The Neural Crest and Neurocristopathies

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MILESTONES

Le Douarin N: **Particularites du noyau interphasique chez la Caille japonaise (Corurnix coturnix japonica). Utilisation de ces partiuclarites comme "marque biologique" dans des recherches sur les interactions tissularires et les migrations cellulaires au cours l'ontogeenese** *Bulletin Biologique de la France et de la Belgique* 1969, 103:435-452

Le Douarin N: **A biological labeling technique and its use in experimental embryology**. *Developmental Biology* 1973, 30:217-222

Nicole Le Douarin

The neural crest, initially described by Wilhelm His in 1868 as a novel longitudinal band of cells dorsal to the spinal cord[3], is a fascinating and unique progenitor tissue. Fascinating because it gives rise to a surprising diversity of specialized cells and tissues. Unique because it is an embryonic germ cell structure that disappears.

Abnormalities of neural crest development result in a bewildering myriad of diseases, some involving complex syndromes of seemingly unrelated lesions that until recently defied any unifying explanation. Groundbreaking investigations by the French embryologist Nicole Le Douarin[1,2] launched decades of research, initially in birds, and later in mice, that uncovered the startling dimensions of the neural crest lineage. Ongoing studies in genetically engineered mice and in humans continue to identify the molecular defects underlying the constellation of diseases termed neurocristopathies by Bolande[4].

The pioneering discoveries of Le Douarin and colleagues began with a simple observation made shortly after obtaining her PhD. Her dissertation research proved that mesoderm and endoderm participated in the development of the avian liver. She became interested in whether mesoderm and endoderm from different species could productively interact during hepatogenesis. To test this possibility, she cultured quail liver mesoderm with chick prehepatic endoderm and found that interspecies cooperation took place. While analyzing the histological sections from those experiments, Le Douarin noticed that the interphase nuclei of quail cells were easily distinguished from those of chick cells. Quail cells had strikingly large nucleoli[1], eye-catching because of their abundant chromatin. She immediately recognized the potential of quail/chick chimeras for investigating highly migratory cells[2] and turned her attention to the neural crest.

Avian species formed ideal subjects for her work. Eggs afforded easy access to the embryo and allowed experimentation during the entire period of development. Earlier embryologists had described the morphology of the neural crest and identified some of its migratory properties. Prior to Le Douarin's observation no reliable method existed for labeling embryonic cells to study their migration. Techniques based on tritiated thymidine incorporation suffered from rapid dilution of the marker in highly proliferating embryonic cells. The quail/chick chimera system devised by Le Douarin revolutionized experimental embryology and launched an era of unprecedented discovery in developmental biology. The impact of those discoveries continues to enrich our understanding of developmental diseases and their pathogenesis.

Research during the first half of the 20th century, mostly studies of lower vertebrates, established that neurocrest cells delaminate from the primitive neural folds early in embryonic life and coalesce above the neural tube to form the neural crest. Le Douarin and colleagues, using microsurgical techniques, removed the neural folds from chick embryos before the onset of migration and replaced them with neural folds from quail embryos matched for developmental stage. Exploiting the biological marker in quail cells, they showed that virtually every tissue in the embryonic and adult body contains cells originating in the highly invasive and pluripotent neural crest.

In a series of investigations Le Douarin and co-workers extirpated segments of chick neural crest and replaced them with the corresponding quail segments. This approach allowed them to map the destination of neural crest cells at different locations along the neural crest. Their experiments established that the axial level of origin in the neural crest determined the migratory pathways taken.

Four major regions of the neural crest were recognized: *cephalic, vagal, truncal* and *sacral.*

Le Douarin's group established that cephalic neural crest cells formed most of the bones of

the head and facial skeleton. They also formed the connective tissue associated with striated muscles of the head and neck, the buccal and pharyngeal glands, the parathyroid glands, the thymus, and the conotruncal region of the heart. These findings would later provide strong clues about the pathogenesis of complex developmental disorders, such as DiGeorge syndrome.

Humans with DiGeorge syndrome exhibit agenesis or hypoplasia of the thymus and parathyroids, conotruncal abnormalities of the heart, and craniofacial dysmorphism. Each of these lesions occurs in tissues derived from cephalic neural crest. Experimental ablation of the cephalic neural crest in chick embryos results in an identical combination of defects.

DiGeorge syndrome maps to a locus on chromosome 22 that encodes a protein with the structural features of a transcriptional regulator. Mice homozygous for a targeted null mutation of the homologue have defects in multiple cranial and cardiac neural crest derivatives, including the cranial ganglia, aortic arch arteries, cardiac outflow tract, thymus, parathyroid glands and craniofacial structures.

The Le Douarin group showed that cells from the vagal crest colonized the gut to form the enteric nervous system.

They also showed that the truncal crest gave rise to pigment cells of the skin, spinal dorsal root ganglia, paravertebral sympathetic ganglia, Schwann cells of all peripheral nerves, the adrenal medulla and paraganglia. The sacral crest contributed to the innervation of the distal hindgut.

Some neurocristopathies occur as tumors, others as malformations; some as isolated lesions, others as combinations of lesions clustered in a syndromic pattern. Isolated and combinatorial lesions occur in both sporadic and familial forms.

Tumors derived from neural crest cells include neurofibroma, neuroblastoma, medullary thyroid cancer, pheochromocytoma, melanoma and several less common neoplasms.

Neural crest malformations include aganglionic megacolon (Hirschsprung's disease), congenital nevi, albinism, cleft lip and/or palate, conotruncal heart malformations and the lesions in fetal alcohol syndrome.

Our current knowledge of neural crest ontogeny, the genetic and microenvironmental factors that govern its properties, and the developmental defects that give rise to its disorders all trace their pedigree to the milestone research of Nicole Le Douarin. Reviews of these advances have been published[4, 5].

The tools of molecular biology made labeling of the neural crest in mice a reality. Neural crest-derived cells are readily identified in transgenic mice in which the expression of beta-galactosidase or green fluorescent protein is driven by promoters of genes expressed in neural crest cells. Permanent long-term labeling occurs in double transgenic mice in which the production of Crerecombinase is conditioned upon expression of either *Wnt1* or *Sox10* genes.

Genetic studies continue to identify pivotal mutations that clarify the previously baffling combination of pathologic findings in syndromic neurocristopathies. Mutations in the receptor tyrosine kinase RET and the endothelin-β receptor underlie two familial forms of aganglionic megacolon (Hirschsprung's disease). Targeted disruption of the mouse endothelin-3 ligand results in megacolon and pigment disorders, the latter also seen in some patients with Hirschsprung's Disease and a hallmark of Von Recklinghausen's disease. Some Hirschsprung patients develop neuroblastomas or other neoplastic forms of neurocristopathy. All of these combinations have in common a linkage with *vagal neural* crest.

Neurofibromatosis Type 1 (*NF1* gene) is one of the most common genetic diseases and neurocristopathies. Affected individuals develop a multiplicity of cutaneous and visceral neurofibromas, pigmented lesions of the skin, and one or more pigmented iris hamartomas. Some patients develop adrenal gland pheochromatcytomas. At first glance these seem esoteric combinations of lesions, but when considered in terms of neural crest ontogeny, the relationships of the lesions become obvious.

The impact of Le Douarin's research continues to grow with recent findings showing that environmental agents can cause neurocristopathic effects in humans. The frequent occurrence of non-familial neurocristopathies suggested that environmental factors played a causal role. Several studies have now shown that the consumption of alcohol during early pregnancy and the dermatological use of 13-*cis*-retinoic acid for the treatment of severe acne in pregnant women produce characteristic malformations in neural crest-derived tissues.

Le Douarin took a simple observation about quail nucleoli and transformed it into a brilliant discovery. That leap forward resonates with the definition of discovery suggested by the Hungarian physiologist and Nobel laureate Albert Szent-Georgy: "…seeing what others have seen, but thinking what others have not."

References

1. Le Douarin N: *Bull Biol Fr Belg* 1969, 103:435-452
2. Le Douarin N: *Dev Biol* 1973, 30:217-222
3. His W: Untersuchungen ueber die erste Anlage der Wirtbeltierleibes die erste Entwickelung des Hunchens im Ei. Leipzig. *Vogel,* 1868
4. *Adv Exp Med Biol* 2006, 589: 1-244
5. Bolande, RP: *Pediatr Pathol Lab Med* 1997, 17: 1-25