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Notch Pathway Plays an Important Role in the Formation of Xenograft Model with Original Spinal Cord Diffuse Astrocytoma Cells

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This study was designed to investigate whether the Notch pathway is involved in the development of diffuse spinal cord astrocytomas. BALB/c nude mice received injections of CD133+ and CD133- cell suspensions prepared using human recurrent diffuse spinal cord astrocytoma tissue through administration into the right parietal lobe. After 7–11 weeks, magnetic resonance imaging was performed weekly. Xenografts were observed on the surfaces of the brains of mice receiving the CD133+ cell suspension, and Notch-immunopositive expression was observed in the xenografts. By contrast, no xenografts appeared in the identical position on the surfaces of the brains of mice receiving the CD133- cell suspension, and Notch-immunopositive expression was hardly detected either. Hematoxylin–eosin staining and immunohistochemical staining revealed xenografts on the convex surfaces of the brains of mice that underwent CD133+ astrocytoma transplantation. Some sporadic astrogloma cells showed pseudopodium-like structures, which extended into the cerebral white matter. However, it should be emphasized that the subcortex xenograft with Notch-immunopositive expression was found in the fourth mouse received injection of CD133- astrocytoma cells. So, these findings suggest that the Notch pathway plays an important role in the formation of astrocytomas, and can be considered a novel treatment target for diffuse spinal cord astrocytoma.

Key words: diffuse astrocytoma, mice, immunodeficiency (BALB/c) mice, CD133, Notch pathway, spinal cord tumor, brain model

Declaration from correspondence author (Jianjun Sun): Based on traditional friendship and communicating between Peking University and Juntendo University, our paper was accepted as a part of memorial Journal. However, this paper contains extracts from “Jian-jun Sun, et al. Prevention against diffuse spinal cord astrocytoma: can the Notch pathway be a novel treatment target? Neural Regen Res, 2015; 10: 244–251”.

A specific marker of glioma stem cells (GSCs) has been widely searched for, but the results have been inconclusive. CD133 is the most widely used antigen for enrichment of GSCs1, and has been repeatedly validated in freshly isolated patient specimens2–3. It is the consensus that GSCs have been prospectively enriched by selection of the CD133 cell surface marker4–5. CD133 has been used as a GSC marker to identify and isolate a small fraction of cells in gliomas with a significantly increased potential to generate tumor neurospheres1–6–7. However, several studies4–8–11 have
found that CD133- cells may still have the characteristics of GSCs, and that CD133 is not a unique marker for GSCs. Therefore, some authors have suggested that CD133 should not be used as a unique marker for collecting glioma stem cells.

Notch activity is critically implicated in the radioresistance of GSCs. The Notch pathway can promote or repress tumorigenesis in a context-dependent manner. The Notch pathway has functions in cell growth, proliferation, and survival. A new hypothesis was proposed by some scientists that glioma cells would only become cancer stem cells if Notch is expressed, leading to sustained self-renewal and potent tumorigenicity, regardless of the expression of CD133 or other markers.

Different sub-types of tumors may also have different markers for CSCs. Although many studies have investigated cell markers for GSCs in the brain, none have focused on GSCs isolated from recurrent spinal cord astrocytomas.

In this study, we focused on identifying a marker for the isolation and generation of xenografts with a small population of original intramedullary diffuse spinal cord astrocytoma cells, and on the key role of the Notch pathway in the development of cerebral xenografts from only a few original spinal cord intramedullary astrocytoma cells.

Whether CD133 or Notch can be considered a marker of GSCs remains inconclusive. We found that CD133- original astrocytoma cells formed xenografts in immune-deficient mouse brain. Moreover, the xenografts still showed negative expression for CD133 staining. However, all xenografts showed positive expression for Notch. It was hypothesized that Notch played a key role in the formation of xenografts from the original diffuse spinal cord astrocytoma cells.

The definition of CSCs remains functional, requiring sustained self-renewal and tumor propagation. In our study, the growth of astrocytoma xenografts was closely related to positive expression of Notch. The Notch pathway is a key signaling pathway for GSCs. Moreover, CD133+ is not a representative marker for GSCs. In addition, positive expression of Notch pathway might be correlated with the vitality of GSCs. Once Notch is expressed, regardless of the expression of CD133 or nestin, these astrocytoma cells are activated from the dormant state. These active glioma (astrocytoma) cells began to become active and proliferate, acting as truly functional GSCs.

Figure 1: Gross changes of mouse brain after xenograft implantation

A, B. At 8 weeks after transplantation of CD133+ cells, a red xenograft nodule was observed on the surface of one mouse brain. Original magnification: 4× (A) and 10× (B). C, D. At 11 weeks after transplantation of CD133+ cells, a red-brown lesion was observed on the cortex of another mouse brain, indicating the presence of a xenograft. Original magnification: 6× (C) and 10× (D). E, F. At 10 weeks after transplantation of CD133- astrocytoma cells, a blurry insert needle spot (arrow) was left, without any abnormal lesions on the surface of the mouse brain. A xenograft was present in the subcortex. Original magnification: 6× (E) and 12× (F).

(Sun JJ, et al: Neural Regen Res, 2015; 10: 244-251)
Figure 2  Histological changes to xenografts after transplantation of CD133+ astrocytoma cells
A. At 8 weeks after cell transplantation, xenografts were observed outside the cerebral cortex of CD133+ mice by hematoxylin–eosin staining. A large tumor nodule protrudes from the surface of cortex, in which there are atypical cells of different sizes (red oval circle). There are several vessels that supply blood to the tumor nodule in the juncture between the nodule and cortex (as shown by green arrows). Some diffuse astrocytoma cells like pseudopodia infiltrated the surrounding subcortical white matter (as shown by yellow arrows). Original magnification: × 100.
B. Many glioma cells on the surface of cortex of CD133+ mouse showed strong GFAP-positive expression, several cells infiltrated deeply into the white matter (pointed by arrow). Original magnification: × 400.
C. Nestin immunoreactivity was seen in vascular cavity, wall, and extracellular matrix (arrow). Original magnification: × 200.
D. Strong Notch immunoreactivity was seen in the cell cytoplasm. Proliferating astrocytoma cells accumulating around the stem cell niche were observed on the same slide. Original magnification: × 400.
E. CD133 immunoreactivity was observed on the astrocytoma cell membrane (arrow). Original magnification: × 200.
F. At 11 weeks after transplantation of CD133+ diffuse astrocytoma cells into mouse brain, a xenograft like a lotus root was found in the cortex (green circle). Original magnification: × 100.
G. GFAP immunoreactivity on the extracellular matrix of diffuse astrocytoma cells (arrow). Original magnification: × 40.
H. Nestin immunoreactivity on the pathological vascular cavity (arrow). Extracellular matrix infiltrated deep into the white matter, and a pathological vascular structure was seen inside the xenograft. Original magnification: × 100.
I. Cluster-like Notch immunoreactivity on the astrocytoma cell membrane and cytoplasm in the adjacent subcortex (black circle). Original magnification: × 100.
J. CD133 immunoreactivity was detected on the cell membrane of some scattered astrocytoma cells in a cell niche (arrow). Original magnification: × 400.
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Figure 3  Histological changes to xenografts after transplantation of CD133- astrocytoma cells
A. At 10 weeks after transplantation of CD133- diffuse astrocytoma cells, an intra-cortex xenograft around the insertion site (arrow) was observed by hematoxylin-eosin staining. Original magnification: × 200.
B. Weak Notch immunoreactivity on partial cytoplasm and extracellular matrix of diffuse astrocytoma cells (arrow). Original magnification: × 400.
C. The xenograft cells showed negative immunoreactivity for CD133 (black circle). Original magnification: × 200.
(Sun JJ, et al: Neural Regen Res, 2015; 10: 244-251)
References


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