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Angiotensinogen and HLA class II predict bevacizumab response in recurrent glioblastoma patients

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ABSTRACT

Background: Bevacizumab combination therapy is among the most frequently used treatments in recurrent glioblastoma and patients who achieve response to bevacizumab have improved survival as well as quality of life. Accordingly, the aim of this study was to identify predictive biomarkers for bevacizumab response in recurrent glioblastoma patients.

Methods: The study included a total of 82 recurrent glioblastoma patients treated with bevacizumab combination therapy whom were both response and biomarker evaluable. Gene expression of tumor tissue was analyzed by using a customized NanoString platform covering 800 genes. Candidate gene predictors associated with response were analyzed by multivariate logistic and Cox regression analysis.

Results: Two genes were independently associated with response: Low expression of angiotensinogen (2-fold decrease in *AGT*; OR = 2.44; 95% CI: 1.45–4.17; P = 0.0009) and high expression of a HLA class II gene (2-fold increase in *HLA-DQA1*; OR = 1.22; 95% CI: 1.01–1.47; P = 0.04). These two genes were included in a model that is able predict response to bevacizumab combination therapy in clinical practice. When stratified for a validated prognostic index, the predictive model for response was significantly associated with improved overall survival.

Abbreviations: VEGF, vascular endothelial growth factor A; C-index, concordance index; AGT, angiotensinogen; HLA-DQA1, human leukocyte antigen complex class II DQ alpha 1; IDH1, isocitrate dehydrogenase 1.

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Conclusion: Two genes (low angiotensinogen and high HLA-class II expression) were predictive for bevacizumab response and were included in a predictive model for response. This model can be used in clinical practice to identify patients who will benefit from bevacizumab combination therapy.

1. Introduction

Glioblastoma is the most common primary malignant brain tumor in adults. Despite aggressive standard treatment, including maximal surgical resection and post-operative radiochemotherapy with temozolomide concomitantly and as maintenance, newly diagnosed patients have a median overall survival (OS) of less than 15 months (Stupp et al., 2005). At tumor recurrence no standard treatment is available and most known options have limited clinical effect.

Glioblastoma is characterized by increased angiogenesis and abnormal network of blood vessels. Anti-angiogenic agents inhibiting vascular endothelial growth factor A (VEGF) have been shown to normalize the tumor vasculature and improve blood flow, emphasizing the potential value of combining anti-angiogenic therapy with drugs targeting the tumor (Batchelor et al., 2013; Lu-Emerson et al., 2015). However, recent results from the first randomized phase III trial investigating chemotherapy with or without the VEGF-antibody bevacizumab did not demonstrate any difference in OS when considering the whole group of recurrent glioblastoma patients (Wick et al., 2015). Still, approximately 30% of patients achieve durable bevacizumab response and this group of patients has demonstrated improved survival as well as quality of life (Henriksson et al., 2011; Huang et al., 2016; Moller et al., 2012). This underscores the importance of identifying patients who will benefit from bevacizumab combination therapy. To date, no validated predictive tumor markers of a durable bevacizumab response have been identified. By analyzing gene expression profiles of glioblastoma patient tumors, the aim of this study was to identify predictive factors for bevacizumab response in recurrent glioblastoma patients.

2. Patients and methods

2.1. Patients

All patients with pathologically confirmed glioblastoma (WHO grade IV) who were treated at recurrence with bevacizumab plus irinotecan between May 2005 and December 2011 at Rigshospitalet were assessed for eligibility. During this period, bevacizumab (10 mg/kg) and irinotecan (125 mg/m^2), administered every two weeks, could be prescribed to all recurrent glioblastoma patients in WHO performance status 0–2 according to a published treatment protocol (Poulsen et al., 2009). Alternatively, both agents were combined with cetuximab in a phase 2 trial (Hasselbalch et al., 2010). Bevacizumab monotherapy was not administered at our center. Eligibility criteria for this study were response evaluability and biomarker assessable tissue from the time of glioblastoma diagnosis. The criteria are specified in Section 2.2–2.4 and a REMARK diagram is shown in Supplementary Figure S1. The study was conducted in accordance with the Helsinki Declaration and was approved by the Danish Ethical Committee (H-2-2012-069).

2.2. Clinical follow-up

According to the treatment protocol, patients had to have measurable progressive disease by contrast-enhanced MRI after standard therapy and be at least 4 weeks from prior chemotherapy and 3 months from completion of radiation therapy. For patients who had undergone relapse surgery a post-surgical MRI was performed prior to treatment initiation. Clinical follow-up was performed every 4-weeks and MRI every 8 weeks. Treatment response was evaluated based on the RANO criteria (Wen et al., 2010). Patients were categorized according to their best response; patients who achieved complete response (CR) or partial response (PR) were classified as responders, while patients with stable disease (SD) or progressive disease (PD) were classified as non-responders. Patients not evaluable by MRI at first response evaluation (week 8) due to early toxicity, progression or death were classified as non-evaluable and excluded.

2.3. Sample acquisition and RNA preparation

A total of 90 archived formalin-fixed, paraffin-embedded tissue samples from time of initial glioblastoma diagnosis were collected and freshly cut sections (5 microns) were sent to HistogeneX, Belgium, and stored at 2–8 °C. Tissue review was conducted by a pathologist blinded to identifiers and clinical outcome, and areas containing representative tumor cells were marked on hematoxylin and eosin-stained slides. Five samples with insufficient tumor tissue area for RNA analysis were excluded. Tumors were microdissected to enrich tumor cell RNA in the gene expression analyses. RNA was extracted using the High Pure RNA Paraffin Isolation kit (Roche, Ca. No. 03 270 289 001) and RNA extracts were stored at −80 °C.

2.4. Gene expression data generation

The platform consisted of 800 genes selected by Genentech using a custom code set for the NanoString gene expression platform (NanoString Technologies, Seattle, WA) (Geiss et al.,