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A comprehensive analysis of the angiogenesis-related genes in glioblastoma multiforme vs. brain lower grade glioma

Uma análise abrangente dos genes relacionados à angiogênese no glioblastoma multiforme vs. glioma cerebral de baixo grau

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ABSTRACT

Brain tumors are one of the most common causes of cancer-related deaths around the world. Angiogenesis is critical in high-grade malignant gliomas, such as glioblastoma multiforme. **Objective:** The aim of this study is to comparatively analyze the angiogenesis-related genes, namely VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 in LGG vs. GBM to identify molecular distinctions using datasets available on The Cancer Genome Atlas (TCGA). **Methods:** DNA sequencing and mRNA expression data for 514 brain lower grade glioma (LGG) and 592 glioblastoma multiforme (GBM) patients were acquired from The Cancer Genome Atlas (TCGA), and the genetic alterations and expression levels of the selected genes were analyzed. **Results:** We identified six distinct KDR mutations in the LGG patients and 18 distinct KDR mutations in the GBM patients, including missense and nonsense mutations, frame shift deletion and altered splice region. Furthermore, VEGFA and CXCL8 were significantly overexpressed within GBM patients. **Conclusions:** VEGFA and CXCL8 are important factors for angiogenesis, which are suggested to have significant roles during tumorigenesis. Our results provide further evidence that VEGFA and CXCL8 could induce angiogenesis and promote LGG to progress into GBM. These findings could be useful in developing novel targeted therapeutics approaches in the future.

Keywords: brain tumor; angiogenesis; genetics.

RESUMO

Os tumores cerebrais são uma das causas mais comuns de mortes relacionadas ao câncer em todo o mundo. A angiogênese tem caráter crítico em gliomas malignos de alto grau, como o glioblastoma multiforme. **Objetivo:** O objetivo deste estudo foi analisar comparativamente os genes relacionados à angiogênese, VEGFA, VEGFB, KDR, CXCL8, CXCR1 e CXCR2 em GBG *vs*. GBM para identificar distinções moleculares usando conjuntos de dados disponíveis no *The Cancer Genome Atlas* (TCGA). **Métodos:** Os dados de sequenciamento de DNA e expressão de mRNA para 514 pacientes com glioma cerebral de baixo grau (GBG) e 592 pacientes com glioblastoma multiforme (GBM) foram adquiridos do TCGA e as alterações genéticas e os níveis de expressão dos genes selecionados foram analisados. **Resultados:** Identificamos seis mutações KDR distintas nos pacientes GBG e 18 mutações KDR distintas nos pacientes GBM, incluindo mutações *missense* e *nonsense*, exclusão de mudança de quadro e região de emenda alterada. Além disso, VEGFA e CXCL8 foram significativamente super-expressos nos pacientes com GBM. **Conclusões:** VEGFA e CXCL8 são fatores importantes para a angiogênese, os quais parecem ter um papel significativo durante a tumorigênese. Nossos resultados fornecem evidências adicionais de que o VEGFA e o CXCL8 podem induzir a angiogênese e promover o GBG a progredir no GBM. Esses achados podem ser úteis no desenvolvimento de novas abordagens terapêuticas direcionadas no futuro.

Palavras-chave: neoplasias encefálicas; angiogênese; genética.

Central nervous system cancers are rare, but present high mortality and morbidity rates worldwide. Gliomas are the second most common brain tumors, accounting for almost one fourth of all brain tumors in adults[1]. Based on histopathological analysis, gliomas are classified into 4 grades (I-IV). Grade I: gliomas are usually benign and easily curable. Lower grade gliomas (LGG) are grade II, which are often encountered in young adults (mean age of 35), presented with lesions in the temporal, insular or frontal lobes, as well as seizure disorders[2]. Glioblastomas, which

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constitute half of all glioma cases, are grade IV gliomas[3]. In adults, glioblastoma multiforme (GBM) is the most common and most aggressive type of primary brain tumor, which usually occurs in elderly people (mean age of 65). Accordingly, 5-year post-diagnosis survival rates are as low as 5.1% in GBM patients, whereas 90% of LGG patients survive 10 years post-diagnosis[1].

Angiogenesis, which is the formation of new blood vessels, is a typical feature of solid tumors. In order to compensate the nutrition and oxygen need of highly proliferating tumor cells, a new network of blood vessels needs to be established within the tumor microenvironment. This is often achieved with an increased secretion of growth factors, including vascular endothelial growth factor (VEGF) family proteins, causing abnormal endothelial proliferation[4,5]. VEGFA and VEGFB are important drivers of vasculogenesis, cell migration and permeabilization of blood vessels, all of which are hallmarks of malignant cancers[6]. KDR, which is also known as VEGFR2, is a tyrosine kinase with a weak kinase activity and preferentially acts as a cell-surface receptor for VEGF family proteins. The inflammatory response is also frequently adjusted in favor of tumor angiogenesis[7]. Studies show that interleukin IL-8 (CXCL8) is secreted to the GBM tumor microenvironment in high levels[8]. Receptors for CXCR8 signalling are CXCL1 and CXCL2, which play critical roles in microvascular endothelial cells[9].

Vascularization is one of the major pathological distinctions between GBM and LGG. Studies have suggested that the high mortality of GBM is strongly influenced by tumor angiogenesis, which provided the tumor with the ability to infiltrate throughout the brain tissue and persist through drug therapies[10,11]. Therefore, this study aims to comparatively analyze the angiogenesis-related genes, namely VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 in LGG vs. GBM using DNA sequencing and mRNA expression data available on The Cancer Genome Atlas (TCGA) to identify molecular distinctions that could explain the progress from LGG to GBM.

METHODS

Mutation analysis

The cBio Cancer Genomics Portal (http://cbioportal.org) is an open-access tool that provides mutation data, copy number alterations, microarray-based and RNA sequencing-based mRNA expression changes, DNA methylation values, protein and phosphoprotein levels based on the TCGA-derived data[12,13]. Seeking to comparatively study the mutations in VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 genes in brain lower grade glioma (LGG) and glioblastoma multiforme (GBM), we first selected our cancer studies of interest on the web interface. The TCGA LGG and GBM data sets we selected comprised of genome wide sequencing and mRNA expression data for a cohort of 514 and 592 cancer patients respectively, among which we carried on analyzing complete tumor samples that had mRNA, copy number alteration and sequencing data[14]. We then selected VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 genes and determined their mutational status using the *OncoPrint* feature.

mRNA expression analysis

GEPIA (http://gepia.cancer-pku.cn/index.html) is an online tool that provides differential expression analysis between tumor vs. normal samples based on RNA expression data obtained from 9,736 tumor and 8,587 normal samples from TCGA and the Genotype-Tissue Expression (GTEx) projects[15]. Dot plots and box plots of gene expression profiles of the selected genes across all tumor samples and paired normal tissues were generated on GEPIA. Finally, p-values were automatically calculated by the tool, and p-values below 0.05 (%5) were considered significant.

RESULTS

Aiming to identify genetic alterations within angiogenesis-related genes in LGG and GBM patients, we analyzed the DNA sequencing and mRNA expression data for 507 glioma and 360 hepatocellular carcinoma patients, available on The Cancer Genome Atlas (TCGA) through the cBioPortal interface. We found that 7.2% of all LGG patients and 14.5% of all GBM patients were subjected to at least one genetic alteration (amplifications, deletions and point mutations) in VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 genes (Figure 1). Among the selected genes we analyzed, we identified KDR (VEGFR2) as the most commonly altered gene within the patient groups, with 4 and 11% alteration percentages in LGG and GBM, respectively. Most of these alterations were gene amplifications.

Considering KDR was the most frequently altered gene, we investigated the point mutations within the KDR gene in more detail and compared whether LGG and GBM patients shared some common point mutations. There were six distinct KDR mutations in the LGG patients, five of which resulted in amino acid substitutions (missense mutations: E207G, R347H, V398A, T723I and K981N), as well as a nonsense mutation at position R944*, that resulted in a truncated protein (Figure 2, upper panel). On the other hand, we detected a total of 18 distinct KDR mutations in the GBM patients, including missense (I36M, R347H, A352V, A505T, S620N, A632T, D636N, E759K, R961W, R962C, V1012M, V1093I, S1104F, P1243L, S1290R) and nonsense (W63*, R1032*) mutations, frame shift deletion (D703Ifs*6) and altered splice region (G23=) (Figure 2, lower panel). Of all the mutations that we detected, only R347H amino acid substitution was common in both patient groups.

Cancer is a disease characterized by aberrant protein expression. In order to determine whether the gene expression profiles of angiogenesis-related genes display alterations in LGG and GBM patients, we investigated the expression levels of VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 genes in the GBM or LGG tumor samples in comparison to the paired normal tissues (Figure 3). Results showed that KDR was significantly upregulated in both tumors when compared to the normal tissues. Furthermore, the expression levels of KDR were similar for both LGG and GBM. We did not

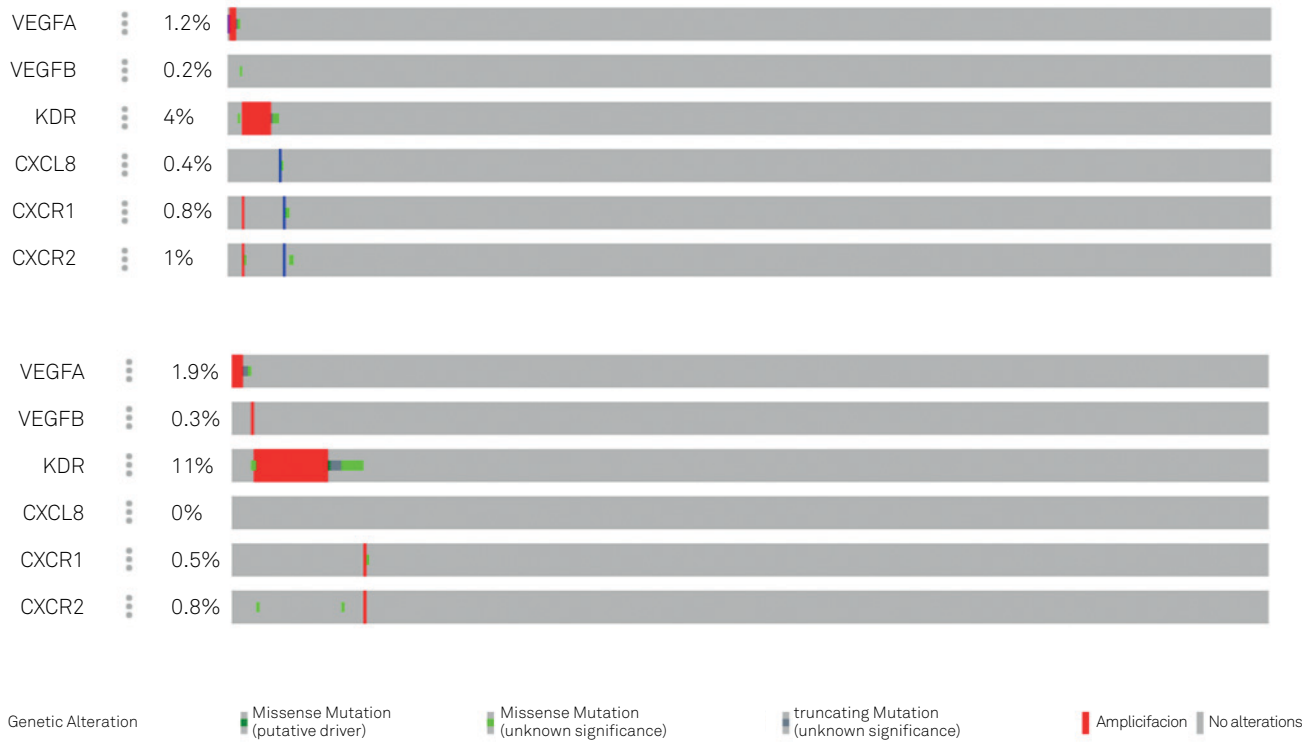

Figure 1. Genetic alterations within the angiogenesis-related genes VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 in the LGG (upper panel) and GBM (lower panel) patient groups. Each bar represents a patient. LGG: Brain Lower Grade Glioma; GBM: Glioblastoma Multiforme.

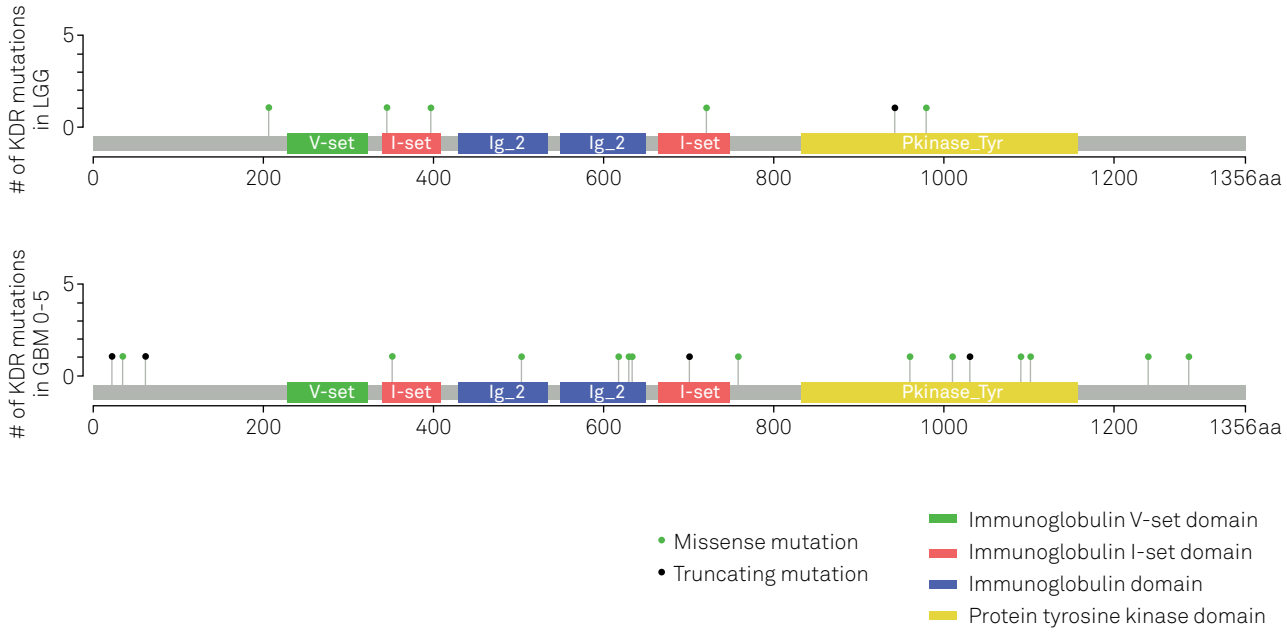

Figure 2. KDR domain structures and the point mutations detected within the LGG and GBM patient cohorts.

detect any significant changes in VEGFB, CXCR1 and CXCR2 between LGG and GBM or tumor and normal tissue samples. Interestingly, however, VEGFA and CXCL8 were significantly upregulated in GBM tumors in comparison to the paired normal tissue, as well as the LGG tumor tissue, which did not show any significant alterations at the expression level when compared to the normal tissue.

DISCUSSION

It is highly suspected in the literature that angiogenesis is a key feature of GBM, thus explaining its aggressiveness and higher mortality over LGG. Although GBM is characterized as a genetically heterogeneous disease with patients carrying one or more genetic alterations at their EGFR, PTEN, RB1 and NF1 genes[16], there are no current studies that comprehensively investigate the genetic alterations in angiogenesis-related genes in GBM in comparison to LGG, which is a less aggressive brain tumor type. Therefore, in the present study, we aimed to identify molecular distinctions within the angiogenesis-related genes between GBM and LGG, and provide evidence for the role of angiogenesis in brain cancer progression from LGG to GBM.

We found that the alterations within all the selected genes, except for KDR, were rare changes (<2%). The most frequently altered gene within both patient cohorts, KDR, was subjected to several point mutations. R347H amino acid substitution, which was the only common mutation type between the LGG and GBM patients, is a mutation that has been linked with several cancer types, including leukemia, colorectal carcinoma, brain lower grade glioma and lung cancer[14-19], whereas its function is yet to be characterized. Similarly, although the other KDR point mutations were identified in several genome-wide cancer studies, they have not been linked with any function or pathology so far. Therefore, at this point, we can only speculate that these mutations have cancer-driving roles.

Our analysis on the expression profiles of the angiogenesis-related genes revealed that KDR was upregulated in both gliomas when compared to the normal tissue counterparts. This could be directly reflected by the fact that KDR gene was amplified in several LGG and GBM patients, resulting in an overexpressed protein. We did not detect significant changes in VEGFB, CXCR1 and CXCR2 expression levels. VEGFA was suggested as a prime candidate driving angiogenesis in GBM by several studies[20]. In line with this, we found that its expression levels were significantly higher in the GBM tumor tissues than both its paired healthy tissue and LGG tumor and normal samples; suggesting that it could be an important inducer of angiogenesis in GBM. A similar trend was observed for the chemokine CXCL8 as well; because its expression levels were significantly upregulated specifically in the GBM tumor tissue. Previous studies

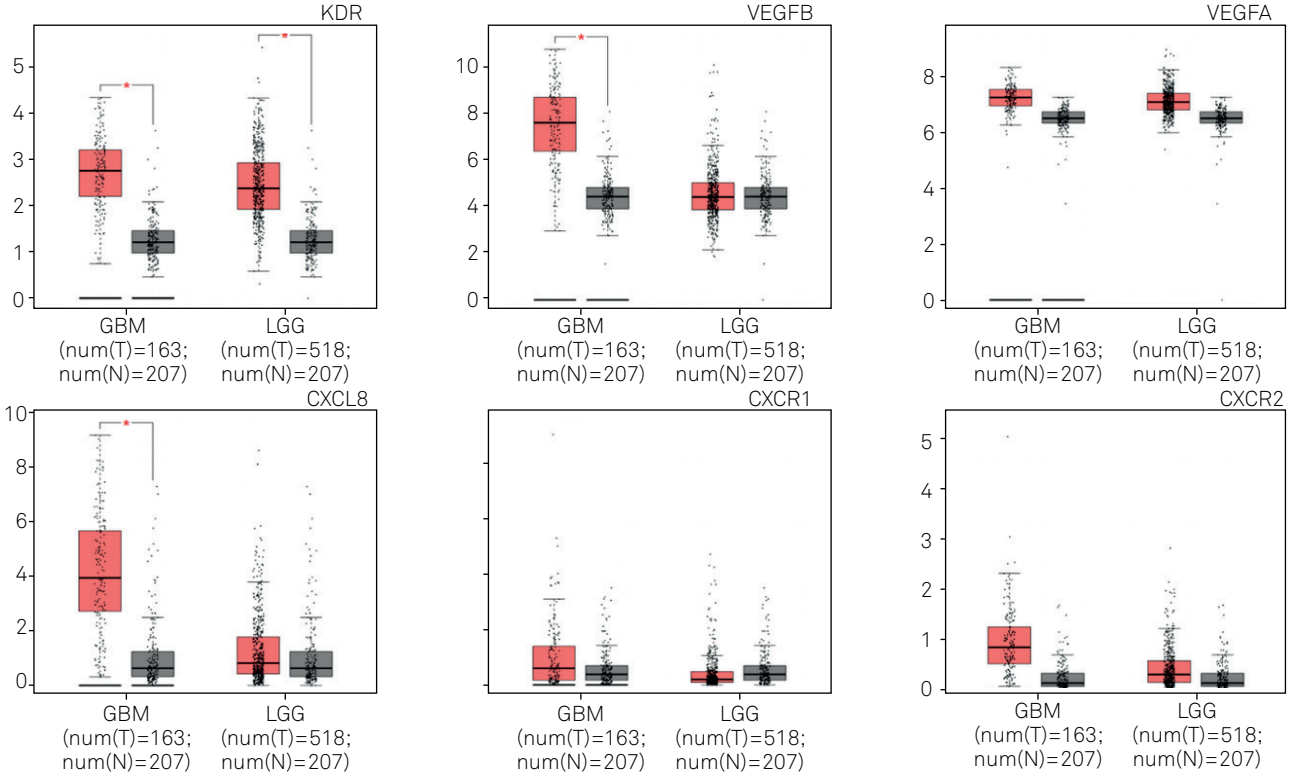

Figure 3. Box plots showing gene expression profiling of VEGFA, VEGFB, KDR, CXCL8, CXCR1 and CXCR2 genes in the GBM or LGG tumor samples (red) in comparison to the paired normal tissues (grey). (*indicates p<0.05).

attributed important roles for CXCL8 in the proliferation, survival, invasion, and angiogenesis in breast cancer[21], melanoma[22] and glioblastoma[23] and suggested it as a pro-angiogenic factor during carcinogenesis[24]. These results are consistent with our findings, showing that angiogenesis-related genes, VEGFA and CXCL8, are significantly overexpressed within the GBM patients. Bearing in mind the proposed role of angiogenesis in its progression to GBM, this study provides further evidence that VEGFA and CXCL8 could induce angiogenesis and promote LGG to progress to GBM. Identifying the molecular distinctions between LGG and GBM could prove to be useful in developing novel targeted therapeutics approaches in the future.

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