Neurosurgical Forum

LETTERS TO THE EDITOR

MicroRNA levels and anti-VEGF therapy in glioblastoma

TO THE EDITOR: We read with interest the recent article by Siegal et al.⁴ (Siegal T, Charbit H, Paldor I, et al: Dynamics of circulating hypoxia-mediated miRNAs and tumor response in patients with high-grade glioma treated with bevacizumab. *J Neurosurg* [epub ahead of print January 22, 2016. DOI: 10.3171/2015.8.JNS15437]). The authors found that serum levels of hypoxia-associated microRNAs (miRNAs) miR-10b and miR-21 were elevated in patients with glioblastoma (GBM) during therapy with the monoclonal antibody bevacizumab, which targets vascular endothelial growth factor (VEGF). They concluded that the circulating levels of both miRNAs might reflect the angiogenic effect of therapy. However, the role of increased miR-10b and miR-21 in patients undergoing treatment with bevacizumab remains unclear.

When discussing their findings, Siegal et al.⁴ did not address some aspects of the interactions between miR-21 and VEGF, and the possible role of miR-21 in angiogenesis, which may be relevant for interpreting the data and raising hypotheses. For example, miR-21 co-localizes with VEGF in astrocytomas.¹ In bladder cancer, increased miR-21 mRNA expression is accompanied by higher VEGF-C content, and overexpression of both miR-21 and VEGF-C is associated with a poorer prognosis.⁵ In human umbilical vein endothelial cells, VEGF upregulates miR-21 levels, and overexpression of miR-21 supports VEGF-mediated angiogenesis, whereas miR-21 inhibition contributes to impairing angiogenesis.² Experiments using transformed human bronchial epithelial cells have shown that miR-21 from exosomes increases VEGF levels through STAT3 activation, leading to increased angiogenesis, whereas knockdown of STAT3 reduced miR-21 levels and inhibited angiogenesis.³ Finally, in VEGFR2-luc transgenic mice implanted with breast cancer cells, miR-21 knockdown impaired angiogenesis through suppression of the HIF-1 α /VEGF/VEGFR2 pathway.⁶

This evidence indicates that, beyond being a hypoxia marker upregulated in GBM, miR-21 expression is closely related to VEGF levels, and, most importantly, miR-21 displays a crucial functional interaction with VEGF in mediating angiogenesis. This further supports the possibility raised by Siegal et al.⁴ that the increase in miR-21 levels during treatment with bevacizumab observed in

their study may be a compensatory response to overcome therapy-induced inhibition of VEGF-mediated angiogenesis. If that hypothesis is confirmed by further research, combining anti-VEGF therapy with miR-21 inhibitors may represent a novel opportunity to increase the efficacy of antiangiogenic therapy in patients with brain tumors.

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Disclosures

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Response

No response was received from the authors of the original article.

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Should osseointegration be a target to achieve during cranioplasty?

TO THE EDITOR: We read with great interest the paper by Schwarz et al.³ (Schwarz F, Dünisch P, Walter J, et al: Cranioplasty after decompressive craniectomy: is there a rationale for an initial artificial bone-substitute implant? A single-center experience after 631 procedures. *J Neurosurg* 124:710–715, March 2016). Based on a retrospective series of patients who underwent cranioplasty after decompressive craniotomy, the authors suggest that patients younger than 30 years of age and older patients with a fragmented bone flap may be candidates for an initial artificial bone substitute, rather than an autograft, because of the high incidence of bone flap necrosis. We believe that the authors missed the important concept of achieving osseointegration between the bone flap and the skull.

The term osseointegration was used for the first time by Albrektsson et al. in 1986¹ in the field of otitis, to define a “direct structural and functional connection between vital bone and the surface of an implant subjected to load.” The process of osseointegration is well researched in the fields of orthopedics, maxillofacial surgery, and spine surgery, but may not have been adequately considered in cranial neurosurgery. The facilitation of osseointegration means understanding bone biology and physiology, as well as the various factors that influence bone healing and related technical measures. Fractured bone is genetically programmed to heal without the interposition of connective scar tissue. This is unique to bone tissue and is probably linked to the role of support tasks and protection played by bone itself. These functions would be at risk in the case of a fractured bone that had healed with an interposed scar.

We can easily compare the healing process of a bone fracture with the healing process of a craniolacunia when an operculum bone is placed. This process occurs both during the cranioplasty and at the end of the surgical procedure after the dura mater is closed, along with the bone operculum repositioning.

The healing of the skull and the restoration of its integrity depends on both biological and mechanical requirements. The biological requirements depend on the presence of bone cells that are capable of supporting healing and an adequate vascular supply. The mechanical requirements obey the degree of rigidity and stability of the “craniolacunia–bone flap” system, which mainly depends on the surgical fixation technique. Rigidity is defined as the

ability of the system to withstand forces that may deform it, whereas stability is the absence of either micro- or macromovements of the bone flap.

The system’s stability can also be influenced by the intracranial pressure, as well as by external pressures that can be transferred directly onto the bone flap. Based on the mechanical stability and resistance conditions, the healing of fractured bone can occur in either a direct or indirect manner. When absolute and adequate stability exists, surgeons can implement direct healing, whether or not there is a gap between the skull and bone operculum. When the edges between the bone flap and skull are aligned to the point of compression, healing can be direct and does not lead to the formation of a callus. The osteoclasts behave like the head of a drill, creating channels in the interface between the skull and bone flap. Thereafter, these channels are colonized by osteoblasts to form osteons that are considered bone bridges between the 2 bone fragments.

However, the most common condition of healing between the craniolacunia and the bone operculum, in conditions of stability, is the presence of a gap in the interface between the craniolacunia and bone flap. The osteoblasts that deposit osteoid tissue in the gap promote the formation of lamellar bone that is transversely oriented. This process is favored by the positioning within the gap of osteoconductive material, such as bone dust, and promoted by the presence of blood. The insertion of hemostatic material in the gap is not encouraged because it supports the formation of fibrous tissue.

Indirect healing takes place when adequate rigidity and stability exists between the craniolacunia and the bone flap. For this reason, the body implements mechanisms that lead from a biomechanical point of view to the progressive reduction of movement between bone and implants (Perren’s theory).² The biological steps start from the formation of a hematoma in the soft tissues that surround the gap between the skull and bone flap, followed by replacement by granulation tissue and bone reabsorption of margins as a result of the action of osteoclasts. The granulation tissue remodels the connective tissue, whose cells have the highest capacity for proliferation within a moving environment. The connective tissue remodels fibrocartilage tissue that can partially calcify as a consequence of the osteoblasts to obtain osteofibrointegration (with a low capacity for resistance to mechanical forces). In case of abnormal osteoclast activity, bone reabsorption is established.

In conclusion, to obtain osseointegration, the bone flap and the margins of the craniolacunia must be viable without interposed fibrous tissue,⁴ and appropriate biological and mechanical factors are required. We encourage neurosurgeons to obtain osseointegration with autologous bone both during cranioplasty procedures and in the surgical closing phase after craniotomy. The bone flaps have to be placed to sustain and assist extensive cell colonization and anchorage to the existing bone, ultimately leading to osseointegration, which also allows biomechanical competence to be gained. Osteoconductivity ensures physical and mechanical integration with the surrounding bone, which prevents micromovements, bone flap necrosis, and bone reabsorption.

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Disclosures

The authors report no conflict of interest.

Response

We thank Drs. Stefini and Zanetti for their careful analysis of our paper. The authors have stated that we have not gone into detail about osteointegration. They state that different mechanical and biological factors (e.g., a mixture of bone dust and blood) seem to be responsible for bone healing and the consolidation of the bone flap with the skull. Also they discuss their belief that the reduction of macro- and micromovement of the bone flap should result in an improvement of bone healing. They have mainly referenced Perren's 1979 study.²

Evidence in the current literature differs from Drs. Stefini and Zanetti's opinion regarding the role of micro-

movement. Kassis et al. and Yamaji et al. have shown that micromovement does improve bone healing.^{1,3} These authors used long bones as study objects. To the best of our knowledge, there are no comparable studies in which the skull is used as a study object. In relation to macromovement, in the section "Operation Procedures" we did state that every bone had been fixed with rigid titanium plates.

Regarding biological factors, the use of bone dust was not possible in our operations, because all cranioplasties done with an autograft were operations that were performed after cryoconservation of the bone flap. Cryoconservation of bone dust was not done. To compare the different surgical techniques, a prospective randomized study would be beneficial.

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