**Supplemental Table S1: CAI2 sequence and features**

|  |
| --- |
| **GGGAG**TAAAACTACTGAATTGGGCCACAAGCAAATGAATAAACTGAACGACTCTTAACCAAACCTAATATATTTAATCCAAACACACAAGTCTTTCATTTCTTCCCTCCTCCCTTCCTTCTCTTACTCCCCAACACCCCCTCTTCAAGCACAATTAATTAT**atg**gttagattctactgcgtgatcagccctgttctaggtggtgggcacgccaaggtgaatgagaccaaacaagagtcttgccctcatggggtttacatttggagacagagtcgatctgttgcccaacctggagtgcagtggcgcgatcacagctcactgcagcctcaaactccctggctcaaggggttctcccacctgagcctcccgactagctgggaccacaggtgcacgccacgacgcctgggtttgtttgtttgtttaatagagacgaaggtctcaccatgttatctgggctcaagcgatcatcccccctcctcctcc**taa**AGTACTGGGATTACAGTCCCAAGCTATCTTGCCCGACCTGGGAAACAGACGTTAAGGAAGATAACAATCTATTTTCAGAGAGCGAGTTTATAAAACCAATGCAATGGGTAAATATGAAGTGTGAATAGGAGGAGAAGCTAAAGAGTGGTCGGAGAATCTAATGCAAGCTACGGGAGAAAGAAACTCAAGTGCAAATGCTGCCTCAGGAATAAACGTAAAAAGAGACTTTCAAGTGCAAATGCTCCCTCAGGAATAAAATAATCTTGAGACTCTCAAGTGTAAATGCTGCCTCGGGAGAACCGAACGGCGAGCTGGAGCCCATACGCAACGAGATTAGAGAGGAAGGCAGAAGCCAGAGCACATGAATAAATGAGCATCCATTTTGTTTCAGAAATGATCGGAAACCATTTGTGGGTTTGTAGAAGCAGGCATGCGTAGGGAAGCTACGGGATTCCGCCGAGGAGCGCCAGAGCCTGAGGCGCCCTTTGGTTATCGCAAGCTGGCTGGCTCACTCCGCACCAGGTGCAAAAGATGCCTGGGGATGCGGGAAGGGAAAGGCCACATCTTCACGCCTTCGCGCCTGGCATTGTGAGCAACCACTGAGACTCATTATATAACACTCGTTTTCTTCTTGCAACCCTGCGGGCCGCGCGGTCGCGCTTTCTCTGCCCTCCGCCGGGTGGACCTGGAGCGCTTGAGCGGTCGGCGCGCCTGGAGCAGCCAGGCGGGCAGTGGACTAGCTGCTGGACCAGGGAGGTGTGGGAGAGCGGTGGCGGCGGGTACATGCACGTGAAGCCATTGCGAGAACTTTATCCATAAGTATTTCAATGCCGGTAGGGACGGCAAGAGAGGAGGGCGGGATGTGCCACACATCTTTGACCTCAGGTTTCTAACGCCTGTTTTCTTTCTGCCCTCTGCAGACATCCCCGAT**TGA**AAGAACCAGAGAGGCTCTGAGAAACCTCGGGAAACTTAGATCATCAGTCACCGAAGGTCCTACAGGGCCACAACTGCCCCCGCCACAACCCACCCCGCTTTCGTAGTTTTCATTTAGAAAATAGAGCTTTTAAAAATGTCCTGCCTTTTAACGTAGATATATGCCTTCCCCCACTACCGTAAATGTCCATTTATATCATTTTTTATATATTCTTAT**AAAAATGT**AAAAAAGAAAAACACCGCTTCTGCCTTTTCACTGTGTTGGAGTTTTCTGGAGTGAGCACTCACGCCCTAAGCGCACATTCATGTGGGCATTTCTTGCGAGCCTCGCAGCCTCCGGAAGCTGTCGACTTCATGACAAGCATTTTGTGAACTAGGGAAGCTCAGGGGGGTTACTGGCTTCTCTTGAGTCACACTGCTAGCAAATGGCAGAACCAAAGCTCAAATAAA**AATAAA**ATAATTTTCATTCATTCACTC**A** |

**Supplemental Table S1: CAI2 sequence and features*.***

*CAI2* sequence is shown in relationship to exon γ and p16 exon 3. It is annotated as follows:

1. **Boxed and bold** sequences are the determined 5’ and 3’ RACE ends of the 1643 base *CAI2* transcript. Sequence analysis revealed *CAI2* extended 875 bases 5' from the beginning of exon γ (1255 bases from the 3’ end of p16 exon 2) and terminated at a poly A site in p16/ARF exon 3 that is 224 bases after the end of p16 stop signal codon (253 bases before the p16 poly A transcription termination site).
2. Exon gamma is double underlined.
3. ORF2 is shown in lower case with its putative ATG start codon and its putative TAA termination codon site in **bold**;
4. P16/ARF exon 3 is squiggly underlined, with the p16 TGA stop site near the beginning of the exon in **bold**;
5. The p16/ARF poly A signal and the poly A site are **bolded** and highlighted in grey.

**Supplemental Table S2: *CAI2, ARF* and *p16* expression in various tumor cell lines and normal cells**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Cell Line** | **Type** | **ARF** | **p16** | **CAI2** |
| 1 | CEM | T-ALL | - (del) | - (del) | - (del) |
| 2 | CEM-VLB100 | T-ALL | - (del) | - (del) | - (del) |
| 3 | Jurkat | T-ALL | - (del) | - (del) | - (del) |
| 4 | Molt4 | T-ALL | - (del) | - (del) | - (del) |
| 5 | Molt16 | T-ALL | - (del) | - (del) | - (del) |
| 6 | SB | B-ALL | +++ | +++ | +++ |
| 7 | K562 | CML | - (del) | - (del) | - (del) |
| 8 | Ramos | Burkitt's | +++ | - (+CH3) | +++ |
| 9 | HL60 | AML | +++ | +++ | +++ |
| 10 | SJ-SA-1 | Osteosarcoma | +++ | +++ | +++ |
| 11 | Saos-2 | Osteosarcoma | +++ | +++ | +++ |
| 12 | U2OS | Osteosarcoma | - (+CH3) | - (+CH3) | - |
| 13 | HeLa | Cervical | +++ | +++ | +++ |
| 14 | LS174T | Colon | - (+CH3) | - (+CH3) | - |
| 15 | LoVo | Colon | ++ (-/+CH3) | - | + |
| 16 | HT29 | Colon | ++ | -/+ (+CH3) | ++ |
| 17 | HCT116 | Colon | +++ | ++ (-/+CH3) | +++ |
| 18 | MCF7 | Breast | - (del) | - (del) | - (del) |
| 19 | T47D | Breast | +++ | - (+CH3) | ++ |
| 20 | MN1 | Melanoma | +++ | +++ | +++ |
| 21 | M2 | Melanoma | +++ | +++ | ++ |
| 22 | M14 | Melanoma | +++ | +++ | +++ |
| 23 | M21 | Melanoma | +++ | +++ | +++ |
| 24 | SK-MEL-5 | Melanoma | - (del) | - (del) | - (del) |
| 25 | A2058 | Melanoma | - (del) | +++ | +++ |
| 26 | A375 | Melanoma | - (del) | +++ | +++ |
| 27 | Be2C | Neuroblastoma | +++ | +++ | +++ |
| 28 | Be2C/ADR5 | Neuroblastoma | - (del) | - (del) | - (del) |
| 29 | NMB7 | Neuroblastoma | +++ | + | + |
| 30 | NB4 | Neuroblastoma | +++ | +++ | +++ |
| 31 | NB5 | Neuroblastoma | +++ | +++ | +++ |
| 32 | NB14 | Neuroblastoma | +++ | +++ | +++ |
| 33 | NB20 | Neuroblastoma | +++ | +++ | +++ |
| 34 | PCL1643 | Neuroblastoma | +++ | + | + |
| 35 | PCL1691 | Neuroblastoma | +++ | +++ | +++ |
| 36 | PCL2021 | Neuroblastoma | +++ | +++ | +++ |
| 37 | PCL4199 | Neuroblastoma | +++ | +++ | +++ |
| 38 | PCL3014 | Neuroblastoma | +++ | + | +++ |
| 39 | SMS-KAN | Neuroblastoma | +++ | +++ | +++ |
| 40 | SMS-KCN | Neuroblastoma | +++ | + | ++ |
| 41 | SMS-SAN | Neuroblastoma | ++ | + | +++ |
| 42 | IMR32 | Neuroblastoma | ++ | + | + |
| 43 | U251 | Glioblastoma | - (del) | - (del) | - (del) |
| 44 | U373 | Glioblastoma | +++ | +++ | +++ |
| 45 | PC3 | Prostate | - (+CH3) | - (+CH3) | - |
| 46 | Detroit 562 | Head and neck | +++ | - (del) | +++ |
| **Non-tumor cells and cell lines** | | | | | |
| 47 | WI38 | Fibroblast | ++ | ++ | + |
| 48 | IMR90 | Fibroblast | ++ | ++ | + |
| 49 | FS15 | 1o Fibroblast | ++ | ++ | + |
| 50 | MNC | Normal | + | + | + |
| 51 | Thymocytes | Normal | + | + | + |
| **Table 1** | **Cell Line** | **Type** | **ARF** | **p16** | **CAI2** |

**Supplemental Table S2: *CAI2, ARF* and *p16* expression in various tumor cell lines and normal cells.**

Expression of ARF, p16 and CAI2 was determined in multiple experiments by PCR and/or qRT-PCR and relative expression qualitatively summarized here from non-detectable (-), barely detectable (-/+), and low (+), moderate (++) and robust (+++) expression. Of the 46 tumor cell lines, there were 25 with all three genes intact, non-hypermethylated and expressed (SB, HL60, SJ-SA, Saos-2, HeLa, MN1, M2, M14, M21, Be2C, NMB7, NB4, NB5, NB14, NB20, PCL1643, PCL1691, PCL2021, PCL4199, PCL3014, SMS-KAN, SMS-KCN, SMS-SAN, IMR32), 13 do not express any of the three genes due to hypermethylation, deletion or undetermined (CEM, CEM-VLB100, Jurkat, Molt4, Molt16, K562, U2OS, LS174T, MCF7, SK-MEL-5, Be2C-ADR5, U251 and PC3), and 7 are hypermethylated or deleted for at least one of the three genes (Ramos, LoVo, HT29, T47D, A2058, A375 and Detroit 562), and one (HCT116) hypermethylated for one gene but nevertheless expressed. Note that none of the 5 non-tumor samples expressed all three genes robustly, a pattern not seen in any of the 25 cell lines with all three genes intact and expressed nor even in the intact genes from the 8 cell lines with *CAI2* and one other gene expressed. Cell line source and p16 status for some of the cell lines has been documented previously ([1-4](#_ENREF_1)). MNC, Peripheral blood mono nuclear cells; del, deleted; +CH3, hypermethylated; -/+CH3, partially methylated.

1. Diccianni MB, Chau LS, Batova A, Vu TQ, Yu AL. The p16 and p18 tumor suppressor genes in neuroblastoma: implications for drug resistance. Cancer Letters. 1996;104:183-92.

2. Diccianni MB, Omura-Minamisawa M, Batova A, Le T, Bridgeman L, Yu AL. Frequent deregulation of p16 and the p16/G1 cell cycle-regulatory pathway in neuroblastoma. Int J Cancer. 1999;80:145-54.

3. Diccianni MB. The enigmatic role of p16CDKN2/INK4a in neuroblastoma. Recent Advances and Research Updates. 2006;7:113-26.

4. Diccianni MB, Chilcote RR, Yu A. The genes of chromosomes 9p21 (p16, p15, ARF, MTAP) in pediatric acute leukemia: Inactivation and exploitation for tumor-targeted therapeutics. Trends In Cancer Research. 2007;2:135-49.

**Supplemental Table S3: Morphological assessment of low and high passage NMB7 cells after treatment by serum starvation or RA**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **#** | **Cell Line**  **passage** | **Treatment, % Serum** | **Observation at T=24h** | **Observation at T=72h** |
| 1 | NMB7,  pass 16 | Untreated, **0%** | very healthy, elongated phenotype with long cell processes | very healthy, elongated phenotype with long cell processes |
| 2 | NMB7,  pass 16 | 0.5% DMSO, **10% (control)** | very healthy, elongated phenotype with long cell processes | very healthy, elongated phenotype with long cell processes |
| 3 | NMB7,  pass 16 | 5 µM RA, **10%** | very healthy, elongated phenotype with long cell processes | very healthy, elongated phenotype with long cell processes |
| 4 | NMB7,  pass 5 | Untreated, **0%** | unhealthy, cells “stringy”, many detaching | unhealthy, few remain attached |
| 5 | NMB7,  pass 5 | 0.5% DMSO, **10% (control)** | very healthy, rounded, trapezoidal-like | cells very healthy, rounded, trapezoidal-like |
| 6 | NMB7,  pass 5 | 5 µM RA, **10%** | unhealthy, cells are smaller, skinnier and detaching | Unhealthy, round and grainy, most detaching/detached, phenoype is round/cylindrical, not "trapezoidal" |
| 7 | NMB7,  pass 16 | Untreated, **0%** | see *#1, above* | see *#1, above* |
| 8 | NMB7,  pass 16 | 0.5% DMSO, **0%** | n/d | very healthy, elongated phenotype with long cell processes |
| 9 | NMB7,  pass 16 | 5 µM RA, **0%** | n/d | very unhealthy, cells round and grainy |
| 10 | NMB7,  pass 5 | Untreated, **0%** | see *#4, above* | see *#4, above* |
| 11 | NMB7,  pass 5 | 0.5% DMSO, **0%** | n/d | unhealthy, few remain attached |
| 12 | NMB7,  pass 5 | 5 µM RA, **0%** | n/d | unhealthy, few remain attached |

**Supplemental Table S3: Morphological assessment of low and high passage NMB7 cells after treatment by serum starvation or RA.**

Low and high passage NMB7 cells were cultured in complete RPMI media for 24h – 72h with the above treatments and observed. In independent experiments, cells were photographed and RNA extracted (Figure 4B and Supplemental Figure S3). RA only influenced the high passage cells when they were cultured in the absence of serum. pass: passage