

The contribution of proteoglycan to heterogeneity and temozolomide resistance of glioblastoma cells

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Supplementary Table 1. Cell lines characterization.

| Cell line | Grade | Morphology | Patient | Karyotype | Reference |
|-------------------|---|-------------------------|-------------------------------------|------------------|---|
| U343MG | Glioblastoma | | 54-year-old male | | https://www.cellosaurus.org/CVCL_S471 |
| U343-MG (Uppsala) | Glioblastoma astrocytoma | Pleomorphic/astrocytoid | | 2n | https://www.sigmaaldrich.com/SE/en/product/sigma/08061901?gclid=EAIaIQobChMIImLSZ5I2Y_wIVAv9RC h0SMwBsEAAYASAAEgL3oPD_BwE&gclidsrc=aw.ds |
| LN215 | Diffuse glioma (glioblastoma) | | | | https://depmap.org/portal/cell_line/ACH-000328?tab=overview |
| LN215 | Astrocytoma | | 60-year-old female | | https://www.cellosaurus.org/CVCL_3954 |
| HS683 | Glioma | fibroblastic | White, 76 year old male (Caucasian) | Hyper triploid | https://www.atcc.org/products/htb-138 |
| U87 | Likely glioblastoma | epithelial | male | Hypo diploid | https://www.atcc.org/products/htb-14 |
| LN71 | Glioblastoma | | 67-year-old male | | https://www.cellosaurus.org/CVCL_6842 |
| LN405 | Glioblastoma | | 61-year-old female | | https://www.cellosaurus.org/CVCL_1378 |
| LN405 | Astrocytoma glioblastoma cells established from an astrocytoma tumor (grade IV) | | | Hyper tetraploid | https://www.dsmz.de/collection/catalogue/details/culture/ACC-189 |
| LN18 | Glioblastoma | epithelial-like | White, 65-year-old male | | https://www.atcc.org/products/crl-2610 |

Supplementary Table 2. Sequences of primers for human PG core proteins used in this study.

| Proteoglycan | GeneBank | Gene | Sequence |
|--------------|----------|------|----------|
|--------------|----------|------|----------|

| | | | |
|------------|----------------|--------|---|
| Syndecan-1 | NM_002997 | SDC1 | F 5'-CTCTGGCTCTGGCTGTGC-3' R 5'-AGCCATCTTGATCTTCAGGG-3' |
| Syndecan-3 | NM_014654.4 | SDC3 | F 5'- CACAACCAGACACAGCCAATG – 3' R 5'- CACAATCACAGCTACGAGCA – 3' |
| Glypican-1 | NM_002081 | GPC1 | F 5'-AGATCTACGGAGCCAAGGG-3' R 5'-TGTAGCCCTGGGGACAGAT-3' |
| Perlecan | NM_001291860 | HSPG2 | F 5'-TCGGCCATGAGTCCTTCTAC-3' R 5'-CCTGCTGTGTAGGAGAGGGT-3' |
| Versican | NM_004385 | VCAN | F 5'-TCTCCCCAGGAACTTACGA-3' R 5'-CACTCTTTTGCAGCCTCCTC-3' |
| Aggrecan | NM_001135 | ACAN | F 5'-TTCTTGAGAAAGGGAGTCCA-3' R 5'-ACAGCTGCAGTGATGACCCT -3' |
| Brevican | NM_021948 | BCAN | F 5'-ACCCAGCCCTGGGTAG-3' R 5'-GTCTCCTCCAGAACATCTGC-3' |
| Neurocan | NM_004386.3 | NCAN | F 5'- TGGTCCCAAAGAGTGGATGC-3' R 5'-CCTGTCCTGTTGGGGTCAAA -3' |
| NG2/CSPG4 | NM_001897 | CSPG4 | F 5'-TATGTTGGCCAGACTTGCAT-3' R 5'-TGCAGGTCTATGTCCGGTCAG-3' |
| CD44 | NM_000610 | CD44 | F 5'-GACAAGTTTTGGTGGCACG-3' R 5'-CACGTGGAATACACCTGCAA-3' |
| Decorin | NM_001920 | DCN | F 5'-AATGCCATCTTCGAGTGGTC-3' R 5'-TGCAGGTCTAGCAGAGTTGTGT-3' |
| Biglycan | NM_001711 | BGN | F 5'-AACTAGTCAGCCTGCGCCT-3' R 5'-CTCCCAGAAGCCTCTCTGCT-3' |
| Lumican | NM_002345 | LUM | F 5'-ACCAACTGTCAATGAAAACCTTG-3' R 5'-AGTAGGATAATGGCCCCAGG-3' |
| CSPG5 | NM_006574.4 | CSPG5 | F 5'- ACATCTGGCACAAGGGGATG – 3' R 5'- TGGGGTCCGGAATTTGTTGG – 3' |
| Phosphacan | NM_002851.3 | PTPRZ1 | F 5'- TGTTCCACGTTTCAGAGGCA – 3' R 5'- AGGGCTGACACGATCACAAG – 3' |
| GR | NM_001204264.2 | NR3C1 | 5-GTCATTCCACCAATTCCCGT-3 5-AACACTTGATCTTACCTTGAATAGC-3 |
| GAPDH | NM_002046 | GAPDH | F 5'-AAGGTGAAGGTCCGAGTCAA-3' R 5'-AATGAAGGGGTCATTGATGG-3' |

Supplementary Table 3. TMZ resistance of various human GB cell lines determined as a TMZ concentration leading to 2-fold decrease of proliferation rate of the studied cells (IC₅₀). Data are expressed as the means ± SD.

| Cell line | IC ₅₀ for TMZ, μM |
|--------------|------------------------------|
| U343 | 8.51 ± 3.16 |
| LN215 | 32.53 ± 2.07 |
| HS683 | 96.60 ± 51.37 |

| | |
|--------------|------------------------|
| U87 | 182.59 ± 6.02 |
| LN71 | 309.92 ± 221.36 |
| LN405 | 318.48 ± 68.31 |
| LN18 | 369.59 ± 42.85 |

Supplementary Table 4. Expression of PG core proteins in human GB cell lines. The data show the average expression level from triplicate experiments. Statistical analysis was performed using Origin 8.1 (data represent the mean ± SD).

| | U343 | LN215 | HS683 | U87 | LN71 | LN405 | LN18 |
|-------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Syndecan-1 | 0.0005±0.0001 | 0.0004±0.0001 | 0.0002±0.0002 | 0.0004±0.0001 | 0.0002±0.0007 | 0.0001±0.0005 | 0.0002±0.0005 |
| Syndecan-3 | 11.15±1.15 | 12.61±4.05 | 20.24±3.87 | 2.12±0.225 | 10.43±1.785 | 10.39±1.782 | 3.85±0.358 |
| Glypican-1 | 4.01±0.52 | 10.93±7.75 | 2.32±0.39 | 2.83±0.0966 | 6.76±0.679 | 2.34±0.561 | 0.85±0.8 |
| Perlecan | 0.009±0.002 | 0.073±0.008 | 0.013±0.003 | 0.190±0.051 | 0.043±0.016 | 0.007±0.002 | 0.029±0.027 |
| Neurocan | 0.005±0.001 | 0.002±0.0003 | 0.002±0.001 | 0.002±0.0001 | 0.003±0.0003 | 0.002±0.001 | 0.002±0.001 |
| Versican | 0.54±0.07 | 1.02±0.078 | 2.22±0.0478 | 1.11±0.0793 | 0.08±0.0189 | 0.39±0.096 | 0.31±0.013 |
| Brevican | 0.04±0.006 | 0.05±0.015 | 0.03±0.0085 | 0.04±0.0149 | 0.03±0.0004 | 0.02±0.003 | 0.02±0.005 |
| Aggrecan | 0.37±0.17 | 0.22±0.01 | 0.09±0.0024 | 0.67±0.088 | 0.0001±0.0001 | 0.03±0.006 | 0.10±0.02 |
| CD44 | 122.95±10.32 | 99.10±16.03 | 267.98±35.91 | 259.56±20.87 | 233.68±7.408 | 186.01±25.02 | 343.98±13.15 |
| NG2/CSPG4 | 0.73±0.02 | 3.12±0.26 | 0.02±0.013 | 6.94±0.178 | 0.08±0.065 | 21.45±3.84 | 0.01±0.008 |
| CSPG5 | 0.16±0.08 | 7.72±0.97 | 1.55±0.43 | 0.18±0.062 | 0.70±0.045 | 0.12±0.009 | 1.06±0.019 |
| Decorin | 0.0005±0.0002 | 0.001±0.001 | 0.0003±0.0001 | 0.524±0.051 | 0.030±0.004 | 0.018±0.002 | 0.003±0.0003 |
| Biglycan | 7.78±1.54 | 3.77±0.79 | 4.87±0.85 | 2.91±0.0739 | 27.24±0.274 | 6.81±0.044 | 13.01±0.483 |
| Lumican | 0.45±0.16 | 4.16±0.49 | 0.36±0.34 | 6.65±0.855 | 11.57±1.756 | 1.46±0.158 | 1.00±0.002 |
| PTPRZ1 | 0.03±0.01 | 0.01±0.008 | 0.49±0.13 | 0.05±0.039 | 0.01±0.001 | 0.17±0.023 | 0.01±0.007 |