Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Cellular plasticity in the neural crest and cancer

Zhiyuan Hu* and Tatjana Sauka-Spengler*

In vertebrates, neural crest cells (NCCs) are a multipotent embryonic population generating both neural/neuronal and mesenchymal derivatives, and thus the neural crest (NC) is often referred to as the fourth germ layer. NC development is a dynamic process, where NCCs possess substantial plasticity in transcriptional and epigenomic profiles. Recent technical advances in single-cell and low-input sequencing have empowered fine-resolution characterisation of NC development. In this review, we summarise the latest models underlying NC-plasticity acquirement and cell-fate restriction, outline the connections between NC plasticity and NC-derived cancer and envision the new opportunities in studying NC plasticity and its link to cancer.

Address
MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 9DS, UK

Corresponding author:
Tatjana Sauka-Spengler (tatjana.sauka-spengler@imm.ox.ac.uk)
* Twitter account: (@zhi_yuan_hu), (@saukaspengler)

Current Opinion in Genetics and Development 2022, **75**:101928

This review comes from a themed issue on **Cell Reprogramming, regeneration and repair**

Edited by **Ophir Klein** and **Ryuichi Nishinakamura**

For complete overview of the section, please refer to the article collection, "Cell Reprogramming, regeneration and repair"

Available online 21th June 2022

https://doi.org/10.1016/j.gde.2022.101928

Introduction

Cellular plasticity is a broad term indicating the cell identity change [1], which has been studied intensively in scenarios of normal development and cancer. In all vertebrates, unlike the ectodermal neural plate, which gives rise to neural/neuronal fates only, neural crest cells (NCCs) have the potential of contributing to both the neural/neuronal and mesenchymal derivatives (usually originating from mesoderm). NCCs and their progeny *in vivo* are highly heterogeneous in gene expression and epigenomic regulation. This highly multipotent population, most of which originates from the neural-plate border (NPB), can differentiate into a large variety of derivatives [2], which provides therapeutic opportunities for intervention, such as spinal cord injury repair (reviewed in Ref. [3]).

Recently, research on neural crest (NC) development has also entered the genomic era [4–10], enabling the genome-wide characterisation of developmental programmes and underlying regulatory networks. Moreover, whole-organism single-cell sequencing of embryogenesis [11] provided an unprecedently comprehensive view to study not only singular lineages but also the interactions between cells.

In this review, we focus on the latest progress elucidating NC plasticity in three aspects: how NC precursors gain plasticity, how NCCs lose their plasticity during differentiation and how the plasticity is reflected in the NC-derived tumours. We will emphasise the new insights provided by technical advances in NC studies, such as single-cell sequencing and low-input chromatin profiling.

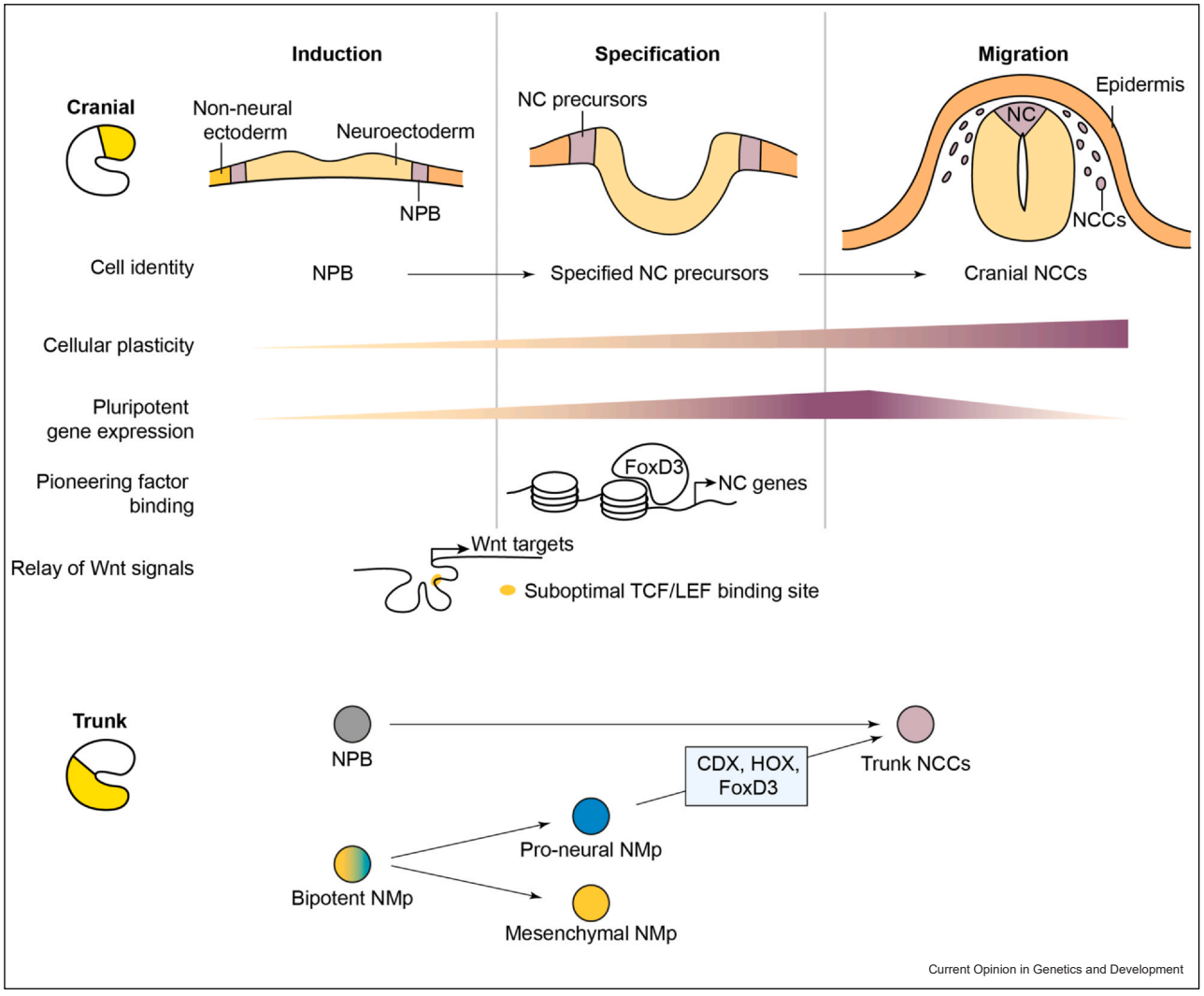
Gaining plasticity of neural crest precursors

The early development of NCC is conventionally conceptualised into three main phases: induction, specification and migration (Figure 1). During the NC formation, a proportion of NC precursor cells procure plasticity of the so-called ectomesenchyme fate. This section reviews how phenotypic shifts and regulatory activities contribute to this process. Additionally, we elucidate a new hypothetic origin of the trunk NC.

Reacquisition of pluripotency in cranial neural crest development

A long-standing question is how NCCs acquire the differentiation potential of ectomesenchyme. The activation of pluripotency-associated genes, *Oct4* and *Nanog*, has been reported in the cranial NC precursors of zebrafish (*pou5f3*, *pou2f1b*; *nanog*) [6] and *Xenopus laevis* (*pou5f3.1/2/3*; *ventx1/2*) [12]. Depleting VENTX/NANOG expression led to the loss of NC skeletogenic potential [13]. However, it remained unclear whether the expression of pluripotency-associated transcription factors (TFs) was maintained from the earlier stages [12] or was transitionally switched on (Figure 1). The comprehensive single-cell RNA-sequencing (scRNA-Seq) analysis of *Xenopus* embryos between zygotic genome activation and early organogenesis disproved the retention hypothesis [11]. The temporal imaging of *Oct4-GFP*/+ murine embryos further supported the reactivation scenario [9••]. The scRNA-Seq (Smart-seq2 [14])

Figure 1



Overview of the cell identity shifts, cellular plasticity levels, transcriptional changes and regulatory factors during NC development. Respecting the cellular plasticity, it is arguable that NC precursors at the induction and specification phases retain higher levels of stemness or potency, whilst cellular plasticity tends to be manifest in the migratory NCCs, which are challenged by diverse environments and are ready to undertake cell identity changes. The schematic diagram for three major phases of cranial NC development exemplifies the hindbrain region.

delineated a set of *Oct4*-expressing *Wnt1*+ precursor cells in murine embryos, and the functions of *Oct4* in cranial NC development were further characterised in mouse embryos using *Wnt1*-Cre conditional knockout [9••]. Moreover, temporal scRNA-Seq data analysis and RNA fluorescence *in situ* hybridisation analysis revealed the progression expression of *Oct4* and *Nanog* along the anterior–posterior axis in the cephalic NC [9••]. Still, the mechanisms driving such a shift remained unclear.

The likeness between cranial *Oct4*-expressing NC and epiblast stem cells is supported by the comparative analysis of open-chromatin landscapes [9••]. Coupled with the finding that epiblast stem cells also resemble the anterior primitive streak [15], these data support the hypothesis that the core *cis*-regulatory programmes are rehashed in similar scenarios, but serve different developmental purposes. The generation of cranial NCCs is likely the consequence of dedifferentiation supported by the reactivation of pluripotency-associated TFs. The critical remaining questions involve the regulatory mechanisms of reactivating the epiblast-like pluripotency programme in the cranial NC and the evolutionary cause of repeatedly using core *cis*-regulatory programmes during development.

From the evolutionary perspective, a comparative analysis elucidated that the potentials of cranial NCCs are

extended along with the course of vertebrate evolution by 'co-opting' other regulatory programmes into the cranial NC circuits [16]. In amniotes, the cranial NC-specific regulatory circuit, composed of TFs *Lhx5*, *Brn3*, *Dmbx1*, *SoxE*, *Tfap2* and *Ets1*, is crucial for skeletal differentiation. In the evolutionary course from sea lamprey, little skate, zebrafish to chicken, *Ets1*, *Ihx5*, *dmbx1* and *BRN3C* were progressively added upon the primitive circuits of *SoxE* and *Tfap2* in the sea lamprey, which confers the potential of certain cranial derivatives, for example, jaws and vagal NC.

Regulatory activities underlying boosted neural crest plasticity

The acquirement of NC plasticity is hypothesised to be modulated by epigenomic remodelling and environmental cues. Recent advances in epigenomic profiling methods, especially for low input, have facilitated the rapidly growing number of catalogued NC enhancers [7,17,18]. Application of chromatin immunoprecipitation followed by sequencing in the *in vitro* human embryonic stem cell differentiation model [18] systematically characterised the regulatory regions by profiling genome-wide histone modifications and TF/co-factor-binding sites. Genome-wide analysis of FoxD3 binding in the wild-type and mutant zebrafish embryos revealed that FoxD3 acts as a pioneer factor to prime distal enhancers for NC specification and then switches to a repressor to modulate differentiation [6] (Figure 1). The extensive profiling of NC development at consecutive developmental stages by profiling transcriptomes and chromatin accessibility uncovered the comprehensive repertoire of NC enhancers and super-enhancers, which were validated in the *in vivo* embryonic model [7]. Furthermore, analysis of this RNA-Seq dataset [7] identified the potential role that an RNA-binding protein Elavl1/HuR plays in cranial NC specification [19]. Another epigenomic profiling technique, Cleavage Under Targets and Release Using Nuclease [20], has been used to delineate the chromatin remodelling during the NC induction and specification [17].

Regarding how the environmental cues, for example, Wnt signals, are relayed into the gene-regulatory networks (GRNs), a recent study [21] proposed a hub-and-spoke model, a term initially used in the field of transport-topology optimisation. Unlike the previous hierarchical model, where the Wnt signalling stands at the top of the NC-GRN, the Wnt signalling pathway in the hub-and-spoke model directly regulates many NC-specific target genes preferably by the suboptimal TCF/LEF-binding motif [21] (Figure 1). The mechanism of Wnt signalling directly regulating *Axud1* was further illustrated in Ref. [22]. Apart from constructing the hub-and-spoke model, this work also indicated the indispensability of using high-resolution chromosome-conformation capture techniques to study developmental plasticity as the tissue-specific 3D genome architecture likely manifests in the level of enhancer–promoter interactions rather than the intra-topologically associating domain interactions [21].

Neuromesodermal progenitors as a potential origin of trunk neural crest

Studies of NC specification were predominantly focused on the cranial NC, while the *in vivo* origin of trunk NCCs is mainly unknown. The long-lasting hypothesis is that the NC specification progresses along the anterior–posterior axis, where ectodermal precursors at the NPB generate trunk NC based on spatial expression analyses [23]. Recently, the neuromesodermal progenitor (NMp) population is proposed to be an alternative hypothetical cellular origin of trunk NCCs. The NMp–NCC transition is supposed to happen much later than the NC specification in the NPB [24].

In the human *in vitro* models, several studies have revealed a transition route from human pluripotent stem cells (hPSCs) first to NMps, then the proneural fate and finally the trunk NC, which implied that one of the developmental trajectories of trunk NC precursors might differ from cranial NC [25–28].

A more recent *in vivo* study suggested that NMps, apart from the NPB epiblast, are another potential progenitor of trunk NCCs [24] (Figure 1). The NMp is a bipotent posterior cell population that can give rise to both spinal cord and paraxial mesoderm derivatives [29], and can potentially be used to treat spinal cord injury (reviewed in Ref. [3]). Recent work in zebrafish used a posterior-specific *foxd3* enhancer to isolate cells, including NMp subpopulations and NCCs, and profile their single-cell transcriptomes. The joint analysis of 10x scRNA-seq and single-nucleus assay for transposase-accessible chromatin using sequencing (snATAC-seq) data reconstructed the NMp–GRNs, where *cdx1a*, *cdx4*, *hoxa13b*, *hoxa10*b and *foxd3* are at the top of this GRN hierarchy. The NMp–GRNs embedded two sets of subcircuits, which modulated the differentiation into neural NMp and mesodermal NMp, respectively. The hypothetical transition from NMp to posterior NC through a proneural NMp state is proposed based on the RNA velocity analysis, which uses the ratio between spliced and unspliced transcripts to predict the directions of cell-state transitions [30]. Moreover, the NMp-population transitions are driven by the posterior expression of CDX genes [24], in line with the finding that Cdx4 regulates posterior NC specification in zebrafish [31]. Yet, this putative development trajectory from NMps to trunk NCCs needs further validation by lineage-tracing experiments and imaging analyses.

The new hypothetical origin of trunk NCCs raises intriguing questions of the trunk NCC plasticity. It remains to be addressed whether the NMp-derived trunk NCCs are more plastic than posterior ectoderm-derived

ones, whether the transition from neural NMps to trunk NCCs is consistent with a decrease in cellular plasticity, and whether at least some trunk NCCs possess latent mesenchymal potential, given their NMp origin.

Plasticity during differentiation

Once NCCs are specified through the elaborately regulated process, they start to migrate and differentiate into specialised lineages. This section discusses the cell-fate-restriction models, the new framework built by single-cell analysis and the environmental factors that influence NC migration and differentiation.

Emerging models of cell-fate restriction

New models detailing how cell fates are restricted from molecular and cellular perspectives have been recently proposed. At the molecular level, a new model for cell-fate bifurcation was proposed based on the integrative analysis of deep scRNA-Seq (Smart-seq2) and 32-gene multiplexed *in situ* sequencing [32], identifying the bipotent progenitor for murine trunk NC differentiation, such as the autonomic–mesenchymal progenitor [8••]. This model defined three phases of the cell-fate bifurcation: initial coactivation of competing transcriptional programmes, gradual biasing towards one fate and cell-fate commitment [8••]. Moreover, this study elaborately predicted the activity of TFs for trunk NC fate commitment based on the co-expression patterns of multiple target genes rather than the expression of TFs *per se*. This is reminiscent of an earlier finding that the positional genes maintain a poised rather than repressed chromatin status during cranial NC development, revealing a potential epigenomic mechanism underlying the initial coactivation of dual regulatory programmes [10]. On the other hand, the scRNA-Seq analysis [8••] showed the continuous trajectory of binary-fate decisions, raising the question of whether progenitor-like cell states at the bifurcation are fate-restricted.

At the cellular level, there are two conventional models of cell-fate restriction, namely direct fate restriction and progressive fate restriction. The key difference between these two is the existence of intermediate progenitors between NCCs and single-fate-restricted cells. The progressive fate-restriction model has been supported by the identification of putative intermediate progenitors [32]. A new 'cyclical fate restriction' hypothesis, which can be viewed as an extension of the direct fate-restriction model, was proposed in the context of pigment-cell development, in which the multipotent progenitors give rise to three pigment-cell populations (melanocytes, xanthophores and iridophores) [33,34]. Akin to the direct fate-restriction model, this cyclical model refutes the existences of restricted intermediate progenitors, chromatoblast and melanoiridoblast, based on the single-cell expression data of 42 genes measured by NanoString. A unique concept of the cyclical model is the 'sub-state', which tends to resemble the combinations of specific fates, but is more plastic than the intermediate progenitors defined in the progressive models. Thus, the critical point to reconcile between the cyclical and progressive models is to evaluate whether the intermediate dual-fate progenitors are convertible or not. However, it is difficult to address this question by merely using phenotypic data, that is, gene or protein expression levels. Chromatin profiling and other epigenomic data coupled with expression data of fine temporal resolution will be informative to define if an intermediate state is an irreversible bipotent progenitor state or a plastic 'sub-state'. Therefore, we envision that synchronous depiction of multiomics in single cells will likely bring in evidence to resolve this dispute.

Compared with the trunk NC, there is much less known about how cranial NC cells are lineage-restricted. The cranial NC contributes to most of the craniofacial skeleton, cranial ganglia and melanocytes. The Spatial Genomic Analysis, which incorporated the sequential single-molecule fluorescence *in situ* hybridisation, profiled the differential expression between premigratory (high levels of *FoxD3*, *Sox9*, *Snai2* and *Myc*) and migratory (high levels of *Msx2*, *Tfap2a*, *Tfap2b* and *Axud1*) cells in the chick dorsal neural tube at the midbrain level [35]. The spatial analysis also characterised the location and gene expression of five lineage-marker-expressing cellular subpopulations, shedding light on the early stages on cranial NC migration and lineage specification. We speculate that systematic characterisation with spatial and single-cell analysis will provide deeper insight into the model of cranial NC-fate restriction.

At the regulatory level, a recently proposed mechanism for *in vitro* differentiation of NCCs into actin-positive smooth muscle cells, a paradigm for the smooth muscle tissue surrounding the aorta and pulmonary artery, was proposed [36]. In this model, Bromodomain-containing protein 4 (BRD4), an epigenetic regulator, interacts with the cohesin agonist NIPBL to regulate 3D genome folding and promote NC differentiation. The murine model demonstrated that *in vivo* BRD4 depletion led to symptoms reassembling cohesinopathies, which are a collection of diseases caused by dysfunctional cohesin proteins and a few other proteins (e.g. BRD4) [36].

Redefined neural crest lineages at single-cell resolution

NC differentiation is a dynamic process, in which NCCs and their progeny are highly heterogeneous in phenotypes, which are often averaged out in bulk sequencing data. High-throughput single-cell sequencing has boosted the systematic documentation of NC lineages and cellular phenotypes and renovated the definitions of cell populations and cell states.

To catalogue the NC derivatives in the head, researchers performed scRNA-Seq and snATAC-seq on the dissected zebrafish heads at embryonic, larval, juvenile and adult phases, in which the NCCs and their progeny have been marked by *sox10* expression at 10 hours post fertilisation (hpf) [37•]. The cell census identified gill derivatives, skeletal derivatives (mainly cartilage, bone, and teeth) and dermal fibroblasts, while the single-cell chromatin-accessibility analysis revealed a list of putative lineage-priming TFs. Another single-cell transcriptomic profiling of post-migratory cranial NCCs in 24-hpf zebrafish embryos characterised anterior arch, frontonasal, posterior-arch populations and melanocytes, identifying the frontonasal marker gene, *alx3*, as an essential factor for frontonasal NCC differentiation validated by mutant analyses [38]. Moreover, specific lineages from cranial NCCs have also been characterised by single-cell sequencing, including teeth development in mice [39] and NC derivatives from the first pharyngeal arch in the zebrafish [40].

Trunk NCCs and progeny have been profiled by scRNA-Seq at 24, 48 and 68 hpf in zebrafish, which documented trunk NC mesenchymal, neuronal, neural, glial, muscle, pigment, and otic cell lineages [41,42]. The single-cell profiling of the melanocyte lineage identified a postembryonic *tfap2b*-expressing melanocyte stem cell population in zebrafish, which could potentially represent cells of origin of melanoma [43].

Whilst the isolation of cranial or trunk NC derivatives can be achieved by dissection, it is challenging to profile the vagal NC differentiation. Recent work has characterised the cell-fate decisions of chicken vagal NC by transcriptomic and epigenomic analysis. The vagal NC, originally located in somites 1–7, can produce the predominant components of the enteric nervous system and derivatives in other organs. A vagal NC-specific enhancer, *EdnrB*-E2, was exploited to isolate vagal NCCs for scRNA-Seq, which identified three lineages, namely neural (*Tfap2a/b* coupled with *Sox10*), neuronal (*Sox2/3*) and mesenchymal (*Prrx1*, *Twist1* with other TF genes) [5]. Despite the capability to form all three lineages (neurogenic, neural, and mesenchymal) in most premigratory vagal NCCs, a small portion of vagal NCCs, marked by low *Sox10* and loss of *Foxd3* expression, were restricted to the neurogenic and mesenchymal fates before delamination [5]. Bulk ATAC-seq identified the set of noncoding, *cis*-regulatory elements specific to the vagal region.

Taken together, scRNA-Seq and snATAC-seq have been powerful tools to document cell states, profile developmental trajectories and identify lineage-specific regulatory elements. Yet, two major limitations of single-cell profiling of NCCs isolated based on the expression of lineage markers are the loss of spatial locations of profiled cells and the missing information of interactions between NCCs and neighbouring non-NC cells. The first can be overcome by integrative analysis of spatial gene expression and single-cell sequencing [32]. The second can be alleviated by performing systematic embryogenesis-level and tissue-specific profiling [11,44–46].

Environmental effects on migration and differentiation

It is crucial to study how NCC ontogeny interacts with other tissues and environments. Herein, we discuss latest progress on chemotaxis, durotaxis and co-development. Chemotaxis routes of the caudal hindbrain NC (also termed as 'vagal' NC) migration have been characterised by perturbation experiments and imaging analysis in the *Gallus gallus* [47]. The caudal hindbrain NC, situated from the mid-otic vesicle axial level to the level of the seventh somite, is conventionally partitioned into two compartments, the 'cardiac' NC (mid-otic vesicle to the third somite) and the 'posterior vagal' NC (somites 4–7). Nevertheless, the dichotomisation of cardiac and vagal NC is not accurate, as cardiac NCCs, despite generating parts in predominantly hearts but also other organs, can intermix with posterior vagal NCCs to populate the enteric nervous system [47]. Initially, the cardiac NCCs migrate towards the pharyngeal arches guided by the FGFR1–FGF8 signalling [48]. After migratory cardiac NCCs reach the perspective positions at pharyngeal arch arteries, they diverge towards the heart outflow tract or the gut guided by CXCR4–SDF1 and RET4–GDNF signalling, respectively, and differentiate in accordance with the local environmental cues [47]. The ligand GDNF emanates from the gut mesenchyme [49], in line with the finding that vagal NC-derived mesenchymal cells are indispensable for the formation of the enteric nervous system [5].

In *Xenopus laevis*, chemotaxis cooperates with durotaxis *in vivo* during cephalic NC migration [50••]. Durotaxis is a type of cell migration in which cells are guided by the stiffness gradient of their extracellular matrix. In this scenario, NCCs first migrate towards chemoattractant Sdf1-secreting placode [51], forming a durotactic gradient by interacting with the adjoining placode via N-cadherin, which in turn modulates NCC migration.

Studying co-development with human cells has been achieved in a self-assembling 3D tissue model, termed elongating multilineage-organised (EMLO) gastruloids [52]. The structure of EMLO gastruloids derived from the human-induced pluripotent stem cells mimics three germ layers and the NC. Using small-molecular inhibitors, this work demonstrated that in EMLO gastruloids, the self-organising primitive gut tube contributes to neuronal development. Moreover, this proof-of-concept study showed that NC development in EMLO gastruloids mimics the one *in vivo* [8••], suggesting opportunities to study interactions between NC development and other tissues [52].

Links between neural crest and neural crest-derived tumours

Neuroblastoma

Neuroblastoma is a paediatric malignancy that presumably originates from the trunk NC derivatives. Xenotransplantation of hPSC-derived NCCs into mouse embryos can give rise to tumours akin to human neuroblastoma [53]. The invasiveness signature derived from NCC migration is associated with neuroblastoma metastasis [46]. However, it has been challenging to pinpoint the exact cells of origin for neuroblastoma due to the dynamic status of NC development.

Extensive single-cell transcriptomic profiling of foetal adrenal glands has identified four major populations (Schwann cell precursors, bridge cells, chromaffin cells, and neuroblasts) and refined the typical developmental trajectories of NCCs in the adrenal medulla [54–56]. Despite the controversy in transitions among various cell states, it was concluded that human Schwann cell precursors could generate at least a proportion of intra-adrenal sympathetic neuroblasts and neurosecretory chromaffin cells.

Although there has been a debate of the closest approximation of neuroblastoma cells in normal development, plausibly caused by inconsistent cell-type annotations, evidence indicates that the neuroblastoma cell states are reminiscent of the normal cell phenotypes from Schwann cell precursor lineage trajectories [55,57••–59]. The latest scRNA-Seq analysis of both normal developmental tissues and tumours has reached a consensus that the expression profiles of neuroblastoma tumour cells resembled the neuroblast-derived transcriptional signature [57••]. The differentiation degree of tumour cells is associated with the clinical prognosis, that is, tumours enriched in more differentiated signatures tend to represent lower risk. Therefore, the transcription regulators (MYCN and TFAP2B) of repressive and permissive roles in differentiation might provide therapeutical values for neuroblastoma [57••]. Future work focussing on how the undifferentiated high-risk subtype is linked to genomic alterations and epigenetic reprogramming would lead to greater insight and potential therapeutics for this malignancy.

Melanoma

Cells of origin of melanoma are presumably the melanocyte stem cells from the NC-derived melanocyte lineage. Based on the transcriptomic study, a melanoma subtype is enriched in the NC-like signature among melanocytic, mesenchymal-like (or undifferentiated) and intermediate signatures [60]. Enhanced plasticity driven by overexpression of the chromatin-binding factor ATAD2 also facilitates the tumorigenesis competence of melanocytes, presumably by reprogramming the chromatin landscape [61••]. Oncogene-expressing melanocytes are reprogrammed into an NC-like progenitor state during the melanoma initiation in the zebrafish melanoma model, while such re-emergence of the NC-like progenitor state involved the activation of NC enhancers [62].

Several other studies have shown that developmental pathways, for example, a developmental LPAR1 axis *in vitro* and the AP-2α/EZH2 pathway, can promote melanoma metastasis and drug resistance [63,64]. Aside from the shared properties of plasticity and invasiveness between development and cancer, the Warburg effect, a metabolic effect of cancer hallmarks, promotes NC migration via the YAP–TEAD signalling [65]. The Warburg effect describes that cancer cells tend to metabolise glucose anaerobically even under aerobic conditions. Metabolic reprogramming should be taken into consideration in future research when studying the connections between NC development and cancer.

Challenges and future perspectives

Recent advances in epigenomic profiling techniques and single-cell sequencing have significantly improved our understanding of the plasticity of NCCs, including NC specification and lineage differentiation. NC development involves complex processes, requiring differential spatial and temporal regulation in the embryo spanning many developmental stages. We speculate that these emerging techniques, including single-cell and spatial multiomics, and integrative analysis of lineage tracing and single-cell sequencing, will enable fine-resolution characterisation and resolve how the cellular plasticity is regulated during NC development. Moreover, the 3D culture models will empower the profiling and perturbing experiments for studying human NC development, which can bridge the findings in model animals and the clinical translation. Apart from the normal development, genomic analyses have provided new opportunities to study developmental primordia and corresponding tumours in parallel to track down genes and *cis*-regulatory circuits critical for tumorigenesis and cancer progression.

Conflict of interest statement

The authors declare no competing interests.

Acknowledgements

Z.H. and T.S.-S. were supported by the Wellcome Trust (ref 215615/Z/19/Z).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Mills JC, Stanger BZ, Sander M: **Nomenclature for cellular plasticity: are the terms as plastic as the cells themselves?** *EMBO J* 2019, **38**:e103148.

2. Le Douarin NM, Creuzet S, Couly GR, Dupin E: **Neural crest cell plasticity and its limits**. *Development* 2004, **131**:4637-4650.

3. Olmsted ZT, Paluh JL: **Stem cell neurodevelopmental solutions for restorative treatments of the human trunk and spine**. *Front Cell Neurosci* 2021, **15**:667590.

4. Hockman D, Chong-Morrison V, Green SA, Gavriouchkina D, Candido-Ferreira I, Ling ITC, Williams RM, Amemiya CT, Smith JJ, Bronner ME, *et al.*: **A genome-wide assessment of the ancestral neural crest gene regulatory network**. *Nat Commun* 2019, **10**:4689.

5. Ling ITC, Sauka-Spengler T: **Early chromatin shaping predetermines multipotent vagal neural crest into neural, neuronal and mesenchymal lineages**. *Nat Cell Biol* 2019, **21**:1504-1517.

6. Lukoseviciute M, Gavriouchkina D, Williams RM, Hochgreb-Hagele T, Senanayake U, Chong-Morrison V, Thongjuea S, Repapi E, Mead A, Sauka-Spengler T: **From pioneer to repressor: bimodal foxd3 activity dynamically remodels neural crest regulatory landscape in vivo**. *Dev Cell* 2018, **47**:608-628.e606.

7. Williams RM, Candido-Ferreira I, Repapi E, Gavriouchkina D, Senanayake U, Ling ITC, Telenius J, Taylor S, Hughes J, Sauka-Spengler T: **Reconstruction of the global neural crest gene regulatory network in vivo**. *Dev Cell* 2019, **51**:255-276.e257.

8. •• Soldatov R, Kaucka M, Kastriti ME, Petersen J, Chontorotzea T, Englmaier L, Akkuratova N, Yang Y, Haring M, Dyachuk V, *et al.*: **Spatiotemporal structure of cell fate decisions in murine neural crest**. *Science* 2019, **364**:eaas9536.

This study characterised the trajectories of murine NC migration and differentiation and proposed a three-phase model of cell fate decisions involving coactivation, biasing and commitment.

9. •• Zalc A, Sinha R, Gulati GS, Wesche DJ, Daszczuk P, Swigut T, Weissman IL, Wysocka J: **Reactivation of the pluripotency program precedes formation of the cranial neural crest**. *Science* 2021, **371**:eabb4776.

This study demonstrates that the NC obtains cellular plasticity by the transient reactivation of pluripotency programs.

10. Minoux M, Holwerda S, Vitobello A, Kitazawa T, Kohler H, Stadler MB, Rijli FM: **Gene bivalency at Polycomb domains regulates cranial neural crest positional identity**. *Science* 2017, **355**:eaal2913.

11. Briggs JA, Weinreb C, Wagner DE, Megason S, Peshkin L, Kirschner MW, Klein AM: **The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution**. *Science* 2018, **360**:eaar5780.

12. Buitrago-Delgado E, Nordin K, Rao A, Geary L, LaBonne C: **Shared regulatory programs suggest retention of blastula-stage potential in neural crest cells**. *Science* 2015, **348**:1332-1335.

13. Scerbo P, Monsoro-Burq AH: **The vertebrate-specific VENTX/NANOG gene empowers neural crest with ectomesenchyme potential**. *Sci Adv* 2020, **6**:eaaz1469.

14. Picelli S, Björklund ÅK, Faridani OR, Sagasser S, Winberg G, Sandberg R: **Smart-seq2 for sensitive full-length transcriptome profiling in single cells**. *Nat Methods* 2013, **10**:1096-1098.

15. Kojima Y, Kaufman-Francis K, Studdert JB, Steiner KA, Power MD, Loebel DAF, Jones V, Hor A, de Alencastro G, Logan GJ, *et al.*: **The transcriptional and functional properties of mouse epiblast stem cells resemble the anterior primitive streak**. *Cell Stem Cell* 2014, **14**:107-120.

16. Martik ML, Gandhi S, Uy BR, Gillis JA, Green SA, Simoes-Costa M, Bronner ME: **Evolution of the new head by gradual acquisition of neural crest regulatory circuits**. *Nature* 2019, **574**:675-678.

17. Rothstein M, Simoes-Costa M: **Heterodimerization of TFAP2 pioneer factors drives epigenomic remodeling during neural crest specification**. *Genome Res* 2020, **30**:35-48.

18. Rada-Iglesias A, Bajpai R, Prescott S, Brugmann SA, Swigut T, Wysocka J: **Epigenomic annotation of enhancers predicts transcriptional regulators of human neural crest**. *Cell Stem Cell* 2012, **11**:633-648.

19. Hutchins EJ, Chacon J, Bronner ME: **RNA-Binding Protein Elavl1/HuR is Required for Maintenance of Cranial Neural Crest Specification**. Cold Spring Harbor Laboratory; 2020.

20. Skene PJ, Henikoff S: **An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites**. *eLife* 2017, **6**:e21856.

21. Azambuja AP, Simoes-Costa M: **The connectome of neural crest enhancers reveals regulatory features of signaling systems**. *Dev Cell* 2021, **56**:1268-1282.e1266.

22. Azambuja AP, Simoes-Costa M: **A regulatory sub-circuit downstream of Wnt signaling controls developmental transitions in neural crest formation**. *PLoS Genet* 2021, **17**:e1009296.

23. Cheung M, Chaboissier M-C, Mynett A, Hirst E, Schedl A, Briscoe J: **The transcriptional control of trunk neural crest induction, survival, and delamination**. *Dev Cell* 2005, **8**:179-192.

24. Lukoseviciute M, Mayes S, Sauka-Spengler T: **Neuromesodermal progenitor origin of trunk neural crest in vivo**. In *biorxiv*; 2021. https://doi.org/10.1101/2021.02.10.430513.

25. Frith TJ, Granata I, Wind M, Stout E, Thompson O, Neumann K, Stavish D, Heath PR, Ortmann D, Hackland JO, *et al.*: **Human axial progenitors generate trunk neural crest cells in vitro**. *eLife* 2018, **7**:e35786.

26. Cooper F, Gentsch GE, Mitter R, Bouissou C, Healy L, Rodriguez AH, Smith JC, Bernardo AS: **Rostrocaudal Patterning and Neural Crest Differentiation of Human Pre-neural Spinal Cord Progenitors In Vitro**. Cold Spring Harbor Laboratory; 2020.

27. Hackland JOS, Shelar PB, Sandhu N, Prasad MS, Charney RM, Gomez GA, Frith TJR, García-Castro MI: **FGF modulates the axial identity of trunk hPSC-derived neural crest but not the cranial-trunk decision**. *Stem Cell Rep* 2019, **12**:920-933.

28. Gomez GA, Prasad MS, Wong M, Charney RM, Shelar PB, Sandhu N, Hackland JOS, Hernandez JC, Leung AW, García-Castro MI: **WNT/β-CATENIN modulates the axial identity of ES derived human neural crest**. *Development* 2019, **146**:dev175604.

29. Tzouanacou E, Wegener A, Wymeersch FJ, Wilson V, Nicolas J-F: **Redefining the progression of lineage segregations during mammalian embryogenesis by clonal analysis**. *Dev Cell* 2009, **17**:365-376.

30. La Manno G, Soldatov R, Zeisel A, Braun E, Hochgerner H, Petukhov V, Lidschreiber K, Kastriti ME, Lönnerberg P, Furlan A, *et al.*: **RNA velocity of single cells**. *Nature* 2018, **560**:494-498.

31. Rocha M, Kushkowski E, Schnirman R, Booth C, Singh N, Beadell A, Prince VE: **Zebrafish Cdx4 regulates neural crest cell specification and migratory behaviors in the posterior body**. *Dev Biol* 2021, **480**:25-38.

32. Ke R, Mignardi M, Pacureanu A, Svedlund J, Botling J, Wählby C, Nilsson M: **In situ sequencing for RNA analysis in preserved tissue and cells**. *Nat Methods* 2013, **10**:857-860.

33. Sutton G, Kelsh RN, Scholpp S: **Review: the role of wnt/beta-catenin signalling in neural crest development in zebrafish**. *Front Cell Dev Biol* 2021, **9**:782445.

34. Kelsh RN, Camargo Sosa K, Farjami S, Makeev V, Dawes JHP, Rocco A: **Cyclical fate restriction: a new view of neural crest cell fate specification**. *Development* 2021, **148**:dev176057.

35. Lignell A, Kerosuo L, Streichan SJ, Cai L, Bronner ME: **Identification of a neural crest stem cell niche by spatial genomic analysis**. *Nat Commun* 2017, **8**:1830.

36. Linares-Saldana R, Kim W, Bolar NA, Zhang H, Koch-Bojalad BA, Yoon S, Shah PP, Karnay A, Park DS, Luppino JM, *et al.*: **BRD4 orchestrates genome folding to promote neural crest differentiation**. *Nat Genet* 2021, **53**:1480-1492.

37. • Fabian P, Tseng K-C, Thiruppathy M, Arata C, Chen H-J, Smeeton J, Nelson N, Crump JG: **Lifelong single-cell profiling of cranial neural crest diversification in zebrafish**. *Nat Commun* 2022, **13**:13.

The study profiled the single-cell atlas of cranial NC derivatives across the zebrafish lifetime and identified putative TFs that primed NC lineages.

38. Mitchell JM, Sucharov J, Pulvino AT, Brooks EP, Gillen AE, Nichols JT: **The alx3 gene shapes the zebrafish neurocranium by regulating frontonasal neural crest cell differentiation timing**. *Development* 2021, **148**:dev.197483.

39. Krivanek J, Soldatov RA, Kastriti ME, Chontorotzea T, Herdina AN, Petersen J, Szarowska B, Landova M, Matejova VK, Holla LI, *et al.*: **Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth**. *Nat Commun* 2020, **11**:4816.

40. Tatarakis D, Cang Z, Wu X, Sharma PP, Karikomi M, Maclean AL, Nie Q, Schilling TF: **Single-cell transcriptomic analysis of zebrafish cranial neural crest reveals spatiotemporal regulation of lineage decisions during development**. *Cell Rep* 2021, **37**:110140.

41. Lencer E, Prekeris R, Artinger KB: **Single-cell RNA analysis identifies pre-migratory neural crest cells expressing markers of differentiated derivatives**. *eLife* 2021, **10**:e66078.

42. Howard AG, Baker PA, Ibarra-Garcia-Padilla R, Moore JA, Rivas LJ, Tallman JJ, Singleton EW, Westheimer JL, Corteguera JA, Uribe RA: **An atlas of neural crest lineages along the posterior developing zebrafish at single-cell resolution**. *eLife* 2021, **10**:e60005.

43. Brombin A, Simpson DJ, Travnickova J, Brunsdon H, Zeng Z, Lu Y, Young AIJ, Chandra T, Patton EE: **Tfap2b specifies an embryonic melanocyte stem cell that retains adult multifate potential**. *Cell Rep* 2022, **38**:110234.

44. Farrell JA, Wang Y, Riesenfeld SJ, Shekhar K, Regev A, Schier AF: **Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis**. *Science* 2018, **360**:eaar3131.

45. Tambalo M, Mitter R, Wilkinson DG: **A single cell transcriptome atlas of the developing zebrafish hindbrain**. *Development* 2020, **147**:dev184143.

46. Morrison JA, McLennan R, Teddy JM, Scott AR, Kasemeier-Kulesa JC, Gogol MM, Kulesa PM: **Single-cell reconstruction with spatial context of migrating neural crest cells and their microenvironments during vertebrate head and neck formation**. *Development* 2021, **148**:dev199468.

47. Tang W, Li Y, Li A, Bronner ME: **Clonal analysis and dynamic imaging identify multipotency of individual *Gallus gallus* caudal hindbrain neural crest cells toward cardiac and enteric fates**. *Nat Commun* 2021, **12**:1894.

48. Sato A, Scholl AM, Kuhn EB, Stadt HA, Decker JR, Pegram K, Hutson MR, Kirby ML: **FGF8 signaling is chemotactic for cardiac neural crest cells**. *Dev Biol* 2011, **354**:18-30.

49. Natarajan D, Marcos-Gutierrez C, Pachnis V, De, Graaff E: **Requirement of signalling by receptor tyrosine kinase RET for the directed migration of enteric nervous system progenitor cells during mammalian embryogenesis**. *Development* 2002, **129**:5151-5160.

50. •• Shellard A, Mayor R: **Collective durotaxis along a self-generated stiffness gradient in vivo**. *Nature* 2021, **600**:690-694.
This study demonstrates that the durotaxis process along the stiffness gradient occurs *in vivo* during the cephalic NC migration in Xenopus laevis and characterised the synergy between chemotaxis and durotaxis.

51. Theveneau E, Steventon B, Scarpa E, Garcia S, Trepat X, Streit A, Mayor R: **Chase-and-run between adjacent cell populations promotes directional collective migration**. *Nat Cell Biol* 2013, **15**:763-772.

52. Olmsted ZT, Paluh JL: **Co-development of central and peripheral neurons with trunk mesendoderm in human elongating multi-lineage organized gastruloids**. *Nat Commun* 2021, **12**:3020.

53. Cohen MA, Zhang S, Sengupta S, Ma H, Bell GW, Horton B, Sharma B, George RE, Spranger S, Jaenisch R: **Formation of human neuroblastoma in mouse-human neural crest chimeras**. *Cell Stem Cell* 2020, **26**:579-592.e576.

54. Olsen TK, Otte J, Mei S, Kameneva P, Björklund Å, Kryukov E, Hou Z, Johansson A, Sundström E, Martinsson T, *et al.*: **Malignant Schwann Cell Precursors Mediate Intratumoral Plasticity in Human Neuroblastoma**. Cold Spring Harbor Laboratory; 2020.

55. Dong R, Yang R, Zhan Y, Lai H-D, Ye C-J, Yao X-Y, Luo W-Q, Cheng X-M, Miao J-J, Wang J-F, *et al.*: **Single-cell characterization of malignant phenotypes and developmental trajectories of adrenal neuroblastoma**. *Cancer Cell* 2020, **38**:716-733.e716.

56. Hanemaaijer ES, Margaritis T, Sanders K, Bos FL, Candelli T, Al-Saati H, Van Noesel MM, Meyer-Wentrup FAG, Van De Wetering M, Holstege FCP, *et al.*: **Single-cell atlas of developing murine adrenal gland reveals relation of Schwann cell precursor signature to neuroblastoma phenotype**. *Proc Natl Acad Sci USA* 2021, **118**:e2022350118.

57. •• Jansky S, Sharma AK, Körber V, Quintero A, Toprak UH, Wecht EM, Gartlgruber M, Greco A, Chomsky E, Grünewald TGP, *et al.*: **Single-cell transcriptomic analyses provide insights into the developmental origins of neuroblastoma**. *Nat Genet* 2021, **53**:683-693.
This study characterised the single-cell transcriptomes of neuroblastomas and normal human developing adrenal glands and revealed that when tumour cells are projected onto the differentiation trajectory of normal cells of origin, less differentiated neuroblastoma is associated with poor prognosis.

58. •• Kameneva P, Artemov AV, Kastriti ME, Faure L, Olsen TK, Otte J, Erickson A, Semsch B, Andersson ER, Ratz M, *et al.*: **Single-cell transcriptomics of human embryos identifies multiple sympathoblast lineages with potential implications for neuroblastoma origin**. *Nat Genet* 2021, **53**:694-706.
This study refined the models for cellular type transitions in the developing human and mouse sympathoadrenal systems by integrative analysis of scRNA-Seq and lineage tracing.

59. Kildisiute G, Kholosy WM, Young MD, Roberts K, Elmentaite R, Van Hooff SR, Pacyna CN, Khabirova E, Piapi A, Thevanesan C, *et al.*: **Tumor to normal single-cell mRNA comparisons reveal a pan-neuroblastoma cancer cell**. *Sci Adv* 2021, **7**:eabd3311.

60. Wouters J, Kalender-Atak Z, Minnoye L, Spanier KI, De Waegeneer M, Bravo González-Blas C, Mauduit D, Davie K, Hulselmans G, Najem A, *et al.*: **Robust gene expression programs underlie recurrent cell states and phenotype switching in melanoma**. *Nat Cell Biol* 2020, **22**:986-998.

61. •• Baggiolini A, Callahan SJ, Montal E, Weiss JM, Trieu T, Tagore MM, Tischfield SE, Walsh RM, Suresh S, Fan Y, *et al.*: **Developmental chromatin programs determine oncogenic competence in melanoma**. *Science* 2021, **373**:eabc1048.
This study shows that under the condition of p53 knockout and BRAF mutations, mature melanocytes, compared with NCCs and melanoblasts, were less likely to develop malignant cells in zebrafish and hPSC-derived cancer models and that the tendency of NCCs and melanoblasts towards tumorigenesis was attributed to the higher plasticity shaped by chromatin landscapes. The authors identified the chromatin-binding factor ATAD2 that was upregulated in melanoblasts compared to melanocytes and amplified or highly expressed in bulk melanomas. Over-expression of atad2 endowed p53-knockout BRAF-mutated melanocytes with tumorigenesis competency presumably by reprogramming the chromatin landscape, providing insights into melanoma initiation.

62. Kaufman CK, Mosimann C, Fan ZP, Yang S, Thomas AJ, Ablain J, Tan JL, Fogley RD, van Rooijen E, Hagedorn EJ, *et al.*: **A zebrafish melanoma model reveals emergence of neural crest identity during melanoma initiation**. *Science* 2016, **351**:aad2197.

63. Liu J, Rebecca VW, Kossenkov AV, Connelly T, Liu Q, Gutierrez A, Xiao M, Li L, Zhang G, Samarkina A, *et al.*: **Neural crest-like stem cell transcriptome analysis identifies LPAR1 in melanoma progression and therapy resistance**. *Cancer Res* 2021, **81**:5230-5241.

64. White JR, Thompson DT, Koch KE, Kiriazov BS, Beck AC, Van Der Heide DM, Grimm BG, Kulak MV, Weigel RJ: **AP-2α–mediated activation of E2F and EZH2 drives melanoma metastasis**. *Cancer Res* 2021, **81**:4455-4470.

65. Bhattacharya D, Azambuja AP, Simoes-Costa M: **Metabolic reprogramming promotes neural crest migration via yap/tead signaling**. *Dev Cell* 2020, **53**:199-211.e196.