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Research Article

Actin cytoskeleton organization, cell surface modification and invasion rate of 5 glioblastoma cell lines differing in PTEN and p53 status

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ABSTRACT

Glioblastoma cells exhibit highly invasive behavior whose mechanisms are not yet fully understood. The present study explores the relationship between the invasion capacity of 5 glioblastoma cell lines differing in *p53* and *PTEN* status, expression of mTOR and several other marker proteins involved in cell invasion, actin cytoskeleton organization and cell morphology. We found that two glioblastoma lines mutated in both *p53* and *PTEN* genes (U373-MG and SNB19) exhibited the highest invasion rates through the Matrigel or collagen matrix. In DK-MG (*p53*wt/PTENwt) and GaMG (*p53*mut/PTENwt) cells, F-actin mainly occurred in the numerous stress fibers spanning the cytoplasm, whereas U87-MG (*p53*wt/PTENmut), U373-MG and SNB19 (both *p53*mut/PTENmut) cells preferentially expressed F-actin in filopodia and lamellipodia. Scanning electron microscopy confirmed the abundant filopodia and lamellipodia in the *PTEN* mutated cell lines. Interestingly, the gene profiling analysis revealed two clusters of cell lines, corresponding to the most (U373-MG and SNB19, *i.e. p53* and *PTEN* mutated cells) and less invasive phenotypes. The results of this study might shed new light on the mechanisms of glioblastoma invasion.

Abbreviations: CGM, complete growth medium; ECM, extracellular matrix; Erk, extracellular signal regulated kinase; FAK, focal adhesion kinase; FBS, fetal bovine serum; GBM, Glioblastoma multiforme; MEK, MAPK/Erk kinase; MMP, matrix metalloproteinase; PTEN, phosphatase and tensin homolog; SEM, scanning electron microscopy

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Introduction

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor in adults associated with a median survival time of 15 months, even after surgical resection, chemotherapy and radiotherapy [1]. Although more long-term survivors have been reported after combined chemoradiotherapy [2], the unusual aggressiveness of GBM due to the diffuse infiltration of single tumor cells into the surrounding brain parenchyma makes complete tumor debulking virtually impossible [3].

GBMs commonly invade and migrate largely within the CNS yet, paradoxically, nearly never metastasize beyond the CNS [4]. Therefore, better therapies for newly diagnosed GBM patients are necessary, involving strategies to prevent cell invasion, which would enhance the tumor response to local treatment. These challenges have led to extensive efforts in elucidating the regulatory mechanisms of GBM cell motility *in vitro* and *in vivo*.

Among others structures, the extracellular matrix (ECM), adhesion proteins and molecular motors such as nonmuscle myosin II have been identified as key regulators of cell motility [3]. *In vitro* studies have also shown the importance of fibronectin, laminin and collagen in stimulating a migratory phenotype of GBM cells [5,6]. In addition, the activation of MMPs (matrix metalloproteinase) and uPA (urinary plasminogen activator) is essential for maintaining the GBM phenotype and mesenchymal migration *in vitro* and *in vivo* (for review, *see* [7]). Strong correlations between MMPs activation, GBM invasion, and poor prognosis indicate that tumor cells are able to extensively remodel the surrounding ECM during invasion [8].

The mechanisms underlying GBM invasion in respect to molecular gene signatures are still unclear [9]. According to the Cancer Genome Atlas Research Network, a typical glioblastoma harbors more than 60 genetic alterations [10]. Despite this large number, the affected genes can be divided into three groups: the receptor tyrosine kinase (RTK)/PTEN/PI3K, the *p53* and the retinoblastoma (RB1) pathways [10]. Frequent mutations in the *p53*, RTK, phosphatase and tensin homolog (PTEN) genes have been reported to contribute to a resistance phenotype to radio- and chemotherapy and also correlate with poor overall survival [11].

The gene *PTEN* is deleted or mutated in 30% of GBMs and at lesser frequencies in a range of other tumors [12]. PTEN inhibits cell migration, spreading, and focal adhesions [12]. However, the exact role of PTEN in tumor invasion and metastasis is still elusive [13].

The second most frequently mutated gene in glioblastomas is *p53* [10]. It has been largely studied in gliomas, but conflicting results can be found in the literature about the impact of *p53* status in the resistance to cancer therapy [14]. Recent data indicate that some of the most common mutant *p53* proteins have, in addition to losing transcriptional function, acquire a gain of function: these mutants drive tumor cell migration and metastasis as a result of their ability to interfere with another *p53* family member, *p63* [15]. The effects of *p53* on cell motility are largely mediated through the regulation of Rho signaling, thereby controlling actin cytoskeletal organization [16] and preventing filopodia formation, cell spreading, migration and invasion. A deficiency in *p53* promotes cell migration by upregulating Rho GTPase activities and fibronectin production [17]. Loss of *p53* function increases the activities of RhoA and Rac (through the activation of the PI3K-pathway), and also causes overabundance of Cdc42-dependent filopodia formation. As a result, activation of this network promotes cell adhesion and migration [17].

In order to assess the impact of *p53* and *PTEN* as the most frequently mutated genes in GBM, we compare in the present study the invasion capacities of five established GBM cell lines. These included a cell line wild type for both genes (DK-MG), along with cell lines mutated either for *p53* (GaMG) or *PTEN* (U87-MG), or both genes (U373-MG and SNB19). The cell lines were analyzed for invasiveness, actin distribution, cell morphology and expression of several marker proteins of the PI3K- and MAPK-pathways, as well as of MMP-2. Proteins responsible for cell adhesion and actin cytoskeleton (FAK/phospho-FAK, RhoA, ILK1, cofilin) were also examined.

Materials and methods

Cell culture

Five GBM cell lines differing in their PTEN or *p53* status were obtained either from the American Type Culture Collection (ATCC, Manassas, VA) or German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The cell lines sample includes a cell line wild type for both PTEN and *p53* (DK-MG), along with cell lines mutated either for *p53* (GaMG) or PTEN (U87-MG), or both genes (U373-MG and SNB19) (Supplementary Table S1). The cells were cultured under standard conditions (5% CO_2, 37 °C) in complete growth medium (CGM) containing either DMEM (DK-MG, GaMG, SNB19) or MEM (U87-MG, U373-MG) supplemented with 10% fetal bovine serum (FBS). The population doubling times were determined to be, respectively, about 50, 40–50, 40, 36 and 36 h for DK-MG, GaMG, U87-MG, U373-MG, and SNB19 cells.

Cell treatment

Inhibitors of *p53* (Pifithrin-α, PFT, S2929, Selleckchem, Munich, Germany) and PTEN (BpV(Hopic), sc-221377, Santa Cruz Biotechnology, Heidelberg, Germany) were added for 24 h to exponentially growing cell cultures at concentrations of 200 nM and 100 nM, respectively. Drugs were freshly diluted from frozen aliquots in DMSO stored at −20 °C.

Two siRNA constructs targeting either *p53* or PTEN were purchased from Cell Signaling (Danvers, MA). Non-silencing siRNA (All Stars Negative Control siRNA, Qiagen, Hilden, Germany) was used as control. The siRNAs were transfected into exponentially growing cells using HiPerfect reagent (Qiagen, Hilden, Germany) at a final concentration of 50 nM according to manufacturer's instructions.

Antibodies

Primary and secondary antibodies are specified in Supplementary information.

Matrigel and collagen invasion assays

The invasion capacity of tumor cells in vitro was examined using either the Matrigel (#354480, BD Biosciences, Heidelberg, Germany) or collagen (CBA-110-COL, Cell Biolabs, San Diego, CA) coated inserts