

## Expression of $\alpha_2$ -macroglobulin receptor/low density lipoprotein receptor-related protein is increased in reactive and neoplastic glial cells

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### Abstract

$\alpha_2$ -Macroglobulin receptor/low density lipoprotein receptor-related protein ( $\alpha_2$ M-R/LRP) is a multi-functional cell-surface receptor that has been implicated in diverse physiologic processes. In normal human brain,  $\alpha_2$ M-R/LRP is expressed principally by pyramidal neurons with localization to cell bodies and proximal processes. By contrast,  $\alpha_2$ M-R/LRP is not present in either the cell bodies or processes of most normal macroglia (including astrocytes). In this investigation, we studied the expression of  $\alpha_2$ M-R/LRP in the brain, following tissue injury or neoplastic transformation, by immunohistochemistry. Significantly increased  $\alpha_2$ M-R/LRP immunoreactivity was identified in reactive astrocytes, indicating that expression of this receptor is regulated in vivo in response to brain injury.  $\alpha_2$ M-R/LRP immunoreactivity was also detected in glial cell tumors, this finding is novel since malignant transformation is typically thought to turn off expression of this receptor.

**Key words:**  $\alpha_2$ -Macroglobulin receptor, Low density lipoprotein receptor-related protein, Tumor invasion, Glioma

### 1. Introduction

$\alpha_2$ -Macroglobulin receptor/low-density lipoprotein receptor-related protein ( $\alpha_2$ M-R/LRP) is a multi-functional plasma membrane receptor which has been implicated in cellular lipoprotein metabolism, cell-surface trafficking of plasminogen activators and their inhibitors, modulation of growth factor responses, and *Pseudomonas* exotoxin A clearance [1–6].  $\alpha_2$ M-R/LRP is synthesized as a 600 kDa precursor protein which undergoes post-translational processing into 515-kDa and 85-kDa subunits [7,8]. The 515-kDa subunit confers ligand binding activity and is entirely extracellular. The 85-kDa subunit has a transmembrane domain that anchors the large subunit to the cell surface by a non-covalent interaction [8,9].  $\alpha_2$ M-R/LRP and its mRNA are distributed through numerous human tissues [7,10], however, the cellular distribution in human brain is selective. Immunoreactivity is restricted almost exclusively to neurons, principally of the pyramidal type in the cerebral cortex, subcortical gray matter, and cerebellum [10,11]. Although  $\alpha_2$ M-R/LRP is not normally expressed by most glia, immunoreactivity is detected in a highly localized perivascular zone which corresponds to the distribution of astrocytic foot processes. Expression of  $\alpha_2$ M-R/LRP in this region suggests a role for the receptor in the function of the blood–brain barrier.

Expression of  $\alpha_2$ M-R/LRP is regulated in vitro by cytokines and mediators of inflammation. In macrophages,  $\alpha_2$ M-R/LRP is up-regulated by colony stimulating factor-1 and down-regulated by interferon- $\gamma$  and bacterial lipopolysaccharide [12,13]. Based on work with fibroblasts in culture, Van Leuven et al. [14] suggested that cancer cells may express greatly decreased levels of  $\alpha_2$ M-R/LRP. More recently, we demonstrated that  $\alpha_2$ M-R/LRP is decreased or absent in hepatocellular carcinomas, as determined by immunohistochemistry [15].

Following brain injury or after neoplastic transformation, astrocytes undergo profound phenotypic changes, associated with increased proliferation and an augmented capacity for tissue invasion. The purpose of the present investigation was to determine whether brain injury or glial neoplastic transformation is associated with altered  $\alpha_2$ M-R/LRP expression.

### 2. Materials and methods

#### 2.1. Materials

Fifty-seven glioma specimens from archives of the University of Virginia Health Sciences Center were examined. The tumors were classified according to the World Health Organization (WHO) criteria [16]. Histopathological examination revealed 4 low-grade astrocytomas, 2 gemistocytic astrocytomas, 11 anaplastic astrocytomas, 13 glioblastomas multiforme, 2 gliosarcomas, 13 pilocytic astrocytomas, 1 pleomorphic xanthoastrocytoma, 1 optic glioma, 3 oligodendrogliomas, and 7 ependymomas. Reactive (non-neoplastic) astrocytes were studied in 5 specimens with acute and chronic infarcts and in the tissue adjacent to tumors.

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## 2.2 Immunohistochemistry

Monoclonal antibody 8G1 directed against the  $\alpha_2$ M-R/LRP 515-kDa chain was kindly provided by Dr Dudley K Strickland (American Red Cross, Rockville, MD). 8G1 was incubated with formalin-fixed, deparaffinized sections for 15–18 h at 4°C. The primary antibody was detected using the avidin-biotin immunoperoxidase technique [11]. Normal non-immune serum was substituted for primary antibody as a negative control. Sections of normal human liver were treated in parallel to provide positive controls.

## 3. Results and discussion

In contrast with quiescent astrocytes in normal brain, activated astrocytes in the gray and white matter were consistently immunoreactive for  $\alpha_2$ M-R/LRP. The pattern of immunoreactivity varied from predominantly perinuclear, punctate accumulations, to a more diffuse cytoplasmic distribution. The first pattern (Fig 1, arrowheads) was highly suggestive of an intracytoplasmic distribution while the diffuse form (Fig 1, inset) could reflect a combination of both surface and cytoplasmic localization. The cytoarchitectural distribution of  $\alpha_2$ M-R/LRP in reactive astrocytes was clearly different from the restricted pattern of immunoreactivity in perivascular astrocytic processes, reported previously [11]. Activated astrocytes were the only reactive glial cells with increased  $\alpha_2$ M-R/LRP. Myelin-forming oligodendrocytes and ependymal cells, which lined the ventricles in zones contiguous with injured brain, did not show  $\alpha_2$ M-R/LRP immunoreactivity.

Neoplastic transformation of macroglial cells resulted in increased expression of  $\alpha_2$ M-R/LRP antigen, regardless of cell type (i.e. astrocytic, oligodendroglial or ependymal) (Table 1); however, the level and cytoarchitectural distribution of  $\alpha_2$ M-R/LRP in the different tumors was variable (Table 2). Astrocytomas which

Table 1

$\alpha_2$ M-R/LRP immunohistochemistry in gliomas

Tumor	Cases (positive/total)
Astrocytoma	4/4
Gemistocytic astrocytoma	2/2
Anaplastic astrocytoma	11/11
Glioblastoma multiforme	12/13
Pleomorphic xanthoastrocytoma	1/1
Pilocytic astrocytoma	12/13
Optic glioma	1/1
Oligodendroglioma	3/3
Ependymoma	7/7

diffusely invade surrounding brain are shown in Fig 2A–C. In many of the tumor cells,  $\alpha_2$ M-R/LRP immunoreactivity was conspicuously localized in the cytoplasm as punctate, perinuclear accumulations (panels A and C). Other tumor cells with larger amounts of cytoplasm showed more diffuse immunoreactivity (Fig 2B and 2C inset). In contrast to the invasive astrocytomas, pilocytic astrocytomas, which have limited capacity to invade, expressed  $\alpha_2$ M-R/LRP primarily in cell processes (Fig 2D).

Most neoplastic oligodendroglial cells (Fig 3) and ependymal cells (not shown) expressed  $\alpha_2$ M-R/LRP, in contrast with their non-malignant (normal and reactive) counterparts, which were immunonegative. The pattern of immunoreactivity in the oligodendrogliomas varied. Most cells expressed  $\alpha_2$ M-R/LRP in a punctate, perinuclear pattern, similar to that observed with reactive and neoplastic astrocytes (panel A). Other cells demonstrated  $\alpha_2$ M-R/LRP immunopositivity in a more diffuse pattern (panel B); however, this distribution was not common.

The results reported in this investigation provide sharp

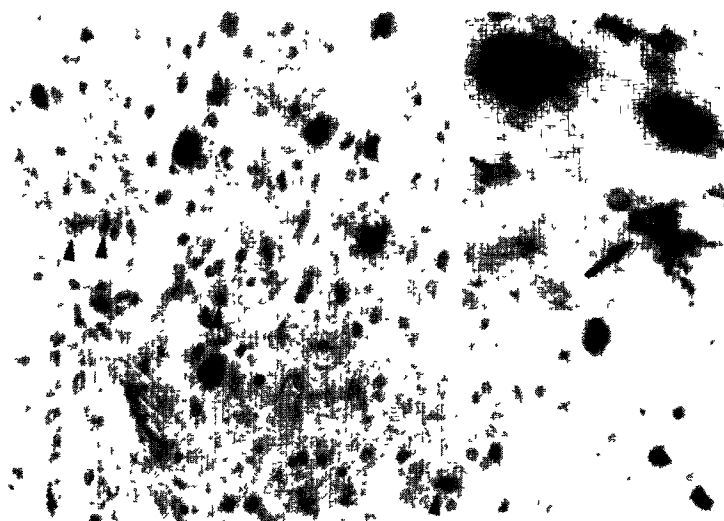


Fig 1 Localization of  $\alpha_2$ M-R/LRP in a brain infarct. Reactive astrocytes on the periphery of the lesion (left) demonstrated cytoplasmic  $\alpha_2$ M-R/LRP immunoreactivity in both a punctate (small astrocytes, arrowheads) and diffuse (inset: gemistocytic astrocytes, upper, and fibrillary astrocyte, arrow) pattern. Fibrillary astrocytes with prominent staining in cellular processes were present in small numbers (inset, arrow). [Monoclonal antibody 8G1 immunoperoxidase, DAB with light hematoxylin counterstain,  $\times 300$ ,  $\times 600$  inset.]

Table 2  
Pattern of  $\alpha_2$ M-R/LRP immunoreactivity in glial cells

Cytoplasmic distribution	
Reactive astrocytes	punctate (circumscribed) > diffuse
Neoplastic macroglia	punctate (circumscribed) > diffuse
Cellular processes	
Reactive astrocytes	rare
Infiltrating astrocytomas	rare
Circumscribed astrocytomas	common
Oligodendrogliomas	rare
Ependymomas	rare
Cell surface	
Reactive astrocytes	none
Astrocytomas	present
Oligodendrogliomas	present
Ependymomas	none

contrast with our previous study of normal human brain [11] Expression of  $\alpha_2$ M-R/LRP in reactive and transformed astrocytes suggests that these cells synthesize  $\alpha_2$ M-R/LRP in response to physiologic signals *in vivo*. The predominant intracytoplasmic localization of  $\alpha_2$ M-R/LRP in reactive and neoplastic macroglia was surprising since  $\alpha_2$ M-R/LRP is a plasma membrane receptor. Moestrup et al. [17] reported a similar finding in their analysis of fibroblasts and attributed the intracytoplasmic distribution of  $\alpha_2$ M-R/LRP to intracellular vesicles in the receptor recycling pathway.

The function of  $\alpha_2$ M-R/LRP in normal brain is still unknown, however, proteinases, proteinase inhibitors and cytokines are important effectors of glial-neuronal cell interactions. Based on its ability to bind diverse ligands (some of which are carriers of other macromole-

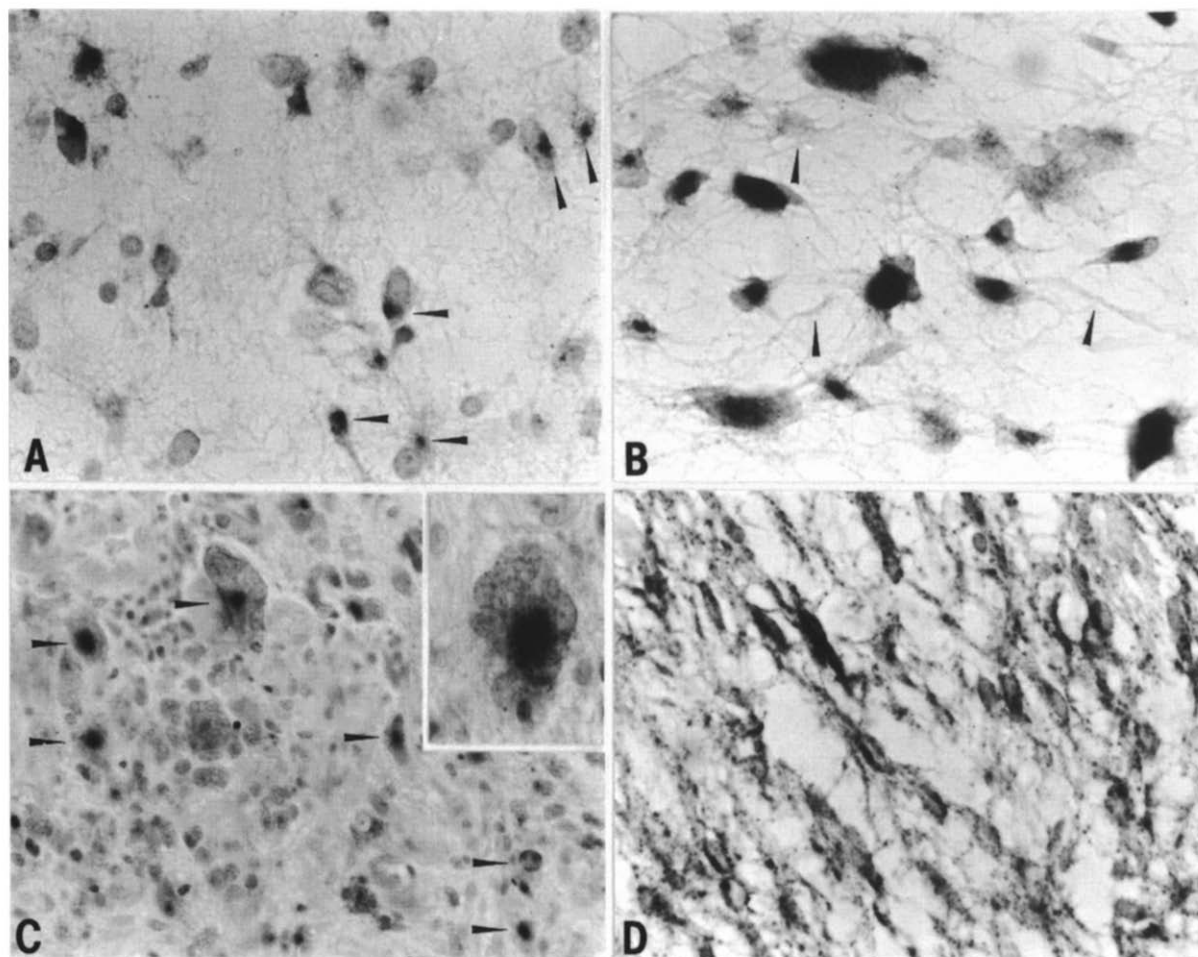


Fig 2 Localization of  $\alpha_2$ M-R/LRP in astrocytic tumors (A–C)  $\alpha_2$ M-R/LRP immunoreactivity was detected in all grades of diffusely infiltrating astrocytomas (A)  $\alpha_2$ M-R/LRP immunoreactivity in low-grade astrocytomas was localized in the cytoplasm as punctate staining (arrowheads) in the perinuclear region ( $\times 600$ ) (B) Tumor cells with increased cytoplasmic size (gemistocytic astrocytes) usually had a more diffuse pattern of staining. Note that the fine fibrillary processes (arrowheads) of the tumor cells are not labelled ( $\times 600$ ) (C) Cells in glioblastoma multiforme also demonstrated a punctate pattern of immunostaining for  $\alpha_2$ M-R/LRP (arrowheads) while the increased cytoplasm of giant, multinucleated cells was more diffusely immunoreactive ( $\times 300$ ,  $\times 600$  inset) (D) Pilocytic astrocytomas demonstrated a striking difference of  $\alpha_2$ M-R/LRP distribution, in contrast to the diffusely invasive astrocytic tumors, with immunoreactivity localized only to cell processes. Note the fine particulate pattern of the staining in the cellular processes ( $\times 600$ ) (Monoclonal antibody 8G1 immunoperoxidase, DAB with hematoxylin counterstain)

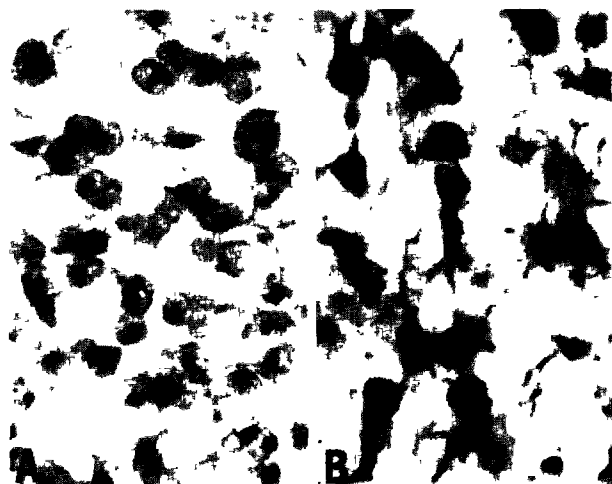


Fig 3 Localization of  $\alpha_2$ M-R/LRP in oligodendrogliomas (A)  $\alpha_2$ M-R/LRP immunoreactivity was conspicuously localized as punctate, per-nuclear accumulations in the scant cytoplasm (B) In focal areas of the same tumor, blunt cellular processes and plasma membrane were also highlighted by the immunostaining ( $\times 600$ ) (8G1 immunoperoxidase)

cules),  $\alpha_2$ M-R/LRP may function in the transfer of critical effectors between cells in the brain. For example, cytokines, such as transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and platelet-derived growth factor (PDGF), bind to activated  $\alpha_2$ M which is a ligand for  $\alpha_2$ M-R/LRP. Since cytokine binding does not inhibit the interaction of  $\alpha_2$ M with its receptor, these complexes can be targeted to cells which express  $\alpha_2$ M-R/LRP [5,18]. In the brain, PDGF is important in gliogenesis [19]. PDGF also supports proliferation of reactive astrocytes following brain injury [20]. PDGF and TGF- $\beta$  influence the growth of malignant gliomas [21,22]. Since  $\alpha_2$ M is present in developing brain [23] and in adult brain during subacute responses to injury and chronic degenerative states [24,25],  $\alpha_2$ M may influence the trafficking of TGF- $\beta$  and PDGF between cells in the brain. The ultimate delivery of  $\alpha_2$ M-TGF- $\beta$  or  $\alpha_2$ M-PDGF complex to a particular brain cell depends on expression of  $\alpha_2$ M-R/LRP. Disease processes which increase  $\alpha_2$ M-R/LRP expression in glial cells might facilitate  $\alpha_2$ M-targeting of TGF- $\beta$  and PDGF to these cells.

Binding of ligands to  $\alpha_2$ M-R/LRP results in receptor clustering in clathrin-coated pits and rapid clearance of the receptor-ligand complexes from the cell surface by endocytosis [26]. The ligands are transferred to lysosomes while the  $\alpha_2$ M-R/LRP typically recycles to the cell surface [27]. Although all types of gliomas exhibited  $\alpha_2$ M-R/LRP immunoreactivity, the more invasive astrocytomas demonstrated a predominantly cytoplasmic pattern of  $\alpha_2$ M-R/LRP staining while the less invasive pilocytic astrocytomas expressed  $\alpha_2$ M-R/LRP primarily in cell processes. Pilocytic astrocytomas express high levels of proteinase inhibitors compared with other glial cell neoplasms [28]. Whether expression of proteinase inhib-

itors and  $\alpha_2$ M-R/LRP supports the non-invasive phenotype of the pilocytic astrocytoma is a topic for future investigation.

In summary, the present study has demonstrated that expression of  $\alpha_2$ M-R/LRP is regulated in glial cells in vivo. Regulated expression and selective cellular localization of  $\alpha_2$ M-R/LRP in reactive and neoplastic glia suggests a role for this receptor in glial cell function.

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