
Article:
Cooper, F. and Tsakiridis, A. (2022) Towards clinical applications of in vitro-derived axial progenitors. Developmental Biology, 489. pp. 110-117. ISSN: 0012-1606

https://doi.org/10.1016/j.ydbio.2022.06.006

Developmental Biology 489 (2022) 110–117

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Towards clinical applications of *in vitro*-derived axial progenitors

Fay Cooper [a,b], Anestis Tsakiridis [a,b,*]

[a] Centre for Stem Cell Biology, School of Bioscience, The University of Sheffield, Western Bank, Sheffield, S10 2TN, United Kingdom
[b] Neuroscience Institute, The University of Sheffield, Western Bank, Sheffield, S10 2TN, United Kingdom

ARTICLE INFO

Keywords:
Neuromesodermal progenitors
Disease modelling
Cell therapy
Axis elongation

ABSTRACT

The production of the tissues that make up the mammalian embryonic trunk takes place in a head-tail direction, via the differentiation of posteriorly-located axial progenitor populations. These include bipotent neuromesodermal progenitors (NMPs), which generate both spinal cord neurectoderm and presomitic mesoderm, the precursor of the musculoskeleton. Over the past few years, a number of studies have described the derivation of NMP-like cells from mouse and human pluripotent stem cells (PSCs). In turn, these have greatly facilitated the establishment of PSC differentiation protocols aiming to give rise efficiently to posterior mesodermal and neural cell types, which have been particularly challenging to produce using previous approaches. Moreover, the advent of 3-dimensional-based culture systems incorporating distinct axial progenitor-derived cell lineages has opened new avenues toward the functional dissection of early patterning events and cell vs non-cell autonomous effects. Here, we provide a brief overview of the applications of these cell types in disease modelling and cell therapy and speculate on their potential uses in the future.

1. Introduction

The *in vitro* differentiation of human pluripotent stem cells (hPSCs), combined with the recent development of genome engineering and reprogramming technologies, has opened unique possibilities in expanding our knowledge of embryonic development and disease. Moreover, hPSC derivatives can potentially form the basis of effective cell-based therapies and the first clinical trials employing midbrain dopaminergic neuron progenitors and retinal pigment epithelium cells produced from hPSCs to treat Parkinson's disease and age-related macular degeneration, respectively, are already underway (da Cruz et al., 2018; Piao et al., 2021; Takahashi, 2020). However, until recently, the efficient production of postcranial cell types, especially those corresponding to the thoracic and lumbosacral levels of the body axis, from hPSCs has been challenging (Cooper and Tsakiridis, 2022; Frith et al., 2018; Metzis et al., 2018), thus precluding their use in downstream *in vitro* modelling/therapy applications.

In vivo, the neural and mesodermal components of the mammalian body trunk are generated by axial progenitors residing in a posterior growth region, which includes the early post-gastrulation caudal lateral epiblast, the primitive streak and the node-streak border, and, later, the tail bud (Cambray and Wilson, 2002, 2007; Mugele et al., 2018; Wymeersch et al., 2016). A fraction of these progenitors is marked by the ability to produce both spinal cord neurectoderm and presomitic mesoderm and therefore are referred to as neuromesodermal progenitors (NMPs) (Tzouanacou et al., 2009). Interestingly, mono-fated axial progenitors in the caudal lateral epiblast can also produce both neural and mesodermal derivatives when transplanted in an environment providing the 'appropriate' extrinsic cues, such as the node-streak border, indicating that neuromesodermal competence in the posterior growth region is more widespread than neuromesodermal fate (Wymeersch et al., 2016). Both neuromesodermal-fated (NMPs) and neuromesodermal-competent (NMCs) axial progenitors (as defined recently by (Binagui-Casas et al., 2021) exhibit co-expression of early neural and mesodermal markers, such as Sox2 and Brachyury (T) respectively, alongside other key posterior regulators (e.g. Cdx2) and Hox gene family members (Amin et al., 2016; Gouti et al., 2017; Koch et al., 2017; Tsakiridis et al., 2014; Wymeersch et al., 2016, 2019). The induction and maintenance of NMPs occur predominantly under the influence of WNT and FGF signalling, which also drive their differentiation toward presomitic mesoderm in conjunction with Notch signalling, following upregulation of T and downstream pro-mesodermal transcription factors, such as Tbx6 and Msgn1 (Amin et al., 2016; Anderson et al., 2020; Chalamalasetty et al., 2011, 2014; Garriock et al., 2015; Gouti et al., 2017; Hofmann et al., 2004; Javali et al., 2017; Koch et al., 2017; Takemoto et al., 2011; Wymeersch et al., 2016, 2019; Young et al.,

* Corresponding author. Centre for Stem Cell Biology, School of Bioscience, The University of Sheffield, Western Bank, Sheffield, S10 2TN, United Kingdom.
E-mail address: a.tsakiridis@sheffield.ac.uk (A. Tsakiridis).

https://doi.org/10.1016/j.ydbio.2022.06.006
Received 27 January 2022; Received in revised form 28 April 2022; Accepted 14 June 2022
Available online 17 June 2022

2009). Conversely, lower levels of/shorter exposure to WNT/FGF signalling promote the transition of NMPs toward Sox2+T-neural progenitors, which subsequently give rise to spinal cord neurectoderm upon exposure to somite-derived retinoic acid (Delfino-Machin et al., 2005; Diez del Corral et al., 2002; Diez del Corral et al., 2003; Gouti et al., 2017; Martin and Kimelman, 2010, 2012; Molotkova et al., 2005; Olivera-Martinez et al., 2012).

Given that NMPs comprise a major source of key posterior tissues in the embryo, the development of protocols for their *in vitro* generation from PSCs over the past few years has been instrumental in the refinement of differentiation strategies aiming to produce previously "hard-to-make" thoracic and lumbosacral cell types. The biology of embryonic NMPs/axial progenitors and their role in body patterning has been covered in detail both in this special issue and elsewhere (Aires et al., 2018; Binagui-Casas et al., 2021; Henrique et al., 2015; Needham and Metzis, 2022; Sambasivan and Steventon, 2020; Wymeersch et al., 2021). In this review, we aim to present recent advances in the use of hPSC-derived NMPs and their downstream products in disease modelling/cell replacement applications and to discuss further potential uses.

2. A brief history of *in vitro*-derived human axial progenitors

Similar to their *in vivo* counterparts, the phenotypic definition of hPSC-derived NMP-like cell populations has relied on the co-expression of TBXT (the human homologues of Brachyury) and SOX2, denoting a prospective mesodermal and neural character respectively. Invariably, all human adherent NMP-like cell induction protocols involve WNT signalling stimulation for 2–4 days, usually through the use of the GSK3 inhibitor CHIR99021 (CHIR), either alone or in combination with FGF signalling agonists such as FGF2 or FGF8b (Cooper et al., 2022; Dady et al., 2022; Diaz-Cuadros et al., 2020; Frith et al., 2018; Gomez et al., 2019; Gouti et al., 2014; Hackland et al., 2019; Kirino et al., 2018; Kumamaru et al., 2018; Lippmann et al., 2015; Olmsted et al., 2021; Verrier et al., 2018) (Fig. 1). In some cases, RA or BMP/TGFβ/Notch signalling inhibition are also included (Cooper et al., 2022; Dady et al., 2022; Denham et al., 2015; Kumamaru et al., 2018; Olmsted et al., 2021; Verrier et al., 2018). Despite their apparent transcriptional heterogeneity, the resulting cultures exhibit expression of several common key markers indicative of an axial progenitor identity such as *NKX1-2*, WNT/FGF components, *CDX2* and HOX gene family members and can be further steered to generate presomitic mesoderm (Diaz-Cuadros et al., 2020; Frith et al., 2018; Gouti et al., 2014) and spinal cord neurectoderm cells (Dady et al., 2022; Frith et al., 2018; Gouti et al., 2014; Kumamaru et al., 2018; Lippmann et al., 2015; Olmsted et al., 2021; Verrier et al., 2018; Wind et al., 2021; Wind and Tsakiridis, 2021), therefore exhibiting neuromesodermal bipotency/competence (Fig. 1). Analysis of expression of various Hox paralogous group (PG) members, which serves as a read-out of anteroposterior (A-P) axial identity, indicated that the neural and mesodermal derivatives of human NMPs predominantly exhibit a posterior brachial/thoracic character, driven primarily by WNT and FGF signals (Cooper et al., 2022; Gogolou et al., 2021; Lippmann et al., 2015; Mouilleau et al., 2021; Wind et al., 2021). Addition of exogenous GDF11, which regulates trunk-to-tail transition *in vivo*, and the upregulation of the most posterior Hox genes (Aires et al., 2019; Jurberg et al., 2013; Liu et al., 2001), appears to promote a lumbosacral axial identity in neurectodermal cell populations obtained from hPSC-derived NMP-like cells (Cooper et al., 2022; Lippmann et al., 2015; Mouilleau et al., 2021).

Recent work revealed that hPSC-derived TBXT + SOX2+ cells are also an efficient source of trunk neural crest and their sympathoadrenal derivatives (Abu-Bonsrah et al., 2018; Denham et al., 2015; Frith et al., 2018; Gomez et al., 2019; Hackland et al., 2019; Kirino et al., 2018) (Fig. 1), reflecting *in vivo* findings showing the close lineage relationship between this cell type and NM-potent axial progenitors (Javali et al., 2017; Lukoseviciute et al., 2021; Shaker et al., 2021; Wymeersch et al., 2016). Moreover, NMP-like cultures generated *in vitro* from PSCs have also been reported to give rise to lateral plate/intermediate mesoderm cells, following BMP signalling stimulation (Edri et al., 2019; Row et al., 2018) (Fig. 1).

The combination of these differentiation strategies with micropatterning platforms allowing control of geometric confinement parameters has further increased differentiation efficiency and enabled the precise dissection of signal-induced morphogenesis (Knight et al., 2018; Seo et al., 2021). Three-dimensional (3D) hPSC aggregate strategies involving WNT stimulation have also been shown to yield NMP-like cell populations (Denham et al., 2015; Mouilleau et al., 2021) and often give rise to sophisticated organoid/gastruloid structures containing a wide variety of cell types representative of all three germ layers and recapitulating early embryonic anteroposterior and dorsoventral patterning events (Dady et al., 2022; Denham et al., 2015; Faustino Martins et al., 2020; Lee et al., 2022; Libby et al., 2021; Mavrommatis et al., 2021; Moris et al., 2020; Mouilleau et al., 2021; Olmsted and Paluh, 2021; Pereira et al., 2021; Seo et al., 2021).

The severity of embryonic phenotypes associated with mutations in key posterior regulators in model organisms and the difficulty in distinguishing between cell vs non cell-autonomous effects have been major obstacles in addressing fundamental questions about axial progenitor biology as well as modelling diseases affecting them and their derivatives. The *in vitro* production of their human counterparts from hPSCs

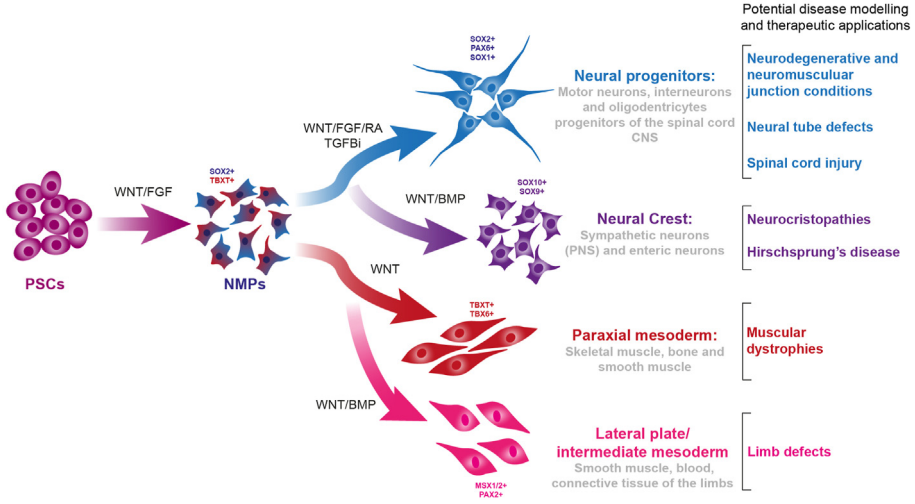

Fig. 1. Diagram showing the key signals/treatments shown to direct adherent pluripotent stem cells (PSC) towards NMPs/axial progenitors and their derivatives. The expression of key transcription factors denoting distinct cell identities is also shown. i, inhibition.

offers an attractive solution to this problem by facilitating the isolation of large numbers of relatively pure posterior cell populations that are amenable to genetic manipulation and can be employed for transcriptome/epigenetic analyses and various clinical applications (Fig. 1). Below, we focus specifically on the progress made in the use of human axial progenitors toward the development of disease modelling and cell replacement strategies.

3. Applications of human axial progenitor derivatives: disease modelling

3.1. Axial defects

A considerable number of human congenital developmental defects affect axial tissues and often reduce dramatically the life quality and expectancy of the affected individuals (Binagui-Casas et al., 2021; Wymeersch et al., 2021) (Table 1). Some of these axial defects are likely to arise during the early stages of posterior elongation as a result of impaired axial progenitor specification/differentiation. For example, some vertebral malformations and congenital scoliosis conditions, such as cases of spondylocostal dysostosis and sacral agenesis, have been linked to mutations in genes that exclusively define NMPs and presomitic/paraxial mesoderm and are associated with the segmentation clock (*TBXT*, *TBX6*, *DLL3*, *LFNG*, *HES7*) (Baschal et al., 2015; Bulman et al., 2000; Ghebranious et al., 2008; Giampietro et al., 2009; Postma et al., 2014; Sparrow et al., 2006, 2008, 2013a). This contrasts similar defects (e.g. Cousin syndrome, spondyloepiphyseal dysplasia congenita and campomelic dysplasia) that appear to arise in later mesodermal derivatives such as the sclerotome (Loh et al., 2016). A number of protocols that lead to the efficient generation of presomitic mesoderm cells from hPSCs via the employment of NMP-inducing culture conditions (WNT/FGF signalling stimulation) have been described over the past few years (Al Tanoury et al., 2020a,b; Chal et al., 2015, 2016; Diaz-Cuadros et al., 2020; Loh et al., 2016; Matsuda et al., 2020). Remarkably, some of these studies have reported the oscillatory expression of segmentation clock genes in hPSC-derived presomitic mesoderm thus facilitating for the first time the *in vitro* dissection of early somitogenesis events as well as their disruption in the case of certain axial defects (Diaz-Cuadros et al., 2020; Matsuda et al., 2020). As a proof-of-principle, Matsuda and colleagues demonstrated that CRISPR-Cas9-induced attenuation of genes linked to vertebral malformations, or precise introduction of pathogenic mutations associated with such defects, result in impaired oscillation patterns of a *HES7* promoter-driven luciferase reporter and dysregulation of various key patterning genes, especially FGF signalling components (Matsuda et al., 2020). Although the actual disease relevance of these results remains to be elucidated, this work clearly shows that *in vitro* generation of early NMP mesodermal derivatives from hPSCs, is likely to be a powerful system for the study of some vertebral defects, especially when complemented with 3D organoid approaches recapitulating formation of somite-like structures (van den Brink et al., 2020; Veenvliet et al., 2020).

Table 1
Examples of developmental defects due to mutations in axial progenitor-associated genes[a].

Gene affected (expression)	Developmental Defect(s)	References
TBX6 (NMPs, presomitic mesoderm)	Spondylocostal dysostosis, Familial Idiopathic Scoliosis, Congenital vertebral malformation	(Baschal et al., 2015; Otomo et al., 2019b; Ren et al., 2020; Sparrow et al., 2013b; Wu et al., 2015)
DLL3 (presomitic mesoderm)	Spondylocostal dysostosis	(Bonafe et al., 2003; Bulman et al., 2000; Turnpenny et al., 2003)
LFNG (presomitic mesoderm)	Spondylocostal dysostosis	(Otomo et al., 2019a; Sparrow et al., 2006)
HES7 (presomitic mesoderm)	Spondylocostal dysostosis	(Sparrow et al., 2008, 2010, 2013a)
TBXT (NMPs, presomitic mesoderm, notochord)	Sacral agenesis, Neural tube defects, Congenital vertebral malformation, Chordoma	(Agopian et al., 2013; Carter et al., 2011; Fellous et al., 1982; Ghebranious et al., 2008; Jensen et al., 2004; Morrison et al., 1996; Pillay et al., 2012; Postma et al., 2014; Shields et al., 2000; Trembath et al., 1999; Yang et al., 2009)
PAX3 (NMPs, presomitic mesoderm, somites, spinal cord neurectoderm, neural crest)	Neural tube defects	Agopian et al. (2013)
HOXB7 (NMPs, presomitic mesoderm, spinal cord neurectoderm)	Neural tube defects	Rochtus et al. (2015)
MNX1 (NMPs, node)	Currarino syndrome	(Hagan et al., 2000; Ross et al., 1998; Wang et al., 2006)

[a] Note: based on published studies focusing on analysis of human patient sequencing data.

3.2. Muscular dystrophies

Presomitic mesoderm cells derived via the initial induction of a transient TBXT + SOX2+ NMP-like entity from hPSCs can be further directed to efficiently produce multinucleated muscle fibres with organized myofibrils as well as satellite-like cells (Al Tanoury et al., 2020a; Chal et al., 2015, 2016; Diaz-Cuadros et al., 2020). Capture of such later myogenic fates has opened new avenues toward the modelling of human muscular dystrophies such as Duchenne Muscular Dystrophy (DMD), an X-linked disorder caused by mutations in the dystrophin gene. Indeed, use of these differentiation protocols together with dystrophin mutant hPSC lines has been shown to recapitulate key aspects of DMD such as mislocalisation of components of the Dystrophin-glycoprotein associated complex, increased fibre branching/fusion, reduced differentiation competence, contraction defects and Ca^{2+} hyperexcitability (Al Tanoury et al., 2020b; Chal et al., 2015; Paredes-Redondo et al., 2021). Strikingly, some of these DMD hallmarks can be rescued by treating cultures with prednisolone, which has been employed in the treatment of DMD, thus providing novel insights into the mechanism of action of this synthetic glucocorticoid hormone (Al Tanoury et al., 2020b). Moreover, DMD patient-derived and CRISPR-corrected myofibres generated from hPSCs via an NMP intermediated have been combined with *in vitro*-derived motor neurons (MNs) to assemble neuromuscular junctions (NMJs) (see also below). In conjunction with sophisticated tools, such as compartmentalised microdevices and optogenetics, this approach has revealed that disease-associated NMJ/myofibre contraction defects can be rescued by TGFβ signalling inhibition and normalisation of axon guidance-linked gene expression (Paredes-Redondo et al., 2021). A complementary NMP-based human skeletal muscle organoid strategy has also been employed to examine the differentiation of human induced PSCs (hiPSCs) derived from patients suffering from Limb Girdle Muscular Dystrophy 2A (LGMD2A), an autosomal recessive disorder marked by progressive, symmetrical weakness of the proximal limb and girdle muscle, caused by mutations in the *CAPN3* gene (Mavrommatis et al., 2021). This study revealed that *CAPN3* mutant myogenic progenitors generated through this approach exhibit differences in mitochondrial protein levels and impaired Ca^{2+} transport.

3.3. Neural tube defects

Certain cases of spinal neural tube defects (NTDs), congenital malformations arising due to incomplete neural tube closure, have also been associated with mutations/misregulation of genes that play a crucial role in the specification and differentiation of NMPs/axial progenitors

(Fig. 1), as revealed by experiments targeting specifically these cell populations (Dady et al., 2014; Economou et al., 2015). Examples include *TBXT* (Agopian et al., 2013; Carter et al., 2011; Fellous et al., 1982; Morrison et al., 1996; Park et al., 1989; Searle, 1966; Shields et al., 2000), CDX factors (Savory et al., 2011; Zhao et al., 2014), PAX3 (Deal et al., 2021; Palmer et al., 2021; Zhao et al., 2014), canonical WNT/FGF signalling components (Anderson et al., 2016; Chang et al., 2019; Palmer et al., 2021; Zhao et al., 2014) and HOX family members (Rochtus et al., 2015; Yu et al., 2019). These genes, together with the Wnt/PCP pathway, heavily influence either the balanced production of NMPs and NMP-derived mesodermal and neurectodermal/neural crest cells in the posterior growth region, or its tight coupling with the biomechanical processes driving neurulation, and thus are critical for proper neural tube closure (Anderson et al., 2016; Dady et al., 2014; Galea et al., 2017, 2021; Poncet et al., 2020).

The generation of NMP-like cells and their derivatives from hPSCs either in an adherent 2D or in a 3D spinal organoid context is an attractive route for the dissection of the cellular and molecular basis of spinal NTDs *in vitro*. For example, recent work has revealed a role for TBXT in orchestrating trunk neural crest patterning by controlling posterior axial identity acquisition/HOX gene activation, as well as guiding early morphogenetic events associated with neural tube formation and extension, shedding light on the potential links between TBXT locus mutations and NTDs (Gogolou et al., 2021; Libby et al., 2021). The combination of such approaches with recently developed, sophisticated biomechanical manipulation, quantitative image analysis and geometric confinement tools (Abdel Fattah et al., 2021; Blin, 2021; Karzbrun et al., 2021; Seo et al., 2021) that allow the dissection of neural tube closure events, is likely to yield further mechanistic insights into the genetic aetiology of spinal NTDs. These strategies can also be employed for assessing environmental assaults on neural tube patterning. In a recent, proof-of-concept study, Lee et al. utilised a hPSC/NMP-derived spinal cord organoid model that recapitulates aspects of neural tube morphogenesis, coupled with an automated deep learning-based image analysis platform, to screen for antiepileptic drugs promoting an NTD-like phenotype (Lee et al., 2022).

3.4. Neuromuscular diseases

NMP-based, 3D hPSC differentiation strategies involving the simultaneous induction of posterior neurectodermal, neural crest and presomitic mesoderm-derived cell types have been shown to be marked by the subsequent self-organised emergence of structures exhibiting features of neuromuscular junctions (NMJs) and muscle contraction (Faustino Martins et al., 2020; Pereira et al., 2021). Hence, use of these protocols opens new promising avenues for the *in vitro* modelling of neuromuscular diseases. One example is myasthenia gravis, an autoimmune condition caused by autoantibodies targeting NMJ components and, in turn, affecting neuromuscular transmission. Treatment of trunk neuromuscular organoids, generated from hPSCs via NMPs, with purified immunoglobulin G (IgG) fractions obtained from myasthenia gravis patients and containing autoantibodies against the nicotinic acetylcholine receptor (AChR) (occurring in ~85% of cases), was found to recapitulate pathological disease hallmarks, such as reduction in the number of NMJ-like structures as well as muscle contraction rate/amplitude (Faustino Martins et al., 2020). Another disease marked by NMJ degeneration is amyotrophic lateral sclerosis (ALS), a fatal disease affecting the motor nervous system. Production and analysis of NMJ structure-containing, neuromuscular organoids from hiPSCs derived from ALS patients via a similar NMP-based differentiation strategy revealed that ALS organoids exhibit impaired muscle contractions and, interestingly, distinct patient genotype-specific NMJ phenotypes (Pereira et al., 2021). Further use of such models and their expansion to include other neuromuscular conditions, such as spinal muscular atrophy, will improve our understanding of their causes and allow the development of screening platforms aiming to identify novel therapeutic agents for their treatment. Moreover, further refinement of protocols for the generation of neuromuscular organoids corresponding to distinct axial levels (e.g. brachial vs thoracic vs lumbosacral) will potentially shed light on the influence of anteroposterior regional identity on the selective vulnerability often exhibited by the cell types affected by these degenerative conditions (Brockington et al., 2013; Gerardo-Nava et al., 2013; Kaplan et al., 2014).

4. Applications of human axial progenitor derivatives: cell therapy

4.1. Spinal cord derivatives

The anteroposterior axial identity of cells in the nervous system is a major determinant of their differentiation potential and functionality. Classic heterotopic transposition experiments between different segments of the neural tube in chick/quail embryos have shown that grafted donor early neural cells are competent to "reprogram" their axial identity (as defined by region-specific Hox gene expression/differentiation potential), depending on the location of their origin and the A-P level of their host environment following transplantation (Grapin-Botton et al., 1995, 1997; Itasaki et al., 1996). However, this plasticity in regional identity appears to be a property of "young" neural tube fragments and disappears at later stages (Ensini et al., 1998). Moreover, it has been unclear whether the axial character of neural derivatives of hPSCs influences their behaviour *in vivo* and therefore is a critical parameter to take into account during the design of cell replacement therapies e.g. for the treatment of spinal cord injury. Early experiments involving grafting of different mouse PSC-derived motor neuron populations into the spinal cord of chick embryos indicated that anteroposterior regionalisation *in vitro* may affect donor cell behaviour *in vivo* (Peljto et al., 2010; Soundararajan et al., 2006). Subsequent studies, based on the transplantation of trunk neural crest and thoracic motor neuron progenitors generated from hPSC-derived NMP-like cells, into the posterior neural tube of chick embryos, revealed that these can incorporate and differentiate quite efficiently within the host environment (Frith et al., 2018; Wind et al., 2021), although a longer embryo culture period appears to correlate with deterioration in the differentiation efficiency of human donor cells *in vivo* (Dady et al., 2022). However, these studies did not examine the integration capacity of the anterior counterparts of these NMP-derived cell populations in the same transplantation context. Such comparison (and with more relevance to cell therapy applications) was conducted in a rat model of spinal cord injury, which assessed the ability of foetal rat spinal cord progenitors derived from different A-P regions of the central nervous system (telencephalon, hindbrain, spinal cord) as well as anterior (forebrain) and posterior (hindbrain/cervical spinal cord) hPSC-derived neural progenitors and revealed that axial level homology between donor cell and spinal cord transplantation sites is a crucial factor affecting functional recovery (Kadoya et al., 2016). Importantly, in a follow-up study, the same group reported the generation of self-renewing posterior spinal cord progenitors capable of producing various neuronal and glial cell types, from hPSCs via an NMP intermediate. After grafting into a similar rat spinal cord injury model, these were found to mediate corticospinal regeneration into the injury site, establish synapse-forming human axons within the host spinal cord and trigger improvement in hindlimb function (Kumamaru et al., 2018). These findings suggest that NMP-derived spinal cord progenitors and their downstream products are a promising starting point for the development of cell transplantation-based therapies against spinal cord injury, especially in combination with novel encapsulation technologies and other cell types, such as oligodendrocyte progenitor cells, to ensure optimal delivery, survival of donor cells and subsequent functional integration (Olmsted et al., 2021).

4.2. Mesodermal derivatives

Muscle satellite cells marked by expression of the key myogenic transcription factor PAX7, are an attractive cell population to employ in transplantation approaches aiming to treat muscle injuries and dystrophies (Collins et al., 2005; Montarras et al., 2005; Sacco et al., 2008). Such PAX7+ satellite-like cells can be generated *in vitro* from hPSCs via the induction of a transient TBXT + SOX2+ intermediate, which gives rise to presomitc mesoderm cells (Al Tanoury et al., 2020a; Chal et al., 2015, 2016; Diaz-Cuadros et al., 2020). A proof-of-principle report demonstrated that, when engrafted into a mouse model of Duchenne muscular dystrophy (*mdx* mice), these cells exhibit behaviour similar to endogenous muscle satellite cells *in vivo,* generating both muscle fibres and satellite cells (Chal et al., 2015). This result indicates that NMP-derived presomitic mesoderm may be the optimal population to use in cell therapy approaches aiming to treat muscular dystrophies.

5. Future perspectives

The recent interest in axial progenitor biology and the proliferation of increasingly sophisticated protocols for the generation of previously refractory posterior mesodermal and neural cell types from hPSC-derived axial progenitors, has opened new frontiers in disease modelling and regenerative medicine. Although the research focus so far has been on the use of central nervous system spinal cord and mesodermal derivatives of NMPs, recent studies reporting the efficient induction of posterior neural crest cells from hPSC-derived axial progenitors have also laid the foundations for the *in vitro* modelling of neurocristopathies (neural crest-associated diseases) arising at the trunk axial level. Examples of such conditions include neuroblastoma and pheochromocytoma, solid tumours, which typically arise in the trunk neural crest-derived sympathetic ganglia and adrenal gland. Interrogation of phenotypes during the induction of these sympathoadrenal cell types through the use of recently described NMP-based protocols (Abu-Bonsrah et al., 2018; Frith et al., 2018; Frith and Tsakiridis, 2019; Gomez et al., 2019; Hackland et al., 2019) from hPSCs carrying tumour-promoting mutations (e.g. *MYCN* oncogene amplification and *ALK* gain-of-function mutations in the case of neuroblastoma) (Cohen et al., 2020; Marin Navarro et al., 2019; Matthay et al., 2016; Weng et al., 2021) can potentially be a powerful tool for dissecting the cellular and molecular basis of oncogenic transformation *in vitro*. hPSC-derived sympathetic neurons have also been shown to modulate cardiomyocyte beating (Oh et al., 2016; Takayama et al., 2020) and co-culture systems involving these cell types as well as other autonomic nervous system targets, can be utilised for modelling autonomic neuropathies and screening of candidate drugs for their treatment. Finally, defining precisely the optimal culture conditions for the generation of sacral neural crest cells and their downstream enteric nervous system derivatives (Burns and Douarin, 1998; Le Douarin and Teillet, 1973), from hPSC-derived axial progenitors, would expand the range of candidate cell types for use in cell therapy-based approaches aiming to treat enteric neuropathies, such as Hirschsprung disease.

Acknowledgements

We would like to thank Rebecca Lea for critical reading of the manuscript. Our work is supported by the Biological Sciences Research Council (New Investigator Research Grant BB/P000444/1), H2020 European Research Council (824070), Children's Cancer and Leukaemia Group/Neuroblastoma U.K. (CCLGA 2019 28) and the Medical Research Council (MR/V002163/1).

References

Abdel Fattah, A.R., Daza, B., Rustandi, G., Berrocal-Rubio, M.A., Gorissen, B., Poovathingal, S., Davie, K., Barrasa-Fano, J., Condor, M., Cao, X., Rosenzweig, D.H., Lei, Y., Finnell, R., Verfaillie, C., Sampaolesi, M., Dedecker, P., Van Oosterwyck, H., Aerts, S., Ranga, A., 2021. Actuation enhances patterning in human neural tube organoids. Nat. Commun. 12, 3192.

Abu-Bonsrah, K.D., Zhang, D., Bjorksten, A.R., Dottori, M., Newgreen, D.F., 2018. Generation of adrenal chromaffin-like cells from human pluripotent stem cells. Stem Cell Rep. 10, 134–150.

Agopian, A.J., Bhalla, A.D., Boerwinkle, E., Finnell, R.H., Grove, M.L., Hixson, J.E., Shimmin, L.C., Sewda, A., Stuart, C., Zhong, Y., Zhu, H., Mitchell, L.E., 2013. Exon sequencing of PAX3 and T (brachyury) in cases with spina bifida. Birth Defects Res A Clin Mol Teratol 97, 597–601.

Aires, R., de Lemos, L., Novoa, A., Jurberg, A.D., Mascrez, B., Duboule, D., Mallo, M., 2019. Tail bud progenitor activity relies on a network comprising Gdf11, Lin28, and Hox13 genes. Dev. Cell 48, 383–395 e388.

Aires, R., Dias, A., Mallo, M., 2018. Deconstructing the molecular mechanisms shaping the vertebrate body plan. Curr. Opin. Cell Biol. 55, 81–86.

Al Tanoury, Z., Rao, J., Tassy, O., Gobert, B., Gapon, S., Garnier, J.M., Wagner, E., Hick, A., Hall, A., Gussoni, E., Pourquie, O., 2020a. Differentiation of the human PAX7-positive myogenic precursors/satellite cell lineage in vitro. Development 147.

Al Tanoury, Z., Zimmermann, J.F., Rao, J., Sieiro, D., McNamara, H., Cherrier, T., Hick, A., Bousson, F., Fugier, C., Marchiano, F., Habermann, B., Chal, J., Nesmith, A.P., Gapon, S., Wagner, E., Bassel-Duby, R., Olson, E., Cohen, A.E., Parker, K.K., Pourquié, O., 2020b. Prednisolone rescues Duchenne Muscular Dystrophy phenotypes in human pluripotent stem cells-derived skeletal muscle in vitro. bioRxiv.

Amin, S., Neijts, R., Simmini, S., van Rooijen, C., Tan, S.C., Kester, L., van Oudenaarden, A., Creyghton, M.P., Deschamps, J., 2016. Cdx and T Brachyury Co-activate growth signaling in the embryonic axial progenitor niche. Cell Rep. 17, 3165–3177.

Anderson, M.J., Magidson, V., Kageyama, R., Lewandoski, M., 2020. Fgf4 maintains Hes7 levels critical for normal somite segmentation clock function. Elife 9.

Anderson, M.J., Schimmang, T., Lewandoski, M., 2016. An FGF3-BMP signaling Axis regulates caudal neural tube closure, neural crest specification and anterior-posterior Axis extension. PLoS Genet. 12, e1006018.

Baschal, E.E., Swindle, K., Justice, C.M., Baschal, R.M., Perera, A., Wethey, C.I., Poole, A., Pourquie, O., Tassy, O., Miller, N.H., 2015. Sequencing of the TBX6 gene in families with familial idiopathic scoliosis. Spine Deform 3, 288–296.

Binagui-Casas, A., Dias, A., Guillot, C., Metzis, V., Saunders, D., 2021. Building consensus in neuromesodermal research: current advances and future biomedical perspectives. Curr. Opin. Cell Biol. 73, 133–140.

Blin, G., 2021. Quantitative developmental biology in vitro using micropatterning. Development 148.

Bonafe, L., Giunta, C., Gassner, M., Steinmann, B., Superti-Furga, A., 2003. A cluster of autosomal recessive spondylocostal dysostosis caused by three newly identified DLL3 mutations segregating in a small village. Clin. Genet. 64, 28–35.

Brockington, A., Ning, K., Heath, P.R., Wood, E., Kirby, J., Fusi, N., Lawrence, N., Wharton, S.B., Ince, P.G., Shaw, P.J., 2013. Unravelling the enigma of selective vulnerability in neurodegeneration: motor neurons resistant to degeneration in ALS show distinct gene expression characteristics and decreased susceptibility to excitotoxicity. Acta Neuropathol. 125, 95–109.

Bulman, M.P., Kusumi, K., Frayling, T.M., McKeown, C., Garrett, C., Lander, E.S., Krumlauf, R., Hattersley, A.T., Ellard, S., Turnpenny, P.D., 2000. Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. Nat. Genet. 24, 438–441.

Burns, A.J., Douarin, N.M., 1998. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development 125, 4335–4347.

Cambray, N., Wilson, V., 2002. Axial progenitors with extensive potency are localised to the mouse chordoneural hinge. Development 129, 4855–4866.

Cambray, N., Wilson, V., 2007. Two distinct sources for a population of maturing axial progenitors. Development 134, 2829–2840.

Carter, T.C., Pangilinan, F., Troendle, J.F., Molloy, A.M., VanderMeer, J., Mitchell, A., Kirke, P.N., Conley, M.R., Shane, B., Scott, J.M., Brody, L.C., Mills, J.L., 2011. Evaluation of 64 candidate single nucleotide polymorphisms as risk factors for neural tube defects in a large Irish study population. Am. J. Med. Genet. 155A, 14–21.

Chal, J., Al Tanoury, Z., Hestin, M., Gobert, B., Aivio, S., Hick, A., Cherrier, T., Nesmith, A.P., Parker, K.K., Pourquie, O., 2016. Generation of human muscle fibers and satellite-like cells from human pluripotent stem cells in vitro. Nat. Protoc. 11, 1833–1850.

Chal, J., Oginuma, M., Al Tanoury, Z., Gobert, B., Sumara, O., Hick, A., Bousson, F., Zidouni, Y., Mursch, C., Moncuquet, P., Tassy, O., Vincent, S., Miyanari, A., Bera, A., Garnier, J.M., Guevara, G., Hestin, M., Kennedy, L., Hayashi, S., Drayton, B., Cherrier, T., Gayraud-Morel, B., Gussoni, E., Relaix, F., Tajbakhsh, S., Pourquie, O., 2015. Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy. Nat. Biotechnol. 33, 962–969.

Chalamalasetty, R.B., Dunty Jr., W.C., Biris, K.K., Ajima, R., Iacovino, M., Beisaw, A., Feigenbaum, L., Chapman, D.L., Yoon, J.K., Kyba, M., Yamaguchi, T.P., 2011. The Wnt3a/beta-catenin target gene Mesogenin1 controls the segmentation clock by activating a Notch signalling program. Nat. Commun. 2, 390.

Chalamalasetty, R.B., Garriock, R.J., Dunty Jr., W.C., Kennedy, M.W., Jailwala, P., Si, H., Yamaguchi, T.P., 2014. Mesogenin 1 is a master regulator of paraxial presomitic mesoderm differentiation. Development 141, 4285–4297.

Chang, S., Lu, X., Wang, S., Wang, Z., Huo, J., Huang, J., Shangguan, S., Li, S., Zou, J., Bao, Y., Guo, J., Wang, F., Niu, B., Zhang, T., Qiu, Z., Wu, J., Wang, L., 2019. The effect of folic acid deficiency on FGF pathway via Brachyury regulation in neural tube defects. Faseb. J. 33, 4688–4702.

Cohen, M.A., Zhang, S., Sengupta, S., Ma, H., Bell, G.W., Horton, B., Sharma, B., George, R.E., Spranger, S., Jaenisch, R., 2020. formation of human neuroblastoma in mouse-human neural crest chimeras. Cell Stem Cell 26, 579–592 e576.
Collins, C.A., Olsen, I., Zammit, P.S., Heslop, L., Petrie, A., Partridge, T.A., Morgan, J.E., 2005. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122, 289–301.
Cooper, F., Gentsch, G.E., Mitter, R., Bouissou, C., Healy, L.E., Rodriguez, A.H., Smith, J.C., Bernardo, A.S., 2022. Rostrocaudal patterning and neural crest differentiation of human pre-neural spinal cord progenitors in vitro. Stem Cell Rep.
Cooper, F., Tsakiridis, A., 2022. Shaping axial identity during human pluripotent stem cell differentiation to neural crest cells. Biochem. Soc. Trans. 50, 499–511.
da Cruz, L., Fynes, K., Georgiadis, O., Kerby, J., Luo, Y.H., Ahmado, A., Vernon, A., Daniels, J.T., Nommiste, B., Hasan, S.M., Gooljar, S.B., Carr, A.F., Vugler, A., Ramsden, C.M., Bictash, M., Fenster, M., Steer, J., Harbinson, T., Wilbrey, A., Tufail, A., Feng, G., Whitlock, M., Robson, A.G., Holder, G.E., Sagoo, M.S., Loudon, P.T., Whiting, P., Coffey, P.J., 2018. Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. Nat. Biotechnol. 36, 328–337.
Dady, A., Davidson, L., Halley, P.A., Storey, K.G., 2022 Feb 21. Human spinal cord in vitro differentiation pace is initially maintained in heterologous embryonic environments. Elife 11, e67283. https://doi.org/10.7554/eLife.67283.
Dady, A., Havis, E., Escriou, V., Catala, M., Duband, J.L., 2014. Junctional neurulation: a unique developmental program shaping a discrete region of the spinal cord highly susceptible to neural tube defects. J. Neurosci. 34, 13208–13221.
Deal, K.K., Chandrashekar, A.S., Beaman, M.M., Branch, M.C., Buehler, D.P., Conway, S.J., Southard-Smith, E.M., 2021. Altered sacral neural crest development in Pax3 spina bifida mutants underlies deficits of bladder innervation and function. Dev. Biol. 476, 173–188.
Delfino-Machin, M., Lunn, J.S., Breitkreuz, D.N., Akai, J., Storey, K.G., 2005. Specification and maintenance of the spinal cord stem zone. Development 132, 4273–4283.
Denham, M., Hasegawa, K., Menheniott, T., Rollo, B., Zhang, D., Hough, S., Alshawaf, A., Febbraro, F., Ighaniyan, S., Leung, J., Elliott, D.A., Newgreen, D.F., Pera, M.F., Dottori, M., 2015. Multipotent caudal neural progenitors derived from human pluripotent stem cells that give rise to lineages of the central and peripheral nervous system. Stem Cell. 33, 1759–1770.
Diaz-Cuadros, M., Wagner, D.E., Budjan, C., Hubaud, A., Tarazona, O.A., Donelly, S., Michaut, A., Al Tanoury, Z., Yoshioka-Kobayashi, K., Niino, Y., Kageyama, R., Miyawaki, A., Touboul, J., Pourquie, O., 2020. In vitro characterization of the human segmentation clock. Nature 580, 113–118.
Diez del Corral, R., Breitkreuz, D.N., Storey, K.G., 2002. Onset of neuronal differentiation is regulated by paraxial mesoderm and requires attenuation of FGF signalling. Development 129, 1681–1691.
Diez del Corral, R., Olivera-Martinez, I., Goriely, A., Gale, E., Maden, M., Storey, K., 2003. Opposing FGF and retinoid pathways control ventral neural pattern, neuronal differentiation, and segmentation during body axis extension. Neuron 40, 65–79.
Economou, C., Tsakiridis, A., Wymeersch, F.J., Gordon-Keylock, S., Dewhurst, R.E., Fisher, D., Medvinsky, A., Smith, A.J., Wilson, V., 2015. Intrinsic factors and the embryonic environment influence the formation of extragonadal teratomas during gestation. BMC Dev. Biol. 15, 35.
Edri, S., Hayward, P., Baillie-Johnson, P., Steventon, B.J., Martinez Arias, A., 2019. An epiblast stem cell-derived multipotent progenitor population for axial extension. Development 146.
Ensini, M., Tsuchida, T.N., Belting, H.G., Jessell, T.M., 1998. The control of rostrocaudal pattern in the developing spinal cord: specification of motor neuron subtype identity is initiated by signals from paraxial mesoderm. Development 125, 969–982.
Faustino Martins, J.M., Fischer, C., Urzi, A., Vidal, R., Kunz, S., Ruffault, P.L., Kabuss, L., Hube, I., Gazzerro, E., Birchmeier, C., Spuler, S., Sauer, S., Gouti, M., 2020. Self-organizing 3D human trunk neuromuscular organoids. Cell Stem Cell 26, 172–186 e176.
Fellous, M., Boue, J., Malbrunot, C., Wollman, E., Sasportes, M., Van Cong, N., Marcelli, A., Rebourcet, R., Hubert, C., Demenais, F., Elston, R.C., Namboodiri, K.K., Kaplan, E.B., Fellous, M., 1982. A five-generation family with sacral agenesis and spina bifida: possible similarities with the mouse T-locus. Am. J. Med. Genet. 12, 465–487.
Frith, T.J., Granata, I., Wind, M., Stout, E., Thompson, O., Neumann, K., Stavish, D., Heath, P.R., Ortmann, D., Hackland, J.O., Anastassiadis, K., Gouti, M., Briscoe, J., Wilson, V., Johnson, S.L., Placzek, M., Guarracino, M.R., Andrews, P.W., Tsakiridis, A., 2018. Human axial progenitors generate trunk neural crest cells in vitro. Elife 7.
Frith, T.J.R., Tsakiridis, A., 2019. Efficient generation of trunk neural crest and sympathetic neurons from human pluripotent stem cells via a neuromesodermal axial progenitor intermediate. Curr Protoc Stem Cell Biol 49, e81.
Galea, G.L., Cho, Y.J., Galea, G., Mole, M.A., Rolo, A., Savery, D., Moulding, D., Culshaw, L.H., Nikolopoulou, E., Greene, N.D.E., Copp, A.J., 2017. Biomechanical coupling facilitates spinal neural tube closure in mouse embryos. Proc. Natl. Acad. Sci. U. S. A. 114, E5177–E5186.
Galea, G.L., Maniou, E., Edwards, T.J., Marshall, A.R., Ampartzidis, I., Greene, N.D.E., Copp, A.J., 2021. Cell non-autonomy amplifies disruption of neurulation by mosaic Vangl2 deletion in mice. Nat. Commun. 12, 1159.
Garriock, R.J., Chalamalasetty, R.B., Kennedy, M.W., Canizales, L.C., Lewandoski, M., Yamaguchi, T.P., 2015. Lineage tracing of neuromesodermal progenitors reveals novel Wnt-dependent roles in trunk progenitor cell maintenance and differentiation. Development 142, 1628–1638.
Gerardo-Nava, J., Mayorenko, I.I., Grehl, T., Steinbusch, H.W., Weis, J., Brook, G.A., 2013. Differential pattern of neuroprotection in lumbar, cervical and thoracic spinal cord segments in an organotypic rat model of glutamate-induced excitotoxicity. J. Chem. Neuroanat. 53, 11–17.
Ghebranious, N., Blank, R.D., Raggio, C.L., Staubli, J., McPherson, E., Ivacic, L., Rasmussen, K., Jacobsen, F.S., Faciszewski, T., Burmester, J.K., Pauli, R.M., Boachie-Adjei, O., Glurich, I., Giampietro, P.F., 2008. A missense T (Brachyury) mutation contributes to vertebral malformations. J. Bone Miner. Res. 23, 1576–1583.
Giampietro, P.F., Dunwoodie, S.L., Kusumi, K., Pourquie, O., Tassy, O., Offiah, A.C., Cornier, A.S., Alman, B.A., Blank, R.D., Raggio, C.L., Glurich, I., Turnpenny, P.D., 2009. Progress in the understanding of the genetic etiology of vertebral segmentation disorders in humans. Ann. N. Y. Acad. Sci. 1151, 38–67.
Gogolou, A., Souilhol, C., Granata, I., Wymeersch, F.J., Manipur, I., Wind, M., Frith, T.J.R., Guarini, M., Bertero, A., Bock, C., Halbritter, F., Takasato, M., Guarracino, M.R., Tsakiridis, A., 2021. Early anteroposterior regionalisation of human neural crest is shaped by a pro-mesodermal factor. bioRxiv.
Gomez, G.A., Prasad, M.S., Wong, M., Cherney, R.M., Shelar, P.B., Sandhu, N., Hackland, J.O.S., Hernandez, J.C., Leung, A.W., Garcia, M.I., 2019. WNT/β-CATENIN Modulates the Axial Identity of ES Derived Human Neural Crest.
Gouti, M., Delile, J., Stamataki, D., Wymeersch, F.J., Huang, Y., Kleinjung, J., Wilson, V., Briscoe, J., 2017. A gene regulatory network balances neural and mesoderm specification during vertebrate trunk development. Dev. Cell 41, 243–261 e247.
Gouti, M., Tsakiridis, A., Wymeersch, F.J., Huang, Y., Kleinjung, J., Wilson, V., Briscoe, J., 2014. In vitro generation of neuromesodermal progenitors reveals distinct roles for wnt signalling in the specification of spinal cord and paraxial mesoderm identity. PLoS Biol. 12, e1001937.
Grapin-Botton, A., Bonnin, M.A., Le Douarin, N.M., 1997. Hox gene induction in the neural tube depends on three parameters: competence, signal supply and paralogue group. Development 124, 849–859.
Grapin-Botton, A., Bonnin, M.A., McNaughton, L.A., Krumlauf, R., Le Douarin, N.M., 1995. Plasticity of transposed rhombomeres: Hox gene induction is correlated with phenotypic modifications. Development 121, 2707–2721.
Hackland, J.O.S., Shelar, P.B., Sandhu, N., Prasad, M.S., Charney, R.M., Gomez, G.A., Frith, T.J.R., Garcia-Castro, M.I., 2019. FGF modulates the axial identity of trunk hPSC-derived neural crest but not the cranial-trunk decision. Stem Cell Rep. 12, 920–933.
Hagan, D.M., Ross, A.J., Strachan, T., Lynch, S.A., Ruiz-Perez, V., Wang, Y.M., Scambler, P., Custard, E., Reardon, W., Hassan, S., Nixon, P., Papapetrou, C., Winter, R.M., Edwards, Y., Morrison, K., Barrow, M., Cordier-Alex, M.P., Correia, P., Galvin-Parton, P.A., Gaskill, S., Gaskin, K.J., Garcia-Minaur, S., Gereige, R., Hayward, R., Homfray, T., 2000. Mutation analysis and embryonic expression of the HLXB9 Currarino syndrome gene. Am. J. Hum. Genet. 66, 1504–1515.
Henrique, D., Abranches, E., Verrier, L., Storey, K.G., 2015. Neuromesodermal progenitors and the making of the spinal cord. Development 142, 2864–2875.
Hofmann, M., Schuster-Gossler, K., Watabe-Rudolph, M., Aulehla, A., Herrmann, B.G., Gossler, A., 2004. WNT signaling, in synergy with T/TBX6, controls Notch signaling by regulating Dll1 expression in the presomitic mesoderm of mouse embryos. Genes Dev. 18, 2712–2717.
Itasaki, N., Sharpe, J., Morrison, A., Krumlauf, R., 1996. Reprogramming Hox expression in the vertebrate hindbrain: influence of paraxial mesoderm and rhombomere transposition. Neuron 16, 487–500.
Javali, A., Misra, A., Leonavicius, K., Acharyya, D., Vyas, B., Sambasivan, R., 2017. Co-expression of Tbx6 and Sox2 identifies a novel transient neuromesoderm progenitor cell state. Development 144, 4522–4529.
Jensen, L.E., Barbaux, S., Hoess, K., Fraterman, S., Whitehead, A.S., Mitchell, L.E., 2004. The human T locus and spina bifida risk. Hum. Genet. 115, 475–482.
Jurberg, A.D., Aires, R., Varela-Lasheras, I., Novoa, A., Mallo, M., 2013. Switching axial progenitors from producing trunk to tail tissues in vertebrate embryos. Dev. Cell 25, 451–462.
Kadoya, K., Lu, P., Nguyen, K., Lee-Kubli, C., Kumamaru, H., Yao, L., Knackert, J., Poplawski, G., Dulin, J.N., Strobl, H., Takashima, Y., Biane, J., Conner, J., Zhang, S.C., Tuszynski, M.H., 2016. Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. Nat. Med. 22, 479–487.
Kaplan, A., Spiller, K.J., Towne, C., Kanning, K.C., Choe, G.T., Geber, A., Akay, T., Aebischer, P., Henderson, C.E., 2014. Neuronal matrix metalloproteinase-9 is a determinant of selective neurodegeneration. Neuron 81, 333–348.
Karzbrun, E., Khankhel, A.H., Megale, H.C., Glasauer, S.M.K., Wyle, Y., Britton, G., Warmflash, A., Kosik, K.S., Siggia, E.D., Shraiman, B.I., Streichan, S.J., 2021. Human neural tube morphogenesis in vitro by geometric constraints. Nature 599, 268–272.
Kirino, K., Nakahata, T., Taguchi, T., Saito, M.K., 2018. Efficient derivation of sympathetic neurons from human pluripotent stem cells with a defined condition. Sci. Rep. 8, 12865.
Knight, G.T., Lundin, B.F., Iyer, N., Ashton, L.M., Sethares, W.A., Willett, R.M., Ashton, R.S., 2018. Engineering induction of singular neural rosette emergence within hPSC-derived tissues. Elife 7.
Koch, F., Scholze, M., Wittler, L., Schifferl, D., Sudheer, S., Grote, P., Timmermann, B., Macura, K., Herrmann, B.G., 2017. Antagonistic activities of Sox2 and brachyury control the fate choice of neuro-mesodermal progenitors. Dev. Cell 42, 514–526 e517.
Kumamaru, H., Kadoya, K., Adler, A.F., Takashima, Y., Graham, L., Coppola, G., Tuszynski, M.H., 2018. Generation and post-injury integration of human spinal cord neural stem cells. Nat. Methods 15, 723–731.
Le Douarin, N.M., Teillet, M.A., 1973. The migration of neural crest cells to the wall of the digestive tract in avian embryo. J. Embryol. Exp. Morphol. 30, 31–48.
Lee, J.H., Shin, H., Shaker, M., Kim, H.J., Park, Si-Hyung, Kim, J.H., Lee, N., Kang, M., Cho, S., Kwak, T.H., Kim, J.W., Song, M.-R., Kwon, S.-H., Han, D.W., Lee, S., Choi, S.-Y., Rhyu, I.J., Kim, H., Geum, D., Cho, I.-J., Sun, W., 2022 Apr. Production of human

spinal-cord organoids recapitulating neural-tube morphogenesis. Nat. Biomed. Eng. 6 (4), 435–448. https://doi.org/10.1038/s41551-022-00868-4.
Libby, A.R.G., Joy, D.A., Elder, N.H., Bulger, E.A., Krakora, M.Z., Gaylord, E.A., Mendoza-Camacho, F., Butts, J.C., McDevitt, T.C., 2021. Axial elongation of caudalized human organoids mimics aspects of neural tube development. Development 148.
Lippmann, E.S., Williams, C.E., Ruhl, D.A., Estevez-Silva, M.C., Chapman, E.R., Coon, J.J., Ashton, R.S., 2015. Deterministic HOX patterning in human pluripotent stem cell-derived neuroectoderm. Stem Cell Rep. 4, 632–644.
Liu, J.P., Laufer, E., Jessell, T.M., 2001. Assigning the positional identity of spinal motor neurons: rostrocaudal patterning of Hox-c expression by FGFs, Gdf11, and retinoids. Neuron 32, 997–1012.
Loh, K.M., Chen, A., Koh, P.W., Deng, T.Z., Sinha, R., Tsai, J.M., Barkal, A.A., Shen, K.Y., Jain, R., Morganti, R.M., Shyh-Chang, N., Fernhoff, N.B., George, B.M., Wernig, G., Salomon, R.E.A., Chen, Z., Vogel, H., Epstein, J.A., Kundaje, A., Talbot, W.S., Beachy, P.A., Ang, L.T., Weissman, I.L., 2016. Mapping the pairwise choices leading from pluripotency to human bone, heart, and other mesoderm cell types. Cell 166, 451–467.
Lukoseviciute, M., Mayes, S., Sauka-Spengler, T., 2021. Neuromesodermal progenitor origin of trunk neural crest in vivo. bioRxiv.
Marin Navarro, A., Day, K., Kogner, P., Wilhelm, M., Falk, A., 2019. Generation of induced pluripotent stem cell lines from two Neuroblastoma patients carrying a germline ALK R1275Q mutation. Stem Cell Res. 34, 101356.
Martin, B.L., Kimelman, D., 2010. Brachyury establishes the embryonic mesodermal progenitor niche. Genes Dev. 24, 2778–2783.
Martin, B.L., Kimelman, D., 2012. Canonical Wnt signaling dynamically controls multiple stem cell fate decisions during vertebrate body formation. Dev. Cell 22, 223–232.
Matsuda, M., Yamanaka, Y., Uemura, M., Osawa, M., Saito, M.K., Nagahashi, A., Nishio, M., Guo, L., Ikegawa, S., Sakurai, S., Kihara, S., Maurissen, T.L., Nakamura, M., Matsumoto, T., Yoshitomi, H., Ikeya, M., Kawakami, N., Yamamoto, T., Woltjen, K., Ebisuya, M., Toguchida, J., Alev, C., 2020. Recapitulating the human segmentation clock with pluripotent stem cells. Nature 580, 124–129.
Matthay, K.K., Maris, J.M., Schleiermacher, G., Nakagawara, A., Mackall, C.L., Diller, L., Weiss, W.A., 2016. Neuroblastoma. Nat. Rev. Dis. Prim. 2, 16078.
Mavrommatis, L., Jeong, H.-W., Gomez-Giro, G., Stehling, M., Kienitz, M.-C., Psathaki, O.E., Bixel, M.G., Morosan-Puopolo, G., Gerovska, D., Araúzo-Bravo, M.J., Schwamborn, J.C., Schöler, H.R., Adams, R.H., Vorgerd, V., Brand-Saberi, B., Zaehres, H., 2021. Human skeletal muscle organoids model fetal myogenesis and sustain uncommitted PAX7 myogenic progenitors. bioRxiv.
Metzis, V., Steinhauser, S., Pakanavicius, E., Gouti, M., Stamataki, D., Ivanovitch, K., Watson, T., Rayon, T., Mousavy Gharavy, S.N., Lovell-Badge, R., Luscombe, N.M., Briscoe, J., 2018. Nervous system regionalization entails axial allocation before neural differentiation. Cell 175, 1105–1118 e1117.
Molotkova, N., Molotkov, A., Sirbu, I.O., Duester, G., 2005. Requirement of mesodermal retinoic acid generated by Raldh2 for posterior neural transformation. Mech. Dev. 122, 145–155.
Montarras, D., Morgan, J., Collins, C., Relaix, F., Zaffran, S., Cumano, A., Partridge, T., Buckingham, M., 2005. Direct isolation of satellite cells for skeletal muscle regeneration. Science 309, 2064–2067.
Moris, N., Anlas, K., van den Brink, S.C., Alemany, A., Schroder, J., Ghimire, S., Balayo, T., van Oudenaarden, A., Martinez Arias, A., 2020. An in vitro model of early anteroposterior organization during human development. Nature 582, 410–415.
Morrison, K., Papapetrou, C., Attwood, J., Hol, F., Lynch, S.A., Sampath, A., Hamel, B., Burn, J., Sowden, J., Stott, D., Mariman, E., Edwards, Y.H., 1996. Genetic mapping of the human homologue (T) of mouse T(Brachyury) and a search for allele association between human T and spina bifida. Hum. Mol. Genet. 5, 669–674.
Mouilleau, V., Vaslin, C., Robert, R., Gribaudo, S., Nicolas, N., Jarrige, M., Terray, A., Lesueur, L., Mathis, M.W., Croft, G., Daynac, M., Rouiller-Fabre, V., Wichterle, H., Ribes, V., Martinat, C., Nedelec, S., 2021. Dynamic extrinsic pacing of the HOX clock in human axial progenitors controls motor neuron subtype specification. Development 148.
Mugele, D., Moulding, D.A., Savery, D., Mole, M.A., Greene, N.D.A., Martinez-Barbera, J.P., Copp, A.J., 2018. Genetic approaches in mice demonstrate that neuro-mesodermal progenitors express T/Brachyury but not Sox2. bioRxiv.
Needham, J., Metzis, V., 2022. Heads or tails: making the spinal cord. Dev. Biol. 485, 80–92.
Oh, Y., Cho, G.S., Li, Z., Hong, I., Zhu, R., Kim, M.J., Kim, Y.J., Tampakakis, E., Tung, L., Huganir, R., Dong, X., Kwon, C., Lee, G., 2016. Functional coupling with cardiac muscle promotes maturation of hPSC-derived sympathetic neurons. Cell Stem Cell 19, 95–106.
Olivera-Martinez, I., Harada, H., Halley, P.A., Storey, K.G., 2012. Loss of FGF-dependent mesoderm identity and rise of endogenous retinoid signalling determine cessation of body axis elongation. PLoS Biol. 10, e1001415.
Olmsted, Z.T., Paluh, J.L., 2021. Co-development of central and peripheral neurons with trunk mesendoderm in human elongating multi-lineage organized gastruloids. Nat. Commun. 12, 3020.
Olmsted, Z.T., Stigliano, C., Scimemi, A., Wolfe, T., Cibelli, J., Horner, P.J., Paluh, J.L., 2021. Transplantable human motor networks as a neuron-directed strategy for spinal cord injury. iScience 24, 102827.
Otomo, N., Mizumoto, S., Lu, H.F., Takeda, K., Campos-Xavier, B., Mittaz-Crettol, L., Guo, L., Takikawa, K., Nakamura, M., Yamada, S., Matsumoto, M., Watanabe, K., Ikegawa, S., 2019a. Identification of novel LFNG mutations in spondylocostal dysostosis. J. Hum. Genet. 64, 261–264.
Otomo, N., Takeda, K., Kawai, S., Kou, I., Guo, L., Osawa, M., Alev, C., Kawakami, N., Miyake, N., Matsumoto, N., Yasuhiko, Y., Kotani, T., Suzuki, T., Uno, K., Sudo, H., Inami, S., Taneichi, H., Shigematsu, H., Watanabe, K., Yonezawa, I., Sugawara, R., Taniguchi, Y., Minami, S., Kaneko, K., Nakamura, M., Matsumoto, M., Toguchida, J., Watanabe, K., Ikegawa, S., 2019b. Bi-allelic loss of function variants of TBX6 causes a spectrum of malformation of spine and rib including congenital scoliosis and spondylocostal dysostosis. J. Med. Genet. 56, 622–628.
Palmer, A.J., Savery, D., Massa, V., Copp, A.J., Greene, N.D.E., 2021. Genetic interaction of Pax3 mutation and canonical Wnt signaling modulates neural tube defects and neural crest abnormalities. Genesis 59, e23445.
Paredes-Redondo, A., Harley, P., Maniati, E., Ryan, D., Louzada, S., Meng, J., Kowala, A., Fu, B., Yang, F., Liu, P., Marino, S., Pourquie, O., Muntoni, F., Wang, J., Lieberam, I., Lin, Y.Y., 2021. Optogenetic modeling of human neuromuscular circuits in Duchenne muscular dystrophy with CRISPR and pharmacological corrections. Sci. Adv. 7, eabi8787.
Park, C.H., Pruitt, J.H., Bennett, D., 1989. A mouse model for neural tube defects: the curtailed (Tc) mutation produces spina bifida occulta in Tc/+ animals and spina bifida with meningomyelocele in Tc/t. Teratology 39, 303–312.
Peljto, M., Dasen, J.S., Mazzoni, E.O., Jessell, T.M., Wichterle, H., 2010. Functional diversity of ESC-derived motor neuron subtypes revealed through intraspinal transplantation. Cell Stem Cell 7, 355–366.
Pereira, J.D., DuBreuil, D.M., Devlin, A.C., Held, A., Sapir, Y., Berezovski, E., Hawrot, J., Dorfman, K., Chander, V., Wainger, B.J., 2021. Human sensorimotor organoids derived from healthy and amyotrophic lateral sclerosis stem cells form neuromuscular junctions. Nat. Commun. 12, 4744.
Piao, J., Zabierowski, S., Dubose, B.N., Hill, E.J., Navare, M., Claros, N., Rosen, S., Ramnarine, K., Horn, C., Fredrickson, C., Wong, K., Safford, B., Kriks, S., El Maarouf, A., Rutishauser, U., Henchcliffe, C., Wang, Y., Riviere, I., Mann, S., Bermudez, V., Irion, S., Studer, L., Tomishima, M., Tabar, V., 2021. Preclinical efficacy and safety of a human embryonic stem cell-derived midbrain dopamine progenitor product, MSK-DA01. Cell Stem Cell 28, 217–229 e217.
Pillay, N., Plagnol, V., Tarpey, P.S., Lobo, S.B., Presneau, N., Szuhai, K., Halai, D., Berisha, F., Cannon, S.R., Mead, S., Kasperaviciute, D., Palmen, J., Talmud, P.J., Kindblom, L.G., Amary, M.F., Tirabosco, R., Flanagan, A.M., 2012. A common single-nucleotide variant in T is strongly associated with chordoma. Nat. Genet. 44, 1185–1187.
Poncet, N., Halley, P.A., Lipina, C., Gierlinski, M., Dady, A., Singer, G.A., Febrer, M., Shi, Y.B., Yamaguchi, T.P., Taylor, P.M., Storey, K.G., 2020. Wnt regulates amino acid transporter Slc7a5 and so constraints the integrated stress response in mouse embryos. EMBO Rep. 21, e48469.
Postma, A.V., Alders, M., Sylva, M., Bilardo, C.M., Pajkrt, E., van Rijn, R.R., Schulte-Merker, S., Bulk, S., Stefanovic, S., Ilgun, A., Barnett, P., Mannens, M.M., Moorman, A.F., Oostra, R.J., van Maarle, M.C., 2014. Mutations in the T (brachyury) gene cause a novel syndrome consisting of sacral agenesis, abnormal ossification of the vertebral bodies and a persistent notochordal canal. J. Med. Genet. 51, 90–97.
Ren, X., Yang, N., Wu, N., Xu, X., Chen, W., Zhang, L., Li, Y., Du, R.Q., Dong, S., Zhao, S., Chen, S., Jiang, L.P., Wang, L., Zhang, J., Wu, Z., Jin, L., Qiu, G., Lupski, J.R., Shi, J., Zhang, F., Liu, P., 2020. Increased TBX6 gene dosages induce congenital cervical vertebral malformations in humans and mice. J. Med. Genet. 57, 371–379.
Rochtus, A., Izzi, B., Vangeel, E., Louwette, S., Wittevrongel, C., Lambrechts, D., Moreau, Y., Winand, R., Verpoorten, C., Jansen, K., Van Geet, C., Freson, K., 2015. DNA methylation analysis of Homeobox genes implicates HOXB7 hypomethylation as risk factor for neural tube defects. Epigenetics 10, 92–101.
Ross, A.J., Ruiz-Perez, V., Wang, Y., Hagan, D.M., Scherer, S., Lynch, S.A., Lindsay, S., Custard, E., Belloni, E., Wilson, D.I., Wadey, R., Goodman, F., Orstavik, K.H., Monclair, T., Robson, S., Reardon, W., Burn, J., Scambler, P., Strachan, T., 1998. A homeobox gene, HLXB9, is the major locus for dominantly inherited sacral agenesis. Nat. Genet. 20, 358–361.
Row, R.H., Pegg, A., Kinney, B.A., Farr 3rd, G.H., Maves, L., Lowell, S., Wilson, V., Martin, B.L., 2018. BMP and FGF signaling interact to pattern mesoderm by controlling basic helix-loop-helix transcription factor activity. Elife 7.
Sacco, A., Doyonnas, R., Kraft, P., Vitorovic, S., Blau, H.M., 2008. Self-renewal and expansion of single transplanted muscle stem cells. Nature 456, 502–506.
Sambasivan, R., Steventon, B., 2020. Neuromesodermal progenitors: a basis for robust axial patterning in development and evolution. Front. Cell Dev. Biol. 8, 607516.
Savory, J.G., Mansfield, M., Rijli, F.M., Lohnes, D., 2011. Cdx mediates neural tube closure through transcriptional regulation of the planar cell polarity gene Ptk7. Development 138, 1361–1370.
Searle, A.G., 1966. Curtailed, a new dominant T-allele in the house mouse. Genet. Res. 7, 86–95.
Seo, K., Cho, S., Lee, J.-H., Kim, J.H., Lee, B., Jang, H., Kim, Y., Cho, H.M., Lee, S., Park, Y., Kim, H.Y., Lee, T., Park, W.-Y., Kim, Y.J., Yang, E., Geum, D., Kim, H., Jae, R.R., Sun, W., 2021. Symmetry breaking of hPSCs in micropattern generates a polarized spinal cord-like organoid (pSCO) with dorsoventral organization. bioRxiv.
Shaker, M.R., Lee, J.H., Kim, K.H., Ban, S., Kim, V.J., Kim, J.Y., Lee, J.Y., Sun, W., 2021. Spatiotemporal contribution of neuromesodermal progenitor-derived neural cells in the elongation of developing mouse spinal cord. Life Sci., 119393
Shields, D.C., Ramsbottom, D., Donoghue, C., Pinjon, E., Kirke, P.N., Molloy, A.M., Edwards, Y.H., Mills, J.L., Mynett-Johnson, L., Weir, D.G., Scott, J.M., Whitehead, A.S., 2000. Association between historically high frequencies of neural tube defects and the human T homologue of mouse T (Brachyury). Am. J. Med. Genet. 92, 206–211.
Soundararajan, P., Miles, G.B., Rubin, L.L., Brownstone, R.M., Rafuse, V.F., 2006. Motoneurons derived from embryonic stem cells express transcription factors and develop phenotypes characteristic of medial motor column neurons. J. Neurosci. 26, 3256–3268.
Sparrow, D.B., Chapman, G., Wouters, M.A., Whittock, N.V., Ellard, S., Fatkin, D., Turnpenny, P.D., Kusumi, K., Sillence, D., Dunwoodie, S.L., 2006. Mutation of the LUNATIC FRINGE gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype. Am. J. Hum. Genet. 78, 28–37.

Sparrow, D.B., Faqeih, E.A., Sallout, B., Alswaid, A., Ababneh, F., Al-Sayed, M., Rukban, H., Eyaid, W.M., Kageyama, R., Ellard, S., Turnpenny, P.D., Dunwoodie, S.L., 2013a. Mutation of HES7 in a large extended family with spondylocostal dysostosis and dextrocardia with situs inversus. Am. J. Med. Genet. 161A, 2244–2249.
Sparrow, D.B., Guillen-Navarro, E., Fatkin, D., Dunwoodie, S.L., 2008. Mutation of Hairy-and-Enhancer-of-Split-7 in humans causes spondylocostal dysostosis. Hum. Mol. Genet. 17, 3761–3766.
Sparrow, D.B., McInerney-Leo, A., Gucev, Z.S., Gardiner, B., Marshall, M., Leo, P.J., Chapman, D.L., Tasic, V., Shishko, A., Brown, M.A., Duncan, E.L., Dunwoodie, S.L., 2013b. Autosomal dominant spondylocostal dysostosis is caused by mutation in TBX6. Hum. Mol. Genet. 22, 1625–1631.
Sparrow, D.B., Sillence, D., Wouters, M.A., Turnpenny, P.D., Dunwoodie, S.L., 2010. Two novel missense mutations in HAIRY-AND-ENHANCER-OF-SPLIT-7 in a family with spondylocostal dysostosis. Eur. J. Hum. Genet. 18, 674–679.
Takahashi, J., 2020. iPS cell-based therapy for Parkinson's disease: a Kyoto trial. Regen Ther 13, 18–22.
Takayama, Y., Kushige, H., Akagi, Y., Suzuki, Y., Kumagai, Y., Kida, Y.S., 2020. Selective induction of human autonomic neurons enables precise control of cardiomyocyte beating. Sci. Rep. 10, 9464.
Takemoto, T., Uchikawa, M., Yoshida, M., Bell, D.M., Lovell-Badge, R., Papaioannou, V.E., Kondoh, H., 2011. Tbx6-dependent Sox2 regulation determines neural or mesodermal fate in axial stem cells. Nature 470, 394–398.
Trembath, D., Sherbondy, A.L., Vandyke, D.C., Shaw, G.M., Todoroff, K., Lammer, E.J., Finnell, R.H., Marker, S., Lerner, G., Murray, J.C., 1999. Analysis of select folate pathway genes, PAX3, and human T in a Midwestern neural tube defect population. Teratology 59, 331–341.
Tsakiridis, A., Huang, Y., Blin, G., Skylaki, S., Wymeersch, F., Osorno, R., Economou, C., Karagianni, E., Zhao, S., Lowell, S., Wilson, V., 2014. Distinct Wnt-driven primitive streak-like populations reflect in vivo lineage precursors. Development 141, 1209–1221.
Turnpenny, P.D., Whittock, N., Duncan, J., Dunwoodie, S., Kusumi, K., Ellard, S., 2003. Novel. Mutations in DLL3, a somitogenesis gene encoding a ligand for the Notch signalling pathway, cause a consistent pattern of abnormal vertebral segmentation in spondylocostal dysostosis. J. Med. Genet. 40, 333–339.
Tzouanacou, E., Wegener, A., Wymeersch, F.J., Wilson, V., Nicolas, J.F., 2009. Redefining the progression of lineage segregations during mammalian embryogenesis by clonal analysis. Dev. Cell 17, 365–376.
van den Brink, S.C., Alemany, A., van Batenburg, V., Moris, N., Blotenburg, M., Vivie, J., Baillie-Johnson, P., Nichols, J., Sonnen, K.F., Martinez Arias, A., van Oudenaarden, A., 2020. Single-cell and spatial transcriptomics reveal somitogenesis in gastruloids. Nature 582, 405–409.
Veenvliet, J.V., Bolondi, A., Kretzmer, H., Haut, L., Scholze-Wittler, M., Schifferl, D., Koch, F., Guignard, L., Kumar, A.S., Pustet, M., Heimann, S., Buschow, R., Wittler, L., Timmermann, B., Meissner, A., Herrmann, B.G., 2020. Mouse embryonic stem cells self-organize into trunk-like structures with neural tube and somites. Science 370.
Verrier, L., Davidson, L., Gierlinski, M., Dady, A., Storey, K.G., 2018. Neural differentiation, selection and transcriptomic profiling of human neuromesodermal progenitor-like cells in vitro. Development 145.
Wang, R.Y., Jones, J.R., Chen, S., Rogers, R.C., Friez, M.J., Schwartz, C.E., Graham Jr., J.M., 2006. A previously unreported mutation in a Currarino syndrome kindred. Am. J. Med. Genet. 140, 1923–1930.
Weng, Z., Lin, J., He, J., Gao, L., Lin, S., Tsang, L.L., Zhang, H., He, X., Wang, G., Yang, X., Zhou, H., Zhao, H., Li, G., Zou, L., Jiang, X., 2021. Human embryonic stem cell-derived neural crest model unveils CD55 as a cancer stem cell regulator for therapeutic targeting in MYCN-amplified neuroblastoma. Neuro Oncol.
Wind, M., Gogolou, A., Manipur, I., Granata, I., Butler, L., Andrews, P.W., Barbaric, I., Ning, K., Guarracino, M.R., Placzek, M., Tsakiridis, A., 2021. Defining the signalling determinants of a posterior ventral spinal cord identity in human neuromesodermal progenitor derivatives. Development 148.
Wind, M., Tsakiridis, A., 2021. In vitro generation of posterior motor neurons from human pluripotent stem cells. Curr Protoc 1, e244.
Wu, N., Ming, X., Xiao, J., Wu, Z., Chen, X., Shinawi, M., Shen, Y., Yu, G., Liu, J., Xie, H., Gucev, Z.S., Liu, S., Yang, N., Al-Kateb, H., Chen, J., Zhang, J., Hauser, N., Zhang, T., Tasic, V., Liu, P., Su, X., Pan, X., Liu, C., Wang, L., Shen, J., Shen, J., Chen, Y., Zhang, T., Zhang, J., Choy, K.W., Wang, J., Wang, Q., Li, S., Zhou, W., Guo, J., Wang, Y., Zhang, C., Zhao, H., An, Y., Zhao, Y., Wang, J., Liu, Z., Zuo, Y., Tian, Y., Weng, X., Sutton, V.R., Wang, H., Ming, Y., Kulkarni, S., Zhong, T.P., Giampietro, P.F., Dunwoodie, S.L., Cheung, S.W., Zhang, X., Jin, L., Lupski, J.R., Qiu, G., Zhang, F., 2015. TBX6 null variants and a common hypomorphic allele in congenital scoliosis. N. Engl. J. Med. 372, 341–350.
Wymeersch, F.J., Huang, Y., Blin, G., Cambray, N., Wilkie, R., Wong, F.C., Wilson, V., 2016. Position-dependent plasticity of distinct progenitor types in the primitive streak. Elife 5, e10042.
Wymeersch, F.J., Skylaki, S., Huang, Y., Watson, J.A., Economou, C., Marek-Johnston, C., Tomlinson, S.R., Wilson, V., 2019. Transcriptionally dynamic progenitor populations organised around a stable niche drive axial patterning. Development 146.
Wymeersch, F.J., Wilson, V., Tsakiridis, A., 2021. Understanding axial progenitor biology in vivo and in vitro. Development 148.
Yang, X.R., Ng, D., Alcorta, D.A., Liebsch, N.J., Sheridan, E., Li, S., Goldstein, A.M., Parry, D.M., Kelley, M.J., 2009. T (brachyury) gene duplication confers major susceptibility to familial chordoma. Nat. Genet. 41, 1176–1178.
Young, T., Rowland, J.E., van de Ven, C., Bialecka, M., Novoa, A., Carapuco, M., van Nes, J., de Graaff, W., Duluc, I., Freund, J.N., Beck, F., Mallo, M., Deschamps, J., 2009. Cdx and Hox genes differentially regulate posterior axial growth in mammalian embryos. Dev. Cell 17, 516–526.
Yu, J., Wang, L., Pei, P., Li, X., Wu, J., Qiu, Z., Zhang, J., Ao, R., Wang, S., Zhang, T., Xie, J., 2019. Reduced H3K27me3 leads to abnormal Hox gene expression in neural tube defects. Epigenet. Chromatin 12, 76.
Zhao, T., Gan, Q., Stokes, A., Lassiter, R.N., Wang, Y., Chan, J., Han, J.X., Pleasure, D.E., Epstein, J.A., Zhou, C.J., 2014. beta-catenin regulates Pax3 and Cdx2 for caudal neural tube closure and elongation. Development 141, 148–157.