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Proteomic analysis underlines the usefulness of both primary adherent and stem-like cell lines for studying proteins involved in human glioblastoma

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ABSTRACT

Primary cell lines derived as neurospheres, enriched in cancer stem cells, are currently the focus of interest in glioblastoma to test new drugs, because of their tumor initiating abilities and resistance to conventional therapies. However, not all glioblastoma samples are propagatable under neurosphere culture and not all neurosphere cell lines are tumorigenic. These cells therefore cannot recapitulate the heterogeneity of glioblastoma samples. We have conducted a proteomic analysis of primary glioblastoma cell lines derived either as adherent cells in the presence of serum (n = 11) or as neurospheres (n = 12). A total of 963 proteins were identified by nano-LC/Q-TOF MS: 342 proteins were found only in neurosphere lines and were mostly implicated in various metabolic and cellular processes, while 112 proteins were found only in adherent cells and mostly linked to cell adhesion. A protein signature of 10 proteins, 9 of them involved in a cell adhesion pathway, characterized adherent lines. Neurospheres were characterized by 73 proteins mostly linked to DNA metabolic processes associated to cell cycle and protein metabolism. In the Repository of Molecular Brain Neoplasia Data, expression of genes coding for several proteins related to adherent cells or neurospheres were of prognostic relevance for glioblastoma.

Biological significance
Primary cell lines enriched in cancer stem cells (CSC) have become popular models for testing new drugs for glioblastoma. In this proteomic study on an important number of cell lines obtained either as adherent cells in the presence of serum (a classic way to derive cell lines) or as neurospheres (enriched in CSC), we show that each type of cell line displays

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different GBM-specific features, highlighting that these two culture types are complementary tools for drug screening.

1. Introduction

Glioblastoma (GBM), the most malignant and common form of primary brain tumor in adults, still has a very poor prognosis, despite a multimodal treatment with surgery, radiation and chemotherapy with temozolomide (TMZ). Therefore, there is an acute need for the development of novel therapies, which must be tested on pre-clinical models. In vitro and in vivo human cancer cell line models remain very important tools for testing anticancer drugs. The established cell lines U251, SF-268, SF-539 and SNB-75, which belong to a panel of 60 cell lines from the National Cancer Institute (NCI60), as well as other glioma cells, such as U87-MG or T98G, are frequently used for glioma studies. All these cells are used after an extended period of culture. However, the extended culture conditions generate a mutational and gene expression profile different from the one observed in the clinical sample that the models are intended to mimic. This has led some authors to question the relevance of using these cells to study the mechanisms of clinical anti-cancer drug resistance [1].

According to the cancer stem cell hypothesis, a tumor is composed of differentiated tumor cells and a limited number of stem cells. Cancer stem cells (CSC) are capable of self-renewal and are the only cells able to initiate and maintain tumor progression. Furthermore, CSC are resistant to conventional radiotherapy and chemotherapy, explaining the relative inefficacy of current treatment. In a genetically engineered mouse model of glioma, TMZ induces an arrest of tumor cell proliferation, which is followed by a repopulation with CSC. Combining TMZ with a treatment targeting CSC impeded tumor development [2]. CSC can be enriched under neurosphere (NS) culture conditions previously developed for the expansion of normal neural stem cells. Several studies have shown that NS culture allows researchers to obtain cells that well represent the genotype and phenotype of GBM tumors [3–5]. Accordingly, cell lines cultivated as NS have become very popular models.

Using only tumorspheres as a model may have some limitations. The efficiency of NS culture to establish CSC lines is low and variable from study to study, ranging from 1% to 50% [6,7]. NS cultures grow only from GBM displaying PTEN deficiency, with two-thirds of PTEN-deficient GBM generating CSC lines, while no cell lines are generated from GBM without PTEN deficiency [7]. Another study showed that only tumors that are isocitrate dehydrogenase 1 (IDH1) wild type, chromosome 7 amplified, and chromosome 10q deleted can give rise to tumorspheres [8]. Using only CSC lines may therefore lead to study only types of GBM with the ability to generate tumorspheres in vitro. Furthermore, the concept that tumor initiating cells (TIC) are found only among CSC is evolving. This clear-cut concept was driven by the first observations that CD133+ cells, displaying stem cell properties in vitro, produced a tumor after orthotopic injection in immunocompromised mice, in contrast to CD133– cells [9]. Since then, it has been shown that CD133– may also initiate tumorspheres and be tumorigenic in vivo [7,10,11]. More importantly, a recent study in a model of mice glioma has shown that self-renewal, one of the hallmarks of CSC reflected by their ability to grow in NS assays, does not predict their tumorigenicity [12]. This observation is also frequently observed in laboratories developing human tumorspheres: not all CSC lines are tumorigenic. With their mice model, Barrett et al. found that in experimental tumors, cells enriched in expression of stem cell markers and very efficient at sphere formation were far less tumorigenic than the more differentiated counterpart cells [12]. Thus, some TIC never grow as tumorspheres, at least under the culture system commonly used, and for some tumors, the cells growing as tumorspheres may not be representative of the tumorigenicity of the parental tumor [13]. This highlights the importance of not relying only on tumorspheres to screen drugs to avoid a selection bias. CSC are also found in classic adherent cell lines [14,15] and this type of culture could be the only way to propagate them in vitro in some cases. Furthermore, these cell lines may be enriched in more differentiated and proliferating cells that must also be killed in addition to CSC to eradicate tumors. These two types of culture could therefore be seen as complementary tools for drug screening.

In this study, we compared a series of adherent (ADH) and NS cell lines with a proteomic approach. In the latter cells, the great majority of proteins were related to stem cells and to the regulation of different intracellular processes. Proteins found in the ADH cells were involved in cell adhesion/invasion. We highlighted some proteins preferentially expressed in ADH or NS cells that could play a crucial role in GBM progression and could therefore be targeted by drugs in relevant in vitro models.

2. Materials and methods

2.1. Reagents

Unless otherwise specified, all the reagents were purchased from Sigma-Aldrich (St Quentin Fallavier, France).

2.2. Cell lines

GBM samples were obtained after informed consent from patients admitted to the Neurosurgery Department at Rennes University Hospital for surgical resection in accordance with the local ethical committee. All tumors were histologically diagnosed as grade IV astrocytoma according to the WHO criteria (Table 1).

GBM cell lines were obtained as previously described [16,17]. Briefly, NS cell lines enriched in GBM-stem like cells were grown in DMEM:F12 (Lonza, Levallois-Perret, France) supplemented with B27 and N2 additives (Invitrogen, Illkirch, France) and basic FGF and EGF growth factors (20 ng/ml) (Peprotech, Neuilly-sur-Seine, France). ADH cell lines were amplified in DMEM 10% FSC (Lonza). For protein preparations,