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Review

The development of the chromaffin cell lineage from the neural crest

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ABSTRACT

Chromaffin cells are neuroendocrine cells, which are highly specialized for the synthesis and release of multiple hormones. Like sympathetic neurons, which are essential, *inter alia*, for neural control of vascular tone, they are derivatives of the neural crest, a transient structure at the dorsal surface of the embryonic neural tube. Chromaffin cells and sympathetic neurons have many features in common, but are also distinct in several respects. This review provides a summary of similarities and differences regarding the development of chromaffin cells and sympathetic neurons, viewed from molecular and morphological perspectives. Two major, still not finally settled issues, are whether (1) the two related cell types arise from one common or two separate cell lineages of delaminating neural crest cells, (2) in the former case when does lineage segregation occur, and what are the molecules underlying their phenotypic diversification.

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1. Introduction

The neuroendocrine chromaffin cells are located in the adrenal gland and in "extradrenal" paraganglia, as e.g. in the carotid body and "organ of Zuckerkandl". The term "chromaffin" was coined by the Prague histologist Alfred Kohn (1867–1959) and refers to the cells' characteristic staining properties with chromium salts. By their development, biochemistry, and physiology, chromaffin cells are closely related to sympathetic neurons, but are morphologically clearly distinct. Both cell types possess the machineries to synthesize, store, release, and take up catecholamines, including the enzymes for noradrenaline synthesis, i.e. tyrosine hydroxylase (TH), and dopamine-ß hydroxylase (DBH), as well as vesicular monoamine transporters (VMAT). In contrast to sympathetic neurons, chromaffin cells lack axons and dendrites; their most impressive morphological feature are the large "chromaffin" granules (about 130–230 nm in diameter, depending on species), which are significantly larger than the so-called dense-core vesicles in sympathetic neurons. Also distinct from sympathetic neurons, a major subpopulation of adrenal chromaffin cells synthesizes adrenaline. Similar to sympathetic neurons, however, chromaffin cells are innervated by "preganglionic" axons which originate from cell bodies located in the intermediolateral column of the spinal cord (Schober and Unsicker, 2001). A cell type, which shares features of sympathetic neurons and chromaffin cells, has been termed "small granule containing" or "small

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intensely fluorescent" (SIF) cell (referring to the intense catecholamine-specific histofluorescence). SIF cells occur in the adrenal gland, in paraganglia, and sympathetic ganglia. Comparative structural and biochemical analyses of chromaffin tissues and sympathetic ganglia in a large number of vertebrate species from fish to mammals have clearly shown that chromaffin cells and sympathetic neurons just occupy the extreme ends of a wide spectrum of so-called "sympathoadrenal" cells with specific phenotypes (cf. Unsicker, 1973; Unsicker et al., 1978a,b).

For comprehensive reviews on chromaffin and SIF cells, see Coupland (1965, 1972); Blaschko et al. (1975); Böck (1982); Unsicker (1993); Unsicker and Krieglstein (1996); and O'Connor and Eiden, (2002).

This review summarizes recent advances in understanding the development of chromaffin cells from the neural crest. Several of the previous enigmas, as e.g. the role of glucocorticoid signaling, transcription factors operating in chromaffin cell development, and the development of chromaffin cells and sympathetic neurons from a common cell lineage (vs. two cell lineages that are already distinct at the time point of delamination of the neural crest) have either been resolved now, or are close to being unraveled. As expected, answers to previously open issues have generated new questions, including the probably most important one, how the two distinct morphological and biochemical cellular phenotypes are generated from a common cell lineage.

2. The origin of chromaffin cells from the neural crest

Classical studies uncovered that chromaffin cells originate from the neural crest (Le Douarin and Teillet, 1974; Teillet and Le Douarin, 1974), a transient group of progenitors that, among many neural and non-neural derivatives, also generate the entire autonomic nervous system, including the enteric branch. In the avian embryo, adrenal chromaffin cells derive from crest cells exiting the dorsal tube at somitic levels 18–24, also termed the "adrenomedullary" level of the neural crest (although in the avian adrenal gland chromaffin cells do not form a "medulla"; reviewed in Le Douarin and Kalcheim (1999)). After delamination the first emigrating neural crest cells take the ventral migration route, passing first through the intersomitic spaces. Then, upon dissociation of the somites, crest cells begin invading the rostral halves of each segment while migrating first between dermomyotome and sclerotome and later through the rostral sclerotomal mesenchyme until arriving at para-aortic sites (Loring and Erickson, 1987; Teillet et al., 1987).

A variety of transcription factors have been identified to be expressed by neural crest cells, including SoxE transcription factors (for review see Cheung et al., 2005). Neural crest cells migrating to the adrenal gland express Sox8 and Sox10 (Reiprich et al., 2008). It has been shown recently that lack of Sox10, but not Sox8, leads to a complete agenesis of the adrenal medulla due to cell death during migration (Reiprich et al., 2008). Sympathetic ganglia of the same axial level are also absent in Sox10 deficient mice, but interestingly more rostral ganglia are only reduced in size (Britsch et al., 2001; Reiprich et al., 2008), indicating a cranio-caudal increase of the importance of Sox10 in sympathetic neuron development.

Based on antibody studies, it had been proposed that sympathetic neurons and chromaffin cells develop from a common pool of catecholaminergic neuronal progenitors–the so-called sympathoadrenal progenitors (SA)–that first aggregate in the vicinity of the dorsal aorta (around E2.5 chick, E10 mouse) to form the primary sympathetic ganglia (Anderson and Axel, 1986; Anderson et al., 1991). Key results of these marker studies included the observation that in rat embryos cells populating the primary sympathetic ganglia co-express neuronal markers and the chromaffin cell specific markers SA1–5. In addition, it was reported that SA cells colonizing the adrenal gland initially express neuron-specific markers like neurofilament and SCG10, which are downregulated subsequently (Anderson and Axel, 1986).

It is well documented that BMP-2/4/7, which are synthesized by the wall of the dorsal aorta, play a central role in instructing neural crest cells towards a catecholaminergic and neuronal fate (Reissmann et al., 1996; Shah et al., 1996; Varley et al., 1995; Schneider et al., 1999; Bilodeau et al., 2001). The *in vivo* requirement of BMP-4/7 for the development of sympathetic neurons has been convincingly demonstrated by gain- and loss-of-function experiments in chick embryos (Reissmann et al., 1996; Schneider et al., 1999). In contrast with the precedent, a recent study has reported the surprising result that canonical TGFß and BMP-signalling is not required for the acquisition of any neural crest cell fate, including autonomic neurons, except for trigeminal sensory neurons (Büchmann-Moller et al., 2009). These results were obtained in mice with a wnt1-Cre conditional deletion of Smad 4 and interpreted on the basis of the assumptions that (1) Smad 4, without any exception, is really essential in BMP signalling and (2) Smad 4 was fully inactivated by the time crest cells reach the dorsal aorta and (3) no compensation of the knockout occurred.

It should be noted that the assumption that chromaffin cells, like sympathetic neurons, require BMPs for their early differentiation is mainly based on the notion that the two cell types are closely related and share similar developmental mechanisms. However, in a recent study we could provide, for the first time, direct evidence that at least a part of the chromaffin cell population requires BMP-4 for their catecholaminergic differentiation (Huber et al., 2008).

In response to BMPs neural crest cells activate a network of transcription factors, including, Mash1/Cash1, Phox2A/B, Hand 2, Gata2/3 and Insm1, that promotes their further development (Guillemot et al., 1993; Schneider et al., 1999; Pattyn et al., 1999; Howard et al., 2000; Lim et al., 2000; Tsarovina et al., 2004; Wildner et al., 2008). The cells initiate the expression of neural markers as e.g. neurofilament (NF), the growth-associated protein SCG10, neuron specific tubulin (Cochard and Paulin, 1984; Groves et al., 1995; Schneider et al., 1999; Sommer et al., 1995), along with TH and DBH, the enzymes for noradrenaline synthesis (Cochard et al., 1978; Ernsberger et al., 1995, 2000).

Originally, it was believed that SA cells migrate to their final destination, i.e. the secondary sympathetic ganglia or the adrenal anlage, after the acquisition of neuronal and catecholaminergic traits (Anderson and Axel, 1986; Anderson et al., 1991). In the light of more recent studies the concept of common SA progenitors that are uniformly specified at the dorsal aorta seems unlikely. Detailed gene expression analyses in chick embryos using a broad repertoire of early markers have suggested that chromaffin progenitors undergo catecholaminergic differentiation in the presumptive adrenal region, rather than in the primary sympathetic ganglia. In addition, it was observed that the majority of these cells, unlike cells in the primary sympathetic ganglia, express undetectable or low levels of neurofilament M and other neuronal markers at the onset of TH expression and throughout further development (Fig. 1; Ernsberger et al., 2005; Gut et al., 2005). These findings suggest that the segregation between the two lineages occurs upstream/prior to catecholaminergic differentiation.

Likewise, recent observations in mouse embryos support the notion that many, if not all progenitors of chromaffin cells, may migrate to the adrenal gland as "undifferentiated" neural crest cells expressing Sox10, a general neural crest marker, but still lacking SA-markers (Gut et al., 2005; Reiprich et al., 2008). Although cells expressing SA-markers like TH, Phox2B and Mash1 have been observed *en route* to the adrenal gland (Anderson and Axel, 1986; Gut et al., 2005; Reiprich et al., 2008) it is not clear whether these cells give rise to chromaffin cells or to sympathetic neurons of the nearby located suprarenal ganglion. We have identified additional sources of BMP-4 in periadrenal tissues (mouse, Gut et al., 2005) and adrenocortical cells (chick, Huber et al., 2008; cf. Fig. 2), suggesting that chromaffin progenitors may receive an instructive BMP-4 signal from tissues other than the dorsal aorta. Recently, we have shown by using chick adrenal explants that at least a part of chromaffin cells depends