Chapter 23

Nestin: Neural Stem/Progenitor Cell Marker in Brain Tumors

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1. Introduction

Glioblastomas are the most common primary malignant neoplasms in the adult brain, and have the characteristics of glial cells [1]. The WHO histopathological classification guidelines categorize gliomas according to their histopathological grades; low-grade gliomas are not anaplastic and are associated with a favorable patient prognosis, while high-grade gliomas exhibit increased cellularity, nuclear atypia, mitotic activity, microvascular proliferation, and necrosis. Glioblastomas are the highest grade of the gliomas. Surgical treatment is the main therapy used for glioblastomas, with radiotherapy and chemotherapy performed as adjuvant care. Despite intensive research and recent advances in treatment, the prognosis for patients with glioblastoma remains poor, with a five-year survival rate of approximately 3% [2, 3]. In addition to having rapid growth rates, glioblastomas aggressively invade the adjacent normal brain tissues, they are often surgically unresectable, and recurrent glioblastomas are resistant to conventional radiotherapy and chemotherapy.

Nestin is a class VI intermediate filament protein that was first described as a neural stem/progenitor cell marker [4, 5]. Neuroepithelial stem cells can differentiate into neurons, oligodendrocytes, and astrocytes, and nestin has been shown to be down-regulated or to completely disappear during such differentiation. Nestin-positive neuroepithelial stem cells are detected in the subventricular zone of the human adult brain and they remain mitotically active throughout adulthood [6]. Unlike other intermediate filament proteins, nestin plays important roles in cellular processes, including stemness, migration, and cell cycle regulation.

Nestin expression has been reported in various types of tumor cells originating from the central nervous system, including glioblastomas. Several reports have indicated a close rela-

tionship between neuroepithelial stem cells and glioblastoma cells at their origin because both cell types express the same stem cell markers, such as CD133 and nestin. High-grade gliomas express higher nestin levels compared to low-grade gliomas [7, 8]. We have reported that knockdown of nestin using short hairpin RNA (shRNA) suppressed cell growth, migration, and invasion [9]; therefore, nestin may serve as a novel candidate for molecular targeted therapy for glioblastomas. In the present chapter, we summarize the available data regarding the expression and roles of nestin in normal brain tissues and brain tumor tissues, and discuss the possibility of using nestin as a novel therapeutic target in brain tumors, mainly for glioblastomas.

2. Structure and characterization of nestin

Nestin is a large protein (>1600 amino acids) that contains a short N-terminal and an unusually long C-terminal. It interacts with other intermediate filament proteins, including vimentin, desmin, and internexin, to form heterodimers and mixed polymers; however, in contrast to other intermediate filament proteins, nestin cannot form homopolymers [10]. The nestin gene has four exons and three introns; in humans, neural cell-specific expression is reportedly regulated by the second intron, whereas nestin expression in tumor endothelium is enhanced by the first intron [11]. Nestin is known to be phosphorylated on Thr316 by cdc2 kinase [12] and/or cyclin-dependent kinase 5 [13], and to modulate mitosis-associated cytoplasmic reorganization during mitosis. However, the roles of glycosylation of nestin have not been closely examined [14].

During early stages of development, nestin is expressed in dividing cells in the central nervous system (CNS), peripheral nervous system, and in myogenic and other tissues. During differentiation in normal brain tissue, nestin expression is downregulated and replaced by expression of tissue-specific intermediate filament proteins; therefore, nesting is widely used as a neuronal stem cell marker. Nestin is also expressed in immature non-neuronal cells and progenitor cells in normal tissues [15-17]. High levels of nestin expression have been detected in oligodendroglial lineage cells, ependymocytes, Sertoli cells, enteroglia, hair follicle cells, podocytes of renal glomeruli, pancreatic stellate cells, pericytes, islets, optic nerve, and odontoblasts [18-23].

In pathological conditions, nestin is re-expressed during repair processes, as well as in various neoplasms and proliferating endothelial cells. Nestin expression has been observed in repair processes in the CNS, muscle, liver, and infarcted myocardium [24-26]. Furthermore, increased nestin expression has been reported in various tumor cells, including CNS tumors, pancreatic cancer, gastrointestinal stromal tumors (GISTs), prostate cancers, breast cancers, malignant melanomas, dermatofibrosarcoma protuberances, and thyroid tumors [27-31]. In several tumors, expression of nestin has been reported to be closely correlated with poor prognosis. Nestin is specifically expressed in proliferating small-sized vascular endothelial cells in glioblastomas and in colorectal, prostate, and pancreatic cancers [7, 32-34].

3. Nestin in normal fetal and adult brain tissues

Many lines of evidence have shown nestin-positive brain cells to be neural stem/progenitor cells; therefore, a great deal of research has involved the use of nestin to detect neural stem cells [35-37]. Children, but not adult humans, exhibit nestin-positive cells in the subventricular zone of the third ventricle [6], and the human embryonic midbrain stem cell line NGC-407 showed degradation of nestin after induction of differentiation [38]. However, in adult mice, nestin-positive cells were detected in CA2 lesions of the hippocampus after transient ischemia [39]. Another study reported that nestin-positive neuroepithelial stem cells are detected in the subventricular zone of the human adult brain and remain mitotically active throughout adulthood [40]. Nestin has been used for research in the field of neural progenitor cells; for example, neural progenitor cell-specific gene transfection was successfully performed using a nestin-driven gene transfection system [41-46]. A recent study has shown that nestin is also a stem/progenitor cell marker in the pituitary gland [47].

4. Nestin in various types of brain tumors

Nestin expression in brain tumor cells has been reported in schwannomas [48], ependymomas [49, 50], neurocytomas [51], adamantinomatous craniopharyngiomas [52], pituitary adenomas [53], medulloblastomas [54-59], oligodendrogliomas [60], and glioblastomas [7, 8, 48, 61] (Table 1). Tissue microarrays of 257 brain tumors have revealed frequent nestin expression in gliomas and schwannomas [48]. Another analysis included 379 tumors, and the results further revealed that nestin immunoreactivity is associated with poor outcome in intracranial ependymomas, and that nestin is an independent marker for poor progression-free survival and overall survival [49].

Expression of nestin has also been reported in tanycytic ependymoma, a rare variant of ependymoma [50], and central neurocytoma cases express nestin, as determined by PCR [51]. Co-expression of nestin, microtubule-associated protein 2 (MAP2), and GFAP has been reported in adamantinomatous craniopharyngiomas [52]. In pituitary adenomas, CD133-positive cells ubiquitously co-express CD34, nestin, and VEGFR2, and may play a role in the neovascularization of tumors [53]. Human medulloblastoma cell lines [54] and medulloblastoma stem cells [55-58] express nestin, and secreted protein acidic and rich in cysteine (SPARC) has been shown to induce neuronal differentiation in medulloblastoma cells with elevations of nestin, NeuN, and neurofilament [59]. One study found that oligodendrogliomas express no or weak nestin, but high Olig2 and alpha-internexin [60]. Oligoastrocytomas moderately express nestin, while astrocytoma and glioblastoma strongly express nestin. Nestin is an intermediate filament protein and is localized in the cytoplasm in most brain tumors; however, in human neuroblastoma and medulloblastoma cell lines, nestin has been observed in nuclei [62], suggesting that nestin may directly bind to DNA or intranucleic proteins. Altogether, these findings demonstrate that nestin is expressed in a wide variety of brain tumors and that this expression correlates with their functions or cell behaviors.

Brain tumors	Expression pattern and roles
Schwannomas	Frequent nestin expression [48]
Ependymomas	Poor progression-free survival and overall survival [49]
Neurocytomas [51]	N/D
Adamantinomatous craniopharyngiomas	Expressed in the invasion niche [52]
Pituitary adenomas	Coexpressed with CD133 [53]
Medulloblastomas	Expressed in tumor stem cells [55-58]
Oligodendrogliomas [60]	N/D
Gliomas	High grade [7,8]
	Worse overall survival [48,61]
Glioblastomas	Infiltration into surrounding tissue [8]
	Tumor stem cells [72-77]
N/D: Not determined.	

Table 1. Expression and roles of nestin in brain tumors

5. Nestin in glioblastoma

5.1. Nestin in low-grade gliomas and glioblastomas

Immunohistochemical analysis has demonstrated nestin expression in the cytoplasm of glioblastoma cells (Figure 1). Large-scale and multicenter studies have shown high immunoreactivity of nestin in glioma cases to be correlated with high grade [7, 8] and worse overall survival [48, 61] (Table 1). Furthermore, expression of nestin and MIB-1 labeling indices in immunohistochemical analyses may correlate with aggressiveness of pilocytic astrocytoma and pilomyxoid astrocytoma [63]. An analysis of several stem cell markers—including CD133, nestin, B lymphoma Mo-MLV insertion region 1 homolog (BMI-1), Maternal embryonic leucine zipper kinase (MELK), and Notch 1-4—was performed using quantitative RT-PCR in 42 glioblastoma samples; MELK was most upregulated, followed by nestin [64]. In contrast, others have reported that nestin immunoreactivity is mostly due to an acute glial reaction and is not specific to the neoplasm [65], and that nestin expression in gliomas does not correlate with prognosis [66].

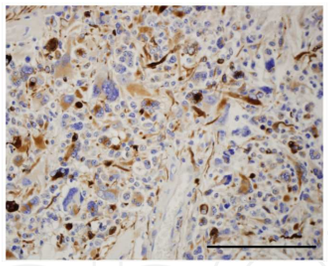

Figure 1. Expression of nestin in glioblastomas. Bar, 100 μm

Immunostaining of nestin in glioblastoma cells has been demonstrated to delineate between invading tumor and the adjacent gray and white matter; therefore, nestin is considered to be a useful marker for examining the infiltration of glioblastomas into surrounding tissues [8]. Furthermore, knockdown of nestin in human glioblastoma cells has been shown to suppress cell migration and invasion, and to increase F-actin expression and cell adhesion to extracellular matrices [9].

Nestin-positive non-tumorous brain cells migrate into the glioblastoma cells and delay astrocytic or elongated bipolar morphology and glomerulus-like microvasculature [67]; therefore, nestin-positive cells have been considered an important component of the tumor microenvironment. CD133-positive and nestin-positive niches are perivascularly localized in all glioma tissues, and the presence of these niches increases significantly with increasing tumor grade [68]. Mice were engineered to co-express platelet-derived growth factor B receptor and Bcl-2 under the control of the glioneuronal-specific nestin promoter, and this resulted in the development of low- and high-grade gliomas [69]. Another study found that human glioblastoma subclones characterized by high nestin levels formed tumors in vivo at a significantly faster rate than subclones with low nestin expression, suggesting that induction of nestin plays an important role in glioblastoma carcinogenesis [70]. However, the opposite result has also been reported [71].

5.2. Nestin in glioma stem cells

Cancer stem cells appear to be responsible for tumor metastasis, resistance to radiotherapy and chemotherapy, and disease relapse; thus, their analysis and therapeutic targeting are believed to be crucial. Many studies have shown that there is a small population of cancer stem cells in glioblastomas, and that nestin is one of the stem/progenitor cell markers of glioblastomas [72-77]. CD133, Oct4, Sox2, and Nanog have also been considered to be stem cell markers in glioblastomas [78, 79]. However, CD133-negative and nestin-negative glioblastoma cells show tumorigenic potential in vivo [71]; thus, there remains some controversy over which specific markers should be used to detect glioblastoma stem cells. An in vitro study has shown that neurospheres of glioblastoma cells exhibit high expressions of nestin, CD133, and Oct4 compared to the expressions in monolayer cells [80]. One study reported that radiation induces increased expressions of stem cell markers, including nestin, CD133, and Musashi [81]; in contrast, another study has shown that radiation induced accumulation of CD133-positive glioblastoma cells, but not nestin [82]. Glioblastoma stem cells are main-

tained in vivo in a niche characterized by hypoxia, and hypoxia reportedly increases the expressions of nestin, CD133, podoplanin, and Bmi-1 [83]. Together, these available data suggest that there is close relationship between nestin and stemness in glioblastoma.

Expression of nestin in cancer stem cells of glioblastoma may indicate the origin and function of these cells. Potential cancer stem cell origins include migration of neural stem cells toward the tumor, migration of mesenchymal stem cells from bone marrow, or dedifferentiation of tumor cells [84]; each of these hypotheses have been proven experimentally. In brain tumors, long-term cultured human neural stem cells undergo spontaneous transformation to tumor-initiating cells [37]. In contrast, Nanog promotes dedifferentiation of p53-deficient mouse astrocytes into glioblastoma stem cells [85]. These results indicate that glioblastoma stem cells may arise from both the transformation of nestin-positive neural stem cells and differentiated astrocytes. Retinoic acid treatment for glioblastoma stem cells was demonstrated to reduce the expression of neural stem cell markers, such as nestin, CD133, Msi-1, and Sox-2 [86].

Xenografts developed from human anaplastic astrocytoma and glioblastoma tumor-derived spheres in the brain of a nude mouse revealed co-expression of PCNA, VCAM-1, caspase-3, and nestin [87]. Cells positive for both caspase-3 and nestin were located adjacent to or around the blood vessels. Glioblastoma stem cells expressed nestin/CD31 or CD133/CD31, and these cells were capable of differentiating into endothelial cells [88]. Dong et al. have shown that human glioma stem/progenitor cells transdifferentiate into vascular endothelial cells in vitro and in vivo [89]. Glioblastoma stem cells have close relationships with the angiogenic switch, intratumor hypoxia, and the neoplastic microvascular network. These findings provide new insights for targeted therapy against glioblastomas.

5.3. Regulation of nestin in glioblastoma cells

Glioblastomas usually show hyperactivation of the PI3K-Akt pathway. Exogeneous expression of the Akt-binding domain of Girdin inhibits its Akt-mediated phosphorylation, and reportedly diminishes migration and the expression of the stem cell markers nestin and SOX2 [90]. Nestin expression in glioblastomas is correlated with proangiogenic chemokines (CXCL12 and its receptor CXCR4) and growth factors (VEGF and PDGF-B and its receptor PDGFRbeta) [91]. Hypoxia and radiation are both inducers of stem cells, and were associated with increased expression of nestin [81, 83]. In glioblastoma cases, a 9-gene profile that included podoplanin and insulin-like growth factor binding protein 2 was found to predict the prognosis, and was also positively associated with expressions of nestin and CD133 [92]. Additionally, the enhancer lesion of nestin is known to be located in the second intron in neural cells, and this lesion is highly conserved in mouse, rat, and human [93].

5.4. Nestin in interstitial tissues and angiogenesis of glioblastoma

Glioblastoma-conditioned medium has been shown to induce human mesenchymal stem cells (hMSCs) to increase expressions of nestin, CD151, VE-cadherin, desmin, α-smooth muscle actin, and nerval/glial antigen 2—indicating pericyte-like differentiation, rather than

differentiation to endothelial cells or smooth muscle cells [94]. hMSCs migrate towards glioblastoma and are incorporated into tumor microvessels.

Much evidence has shown that expression of nestin in vascular endothelial cells is associated with proliferation and angiogenesis [32, 95-98]. In glioblastomas, expression of nestin in both tumor cells and endothelial cells was increased according to increasing tumor grade [7]. A recent study has indicated that the capillaries in gliomas may come from the differentiation of glioblastoma stem cells, and that the glioblastoma stem cells are accumulated around the capillaries [99]. In contrast, CD105 has been proposed to be a more useful marker of tumor angiogenesis in glioblastomas than nestin [100]. The morphology of nestin-positive cells in brain tumors is reportedly more typical of neural stem cells, and less than 0.1% of these cells co-express the endothelial marker CD34 [101].

5.5. Nestin as a therapeutic target for glioblastoma

We have reported that knockdown of nestin using shRNA suppresses cell migration and invasion [9]. Lu et al. demonstrated that blocking the expression of nestin in glioblastomas via intratumor injection of shRNA significantly slowed tumor growth and volume [70]; therefore, nestin may serve as a novel candidate for molecular targeted therapy for glioblastomas [9]. The phytoalexin resveratrol suppresses cell growth, migration, invasion, and expression of nestin in glioblastoma cells [102]. It has been shown that peptides can bind to a nestin isotype that is specifically expressed in glioma stem cells, which enables them to target nestin-positive cells in human glioma tissue [103]. Future studies should focus on developing delivery systems to target these anti-nestin reagents to brain tumors, and on the estimation of the side-effects for normal brain stem cells that express nestin.

6. Conclusion

The neuronal stem cell marker nestin regulates cell growth, migration, invasion, and stemness, and has been found to be expressed in a wide variety of brain tumors. Nestin may be a candidate for the development of promising therapeutic and diagnostic modalities for glioblastoma.

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