Midline1 and the development of the cranial peripheral nervous system

Conference or Workshop Item

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Knockdown of Endogenous Mid1

- Chick embryos were electroporated with a Dominant Negative – Mid1 (DN-Mid1) expression construct at 8ss and incubated to 23ss.
- Neurofilament staining for neurons showed a reduction in the size of the trigeminal ganglia.

Figure 3: An embryo electroporated unilaterally, targeting rhombomere 2 with a DN-Mid1 expressing construct at 8ss and then incubated to 23ss. Embryo was stained for neurofilament (red) and GFP (green).

Over-expression of PP2A in r2

- One function of Mid1 is to target PP2A for destruction. Therefore to determine whether excess PP2A could explain the DN-Mid1 phenotype, we over expressed PP2A in r2.
- Embryos were electroporated unilaterally into rhombomere 2 at 8ss and incubated to 23ss.
- Over-expression of PP2A in r2 gave the same ganglia phenotype as the DN-Mid1 construct, therefore implying that Mid1 is acting through its PP2A ubiquitination function to affect the development of the ganglia.

Figure 4: An embryo electroporated unilaterally targeting rhombomere 2 with a PP2A expressing construct at 8ss and then incubated to 23ss. Embryo was stained for Neurofilament (red) and GFP (green).

PP2A inhibition In Vitro

- Okadaic Acid (OA) is an inhibitor of PP2A. Therefore exposure of r4 cells to OA should mimic the effect of Mid1 expression. When the r4 NCC’s are exposed to OA, the same Mid1 ganglia phenotype is observed.
- Cranial neural crest cells from rhombomere 4 were grown in culture with Okadaic Acid (OA) and time-lapsed for 8 hours.
- The speed of the cells were measured using the Mtrack application in ImageJ.
- The results show that on addition of OA the r4 NCC’s speed up significantly in the first 2 hours of culture and the speed remains high for the rest of the culture period. In comparison, when r2 NCC’s are cultured with OA there is no significant difference in the speed of the cells compared to untreated control cells.

Figure 5: Graph showing speed of r2 and r4 cranial neural crest cells before and after the addition of Okadaic acid (OA) in the cell culture media. P<0.001 (***)

Mid1 and Neural Crest Cell Delamination

- In order to investigate if Mid1 was affecting NCC delamination from the neural tube embryos were electroporated with the Mid1 and GFP constructs and stained for Pax3, a neural crest marker.
- Confocal scans of transverse sections through r4 show that there are fewer NCC’s on the Mid1 electroporated side of the neural tube, which is not seen on the GFP electroporated sections. No differences were detected in cell death or birth within the neural tube or the crest streams (not shown).
- Taken in conjunction with our data on neural crest speed, it would appear that Mid1 acts to promote delamination of NCC’s from the neural tube.

Ectopic Expression of Mid1

- To investigate if Mid1 could promote gangliogenesis in a neural crest population that does not normally express Mid1, embryos were electroporated in r4 with a Mid1 expressing construct at 10ss and incubated to 25ss.
- The expression of Mid1 in r4 and r4 NCC’s resulted in premature development of the facial-acoustic ganglia.
- These results further support the theory that Mid1 has a role in the development of the cranial ganglia.

Figure 5: An embryo electroporated unilaterally targeting rhombomere 4 with a Mid1 expressing construct at 10ss incubated to 25ss. Embryo was stained for Neurofilament (red) and GFP (green).

Expression Pattern of Mid1

- Embryos were processed by in-situ hybridisation (ISH) for Mid1 and Sox10 (neural crest cell marker).
- The results showed that at the 13 somite stage (ss) Mid1 is strongly expressed in rhombomere 2 and the mesencephalon adjacent to r1-r2 and the midbrain.
- Sox10 staining showed the mesenchymal Mid1 staining overlaps with the Sox10.
- Transverse sections through these embryos showed that the Sox10-Mid1 expression does overlap close to the neural tube, therefore implying that r1/r2 NCC’s express Mid1, but as the neural crest migrates into the branchial arch it down-regulates Mid1 expression.

Figure 2: In situ hybridisation on 13 somite stage chick embryos. (A) Mid1, (B) Sox10 stained sections through the branchial arches. (C) Embryos electroporated with a Mid1 construct at 10ss with OA. Immunohistochemistry of Pax3 is used to enhance the sensitivity of Fast Red staining of Sox10 mRNA. The fluorescent signal is masked in cells that co-stain with BM Purple for Mid1 mRNA.

Figure 7: Neural crest cell staining using Pax3 on sections through r4. Pax3 (red) and GFP (green). Figure shows sections through r4 of embryos electroporated with Mid1 (A,B) and the GFP control construct (C,D).