Journal of Pediatric Surgery (2011) **46**, 1197–1200

ELSEVIER

Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Brain lipid–binding protein: a marker of differentiation in neuroblastic tumors

Giuseppe Retrosi [a], Neil J. Sebire [b], Mark Bishay [a], Edward M. Kiely [c], John Anderson [d], Paolo De Coppi [a], Elisa Resca [a], Dyane Rampling [b], Nelly Bier [b], Kevin Mills [e], Simon Eaton [a], Agostino Pierro [a,*]

[a]*Surgery Unit, University College London Institute of Child Health and Great Ormond Street Hospital for Children, WC1N 1EH London, United Kingdom*
[b]*Histopathology Department, Great Ormond Street Hospital for Children,WC1N 1EH London, United Kingdom*
[c]*General and Neonatal Surgery, Great Ormond Street Hospital for Children, WC1N 1EH London, United Kingdom*
[d]*Molecular Haematology and Cancer Biology Unit, University College London Institute of Child Health and Great Ormond Street Hospital for Children, WC1N 1EH London, United Kingdom*
[e]*Clinical and Molecular Genetics Unit, University College London Institute of Child Health and Great Ormond Street Hospital for Children, WC1N 1EH London, United Kingdom*

Received 16 March 2011; accepted 26 March 2011

Key words:
BLBP;
B-FABP;
FABP7;
Neuroblastoma;
Ganglioneuroblastoma;
Ganglioneuroma

Abstract

Purpose: Neuroblastoma (NB), ganglioneuroblastoma (GNB), and ganglioneuroma (GN) are neuroblastic tumours (NT) of sympathetic nervous system origin. Brain lipid-binding protein (BLBP) has potential morphogenic activity during nervous system development but has not been studied in these tumours. We analyzed the expression of BLBP in NT according to histological subtypes and extent of differentiation.

Methods: Thirty cases of NT (10 each of NB, intermixed GNB, and GN) were identified from the histopathology archive of a single center. Tissue sections were obtained from representative paraffin blocks and immunohistochemistry for BLBP performed.

Results: Brain lipid-binding protein was not expressed in any NB case. In all cases of GN, BLBP was strongly expressed in the cytoplasm of mature ganglion cells but negative in Schwannian stroma. In the intermixed GNB, there was similar strong BLBP immunoreactivity in the cytoplasm of fully differentiated and differentiating ganglion cells but no BLBP expression in immature neuroblasts.

Conclusion: Brain lipid-binding protein is strongly expressed in mature and maturing ganglion cells in NT (GN and GNB), whereas it is absent in poorly differentiated neuroblasts of GNB and NB. Cytoplasmic expression of BLBP in NT increases as the cells undergo neural differentiation and is therefore associated with the extent of tumour differentiation and favorable histology.

* Corresponding author. Department of Paediatric Surgery, UCL Institute of Child Health and Great Ormond Street Hospital for Children, WC1N 1EH London, United Kingdom. Fax: +44 20 7404 6181.
E-mail addresses: pierro.sec@ich.ucl.ac.uk, a.pierro@ich.ucl.ac.uk (A. Pierro).

0022-3468/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2011.03.053

Neuroblastic tumors are the most common solid tumors in childhood [1], and they comprise a spectrum of neural crest–derived neoplasms that occur along the sympathetic chain, ranging in their state of differentiation and malignancy. Ganglioneuroma (GN) is the benign form representing the most differentiated phenotype, composed of mature ganglion cells, surrounded by Schwannian stroma. Ganglioneuroblastoma (GNB) and neuroblastoma (NB) vary in malignant potential and display variable degrees of neuroblastic differentiation [2]. The characteristics of GNB and NB may change over time because metastatic, poorly differentiated tumors can undergo regression or maturation [1]. Neuroblastic tumors thus demonstrate a dynamic inverse correlation between differentiation and malignant potential, suggesting that inducing differentiation may be a viable therapeutic strategy [3].

Brain lipid–binding protein (BLBP) is a member of the fatty acid–binding protein (FABP) family and has previously been implicated in neuron-radial glial cell signaling in the developing central nervous system [4,5]. It is considered a marker for some brain tumors [6] and may have a role in tumorigenesis in the peripheral nervous system [7] and in several extra nervous system neoplasms such as breast cancer, renal cancer, and melanoma [8-11].

We hypothesized that BLBP may be differentially expressed in neuroblastic tumors, and the aim of the study was to determine the expression of BLBP in these neoplasms according to their histologic type and extent of differentiation.

1. Materials and methods

The tumor specimens of 30 children with neuroblastic tumors (representing 10 NBs, 10 intermixed GNBs, and 10 GNs) were identified from the routine diagnostic archive of the histopathology department of our institution and reviewed to confirm their diagnosis according to standard published criteria for neuroblastic tumors. Institutional R&D/ethical approval was obtained for this study (10SG11).

Hematoxylin and eosin–stained slides were reviewed by a consultant pediatric pathologist (N.J.S.) to verify the diagnosis and choose the representative sections for further evaluation. Immunohistochemical staining was performed on 4-μm tissue sections obtained from representative paraffin blocks cut onto superfrost slides. The tissue sections were dried at 37°C overnight and placed in the 60°C oven for 30 minutes before staining, treated with endogenous peroxidase (10% H_2O_2 in phosphate-buffered saline) for 20 minutes, and washed in water. A pretreatment in a pressure cooker in citrate buffer was performed for 20 minutes. Polyclonal rabbit antihuman BLBP antibody (dilution 1:1000, ab32423; ABCAM, Cambridge, UK) was used for immunohistochemical evaluation with appropriate positive and negative controls. Slides were incubated with the antibody for 30 minutes at room temperature. A Dako Real Envision detection kit (Dako UK Ltd, Ely, Cambs., UK) was used for visualization. Immunohistochemical staining results were evaluated by 2 observers (N.J.S./G.R.). Intensity of immunoreactivity was scored as negative, positive, or strongly positive, with consensus decisions being made for each case. Cases were labeled by study number only to minimize bias. CD56 immunostaining was also carried out in each case to exclude erroneous negative staining results caused by technical issues.

2. Results

There were marked differences in BLBP immunoreactivity among the neuroblastic tumors analyzed (Table 1). In all specimens of NB, expression was completely absent, whereas positive staining was observed in all the GNB and GN samples. The distribution of immunoreactivity within each tumor was further analyzed and summarized in Table 1 and Fig. 1. In NB specimens, there was no expression in any cell type regardless of the area analyzed (Fig. 1A). In contrast, in GN specimens, strong BLBP expression was detected in the cytoplasm of mature ganglion cells, whereas it was absent in the Schwannian stroma (Fig. 1B). In the intermixed GNB samples, there was positive BLBP immunoreactivity in the cytoplasm of fully differentiated and differentiating ganglion cells but no BLBP expression in the poorly differentiated adjacent neuroblasts (Fig. 1C, D). CD56 staining was appropriately positive in all cases.

3. Discussion

The present study demonstrates that BLBP is significantly expressed in mature and maturing ganglion cells in the neuroblastic tumors GN and intermixed GNB, with the extent and intensity of expression depending on cellular morphological maturity. Hence, BLBP represents a reliable marker of neuroblastic differentiation in this setting.

Neuroblastic tumors represent the most frequent extracranial childhood solid tumors and are neoplasms of the sympathetic ganglia and adrenal medulla [1,2,12]. They are thought to develop from the persistent population of embryonal neural crest cells that would otherwise either differentiate or undergo apoptosis [1,2,12]. Neuroblastic tumors are remarkable for their diverse pathological features, with a wide range of neuroblastic differentiation and amount

Table 1 BLBP immunoreactivity in neuroblastic tumors

	Neuroblastic cells	Ganglion cells	Schwann cells
GN (n = 10)	Not applicable	Strongly positive	Negative
GNB (n = 10)	Negative	Positive	Negative
NB (n = 10)	Negative	Not applicable	Not applicable