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A glioblastoma neurosphere line with alternative lengthening of telomeres

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Neoplastic cells must maintain telomeres in order to replicate extensively, often by inducing telomerase activity. However, some tumors use a telomerase-independent mechanism dependent upon homologous recombination known as alternative lengthening of telomeres (ALT) [1]. ALT has been identified in 11–24 % of glioma tissue samples [2–4], but has been demonstrated in only one glioma cell line (TG20) [5]. In this brief letter, we describe a second ALT-positive glioblastoma (GBM) cell line.

We examined five stem-like GBM neurosphere lines (HSR-GBM1, 040821, 040622, JHH-GBM10, and JHH-GBM14) by telomere-specific FISH and identified the ultra-bright telomeric DNA foci indicative of ALT [6] in JHH-GBM14 (Fig. 1a), which also contained ALT-associated PML bodies (APBs; inset). These neurospheres were isolated from an untreated primary frontal lobe glioblastoma in a 69-year-old male [7], and examination of the surgical specimen also revealed ALT (Fig. 1b). The percentage of cells displaying ultra-bright telomeric foci varied within the tumor, but was low (1–5 %) in both the surgical specimen and JHH-GBM14. In our prior study of 40 ALT-positive high-grade astrocytomas, the percentage of cells exhibiting the ALT phenotype varied significantly from case to case with the majority containing >30 % positive cells, while some displayed a smaller fraction as seen with JHH-GBM14 [2]. Southern blotting showed the highly heterogeneous telomere length distribution typical of ALT (Fig. 1c) [5]. PCR-based TRAP assays revealed low-level telomerase activity, possibly representing a mechanism concentrated in ALT-negative cells (data not shown).

DNA sequencing revealed no mutations in *TP53* exons 5–8, and PCR failed to detect *EGFR*vIII. A Y183C point mutation in *IDH1* associated with familial osteoarthritis [8], but not with glioblastoma or ALT [9], was identified. Methylation-specific PCR analysis of the MGMT promoter revealed complete methylation (Fig. 1d), and treatment with temozolomide caused a significant (>75 %) decrease in culture growth.

Mutations in *ATRX* and *DAXX* have been implicated in ALT [6, 10]. Both were sequenced in JHH-GBM14 cells but no mutations were found, consistent with a number of previously documented adult GBM cases [6]. Interestingly, immunostaining revealed that approximately 30 % of the JHH-GBM14 cell population was ATRX negative, and ATRX expression was absent in ALT-positive cells (Fig. 1e). ATRX protein expression was also

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lost in a significant proportion of the glioma cells in the surgical specimen (Fig. 1f). Nuclear DAXX protein expression was conserved in the JHH-GBM14 line (data not shown).

Having characterized the line in vitro, we injected cells into the flanks and brains of athymic mice to evaluate the potential for xenograft formation. Tumors developed in the majority within 6 months. Intracranial tumors were small but diffusely infiltrative and expressing human-specific nestin (Fig. 1g, h), with a Ki67 proliferation index of over 20 % (data not shown).

In summary, ALT is a telomere maintenance mechanism common in gliomas, but to date only one ALT-positive glioma cell line has been documented. Here, we describe a second ALT-positive GBM-derived neurosphere line with intact *ATRX* and *DAXX* genetic loci and focal ATRX protein loss corresponding to the characteristic telomere changes. The neurosphere line generates intracranial xenografts, and represents a valuable research tool for investigating ALT in the subset of GBM with loss of ATRX protein but no *ATRX/DAXX* mutation.

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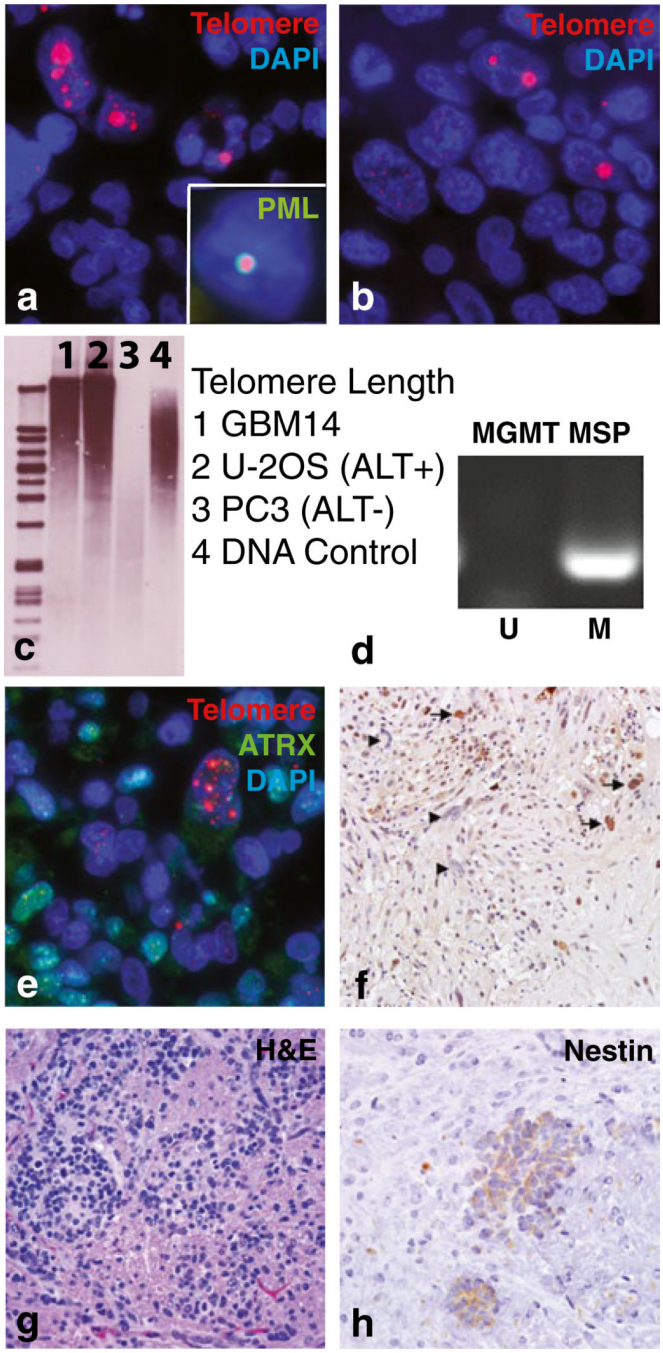

Fig. 1.

ALT characterization in a glioblastoma neurosphere line. **a** Telomere-specific FISH analysis in JHH-GBM14 and **b** primary tumor, as well as concurrent telomere FISH and PML immunofluorescence (*inset* in **a**). **c** Highly heterogeneous telomere length distribution typical of ALT was seen by Southern blot analysis in JHH-GBM14 and U-2 OS ALT(+) cells, while ALT(−) PC3 cells lacked this distribution. **d** MGMT promoter analysis via methylation-specific PCR. **e** JHH-GBM14 cells showing loss of ATRX protein demonstrate the ALT phenotype. **f** Primary tumor, *arrows*: ATRX positive; *arrowheads*: ATRX negative

tumor cells. **g** JHH-GBM14 intracranial xenografts, H&E. **h** Human-specific nestin immunohistochemistry