Sequencing of ACVR1 mutation sites in DIPG cell lines

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Aims

Verify identity of cell lines, and exact DNA sequence surrounding known DIPG ACVR1 mutations for future reference.

Procedure

Primer design

Primers were designed for the 50-200bp from the ends of each exon of ACVR1 (ENSEMBL accession code: ENSG00000115170) bearing a recorded DIPG mutation (i.e. exons 4-7) using NCBI primer blast according to the following requirements of the commercial sequencing service:

- 18-23bp in length
- Start primer is >50bp from area of interest
- Primer T_m = 55-60^oC
- Primer GC content = 40-60%

The primer sequences were as follows:

Primer ID	Sequence	Tm
ACVR1_exon4_f001	GCTGCCCTTCATGTGAGTTA	55
ACVR1_exon4_r001	GCAGATTTTCCAAGTTCCATCT	56
ACVR1_exon5_f001	CTCCAGTAAGCAATGGAGGG	58
ACVR1_exon5_r001	CACTCTCGAATTCACATTCACC	55
ACVR1_exon6_f001	CTCCTCTTAGGGCAATTGGT	56
ACVR1_exon6_r001	GAGATGCAACTCACCTAACC	54
ACVR1_exon7_f001	TGTATTGCAACAGTGACCCT	55
ACVR1_exon7_r001	TTCAATAGTCCCTTCAGCCC	55

DNA extraction

$5x10^6$ cells from each cell line were pelleted and flash frozen. Genomic DNA was isolated using the Qiagen QIAamp DNA mini kit following manufacturer's instructions for culture cells. This yielded the following samples:

Cell line	**Total DNA collected**
HSJD-GBM-02	25.75ug
SU-DIPG-IV	41.04ug
SU-DIPG-006	11.86ug
HSJD-DIPG-007	27.42ug
HSJD-DIPG-011	14.01ug

SU-DIPG-XXI 25.39ug

Each DNA sample was diluted to a concentration of 150ng/5µl in RNase free water for PCR.

PCR of exons

Primers were diluted to 10µM each in 50µL RNase free water. Genomic DNA from cells was all made up to 150ng/5µL in RNase free water (enough for 5 rx).

The following reaction master mix was made up:
(For targets <1kbp)

	stock concentration	**volume added**
Herculase II reaction buffer	5x	10µL
dNTP mix	25mM each nucleotide	0.5µL
DNA template	150ng/5µL	5µL
Primers	10µM each primer	2.5µL
Herculase II fusion DNA polymerase		0.5µL
Distilled water (to final volume of 50µL)		31.5µL

No DMSO was included in order to avoid the associated increase in error rate.

The following PCR cycle was used:

Temperature	**Time**		**Note**
95°C	3 min		Allow lid to reach 98°C before placing in tubes
95°C	15 s	< Repeat 35x	Melt
55°C	15 s		Annealing (temperature at average melting temp of primers)
72°C	30 s		Extension (Temperature according to enzyme used, time according to length of product)
72°C	3 min		Final extension

PCR product was frozen at -20oC and later run on 1% agarose gel in TAE buffer to confirm the presence of one band only, at the expected mass. Samples were sent to Source BioScience for overnight Sanger sequencing.

Results

All expected ACVR1 mutations were present in the expected cell lines (and each heterozygous), and expected wild-type cell lines did not carry mutations in either exon 4, 5, 6 or 7. Sequencing files have also been uploaded.