Supplementary material

Verification of neoplastic nature of glioblastoma-derived cell cultures (G113, G114, G116) – Fig. S1, Fig.S2

Immunofluorescence results showed G113-, G114- and G116-derived cells as positive for AAAs (IL13Rα2, Fra-1). The possible overgrowth by non-tumoral cells were monitored with the use of appropriate markers (CD31, CD34, vWF for endothelial cells; α-SMA, FSP for GASCs - glioblastoma associated stromal cells). The examined cultures were almost negative for tested endothelial markers (CD34, vWF) with exception of single weakly positive cells (in circles) and presented weak immunoreactivity for CD31 antigen co-expressed with IL13Rα2 (Fig.S1). The putative markers (α-SMA, FSP) of GASCs (there is no marker absolutely specific to these cells) described by Claverul et al. [14] were detected at modest level in the G113-, G114- and G116-derived cells positive for AAAs (IL13Rα2, Fra-1). Co-expression of astrocytoma associated markers (Fra-1, EphA2) with α-SMA and FSP was also observed in U87MG (commercially available glioblastoma cell line). In contrast to G113, G114 and G116 cultures, immunofluorescence analysis revealed the case of glioblastoma culture (derived from other tumour) presenting strong expression of α-SMA and FSP and almost undetectable level of AAAs (Fra-1, EphA2). This tumour-derived culture possibly overgrown (OG) by non-tumoral cells was excluded from further experiments (Fig. S2).

The immunofluorescence assays were performed with the use of the following primary antibodies: anti-CD34 (M7165, Dako), anti-CD31 (PAA363Hu01, Cloud-Clone Corp.) anti-vWF (A0082, Dako), anti-α-SMA (MA1-06110,Thermo Scientific), anti-FSP (ab11333, Abcam), anti-IL13 receptor alpha 2 (ab55275, Abcam; WH0003598M1, Sigma-Aldrich), anti-Fra-1 (sc-28310, Santa Cruz Biotech.; PAJ089Hu01, Cloud-Clone Corp), anti-EphA2 (sc-924, Santa Cruz Biotech.) according to protocol described in Material and Methods.

Additionally, the immunofluorescence results were verified by analysis at DNA level (data not shown) enabling detection of anomalies typical for glioblastoma (e.g. LOH analysis) according to the scheme (and methods) described in our previous report [11].
Characteristics of EMT status in G114-derived cell cultures – Fig. S3

Immunofluorescence photomicrographs showing the expression pattern of EMT-associated protein (E-cadherin, N-cadherin, Twist 1, fibronectin, vimentin) in G114-derived cell cultures (10% adh, 0% adh, 0% sph).