Promoted growth of brain tumor with severe hemorrhage by the transplantation of neural progenitor cells facilitated by SDF-1

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Introduction

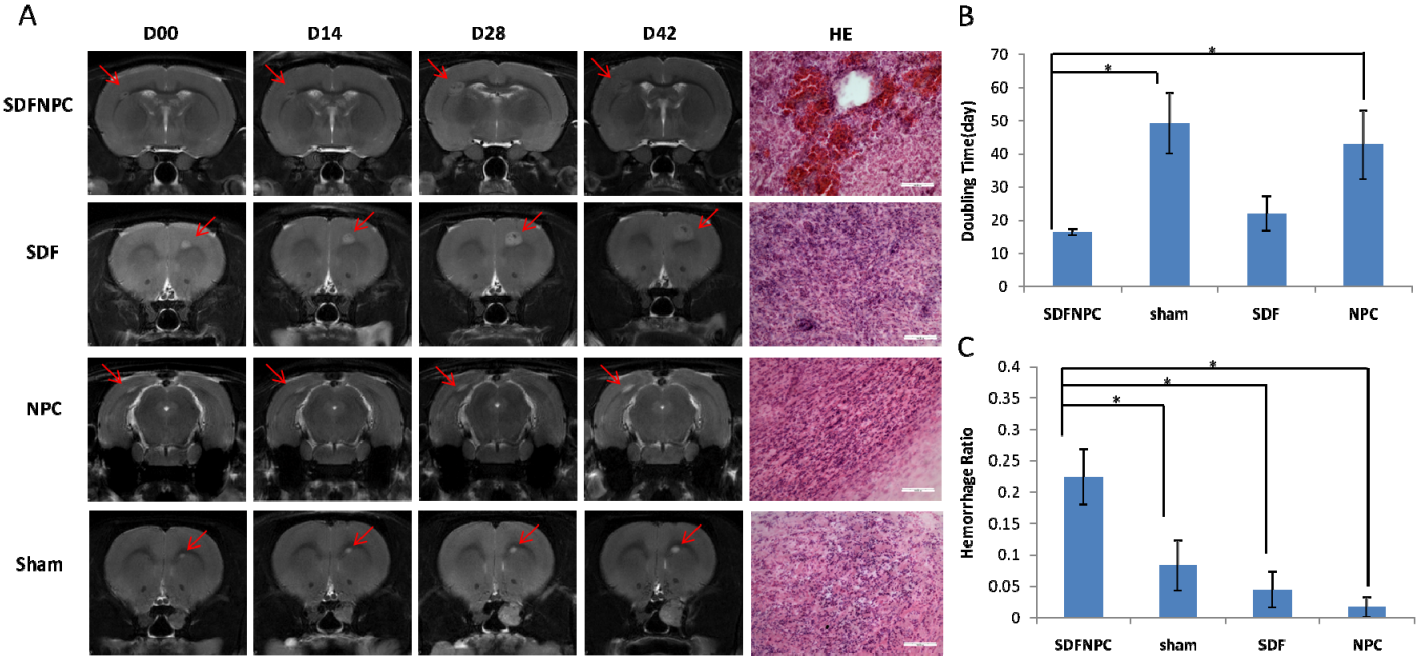
Neural progenitor cells (NPCs) tend to migrate specifically to sites of brain injuries. Such behavior is primarily mediated by the signaling of the chemokine SDF-1 and its receptor CXCR4 [1]. Glioma releases high levels of SDF-1, which may attract the migration of NPCs, which express CXCR4 intrinsically [2]. The migration of NPCs toward gliomas may change the growth pattern of a tumor. To test the possibility, the present study examined whether NPCs migration facilitated by the SDF-1/CXCR4 signaling affected the growth and other characteristics of tumors. NPCs were transplanted into the lateral ventricle while an injection of SDF-1 was made near the tumor site. T2-weighted imaging (T2WI) was repeatedly acquired before, and after the treatment. Our investigations directly provide evidence regarding the role of NPCs and SDF-1 in tumor progression.

Material and Method

Pregnant Sprague–Dawley rats were injected intraperitoneally with 50 mg/kg ethylnitrosourea (ENU) at 18–19 days of gestation. The offspring generated brain tumors spontaneously as early as 3 month old. NPCs were prepared from primary subventricular zone (SVZ) cells as described in the below. The brain of SD neonatal rat was taken off and the tissues nearby SVZ were collected. The tissues were mechanically dissociated, filtered, and seeded in the serum free medium. The cell density was $1.5*10^6$ cells/ml and cultured condition was controlled at 37°C, 5% CO_2 with humidity. The NPCs gradually developed to neurospheres and were then passaged to single cells when the average diameter reached 150um. NPCs were transplanted to the lateral ventricle (Bregma = -0.5 mm; Lateral = 1.5 mm; Depth 3.5 mm.) ipsilateral to the tumor location and SDF-1 was injected near the tumor site. The injected NPCs were $1*10^6$ cells at 5 ul and the SDF-1 concentration was 10 μ g/ml. The rats were scanned for tumors by a 7-T MRI system. Rats were fitted in a custom designed headholder and anesthetized with isoflurane flowed in oxygen (isoflurane at 5% for induction and 2% for maintenance). T2WIs were acquired using a fast spin echo sequence, with a field of view of 3 cm, slice thickness of 1 mm, 28 slices with no gap to cover the entire brain, a TR of 5100 ms, a TE of 70 ms, echo train length of 8, number of excitations being 6, and a matrix size of 256*256. Rats detected with tumors by T2WI were subject to the transplantation of NPCs combined with SDF or vehicles of both. The scan time points were Day0 (before the injection), Day7, Day14, Day28, and Day42. Because tumors are readily distinguishable from surrounding tissues owing to the hyper/hypo signal intensities, the measurement of tumor volume was based upon the summation of these pixels using MR Vision (MR Vision Co.). The doubling time (Td) of the tumor was calculated by customized Matlab codes. Analysis of variance (ANOVA) was used to test the doubling time and the ratio of hemorrhagic area using STATVIEW. Hemorrhage appeared as hypointensities in tumors, and thus could be distinguished using the software Avizo. The hemorrhage ratio is calculated based upon the area of hemorrhage out of the entire tumor.

Results and discussion

Fig. A shows the T2WI of the four groups, SDFNPC (transplanted with NPC and SDF at the same time), NPC, SDF and sham. Arrows indicate the tumor location. The HE staining was obtained from the brain tissues perfused on D42. Two major changes caused by the treatment were observed: the size and the severity of hemorrhage, as shown in Fig. B and C respectively. The doubling time of the tumors was summarized in Fig. B. The Td was 16.46 ± 0.9 days for the SDFNPC group, 22.06 ± 5.25 days for the SDF group, 42.74 ± 10.4 days for the NPC group and 49.16 ± 9.18 days for the sham group. Although the doubling time was not statistically different between the SDFNPC and SDF groups, a significant increase in the severity of hemorrhage was noted in the SDFNPC as shown in Figure C (the severity was four times as much in the SDFNPC group). The results suggest that the transplantation of NPC with promoted migration by SDF may lead to rapid growth of tumors with more hemorrhage formation.



Reference:[1] Imitola J et al., *Proc. Natl. Acad. Sci.*,101;18117–18122, 2004. **[2]** Zhao D et al., *Mol Cancer Res.*,6;1819-1829, 2008.