Journal of Neuro-Oncology (2005) 73: 101–108
DOI 10.1007/s11060-004-4598-2

Laboratory Investigation

CIC, a gene involved in cerebellar development and ErbB signaling, is significantly expressed in medulloblastomas

Ching-Jung Lee[1], Wai-In Chan[2,3] and Paul J. Scotting[2]
[1] *EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, UK;* [2]*Children's Brain Tumour Research Centre, Institute of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham, UK;* [3]*Current address: The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK*

Key words: capicua, cerebellum, ErbB, HMG, medulloblastoma

Summary

In children, the majority of brain tumors arise in the cerebellum. Medulloblastomas, the most common of these, are believed to originate from the granule cell lineage. We have recently identified a mammalian gene, *capicua* (*Cic*), the ortholog of a *Drosophila* gene implicated in c-erbB (Egfr) signaling, which is predominantly expressed during mouse granule cell development. Its expression in medulloblastoma is therefore of particular interest. In the present study the expression of human *CIC* in medulloblastoma was analyzed. *In silico* SAGE analysis demonstrated that medulloblastomas exhibited the highest level of *CIC* expression and expression was most common in tumors of the CNS in general. RT-PCR and *in situ* hybridization verified the expression of *CIC* in tumor cells, although the level of expression varied between different medulloblastoma subtypes. The expression of *CIC* did not correlate with other markers, such as neurofilament, GFAP and Mib-1. In postnatally developing cerebellum, *in silico* analysis and *in situ* hybridization both indicated a strong correlation between Cic expression and the maturation profile of cerebellar granule cell precursors. Expression of *CIC* is therefore a feature shared between immature granule cells and the tumors derived from them. Cic has been implicated as a mediator of ErbB signaling and this pathway has been associated with a poor prognosis for medulloblastomas. Therefore, further analysis of the role of Cic is likely to provide valuable insight into the biology of these tumors. Additionally, study of genes such as *CIC* should provide objective criteria by which, in combination with other markers and clinical data, to categorize these tumors into subgroups that might allow better allocation into specific treatment regimes.

Introduction

Primitive neuroectodermal tumors (PNETs) are a class of pediatric brain tumor composed of immature neural precursor cells and, in some cases, more mature neuronal cell types [1]. PNETs represent a wide range of tumors, composed of neuronal cells, astrocytes, oligodendrocytes, ependymal cells, other cell types (melanocytic and mesenchymal) and mixed cellular elements [2]. These PNETs occur in many locations in the CNS but most frequently arise in the cerebellum in which they are commonly referred to as medulloblastomas. Medulloblastomas constitute approximately 20% of brain tumors in children [3,4]. The tumors are believed to originate from cerebellar granule cell precursors that become transformed and fail to undergo normal differentiation [5]. However, alternative cells of origin have been suggested, which include the subependymal cells of the medullary velum region and possibly even the internal granule cell layer [6]. Medulloblastomas are broadly classified into two subtypes: classical and desmoplastic [7]. In classical medulloblastoma, tumor cells have a high nuclear cytoplasmic ratio and are associated with marked nuclear polymorphism and high mitotic activity. Desmoplastic medulloblastoma shows nodular, reticulin-free zones (known as 'pale islands' containing more differentiated cells) surrounded by densely packed, highly proliferative cells that produce a dense intercellular reticulin fiber network [7]. Recent studies reclassified these medulloblastomas and two more tumor variants, the 'medulloblastoma with extensive nodularity and advanced neuronal differentiation' [8] and 'large cell medulloblastoma' [9], were added. These tumor subtypes show different histological characteristics and malignant levels, however more molecular markers that indicate the cellular composition and tumor biology are needed for better classification and treatment planning.

It has been shown that neoplastic cells in these tumors recapitulate stages in maturation of normal human neuroblasts, therefore embryological studies of the earliest events in the development of the cerebellum may provide useful information about the molecular behavior of the tumor. Transcription factors such as Sox proteins, involved in cerebellar development, have been found to be highly expressed in medulloblastomas [10–12]. Significant *SOX4* and *SOX11* expression in classical medulloblastomas particularly reflects their maturation-dependent expression in normal cerebellar development [11]. The expression patterns of these developmentally regulated genes thus provide an indicator of cellular maturation.

We have previously identified the mammalian ortholog of a *Drosophila* gene, *capicua* (*Cic*), containing an HMG box that encodes an HMG domain most similar to that found in the *Sox* genes [13]. *Drosophila Cic* has

previously been shown to mediate c-erbB (Egfr) signaling via transcriptional repression [14,15]. This pathway has also been implicated in human medulloblastoma biology, where high levels of ERBB2 and ERBB4 correlate with poor prognosis [16,17]. Drugs that block ErbB signaling are now entering clinical trials [18]. Like several *Sox* genes, *Cic* is also predominantly expressed in the developing cerebellum [13]. Thus, analysis of *Cic* in medulloblastoma could provide valuable insight into the mechanism of ErbB signaling and provide a potential therapeutic target for selective modification of ErbB signaling. In this study, we analyzed the *CIC* expression in human medulloblastomas. *In silico* expression analysis demonstrated a highest *CIC* representation in medulloblastomas. *In situ* hybridization and RT-PCR showed that *CIC* was differentially expressed between various subtypes of medulloblastomas. The expression of *CIC* did not match that of the markers, neurofilament, GFAP and Mib-1 (Ki-67). The expression patterns of *CIC* and other markers suggest a complexity of cell composition and maturation levels in these tumors, consistent with the previous observation [19]. During post-natal cerebellum development, *in silico* analysis and *in situ* hybridization both indicated a strong correlation between *Cic* expression and the maturation profile of cerebellar granule cell precursors, reflecting the cellular composition and maturation of medulloblastoma. More comprehensive expression analysis of genes or markers involved in normal CNS development is in particular helpful for characterization of tumor biology as well as tumor classification.

Materials and methods

In silico *serial analysis of gene expression (SAGE)*

Serial analysis of gene expression (SAGE), described by Velculescu et al. [20] is based on the principle that a tag (a 9–10 nt nucleotide sequence) can uniquely identify a transcript, if the position of the sequence within it is known. The gene expression levels in different tissues can be estimated by the frequency of its tag numbers in the libraries derived from those tissues [21]. Data of 250 human and 40 mouse SAGE libraries derived from a number of normal and diseased tissues has been constructed and published in the weekly updated SAGEmap website (http://www.ncbi.nlm.nih.gov/SAGE/). We thus used the searching programs provided on this website to carry out an intact 3′ end sequence-based query mechanism into the experimentally derived SAGE library data and the SAGE gene to tag mapping by the UniGene clusters (human *CIC*: Hs.388236 using the reliable tag 5′-TGCAATATTT-3′; mouse *Cic*: Mm.28833 using the reliable tag 5′-TGCAATATTT-3′). *Cic* expression in normal and tumor SAGE libraries was then calculated and compared by the occurrence of *Cic*-specific tags in these libraries.

Tumor samples and cell lines

Twelve tumor samples from patients with diagnosed medulloblastoma were obtained from the Department of Pathology, Queens Medical Centre. This study was carried out with the Hospital approval. After histological examination, samples were either immediately fixed in formaldehyde and embedded in paraffin, or they were snap frozen in liquid nitrogen and stored at −80 °C for further RNA extraction. The pathological diagnosis was based on the criteria of WHO histological classification of CNS tumors [22]. cDNA from three medulloblastoma cell lines, D283, TE671 and DAOY, (kindly provided by Dr. Denise Sheer, ICRF, London [23]) were also analyzed. *In situ* hybridization and immunohistochemistry were used to analyze the expression of *CIC* and other markers. PCR was used to confirm data of *in situ* hybridization and to test the gene expression in cell lines.

Polymerase Chain Reaction (PCR) analysis

PCR was performed using Qiagen® Taq DNA Polymerase with primer pairs CIC-F 5′-CCCCTG AAGAAGACCTTTGAC-3′ and CIC-R 5′-GCA CTCTTGGGGGTGTTG-3′. Primers were designed spanning the last three predicted exons to analyze *CIC* expression. PCR conditions were: 1 cycle of 94 °C for 3 min; 40 cycle of 94 °C for 20 sec, 60 °C for 20 sec, 72 °C for 50 sec; 1 cycle of 72 °C for 3 min.

In situ *hybridization*

In situ hybridization was performed as described by Rex et al. [24]. Wax embedded human tumor sections and mouse cerebellum sections were dewaxed in Histolene clearing agent (CellPath, UK), rehydrated, treated with proteinase K (10 mg/ml pre-warmed to 37 °C) at room temperature for 15 min and then fixed with 4% PFA. The plasmid pΔKIAA0306-ApaI (containing a 764 bp portion of the 3′ UTR of KIAA0306) subcloned from the KIAA0306 clone and a mouse IMAGE EST clone (GenBank AF363690) highly homologous to human and mouse *Cic* were used as templates for digoxigenin-labeled riboprobe synthesis (Gibco BRL). Slides were incubated with anti-sense or sense (control) riboprobes at 70 °C overnight, then washed and submerged in a 1/5000 dilution of pre-absorbed sheep anti-digoxigenin-alkaline phosphatase Fab fragments (Roche) and incubated at 4 °C overnight. Alkaline phosphatase activity was detected using 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate) and nitroblue tetrazolium salt (NBT).

Immunohistochemistry

Immunohistochemical analysis of the markers, neurofilament, Glial fibrillary acidic protein (GFAP) and Mib-1 (Ki-67), was performed on the sections after color detection of the *in situ* hybridization signal. Slides were blocked with 20% serum in PBS solution and then incubated with primary antibody: anti-neurofilament (Dako), anti-GFAP (Dako) and anti-Mib-1 (Dako), in PBS with 5% serum in skimmed milk solution for 1–2 h. A biotinylated secondary antibody (sheep anti-mouse,

Amersham) diluted in PBS with serum was then used followed by alkaline phosphatase-conjugated streptavidin (Vector laboratories). Alkaline phosphatase activity was detected using Fast Red (Sigma).

Results

SAGE analysis of CIC expression in human tissues

In silico SAGE analysis (see Materials and methods) was performed to predict the expression profile of the human *CIC* gene in normal and tumor tissues. The SAGE gene to tag mapping program was used as previously described [11]. The *CIC* transcript was identified in 165 of a total 250 human SAGE libraries (Figure 1(a)). The 165 *CIC* positive SAGE libraries included 10 (100%) of the normal brain libraries, 57 (89%) of the brain tumor libraries, 50 (53%) of the non-brain normal libraries and 48 (58%) of the non-brain tumor libraries. The 57 brain tumor libraries showing *CIC* expression were 23 (92%) of the medulloblastoma libraries, 10 (91%) of the ependymoma libraries and 24 (86%) of the glial tumor libraries (Figure 1(a)).

Given the high *CIC* representation in the brain libraries, expression level of *CIC* in these tissues was examined. The gene expression level was indicated by the occurrence of its tag in SAGE libraries. These normal and malignant brain libraries contained variable *CIC* tag numbers and the highest expression of *CIC* in medulloblastomas was of particular interest (Figure 1(b)). We investigated *CIC* expression between 25 medulloblastoma and 10 normal brain SAGE libraries. *CIC* expression among medulloblastoma and normal brain libraries was dramatically variable (tag counts between ~0 and 227 per million) (Figure 1(c)). Medulloblastoma libraries exhibited highest levels of *CIC* expression, with 8 out of 25 libraries exhibiting higher levels than the highest level seen in a normal brain library (Figure 1(c)). Among 25 medulloblastoma libraries, the *CIC* tag count was highest (227 tags per million) in medulloblastoma B 98 05 P608, but absent in

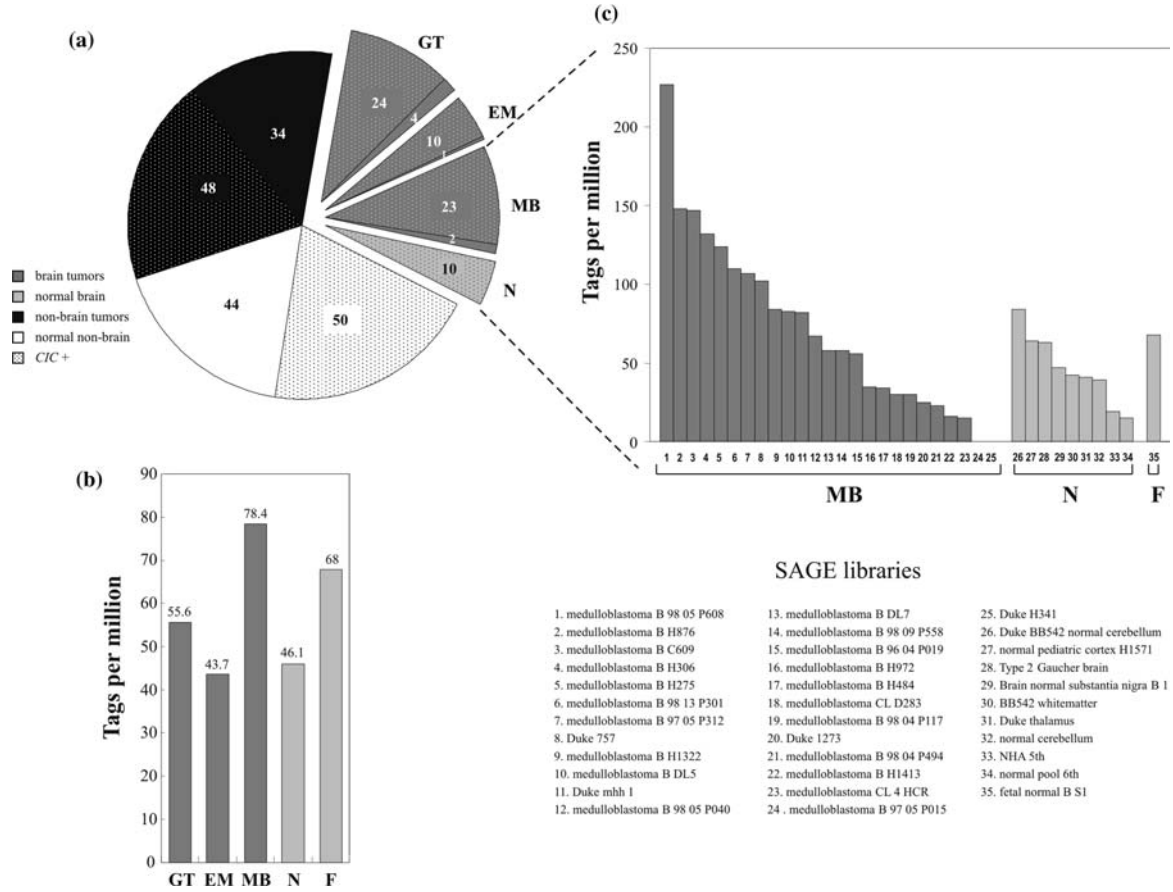

Figure 1. Human *CIC* representation in SAGE libraries. The on-line SAGE gene-to-tag program at the NCBI SAGEmap website was used to predict the expression profile of human *CIC*. The *CIC* UniGene cluster number, Hs.388236, and its reliable tag, 5′-TGCAATATTT-3′, were entered into the program. (a) *CIC* transcripts were detected in 165 of 250 SAGE libraries derived from various tissues (different classes by colors). Speckles indicate the libraries containing *CIC* specific tags. Numbers represent the number of libraries in each category. Among the human *CIC* positive libraries shown, 67 are from neoplastic or normal brains (i.e. 24 from glial tumors (GT), 10 from ependymoma (EM), 23 from medulloblastoma (MB) and 10 from normal brain (N)). (b) The expression level of *CIC* in 67 brain SAGE libraries is indicated by occurrence rate of the gene-specific tags in libraries. Each bar represents the average occurrence rate of *CIC* tags in the libraries derived from each tissue (also shown as numbers on the top of bars). The 10 normal brain libraries showing the *CIC* expression include 9 from adult and 1 from fetus. (c) *CIC* expression profile between 25 medulloblastomas and 10 normal brains. The names of SAGE libraries are also shown. More information about these libraries can be found at the SAGEmap website (http://www.ncbi.nlm.nih.gov/SAGE/index.cgi?cmd = printstats). GT, glial tumors; EM, ependymoma; MB, medulloblastoma; N, normal brain in (a) or normal adult brain in (b) and (c); F, fetal brain.

medulloblastoma B 97 05 P015 and Duke H341 (Figure 1(c)). A high *CIC* tag count was also observed in some normal brain libraries, particularly in Duke BB542 normal cerebellum (84 per million), derived from greater than 95% cerebellar whitematter, i.e. mostly glial cell types. Consistent with our previous observation of significant mouse *Cic* expression in the developing brain [13], SAGE analysis also indicated that human *CIC* was expressed in a fetal brain library, fetal normal B S1 (Figure 1(b and c)).

Human CIC expression in tumors

In order to verify the expression of *CIC* in medulloblastomas, we used RT-PCR to analyze the expression of *CIC* in three cell lines derived from medulloblastomas, D283 (metastatic medulloblastoma), TE671 (subline No.2) and DAOY (desmoplastic cerebellar medulloblastoma). All cell lines examined gave positive signals, with the strongest expression in D283 and DAOY cells (Figure 2).

To gain more detailed information about the nature of *CIC* expression, we analyzed *CIC* expression in various subtypes of medulloblastomas using non-isotopic *in situ* hybridization. Of the 12 tumors examined, 8 were classified as classical medulloblastoma (CMB), 3 as desmoplastic medulloblastoma (DMB), and 1 as medulloblastoma with desmoplastic components (MDC, mostly CMB appearance with some typical foci of DMB) according to the WHO classification of brain tumors [16]. *In situ* hybridization using anti-sense RNA probes derived from the 3′-UTR region of *CIC* revealed that 9 of 12 tumors were positive for *CIC* expression (Table 1). The 9 *CIC* expressing tumors included all (3) DMBs and 6 CMBs. No expression was detected in the MDC. The *CIC* expressing tumors showed highly variable *CIC* expression pattern (Figure 3). For instance, in one tumor, PN17, punctuate *CIC* expression was found in a small area (Figure 3(b)). In another tumor, PN14, *CIC* was strongly and widely expressed (Figure 3(c)). Even within the same tumor, such as in PN22 and in PN28, *CIC* showed differential expression levels in various regions (Figure 3(a and d)). These data together with the differential *CIC* expression profiles between medulloblastomas detected by *in silico* SAGE analysis demonstrate a significant difference of cellular composition and maturation level between these tumors, consistent with the previous observation [19]. No staining was observed when a sense control probe was used (data not shown).

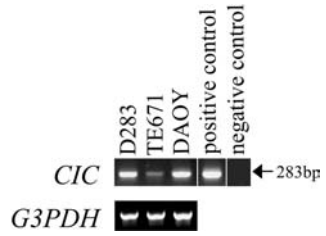

Figure 2. RT-PCR analysis showing human *CIC* expression. All three medulloblastoma cell lines (D283, TE671 and DAOY) showed *CIC* expression (upper panel). *G3PDH* gene expression was used as a control for RNA quality and quantity (lower panel). Reactions lacking cDNA and using DNA from KIAA0306 cDNA clone were performed as negative and positive controls, respectively. The authenticity of the PCR products was verified by sequencing the RT-PCR product from D283.

Comparison of the expression of CIC and other markers

Histological tumor samples were analyzed immunohistochemically using a neuronal marker (neurofilament), a glial marker (GFAP) and a proliferation marker (Mib-1) to characterize the cell types and the cellular maturation within the *CIC* positive regions. The expression pattern of these markers in tumors was compared to that of the *CIC* gene. Neurofilament (NF) proteins in embryonic CNS cells are a specific indicator of commitment to the neuronal lineage and are used to identify neuronal differentiation in tumors. Using an antibody recognizing total NF proteins, we found that a number of our medulloblastoma samples were NF positive (Table 1). In some NF expressing tumors, *CIC* expression was also detected, however, with different spatial expression patterns (Figure 4(a)). The regions with strong NF signals showed little or no *CIC* expression, whereas within the *CIC* positive regions very weak or no NF expression was detected (Figure 4(a)). It seems that *CIC* was expressed in a reciprocal pattern to NF reflecting areas of relatively undifferentiated cells. GFAP is involved in glial differentiation. Like NF, GFAP was found to be expressed in many of our medulloblastoma samples (Table 1). Within the same tumor section, only a small population of GFAP positive cells showed *CIC* expression (Figure 4(b)). Mib-1 (Ki-67) is a commonly used proliferative marker. Our result showed that the *CIC* expressing regions contained Mib-1 negative cells and a small number of Mib-1 positive cells (Figure 4(c)). Therefore, *CIC* was expressed both in proliferating and non-proliferating cells.

The comparative analysis of *CIC* and the markers, NF, GFAP and Mib-1 indicated that, although *CIC* was

Table 1. Summary of *CIC* expression in 12 medulloblastomas

Sample no.	Tumor type	*CIC*	Other markers
PN14	CMB	+	Mib-1+, NF+, GFAP+
PN17	CMB	+	Mib-1+, NF+, GFAP+
PN25	CMB	+	GFAP+
PN22	CMB	+	Mib-1+
PN31	CMB	–	ND
PN9	CMB	–	ND
PN28	CMB	+	GFAP+
PN29	CMB	+	ND
PN21	DMB	+	Mib-1-, GFAP+
PN32	DMB	+	ND
PN4	DMB	+	GFAP+
PN23	MDC	–	NF+

CMB, classical medulloblastoma; DMB, desmoplastic medulloblastoma; MDC, mostly CMB appearance with some typical foci of DMB; ND, not done. *CIC* expression was examined by *in situ* hybridization. Expression of markers, such as Mib-1, neurofilament and GFAP, was examined by immunohistochemistry.

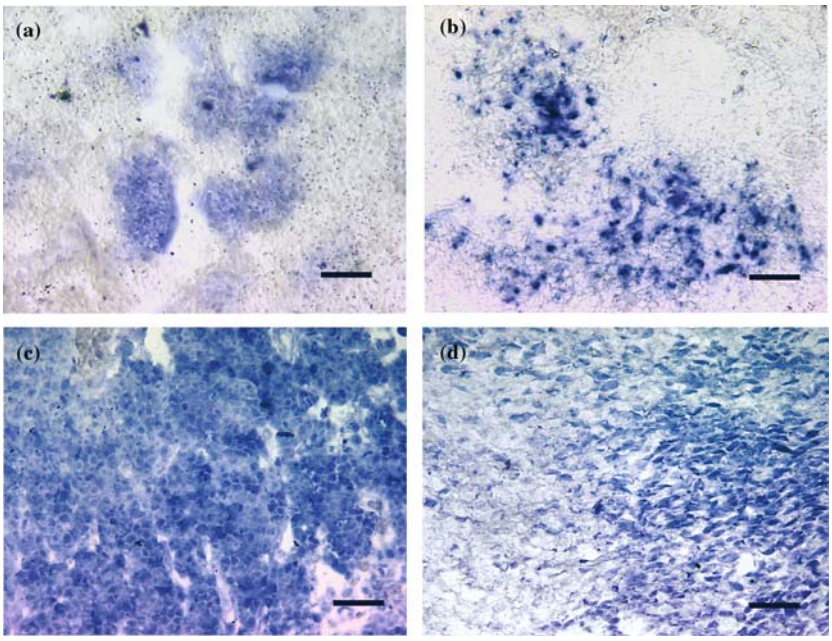

Figure 3. Human *CIC* expression in tumor sections. The *CIC* transcript was detected in blue by *in situ* hybridization. Panels a–d represent four tumor samples PN22, PN17, PN14 and PN28, respectively, showing differential *CIC* expression. Scale bars represent 25 μm.

widely expressed in tumors as well as in normal brains (see Results, *SAGE analysis of CIC*), its expression was primarily in immature cells, including both proliferating and post-mitotic cells. The variable expression patterns of *CIC* and other markers in medulloblastomas also suggested a wide range of cell composition in these tumors.

Cic expression in developing cerebellum

We have previously shown the predominant *Cic* expression in the post-natal immature cerebellum [13]. Given that the *Cic* expression pattern during normal cerebellar development may provide clues to the cell types and maturation level of the *CIC* expressing cells in medulloblastoma, here we carried out a more detailed analysis of the *Cic* expression in mouse developing and adult cerebellum using *in silico* SAGE analysis and *in situ* hybridization. *In silico* SAGE analysis revealed that *Cic* was highly expressed in all (3) libraries derived from the mouse post-natal day (P)8 primary cerebellar granule cell precursors (113–148 tags per million), the progenitor cells of medulloblastoma (Figure 5(a)). Interestingly, a mouse medulloblastoma also significantly expressed *Cic* (115 tags per million) (Figure 5(a)), consistent with its expression found in the human tumor tissues (Figure 1(c)). The *Cic*-specific tag count decreased in the P23 cerebellum (51 tags per million) and was absent in the adult whole brain library although a library from adult hippocampus did include the *Cic* transcripts (32 tags per million) (Figure 5(a)). Expression in mouse adult cerebellum was not analyzed by SAGE due to lack of the library data derived from that tissue in the database. *In situ* hybridization was performed using mouse dissected cerebellum tissues from P3, P7, P15 and adult. *Cic* expression was weakly detected at P3, strongest at P7, weaker again at P15, and then dramatically decreased by adult, accompanied by a migrating pattern from external granular layer (EGL) into the internal granular layer (IGL) (Figure 5(b)). These data suggest that the high *Cic* representation in medulloblastomas might reflect the immature state of the cells.

Discussion

Medulloblastoma, a pediatric brain tumor, is believed to originate from the undifferentiated cerebellar external granule layer [5]. Studies have shown that a number of genes involved in normal granule cell development are also expressed in medulloblastomas and may reflect the malignant potential of medulloblastomas [25,26]. The expression analysis of these genes in tumors could thus help gaining understanding of tumor biology. In this study, *in silico* SAGE analysis, RT-PCR and *in situ* hybridization all demonstrated *CIC* expression in medulloblastoma. The expression of this gene, predominantly expressed in immature granule cells [13], provides further evidence that this cell type is the origin of medulloblastoma. The expression pattern of *CIC* was particularly variable in these tumors. Comparison with other markers, such as NF, GFAP and Mib-1, shows that *CIC* is expressed in immature cells including mitotic and post-mitotic populations. *CIC* expression profile may therefore indicate the cellular composition and cell maturation level of medulloblastomas.

Several developmentally regulated HMG box proteins have been implicated in malignancies. For instance, HMGI/Y (HMGA) proteins are abundantly transcribed in early embryonic and rapidly proliferating cells [27]. Dysregulation of HMGI/Y is involved in a variety of benign tumors, such as uterine leiomyomas, endometrial polyps, lipomas and pulmonary hamartomas [28]. The other HMG box proteins, the TCF/LEF family, were

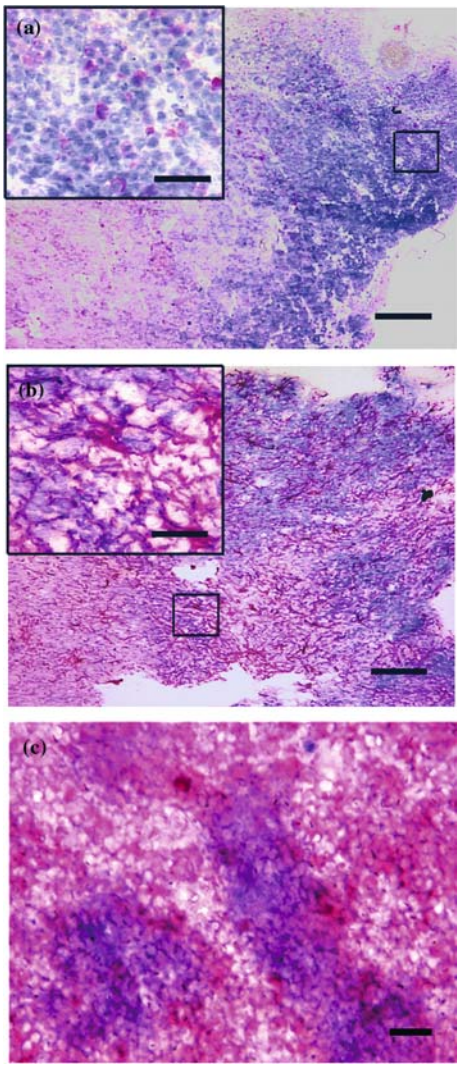

Figure 4. Comparison of *CIC* expression with others markers, neurofilament, GFAP and Mib-1, in medulloblastomas. *CIC* expression was detected by *in situ* hybridization, while the expression of other markers was analyzed by immunohistochemistry. (a) *CIC* (blue) and neurofilament (red) expression in a medulloblastoma sample, PN14. (b) *CIC* (blue) and GFAP (red) expression in a medulloblastoma sample, PN28. Inserts in panels A and B show higher power view of the boxed areas. (c) *CIC* (blue) and Mib-1 (red) in a medulloblastoma sample, PN22. In panels A and B, scale bars represent 25 μm in inserts and 100 μm in the low power views. Scale bar in panel C represents 10 μm.

found to associate with β-catenin and act as components in the Wnt signal transduction pathway [29,30]. This pathway has been shown to play a central role in many differentiation events during embryonic development and in neoplastic transformation after aberrant activation of its components [29–33]. In the Wnt signaling pathway, the APC protein forms a complex with GSK-3β which targets β-catenin for degradation. Wnt signaling inhibits the activity of GSK-3β resulting in an increase in the cytoplasmic-free pool of β-catenin and translocation into the nucleus. The LEF-1/TCF may mediate a nuclear response to Wnt signals by interacting with β-catenin [34]. Interestingly, the transcriptional activity of the TCF/β-catenin complexes is blocked by the interaction of TCFs with transcriptional co-repressors such as the TLE/Groucho family [29]. It has been shown that the *Drosophila* Cic protein can interact with Groucho and repress genes involved in *Drosophila* development [14,15,35]. The *Drosophila* Cic interacts with Groucho via its C-terminal region. This region is conserved in the *C. elegans*, mouse and human orthologs [13] and it therefore seems likely that this mode of action might be conserved. In addition to the Wnt signaling pathway, Cic–Groucho repression has been shown to be involved in the receptor tyrosine kinase (RTK) pathways. The *Drosophila* Cic–Groucho repression is regulated by two RTK pathways, Torso and c-erbB (Efgr) signaling systems, which cause Cic protein degradation and thus release the repression [14,15,35,36]. Cic therefore works as a sensor for these two RTK pathways that control early embryonic development and morphogenesis [14,15,35]. Signaling from RTKs is transduced via a common Ras/Raf/MAPK cascade to regulate the distinct downstream targets. The degradation of *Drosophila* Cic may be mediated via MAPK phosphorylation, consistent with the presence of 14 consensus sites for MAPK phosphorylation [14,35]. Human and mouse Cic proteins also contain 9 consensus MAPK phosphorylation sites. Like their *Drosophila* ortholog, human and mouse proteins may be post-translationally modified by MAPK and their involvement in RTK signaling may be conserved. Given the regulation of Cic at the post-translational level, it appears likely that studies of protein levels will be critical to determining a role for human CIC in these cancers; to this end we are now developing antibodies for its study.

In mammals, one of the RTKs, ErbB, has been implicated in the cerebellar development and medulloblastoma tumorigenesis [37]. ErbB also interacts with β-catenin in Wnt-mediated tumor progression suggesting the crosstalk between ErbB and Wnt pathways in malignancy [38]. Therefore, it will be very interesting to investigate the correlation between the RTK–Cic–Groucho pathway and Wnt signaling pathway, and the possible mechanism in tumorigenesis. As a first step towards this answer we have shown that mammalian Cic does indeed physically interact with mammalian Groucho orthologs (Chan et al., paper in preparation).

In conclusion, we have detected the differential expression pattern of the human *CIC* gene in the different subtypes of medulloblastomas. *Cic* is predominantly expressed in granule cells and maturing neuronal populations and appears to be tightly regulated during the maturation of granule neurons. Its variable expression in medulloblastomas may therefore reflect the complexity of tumor biology and, when combined with the expression of other markers and clinical data, may help to divide the tumors into the clinical subgroups. This gene is of particular interest since it is involved in two oncogenic signaling systems, the RTK (ErbB) and Wnt pathways, which contribute to medulloblastoma formation. Controlling the aberrant expression of these pathways is one of the major current strategies for identification of new treatments and improvements of existing therapies. Drugs that inhibit ErbB signaling are now entering phase I and II clinical trials for the treat-

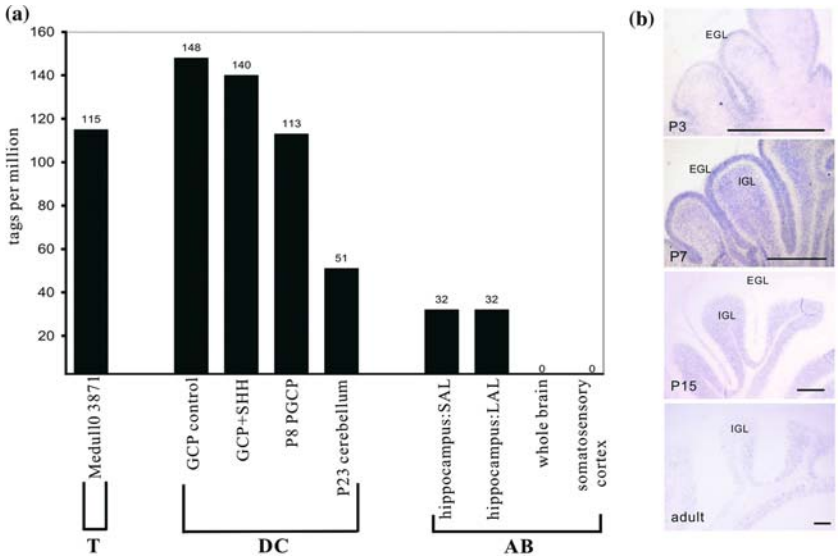

Figure 5. Mouse *Cic* expression in the developing brain. (a) The SAGE gene-to-tag program was used to predict the *Cic* expression in mouse CNS libraries using the *Cic* UniGene cluster number, Mm.28833, and its reliable tag, 5′-TGCAATATTT-3′. The expression in 4 developing cerebellum (DC) libraries (GCP control, GCP + SHH, P8 PGCP and P23 cerebellum), 1 cerebellar tumor (T), medulloblastoma (medullo 3871), and 4 normal adult brain (AB) libraries (hippocampus:SAL, hippocampus:LAL, whole brain and somatosensory cortex) was analyzed. Numbers on the top of bars represent the expression level of *Cic* in libraries (measured by gene-specific tag count per million). Significant *Cic* expression was observed in all three P8 cerebellar granule cell precursor (GCP) libraries (GCP control, GCP + SHH and P8 PGCP) and medulloblastoma. Information about these libraries can be found at http://www.ncbi.nlm.nih.gov/SAGE/index.cgi?cmd = printstats (b) Expression in the mouse cerebellum is shown in blue at stages P3, P7, P15 and adult by *in situ* hybridization on sagittal sections. *Cic* was expressed in the cerebellum throughout the post-natal development and at the adult stage, with strongest signal at P7. At P3, expression was predominantly in the external granular layer (EGL). At P7, expression was seen in the EGL and internal granular layer (IGL). At P15, expression was mostly detected in IGL and by adult only the IGL was weakly stained. Scale bars represent 500 μm.

ment of brain tumors [18]. Future studies on the detailed mechanisms of how these pathways mediate medulloblastoma development and the involvement of Cic in the pathways or in pathway crosstalk should provide a clearer insight into the medulloblastoma biology.

Acknowledgements

We thank Dr. Denise Sheer for cDNA samples and Jonathan Punt and David Walker of the Nottingham Children's Brain Tumour Research Centre for tumor samples. We are deeply indebted to the Kazusa DNA Research Institute for their KIAA0306 clone and many others who have contributed information to the public databases. This work was supported by a grant from the Trent NHS Research executive and the Children's Brain Tumour Research Centre.

References

1. Hart MN, Earle KM: Primitive neuroectodermal tumors of the brain in children. Cancer 32: 890–897, 1973
2. Deutsch M: Management of Childhood Brain Tumours. Kluwer Academic Publishers, USA, 1990, pp 1–3
3. Yachnis AT, Rorke LB, Trojanowski JQ: Cerebellar dysplasias in humans: development and possible relationship to glial and primitive neuroectodermal tumors of the cerebellar vermis. J Neuropathol Exp Neurol 53: 61–71, 1994
4. Russell DS, Rubenstein LJ: Pathology of Tumors of the Nervous System, 5th edn. Williams and Wilkins, Baltimore, 1989, pp 251–279
5. Buhren J, Christoph AH, Buslei R, Albrecht S, Wiestler OD, Pietsch T: Expression of the neurotrophin receptor p75NTR in medulloblastomas is correlated with distinct histological and clinical features: evidence for a medulloblastoma subtype derived from the external granule cell layer. J Neuropathol Exp Neurol 59: 229–240, 2000
6. Provias JP, Becker LE: Cellular and molecular pathology of medulloblastoma. J Neuro-Oncol 29: 35–43, 1996
7. Rubinstein LJ, Northfield DWC: The medulloblastoma and the so called "arachnoidal cerebellar sarcoma". Brain 87: 379–412, 1964
8. Giangaspero F, Perilongo G, Fondelli MP, Brisigotto M, Carollo C, Burnelli R, Burger PC, Garre ML: Medulloblastoma with extensive nodularity: a variant with favorable prognosis. J Neurosurg 91: 971–977, 1999
9. Giangaspero F, Rigobello L, Badiali M, Loda M, Andreini L, Basso G, Zorzi F, Montaldi A: Large cell medulloblastomas. A distinct variant with highly aggressive behavior. Am J Surg Pathol 16: 687–693, 1992
10. Cheng YC, Lee CJ, Badge RM, Orme AT, Scotting PJ: Sox8 gene expression identifies immature glial cells in developing cerebellum and cerebellar tumours. Brain Res Mol Brain Res 92: 193–200, 2001
11. Lee CJ, Appleby V, Orme A, Chan WI, Scotting PJ: Differential expression of SOX4 and SOX11 in medulloblastoma. J Neuro-Oncol 57: 201–214, 2002
12. Scotting P, Thompson S, Punt J, Walker D: Paediatric brain tumours: an embryological perspective. Child Nerv Syst 16: 261–268, 2000
13. Lee CJ, Chan WI, Cheung M, Cheng YC, Appleby V, Orme A, Scotting P: CIC, a member of a novel subfamily of the HMG-box superfamily, is transiently expressed in developing granule neurons. Brain Res Mol Brain Res 106: 151–156, 2002
14. Goff DJ, Nilson LA, Morisato D: Establishment of dorsal–ventral polarity of the Drosophila egg requires capicua action in ovarian follicle cells. Development 128: 4553–4562, 2001

15. Roch F, Jimenez G, Casanova J: EGFR signalling inhibits Capicua-dependent repression during specification of Drosophila wing veins. Development 129: 993–1002, 2002
16. Gilbertson RJ, Perry RH, Kelly PJ, Pearson AD, Lunec J: Prognostic significance of HER2 and HER4 coexpression in childhood medulloblastoma. Cancer Res 57: 3272–3280, 1997
17. Gilbertson RJ, Pearson AD, Perry RH, Jaros E, Kelly PJ: Prognostic significance of the c-erbB-2 oncogene product in childhood medulloblastoma. Br J Cancer 71: 473–477, 1995
18. Gilbertson RJ: Medulloblastoma: signalling a change in treatment. Lancet Oncol 5: 209–218, 2004
19. Adesina AM: Molecular heterogeneity in medulloblastoma with implications for differing tumor biology. J Child Neurol 14: 411–417, 1999
20. Velculescu VE, Zhang L, Vogelstein B, Kinzler KW: Serial analysis of gene expression. Science 270: 484–487, 1995
21. Lash AE, Tolstoshev C, Wagner L, Schuler G, Strausberg R, Riggins G, Altschul S: SAGEmap: a public gene expression resource. Genome Res 10: 1051–1060, 2000
22. Kleihues P, Burger PC, Scheithauer BW: Histological Typing of Tumors of the Central Nervous System. Springer, New York, 1993
23. Jones TA, Flomen RH, Senger G, Nizetic D, Sheer D: The homeobox MEIS1 is amplified in IMR-32 and highly expressed in other neuroblastoma cell lines. Eur J Cancer 36: 2368–2374, 2000
24. Rex M, Church R, Tointon K, Scotting PJ: Combination of non-isotopic *in situ* hybridisation with detection of enzyme activity, bromodeoxyuridine incorporation and immunohistochemical markers. Histochem Cell Biol 107: 519–523, 1997
25. Lee Y, Miller HL, Jensen P, Hernan R, Connelly M, Wetmore C, Zindy F, Roussel MF, Curran T, Gilbertson RJ, McKinnon PJ: A molecular fingerprint for medulloblastoma. Cancer Res 63: 5428–5437, 2003
26. Leung C, Lingbeek M, Shakhova O, Liu J, Tanger E, Saremaslani P, Van Lohuizen M, Marino S: Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. Nature 428: 337–341, 2004
27. Reeves R, Beckerbauer L: HMGI/Y proteins: flexible regulators of transcription and chromatin structure. Biochim Biophys Acta 1519: 13–29, 2001
28. Wisniewski JR, Schwanbeck R: High mobility group I/Y: multifunctional chromosomal proteins causally involved in tumor progression and malignant transformation (review). Int J Mol Med 6: 409–419, 2000
29. Seidensticker MJ, Behrens J: Biochemical interactions in the wnt pathway. Biochim Biophys Acta 1495: 168–182, 2000
30. Novak A, Dedhar S: Signaling through beta-catenin and Lef/Tcf. Cell Mol Life Sci 56: 523–537, 1999
31. Eberhart CG, Tihan T, Burger PC: Nuclear localization and mutation of beta-catenin in medulloblastomas. J Neuropathol Exp Neurol 59: 333–337, 2000
32. Huang H, Mahler-Araujo BM, Sankila A, Chimelli L, Yonekawa Y, Kleihues P, Ohgaki H : APC mutations in sporadic medulloblastomas. Am J Pathol 156: 433–437, 2000
33. Zurawel RH, Chiappa SA, Allen C, Raffel C: Sporadic medulloblastomas contain oncogenic beta-catenin mutations. Cancer Res 58: 896–899, 1998
34. Eastman Q, Grosschedl R: Regulation of LEF-1/TCF transcription factors by Wnt and other signals. Curr Opin Cell Biol 11: 233–240, 1999
35 Jimenez G, Guichet A, Ephrussi A, Casanova J: Relief of gene repression by Torso RTK signalling: role of capicua in Drosophila terminal and dorsoventral patterning. Genes Dev 14: 224–231, 2000
36. Furriols M, Casanova J: In and out of Torso RTK signalling. EMBO J 22: 1947–1952, 2003
37. Gilbertson RJ, Clifford SC, MacMeekin W, Meekin W, Wright C, Perry RH, Kelly P, Pearson AD, Lunec J: Expression of the ErbB-neuregulin signaling network during human cerebellar development: implications for the biology of medulloblastoma. Cancer Res 58: 3932–3941, 1998
38. Schroeder JA, Adriance MC, McConnell EJ, Thompson MC, Pockaj B, Gendler SJ: ErbB-beta-catenin complexes are associated with human infiltrating ductal breast and murine mammary tumor virus (MMTV)-Wnt-1 and MMTV-c-Neu transgenic carcinomas. J Biol Chem: 277: 22692–22698, 2002

Address for offprints: Paul J. Scotting, Institute of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK.; Tel.: +44-115-970-9367; Fax: +44-115-970-9906; E-mail: paul.scotting@nottingham.ac.uk