The Neural Crest: its Derivatives and Stem Cells

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W. His (1868)
The structure of the lecture:

1) Why to study the neural crest (NC)
2) Origin and development of the NCCs (epithelo-mesenchymal transition, migration, differentiation)
3) Methods of labeling of the NC cells
4) Cell types differentiated from the NC
5) Developmental disorders of the NC
6) NC cells in epidermis
7) Neural crest stem cells (NCSCs) in the hair follicle
Why to study the neural crest (NC) cells?

- Cells of NC participate in development of almost all organs originating from ectoderm, mesoderm and endoderm
- NC formation enabled the expansion of vertebrates "... shift from filter feeding to active predation.
- NC is the 4th embryonic layer of craniates
- NC is a model system for studies of embryonic induction, cell determination, differentiation and epithelial-mesenchymal transition
- NC is a model system for the study of cell invasivity – development of carcinoma metastasis
- Defective development of the NC leads to developmental malformations
Origin and development of the NC
Induction of the NC
Epithelial-mesenchymal transition
Migration
Differentiation
Induction of the neural plate and epithelial-mesenchymal transition of NC cells

Noggin, Chordin, Follistatin

BMP4

Neural plate

Notochord

Future neural crest

Shh

Slug

BMP-4, BMP-7

BMP-4, BMP-7
Induction and epithelial-mesenchymal transition of NC cells

B.M. Carlson (1999)

Epithelial-mesenchymal transition of NC cells

ISH of mRNA for Slug

HH 10
Beginning of migration of neural crest cells
Migration of NC cells *in vivo* and *in vitro*
Molecular mechanisms of NC cells migration

- Permissive contact-guidance + chemorepellent molecules
- Scatter factor / c-met receptor, Pax3
- Steel factor (stem cell factor) / c-kit receptor
- Chemokin SDF-1 / CXCR4 receptor

Paracrine signaling systems:

- fibronectin
- tenascin
- HNK-1
Epigenetic landscape was originally a metaphor for biological development. Its originator, Conrad Hal Waddington (1905-1975), said that cell fates were established in development much like a marble rolls down to the point of lowest local elevation.
Regional identity and differentiation of NC cells

Local signaling during migration of NC cells induces differentiated gene expression. Pluripotent NC cells successively differentiate in bipotent and unipotent cell types (marshalling yard as a metaphor for cell differentiation)
Figure 5.2  Schematic representation of types of developmentally important molecules and their sites of action.
Methods of labeling of the NC cells
Ambystoma mexicanum (Černý et al., 2004)
Neural crest cells in embryonic chimeras of Japanese quail and white Leghorn chicken

Embryonic hимерas of Japanese quail (Q) and white Leghorn chick (C)

Feulgenova reakce
Labeling system exploring different organisation of perinucleolar chromatine in chick (C) and Japanese quail (Q) in their embryonic chimeras

Migration of NC cells of the head

Ectomesenchyme:
osteoblasts, fibroblasts, chondroblasts, smooth muscle cells, odontoblasts
Cardiac NC (R4-R8): for cardiac outflow tract
Labeling of NC cells in mammals

- using anti p75 Ab in mouse
- using reporter gene $lacZ$ (E. coli) in transgenic mouse $Wnt1/lacZ$
- permanent expression of $lacZ$ gene in cre-lox system in transgenic mouse $Wnt1$-cre/R26R

- detection of $lacZ$ gene expression indigogenic method ($Xgal$) at pH 7.5 immunohistochemically ($Bgal$) (rabbit anti-E. coli $\beta$-galaktosidase; Chemicon)
Labeling of neural crest cells in \textit{Wnt1-lacZ/}^{+} \textit{mouse}
The Wnt1-cre / R26R two-component system to label neural crest cells

Wnt1-cre / + mouse

- Wnt-1 promoter (neural crest specific)
- Cre-recombinase

R26R / + mouse

- loxP
- β-gal
- R26R promoter (ubiquitous)
- STOP

Wnt1-cre / R26R mouse

- loxP
- loxP
- β-gal

Cre-recombinase recognizes loxP sites and cuts
β-galactosidase is expressed permanently
NCCs in *Wnt1-cre/R26R* mouse

ED13, X gal

Hair follicles, ED 17.5
Cell types differentiated from the NC
Derivatives of trunk NC: HNK-1 Ab, chick embryo
Derivatives of trunk and head NC

Neurons of spinal and autonomic ganglia, enteric eurons, Schwann cells, pigment cells, cells of adrenal medulla

Ectomesenchyme:
- osteoblasts
- fibroblasts
- chondroblasts
- smooth muscle cells
- odontoblasts

Cardiac NC (R4-R8):
- for cardiac outflow tract
Derivatives of the NC in the head

- a part of sensory ganglia of V., VII., IX., X.
- parasymp. ganglia and their satellite cells
- Schwann cells of glomus caroticum
- parafolicullar C – cells (calcitonin),
- melanocyte,
- **Ectomesenchyme** – osteoblasts, fibroblasts, chondroblasts, smooth muscle cells in anterior part of the head, odontoblasts, pia mater, arachnoidea, stromal cells of cornea, smooth muscle cells of iris stromal cells of thymus, thyroid and parathyroid gland, salivary glands, lacrimal gland
- **Cardiac neural crest** – outflow tract, wall of large branches of aortic arch
Migration of cranial neural crest cells

B.M. Carlson (1999)

Wnt1-cre/R26R mouse

ED 9.5
Xgal
Developmental origin of the skull bones:

from the neural crest (green),

from occipital somites (pink)

from nonsegmented paraxial mesoderm (red)

membranous ossification (grey).
Tooth development (Wnt1-cre/R26R) (Chai et al. Development 127:1671, 2000)
From the NC originate odontoblasts, cementoblasts, periodontium
Fate Maps of Neural Crest (red) and Mesoderm (blue) in the Mammalian Eye. P. J. Gage, W. Rhoades, S. K. Prucka and T. Hjalt, Invest Ophthalm & Vis Sci. 46:4200 – 8, 2005
Cardiac neural crest
R 4. – R 8.

Wnt1-cre/R26R transgenic mouse, Xgal, 7. – 9. week
Developmental disorders of the NC
Developmental defects of the NC

- **CHARGE syndrom** (Coloboma iridis, Heart defects, Atresia choanae, Retardation of development, Genital hypoplasia in males, Ear anomalies)

- **DiGeorge syndrom** (hypofunction of parathyroid and thyroid gland, thymus hypoplasia, defects of septation of aorta and pulmonary trunk)

- anomalies of teeth • albinism

- **Waardenburg syndrom** (Pax3 mutation – pigmentation defects, defects of limb muscles, cleft palate, cardiovascular defects, hypertelorism)

- Hirschsprung disease • Piebaldism
*Pax3* mutation in mouse (splotch mutation)
Piebaldism (pigmentation defects, sterility, anemia) mutation of *KIT* gene in man and *Kit* gene in mouse
Neural crest cells in epidermis

Merkel cells, melanocytes, stem cells
Melanocytes
Neural crest cells in epidermis – in hair follicle

**Bgal/H**  Wnt1-cre/R26R
NCCs in hair follicles, back skin, Wnt1-cre/ R26R mouse

Xgal / CK8

Xgal / nucl. red

c-kit

Xgal
Merkel cells - large light cells in the basal layer of epidermis and in mucous membranes of ectodermal origin in vertebrates
F. S. Merkel (1875) : „Tastzellen“ of the skin of birds and mammals
Merkel cells are transducers of tactile stimuli in slowly adapting mechanoreceptors of the skin.

Human MCs represent 3.6 - 5.7% of basal epidermal cells from glabrous and hairy skin (Fradette et al., 2003)

Merkel cells in whisker hair follicle
Whisker hair follicle, blood sinus, innervation, bulge
Merkel cell carcinoma
Merkel cell carcinoma
Merkel cell carcinoma is a rare and highly aggressive skin cancer, which, in most cases, is caused by the Merkel cell polyoma virus discovered at the University of Pittsburg 2008.

It is also known as cutaneous neuroendocrine carcinoma of the skin, primary small cell carcinoma of the skin, and trabecular carcinoma of the skin.

It has 4 times greater mortality than the melanoma and its incidence is rising. Now accounts for 5-6 cases per 1 million inhabitants.

MCC cells express cytokeratins 8, 18, 19 and 20, and neuroendocrine markers as Merkel cells.
Scheme of potential cells of origin of Merkel cell carcinoma (MCC) from an ontogenetic perspective. Arrows: hypothetic lineage relationships.
Neural crest stem cells in hair follicles of the mouse
Dissection of the bulge from adult whisker follicle

Dev Dyn 231:258-269, 2004;

Xgal + NCCs emigrated 4 days after explantation
Bulge explant-derived NCCs are pluripotent

Smooth muscle cells  Neurons

anti-SMA  anti-ß-III tubulin  DAPI

Schwann cells

anti-S-100

Melanocytes  Chondrocytes

Anti-collagen II Ab

MeIEM

Dev Dyn 231:258-269, 2004; Embryo Today 72:162-172, 2004
Supp. by LN 00A065 and VZ 111100003-3
Bulge-derived NCCs undergo self-renewal (determined by serial cloning)

5-day-old secondary clone

Dev Dyn 231:258-269, 2004; cells from 2-weeks secondary clones
Morphology of EPI-NCSC implants in the lesioned spinal cord

Nestin

GAD67

RIP

Mol Cell Neurosci 32: 67 - 81, 2006
ABSTRACT

The present invention describes novel methods for isolating a substantially pure cell population of non-embryonic epidermal neural crest stem cells from the bulge-region of mammalian hair follicles. Also disclosed is the substantially pure cell population of follicular bulge-derived neural crest stem cells for medical research and therapeutic use.
The use of NCSCs in regenerative medicine 2009 – 2014

Epidermal NCSCs are capable of differentiating into cells of mesodermal and ectodermal cell line. Epidermal NCSCs express neurotrophins, angiogenic factors and metalloproteinases. Their transplantation in damaged mouse spinal cord show improvement in sensory function ... but there is no use of NCSCs in clinical medicine up to now...
Neural crest stem cells in human hair follicles
Growth cycle of mammalian hair
Isolation of human epidermal neural crest stem cells (hEPI- NCSCs) from hair follicles

Tissue source: skin biopsy from different body locations

Epidermis with follicles after dispase treatment
Primary culture cells after emigration from isolated follicle
Sphere after readhesion

Coexpression of Sox10 and Nestin, Expression of Nanog

**Sox10 + Nestin**

**Nanog + DAPI**

hEPI-NCSCs in tissue culture after emigration from back skin follicles

Differentiation of human epidermal neural crest stem cells from hair follicles into neural crest progeny

Folia Biol. (Praha) 56, 149-157, 2010

- Schwann cells
- Neurons
- Smooth muscle cells
- Smooth muscle actin
- GFAP
- Beta III tubulin
- Schwann cells
- S100
Isolation and Characterization of Neural Crest Stem Cells from Adult Human Hair Follicles

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Abstract. Neural crest (NC) is a transient embryonic tissue, whose cells are motile and multipotent until they reach their destination and differentiate according to microenvironmental cues into a variety of cell types. However, a subpopulation of these cells remains multipotent. They were found, among other locations, in a bulge of adult murine whisker follicle and were designated epidermal neural crest stem cells (EPI-NCSCs). The aim of this work is to ascertain whether the EPI-NCSCs could be isolated from human hair follicles as well. Due to their exceptional properties, they could represent potential candidates for stem cell therapy. The presented work focuses on the isolation and characterization of EPI-NCSCs from human skin. We obtained a population of cells that expressed markers of NC, NC progeny and general stem cell markers. After prolonged cultivation, the subpopulation of cells spontaneously differentiated into some of NC derivatives, i.e. neurons, smooth muscle cells and Schwann cell progenitors. Targeted differentiation with neuregulin 1 highly increased the number of Schwann cells in the culture. Human EPI-NCSCs could also grow under non-adherent conditions and form 3-dimensional spheres. Microarray analysis was performed and gene profile of human EPI-NCSCs was compared with the list of key genes of murine EPI-NCSCs and the list of genes up-regulated in newly induced NC cells. This revealed 94% and 88% similarity, respectively. All presented results strongly support the NCSC identity and multipotency of isolated human cells. These cells could thus be used in regenerative medicine, especially because of the easy accessibility of donor tissue.

Introduction

Tissue-specific adult stem cells have long attracted attention, especially since they could be isolated from one individual, expanded and eventually differentiated in vitro and transplanted back into the same individual. Accessibility of donor tissue is of considerable importance in such case. Compared to embryonic stem cells, there is no need to use immunosuppressive medication since they are the patient’s own cells and there will therefore be no graft rejection and no problems of ethical nature. Compared to induced pluripotent stem cells (Takahashi and Yamanaka, 2006) or nuclear transfer, there is no need for genetic or mechanistic manipulation.

Neural crest (NC) is a transient embryonic tissue that arises at the border between neural and non-neural ectoderm in early stages of development. Following neural tube closure, NC cells (NCCs) undergo epithelial-mesenchymal transition and migrate along defined pathways to populate various regions of the embryo (reviewed in Le Douarin and Kalcheim, 1999). NCCs contribute to a diverse array of cell types, including multiple skeletal


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