Using Genetics to Develop New Cancer Treatments

Genome-wide comparison of paired diagnostic and relapsed neuroblastomas

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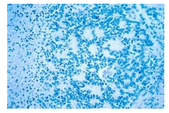
Introduction

Neuroblastoma is the most common extracranial solid tumour in children. Neuroblastoma develops from cells called neuroblasts which form the sympathetic nervous system.[1]



Neuroblastoma cells[2]

For low risk patients the survival rate after 5 years is 95%, however for high risk cases the survival rate after 5 years is only 50%. High risk cases are characterised by amplification of the *MYCN* gene, patient aged over 1 year old and metastasis of the tumour. Relapse of the tumour occurs in 50% of high risk cases.

It has previously been shown that there are more genetic mutations at relapse of neuroblastoma than at diagnosis. This is due to evolution of the tumour. Cells that are resistant to the initial chemotherapy at diagnosis survive then cause the patient to relapse.

Copy number alterations (CNAs) can be detected using a SNP array. A SNP is an alternative form of a single base of DNA. A SNP array uses target probes to bind to and detect these SNPs.

Aims

- To compare genetics of diagnostic and relapsed neuroblastoma cell lines and tumour cells by SNP array
- To test the sensitivity of SNP array technology using spiking experiments

Methods

- **DNA extraction from tissue**: DNA was extracted using the DNA prep kit after lysing the tissue. The DNA was then bound to a membrane, eluted and collected.
- **DNA quantification**: DNA was quantified using a Qubit flurometer after it has been extracted. A dye was used which only becomes fluorescent when it is bound to DNA. The dye's intensity was then detected and measured.
- **SNP arrays**: SNP arrays were carried out on paired cell lines. Three taken at diagnosis (NBLW, PER106, BE1N), three at relapse (NBLW-R, PER107, BE2C) and one at further relapse (PER108). The NBLW and NBLW-R cell lines were taken from the same patient, the PER cell lines were also taken from the same patient. We also carried out SNP arrays on 3 neuroblastoma tumour samples 20/038 (post-chemo), 4/148 (relapse) and 4/50 (post-chemo). In a SNP array single strands of DNA bind to unique probe sequences. Each probe binds to a target sequence. The signal from each probe can then be detected.[4]
- **Spiking:** To find out the level at which CNAs could no longer be detected we set up 7 SNP arrays. Each sample had a different percentage of tumour DNA in. The lowest percentage of tumour content at which CNAs can still be detected shows the sensitivity of the SNP array.

DNA extraction procedure[3]

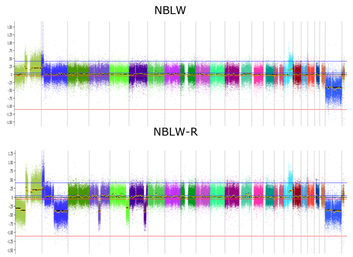
Sample	NBLW-R tumour content (%)	Normal DNA content (%)
1	100	0
2	0	100
3	50	50
4	40	60
5	30	70
6	20	80
7	10	90

Percentages of DNA used for spiking experiment

Results

Comparison of Cell Lines and Tumour Samples

We identified several genetic differences between the NBLW and NBLW-R cell lines at diagnosis and relapse.
We detected more CNAs at relapse than at diagnosis. The locations of these changes can be seen in the table below.



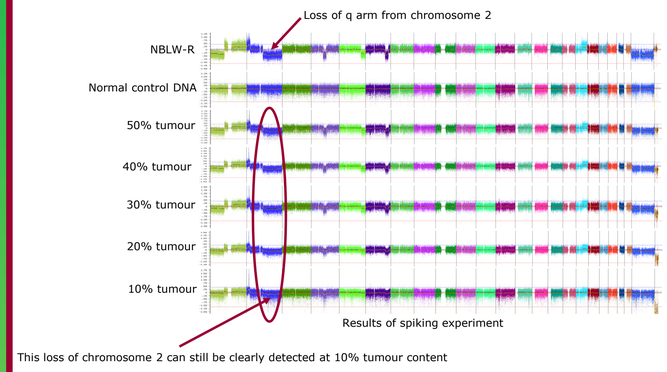
Comparison of NBLW and NBLW-R cell lines

	NBLW		NBLW-R		PER106		PER107		PER108		20/038	4/148		4/50		BE1N		BE2C	
	Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss	Gain	Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
Chromosome 1	p21.3-q44	p36.33-p21.3	p21.3-q44	p36.33-p21.3	p13.3-q24.2	p36.33-p13.3	-	-	-	-	-	-	-	-	231,103,786-249,250,621	142,971,168-249,250,621	0-97,044,239		1,968,138-97,001,088
Chromosome 2	p25.3-p24.1	-	p25.3-p24.1	q13-q37.3	-	-	-	-	-	-	155707 74-167960 68	790,981-42,909,768	-	0-34,092,103	227,054,828-243,199,373	0-48,557,574	-	1,783,188-48,008,900	0-89,566,573-242,650,700	1,992,924	47,871,732-190,252,414
Chromosome 3	-	-	-	-	q26.1-q29	-	q26.1-q29	-	q26.1-q29	-	-	-	-	-	0-66,146,639	62,180,359-89,625,231	0-62,321,780	-	0-61,651,653
Chromosome 4	-	-	-	q21.22-q24					-	q13.1-q27	-	-	-	-	-	-	-	-	-
Chromosome 5	-	-	-	q33.1-q35.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chromosome 6	-	-	-	q23.3-q25.2	-	-	-	-	-	-	-	590605 4-61776 86	-	-	-	-	-	-	-
Chromosome 7	-	-	-	-	-	-	-	-	q36.1-q36.3	-	-	-	0-23,785,531	-	-	-	-	-	-
Chromosome 8	-	-	-	-	-	-	-	p23.3-p21.3	-	p23.3-p21.3	-	-	-	-	-	-	-	-	145,137,155-146,364,022
Chromosome 9	-	-	-	-	p24.3-p22.3	-	p24.3-p22.2	-	p24.3-p22.2	-	-	79,647-25,805,500	-	-	398,233-39,106,483	-	-	-	0-35,602,033
Chromosome 11	-	-	-	-	-	-	-	q23.2-q25	-	q23.2-q25	-	-	-	-	0-8,604,476 57,337,793-71,653,204 67,710,534-135,006,516	-	-	-	-
Chromosome 12	-	-	-	-	-	-	-	-	-	-	-	91,574,929-133,851,895	-	114,753,128-133,851,895	-	-	-	-	-
Chromosome 13	-	-	-	-	-	-	-	-	q12.11-q33.2	-	-	-	-	-	-	-	-	-	-
Chromosome 14	-	-	-	-	-	-	-	-	-		-	-	-	-	82,680,561-107,264,377	-	-	-	-
Chromosome 16	-	p13.3	-	p13.3	-	-	-	-	-	-	-	-	-	69,759,348-90,342,278	-	-	-	-	-
Chromosome 17	-	q11.2-q25.3	q21.31-q25.3	-	q11.2-q25.3	-	q11.2-q25.3	-	q11.2-q25.3	-	-	349493 61-80918696	-	32,530,812-81,195,210	-	30,957,677-81,195,210	-	-	0-31,324,040
Chromosome 18	-	-	-	-	-	-	-	-	-	q21.1-q22.2	-	-	-	-	-	-	-	-	-
Chromosome 19	-	q13.2-q13.43	q13.43	-	p13.2-q13.11	p13.3-p13.2-q13.11-q13.43	-	p13.3-p13.2-q13.11-q13.43	-	q13.11-q13.43	-	0-16,474,742	-	-	0-3,849,716 57,627,345-59,122,287	-	-	-	-
Chromosome 20	-	-	-	-	-	-	q13.33	-	q13.33	-	-	-	-	-	-	-	-	-	0-3,049,417
Chromosome 22	-	-	-	-	-	q11.21-q13.33	-	-	-	-	-	-	-	-	-	-	-	-	-

Comparison of cell lines and tumour samples

Spiking Experiment

The data from the SNP array can be seen in the figure in the column opposite. This shows that CNAs can be detected down to 10% tumour content. In a SNP array analysis, normal chromosome copy numbers are shown along the 0 log2 ratio line. Any SNP probes that are below the line represent a loss of copy number and any that are above the line represent a gain.



Results of spiking experiment

Table showing genetic abnormalities in the PER106, PER107 and PER108 cell lines

Discussion

- One of the key genetic abnormalities in neuroblastoma is amplification of the *MYCN* gene. We found that *MYCN* amplification is maintained at diagnosis and at relapse. Across all the samples there were more gains of copy number than losses of copy number. Cases of uniparental disomy were also seen in the cell lines. This occurs when both chromosomes are from the same parent instead of having one from each parent. Cells with genetic changes that make them resistant to the initial chemotherapy survive and form the relapsed tumour. Detection of genomic changes in relapsed tumours may identify new treatment targets which could be developed.

- Some tumours that need to be analysed by SNP array have a low tumour content, some as low as 10%. This is why it was important to measure the sensitivity of the CytoSNP-850K BeadChip SNP array. From these results we can be confident that CNAs can be detected down to 10% tumour content.

Conclusions

- Our data shows that evolution of the tumour can be tracked between diagnostic and relapsed cell lines and neuroblastoma tumours
- It is possible to confidently detect CNCs down to 10% tumour content using the CytoSNP-850K BeadChip Illumina SNP array

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