*Supplementary Data*

**Altered mRNA expression of genes involved in endocannabinoid signalling in in squamous cell carcinoma of the oral tongue**

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**Supplementary Table S1**. Target sequences and efficiencies for the qPCR primer pairs used in the present study. Product lengths >700 base pairs are not shown in the Table.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| Gene primers for | Efficiency(%)a | Target sequence | Product length (bp) | Intron skipping |
|  |  |  |  |  |
| *ABHD6* | 110 | NM\_001320126.1 *ABHD6* transcript variant 1 | 145 (1205-1349) | Yes |
|  |  | NM\_020676.6 *ABHD6* transcript variant 2 | 145 (1140-1284) | Yes |
|  |  |  |  |  |
| *ABHD12* | 103 | NM\_001042472.3 *ABHD12* transcript variant 1 | 93 (1153-1245) | Yes |
|  |  | NM\_015600.4 *ABHD12* transcript variant 2 | 93 (1300-1392) | Yes |
|  |  |  |  |  |
| *CD36* | 96.3 | NM\_001001548.2 *CD36* transcript variant 1 | 122 (1658-1779) | Yes |
|  |  | NM\_001001547.2 *CD36* transcript variant 2 | 122 (1619-1740) | Yes |
|  |  | NM\_000072.3 *CD36* transcript variant 3 | 122 (1658-1779) | Yes |
|  |  | NM\_001127443.1 *CD36* transcript variant 4 | 122 (1364-1485) | Yes |
|  |  | NM\_001127444.1 *CD36* transcript variant 5 | 122 (1539-1660) | Yes |
|  |  | NM\_001289908.1 *CD36* transcript variant 6 | 122 (1215-1336) | Yes |
|  |  | NM\_001289909.1 *CD36* transcript variant 7 | 122 (1243-1364) | Yes |
|  |  | NM\_001289911.1 *CD36* transcript variant 8 | 122 (1297-1418) | Yes |
|  |  |  |  |  |
| *CNR1*b |  | NM\_016083.5 *CNR1* transcript variant 1 [a] | 166 (290-455) | No |
|  |  | NM\_033181.4 *CNR1* transcript variant 2 [b] | 67 (96-162) | Yes |
|  |  | NM\_001160226.2 *CNR1* transcript variant 3 [a] | 166 (433-598) | No |
|  |  | NM\_001160258.2 *CNR1* transcript variant 4 [a] | 166 (471-636) | No |
|  |  | NM\_001160259.2 *CNR1* transcript variant 5 [a] | 166 (346-511) | No |
|  |  | NM\_001365869.1 *CNR1* transcript variant 6 [a] | 166 (328-493) | No |
|  |  | NM\_001365870.1 *CNR1* transcript variant 7 [a] | 166 (481-646) | No |
|  |  | NM\_001365872.1 *CNR1* transcript variant 8 [a] | 166 (677-842) | No |
|  |  | NM\_001365874.1 *CNR1* transcript variant 9 [a] | 166 (253-418) | No |
|  |  |  |  |  |
| *CNR2* |  | NM\_001841.3 *CNR2* | 76 (57-132) | Yes |
|  |  |  |  |  |
| *DAGLA* | 95.3 | NM\_006133.3 *DAGLA* | 135 (1809-1943) | Yes |
|  |  |  |  |  |
| *DAGLB* | 91.5 | NM\_139179.4 *DAGLB* transcript variant 1 | 120 (1500-1619) | Yes |
|  |  | NM\_001142936.1 *DAGLB* transcript variant 2 | 120 (1171-1290) | Yes |
|  |  |  |  |  |
| *FAAH* | 101.5 | NM\_001441.3 *FAAH* | 125 (1104-1228) | Yes |
|  |  |  |  |  |
| *MGLL* (1) | 95 | NM\_007283.6 *MGLL* transcript variant 1 | 89 (1366-1454) | Yes |
|  |  | NM\_001003794.2 *MGLL* transcript variant 2 | 89 (959-1047) | Yes |
|  |  | NM\_001256585.1 *MGLL* transcript variant 3 | 89 (1276-1364) | Yes |
|  |  |  |  |  |
| *MGLL* (2) | 104.7 | NM\_007283.6 *MGLL* transcript variant 1 | 113 (567-679) | Yes |
|  |  | NM\_001003794.2 *MGLL* transcript variant 2c | 113 (160-272) | Yes |
|  |  | NM\_001256585.1 *MGLL* transcript variant 3 | 113 (567-679) | Yes |

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Supplementary Table S1 (cont.)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
| Gene primers for | Efficiency(%)a | Target sequence | Product length (bp) | Intron skipping |
|  |  |  |  |  |
| *NAAA* | 99.2 | NM\_014435.4 *NAAA* transcript variant 1 | 121 (897-1017) | Yes |
|  |  | NM\_001042402.1 *NAAA* transcript variant 2 | 121 (897-1017) | Yes |
|  |  | NM\_001363719.1 *NAAA* transcript variant 3 | 121 (897-1017) | Yes |
|  |  |  |  |  |
| *NAPEPLD* | 91.2 | NM\_001122838.1 *NAPEPLD* transcript variant 1 | 138 (1181-1318) | Yes |
|  |  | NM\_198990.4 *NAPEPLD* transcript variant 2 | 138 (1181-1318) | Yes |
|  |  |  |  |  |
| *PLA2G4E* | 107 | NM\_001206670.1 *PLA2G4E* | 118 (1926-2043) | Yes |
|  |  |  |  |  |
| *PTGS2* | 92.6 | NM\_000963.4 *PTGS2* | 164 (1454-1617) | Yes |
|  |  |  |  |  |
| *RPL13* | 96.8 | NM\_012423.4 *RPL13A* transcript variant 1 | 157 (749-905) | No |
|  |  | NM\_001270491.1 *RPL13A* transcript variant 2 | 157 (727-883) | No |
|  |  | NM\_020807.2 zinc finger protein 319 *(ZNF319)d* | 557 (3240-3796) | No |
|  |  |  |  |  |
| *RPL19* | 102.7 | NM\_000981.4 *RPL19* transcript variant 1 | 93 (483-575) | Yes |
|  |  | NM\_001330200.1 *RPL19* transcript variant 2 | 93 (837-929) | Yes |
|  |  |  |  |  |
| *RPS12* | 93 | NM\_001016.4 *RPS12* | 164 (231-394) | Yes |
|  |  |  |  |  |
| *TRPV1* | 90.3 | NM\_080704.3 *TRPV1* transcript variant 1 | 137 (1870-2006) | Yes |
|  |  | NM\_018727.5 *TRPV1* transcript variant 2 | 137 (1753-1889) | Yes |
|  |  | NM\_080706.3 *TRPV1* transcript variant 3 | 137 (2122-2258) | Yes |
|  |  | NM\_080705.3 *TRPV1* transcript variant 4 | 137 (1796-1932) | Yes |
|  |  |  |  |  |

The primer specificities were checked using Use Primer-BLAST at <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>. Product length shows the number (and position in brackets of the mRNA nucleotides) of base pairs (bp). For readers not familiar with qPCR and primer design, a detailed example for *CNR1* transcript variant 2 is described below.

aDue to the paucity of available biopsy extract material, primer efficiencies were undertaken using human cancer cell lines (DU145 and T84). mRNA was extracted using the Dynabeads® mRNA DIRECT™ Purification Kit. 5 µg of mRNA was used for reverse transcription using the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Thermo Fisher Scientific). Considering undiluted cDNA as a first point, serial dilutions of cDNA were used to create a standard curve with 5 points. qPCR reactions were run on the Illumina Eco Real Time PCR system (Illumina Inc, San Diego, CA, USA) with an initial denaturation time of 10 minutes at 95˚C and 45 cycles of 10 seconds at 95˚C and 30 seconds at 60˚C. Each dilution was run in duplicate. The average replicate Ct values were plotted against the logarithm of the dilution. The slope of the resulting line was used to calculate the efficiency as follows:

$$Efficiency (\%)=(10^{\frac{-1}{slope}}-1)x100\_{}^{}$$

Efficiencies were considered satisfactory when values were between 90 and 110%.

Note that expression of *CNR1* and *CNR2* in these cell lines was insufficient for reliable efficiency data to be obtained

bAll variants except the transcript variant 2 encode isoform a. The variant 2 lacks a segment near the 5’-end of the coding region. In general the *CNR1* variants have a few small exon regions at the 5’- end followed by a very large exon. Thus, for example, *CNR1* transcript variant 7 has exons at bp 1-194, 195-385 and 386-5859 (<https://www.ncbi.nlm.nih.gov/nucleotide/1476411805>). This rendered impossible the design of a primer pair both crossing exons, and recognising all the transcript variants. The only variant where the primer pair crossed exons was for the CNR1 transcript variant 2, which has exons at bp 1-125 and 126-5375 (<https://www.ncbi.nlm.nih.gov/nucleotide/1476411820> ). Thus, the forward primer, which binds to bp sequence 96-116 and the reverse primer which binds to bp sequence 162-139 give a product length of 67 bp from 96-162, hence both exons.

cPrimer blast is inconsistent: in a previous search from 2018 (1), this transcript was not returned. Here it is returned, albeit with mismatches.

dThis target has been included since the product length was <700 bp, but there are mismatches and it was not ampified as adjudged by the melt curves.

**Supplementary Table S2**. ANOVA P values for the matched array data.

|  |  |  |
| --- | --- | --- |
|  |  | P values |
| Gene | log2FC | cohort | diagnosis | cohort x diagnosis |
|  |  |  |  |
| *Synthetic enzymes* |  |  |  |
| *PLA2G4E* | 0.84 | 0.49 | 0.0062 | 0.98 |
| *HRASLS5* | 0.0068 | 0.66 | 0.77 | 0.44 |
| *NAPEPLD* | 0.55 | 0.97 | 1.8x10-6 | 0.17 |
| *DAGLA* | -0.55 | 0.18 | 2.9 x10-4 | 0.27 |
| *DAGLB* | 0.092 | 5.8 x10-5 | 0.30 | 0.35 |
|  |  |  |  |  |
| *Target receptors* |  |  |  |
| *CNR1* | 0.074 | 0.98 | 0.065 | 0.45 |
| *CNR2* | -0.0035 | 0.58 | 0.78 | 0.96 |
| *TRPV1* | -0.19 | 0.0095 | 0.022 | 0.11 |
|  |  |  |  |  |
| *Catabolic enzymes* |  |  |  |
| *FAAH* | -0.23 | 0.014 | 0.049 | 0.20 |
| *NAAA* | -1.14 | 0.0060 | 4.0x10-11 | 0.77 |
| *MGLL* | -0.60 | 9.3x10-4 | 2.0x10-4 | 0.17 |
| *ABHD6* | -0.17 | 0.11 | 0.075 | 0.77 |
| *ABHD12* | -1.32 | 0.97 | 7.6x10-9 | 0.18 |
| *PTGS2* | 3.75 | 0.83 | 8.0x10-10 | 0.53 |
|  |  |  |  |  |
| *Other genes* |  |  |  |
| *CD36* | -0.14 | 0.41 | 0.62 | 0.17 |
| *FABP5* | 0.89 | 0.0026 | 2.7x10-4 | 0.96 |

Log2FC refers to the mean array score for the tumour tissues minus the corresponding mean score for the non-malignant tissues. Data are for 21 matched pairs (9 from the Tri cohort and 12 from the Qia cohort). P values were determined using two-way mixed ANOVA with diagnosis (tumour vs non-malignant tissue) as matched (”within”) factor and cohort as independent (”between”) factor. The critical value of P for these data at a 5% false discovery rate was 0.014.

**Supplementary Table S3**. ANOVA P values for the matched qPCR data.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  | P values |
| Gene | no. of pairs | log2FC | cohort | diagnosis | cohort x diagnosis |
| *Anabolic enzymes* |  |  |  |  |
| *PLA2G4E* | 15 | -2.02 | 0.45 | 1.8x10-4 | 0.40 |
| *NAPEPLD* | 15 | -1.88 | 0.0094 | 8.8 x10-8 | 0.53 |
| *DAGLA* | 14 | -0.49 | 0.93 | 0.47 | 0.37 |
| *DAGLB* | 11 | -1.01 |  | 4.1E-5 |  |
|  |  |  |  |  |  |
| *Target receptors* |  |  |  |  |
| *CNR1* | 13 | -1.04 | 2.3E-05 | 0.074 | 0.0036 |
| *TRPV1* | 12 | -1.50 | 0.89 | 5.6 x10-3 | 0.99 |
|  |  |  |  |  |  |
| *Catabolic enzymes* |  |  |  |  |
| *FAAH* | 15 | -0.14 | 0.71 | 0.74 | 0.31 |
| *NAAA* | 15 | 0.43 | 0.076 | 0.079 | 0.42 |
| *MGLL(1)* | 15 | 2.05 | 0.63 | 1.5 x10-8 | 0.86 |
| *MGLL(2)* | 15 | 1.68 | 0.14 | 2.8 x10-7 | 0.81 |
| *ABHD6* | 13 | -0.73 | 0.0011 | 0.0041 | 0.77 |
| *ABHD12* | 15 | 0.29 | 0.39 | 0.040 | 0.87 |
| *PTGS2* | 12 | -5.65 | 0.0036 | 2.4E-7 | 0.70 |
|  |  |  |  |  |  |
| *Other gene* |  |  |  |  |
| *CD36* | 15 | -1.23 | 0.41 | 0.12 | 0.067 |
| *RPL13* | 15 | 0.48 | 0.16 | 1.3 x10-4 | 0.029 |

Log2FC refers to the mean qPCR ∆Ct for the tumour tissues minus the corresponding mean score for the non-malignant tissues for the number of matched pairs shown. P values were determined using two-way mixed ANOVA with diagnosis (tumour vs non-malignant tissue) as matched (”within”) factor and cohort as independent (”between”) factor. The critical value of P for these data at a 5% false discovery rate was 0.015 regardless as to which of the two *MGLL* primer pairs was included. †For *DAGLB*, there were only two matched pairs from the Tri cohort, and so an ANOVA was not used. The P value shown (which was not used for the calculation of the critical value of P above) is from a two-tailed paired t-test.

**Reference**

1. Szeremeta J, Karlsson J, Alouayek M, Fowler CJ. Low mRNA expression and activity of monoacylglycerol lipase in human SH-SY5Y neuroblastoma cells. Prostagl Oth Lipid Med 2019;142:59-67