The transcription factor Zfhx1a is expressed in the embryonic chick spinal cord glial cells

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ABSTRACT

In Drosophila, the zing-finger homeodomain transcription factors zfh are expressed in many tissues including nervous system. Two vertebrate homologues, Zfhx1a and Zfhx1b have been characterized. These genes have been implicated in epithelial-mesenchymal transitions (EMT) in normal development as well as in tumorigenesis. However, the expression and the role of these genes in developing vertebrates nervous system is unknown. Here we describe the expression patterns of Zfhx1a in the embryonic chick spinal cord as determined by immunohistochemistry from days 1.5 (E1.5) to 14 (E14) of incubation. We have shown that Zfhx1a is feebly expressed in the neural progenitors in the ventricular zone (VZ) at around day 4. This expression becomes strong at E6. Starting from E7, we have observed that ZFHx1a+ cells stream away from the VZ and migrate to the mantle layer of the spinal cord. Double immunostaining shows no overlap between Zfhx1a and pan-neuronal markers (NeuN and HuC/D). However, there was extensive overlap between Zfhx1a and either oligodendrocyte markers (Olig2 and Nkx2.2) or astrocyte marker (GFAP). We conclude that Zfhx1a is expressed in glial cells in the chick embryo spinal cord.

Keywords: Zfhx1a, Transcription factor, Spinal cord, Chick embryo.

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التعبير عن عامل الانتساخ Zfhx1a في الخلايا الدبقية للنخاع الشوكي في جنين الدجاج

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الملخص

في ذبابة الفاكهة، يعبّر عن عوامل الانتساخ Zfh، الحاوية على إصبع الزنك والمجال المثلي، في العديد من الأنسجة بما فيها الجهاز العصبي. وصف في الفقاريات مثيلان لهذه العوامل، وهما Zfhx1a وZfhx1b، وتبين أنهما يشتركان في التحول الظهاري الميزنشيمي (EMT) في أثناء التنامي الطبيعي وفي أثناء تشكل الأورام، إلا أن التعبير عن هذين الجينين غير معروف في أثناء تنامي الجهاز العصبي للفقاريات. وصفت في هذه الدراسة نماذج التعبير عن Zfhx1a في النخاع الشوكي الجنيني للدجاج ابتداءً من اليوم 1.5 من الحضن (E1.5) إلى اليوم 14 من الحضن (E14) باستعمال الكيمياء النسيجية المناعية. إذْ تبيّن أن Zfhx1a يعبّر عنه بشكلٍ ضعيف في الخلايا السلفية العصبية في المنطقة البطينية (VZ) في اليوم الرابع من الحضن تقريباً. ويزداد هذا التعبير في اليوم السادس، ويلاحظ، ابتداءً من اليوم السابع، خلايا +Zfhx1a تترك (VZ) وتهاجر إلى منطقة المعطف من النخاع الشوكي. يظهر الوسم المناعي المضاعف عدم وجود أي تراكب بين Zfhx1a وواسمات عصبية عامة مثل NeuN وHuC/D، في حين يوجد تراكب واسع بين Zfhx1a وواسمات الخلايا قليلات التغصن Olig2 وNkx2.2 من جهة وواسمات الخلايا النجمية GFAP من جهة أخرى. ومنه نستنتج أنه يعبّر عن Zfhx1a في الخلايا الدبقية للنخاع الشوكي الجنيني في الدجاج.

الكلمات المفتاحية: Zfhx1a، عامل الانتساخ، النخاع الشوكي، جنين الدجاج.

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Introduction

The zinc finger homeodomain (Zfh) proteins constitute an atypical family of transcription factors - conserved throughout evolution - containing two separate krüppel-like zinc finger clusters, and a homeodomain similar to that found in the LIM proteins [1]. Drosophila has two (ZFH) proteins: ZFH-1 and ZFH-2 [2]. Two vertebrate homologues, Zfhx1a (also known as delta-EF1, Zfhep, ZEB1, AREB6 or TCF8) and Zfhx1b (alias SIP1 or ZEB2) have been characterized. ZFH-1 and ZFHX1a each have a central homeodomain and N - terminal and C - terminal clusters of zinc fingers. Drosophila ZFH - 2 has 3 homodomains and 17 zinc - finger domains, while a vertebrate homolog of Drosophila Zfhx1b contains four homeodomains and 23 zinc - finger motifs.

Detailed studies showed that Zfhx1a and Zfhx1b share DNA-binding sequence specificity for the E2-box-like motif and can either repress or activate the expression of several identified target genes. Both zfh genes have been found to represent major regulators of the Epithelial–mesenchymal transition (EMT), an embryonic programme that changes the adhesive properties of cells and rendering them more mobile (for review see [3]), notably induced by TGF-beta family members which explains their importance in many developmental and pathological processes such as neural crest migration and in cancer [4-9].

The mechanism of action of this class of transcription factors in these processes is beginning to be explored. In neural crest cells and in several tumoral cell lines, they have been shown to directly repress the expression of E-cadherin, a key event in the loss of cell-cell contacts that facilitates migration and metastasis [10]. Conversely, loss of Zfhx1a leads to a mesenchyme - epithelial transition (MET) accompanied by increased E-cadherin expression and reduced proliferation of progenitors in several tissues [11].

Mutations in the human ZFHX1A gene are associated with Fuch’s posterior corneal dystrophy in which neural crest-derived endothelial cells undergo premature apoptosis [12, 13]. Heterozygous deletions or mutations in the human ZFHX1B gene have been recently identified as the aetiology of the Mowat-Wilson Syndrome (MWS) which is characterized by a wide range of clinically heterogeneous congenital abnormalities including mental retardation, microcephaly, agenesis of the corpus callosum, poor hippocampal formation, facial dysmorphy, Hirschprung disease (i.e absence of enteric neurons in the bowel), cardiac defects and anomalies of the skeleton and urogenital system [14-20].

In Drosophila nervous system (NS), zfh1 specifies the identity of motoneurons and interneurons, and requires in the survival of a subset of glial cells [21, 22]. However, the expression of the homologues of this gene in developing vertebrates nervous system is unknown. Here we show that in the chick developing spinal cord, Zfhx1a is expressed in neural progenitors within the ventricular zone (VZ) and in glial cells of the astrocyte and oligodendrocyte lineages but not in the neuronal cells.

Material and methods

Animals and tissue preparation

Fertilized White Leghorn chick eggs obtained from commercial source were incubated at 38°C in a humidified incubator and staged according to the series of Hamburger and Hamilton [23]. Two embryos of each stage have been dissected.

Staining procedures

For immunohistochemical analyses, Spinal cords were dissected in ice cold Phosphate buffered saline (PBS), were fixed in 3.7% formaldehyde (Sigma) in PBS overnight at 4°C. Tissues were then rinsed twice in PBS and sectioned at 60-80 μm on a vibratome (Leica) before being processed. Sections were first permeabilized using Triton-X-100 (0.5% in PBS). Then they were blocked using BSA (1% in PBS) and primary antibodies were applied at the appropriate dilution in 0.1% Triton-X-100/BSA 1% in PBS and incubated overnight at 4°C. After rinsing, they were incubated for 1 h with goat anti-mouse or goat anti-rabbit antibodies coupled to Alexa 488 or Alexa 456. Sections were analysed with either Leica SP2 confocal microscope.

Antibodies

Neurones were identified either with NeuN antibody (Chemicon), used at 1:500 or with anti HuC/D (Molecular Probes) used at 1:500. Astrocytes were evidenced by an antiserum directed against Glial Fibrillary Acidic Protein (GFAP; DAKO A/S) used at 1:1000. Oligodendrocytes were visualised with the anti - Nkx2.2 antibody (hybridoma Bank) used at 1:2. The anti - ZFHx1a antiserum (provenance) was used at 1:1000

Results

The expression pattern of Zfhx1a in the embryonic chick spinal cord

We characterized the expression of Zfhx1a in the developing chick spinal cord. Spinal cord neurons and glia are generated in the neuroepithelium in the ventricular zone (VZ) by proliferation of neural progenitors. Precursors migrate out in a regulated fashion and differentiate into the different cell types, motor neurons, interneurons (white matter), oligodendrocytes and astrocytes (grey matter) that form the mature system. The expression of Zfhx1a was examined from E1.5 to E14 in the developing chick spinal cord, by immunohistochemistry with an antiserum against ZFHX1a protein.

From E1.5 to E3 no expression was detected in the ventricular zone VZ of the neural tube (Fig. 1). From E4 to E14 ZFHX1a protein was found throughout the spinal cord VZ, with the exception of the dorsal-most part next to the roof plate (arrow Fig. 1C). Indeed, there was very faint ZFHx1a labeling in the VZ at E4 and E5 (Fig. 1C, 1D). By E6 the VZ becomes intensely labeled (Fig. 1E). Starting from E7, small numbers of ZFHx1a+ cells appeared to be streaming away from the VZ (Fig. 1F), initially from the ventral part (arrowhead Fig. 1F) but later from all parts of the VZ (Fig. 1J). By E10 many scattered ZFHx1a+ cells had settled in both grey and white matter, though the immune-labeling was much stronger in grey matter (Fig. 1I). The immunohistochemistry of ZFHx1a at E9, E11, E12 and E13 was not shown. For ZFHx1a expression at E14 see Fig. 3.

Zfhx1a is not expressed in neurons in the embryonic chick spinal cord

At late stages of the developing chick spinal cord, when immature neurons and glial cells are differentiating and migrating to their final destinations, Zfhx1a+ cell nuclei were found scattered throughout the grey and white matter (see Fig. 1 for overall view of Zfhx1a expression in a section of spinal cord). We tried to establish whether Zfhx1a is expressed by neuronal cells, glial cells or both, by double immune-labeling with antibodies against Zfhx1a and either neuronal antigenic markers or glial antigenic markers. Co-labeling with pan-neuronal markers (HuC/D cytoplasmic antigenic marker (Fig. 2A, B) and NeuN nuclear antigenic marker (Fig. 2C, D)) from E7 to E10

revealed that Zfhx1a is generally not expressed in neurons at any stages of development. Thus, Zfhx1a is expressed in neural precursors in the neuroepithelium, and in non-neuronal migrating cells in the developing spinal cord.

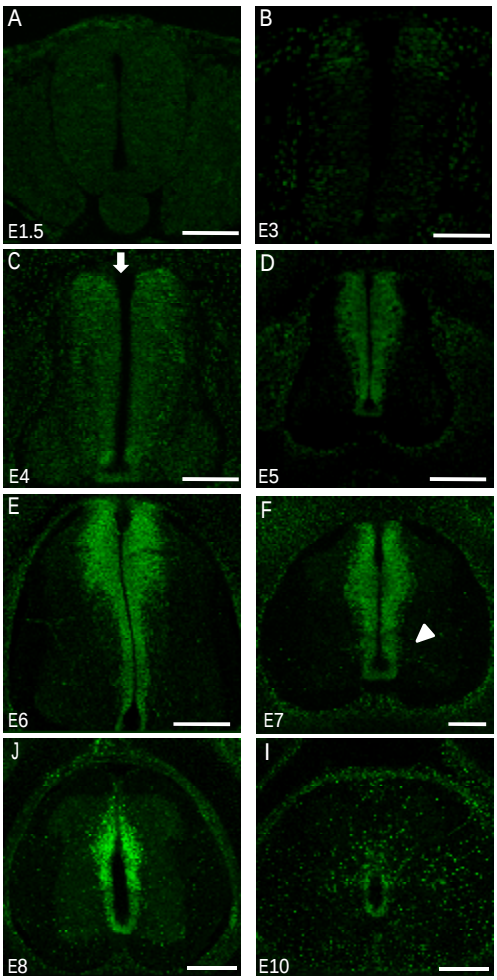

Fig. 1. Expression of ZFHx1a in embryonic chick spinal cord at various ages
(A-I) immunostaining of transversal spinal cord sections with anti-ZFHx1a (green). The ages were indicated in the bottom of each picture. From E1.5 (A) to E3 (B) no labeling was detected. At E4 (C) weak expression was detected in ZV. The arrow indicate the roof plate cells witch are ZFHx1a-. immune-labeling become much stronger at around E6 (E). Starting on E7 (F) ZFHx1a+ cells leave the VZ and migrate in the mantle zone, initially from the ventral part (arrowhead), then from all parts of the VZ (J). At E10 (I) many ZFHx1a +cells had settled in the mantle zone. Scale bars presents 100 µm.

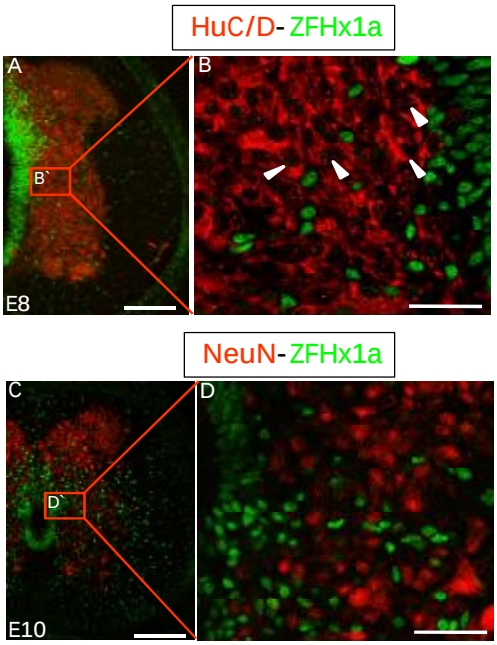

Fig. 2. Expression of Zfhx1a and different pan-neuronal markers in embryonic chick spinal cord
Transverse spinal cord sections from E8 and E10 chick embryos were processed for immunohistochemistry to examine the expression of Zfhx1a with HuC/D (A, B) NeuN (C, D).
(A, B) Double immunostaining for ZFHx1a (green) and HuC/D (red) shows that HuC/D$^+$ neurons (arrowheads) do not express ZFHx1a. (B) presents (B`) at higher magnification. (C, D) Double immunostaining for ZFHx1a (green) and NeuN (red). There is no overlap between ZFHx1a$^+$ nuclei and NeuN $^+$ nuclei (red). (D) presents (D`) at higher magnification. The ages were indicated on the bottom of each picture. Scale bars, 100 µm in A,C, 25 µm in B, D.

Zfhx1a is expressed in glial cells in the embryonic chick spinal cord

To identify the non-neuronal Zfhx1a-expressing cells, we carried out double-labeling experiments with markers of glial cells. Double labelling with Olig2 and Nkx2.2 for immature oligodendrocytes [24] and GFAP for astrocytes showed that Zfhx1a is expressed in these 2 populations (Fig. 3). The oligodendrocyte marker Olig2 labels a subset of the Zfhx1a$^+$ population in the E14 chick spinal cord (Fig. 3A arrows). Similarly, Nkx2.2, a marker of immature oligodendrocytes, labels a subset of Zfhx1a+ cells (Fig. 3B arrows). Most Olig2$^+$ and Nkx2.2$^+$ cells were Zfhx1a positive. GFAP is a marker of maturing astrocytes, and we found the nuclei of many GFAP$^+$ cells contained Zfhx1a (Fig. 3C, D arrowheads). These observations indicate that Zfhx1a is expressed in the majority of maturing glial cells of both the oligodendrocyte and astrocyte lineages.

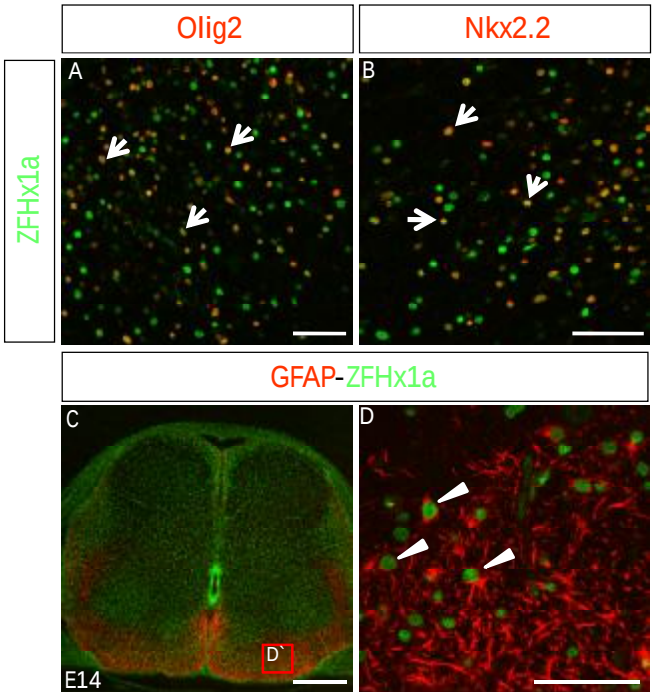

Fig. 3. Expression of Zfhx1a and different glial markers in embryonic chick spinal cord
Transverse spinal cord sections from E14 chick embryos were processed for immunohistochemistry to examine the expression of Zfhx1a with Nkx2.2 (A), Olig2 (B) and GFAP (C, D).
(A, B) Double labeling with the oligodendrocytic lineage markers Olig2 and Nkx2.2shows that a subset of the Zfhx1a population expressed these two markers (yellow nuclei, arrows). (C, D) GFAP staining that labels astrocytes shows that GFAP$^+$ cells expressed Zfhx1a (arrowheads). (D) presents (D`) at higher magnification. Scale bars, 100 µm in C, 25 µm in A, B, D.

Discussion

In this study we show that the zinc finger homeodomain transcription factor Zfhx1a is expressed in neural progenitors and glial cells in the spinal cord during development of the chick embryo. The above results suggest that Zfhx1a may play a role in several aspects of glial cell development.

Neuroepithelium progenitors are “neural”, and can give rise to neuronal and glial cells [25]. We show that, although Zfhx1a is expressed in all cells in the neuroepithelium since E4, its expression is mainly associated with maturing glial cells during later development.

Neural progenitors, like cancer cells, are highly migratory and proliferative. Several studies have shown that Zfhx proteins, by suppressing the expression of E-cadherin, regulate the adhesive

properties of different cell types, allowing them to become more mobile. This is the case in the EMT that takes place when neural crest cells delaminate from the neural tube and migrate out into the periphery. Indeed, in mice lacking Zfhx1b protein, defects in the dorsal neural tube have been reported [26, 27]. Our results showing the expression of Zfhx1a in neural precursors in early neuroepithelium and in the developed spinal cord, suggest that this transcription factor could also be involved in migratory processes of these cells using EMT-like mechanisms. In support of this hypothesis, we observed Zfhx1a^{+} cells in close apposition to vimentin+ radial glia fibres, a known migration route in the developing CNS (data not shown).

In Zfhx1a mutant mice, one might expect changed adhesive properties of such cells leading to altered migration rate or behaviour. Analysis of Zfhx1a knockout mice is hampered by the fact that Zfhx proteins are expressed in a partly overlapping fashion [28] and that Zfhx1a mutant mice die prematurely (embryonic day 18) before the maturation of the different cell types of the spinal cord [29]. It will be interesting to see if this prediction can be verified using conditional knockout strategies.

An interesting feature of Zfhx1a expression in the developed spinal cord is its expression in the majority of glial cells. Expression of Zfhx1a in both astrocytic and oligodendrocytic cells (GFAP^{+} and olig2^{+}) suggests that Zfhx1a may be involved in the maturation or/and the maintenance of adult glial cells.

In the developing oligodendrocytes Zfhx1a is co-expressed with Nkx2.2 and olig2 transcription factors. This suggests that Zfhx1a collaborates with these factors to achieve a common putative cellular function or cell state. Alternatively one of these transcription factors may control expression of the others.

Parallels have been drawn between the processes of neural crest EMT and metastasis in cancer, with several molecules and molecular mechanisms being shared between the two processes. (for discussion see [30]). Many reports have linked Zfhx family members to tumourogenesis in different tissues [31] and likewise one can hypothesise that Zfhx1a may be involved in the formation and invasion of glioblastoma.

Considering the documented role of Zfhx1a in the EMT process, work is in progress to test the possibility of key role of this molecule in the mesenchymal features of glioblastoma.

In conclusion, our present results indicate that Zfhx1a is expressed in neural progenitors and glial cells in the developing chick spinal cord. These results support the notion that in the embryonic spinal cord Zfhx1a may be able to control several aspects of glial cell development

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