Osteopontin is associated with tumor malignancy revealed by multi-parametric MRI assays

Nai-Wei Yao[1,2], Chiao-Chi V. Chen[1], Yi-Hua Hsu[1], Hsiu-Ting Lin[1], Jeou-Yuan Chen[1], and Chen Chang[1]

[1]*Institute of Biomedical Sciences, Academic Sinica, Taipei, Taiwan,* [2]*Department of Zoology, National Taiwan University, Taipei, Taiwan*

Introduction

Malignant tumors are refractory and fatal. Identification of the major molecular player associated with tumor malignancy is essential for the development of possible targeted therapies. Osteopontin (OPN), a member of the small integrin-binding ligand N-linked glycoproteins (SIBLINGs) family, plays important roles in inflammation, immune responses, tumorigenesis and metastasis[1]. OPN is strongly expressed in high-grade and metastatic brain tumors[2] and plays a major role in cancer progression [3,4,5]. In this study, we investigated the role of OPN in association with tumor malignancy in a xenograft glioma model using the rat C6 cells from which OPN expression was knocked down, along with the control knockdown (KD) cells. The effects of OPN KD on tumor formation were assessed by monitoring the tumor size, the integrity of the blood brain barrier (BBB), and the tumor-associated metabolites by T2-weighted imaging (T2WI), dynamic contrast-enhanced (DCE) MRI and magnetic resonance spectroscopy (MRS), respectively. Tumor malignancy was characterized by BBB breakdown and the changes of metabolites. Our data demonstrate a predominant role of OPN in association with tumor malignancy.

Material and Method

The rat C6 cells with OPN-KD or luciferase KD (Luc-KD, serving as control) were established by Lentivirus-based shRNA technology. A volume of 0.6 μL of the cell suspension (1×10^6/10uL in phosphate-beffered saline) was injected into a 9-week-old Sprague–Dawley rat on the stereotaxic apparatus using a 30-gauge needle (Hamilton, NV, USA) and a micro-infusion pump (Model 310; KD Scientific, MA, USA). The injection was made at the left striatum [Bregma=0.2mm, lateral=3.0mm, and depth=5.0 mm]. After recovery from surgery, the rats were scanned by a 7-T MRI system. For scanning, rats were anesthetized with isoflurane flowed in air (isoflurane at 5% for induction and 2% for maintenance), fitted in a custom designed head holder, and inserted into the magnet. T2WIs were acquired at 12, 17, 21, and 24 days after C6 cells implantation using a fast spin echo sequence with field of view (FOV) =2.56 cm, slice thickness=0.5 mm, 8 slices with no gap to cover the whole tumor, TR=4000 msec, TE=70 msec, echo train length=8, number of excitations (NEX)=12, and matrix size=256×128 (zero filled to 256×256). 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 commercial available MR Vision. DCE-MRI experiment was performed at 17 and 21 days after implantation using a dynamic series of 80 T1-weighted gradient-echo images with TR=130.2 ms, TE=4.1 ms, FOV=2.56 cm×2.56 cm, data matrix size=256×128 (zero filled to 256×256), slice thickness=1 mm, and NEX =1. An intravenous bolus injection of 0.2 mmol/kg Gd-DTPA (Magnevist, Schering AG, Germany) was given during acquisition of the eighth image. The vascular permeability coefficient (K^{trans}) map of tumor was calculated by customized Matlab codes. MRS was acquired at 24 days after implantation by the point resolved spectroscopy (PRESS) sequence with voxel size=2.5×2.5×2.5 mm^3, TR=3000ms, TE=136ms, and NEX=256. For each rat, two volume of interest (VOIs) were localized, with one centered on the tumor and the other in the contralateral striatum. MRS data analysis was performed by Bruker ParaVision software for the integral ratio of each spectrum. Analysis of variance (ANOVA) was used to identify group differences using STATVIEW.

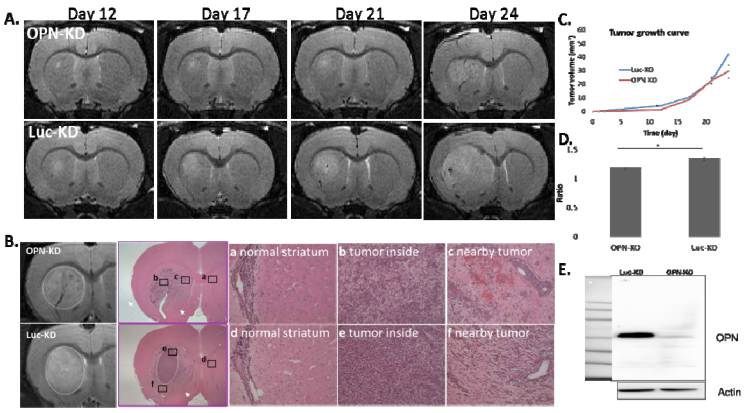

Fig 1. (A) T2WIs of OPN-KD and Luc-KD tumors at different time points. (B) The H&E stain of OPN-KD and Luc-KD tumors. (C) The tumor growth curve. (D) The signal intensity of T2WIs. (E) Western blot verification

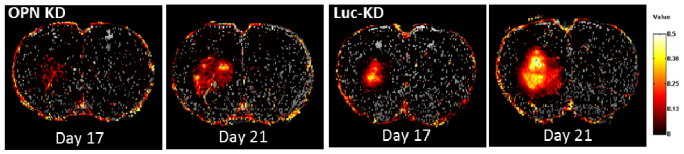

Fig 2. vascular permeability(K^{trans}) map of OPN-KD and Luc-KD tumors on Day 17 and 21

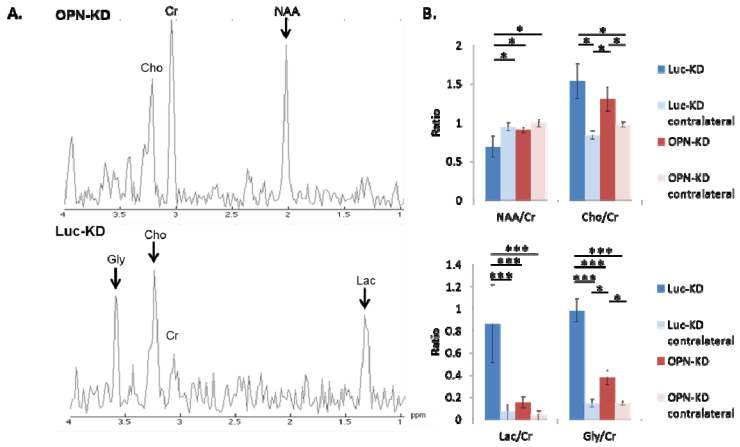

Fig 3. (A) Representative MRS of OPN-KD and Luc-KD tumors. (B) The level of NAA, Cho, Lac, and Gly.

Results and Discussion

The tumor growth was monitored by T2WIs at 12, 17, 21, and 24 days after C6 cells implantation. Fig. 1A shows the T2WI of the two groups injected with OPN-KD and Luc-KD C6 cells, respectively. H&E staining shown in Fig. 1B reveals a dispersed, loose arrangement of tumor cells in OPN-KD tumor as compared to Luc-KD tumors. The tumor volume was measured by T2WIs and the tumor growth curves are presented in Fig. 1C. No statistical difference was seen in the size of tumors formed in the two groups. But analysis of the signal intensity ratio (tumor versus contralateral striatum) showed significantly lower values in tumors with OPN-KD than Luc-KD (Fig. 1D). These data suggest that OPN-KD tumors were less edematous and/or packed with less tumor cells. OPN-KD on C6 cells was verified by western blot (Fig. 1E). Regarding the functionality of BBB, a significant decrease in the severity of vascular permeability (K^{trans}) was noted in the OPN-KD tumor as shown in Figure 2. At both time points, the lower values of K^{trans} were only observed in the OPN-KD tumor but not in Luc-KD tumor. MRS is also used to determine the degree of malignancy. The MR spectra of OPN-KD and control tumors shown in Figure 3A demonstrate decreased Lac, Cho and Gly and increased NAA in the OPN-KD as compared to Luc-KD tumors (Fig. 3B). KD of OPN in C6 glioblastoma leads to decreased tumor malignancy as demonstrated by less severe BBB breakdown and the decreased levels of choline, lactate, and glycine. This finding supports the view that OPN plays a key role in tumor malignancy.

Reference: ***1.*** **Rittling SR et al., 2004.** ***2.*** **Saitoh et al., 1995.** ***3.*** **Saitoh Y et al., 1995.** ***4.*** **Bellahce`ne A et al. 2008.** ***5.*** **Lamour V et al. 2009.**