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Short communication

Immunohistochemical characterization of the rat carotid body

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Abstract

We studied the histochemical phenotype of carotid body (CB) cells in the adult rat. In addition to tyrosine hydroxylase (TH), type I cells expressed numerous growth factors such as glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α), as well as the receptors p75, Ret, epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor-alpha (PDGFR-α). Type II cells expressed the glial fibrillary acid protein (GFAP), vimentin, the trophic factor bFGF and receptors p75, EGFR and PDGFR-α. Both types I and II cells exhibited a positive immunoreaction to markers of neural progenitor cells such as the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) and nestin, respectively, suggesting that CB contain some immature cells even at the adult stage. The possibility that these cells can be expanded and differentiated into mature neurons should be explored.

Keywords: Carotid body; Trophic factors; Progenitor cells

1. Introduction

The mammalian carotid body (CB) is a small, neural crest derivative neuroendocrine organ that detects blood oxygen levels and regulates ventilation. It consists of four principal components: cell clusters, blood vessels, connective tissue, and nerve fibers. Cell clusters comprise groups of type I or glomus cells surrounding of type II or sustentacular cells (Lopez-Barneo et al., 2001). Type I cells are neuron-like cells that express tyrosine hydroxylase (TH) and the early neuronal marker β-III tubulin while type II cells are supporting glia-like cells expressing the glial fibrillary acid protein (GFAP), S-100 protein and vimentin (Kameda, 1996, 2005).

Because glomus cells can survive, proliferate and release dopamine, particularly under hypoxic conditions, CB cell aggregates have been utilized as cell source for transplantation in experimental models of Parkinson's disease (PD) and also in PD patients (Yu et al., 2005). In both cases, the alleviation of parkinsonism observed seems to be due to the trophic effect exerted by the grafted cells on the remaining nigrostriatal pathway since type I cells express, in addition to dopamine, high levels of different growth factors with proven trophic effect on dopaminergic neurons (Toledo-Aral et al., 2003). In addition, Pardal et al. (2007) recently reported the capacity of type II cells to proliferate and originate new dopaminergic cells under hypoxic conditions, results that suggest the presence of stem cells in the adult mouse CB.

In order to afford new insights into the potentiality of CB cells in the treatment of PD, we have studied the histochemical phenotype of the rat CB cells, paying special attention on the expression of different growth factors and markers of neural progenitor cells.

2. Materials and methods

CB was obtained from adult male Wistar rats (body weight 200–250 g). Animals were anesthetized intraperitoneally with a mixture of ketamine (75 mg/kg; Merial) and xylacine (10 mg/kg; Bayer) and intracardially perfused with phosphate buffer saline (PBS) followed by 4% paraformaldehyde. Carotid artery was removed and subsequently the CB was isolated from the carotid bifurcation and cleaned of surrounding connective tissue. The

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whole CB was post-fixed for 30 min at 4 °C and then moved into 30% sucrose in PBS overnight for cryoprotection. Experimental protocols were performed in accordance with European Communities Council Directive 86/609/EC regarding the care and use of animals for experimental procedures.

Tissue sections (20 μm) of CB were obtained using a cryostate (Mycrom, Heidelberg) and processed as free floating sections for double immunofluorescence with TH (Chemicon Intl.), GFAP (Dako) or vimentin (Chemicon Intl.) and the following primary antibodies: β-III tubulin (Babco), the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) (Chemicon Intl.), nestin (Chemicon Intl.), vimentin (Chemicon Intl.), glial cell line-derived neurotrophic factor (GDNF) (Santa Cruz Biotechnology), basic fibroblast growth factor (bFGF) (Upstate), brain-derived neurotrophic factor (BDNF) (Abcam), ciliary neurotrophic factor (CNTF) (Santa Cruz Biotechnology), insulin-like growth factor-I (IGF-I) (Chemicon Intl.), epidermal growth factor (EGF) (Abcam), transforming growth factor-alpha (TGF-α) (Santa Cruz Biotechnology), p75 (Santa Cruz Biotechnology), Ret (R&D Systems), epidermal growth factor receptor (EGFR) (Abcam) and platelet-derived growth factor receptor-alpha (PDGFR-α) (Santa Cruz Biotechnology). The immunoreaction was visualized with the corresponding fluorescent secondary antibodies conjugated with: Cy3 (Sigma) and Alexa Fluor 488 or 568 (Molecular probes). Sections were counterstained with To-pro3 (Molecular probes). The slides were mounted with immumount (Calbiochem) and the fluorescent signal was detected using confocal laser scanning microscope equipped with three lasers (LSM510/Meta; Carl Zeiss).

3. Results

The organization and morphology of the rat CB observed in stained tissue sections were similar to those previously described by other authors. It consisted of cell clusters of type I cells with a large round nucleus and a granular cytoplasm surrounded by type II cells that displayed an elongated nucleus and a thin cytoplasmic layer (Fig. 1A).

A summary of cell markers, growth factors and receptors expression in types I and II CB cells is shown in Table 1. As expected, tissue sections of rat CB processed for immunohistochemistry showed the CB glomus cells (type I cells) selectively stained with anti-TH and β-III tubulin antibodies (Fig. 1B) while type II cells were stained with anti-GFAP and vimentin antibodies (Fig. 1C). Subsequently, we studied whether CB cells expressed the neural progenitor markers PSA-NCAM and nestin. In order to identify the type cell expressing PSA-NCAM or nestin, tissue sections of CB were processed for double immunofluorescence with TH or GFAP and the corresponding marker of immature neuronal cell. We found that TH-positive cells (type I cells) displayed immunoreaction to PSA-NCAM (Fig. 1D and E), while nestin was expressed in GFAP-positive cells (type II cells) (Fig. 1G and H). Confocal imaging analysis demonstrated no cellular co-localization of PSA-NCAM with GFAP (Fig. 1F) and nestin with TH (Fig. 1I). In addition, the majority of types I and II cells expressed PSA-NCAM (Fig. 1D) and nestin, respectively (Fig. 1G).

We further investigated the possibility that growth factors other than those already described could also be expressed in CB cells. For this purpose we performed double immunofluorescence staining with TH (marker of type I cell) and GFAP or vimentin (markers of type II cell) and the antibodies against the following growth factors: GDNF, bFGF, BDNF, CNTF, IGF-I, EGF and TGF-α. Subsequently, the presence in CB cells of some receptors for growth factors (p75, Ret, EGFR and PDGFR-α) was also investigated with double immunofluorescence staining. Using confocal analysis we found that type I cells expressed in addition to TH the growth factors (GDNF, bFGF, BDNF, CNTF, IGF-I, EGF and TGF-α) (Fig. 2A) and receptors analyzed (p75, Ret, EGFR and PDGFR-α) (Fig. 2B). Type II cells were stained with bFGF, p75, EGFR and PDGFR-α as indicated by the co-localization of these molecules with GFAP or vimentin (Fig. 2C). On the contrary, type II cells did not express GDNF, BDNF, CNTF, IGF-I, EGF, TGF-α and Ret, as indicated by the lack of co-localization with GFAP or vimentin (data not shown).

4. Discussion

The CB is a small structure originating from the neural crest and its neural origin can be easily demonstrated by the expression of some neuronal markers such as TH, PGP9.5, β-III tubulin and GFAP in its cells (Kameda, 1996, 2005). Our results confirm and extend previous findings demonstrating two major cell components of the mammalian CB that can be well identifiable on tissue sections according to their specific neurochemical markers. We found that type I cells typically expressed TH, β-III tubulin and numerous growth factors. Interestingly, these type I cells also exhibited a positive immunoreaction to the cell membrane marker of migratory cells PSA-NCAM. By contrast, we detected that type II cells expressed vimentin, GFAP and the

Table 1
Expression of cell markers, growth factors and receptors in type I and type II cells

	TH	GFAP	β-III tubulin	Vimentin	Nestin
Type I cells	+++	−	+++	−	−
Type II cells	−	+++	−	+++	+++

	GDNF	bFGF	BDNF	CNTF	IGF-I	EGF	TGF-α	p75	Ret	EGFR	PDGFR-α
Type I cells	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Type II cells	−	+	−	−	−	−	−	+	−	+	+

−, no positive cells observed; +, <50% positive cells; ++, 50–90% positive cells; +++, >90% positive cells.