# EXPRESSION OF ENTRY RECEPTOR NECTIN-1 OF HERPES SIMPLEX VIRUS 1 AND/OR HERPES SIMPLEX VIRUS 2 IN NORMAL AND NEOPLASTIC HUMAN NERVOUS SYSTEM TISSUES

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**Summary. –** Herpes simplex virus 1 and/or Herpes simplex virus 2 (HSV) are important pathogens of human nervous system (NS) and genetically modified HSV strains have been proposed as vectors for gene therapy targeting the brain and brain tumors. Nectin-1 is an immunoglobulin-like adhesion molecule that participates in the formation of synapses and serves as an entry receptor for HSV. The expression pattern of nectin-1 in normal human NS and brain tumors is not well understood. To better understand the nectin-1 expression in normal and neoplastic human NS, immunohistochemistry was used to detect the nectin-1 expression in sections of normal human brain, spinal cord and trigeminal and dorsal root ganglia (n=10) and in sections of primary NS neoplasms (n=22). In normal human NS, nectin-1 was detected in the soma and processes of central and peripheral neurons, in ependymal cells, choroid plexus epithelial cells, vascular endothelial cells and meningothelial cells. Oligodendrocytes, astrocytes, vascular smooth muscle cells, and Schwann cells showed variable immunoreactivity. Among tumors, schwannoma, fibrous meningioma, and medulloblastoma were nectin-1 negative. Oligodendroglioma, ependymoma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, anaplastic astrocytoma, glioblastoma multiforme and meningothelial meningioma showed weak focal nectin-1-positivity. Ganglion cells of ganglioglioma were strongly positive. These studies provide novel information about the expression of nectin-1 in normal and neoplastic NS, and thus may lead to a better understanding of cell targeting by HSV during HSV-induced neurological disease and during a HSV-based gene therapy.

**Key words:** nectin-1; Herpes simplex virus 1; Herpes simplex virus 2; cell adhesion; brain tumor; glioblastoma multiforme; gene therapy

## Introduction

Infection of central nervous system (CNS) with HSV is an important cause of some neurological diseases, most notably encephalitis and meningitis (Whitley, 2001). HSV can infect a wide range of cell types within CNS including neurons, glial cells and vascular endothelial cells (Whitley, 2001). In HSV encephalitis, there is viral replication within the brain and a necrotizing inflammatory reaction. Survivors may suffer severe neurological sequelae due to permanent damage to neural structures including extensive loss of neurons. How viral and host factors determine which cells and structures in the brain are targeted by HSV is not well understood. A better understanding of the molecular mechanisms by which HSV enter cells and disseminate from cell to cell in NS may provide novel insights into the pathogenesis of HSV infections of the brain, including virus tropism, virus spread from cell to cell, and mechanisms of

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**Abbreviations:** CNS = central NS; 3-OS-HS = 3-O-sulfated heparan; gB, gC and gD = glycoproteins B, C and D, respectively; HSV-1 = Herpes simplex virus 1; HSV-2 = Herpes simplex virus 2; HSV = HSV-1 and/or HSV-2; HVEM = herpes virus entry mediator; NS = nervous system; TNF = tumor necrosis factor

neural injury. In addition, genetically modified HSV strains have been proposed as vectors for gene therapy targeting the brain and for tumor therapy targeting brain tumors, mainly malignant gliomas (Engelhard, 2000; Shah *et al.,* 2003; Glorioso and Fink, 2004; Lou, 2004; Niranjan *et al.,* 2004). Thus, a better understanding of the molecular mechanisms by which HSV target, bind and enter cells of NS and cells of brain tumors may lead to the development of improved therapeutic approaches.

A significant progress has been made in the studies of molecular basis of virus-host interactions, which lead to HSV entry into cells (Spear *et al.,* 2000; Shukla and Spear, 2001). The latter requires an interaction of several viral glycoproteins with specific cell-surface receptors. The current model of virus entry involves the attachment of viral glycoproteins B (gB) and C (gC) to host cell heparan sulfate, allowing glycoprotein D (gD) to engage one of the herpesvirus entry mediators (WuDunn *et al.,* 1989; Herold *et al.,* 1994; Mettenleiter *et al.,* 1990; Shieh *et al.,* 1992; Pertel *et al.,* 2001).

Two cell-surface proteins, herpes virus entry mediator (HVEM) and nectin-1 are known to mediate the entry of HSV into cultured cells by interacting with gD. HVEM is a member of the TNF receptor family (Montgomery *et al.,* 1996). Nectin-1 (CD111), on the other hand, is a member of the immunoglobulin (Ig) superfamily, closely related to the poliovirus receptor CD155 (Eberle *et al.,* 1995; Lopez *et al.,* 1995; Geraghty *et al.,* 1998; Warner *et al.,* 1998; Menotti *et al.,* 2000; Milne *et al.,* 2001). Another member of this family, nectin-2, mediates the cell entry of some mutant strains of HSV-1 and possibly HSV-2 (Eberle *et al.,* 1995; Lopez *et al.,* 1995; Geraghty *et al.,* 1998; Warner *et al.,* 1998; Haarr *et al.,* 2001). In addition, 3-O-sulfated heparan sulfate (3-OS-HS) serves as a gD receptor specific for HSV-1 (Shukla *et al.,* 1999; Tiwari *et al.,* 2004; Xia *et al.,* 2002).

Among the known gD receptors, nectin-1 has been studied most intensively and appears to play a critically important role in HSV infections of NS (Cocchi *et al.,* 1998; Geraghty *et al.,* 1998; Shatoh-Horikawa *et al.,* 2000; Pokutta *et al.,* 2002; Lopez *et al.,* 2001; Richart *et al.,* 2003). Nectin-1 and the related nectin-2 and nectin-3 are cell adhesion molecules found at $Ca^{2+}$-dependent cadherin-based cell adhesion junctions in epithelial and other types of cells (Takahashi *et al.,* 1999; Miyahara *et al.,* 2000; Nishioka *et al.,* 2000; Satoh-Horikawa *et al.,* 2000; Tachibana *et al.,* 2000; Yoon and Spear, 2002). Nectin-1 and nectin-2 are engaged in both homotypic *cis* and *trans* interactions (Miyahara *et al.,* 2000) and in heterotypic *trans* interactions with other members of the nectin family (Takai *et al.,* 2003a). Nectins are involved in the organization of interneuronal synapses, growth cone elongation and signaling leading to reorganization of the cytoskeleton, gene expression and cell polarization (Mizoguchi *et al.,* 2002; Takai *et al.,* 2003b).

Analysis of RNA extracted from various organs or cultured cells has provided an important insight into the patterns of nectin-1 expression in human and animal tissues (Lopez *et al.,* 1995; Geraghty *et al.,* 1998; Menotti *et al.,* 2000; Satoh-Horikawa *et al.,* 2000; Shukla *et al.,* 2000; Haarr *et al.,* 2001; Hung *et al.,* 2002). Expression of nectin-1 is especially prominent in the brain, kidneys, liver, pancreas, skin, cornea and retina, and in cell lines of neuronal and epithelial origin (Lopez *et al.,* 1995; Geraghty *et al.,* 1998; Menotti *et al.,* 2000; Satoh-Horikawa *et al.,* 2000; Haarr *et al.,* 2001; Hung *et al.,* 2002; Richart *et al.,* 2003; Valyi-Nagy *et al.,* 2004; Shukla *et al.,* 2000, 2005). In rats, nectin-1 is expressed at high levels in sensory neurons and their axons and at lower levels in motoric neurons of the spinal cord (Mata *et al.,* 2001). In mice, there is widespread expression of nectin-1 in neurons of peripheral and central NS and in cells of the choroid plexus epithelium, and a less consistent expression of nectin-1 in glial cells (Haarr *et al.,* 2001; Shukla *et al.,* 2005).

The expression pattern of nectin-1 in the human brain, particularly in non-neuronal cells is not well understood. In addition, there is no information about the expression of nectin-1 in tumors of NS, which are potential targets of HSV tumor therapy.

Therefore, in this study, in order to better understand the expression pattern of nectin-1 in normal and neoplastic human NS, we used immunohistochemistry for the detection of nectin-1 expression in normal and neoplastic human brain tissues. We found that (i) nectin-1 was widely expressed in human peripheral and central NS tissues, (ii) the nectin-1 expression was not limited to neurons, (iii) in brain tumors, the nectin-1 expression depended on the histology of the tumor, and (iv) in malignant gliomas, the primary targets of HSV-based oncolytic therapy, the nectin-1 expression was only focal.

## Materials and Methods

*Tissues.* Sections of formalin-fixed, paraffin-embedded archival normal human brain, spinal cord, trigeminal ganglion and dorsal root ganglia (n=10) from autopsies performed at the Department of Pathology, University of Illinois at Chicago, and sections of paraffin-embedded neurosurgical specimens of NS neoplasms (n=22) were examined. Neurosurgical specimens were taken from craniotomies performed at the University of Illinois Hospital, Chicago. Histological diagnostics was done by a board-certified neuropathologist (T.V-N). The use of autopsy and neurosurgical human tissue specimens was approved by the Institutional Review Board of the University of Illinois at Chicago. The tumors examined for nectin-1 expression included oligodendroglioma (WHO grade II, n=2), diffuse astrocytoma (WHO grade II, n=2), anaplastic astrocytoma (WHO grade III, n=2), glioblastoma multiforme (WHO grade IV, n=3), ganglioglioma (WHO grade I, n=1),

pilocytic astrocytoma (WHO grade I, n=1), pleomorphic xanthoastrocytoma (WHO grade II, n=2), ependymoma (WHO grade II, n=2), meningioma (WHO grade I, n=3; two histologal variants: meningothelial (n=2) and fibrous (n=1)), medulloblastoma (WHO grade IV, n=2), and schwannoma (WHO grade I, n=2).

*Immunohistochemical detection* of nectin-1 was performed as described previously (Valyi-Nagy *et al*., 2004).Briefly, tissue sections for immunohistochemistry were deparaffinized with xylene and rehydrated through a series of graded ethanols. Tissue sections were hydrated with distilled water and antigen retrieval was performed using DAKO Target Retrieval Solution 10x Concentrate (DAKO, Carpinteria, CA). Non-specific staining was blocked using an hydrogen peroxide solution for 10 mins followed by a protein block for 10 mins. The sections were incubated with a nectin-1-specific antiserum (1:50 dilution) at room temperature for 1 hr. Nectin-1 staining was don using the DAKO Envision + Kit. The sections were examined by two independent observers and the extent of nectin-1 expression was evaluated using a semi-quantitative grading system. The latter scored the estimated percentage of nectin-1-positive cells from 0 to 4. (0=0%, 1<5%, 50%>2>5%, 3 = 50–95%, 4>95%) and the strength of the nectin-1 signal (increasing from 1+ to 4+). Cell types in nectin-1 stained sections were identified by morphological criteria. Those cell types, which were not readily distinguishable by these criteria, e.g. oligodendrocytes and astrocytes, were considered single groups of cells.

## Results

### *Nectin-1 is expressed in neurons of human central and peripheral NS*

The expression of nectin-1 in human central and peripheral NS was determined immunohistochemically using a nectin-1-specific antiserum. Besides normal and neoplastic human NS tissues also normal human corneal and uterine tissues were examined to check the capability of the technique used to to detect nectin-1 in a pattern consistent with that reported earlier (Haarr *et al.,* 2001; Mata *et al.,* 2001; Hung *et al.,* 2002; Mizuguchi *et al.,* 2002; Richart *et al.,* 2003; Valyi-Nagy *et al.,* 2004; Shukla *et al.,* 2005). The cornea showed a widespread nectin-1 expression in the cells of the epithelium but none in the stroma. Nectin-1 immunostaining was observed in the glands but not in the stroma of human endometrium(data not shown).

Human central NS (CNS) tissues showed a clear nectin-1 expression with prominent staining of neuronal cell bodies and the neuropil-containing neuronal processes (Fig. 1A,C; Table 1). Neurons from all parts of CNS including the cerebral cortex, basal ganglia, diencephalon, brain stem and spinal cord showed a strong nectin-1 immunoreactivity (Fig. 1A,C). Human peripheral NS tissues including dorsal root and trigeminal ganglia were also positive, with prominent staining of cell bodies of ganglion cells (Fig. 1D). A negative control consisting of the assay performed without primary antibody did not yield any staining of human and murine cornea and human brain tissues (Fig. 1B), and human endometrial glands and stroma (data not shown). These findings are consistent with the earlier observations that nectin-1 is expressed in neurons and epithelial tissues (Haarr *et al.,* 2001; Mata *et al.,* 2001; Hung *et al.,* 2002; Mizuguchi *et al.,* 2002; Richart *et al.,* 2003; Valyi-Nagy *et al.,* 2004; Shukla *et al.,* 2005) and indicate that the immunohistochemical assay used is sufficiently sensitive and specific in detecting nectin-1.

### *Nectin-1 expression in non-neuronal cells of human central and peripheral NS*

To determine the expression of nectin-1 in non-neuronal cells of human NS, the nectin-1-specific antiserum was used in probing central and peripheral NS tissues. A widespread strong expression was observed in ependymal cells (Fig. 2A, Table 1), choroid plexus epithelial cells, and vascular endothelial cells (Fig. 2D, Table 1). A widespread but weaker expression was detected in meningothelial cells (Fig. 2B, Table 1). Oligodendrocytes, astrocytes, vascular smooth muscle cells, Schwann cells and satellite cells showed variable immunoreactivity ranging from nil to weak staining (Fig. 2C, 2D, 1D, Table 1). These findings indicate that the expression of nectin-1 in non-neuronal cells of human central and peripheral NS varies among cell types.

### *Nectin-1 expression in primary neoplasms of human NS*

To better understand the expression pattern of nectin-1 in tumors of human NS, the nectin-1-specific antiserum was used in probing surgical specimens of NS neoplasms (n=22) including diffuse astrocytoma, anaplastic astrocytoma, glioblastoma multiforme, oligodendroglioma, gangliоglioma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, ependymoma, meningioma, medulloblastoma, and schwannoma (Table 2). Schwannoma (Fig. 3A), fibrous meningioma, and medulloblastoma (Fig. 3B) were found to be negative (Table 2), while diffuse astrocytoma, anaplastic astrocytoma, glioblastoma multiforme, oligodendroglioma, ependymoma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and meningothelial meningioma showed a weak focal positivity (Fig. 3 C-E, Table 2). In infiltrating astrocytic tumors (diffuse astrocytoma, anaplastic astrocytoma, glioblastoma multiforme) and in oligodendrogliomas, the staining was focal, primarily involving tumor cells with identifiable cytoplasm. Similarly, in pleomorphic xanthoastrocytoma, the expression was primarily detected in tumor cells with abundant cytoplasm. In ependymomas, tumor cells in perivascular pseudorosettes showed a most

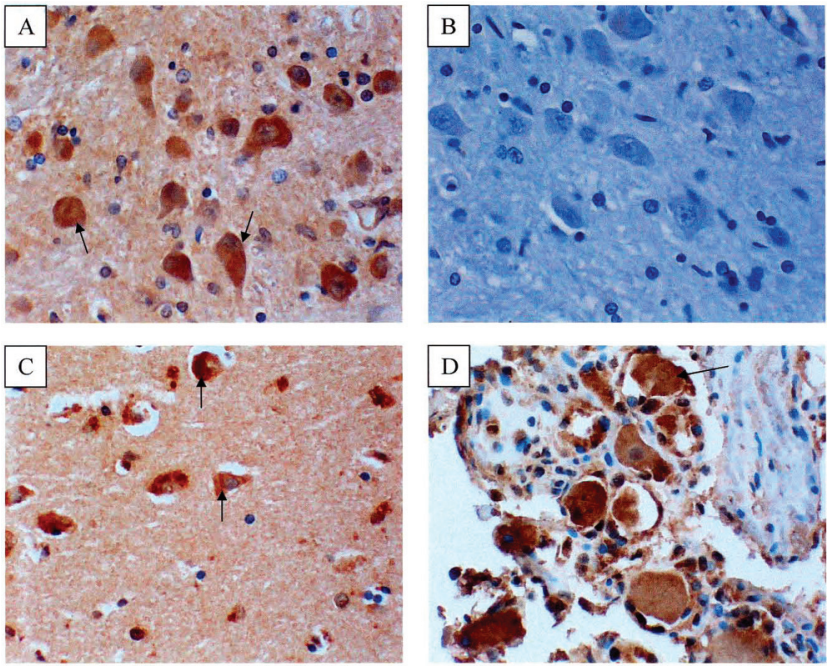


**Fig. 1**

**Nectin-1 expression in the neurons in human basal ganglia (A), cerebral cortex (C) and dorsal root ganglia (D)**

Brown staining indicates nectin-1. Nectin-1-negative cells in human basal ganglia (B) following the immunostaining performed without primary antibody (a negative control). Arrows in panels A, C and D point to some of the nectin-1-positive neurons. Magnification 400x (A, B and D) and 200x (C).

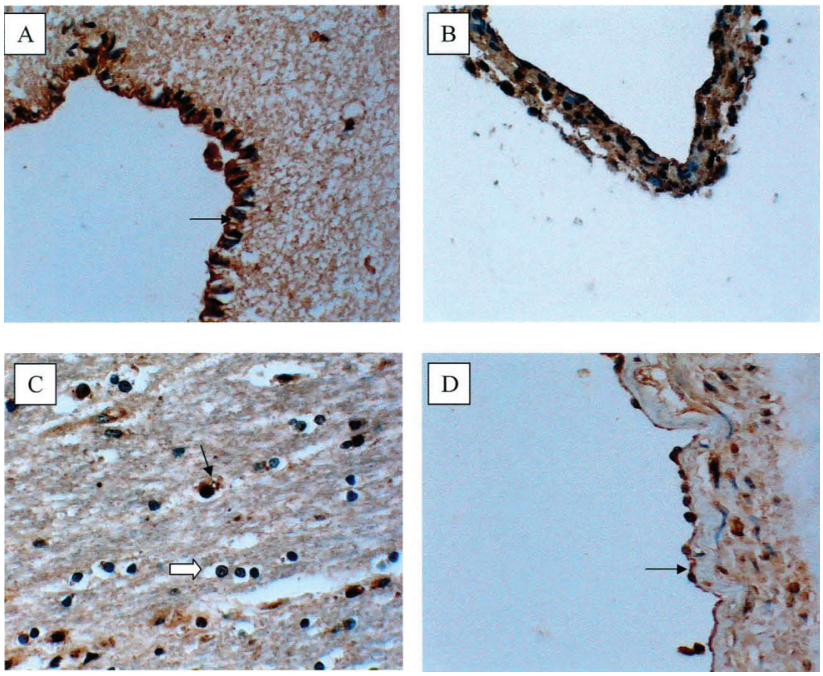


**Fig. 2**

**Nectin-1 expression in non-neuronal cells in human NS**

Brown staining indicates nectin-1. Wall of lateral ventricle (A), cerebral leptomeninges (B), cerebral white matter (C) and leptomeningeal blood vessel (D). The arrow in the panel A indicates a nectin-1-positive ependymal layer. The closed arrow in panel C points to a nectin-1-positive glial cell. The open arrow in panel C points to a nectin-1-negative glial cell. The arrow in panel D points to a nectin-1-positive endothelial layer of leptomeningeal blood vessel. Magnification 400x.

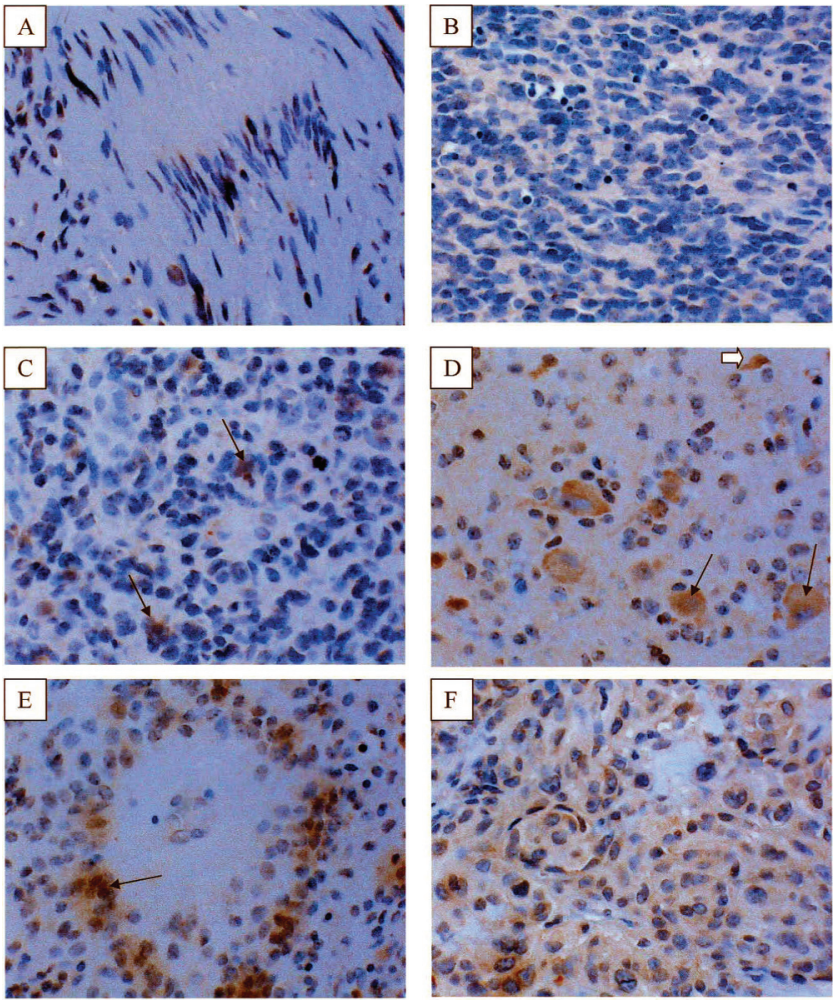


**Fig. 3**

**Nectin-1 expression in primary neoplasms of human NS**

Brown staining indicates nectin-1. Schwannoma (A), medulloblastoma (B), glioblastoma multiforme (C), oligodendroglioma (D), ependymoma (E) and meningothelial meningioma (F). The arrows in panel C indicate rare nectin-1-positive glioma cells in a glioblastoma. The closed arrows in panel D marks nectin-1-positive cortical neurons entrapped by oligodendroglioma cells. The open arrow in panel D points to one of the few nectin-1-positive oligodendroglioma cells. The arrow in panels E indicates an area of nectin-1-posiotive tumor cells in a perivascular pseudorosette in ependymoma. Magnification 400x.

**Table 1. Expression of nectin-1 in various types of cells of human peripheral and central NS**

| Cell type | Nectin-1-positive cells (%) | Nectin-1 expression strength |
|---|---|---|
| Neurons | 4 | 3+ to 4+ |
| Ependymal cells | 4 | 3+ to 4+ |
| Choroid plexus epithelial cells | 4 | 3+ to 4+ |
| Leptomeningeal cells | 4 | 1+ to 3+ |
| Vascular endothelial cells | 4 | 3+ to 4+ |
| Vascular smooth muscle cells | 3 | 1+ to 3+ |
| Oligodendrocytes, astrocytes | 2 | 1+ to 2+ |
| Schwann cells | 1 | 1+ to 2+ |

The percentage of nectin-1-positive cells: **0** = 0%, **1**<5%, 50%>**2**>5%, **3** = 50–95%, **4**>95%.
The strength of the nectin-1 signal varies from 1+ to 4+.

consistent staining (Fig. 3E). Whereas ganglion cells of ganglioglioma were strongly positive, the glial component of ganglioglioma demonstrated a weak focal staining (Table 2). These findings demonstrate that expression of nectin-1 in human brain tumors is dependant on tumor histology.

## Discussion

These data indicate that in normal human NS, there is widespread strong nectin-1 expression in the soma and processes of central and peripheral NS neurons, in ependymal cells, choroid plexus epithelial cells and vascular endothelial cells. There is a widespread but weaker

**Table 2. Expression of nectin-1 in various neoplasms of human NS**

| Tumor type | Nectin-1-positive cells (%) | Nectin-1 expression strength |
|---|---|---|
| Diffuse astrocytoma | 2 | 1+ |
| Anaplastic astrocytoma | 2 | 1+ |
| Glioblastoma multiforme | 2 | 1+ |
| Oligodendroglioma | 2 | 1 to 2+ |
| Pilocytic astrocytoma | 1 | 1+ |
| Pleomorphic xanthoastrocytoma | 2 | 1 to 2+ |
| Ependymoma | 2 | 1 to 2+ |
| Ganglioglioma, ganglion cells | 4 | 3 to 4+ |
| Ganglioglioma, glial component | 2 | 1+ |
| Medulloblastoma | 0 | 0 |
| Schwannoma | 0 | 0 |
| Meningothelial meningioma | 3 | 1+ |
| Fibrous meningioma | 0 | 0 |

The percentage of nectin-1-positive cells: **0** = 0%, **1**<5%, 50%>**2**>5%, **3** = 50–95%, **4**>95%.
The strength of the nectin-1 signal varies from 1+ to 4+.

expression in meningothelial cells. Oligodendrocytes, astrocytes, vascular smooth muscle cells, Schwann cells and satellite cells show variable nectin-1 immunoreactivity ranging from nil to weak. Furthermore, the nectin-1 expression pattern in human brain tumors depends on the histology of the tumor. While some tumors including schwannoma, fibrous meningioma and medulloblastoma do not appear to express nectin-1, oligodendroglioma, ependymoma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, anaplastic astrocytoma, glioblastoma multiforme and meningothelial meningioma show a weak focal expression. In contrast, ganglion cells of ganglioglioma show a strong widespread expression.

Previous studies have demonstrated the presence of nectin-1 RNA and protein in human, mouse and rat neural tissues (Haarr *et al.,* 2001; Mata *et al.,* 2001; Mizuguchi *et al.,* 2002; Richart *et al.,* 2003; Valyi-Nagy *et al.,* 2004; Shukla *et al.,* 2005). However, the distribution of nectin-1 expression in human CNS, including the nectin-1 expression in non-neuronal cells, had not been determined. The distribution observed in this study is similar to that in murine brain (Haarr *et al.,* 2001). HSV can infect different types of cells in the brain including neurons, glial cells, vascular cells and meningothelial cells (Whitley, 2001; Valyi-Nagy and Dermody, 2005). The finding that various non-neuronal cell types in human NS express nectin-1 is novel and raises the possibility that nectin-1 is an important receptor mediating HSV entry into different cells within the brain.

Genetically modified HSV strains are promising vector candidates for gene and tumor therapy targeting NS (Engelhard, 2000; Shah *et al.,* 2003; Glorioso and Fink, 2004; Lou, 2004; Niranjan *et al.,* 2004). A thorough understanding of the expression and distribution of cellular HSV entry receptors in normal and neoplastic human NS tissues may provide a critically important information for the development of novel HSV-based gene and tumor therapy strategies. The primary targets of current HSV-based oncolytic therapy trials are malignant gliomas, most notably recurrent glioblastoma multiforme. Several studies have shown that malignant gliomas are susceptible to HSV infection *in vitro* and *in vivo* (reviewed in Glorioso and Fink, 2004; Lou, 2004; Niranjan *et al.,* 2004). It is currently unknown what type(s) of entry receptors mediate HSV entry into glioma cells. Our findings indicate that nectin-1 is expressed only in a minority of tumor cells in glial tumors including malignant gliomas (anaplastic astrocytoma and glioblastoma multiforme). These finding suggest that the absence of nectin-1 expression in some tumor cells in malignant gliomas may represent a resistance factor to HSV tumor therapy. However, the possibility that HSV can enter glioma cells via receptors other than nectin-1 cannot be excluded.

An interesting aspect of the observations reported here is an apparent lack or paucity of nectin-1 expression in a variety of primary NS tumors including schwannoma, fibrous meningioma, medulloblastoma and gliomas. The relevance of altered expression of cell adhesion molecules to cancer pathogenesis is well demonstrated in the case of the E-cadherin expression (Harrington and Syrigos, 2000; Van Aken *et al.,* 2001; Peinado *et al.,* 2004). Changes in nectin-1 expression in epithelial cells may also play a role in the spread of invasive types of epithelial malignancies. A decreased or nil nectin-1 expression has been found in neoplastic epithelial cells in invasive squamous and basal cell carcinomas of the skin (Matsushima *et al.,* 2003). Our studies raise the possibility that neoplastic transformation of a NS tissue is associated with changes in nectin-1 expression.

Summing up, the observations reported here provide novel information about the expression of nectin-1 in normal and neoplastic human NS tissues and thus may lead to a better understanding of cell targeting by HSV in HSV-induced neurological diseases and HSV-based gene therapy.

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## References

Cocchi F, Menotti L, Mirandola P, Lopez M, Campadelli-Fiume G (1998): The ectodomain of a novel member of the immunoglobulin subfamily related to the poliovirus receptor has the attributes of a bona fide receptor for herpes simplex virus types 1 and 2 in human cells. *J. Virol.* **72**, 9992–10002.

Eberle F, Dubreuil P, Mattei MG, Devilard E, Lopez M (1995): The human PRR2 gene, related to the human poliovirus receptor gene (PVR), is true homolog of the murine MPH gene. *Gene* **159**, 267–272.

Engelhard HH (2000): Gene therapy for brain tumors: the fundamentals. *Surg. Neurol.* **54**, 3-9.

Geraghty R J, Krummenacher C, Cohen GH, Eisenberg RJ, Spear PG (1998): Entry of alphaherpesviruses mediated poliovirus receptor-related protein 1 and poliovirus receptor. *Science* **280**, 1618–1620.

Glorioso JC, Fink DJ (2004): Herpes vector-mediated gene transfer in treatment of diseases of the nervous system. *Annu. Rev. Microbiol*. **58**, 253–271.

Haarr L, Shukla D, Rodahl E, Del Canto MC, Spear PG (2001): Transcription from the gene encoding the herpesvirus entry receptor nectin-1 (HveC) in nervous tissue of adult mouse. *Virology* **287**, 301–309.

Harrington KJ, Syrigos KN (2000): The role of the E-cadherin-catenin complex: more than an intercellular glue? *Ann. Surg. Oncol*. **7**, 783–788.

Herold BC, Visalli RJ, Susmarski N, Brandt CR, Spear PG (1994): Glycoprotein C-independent binding of herpes simplex to cells requires of cell surface heparan sulphate and glycoprotein. *J. Gen. Virol*. **75**, 1211–1222.

Hung SL, Cheng YY, Wang YH, Chang KW, Chen YT (2002): Expression and roles of herpesvirus entry mediators A and C in cells of oral origin. *Oral Microbiol. Immunol*. **17**, 215–23.

Lopez M, Eberle F, Mattei MG, Gabert J, Birg F, Bardin F, Maroc C, Debreuil P (1995): Complementary DNA characterization and chromosomal localization of a human gene related to the poliovirus receptor-encoding gene. *Gene* **155**, 261–265.

Lopez M, Cocchi F, Avitabile E, Leclerc A, Adelaide J, Campadelli-Fiume G (2001): Novel, soluble isoform of the herpes simplex virus (HSV) receptor nectin1 (or PRR1-HIgR-HveC) modulates positively and negatively susceptibility to HSV infection. *J. Virol.* **75**, 5684–5691.

Lou E (2004): Oncolytic viral therapy and immunotherapy of malignant brain tumors: two potential new approaches of translational research. *Ann. Med.* **36**, 2–8.

Mata M, Zhang M, Hu X, Fink DJ (2001): HveC (nectin-1) is expressed at high levels in sensory neurons, but not in motor neurons, of the rat peripheral nervous system. *J. Neurovirol*. **7**, 476–480.

Matsushima H, Utani A, Endo H, Matsuura H, Kakuta M, Nakamura Y, Matsuyoshi N, Matsui C, Nakanishi H, Takai Y, Shiukai H (2003): The expression of nectin-1 alpha in normal human skin and various skin tumours. *Br. J. Dermatol.* **148(4)**, 755–762.

Menotti L, Lopez M, Avitabile E, Stefan A, Cocchi F, Adelaide J, Lecocq E, Dubreuil P, Campedelli-Fiume G (2000): The murine homolog of human nectin1d serves as a species nonspecific mediator for entry of human and animal aherpesviruses in a pathway independent of a detectable binding to gD. *PNAS USA* **97**, 4867.

Mettenleiter TC, Zsak L, Zuckermann F, Sugg N, Kern H, Ben-Porat T (1990): Interaction of glycoprotein gIII with a cellular heparin-like substance mediates adsorption of pseudorabies virus. *J. Virol.* **64**, 278–286.

Milne RSB, Connolly SA, Krummenacher C, Eisenberg RJ, Cohen GH (2001): Porcine HveC, a member of the highly conserved HveC/nectin-1 family, is a functional alphaherpesvirus receptor. *Virology* **281**, 315-328.

Mizoguchi A, Nakanishi H, Kimura K, Matsubara K, Ozaki-Kuroda K, Katata T, Honda T, Kiyohara Y, Heo K, Higashi M, Tsutsumi T, Ide C, Takai Y (2002): Nectin: an adhesion molecule involved in the formation of synapses. *J. Cell. Biol*. **156**, 555–565.

Miyahara M, Nakanishi H, Takahashi K, Miyahara M, Nishimura N, Tachibana K, Mizoguchi A, Takai Y (2000): Interaction of nectin with afadin is necessary for its clustering at cell-cell contact sites but not for its *cis*dimerization or *trans*interaction. *J. Biol. Chem*. **275**, 613–618.

Montgomery RI, Warner MS, Lum BJ, Spear PG (1996): Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* **87**, 427–436.

Niranjan A, Wolfe D, Fellows W, Goins WF, Glorioso JC, Kondziolka D, Lunsford LD (2004): Gene transfer of glial tumors using herpes simplex virus. *Methods Mol. Biol.* **246**, 323–337.

Nishioka H, Mizoguchi D, Nakanishi H, Mandai K, Takahashi K, Kimura K, Satoh-Moriya A, Takai Y (2000): Localization of 1-afadin at puncta adherentia-like junctions between the mossy fiber terminals and the dendritic trunks of pyramidal cells in the adult mouse hippocampus. *J. Comp. Neurol*. **424**, 297–306.

Peinado H, Portillo F, Cano A (2004): Transcriptional regulation of cadherins during development and carcinogenesis. *Int. J. Dev. Biol*. **48**, 365–375.

Pertel P, Fridberg A, Parish ML, Spear PG (2001): Cell fusion induced by herpes simplex virus glycoproteins gB, gD, and gH-gL requires a gD receptor but not necessarily heparan sulfate. *Virology* **279**, 313–324.

Pokutta S, Drees F, Takai Y, Nelson WJ, Weis WI (2002): Biochemical and structural definition of the l-afadin- and actin-binding sites of catenin. *J. Biol. Chem*. **277**, 18868–18874.

Richart SM, Simpson SA, Krummanacher C, Whitbeck JC, Pizer LI, Cohen GH, Eisenberg RJ, Wilcox CL (2003): Entry of herpes simplex virus type 1 into primary sensory neurons in vitro is mediated by Nectin-1/HveC. *J. Virol.* **77**, 3307–3311.

Satoh-Horikawa K, Nakanishi H, Takahashi K, Miyahara M, Nishimura M, Tachibana K, Mizoguchi A, Takai Y (2000): Nectin-3, a new member of immunoglobulin-like cell adhesion molecules that shows homophilic and heterophilic cell-cell adhesion activities. *J. Biol. Chem.* **275**, 10291–10299.

Shah AC, Benos D, Gillespie GY, Markert JM (2003): Oncolytic viruses: clinical applications as vectors for the treatment of malignant gliomas. *J. Neurooncol*. **65**, 203–226.

Shieh M-T, WuDunn D, Montgomery RI, Esko JD, Spear PG (1992): Cell surface receptors for herpes simplex virus

are heparin sulfate proteoglycans. *J. Cell. Biol*. **116**, 1273–1281.

Shukla D, Liu J, Blaiklock P, Shworak NW, Bai X, Esko JD, Cohen GH, Eisenberg RJ, Rosenberg RD, Spear PG (1999): A novel role for 3-*O*-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* **99**, 13–22.

Shukla D, Dal Canto M, Rowe CL, Spear PG (2000): Striking similarity of murine nectin-1a to human nectin-1a (HveC) in sequence and activity as a gD receptor for alpha-herpesvirus entry. *J. Virol*. **74**, 11773–11781.

Shukla D, Spear PG (2001): Herpesviruses and heparan sulfate: an intimate relationship in aid of viral entry. *J. Clin. Invest*. **108**, 503–510.

Shukla D, Scanlan P, Tiwari V, Sheth V, Clement C, Guzman-Hartman G, Dermody TS, Valyi-Nagy T (2006): Expression of nectin-1 in the normal and HSV-1-infected murine nervous system. *Appl. Immunohistochem. Mol. Morphol.* (in press).

Spear PG, Eisenberg RJ, Cohen GH (2000): Three classes of cell surface receptors for entry. *Virology* **275**, 1–8.

Tachibana K, Nakanishi H, Mandai K, Ozaki K, Ikeda W, Yamamoto Y, Nagafuchi A, Tsukita S, Takai Y (2000): Two cell adhesion molecules, nectin and cadherin, interact through their cytoplasmic domain-associated proteins. *J. Cell. Biol*. **150**, 1161–1175.

Takahashi K, Nakanishi H, Miyahara M, Mandai K, Satoh K, Nishioka H, Aoki J, Nomoto A, Mizoguchi A, Takai Y (1999): Nectin/PRR: An immunoglobulin-like cell adhesion molecule recruited to cadherin-based adherens junctions through interaction with afadin, a PDZ domain-containing protein. *J. Cell Biol.* **145**, 539–549.

Takai Y, Irie K, Shimizu K, Sakisaka T, Ikeda W (2003): Nectins and nectin-like molecules: roles in cell adhesion, migration, and polarization. *Cancer Sci*. **94**, 655–667.

Takai Y, Shimizu K, Ohtsuka T (2003): The roles of cadherins and nectins in interneuronal synapse formation. *Curr. Opin. Neurobiol*. **13**, 520–526.

Tiwari V, Clement C, Duncan MB, Chen J, Liu J, Shukla D (2004): A role for 3-O-sulfated heparan sulfate in cell fusion induced by herpes simplex virus type 1. *J. Gen. Virol.* **85**, 805–809.

Xia G, Chen J, Tiwari V, Ju W, Li JP, Malstrom A, Shukla D, Liu J (2002): Heparan sulfate 3-O-sulfotransferase isoform 5 generates both an antithrombin-binding site and an entry receptor for herpes simplex virus, type 1. *J. Biol. Chem*. **277**, 37912-37919.

Yoon M, Spear PG (2002): Disruption of adherens junctions liberates nectin-1 to serve as receptor for herpes simplex virus and pseudorabies virus entry. *J. Virol*. **76**, 7203–7208.

Valyi-Nagy T, Sheth V, Clement C, Tiwari V, Scanlan P, Kavouras J, Leach L, Guzman-Hartman G, Dermody T, Shukla D (2004): Herpes Simplex Virus Entry Receptor Nectin-1 Is Widely Expressed in the Murine Eye. *Curr. Eye Res.* **29**, 303–309.

Valyi-Nagy T, Dermody TS (2005): Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histol. Histopathol*. **20**, 957–967.

Van Aken E, De Wever O, Correia da Rocha AS, Mareel M (2001): Defective E-cadherin/catenin complexes in human cancer. *Virchows Arch.* **439**, 725–751.

Warner MS, Geraghty RJ, Martinez WM, Montgomery RI, Whitbeck JC, Xu R, Eisenberg RJ, Cohen GH, Spear PG (1998): A cell surface protein with herpesvirus entry activity (HveB) confers susceptibility to infection by mutants of herpes simplex virus type 1, herpes simplex virus type 2, and pseudorabies virus. *Virology* **246**, 179–189.

Whitley RJ (2001): Herpes simplex viruses. In Fields BN, Knipe DM, Howley PM (Eds): *Fields Virology*. 4th ed. Philadelphia, Lippincott-Raven Publishers, pp. 2461–2509.

WuDunn D, Spear PG (1989): Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J. Virol*. **63**, 52–58.