VEGF-Dependent Mechanism of Anti-Angiogenic Action of Diamond Nanoparticles in Glioblastoma Multiforme Tumor

M. Grodzik[1], E. Sawosz[2], M. Wierzbicki[3], A. Hotowy[4], M. Prasek[5], S. Jaworski[6] and A. Chwalibog[7]

[1]Warsaw University of Life Sciences, Nowoursynowska 166, 02- 787 Warsaw, Poland, marta_grodzik@sggw.pl
[2]Warsaw University of Life Sciences, Poland, ewa_sawosz@sggw.pl
[3]Warsaw University of Life Sciences, Poland, mateusz_wierzbicki@sggw.pl
[4] University of Copenhagen, Denmark, hotowy@life.ku.dk
[5]Warsaw University of Life Sciences, Poland, martaprasek@gmail.com
[6]Warsaw University of Life Sciences, Poland, jaworski.slawek@gmail.com
[7] University of Copenhagen, Denmark, ac@life.ku.dk

ABSTRACT

Malignant gliomas are highly lethal cancers dependent on angiogenesis. The concept of treating tumors by inhibiting tumor angiogenesis was first articulated almost 30 years ago. Inhibition of tumor angiogenesis suppresses both tumor growth and metastasis. We determined the inhibition effect of diamond nanoparticles on the growth of brain tumor (cultured on CAM membrane) and the development of its blood vessels. We hypothesize that diamond nanoparticles can bind VEGF or their receptors and this way influence of signal transduction between cells. The aim of our study was to evaluate the influence of diamond nanoparticle on VEGF level and inhibition of the brain tumor angiogenesis. We evaluated interaction of VEGF-A and VEGF-receptor proteins with diamond nanoparticles (TEM), visualized lower the permeability of blood vessels after diamond nanoparticles treatment and determined localization in the cell and expression on protein level VEGF-A and VEGF-A-receptor.

Keywords: angiogenesis, cancer, diamond, glioma, nanoparticle, *VEGF*

1 INTRODUCTION

Gliomas are the most common primary brain tumors, glioblastoma multiforme (GBM) being the most aggressive subtype. One of the most effective forms of antiglioma therapy is antiangiogenic therapy. Inhibition of tumor angiogenesis suppresses both tumor growth and metastasis. A large number of pro- and anti-angiogenic cellular factors regulate angiogenesis in glioblastoma. Among them, vascular endothelial growth factor (VEGF) has been implicated as a major mediator in the pathogenesis of glioblastoma. The increased protein level of VEGF is linked to the higher permeability of the blood vessels and their untypical structure, which is characteristic of tumors. Lowering of the VEGF level contributes to the normalization of blood vessels, facilitating the infiltration of other factors into the tumor, while inhibiting VEGF expression limits the transport of nutrients into cancer cells. Recently, the new biologically active substances have appeared that can be useful in antiangiogenic therapy: nanoparticles of carbon allotropes [1] (C60 fullerenes, graphite nanoparticles, multi-walled carbon nanotubes, single-walled carbon nanotubes and diamond nanoparticles).

Our research team determined the effect of carbon nanoparticles manufactured by different methods (detonation method UDD and plasma-assisted chemical vapor deposition process MW-RF) on the growth of brain tumor (cultured on chicken embryo chorioallantoic membrane) and the development of its blood vessels. UDD (diamond nanoparticles) and MW-RF nanoparticles reduced tumor mass and volume and inhibited blood vessels' development in GBM tumors. Moreover, UDD nanoparticles decreased *FGF-2* and *VEGF* expression on mRNA level, while MW-RF nanoparticles only reduced *VEGF* expression, although there was a tendency of reduced *FGF-2* expression too [2]. However, mechanism of action of carbon nanoparticles is unclear.

We hypothesize that diamond nanoparticles can bind VEGF or their receptors and this way influence signal transduction between cells. The aim of our study is to evaluate the influence of diamond nanoparticle on VEGF level and inhibition of the brain tumor angiogenesis.

2 MATERIALS AND METHODS

2.1 Diamond Nanoparticles

Nanoparticles of diamond (size 3 - 4 nm; specific surface area 282 m^2/g; functional group on surface: --OH, --CN, --COOH, -C-O-C, --C=O) were obtained from the Skyspring Nanomaterials Inc (Houston, TX, USA) (Figure 1).

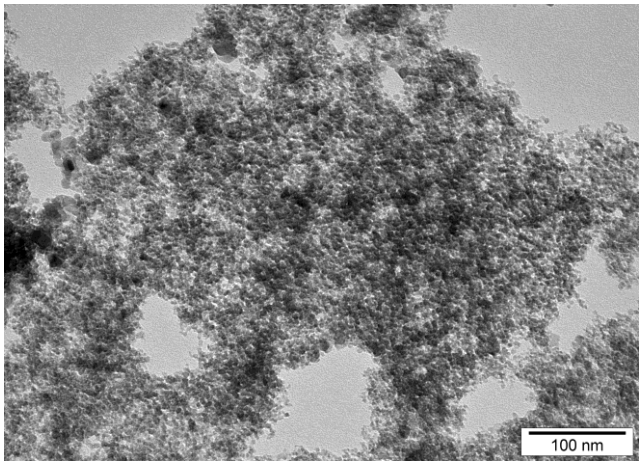

Figure 1 Transmission electron microscope images of diamond nanoparticles.

2.2 Nanoparticles - protein interactions

Hydrocolloid of diamond nanoparticles (50µg/ml) were mixed with proteins: VEGF-A (50 µg/ml; Sigma Aldrich) or VEGF-A receptor (50 µg/ml; Sigma Aldrich) (ratio 1:1) and observed in transmission electron microscope.

2.3 Cells and embryos

Human U87 glioblastoma cells (HTB-14; American Type Culture Collection, Manassas, VA) were maintained in Dulbecco's modified Eagle medium (Sigma-Aldrich Corporation, St Louis, MO) with 10% fetal bovine serum (Sigma-Aldrich). The fertilized eggs (Gallus gallus) (n = 45) were supplied by a commercial hatchery (Debowka, Poland).

2.4 Culture of GBM on chorioallantoic membrane

After 6 days of egg incubation, the silicone ring with the deposited 3–4 × 106 U87 cells suspended in 30 µL of culture medium was placed on the chorioallantoic membrane. The eggs were incubated for 7 days and then 24 eggs with visible tumor development were chosen. Eggs were divided into two groups of twelve: the control group, and diamond nanoparticles group (injected with 200 µL of 500 µg/mL solution of diamond nanoparticles). The solution was added directly into the tumors. After 2 days, the tumors were resected for further analysis.

2.5 Vascular permeability

After 9 days of tumors cultured 100 µl dextran-FITC (0,5mg/ml; 70 000 kDA; Sigma Aldrich) was intravenous injected to 8 chicken embryos (4 per group). Tumours were resected after 30 minutes. Confocal microscope (Olympus FV1000) was used to visualize the tumor tissues.

2.6 Expression of VEGF-A and VEGF-A-receptor protein

Western blotting analyses were performed using following antibodies: human anti-VEGF-A produced in rabbit , anti-KDR antibody produced in rabbit, and anti-b-actin (Sigma-Aldrich). The levels of cellular proteins were visualized with alkaline phosphatase-coupled secondary antibodies (goat anti-rabbit IgG-AP; Sigma Aldrich) using AP Color Development; Bio-Rad, CA, USA)

Immunofluorescence analyses were performed on cryostat sections. Tissue were fixed with 4% PFA, blocked in BSA and then treated with anti-VEGF-A or anti-KDR antibodies (Sigma-Aldrich) at 4 °C overnight. Sections were probed with FITC conjugated secondary antibodies from Molecular Probes. Tissue were counterstained with DAPI and mounted using Prolong Gold Antifade Reagent (Molecular Probes). Images were captured using a confocal microscope (Olympus FV1000).

3 RESULTS AND DISCUSSION

Our previous researches showed that diamond nanoparticles have anti-angiogenic properties in relation to glioblastoma multiforme tumors (Figure 2). Analysis of the antiangiogenic mechanism of diamonds nanoparticles was performed on experimental model in ovo, on the chicken embryo chorioallantoic membrane.

Determination of vascular permeability was made with using dextran-FITC particles. Intravenous injected dextran-FITC particles in chicken embryos from control group was visible in most of the cytoplasm of cancer cells. Carbon nanoparticles treatment reduced vascular permeability in glioblastoma multiforme and spread of fluorescence particle (Figure 3). Decreases of vascular permeability is associated with decrease VEGF expression.

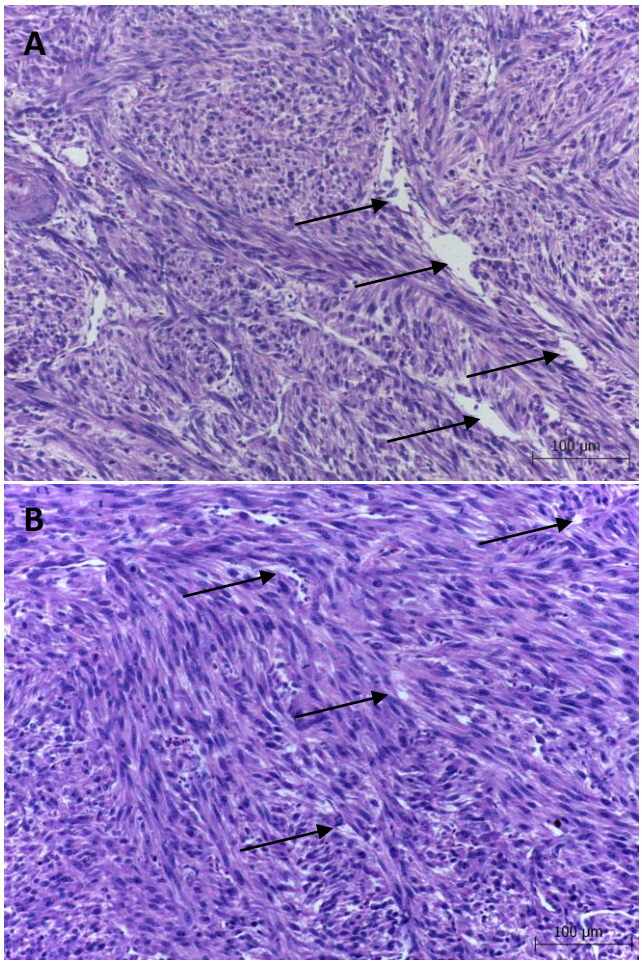

Figure 2. Histology of glioblastoma multiforme tumor: control group (A) and diamond nanoparticle group (B). Notes: Scale bar: 100 µm. Arrows point to blood

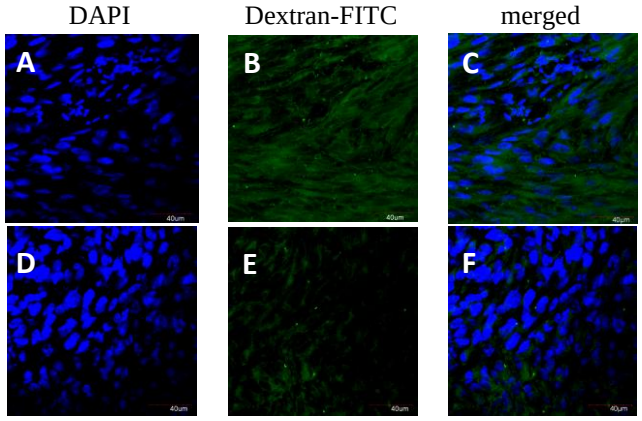

Figure 3 Vascular permeability. Immunofluorescence of glioblastoma multiforme from control group (A, B, C) and after diamond nanoparticle treatment (D, E, F). Notes: Scale bar: 40 µm. Diamond nanoparticles treatment caused reduction spread of dextran-FITC particles in the tissue.

Analysis of VEGF-A and VEGF-A receptor protein expression were performed using immunofluorescence (Figure 4; Figure 5) and western blot methods. Level of expression both protein VEGF-A and their receptor was decreased.

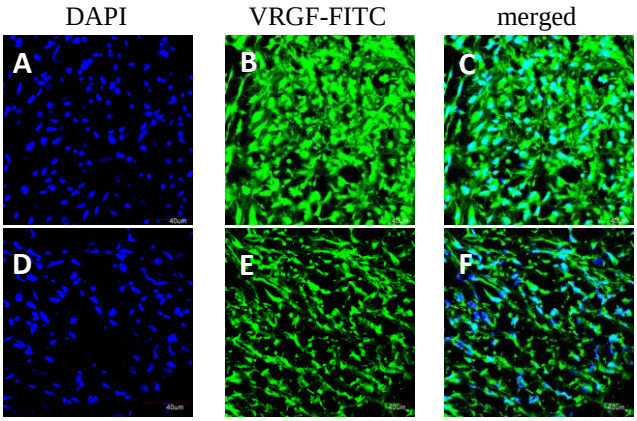

Figure 4 Expression of VEGF protein in glioblastoma multiforme from control group (A, B, C) and after diamond nanoparticle treatment (D, E, F). Notes: Scale bar: 40 µm. Diamond nanoparticles treatment decreased VEGF protein expression.

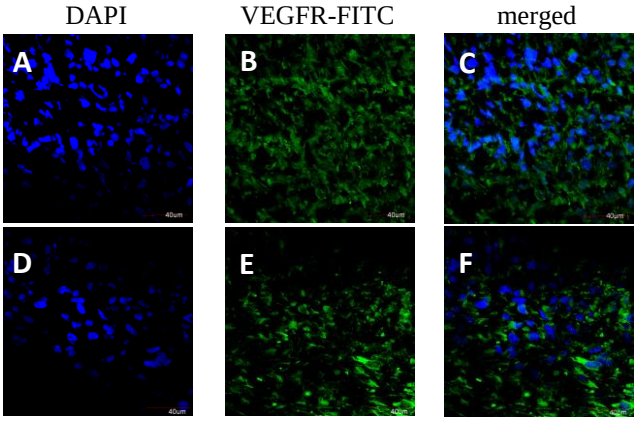

Figure 5 Expression of VEGF receptor protein in glioblastoma multiforme from control group (A, B, C) and after diamond nanoparticle treatment (D, E, F). Notes: Scale bar: 40 µm. Diamond nanoparticles treatment decreased VEGF receptor protein expression.

Diamond nanoparticles treatment caused inhibition of angiogenesis in glioblastoma multiforme tumors cultured on CAM chicken embryo membrane. Complete mechanism of action is still unclear. Decreased levels of expressions VEGF and their receptor suggest that mechanism is associated with this proteins. Diamond nanoparticles can bind with reactive domains of VEGF and VEGF receptor proteins. Decreased VEGF expression affect on cancer vascular permeability and caused vascular normalization.

4 REFERENCES

[1] Murugesan S, MousaSA, O'ConnorLJ, LincolnDW, Linhardt RJ.Carbon inhibits vascular endothelial growth factor- and fibroblast growth factor-promoted angiogenesis. FEBS Lett. 6 1157–1160 2007

[2] GrodzikM, Sawosz E, Wierzbicki M, Orlowski, Hotowy, Urbanska K, Szmidt, Mitura, Chwalibog A. Nanoparticles of carbon allotropes inhibit glioblastoma multiforme angiogenesis in ovo. Int J Nanomedicine 6 3041-3048 2011