Chapter 7
Neural Crest Stem Cell Cultures: Establishment, Characterization and Potential Use

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Abstract The neural crest (NC) is a remarkable structure of vertebrate embryos that continues to fascinate biologists due to its importance in development, evolution and disease. NC cells emerge in the neurulating embryo at the closing borders of the neural plate and, after a phase of extensive migration, settle in different parts of the body and contribute to the formation of a diversity of tissues and organs. Because of their broad potential, NC cells constitute an attractive system to investigate fundamental developmental processes, such as cell commitment and lineage diversification. Moreover, recent evidence for the persistence of multipotent NC-like stem cell in several adult locations opens up opportunities for use of human NC stem cells as a source of material for cell-based transplantation therapies. In this chapter, we describe procedures for the isolation and characterization of NC cells from mouse and avian embryos, as well as adult NC-derived stem cells from the mouse hair follicle. These cultures constitute an accessible *in vitro* system for modeling NC stem cell morphogenesis and have been extensively used to study intrinsic and environmental cues that regulate migration, self-renewal and fate choice.

Keywords Neural crest • NC-like stem cell • Transplantation • Therapies

7.1 Introduction

The neural crest (NC) corresponds to a transient, multi-fated embryonic cell population that emerges at the dorsal margins of the neural folds during development of vertebrates. Upon closure of the neural tube, NC cells undergo epithelial-to-mesenchymal

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transition and, after a phase of extensive migration, become widely distributed within the embryo (Le Douarin and Kalcheim 1999).

The NC is remarkably multipotent giving rise to most of the peripheral nervous system and to a large variety of non-neural cell types, including pigmented cells of the skin, smooth muscle cells of the cardiovascular system, and craniofacial bones, cartilage and connective tissues (Baggiolini et al. 2015; Dupin and Sommer 2012; Le Douarin and Kalcheim 1999). Despite progressive restriction of developmental potential with time, some NC cells are capable of self-renewal—thereby fulfilling the stem cell criteria—and display a developmental repertoire only surpassed by embryonic stem (ES) cells (Baggiolini et al. 2015; Calloni et al. 2007; Douarin and Dupin 2003; Trentin et al. 2004).

Interestingly, NC stem cells are not only present in the developing embryo, but also in various NC-derived tissues in the adult body including gut, cornea, periodontal ligament and the skin (Coura et al. 2008; Fernandes et al. 2006; Kruger et al. 2002; Sieber-Blum et al. 2004; Yoshida et al. 2006). These persistent post-migratory NC stem cells molecularly resemble their embryonic counterparts and also have the ability to differentiate into a wide variety of cell types (reviewed in Dupin and Sommer 2012; Shakhova et al. 2010; Trentin and Calloni 2013).

Because of their broad differential potential, both embryonic NC and adult NC-derived stem cells (NCSCs) represent a valuable model system to study fundamental stem cell and developmental biology. Furthermore, their multilineage potential and easy accessibility in adult tissues open up opportunities for use of human NCSCs as a source of material for pharmaceutical screening, cell-based transplantation therapies and tissue engineering (Sieber-Blum 2014).

In this chapter, we describe protocols and troubleshooting advices for isolating and characterizing NC cells from mouse and avian embryos, as well as adult NCSCs from the mouse hair follicle. The technique involves microdissection of neural tube segments or mouse whisker hair follicles, followed by periods of primary culture in which NC cells migrate onto the culture dish surface. In a second step, tissue explants are removed and isolated NC cells passaged and grown as adherent monolayers. The cells can be further induced to differentiate into specific phenotypes upon appropriated culture conditions and are amenable to chemical screenings and population-based analysis of gene expression. These cultures have been extensively used to model NC morphogenesis and represent an accessible *in vitro* system to investigate cell intrinsic and environmental cues that regulate NC cell migration, self-renewal, lineage commitment and differentiation.

7.2 Embryonic NC Cultures

7.2.1 Reagents

- Gestating mice or fertilized and incubated chicken/quail eggs.
- Alfa-Modified Eagle's Medium (αMEM, Gibco, Cat. No. 11.900-024).

- Phosphate buffer saline (PBS) without Ca^{2+} and Mg^{2+}.
- Pancreatin solution at 6 mg/ml prepared in PBS (Sigma-Aldrich, Cat. No. P3292).
- Fetal calf serum (FCS, e.g. Vitrocell).
- Penicillin-streptomycin solution at 10,000 μ/ml (Invitrogen, Cat. No. 15140148).
- Hydrocortisone (Sigma-Aldrich, Cat. No. H0135).
- Transferrin (Sigma-Aldrich, Cat. No. T5391).
- 3,3,5-Thio-iodo-thyronine (T3, Sigma-Aldrich, Cat. No. T5516).
- Glucagon (Sigma-Aldrich, Cat. No. G3157).
- Epidermal growth factor (EGF, Invitrogen, Cat. No. PHG0314).
- Fibroblast growth factor 2 (FGF2, Invitrogen, Cat. No. PHG0024).
- Insulin (Sigma-Aldrich, Cat. No. I6634).
- Trypsin (0.25 %, wt/vol) 1 mM EDTA in PBS (Invitrogen, Cat. No. 25200-056).
- Rat-tail Collagen I (e.g. Corning, Cat. No. 354236).

7.2.2 Equipment

- Stereomicroscope (e.g., Leica MZ 7.5; Olympus SZ or equivalent).
- Swan-neck fiber optic illumination system.
- Sterile 35 and 100 mm tissue culture dishes.
- 4″ Curved scissor (Fig. 7.1).
- Vannas or Pascheff-Wolff spring scissors (Fig. 7.1).
- Pair of dissecting forceps (e.g. Dumont no. 5) (Fig. 7.1).
- Stainless steel holder with tungsten dissecting needle (Fig. 7.1).
- Perforated spoon (Fig. 7.1).
- Glass Pasteur pipettes.
- Sterile tissue culture hoods (stereomicroscope should be fit in).
- Tissue culture CO_2 incubator.
- Neubauer chamber.
- Inverted phase contrast microscope (e.g., Olympus CK2).

7.2.3 Reagent and Equipment Setup

1. **Embryo preparation**. For mouse NC cell cultures, gestating mice should be plugged 8–10 days prior to the procedure. For avian cultures, freshly laid quail eggs should be incubated at 38 °C with 60 % humidity for 26–55 h. Table 7.1 lists the optimal developmental stages for the preparation of avian and murine NC cell cultures.
2. **Equipment sterilization**. Bake microdissection tools and glass Pasteur pipettes in a dry oven at 150 °C for 2 h. Rinse stereomicroscope and illumination system with

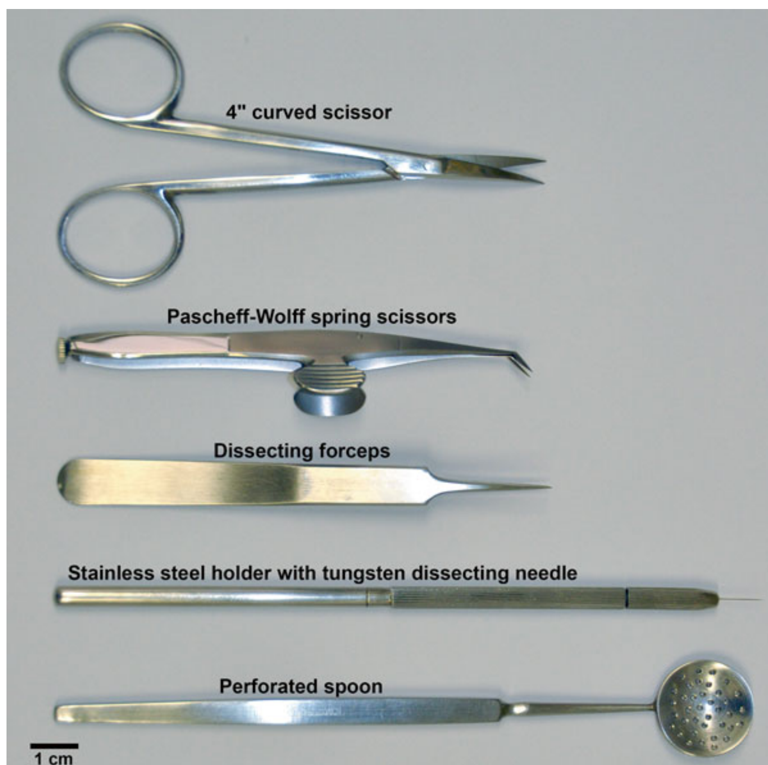

Fig. 7.1 Microsurgery tools required for the protocol. Ideally, instruments should be made of stainless steel and sterilized by dry heat for 2 h at 150 °C before the procedures

Table 7.1 Developmental stages of mouse and quail embryos for NC cell cultures

	Mouse	Quail
Cephalic NC	8.0–8.5 gestation days	26–30 h[a] (5–7 somites)
Trunk NC	9.0–10.0 gestation days	36–46 h[a] (16–26 somites)

Embryonic ages may vary slightly according to the researcher objectives
[a]Incubation time of quail eggs at 38 °C in 60 % humidity

70 % ethanol and fit inside the tissue culture hood. UV-C germicidal light can be used to further sterilize the equipment. Importantly, use sterile technique at all times.

3. **Media preparation.** Before the dissection procedure, prepare the complete NC cell culture medium by combining 9 ml α-MEM, 1 ml FCS and 200 μl chicken embryo extract. Filter the medium through a 0.22 μm filter and add the following components: 100 ng/ml hydrocortisone, 10 μg/ml transferrin, 1 ng/ml insulin, 400 pg/ml T3, 10 pg/ml glucagon, 100 pg/ml EGF, 200 pg/ml FGF2, 200 U/ml penicillin and 10 μg/ml streptomycin. For wash medium, combine 9 ml α-MEM with 1 ml FCS. Media can be stored for up to 2 weeks at 4 °C.
4. **Chicken embryo extract.** Incubate fertilized white chicken eggs for 11–14 days at 38 °C with 60 % humidity. Clean the shell eggs with 70 % ethanol. Break the eggshells and place embryos on 100 mm dishes with ice-cold PBS. Remove the

eyes with the aid of scissors and macerate embryos by passing them through a 50 ml syringe. Transfer material directly to a 50 ml sterile centrifuge tube (use around 10 embryos per tube). Add equal volume of α-MEM medium to the tube. Pass the entire tube contents again through the 50 ml syringe. Centrifuge at 2000 × *g* for 10 min at 4 °C. Filter the supernatant with 0.8 and 0.22 μm strainers sequentially. Aliquot and store at −20 °C.

5. **Culture dish coating with rat-tail collagen I**. Start by diluting cold solubilized collagen with sterile 17 μM acetic acid to 60 mg/ml. This can be stored at 4 °C for 1 week. Cover the bottom of the 35 mm tissue culture dishes with this solution and let sit for 2 h. Make sure the entire surface is covered. Remove the solution and allow plates to dry at room temperature. Wash once with PBS.

7.2.4 Procedure

7.2.4.1 Embryo Isolation

The first step of the protocol consists of isolating embryos from extra-embryonic tissues and other contaminants.

***Note:** Several embryos can be isolated and stored in ice-cold PBS for up to 2 h with no adverse effects before the fine dissection.*

Mouse Embryos

1. Sacrifice mice by cervical dislocation, spray with 70 % ethanol and, with fine scissors, open the peritoneal cavity. Remove both uteri of the animal and place them into a 100 mm Petri dish with ice-cold PBS;
2. Under the stereomicroscope, remove embryos from uterus with blunt forceps and scissors. Hold firmly between individual decidua against the bottom of dish with the forceps, and gently tease uterine tissue until embryo pops out. There are usually 2–4 embryos per uterus in mice;
3. Using a perforated spoon, transfer embryos to a fresh 100 mm dish with PBS. Remove decidua, amniotic and yolk sac membranes with the forceps and dissecting needles. Transfer embryos to a new dish with PBS for the dissection of neural tube.

Quail Embryos

1. Wipe eggs with 70 % ethanol and cut a small hole in the blunt end of eggshell with dissecting scissors;
2. Transfer carefully the content to a 100 mm dish with PBS. Grasp extra-embryonic membranes with forceps. Cut entirely around its perimeter with the Vannas or Pascheff-Wolff spring scissors;
3. Transfer the embryos to a fresh dish with ice-cold PBS. Use only embryos of age-appropriate stage and normal morphology.

7.2.4.2 Neural Tube Isolation

This step consists of isolating the neural tube from surrounding tissues according to specific NC region of interest (Fig. 7.2). During this step, we recommend to place the dishes containing the embryos on a black background under a stereomicroscope. Use the swan-neck fiber optic illumination system to assist the visualization.

Note: *Several isolated neural tube explants can be stored in washing medium on ice for up to 1 h with no adverse effects.*

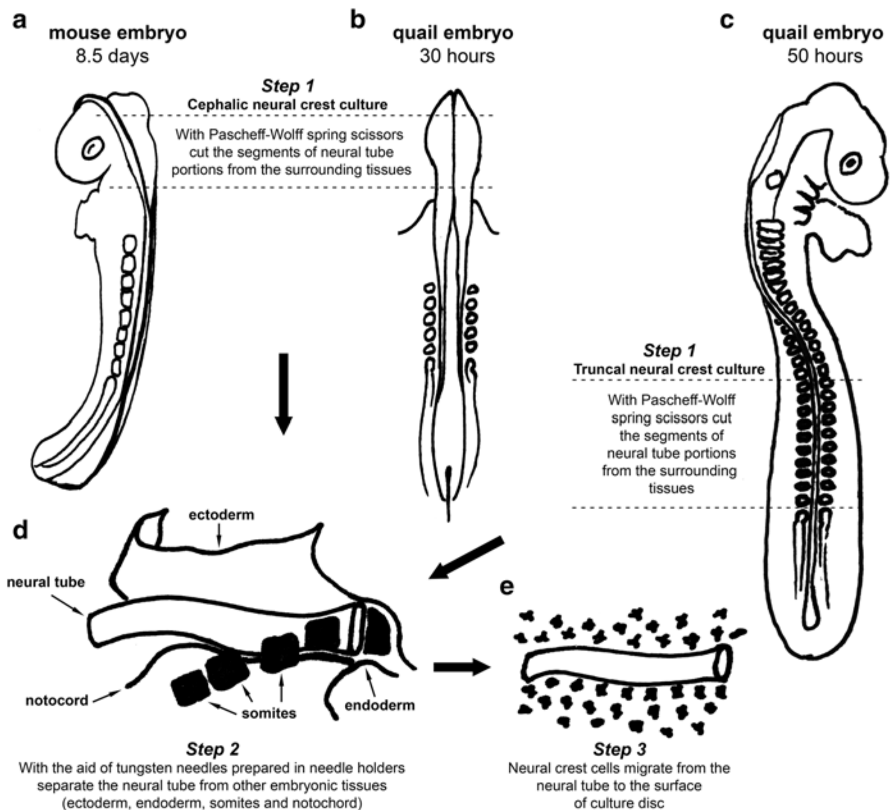

Fig. 7.2 General scheme for embryonic NC cultures. Step 1 for mouse (**a**) and quail (**b**) cephalic NC cultures: cut the anterior region of embryos (*dashed lines* in **a** and **b**) as close as possible of neural tube. Step 2 for quail trunk NC culture: (**c**) cut the truncal region of embryo corresponding to the last 10 somites. Observe the appropriated developmental stage of embryos. Step 2 for mouse trunk NC culture use the same step shown in (**c**) (very similar in quail and mouse at this age). Step 2 for both cephalic and trunk NC cultures: incubate isolated sections in pancreatin solution for 15 min. Subsequently separate neural tube d from other embryonic tissues with aid of tungsten needles (**d**). Step 3: transfer isolated neural tube to culture dish in the desired culture condition and incubate at 37 °C for 24 h in humidified atmosphere of 5 % CO_2 and 10 % O_2 (**e**). NC cells migrate out of neural tubes toward the surface of culture recipient

1. Using Vannas or Pascheff-Wolff spring scissors, make two transversal cuts at the desired levels of neural tube (Fig. 7.2);
2. With a glass pipette, transfer tissue segments to a 35 mm dish containing pancreatin solution and incubate at room temperature for 15 min;
   Note: *Exact time length is empirical and can vary with embryo stage and specific activity of pancreatin. Explants should be removed from the pancreatin solution when the explants become slightly wavy and somites can be separated intact from the ectoderm and endoderm upon gentle agitation with the forceps.*
3. Transfer the tissue segments to a new 35 mm dish containing washing medium. With dissecting needles, gently remove the ectoderm, endoderm, somites and notochord away from the neural tube. Be careful not to damage the neural tube.
   Note: *Neural tube usually bends slightly with dorsal side convex, which helps to identify dorso-ventral polarity.*

7.2.4.3 Primary NC Cell Culture (Migration Phase)

1. Carefully transfer neural tube explants with the glass Pasteur pipette to a 35 mm culture dish containing approximately 800 μl of NC complete medium. For best results, pre-coat culture dish with rat-tail collagen I;
2. Incubate at 37 °C in humidified atmosphere of 5 % CO_2 and 10 % O_2. Culture time may vary from 18 to 48 h according to the experimental design;

Note*: Medium volume is critical. Use just enough so that the neural tubes explants are not exposed. Minimal medium volume increases neural tube contact with culture dish surface facilitating attachment and NC cell emigration.*

7.2.4.4 Secondary NC Cell Cultures (Differentiation Phase)

After NC cells have migrated out of the neural tube explants, secondary cultures can be prepared by dislodging and replating emigrated NC cells at sparse density. At this point, NC cells can also be replated at single cell density, therefore making it possible to perform clonal analysis.

1. Under a stereomicroscope carefully remove neural tubes out of the culture dish surface. Use tungsten needles or aforceps to scrape away explanted neural tubes and neuroepithelial tissue;
2. Wash 3 times with washing medium to remove residual tissue fragments;
3. Incubate with trypsin/EDTA solution at 37 °C for 5 min. Control the reaction in an inverted phase contract microscope;
4. Block trypsin reaction with wash medium. Transfer cells to a 15 ml centrifuge tube and spin at room temperature at $1000 \times g$ for 5 min.

5. Ressuspend the pelleted cells in NC complete medium and count them in a Neubauer chamber. Plate cells into a fresh culture dish. Cell density and media composition may vary according to the experimental design.
6. Incubate at 37 °C in humidified atmosphere of 5 % CO_2 and 10 % O_2. NC cells may be maintained until 15 days under these conditions.

Note: *Secondary cultures may not be required depending on the experimental design. Subculturing, however, allows cell expansion, which is particularly important for experiments that require larger amounts of cells (such as analysis of gene and protein expression). Secondary cultures are also recommended for cell differentiation experiments since it provides a more homogenous cell population. In addition, NC cells can also be plated at single-cell density for clonal analysis (for more details refer to Bittencourt et al.* 2013*; Trentin et al.* 2004*; Calloni et al.* 2007*). For these type of cultures, make sure a single cell solution has been obtained. This can be accomplished by serial of dilution in 96-well plates. Use the inverted phase-contrast microscope to verify the presence of single cells per well.*

7.3 Epidermal Neural Crest Stem Cell (Epi-NCSC) Cultures

Here, we also provide a detailed protocol for preparing mouse EPI-NCSCs cultures from whisker hair follicles, which can also be adapted for isolation of human EPI-NCSCs of scalp hair follicles (Sieber-Blum et al. 2004). Reagents and equipment necessary for this protocol are the same listed above for embryonic NC cell cultures.

7.3.1 Isolation of the Bulge Area of Mouse Whisker Hair Follicles

1. Sacrifice animals by cervical dislocation. We normally use 8- to 12-weeks-old B57BL/6 mice. Other mouse strains can also be used;
2. Rinse facial region with 70 % ethanol;
3. Remove the whisker pads using forceps and dissecting scissors. Avoid cutting into the hair bulbs. Place the isolated pads on ice-cold PBS;
4. Under a stereomicroscope placed into the culture hood, dissect individual whisker follicles. Hold skin next to the follicles with a forceps and lift them out of the whisker pad with another forceps. Poll individual hair follicles in a new dish with fresh PBS;
5. Using Vannas or Pascheff-Wolff spring scissors, make a transverse cut to remove the dermal papilla/bulb of the follicles. Then make a second transverse cut right below the sebaceous gland. The isolated segment corresponds to the bulge area of whisker hair follicle, which is surrounded by a collagen capsule;

6. Grab an end of the capsule with the forceps and roll out the bulge with a bent dissecting needle. You would now be able to see the empty capsule and the isolated bulge;
7. Pool the isolated bulges in a new culture dish with α-MEM. These can be kept on ice for 2 h with no adverse effects.

7.3.2 Culture of Bulge Explants

1. Pre-incubate rat-tail collagen I-coated 35 mm dishes with 500 μl of culture medium in CO_2 incubator for approximately 1 h. Medium consists of 85 % α-MEM and 15 % FCS;
2. Plate 10–15 bulge explants per 35 mm dish. Alternatively, bulges can be plated individually into 24-well plates. Incubate for 2 h at 37 °C in humidified atmosphere of 5 % CO_2 and 10 % O_2. Explants should adhere to the collagen substratum;
3. Gently add 1 ml of fresh culture medium. After that, replace 50 % of the medium every 2–3 days;
4. Within 4–7 days, migratory cells will start emigrating from the bulge explants. These cells display spindle or stellate morphology and lack close cell-to-cell contact. Over the next few days, more cells will migrate out the explants and start to proliferate rapidly;

   Note: *Rare cells with flattened or cobblestone-like morphology might also emerge in some cultures. These cells correspond to putative epidermal stem/progenitor cells and/or follicular keratinocytes. Cultures mixed with these cells should be discarded.*
5. Remove the bulge explants 3–5 days after the onset of cell migration. Cells can be analyzed at this time point by different assays according to the research goal. Avoid keeping the cells for too long in primary cultures, as they tend to differentiate or undergo apoptosis at high cell density. Alternatively, cells can be harvested by tripsinization, replated and further grown as homogenous monolayers. For passaging the cells, we recommend a density of at least 50,000 cells/25 cm^2 flask.

7.4 Maintenance

Once migrated away from the neural tube explants, embryonic NC cells tend to differentiate quickly. Long-term NC cultures may therefore be challenging, as more specific culture conditions may be required for survival and self-renewal of the cells. Moreover, NC cells maintained in culture for longer periods may not faithfully represent their *in vivo* counterparts; therefore, extensive passaging should be avoided. Using the standard conditions described above, we recommend keeping

NC cells for not more than 15 days in secondary cultures. Media should be refreshed every 2–3 days. To avoid spontaneous differentiation and arrest of proliferation, cells must not be more than 70 % confluent.

7.5 Characterization

Embryonic NC cell cultures can be easily characterized by immunostaining for specific cell markers. Conventionally, the carbohydrate epitope HNK-1 is used to identify avian and human NC cells (Bronner-Fraser 1986; Coura et al. 2008; Etchevers 2011) (Fig. 7.3). In mice, the neurotrophin receptor p75 (p75NTR) and the intermediate filament protein nestin are used as general NC markers (Morrison et al. 1999). The transcription factor Sox10, which is required for early development of NC and play different roles throughout its development, is another commonly used NC marker (Sauka-Spengler and Bronner-Fraser 2008). Other transcription factors related to early events of NC morphogenesis include Sox9, Twist, Slug, Snail, FoxD3 and Pax3 (Sauka-Spengler and Bronner-Fraser 2008). The expression of these markers can be identified in NC cultures at the RNA level by reverse transcription (RT)-PCR or at protein level by immunostaining with specific antibodies. Similarly, to embryonic NC

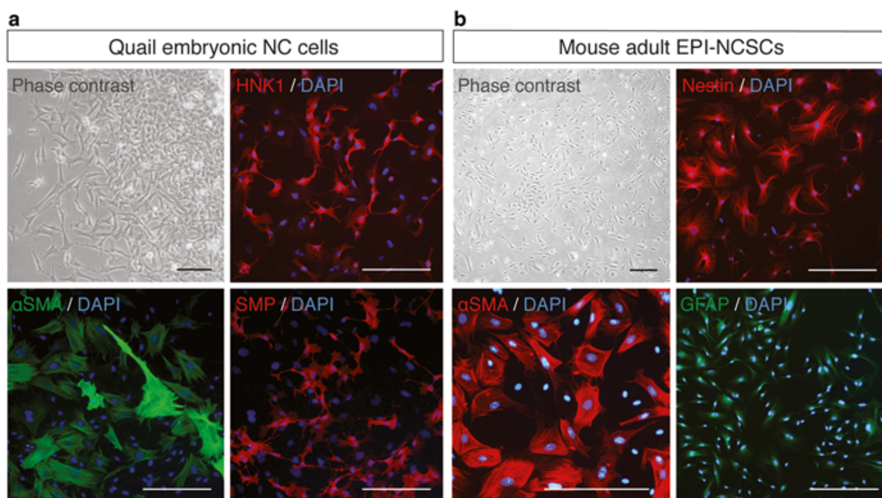

Fig. 7.3 Characterization of NC cell cultures. (**a**) Quail embryonic NC cells and (**b**) adult mouse EPI-NCSCs. Top panel shows phase contrast images of NC cells after emigration from the tissue explants and staining for the common undifferentiated NC markers HNK-1 (quail) and Nestin (mouse). Bottom images show differentiated NC-derived cell types in secondary cultures. Smooth muscle cells are identified by staining for alpha smooth muscle actin (αSMA) in both avian and murine cultures. Glial cells are identified by staining for Schwann myelin protein (SMP) and glial fibrillary acidic protein (GFAP) in avian and murine cultures, respectively. Cell nuclei are stained with DAPI. Scale bar: 200 μm

cells, EPI-NCSC cultures can be characterized by the expression of Nestin (Fig. 7.3), p75NTR, Sox10 and other transcription factors involved in NC determination and migration *in vivo* (Bressan et al. 2014; Sieber-Blum et al. 2004). We recommend the following primary antibodies and dilutions for the characterization of NC cell cultures: HNK-1 (1:50, DHSB, Cat. No. 3H5), p75NTR (1:200, Millipore, Cat. No. Ab1554), Nestin (1:250, Abcam, Cat. No. Ab92391) and Sox10 (1:100, Abcam, Cat. No. Ab27655).

In addition to marker expression, NC cells can be further characterized by their differentiation potential *in vitro*. As the NC originates a wide range of differentiated cell types (e.g. neurons, glial cells, melanocytes, smooth muscle cells, bone, cartilage and adipose tissue), expression of differentiated cell markers are used for functional characterization of NC cultures (Calloni et al. 2007, 2009; Mayor and Theveneau 2013; Trentin et al. 2004) (Fig. 7.3). Furthermore, single cell cultures can be used to determine the differentiation potential and self-renewal capacity of NC cell clones (for further details see Trentin et al. 2004; Bittencourt et al. 2013). In some cases, differentiation into NC-derived cell types may require more specific culture conditions, such as inductive media and culture substratum.

Functionally, NC cells can be also characterized by their migratory behavior *in vitro*. Cultured NC cells display high motility, which is a reminiscent of their migratory ability *in vivo*. Various *in vitro* assays can be used to assess NC cell migration, including time-lapse cell tracking, transwell migration assay or simple measurement of the area occupied by NC cells after emigration from the NT explants in primary cultures (Costa-Silva et al. 2009). A comprehensive review on cell migration assay is provided by (Kramer et al. 2013).

7.6 Potential Use of NC Stem Cell Cultures

The NC cultures described in this chapter have been extensively used as model of NC morphogenesis *in vitro*. The relative ease and high fidelity manipulation render NC cultures an excellent *in vitro* system to study cellular and molecular mechanisms underlying epithelium-to-mesenchyme transition, cell migration, lineage commitment and differentiation (Dupin and Sommer 2012; Trentin and Calloni 2013).

NC cell behavior is highly influenced by environmental signals that these cells encounter along their migratory pathways during development (Le Douarin and Kalcheim 1999). We and others have employed the NC culture system to investigate the effects of environmental cues such as growth factors and extracellular matrix components on NC cell migration, proliferation and differentiation (Bittencourt et al. 2013; Costa-Silva et al. 2009; Garcez et al. 2008; Ito and Morita 1995; Kleber et al. 2005; Lahav et al. 1996).

In addition to the population-based analysis, clonal NC cultures can be used to assess the whole spectrum of developmental potential of single NC cells. By the use of this technique, the presence of several types of multipotent progenitor and stem

cells has been reported in different regions of the avian NC (reviewed by Dupin et al. 2010; Dupin and Sommer 2012). The *in vitro* clonal approach also permits to challenge NC cell differentiation options in order to characterize the influences of extracellular factors on the behavior of specific sets of NC progenitors (Trentin and Calloni 2013). For instance, the influence of soluble factors such as endothelin-3, sonic hedgehog and FGF2 on self-renewal and fate choice have been investigated by series of cloning and subcloning of avian NC cells (Bittencourt et al. 2013; Trentin et al. 2004; Calloni et al. 2007).

Despite its importance to developmental biology, studying the NC is also relevant for the understanding of a diverse range of human pathologies, which are known to be associated with abnormal NC development. Collectively referred as neurocristopathies, these conditions include multiple neoplasia, skin pigmentation disorders and various craniofacial and cardiovascular malformations (Hall 2010). Embryonic NC cell cultures, thus, also represent an accessible system to investigate the etiopathogeny of NC-related human disorders. For instance, mouse and chicken primary NC cultures have been used to study cellular mechanisms involved in cephalic and cardiac malformations associated with folic acid deficiency and/or hyperhomocysteinemia as well as fetal alcohol syndrome (Boot et al. 2003; Jaurena et al. 2011; Rovasio and Battiato 2002).

In addition to the study of fundamental developmental biology questions, the recent identification of NC-derived stem/progenitors cells in several postnatal tissues opens up opportunities for the use of NC stem cells for pharmaceutical screenings, cell-based transplantation therapies and tissue engineering (Sieber-Blum 2014). The protocol described in this chapter allows the isolation of a relatively pure population of adult EPI-NCSCs from mouse whisker follicles (Sieber-Blum et al. 2004). This protocol can also be adapted for the isolation of human EPI-NCSCs from scalp hair follicles (Krejčí and Grim 2010; Clewes et al. 2011). Both mouse and human EPI-NCSCs are easily accessible by a minimally invasive procedure and can generate multiple cells types of clinical relevance *in vitro*, including neurons, glia, myofibroblast/smooth muscle cells, chondrocytes, and melanocytes (reviewed in Sieber-Blum 2014).

The easy isolation together with their multilineage potential render the possibility of using these cells for transplantation in variety of disorders very attractive. For instance, it has been shown in animal models that transplanted mouse EPI-NCSCs are able to integrate into the injured spinal cord tissue and elicit improvement of sensory connectivity and in touch perception. The intraspinal EPI-NCSC did not form tumours and were able to differentiate to some extend into GABAergic neurons and myelinating oligodendrocytes. Besides cell replacement, the transplanted EPI-NCSCs are through to exert other pertinent functions in the contused spinal cord, including neuroprotection, angiogenesis and modulation of scar formation (Sieber-Blum et al. 2006; Hu et al. 2010; Sieber-Blum 2010).

In addition, human EPI-NCSCs have recently been shown as a feasible autologous cell source for future applications in Parkinson's disease and peripheral nerve injury, as they can be used for the generation of highly pure populations of dopaminergic neurons and Schwann cells (Narytnyk et al. 2014; Sakaue and Sieber-

Blum 2015). The ability of isolate these cells together with the application of robust differentiation protocols and *in vivo* injury models should now help to further assess the potential of EPI-NCSCs in the field of regenerative medicine.

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