Short Communication

Glioma cells showing IDH1 mutation cannot be propagated in standard cell culture conditions

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BACKGROUND: It has recently been reported by several sources that original (i.e., present in vivo) glioma cell phenotypes or genotypes cannot be maintained in vitro. For example, glioblastoma cell lines presenting EGFR amplification cannot be established.

METHODS AND RESULTS: IDH1 sequencing and loss of heterozygosity analysis was performed for 15 surgery samples of astrocytoma and early and late passages of cells derived from those and for 11 archival samples. We were not able to culture tumour cells presenting IDH1 mutations originating from currently proceeded 10 tumours; the same results were observed in 7 samples of archival material.

CONCLUSION: The IDH1 mutation is expected to be almost mutually exclusive with EGFR amplification, so glioma cells with IDH1 mutations seem to represent a new group of tumour cells, which cannot be readily analysed in vitro because of their elimination. The reasons for this intriguing phenomenon should be investigated since its understanding can help to define a new therapeutic approach based on simulating in vivo conditions, responsible for tumour cells elimination in vitro. Moreover, a new model for culturing glioma cells in vitro should be designed since the current one does not provide conditions corresponding to in vivo growth.

Keywords: IDH1; astrocytic tumours; glioblastoma; cell line; EGFR; R132H

The discovery of IDH1 mutations was very significant for neuro-oncologists. So far, mutations of IDH1 have been detected almost exclusively in glial tumours (Bleeker et al, 2009), including astrocytomas and secondary glioblastomas, but not in primary glioblastomas (Watanabe et al, 2009). In addition, the function of IDH1 does not seem to be typical for a tumour suppressor, a classical oncogene, or a mutator. The IDH1 protein is required for NADPH production and lipid synthesis (Kloosterhof et al, 2011). All of these factors have attracted a lot of attention to IDH1 analyses, including in vitro investigations. Nevertheless, it appears difficult to propagate cells showing the IDH1 mutation in vitro since they are easily eliminated under standard cell culture conditions.

MATERIALS AND METHODS

Tumour samples

The study included 26 (15 cultured and 11 archival) cases of astrocytic tumours (17 – diffuse astrocytoma, 9 – anaplastic astrocytoma) diagnosed at the Department of Pathomorphology, Medical University of Lodz according to the World Health Organisation criteria for classification of brain tumours. Tissue samples were obtained from patients with astrocytic tumours treated in the Department of Neurosurgery, Medical University of Lodz, Poland.

Cell cultures/DNA isolation

Fifteen specimens were cultured. The tumour cells were dispersed by means of collagenase type IV (200 U ml⁻¹, 37 °C for 6 h). Subsequently, the cells were cultured in alpha MEM media containing 10% FBS. After 48 and 96 h, the medium was changed and part of the cells was isolated for DNA analysis. DNA was isolated from snap-frozen tissues (stored at –80 °C) and from cells harvested after the first and the second passage. On the first and the second day, DNA samples were also isolated from cells floating (dead) in the culture medium for the purpose of the DNA profile analysis. The first harvesting was performed no longer than 2 days after cells culturing. For loss of heterozygosity (LOH) analysis, DNA was isolated from blood samples. All DNA samples were extracted by means of a Macherey–Nagel DNA purification kit (Macherey–Nagel, Düren, Germany). All types of cultured cells, including floating cells, were tested for Trypan blue absorbing.

Archival sample analysis

Eleven samples of astrocytoma with visible karyotypic changes (collected in 1990s) were verified for IDH1 mutation presence.
DNA was extracted from snap-frozen tumour tissues (stored at −80 °C) and from microscopic specimens derived for karyotyping from cell cultures after the second passage. All DNA samples were extracted by means of a Macherey–Nagel DNA purification kit.

**IDH1 sequencing**

Exon 6, including codon 132 of the IDH1 gene, was amplified by PCR and sequenced using the dideoxy termination method and SequiTherm Excel DNA Sequencing Kit (Epicentre Technologies, Madison, WI, USA). The primers used for PCR amplification of the DNA sequences were: IDH1-5'-GGCACCCATCTCTGTTT-3' (sense) and 5'-ATATATGCTTCTCAATTCTCA-3' (antisense). The sequencing primers used were: IDH1 exon 6 – 5'-GCAAAAATATCCC CGGGCTT-3' (sense), and IDH1 exon 6 – 5'-CGGTCCTCTCAGAGACACATT-3' (antisense). A Li-Cor automatic sequencer system (Li-Cor, Lincoln, NE, USA) was applied for the separation and analysis of PCR-sequencing products.

**LOH analysis: microsatellite analysis to classify type of in vitro surviving cells (normal vs tumour)**

The specimens were examined for LOH using pairs of tumour specimens and corresponding peripheral blood samples. The markers were selected using the NCBI database (D1S508, D1S199, D1S197, D1S162, D1S2734, D1S2720, D1S429, D1S510, D1S1709, D1S5290, D1S587, D1S197, D1S1267, D1S587, D1S481, D2S303, D2S257, D2S1163 and D2S1150) and obtained from MWG-Biotech AG (Ebersberg, Germany). PCR products were visualised with a Li-Cor automatic sequencer. Loss of homozygosity was judged to be present if the allelic signal intensity of the tumour sample was reduced by at least 50% relative to the corresponding allele in the patient's control DNA.

**RESULTS**

**IDH1 mutations in frozen tumour cells and corresponding cell cultures**

*Cell cultures* DNA sequencing revealed IDH1 mutations in 10 surgical specimens of astrocytic tumours coming from 15 patients. The mutated template was no longer detectable in adherent cells grown in vitro (in four cases after the first passage and in six cases after the second passage) derived from the tumour samples with IDH1 mutations (Figure 1). Free floating cells (absorbing Trypan blue) from the medium showed IDH1 amplification after the first and the second passage. Our previous investigations showed that none of the tumours presented EGFR amplification, and three showed a mutation of TP53.

In eight cases, cell cultures derived from specimens originally presenting the IDH1 mutation survived at least 10 passages but were never stabilised. In five of those cases, LOH was observed in the initial culture. Only in one case, tumour cells presenting LOH were observed after disappearance of cells presenting IDH1 mutation. It suggested that after elimination of cells with IDH1 mutation only normal cells remained in the majority of the cell cultures. Two out of five astrocytomas presenting lack of IDH1 mutation (after the analysis of surgery sample and early cell culture sample), showed LOH of several markers. Cells coming from one of them cultured in standard cell culture conditions still show LOH of 1p (after 20 passages – possible stabilisation). In the second case, we did not detect in the standard cell culture conditions LOH observed in vivo.

*Archival sample analysis* In all, 7 out of 11 DNA samples isolated from frozen tumour tissues showed IDH1 mutations in the sequencing analysis. The quality and amount of archival DNA was not sufficient to perform other molecular analyses such as LOH, and so on. Nonetheless, in six corresponding DNA samples isolated from cultured cells in 1990s after the second passage, IDH1 mutation was not visible. In one remaining case, mutated bands were significantly weaker (almost invisible) than in the frozen tissue samples.

**DISCUSSION**

IDH1 mutations seem to have a significant role in glial tumours (Ducray et al, 2009; Hartmann et al, 2009). Zhao et al (2009) showed that IDH1 regulates HIF1 expression. Recently, Sanson et al (2009) suggested that the IDH1 mutation might correlate with a glial tumour grade. However, the specific role of the IDH1 mutation in glial tumours, such as astrocytic tumours and secondary glioblastomas is unknown.

In this paper, we described obstacles in culturing glioma cells with IDH1 mutations. What needs to be added, similar problems were described for EGFR amplification analysis in glioblastomas, that is, disappearance of EGFR amplification was observed in standard cell culture conditions. Elimination of EGFR amplification during glioblastoma cells culturing is a well-known phenomenon the causes of which, however, remain enigmatic (Pandita et al, 2004; Witusik-Perkowska et al, 2010). We observed two cases of astrocytoma without IDH1 mutation presenting LOH for several markers in surgery samples (*in vivo*). In *vitro* proliferating (surviving) cells of one of them showed lack of LOH (putative normal) whereas cells of the second astrocytoma presenting LOH survived in standard cell culture conditions for 20 passages so far. In addition, glioma cell culture collections can be analysed, and none of the 15 commercially available cell lines presents IDH1 mutation (Bleeke et al, 2009; Kloosterhof et al, 2011).
All collected data suggest that glioma tumour cells with IDH1 mutation cannot be propagated in vitro in conditions suitable for survival of normal human cells or glioma cells without IDH1 mutation. The IDH1 mutation is expected to be almost mutually exclusive with EGFR amplification, so glioma cells with IDH1 mutation seem to represent a new group of tumour cells, which cannot be readily analysed in vitro because of their elimination. Nonetheless, we do not suggest that IDH1 mutation is responsible for cells in vitro elimination. Apparently, the presence of a TP53 mutation does not protect glioma cells with IDH1 mutation against factors responsible for cells elimination. Although we did not define that beyond any doubt, the cells that survived after the elimination of tumour cells with IDH1 mutation are most likely normal cells. Surprisingly, the kind of cells surviving in vitro in case of gliomas presenting EGFR amplification has not been defined in spite of years of investigation. We did not detect genetic alterations in cells cultured after the elimination of cells showing IDH1 mutation, except for one case. Moreover, IDH1 mutation is probably the earliest stage of glioma tumourigenesis (Kloosterhof et al, 2011).

Defining cell culture conditions that would sustain the original phenotype and genotype of glioma cells is critical. The cell culture conditions currently used are artificial and thus unsuitable for the growth and analysis of glioma cells – causing numerous artefacts. Nonetheless, as normal cells seem to be more congruous to the standard cell culture conditions than glioma cells with IDH1 mutation, the factors responsible for the elimination of glioma cells in vitro should be identified for possible pharmacological use.

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