Presence of neural cell adhesion molecule on human embryonic and brain tumours

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Development and maintenance of tissue organization in complex organisms has long been suspected to involve altered cell surface interactions between adjacent cells. Similarly, the process of tumour metastasis undoubtedly requires the expression of appropriate attachment sites for the embedding and subsequent proliferation of secondary tumours. Exciting developments in molecular cell biology are opening up new avenues for the study of both of these phenomena. The past few years have seen the identification of a whole array of glycoproteins which are involved in cell-cell and cell-substrate adhesion.

One of the best characterized cell adhesion molecules is the neural cell adhesion molecule (N-CAM) [1]. We became interested in this glycoprotein when we showed that monoclonal antibody UJ13A [2], an antibody which has found extensive use in cancer medicine, recognizes N-CAM. This has led to the investigation of N-CAM expression in human embryonic and brain tumours to assess whether lack or abnormal expression contributes to tumour pathology and metastasis. In addition, identification of specific N-CAM isoforms associated with various tumours may allow construction of appropriate probes for differential diagnosis of tumours. Here, we briefly outline the expression of N-CAM in normal tissues and review the available data on its expression in various tumours.

Introduction to N-CAM

N-CAMs are a family of closely related cell surface glycoproteins believed to mediate cell-cell interactions via a homophilic binding site in a Ca²⁺-independent manner. The glycoproteins are coded for by a single gene located on chromosome 11q23 in humans. At least 19 exons have been described in the chicken and by extrapolation it is presumed that there are a similar number of genes in the human. Various species of N-CAM proteins have been identified which are believed to arise through alternative splicing of mRNA and differential usage of multiple polyadenylation sites [3]. The glycoproteins undergo a variety of post-translational modifications such as sulphation, phosphorylation and glycosylation [4]. A particular feature of the protein is the presence of long chains of polysialic acid residues linked by α2–8 linkages [5]. The polysialylation and expression of N-CAM is spatially and temporally controlled.

In the chicken, mRNA size classes of 7.4, 6.7 and 4.2 kb have been identified, whereas in the mouse and human, 7.4, 6.7, 5.2, 4.3 and 2.9 kb species are present. The 7.4 kb and 6.7 kb transcripts have been most clearly shown to code for N-CAM-180 and N-CAM-140, respectively. In addition, a secreted form of molecular mass 115 kDa has been shown to be coded for by 5.2 kb mRNA [6]. It is believed that N-CAM-120 is coded for by 5.2, 4.3 and 2.9 kb transcripts.

N-CAM from various sources has been cloned and sequenced. The molecule belongs to the immunoglobulin supergene family. Members of this family share a common structural domain called the immunoglobulin homology unit.

Five immunoglobulin-like domains have been described in N-CAM [1].

N-CAM expression in normal tissues

During embryogenesis, N-CAM is widely expressed in tissues derived from all three germ layers. These include fetal brain, fetal skeletal and cardiac muscle and fetal kidney. In the adult, N-CAM expression was thought to be restricted to tissues of the nervous system and neuro-muscular junctions, although it is now becoming clear that this is not so. In addition, N-CAM is present in adult regenerating skeletal muscle and diseased cardiac muscle. The N-CAMs in brain and skeletal muscle have been well characterized and are described in detail below.

Brain. In the mouse brain, four main isoforms of N-CAM have been described of 180, 140, 120 and 115 kDa (in their desialo form) [6, 7]. The presence of N-CAM-170 in normal brain tissue has recently been reported by Bhat & Silberberg [9]. Their data suggests that N-CAM-170 arises from a different primary protein product rather than from degradation or post-translational modification of the other isoforms of N-CAM. We have also examined N-CAM expression in human brain during development [10]. The data show that the expression of N-CAM-180 decreases, whereas that of N-CAM-120 increases as development proceeds. In contrast, the expression of N-CAM-140 remains relatively unchanged. Differences in N-CAM expression in different regions of the adult brain were also observed. White matter from the cerebrum and cerebellum expressed only N-CAM-120, whereas the grey matter expressed all three isoforms.

Muscle. Skeletal muscle myoblasts express a transmembrane isoform of 140 kDa. As these cells differentiate and fuse to form myotubes, the 140 kDa isoform is replaced by N-CAM-155 and N-CAM-125 GPI-linked isoforms [10]. A secreted protein of 115 kDa has also been described [6]. The GPI-linked proteins have been shown to contain additional sequences in their extracellular domain which is restricted to skeletal muscle N-CAM and has been termed muscle-specific domain (MSD-1). This sequence is the best-described tissue-specific sequence associated with N-CAM. MSD-1 is comprised of three discrete exons [11]. In addition, the protein backbone translated from these three exons are sites for O-glycosylation [12]. Tissue-specific sequences have also been described in chick cardiac muscle [13].

Abbreviations used: N-CAM, neural cell adhesion molecule; GPI, glycosylphosphatidylinositol; MSD, muscle-specific domain.
Other tissues. It is becoming clear that N-CAM expression in the adult may not be as restricted as previously believed. N-CAM is present in low quantity in a number of non-neuroectodermally derived tissues.

Murine stromal cells have been shown to express a predominant N-CAM of 155 kDa and minor isoforms of 220 and 135 kDa [14]. Unpublished work (K. Patel, F. Gibson & J. T. Kemshead) from this laboratory suggests that N-CAM is present on a small population of human marrow stromal cells grown in Dexter culture. Antibodies UJ13A and ERIC which recognize different epitopes on N-CAM, consistently stain human marrow stromal cells. At present, we do not know whether a low level of N-CAM expression is present on various sources (human, mouse) have also been shown to express N-CAM in developmentally regulated manner [15, 16].

Monoclonal antibody T-199 described by Feickert et al. [17] also probably recognizes N-CAM on human NK cells. Although the glycoprotein identified by this antibody has not been formally proven to be N-CAM, the antigen has similar characteristics to those of N-CAM. Apart from cells of haemopoietic origin, the presence of N-CAM has also been demonstrated in adult rat endocrine cells [18] and chick lung epithelium [19].

N-CAM expression in tumours

We have shown that monoclonal antibody UJ13A, raised against human fetal brain, recognizes N-CAM [2]. This antibody reacts with tumours of neuroectodermal origin, e.g. neuroblastoma and melanoma. In addition, the reagent reacts with rhabdomyosarcoma, Wilms' tumour, leiomyosarcoma, small cell lung carcinoma and occasionally leukaemia. The corollary is that N-CAM is present on these tissues. We have undertaken a detailed study to characterize the N-CAMS present on these tumours since information in this important area of tumour biology is lacking.

Neuroblastoma. Apart from the data from our laboratory, there have been only two reports regarding N-CAM expression in human neuroblastoma. Lipinski et al. [20] reported the presence of N-CAM-180 and N-CAM-140 and their corresponding mRNA transcripts in the human neuroblastoma cell line IMR32. We have confirmed this finding and undertaken a more thorough study of N-CAM expression in human neuroblastoma cell lines. Using a human N-CAM-specific cDNA probe, Northern Blot analysis showed that in general, the 6.7 kb and 5.5 kb species were present in the human neuroblastoma cell lines tested (exceptions were NB-1 and IMR-32 line which expressed the 7.4 kb transcript as well).

The 14.0 kDa protein coded by 6.7 kb mRNA is the earliest N-CAM expressed in embryogenesis and it is therefore not surprising that the 6.7 kb mRNA is predominantly expressed in embryonic tumours such as neuroblastoma. The pattern of mRNA expression in neuroblastoma cell lines is at variance with that seen in fetal brain. RNA transcripts of 7.4, 6.7, 5.5, 4.3 and 2.9 kb were observed in fetal brain. The significance of this observation is unclear at present. Preliminary studies on the protein isoforms present corroborates the above data. Highly sialylated N-CAMs characteristic of the embryonic form are present. This result agrees with that of Livingstone et al. [21] who reported the presence of polysialic acid chains on glycoproteins from human neuroblastoma cell lines. Although the glycoprotein carrying these polysialic acid chains was not identified, it may well be N-CAM since this is the only known glycoprotein in vertebrates that has long chains of polysialic acid attached to it. The type of linkage utilized by N-CAM in neuroblastoma cell lines is being characterized at present. In addition, N-CAM isoforms in neuroblastoma tissues are being investigated since culturing of cells in vitro may have altered N-CAM expression.

Brain tumours. The presence of N-CAM in brain tumours has been assessed in our laboratory by Western blot analysis. Differences in N-CAM isoforms present in any particular tumour were noted. Oligodendrogioma and grade II astrocytoma were shown to express N-CAMs-180, -140 and -120, whereas medulloblastoma, juvenile astrocytoma, Schwannomas and ependymomas expressed only N-CAM-140 and N-CAM-120. Gliomas, on the other hand expressed N-CAM-180 and N-CAM-120. At present, it is not clear whether small differences (10–20 kDa) between N-CAM from normal tissue and tumours is due to post-translational modification or differences in the primary protein product. This is currently under investigation.

Rhabdomyosarcomas. There are no reports regarding N-CAM expression in rhabdomyosarcomas. However, data from this laboratory show that N-CAM can be expressed in this childhood tumour. Transcripts of 6.7 and 5.5 kb have been detected in some of the rhabdomyosarcoma cell lines by Northern Blot analysis. In addition, the JR1 cell line expresses a highly sialylated N-CAM which on neuraminidase treatment resolves into bands of 140 and 120 kDa.

Wilms' tumour. Roth et al. [22] have demonstrated the presence of embryonic N-CAM in Wilms' tumour by immunohistochemistry using an antibody directed against polysialic acid. Data from our laboratory corroborate this finding. Wilms' tissues were shown to express an embryonic N-CAM which a neuraminidase treatment resolved into N-CAM-140 and N-CAM-120.

Haemopoietic cell malignancies. While in general, leukaemias do not react with UJ13A, there are reports suggesting that occasionally leukaemic cell lines can react with UJ13A and therefore express N-CAM. In addition, fresh leukaemias do occasionally react with UJ13A.

In our laboratory, we have shown the presence of N-CAM on Nalm-16 cell line, a pre-B acute lymphoblastic leukaemia cell line. A smear characteristic of embryonic N-CAM is seen in Western blot analysis. On neuraminidase treatment, a major band of 145 kDa and a minor band of 120 kDa were observed. This is corroborated by Northern blot analysis where 6.7 kb and 5.5 kb mRNA size classes are detected. The results of Lanier et al. [16] also show that N-CAM is present on other cell lines, e.g. KG1a, a myeloid leukaemia cell line, which expresses a 145 kDa transmembrane isoform.

Future studies

Although N-CAM has been shown to be present on a number of tumours, it remains a poorly characterized molecule in these tumours. Therefore, future studies will be conducted in this area to understand whether there are any subtle alterations which could play a role in the pathalogy of tumours. In addition, biological studies need to be carried out to show whether the N-CAM from tumours is functional in cell–cell adhesion and subsequent signal transduction pathways, whatever they may be.

From a clinical point of view, if one can show that tissue-specific forms of N-CAM resulting from splicing of the unique sequences are present in different tumours, it should be possible to produce highly specific probes (either oligonucleotides or antisera against the peptide sequence) for differential screening of tumours.

We are currently exploring this possibility and have begun a study to investigate the presence of MSD-1 in rhabdomyosarcoma using the polymerase chain reaction.
Characterization of a regulatory region within the human neural cell adhesion molecule gene

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Surface modulation of cell adhesion molecules is a potential mechanism for the regulation of events contributing to tissue morphogenesis [1]. A group of related cell surface glycoproteins known collectively as the neural cell adhesion molecule (N-CAM) [2] have been functionally implicated in neurogenesis [3], axonal outgrowth and guidance [4], and the development and innervation of skeletal muscle [5]. N-CAM is encoded by a single gene from which several transcripts and polypeptides arise by alternative splicing mechanisms and by use of alternative polyadenylation signals [6–9]. N-CAM expression is regulated throughout embryonic development by both developmental stage- and tissue-specific mechanisms [10–12], though details of these mechanisms are not yet fully defined. Changes in N-CAM expression have been demonstrated during myogenesis [13, 14], and similar events shown in cell culture [13], have provided evidence for modulation of N-CAM isoform usage as well as regulation of N-CAM gene transcription. Similarly, neuron-specific mRNA splicing events and increased overall expression of N-CAM mRNAs have been described during neuronal differentiation [14], and in the pheochromocytoma PC12 cell line in response to treatment with nerve growth factor [15]. Furthermore, modulation of N-CAM levels has been reported during reinnervation of denervated muscle [5, 16, 17], thyroidectomy [18], and in association with disease states including polymyositis [19], small lung cell carcinoma and neuroblastoma [20].

Regulatory cis-acting DNA elements in the 5’ flanking region of a vast number of developmentally regulated mamalian genes [21] are required for both tissue- and stage-specific control of transcriptional activities. To identify and characterize the functions of those elements in the human N-CAM gene that contribute to transcriptional regulation of N-CAM expression, we have isolated genomic DNA spanning the 5’-most exon of the human N-CAM gene.

A cDNA probe from clone A5 containing only 5’ untrans- lated sequence (bases 237 to 145) is found, by Northern blot analysis, to hybridize with transcripts for trans- membrane N-CAM-140/180 kDa isoforms (6.7 and 7.2 kb) found in embryonic human brain, as well as with transcripts isolated from primary human fetal muscle cultures encoding transmembrane 140 and 180 kDa isoforms, 115 kDa secreted (5.2 kb) and 125 kDa glycosylphosphatidylinositol-linked (GPI-linked) (4.3 and 2.9 kb) N-CAM species. Thus, transcripts encoding embryonic brain N-CAMs and all muscle N-CAMs contain common or very similar 5’ sequences. A lack of alternative splicing within the 5’ sequence was shown by S1 nuclease protection using a single-stranded 32P-labelled cDNA probe from A5 (237–115 bases) and spanning the first two exons of the human gene. These data indicate that transcripts for all N-CAM isoforms in muscle and brain are likely to be regulated from a single transcriptional domain and confirm the constitutive utilization of common 5’ sequences.

Genomic clones were isolated from a charon 35 library using a cDNA probe (clone A5 bases 237–115) encoding exons one and two of the human A. hindIII fragment of 2 kb from clone g3, that preferentially hybridized with the 5’ region of the probe (bases 237 to 145), was isolated and analysed by detailed restriction endonuclease mapping (Fig. 1). Sites for transcriptional initiation were determined by S1 nuclease protection using a single-stranded probe from g3 (bases 2015 to +5 relative to the ATG codon) with total RNA samples from human primary muscle cultures. Two major initiation sites were identified – 194 and 188 bases from the ATG codon. These two major sites of transcriptional initiation determined for human muscle RNAs confirm earlier studies [22] for sites of initiation determined using mouse brain RNAs.

The DNA sequence flanking the first exon of the human N-CAM gene was determined by analysis of the 2 kb hindIII


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Abbreviations used: N-CAM, neural cell adhesion molecule; GPI, glycosylphosphatidylinositol; CAT, chloramphenicol acetyltransferase.