



Proteomic comparison of ALT and Telomerase+ Glioma Stem cells

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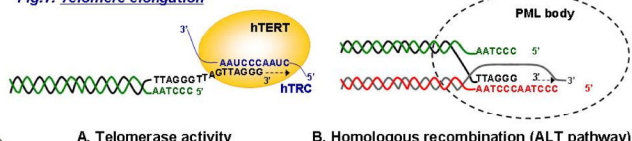
Telomeres are structures located at the end of chromosomes, composed of tandem repeats of a G rich DNA sequence (TTAGGG). Telomeres are subjected to shortening in dividing somatic cells, which results in cell growth arrest. Unlike somatic cells, normal stem cells and germ cells maintain their telomeres in order to keep their self-renewal capacity.

There are two ways for telomere maintenance:

- the first one is well known and happens through the activation of an enzyme called **telomerase**. The telomerase is composed of two sub-units: hTERT, a catalytic sub-unit that elongates the TTAGGG repeats using hTRC, an RNA template sub-unit (Fig.1.A).
- the other mechanism of telomere elongation relies on the **ALT** (alternative lengthening of telomeres) pathway. It is thought that the basics for this pathway are events of recombination between the telomere repetitive DNAs. (Reddel, 2003) (Fig.1.B)

Similar to normal stem cells, 85% of malignant cells show evidence of over expression of telomerase. However, some cancer cells utilize ALT to elongate their telomeres.

Fig.1. Telomere elongation



A. Telomerase activity

B. Homologous recombination (ALT pathway)

Glioblastomas are primary brain tumors particularly aggressive with a median survival of 14.6 months (Stupp et al., 2005). A sub-population of glioma tumor cells was identified to have common characteristics with normal neural stem cells. These cells (known as glioma stem-like cells, GSCs) exhibit specific capacities of self-renewal and are thought to be resistant to conventional treatments, such as radiotherapy (Bao et al., 2006). Given this, only a specific therapy that targets GSCs could efficiently destroy the tumor and prevent a potential relapse. (Fig.2)

Fig.2. Glioma stem-like cells



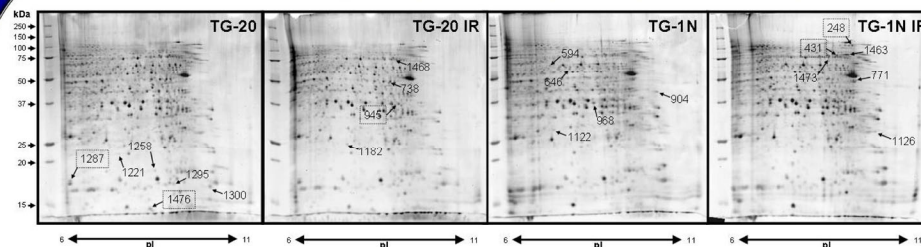
We recently characterized a new GSC line, TG20, that has no detectable telomerase activity but rather shows features of the ALT phenotype (Silvestre et al., 2011). Moreover, we have demonstrated that these cells are more resistant to irradiation compared to their telomerase + counterparts. To better understand the mechanisms governing ALT in GSCs, and the link with resistance to irradiation, we compared the nuclear proteins of these ALT GSCs (TG20) and a telomerase + GSC (TG1N), before and following irradiation, using a gel-based proteomic approach.

METHODS

- Two independent replicates of each GSC cell line were cultured as neurospheres in a serum-free DMEM/F12 medium supplemented with B27, heparin and EGF and FGF-2.
- Irradiation: 2Gy → Cells were pelleted 24hours post-irradiation.
- Nucleus were purified with differential centrifugation in 0.5% NP-40
- Proteins were extracted from nucleus pellet in solubilization buffer (9M urea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT) and 2D electrophoresis was performed with two biological and three technical replicates (n=6) with 6-11 strips for the 1st dimension and a 12% acrylamide gel for the second dimension.

- 2D gels were stained with **Lava-purple** fluorescent dye and the pictures were compared using the **"SameSpots"** software (V 4.1).
- Spots with significant differences between samples ($p < 0.05$; Power > 0.8) were selected and proteins were identified by nano-LC mass spectrometry.
- Proteins were validated by 1D western blotting (12% acrylamide) and 2D western blotting with 3-10 NL strips for the 1st dimension and a 12% acrylamide gel for the second dimension

2D analysis of protein samples (n=6): picture comparison and spot selection

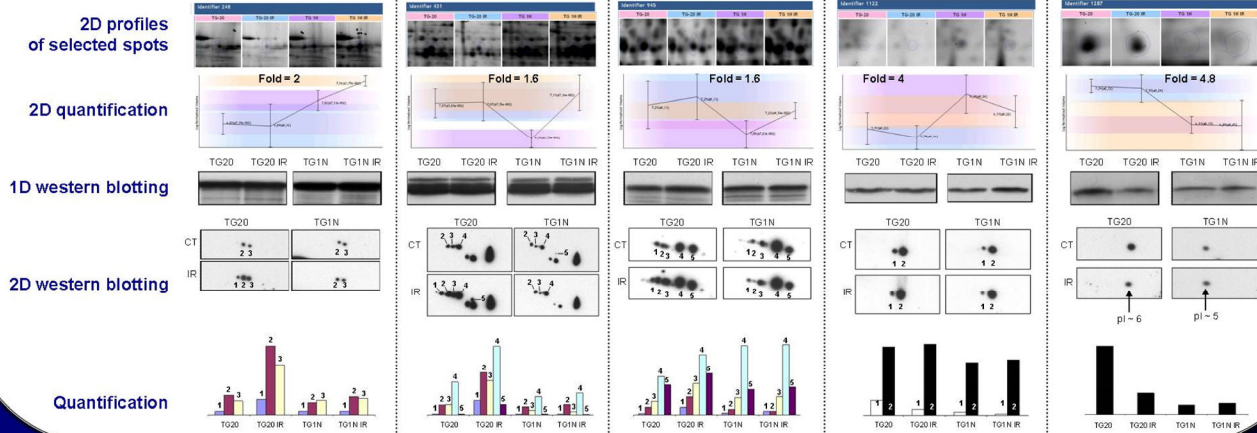


21 spots were differentially expressed between samples and were selected for MS identification.

Among these, 5 proteins were selected according to interesting molecular functions and were subsequently analysed using 1D and 2D western blotting.

#	Assets (in \$)	Field	Average Homologous Features			
			TS-20	TS-20	TS-20	TS-20
1681	34823(4.14)	0.1	1.27(2.09)	1.29(2.09)	6.63(5.05)	6.63(5.05)
738	1777(1.77)	2.0	3.23(2.09)	3.23(2.09)	1.27(2.09)	1.27(2.09)
137	3432(3.14)	3.0	1.27(2.09)	1.27(2.09)	7.36(5.05)	7.36(5.05)
1300	4351(4.14)	3.9	2.25(2.09)	2.25(2.09)	1.27(2.09)	1.27(2.09)
1173	7472(4.14)	5.1	5.81(5.05)	5.81(5.05)	2.79(5.05)	2.79(5.05)
1152	1388(4.14)	5.3	6.78(5.05)	6.78(5.05)	1.27(2.09)	1.27(2.09)
934	1356(4.14)	6.4	1.27(2.09)	1.27(2.09)	2.43(5.05)	2.43(5.05)
1475	848(4.14)	6.8	2.67(2.09)	2.67(2.09)	1.27(2.09)	1.27(2.09)
181	1033(4.14)	6.9	1.27(2.09)	1.27(2.09)	2.38(5.05)	2.38(5.05)
244	1388(4.14)	7.2	7.57(5.05)	7.57(5.05)	1.03(5.05)	1.03(5.05)
137	137(4.14)	7.5	1.27(2.09)	1.27(2.09)	1.27(2.09)	1.27(2.09)
1287	535(4.14)	8.4	3.95(4.07)	3.95(4.07)	8.35(5.05)	8.35(5.05)
1292	1356(4.14)	8.5	8.75(5.05)	8.75(5.05)	2.76(5.05)	2.76(5.05)
1293	2291(4.14)	8.5	1.27(2.09)	1.27(2.09)	1.27(2.09)	1.27(2.09)
1291	2291(4.14)	9.1	1.88(4.07)	1.88(4.07)	6.35(5.05)	6.35(5.05)
1122	4123(4.14)	9.5	8.75(5.05)	8.75(5.05)	2.76(5.05)	2.76(5.05)
1481	740(4.14)	9.5	8.75(5.05)	8.75(5.05)	5.93(5.05)	5.93(5.05)
1477	1333(4.14)	9.7	1.36(4.07)	1.36(4.07)	2.01(5.05)	2.01(5.05)
518	50(2)	1.6	1.887(7.07)	1.887(7.07)	1.27(2.09)	1.27(2.09)

Analysis of differentially expressed protein spots



CONCLUSION

Using a strategy of nucleus enrichment and protein focusing between pI 6-11, we aimed to target nuclear proteins potentially involved in telomere stability and/or response to irradiation. Here, we present 5 protein profiles, according to 2-D gel analysis, 1-D and 2-D western blotting. When quantifying the signals, we found in some cases variable and different protein amounts especially between the 2-D gel analysis and the 2-D western blotting.

This clearly shows the capacities and limits of both techniques to find and analyze protein markers following a classical gel-based proteomic study. Most of analyzed proteins have a complex pattern, with a lot of PTMs.

Many variations were observed, especially with TG20 cells following irradiation. The analyzed proteins seem to be implicated in the metabolism of nucleic acids and further investigation is needed to try to link these proteomic changes with the ALT phenotype and the resistance to irradiation. We found a different profile of PTM of the targeted proteins in TG-20 cells following irradiation compared to the telomerase positive TG1N cells.

Moreover, these results were very encouraging because differences for some proteins were also found in a transcriptomic study that compares TG20 ALT cells and other glioma telomerase positive cells including TG1N.