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

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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 neuro-mesodermal progenitors (NMPs), which generate both spinal cord neuroectoderm 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 neuroectoderm 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 Msn1 (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.,

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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 micro-patterning 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

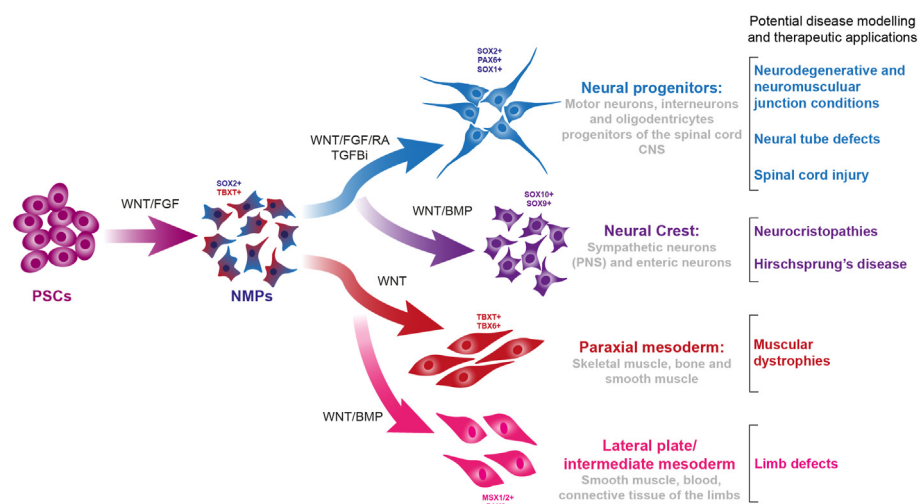


Fig. 1. Diagram showing the key signals/treatments shown to direct adherent pluripotent stem cells (PSC) towards NMPs/axial progenitors and their derivatives. The expression of key transcription factors denoting distinct cell identities is also shown. i, inhibition.

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

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

Gene affected (expression)	Developmental Defect(s)	References
<i>TBX6</i> (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)
<i>DLL3</i> (presomitic mesoderm)	Spondylocostal dysostosis	(Bonafe et al., 2003; Bulman et al., 2000; Turnpenny et al., 2003)
<i>LFNG</i> (presomitic mesoderm)	Spondylocostal dysostosis	(Otomo et al., 2019a; Sparrow et al., 2006)
<i>HES7</i> (presomitic mesoderm)	Spondylocostal dysostosis	(Sparrow et al., 2008, 2010, 2013a)
<i>TBXT</i> (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)
<i>PAX3</i> (NMPs, presomitic mesoderm, somites, spinal cord neuroectoderm, neural crest)	Neural tube defects	Agopian et al. (2013)
<i>HOXB7</i> (NMPs, presomitic mesoderm, spinal cord neuroectoderm)	Neural tube defects	Rochtus et al. (2015)
<i>MNX1</i> (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.

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).

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 TGF β 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 neuroectodermal/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 neuroectodermal, 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 (Ensign 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; Sundararajan 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 presomitic 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.

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