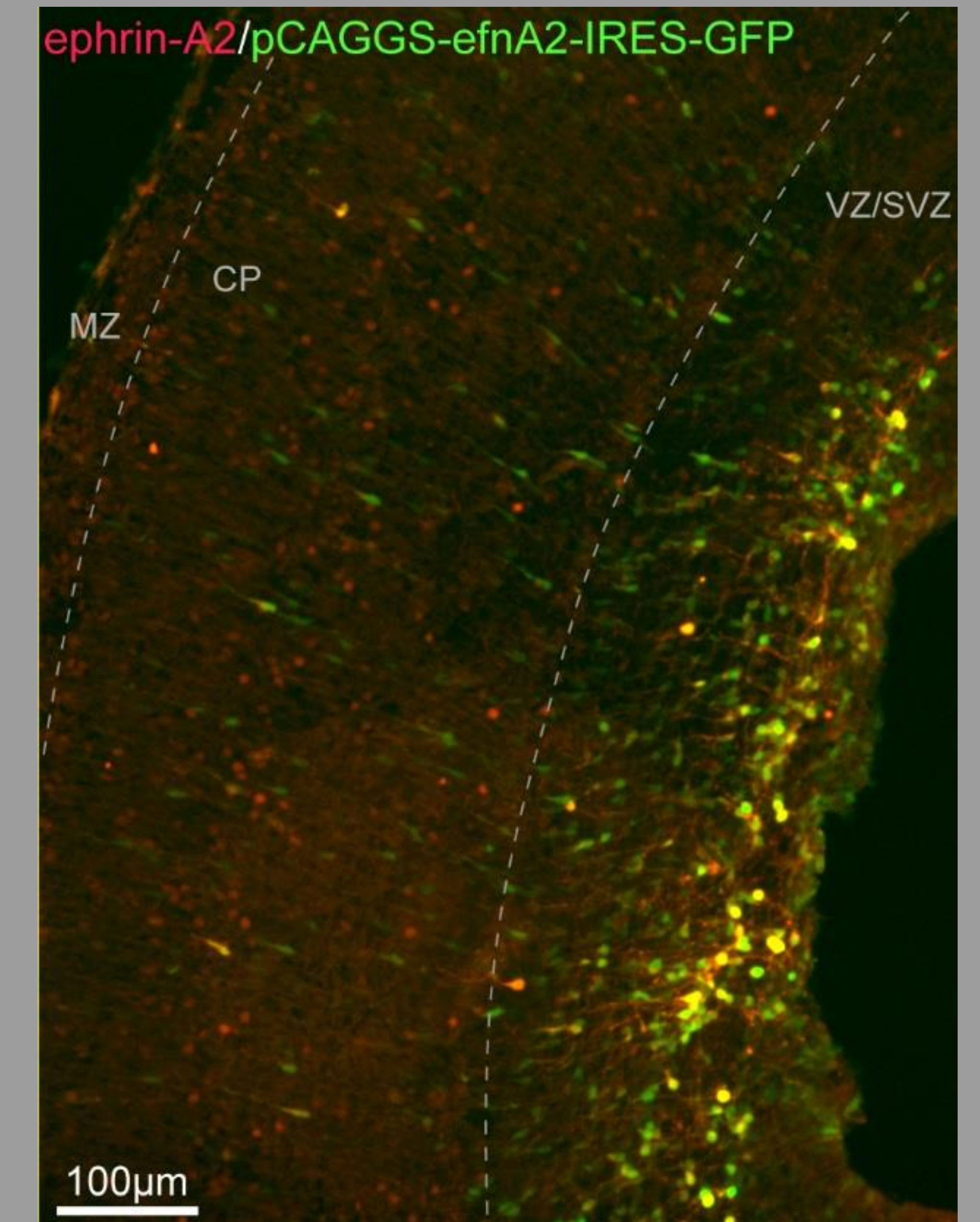

Figure 4. Detection of ephrin-A2 by immunlabelling (red) in cortical neurons electroporated with a pCAGGS-efnA2-IRES-GFP at E14.5 + 2 days post electroporation

CONCLUSION

- The 4 siRNA successfully carried out RNA interference to knockdown expression of *efnA2*. The sequences will be cloned into microRNA vectors to perform in vivo knockdown.
- PCR results showed the combined knockdown effect of all 4 siRNA (Figure 1 and 2)
- Western blot showed combined siRNA and individual siRNA effects of efnA2 expression. Confirmed that all 4 combined had a greater effect on level of knockdown (Figure 3)
- Successful in utero electroporation, validating plasmid construct delivery method (Figure 4).

DISCUSSION

- We could have used another control condition of enfA2 construct and scramble siRNA to show siRNA 09-12 is specific to m_efnA2. As scramble has no target we would expect to see no extinction, same as condition 2A/B.

References

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