Stability of gene expression and epigenetic profiles highlights the utility of patient-derived paediatric acute lymphoblastic leukaemia xenografts for investigating molecular mechanisms of drug resistance

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Abstract

Background: Patient-derived tumour xenografts are an attractive model for preclinical testing of anti-cancer drugs. Insights into tumour biology and biomarkers predictive of responses to chemotherapeutic drugs can also be gained from investigating xenograft models. As a first step towards examining the equivalence of epigenetic profiles between xenografts and primary tumours in paediatric leukaemia, we performed genome-scale DNA methylation and gene expression profiling on a panel of 10 paediatric B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) tumours that were stratified by prednisolone response.

Results: We found high correlations in DNA methylation and gene expression profiles between matching primary and xenograft tumour samples with Pearson’s correlation coefficients ranging between 0.85 and 0.98. In order to demonstrate the potential utility of epigenetic analyses in BCP-ALL xenografts, we identified DNA methylation biomarkers that correlated with prednisolone responsiveness of the original tumour samples. Differential methylation of CAPS2, ARHGAP21, ARX and HOXB6 were confirmed by locus specific analysis. We identified 20 genes showing an inverse relationship between DNA methylation and gene expression in association with prednisolone response. Pathway analysis of these genes implicated apoptosis, cell signalling and cell structure networks in prednisolone responsiveness.

Conclusions: The findings of this study confirm the stability of epigenetic and gene expression profiles of paediatric BCP-ALL propagated in mouse xenograft models. Further, our preliminary investigation of prednisolone sensitivity highlights the utility of mouse xenograft models for preclinical development of novel drug regimens with parallel investigation of underlying gene expression and epigenetic responses associated with novel drug responses.

Keywords: Acute lymphoblastic leukaemia, Xenografts, Genome-wide DNA methylation, Microarray analysis of gene expression, Glucocorticoid resistance

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Background
Despite progress in the treatment of several cancers over recent decades, the lack of clinically relevant tumour models for individual subtypes of human cancer has proven to be a major impediment in the development of effective anti-cancer therapies [1]. Approaches that facilitate development of novel rational therapies targeting specific tumours (or specific features of tumours) remain an urgent priority. Traditional models of human cancer involving the analysis of immortalised cell lines have given way in recent years to more clinically relevant studies in models that mirror the features of primary tumours [2]. The two main approaches have been the generation of primary tumour-derived cell lines, and the generation of mouse models, either via transgenic approaches or through the engraftment of primary human tumour into immune-compromised mouse models [3]. Mouse models have been used extensively in this regard, for preclinical testing of drug efficacy and toxicity prior to establishing clinical trials. A broad panel of xenografts with known treatment responsiveness, and well-defined molecular profiles, would provide an excellent adjunct to these models [4].

Mouse xenograft models of haematological malignancies, established by the transplantation of donor cells into non-obese diabetic/severe combined immunodeficient (NOD/SCID) or NOD/SCID/IL-2 receptor gamma chain−/− (NSG) mice, are recognised as one of the most clinically relevant systems for investigating leukaemia biology and testing new treatments [5-12]. This is due to the faithful recapitulation of many aspects of the human disease, including kinetics of engraftment in the bone marrow (BM), with subsequent infiltration of the spleen, peripheral blood and other organs [10,13,14]. For these reasons, patient-derived xenografts (PDXs) are considered superior to in vitro immortalised cancer cell lines that show many differences to primary tumours, including gene expression, drug responsiveness and epigenetic profiles [15], which is most likely due to the selective processes associated with long term culturing. PDXs have become increasingly popular as evidence mounts that they accurately recapitulate many of the features of patient tumours, such as tumour microenvironment, differentiation state and morphology, architecture and in some instances molecular signatures of the original patient tumour [reviewed in [1,2]].

To establish the relevance of PDX models to primary tumours, high density molecular profiling of gene expression and epigenetic markers should be performed. This was recently demonstrated for gene expression both between two tissue types, bone marrow and spleen and between independently engrafted mice for T-ALL [16].

As a first step towards examining the equivalence of epigenetic profiles between primary tumour and xenograft, we carried out parallel DNA methylation and gene expression profiling on a panel of childhood B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) selected by their clinical responses to prednisolone. This panel consisted of five individuals who had a good response to prednisolone (PGR) and five who had a poor response (PPR). By comparing DNA methylation and gene expression profiles between primary and derived, single-passaged xenograft lines, we report the stability of both gene expression and DNA methylation in the xenograft, further highlighting their potential for exploring gene expression and epigenetic changes associated with responses to established and novel drugs.

Methods
Patient samples, characteristics and xenograft model generation
All experimental studies were approved by the Human Research Ethics Committee and the Animal Care and Ethics Committee of the University of New South Wales. Written informed consent was obtained from the parents or guardians of paediatric ALL patients for use of biopsy samples in research, with the exception of samples obtained prior to May 2003 (ALL-26, ALL-28 and ALL-53), for which a waiver had been issued by the Human Research Ethics Committee. A total of 10 xenograft lines were generated from children diagnosed with BCP-ALL. Individuals were selected based on their response to prednisolone. We classified prednisolone poor responders (PPR) as patients with a peripheral blast count of ≥ 1 × 10⁹/L on day 8 following induction treatment with prednisolone and a single intrathecal dose of methotrexate, while a prednisolone good responder (PGR) demonstrated a day 8 peripheral blast count of < 1 × 10⁹/L (Table 1). Xenografts were established in NOD/SCID or NSG mice using direct explants of patient BM biopsies, exactly as described previously [10,17]. When mice were highly engrafted with leukaemia human CD45+, mononuclear cells were isolated from spleens by FACS at >90% purity and cryopreserved for subsequent experiments.

Genomic DNA and total RNA extraction
Genomic DNA was extracted from the primary bone marrow biopsies used for xenografting and from cells harvested from the spleens of engrafted animals for each xenograft using standard phenol/chloroform extraction and isopropanol precipitation. Total RNA was extracted using TriZol Reagent (Life Technologies, Carlsbad, USA) according to manufacturer’s instructions. Quality and yield were measured using a Nanodrop spectrophotometer.

Sodium bisulphite conversion of genomic DNA
Genomic DNA was converted for DNA methylation analysis using the MethylEasy Xceed Kit (Human Genetic
| Xenograft | Sex | Age (Months) | Cytogenetics | Immunophenotype (Diagnostic patient sample) | Diagnosis WCC $\times 10^9$/L | Diagnosis blasts $\times 10^9$/L | Day 8 blasts $\times 10^9$/L |
|----------|-----|--------------|--------------|---------------------------------------------|-------------------------------|-------------------------------|-----------------------------|
| PPR      | M   | 20           | Hyperdiploid | CD45+/DR+/10+/19+/2-/7-/13-/33+/34+           | 15.0                         | 11.8                          | 1.9                         |
| PPR      | M   | 131          | Normal       | CD45+/DR+/10+/19+/20+                        | 34.6                         | 26.1                          | 5.5                         |
| PPR      | M   | 89           | Normal       | CD45+/DR+/10+/19+/20+/34+/13-/33-            | 185.0                        | 174.8                         | 1.2                         |
| PPR      | M   | 176          | t(9;22)      | CD45+/DR+/10+/19+/13+/33+/34+                | 422.5                        | 388.7                         | 22.6                        |
| PPR      | F   | 72           | t(1;19)      | CD45+/DR+/19+/10+/34-2-/7-                  | 15.9                         | 7.2                           | 1.6                         |
| PGR      | F   | 43           | t(12;21)     | CD45+/DR+/CD19+/10+/22+/3-/34+/117-/Cu-/TdT+| 89.4                         | 80.5                          | 0.0                         |
| PGR      | M   | 19           | dic(7;9)     | CD45+/DR+/CD19+/10+/22+/34+/117-/Cu-/TdT+   | 90.5                         | 76.9                          | 0.0                         |
| PGR      | M   | 138          | t(7;15)      | CD45+/DR+/CD19+/22+/33+/10+/34+/Cu-/TdT+    | 14.4                         | 4.0                           | 0.0                         |
| PGR      | M   | 87           | t(12;21)     | CD45+/DR+/10+/19+/34+                       | 20.3                         | 13.8                          | 0.1                         |
| PGR      | M   | 120          | t(9;22)      | CD45+/DR+/10+/19+/34+/2-/7-/13-/33-         | 8.5                          | 0.1                           | 0.0                         |
Signatures, Sydney, Australia) according to manufacturer’s instructions. Converted DNA was used for downstream Illumina Infinium DNA methylation BeadArray analysis and SEQUENOM EpiTYPER validation.

**Genome-scale DNA methylation analysis**

Converted genomic DNA was processed and analysed for Illumina Infinium HumanMethylation27 BeadArray (Illumina, San Diego, USA) according to manufacturer’s instructions (ServiceXS, Leiden, The Netherlands). This BeadArray platform interrogates 27,578 CpG sites across the human genome. The arrays were scanned using an Illumina BeadArray Reader and subsequently processed using the Illumina GenomeStudio V.1 software package. The Bioconductor Lumi package was used for downstream data processing and normalisation [18]. Briefly, DNA probe methylation data were quality checked and then colour balance adjusted, background corrected and scaled based on the mean of all probes, using the methylation simple scaling normalization (SSN) implemented within the Lumi package. CpG sites with at least one sample having a detection p-value > 0.01 were excluded from subsequent analyses, leaving 27,341 CpG sites. Differential methylation analysis was performed using the LIMMA package from Bioconductor [19]. Significantly differentially methylated probes were selected based on a Benjamini-Hochberg adjusted p-value < 0.05. The methylation microarray data have been deposited into Gene Expression Ominibus (http://www.ncbi.nlm.nih.gov/geo/) with the identifier GSE57581.

**Gene expression Illumina array analysis**

Total RNA was extracted from the primary and xenograft tumours and amplified using the Illumina TotalPrep RNA amplification kit (Ambion, Austin, USA). The amplified total RNA was analysed using Illumina WG-6_V3 chips (Illumina, San Diego, USA) according to manufacturer’s instructions. The sample probe profiles with no normalisation or background correction were exported from BeadStudio (version 3.0.14, Illumina), and the data were pre-processed using quantile normalisation. Probes with detection p-value greater than 0.01 on all arrays were deemed as non-expressed probes and filtered out. Differential gene expression was determined using LIMMA with the positive False Discovery Rate (FDR) correction for multiple testing (Benjamini-Hochberg adjusted p-value < 0.05). The gene expression microarray data have been deposited into Gene Expression Ominibus (http://www.ncbi.nlm.nih.gov/geo/) with the identifier GSE57491.

**SEQUENOM MassArray EpiTYPER analysis**

Primers (detailed in Additional file 1: Table S1) were designed to generate PCR amplicons from bisulphite converted genomic DNA suitable for SEQUENOM EpiTYPER chemistry as per the manufacturer’s protocol. Samples were analysed using MALDI-TOF mass spectrometry, DNA methylation information was collected using EpiTYPER Viewer Software (v 1.0.5). Non-analysable and poor quality CpG sites were removed from downstream analysis as previously described [20].

**Results**

Xenograft models of BCP-ALL are an accurate reflection of DNA methylation and gene expression status of the corresponding primary tumour

One sample in our analysis, ALL28P, failed to meet array quality metrics (low overall signal intensity). Therefore, the matching xenograft pair, ALL28X along with ALL28P gene expression data was removed from subsequent analysis. ALL28 was also removed from the DNA methylation and gene expression correlation analysis herein.

Plotting the beta values of the entire data set revealed similar DNA methylation profiles between primary tumour tissue and the matching xenograft from each of the 10 patients in our study. Similarly, gene expression levels between primary tumour tissue and xenograft were also comparable (Figure 1A). For genome-scale DNA methylation, Pearson’s correlation coefficients between matching primary and xenograft samples ranged between 0.94-0.98 while correlation coefficients between individuals ranged between 0.80-0.91. For genome-wide gene expression, Pearson’s correlation coefficients between primary and xenograft samples ranged between 0.85-0.97 and between individuals was greater than 0.83-0.96 (Figure 1B). Gene expression profiles between individuals were more correlated than their DNA methylation profiles.

Consistent with this observation, unsupervised hierarchical clustering of the most variable DNA methylation and gene expression across all samples revealed clustering of matching primary and xenograft samples. This implies that the profiles from the xenografts recapitulate the profile of the primary tumour (Figure 1C).

To identify differential DNA methylation between primary tumour and matching xenograft samples we applied a linear model with empirical Bayes estimation and found 1564 probes to be differentially methylated between matching primary tumour and xenograft sample after correction for multiple testing (adjusted p-value < 0.05, Additional file 2: Table S2). The majority of these probes demonstrated a small change in DNA methylation with the average difference across individuals ranging from 0.4 to 8.6% (Additional file 3: Figure S1A).

We also looked for differential gene expression between matching primary and xenograft cell lines again applying a linear model with empirical Bayes estimation on the genome-scale gene expression microarray results. We found 3441 probes from 3208 genes to be differentially expressed between primary and xenograft lines
Figure 1 (See legend on next page.)
(adjusted p-value < 0.05, Additional file 4: Table S3). However, as we observed with DNA methylation, the differences in expression of these probes between primary and xenograft were minimal with an average fold difference in expression between primary and xenograft tumours of 1.12 (Additional file 3: Figure S1B).

Using DAVID (http://david.abcc.ncifcrf.gov/), the differentially methylated and differentially expressed genes between primary and matching xenograft lines were found to be mainly involved in haematological and cell signalling processes that could be accounted for given the cellular origins of the primary (bone marrow) and xenograft (spleen) samples.

Given the relatively small number of differentially methylated probes (6%) and differentially expressed probes (17%), and the minimal absolute differences in DNA methylation and expression (Additional file 3: Figure S1A and S1B), our results indicate that xenograft models largely recapitulate the DNA methylation and gene expression profile of the corresponding primary tumour. This highlights the potential utility of xenograft cell lines for modelling primary disease.

**Molecular biomarkers associated with prednisolone response**

We then sought to identify differential DNA methylation and gene expression associated with prednisolone poor (PPR) and prednisolone good (PGR) responders and included primary and xenograft samples in our analysis. After correction for multiple testing, 35 DNA methylation probes were differentially methylated between PPR and PGR (Benjamini-Hochberg adjusted p-value < 0.05, Table 2, Figure 2). Gene expression analysis revealed 23 genes differentially expressed between PPR and PGR (Benjamini-Hochberg adjusted p-value < 0.05, Table 2, Figure 2). From these lists, we did not find any commonly annotated genes associated with prednisolone response between the top differentially methylated and top differentially expressed probes. Differential DNA methylation segregated PPR from PGR by supervised hierarchical clustering and may serve as potential biomarkers for prednisolone response (Figure 2A).

However, interrogating gene expression alone did not accurately segregate PPR from PGR (Figure 2B). Functional annotation of differentially methylated genes annotated to these probes identified a number of apoptotic, cell signalling/structure pathways that did not reach statistical significance (Additional file 5: Table S4).

We then determined the relationship between DNA methylation and gene expression in association with prednisolone response. Plotting the average DNA methylation and gene expression differences between PPR and PGR revealed 22 probes annotated to 12 genes that were more highly expressed and less methylated in PPR samples compared to PGR samples (gene expression cut-off greater than 2 and a DNA methylation cut-off of less than -0.2, Figure 3, Table 3). Conversely, 11 probes annotated to 8 genes were less highly expressed and more methylated in PPR samples compared to PGR (gene expression cut-off of less than −2 and a DNA methylation cut-off greater than 0.2, Figure 3, Table 3). With the exception of expression probes annotated to PAWR, MTX2 and MYO3A no other gene expression and DNA methylation probes reached statistical significance (Table 3). DNA methylation probes associated with PAWR, MTX2 and MYO3A demonstrated an average difference of >0.2 between groups but did not reach significance with LIMMA analysis between PPR and PGR.

**Validation of DNA methylation biomarkers associated with prednisolone response**

From our array analysis, the DNA methylation changes segregated samples by prednisolone response. We validated 17 of these probes using SEQUENOM Epityper chemistry on both primary and xenograft samples by selecting from our LIMMA analysis, those also associated with changes in gene expression (Additional file 6: Figure S2). Of the assays containing the 17 probes of interest, 4 regions continued to discriminate samples according to prednisolone response (Figure 4). These were associated with the genes CAPS2 and ARHGAP21 (less methylated in PPR), ARX and HOX6 (more methylated in PPR). Primary and matching xenograft samples showed similar DNA methylation levels in all cases.

**Discussion**

It is becoming clear that the complexity of genetic, epigenetic, and subsequent gene expression disruption associated with human cancer is immense. As such, many mouse models of tumourigenesis are limited in their...
| Probe type | Probe ID   | Gene symbol | Adjusted p-value | ACC   | DESC                                      |
|------------|------------|-------------|------------------|-------|-------------------------------------------|
| DNA methylation probes | cg02780988  | KRTIA6      | 0.0001           | NM_003771 | keratin 36                               |
|            | cg16848873  | HOXB6       | 0.0001           | NM_018952 | homeobox B6                               |
|            | cg00546897  | LOC284837   | 0.0002           | NM_194310 |                                           |
|            | cg02789485  | MGCS39497   | 0.0007           | NM_152436 | GLI pathogenesis-related 1 like 2        |
|            | cg01605783  | LOC284837   | 0.0010           | NM_194310 |                                           |
|            | cg20291222  | CAPS2       | 0.0012           | NM_032606 | calycyosin 2                              |
|            | cg05724065  | PHKG1       | 0.0019           | NM_006213 | phosphorlyase kinase, gamma 1 (muscle)   |
|            | cg00645579  | IRF7        | 0.0033           | NM_001572 | interferon regulatory factor 7           |
|            | cg20100629  | AMID        | 0.0158           | NM_032797 | apoptosis-inducing factor, mitochondrion-associated, 2 |
|            |            |             |                  |       |                                           |
|            | cg20649991  | LILRB5      | 0.0170           | NM_006840 | keratin 72                                |
|            | cg11952714  | SNX7        | 0.0170           | NM_015976 | sorting nexin 7                          |
|            | cg20050826  | K6RS2       | 0.0170           | NM_080747 | keratin 72                                |
|            | cg21306775  | FLJ44881    | 0.0190           | NM_207461 |                                           |
|            | cg20468883  | BNIP2       | 0.0202           | NM_004330 | BCL2/adenovirus E1B 19kDa interacting protein 2 |
|            | cg08739282  | DHX15       | 0.0202           | NM_001358 | DEAD (Asp-Glu-Ala-His) box polypeptide 15 |
|            | cg03172991  | NFIX        | 0.0211           | NM_002501 | nuclear factor I/X (CCAT-binding transcription factor) |
|            | cg19238840  | GP2         | 0.0211           | NM_001007240 | glycoprotein 2 (zymogen granule membrane) |
|            | cg10148841  | ROBO4       | 0.0223           | NM_019055 | roundabout homolog 4, magic roundabout (Drosophila) |
|            | cg09892390  | ARHGAP21    | 0.0254           | NM_020824 | Rho GTPase activating protein 21          |
|            | cg05961212  | ADPRH       | 0.0254           | NM_001125 | ADP-ribosylarginine hydrolase             |
|            | cg22846623  | GJA12       | 0.0254           | NM_020435 | gap junction protein, gamma 2, 47kDa      |
|            | cg18096388  | PDCD1       | 0.0255           | NM_005018 | programmed cell death                    |
|            | cg05921324  | APOA4       | 0.0255           | NM_000482 | apolipoprotein A-I-V                     |
|            | cg13633560  | LRR3C2      | 0.0270           | NM_005512 | leucine rich repeat containing 32         |
|            | cg19573166  | SLC22A17    | 0.0270           | NM_020372 | solute carrier family 22, member 17      |
|            | cg01410472  | CRISPLD1    | 0.0277           | NM_031461 | cysteine-rich secretory protein LCCL domain containing 1 |
|            | cg26624914  | AQP3        | 0.0377           | NM_004925 | aquaporin 3 (Gill blood group)            |
|            | cg23752985  | VAMP8       | 0.0389           | NM_003761 | vesicle-associated membrane protein 8 (endobrevin) |
|            | cg21148892  | CLEC4F      | 0.0389           | NM_173535 | C-type lectin domain family 4, member F   |
|            | cg00032666  | CXorf6      | 0.0402           | NM_005491 | mastermind-like domain containing 1       |
|            | cg19511844  | ORMOL3      | 0.0418           | NM_139280 | ORM1-like 3 (S. cerevisiae)              |
|            | cg16127900  | GPRC6A      | 0.0466           | NM_148963 | G protein-coupled receptor, family C, group 6, member A |
|            | cg12552392  | NFS1        | 0.0475           | NM_181679 |                                           |
|            | cg22437699  | ARX         | 0.0479           | NM_139058 | aristless related homeobox               |
|            | cg02849695  | CCDC19      | 0.0486           | NM_012337 | coiled-coil domain containing 19          |
| Gene expression probes | ILMN_1806907 | PAWR        | 0.0000           | NM_002583 | PRKC, apoptosis, WT1, regulator           |
|            | ILMN_1794046 | MTX2        | 0.0119           | NM_006534 | Metaxin 2                                |
|            | ILMN_1758128 | CYGB        | 0.0119           | NM_134268 | Cytooglobin                              |
|            | ILMN_1738438 | MAST4       | 0.0157           | NM_198828 | Microtubule associated serine/threonine kinase family member 4 |
capacity to faithfully mimic human disease. In light of this, patient derived tumour tissue xenograft models are increasingly recognised as offering the most robust approach for testing tumour responses to various chemotherapeutic regimens, evaluating the efficacy of novel therapeutic agents, analysing the process of tumour progression at the cellular and molecular level and the identification of new therapeutic targets [2]. However, as with most mouse xenograft models, the stability of molecular profiles (gene expression and epigenetic) that regulate all aspects of

Table 2 Differential probes associated with prednisolone response (Continued)

| Probe ID     | Gene Symbol | Probe ID     | Gene Symbol |
|--------------|-------------|--------------|-------------|
| ILMN_1789384 | QSOX2       | ILMN_181701  | QSOX2       |
| ILMN_2306565 | MTX2        | ILMN_006554  | MTX2        |
| ILMN_2295987 | NBPF1       | ILMN_001006635 | NBPF1       |
| ILMN_1765772 | MYO3A       | ILMN_017940  | MYO3A       |
| ILMN_1713934 | LITAF       | ILMN_004862  | LITAF       |
| ILMN_1668125 | MYRIP       | ILMN_015460  | MYRIP       |
| ILMN_1681888 | PRKAR2A     | ILMN_004157  | PRKAR2A     |
| ILMN_2184966 | ZHK2        | ILMN_014943  | ZHK2        |
| ILMN_1706505 | COL5A1      | ILMN_000093  | COL5A1      |
| ILMN_1656057 | PLAU        | ILMN_002658  | PLAU        |
| ILMN_1761540 | SEMA3F      | ILMN_004186  | SEMA3F      |
| ILMN_1753143 | DKFzp761L1918 | ILMN_033103 | DKFzp761L1918 |
| ILMN_2148944 | ADY4        | ILMN_139247  | ADY4        |
| ILMN_1812618 | ARAP3       | ILMN_022481  | ARAP3       |
| ILMN_1681081 | AGPAT2      | ILMN_006412  | AGPAT2      |
| ILMN_1743275 | SH3RF3      | ILMN_001099289 | SH3RF3      |
| ILMN_1656951 | APCDD1      | ILMN_153000  | APCDD1      |
| ILMN_1719756 | ZAP70       | ILMN_0207519 | ZAP70       |
| ILMN_1768732 | SPAG16      | ILMN_00125436 | SPAG16      |

ILMN_1789384 QSOX2 0.0190 NM_181701 Quiescin Q6 sulfhydryl oxidase 2
ILMN_2306565 MTX2 0.0190 NM_001006635 Metaxin 2
ILMN_2295987 NBPF1 0.0237 NM_017940 Neuroblastoma breakpoint family, member 1
ILMN_1765772 MYO3A 0.0305 NM_017433 Myosin IIIa
ILMN_1713934 LITAF 0.0305 NM_004862 Lipopolysaccharide-induced TNF factor
ILMN_1668125 MYRIP 0.0305 NM_015460 Myosin VI and Rab interacting protein
ILMN_1681888 PRKAR2A 0.0305 NM_004157 Protein kinase, cAMP-dependent, regulatory, type II, alpha
ILMN_2184966 ZHK2 0.0305 NM_014943 Zinc fingers and homeoboxes 2
ILMN_1706505 COL5A1 0.0305 NM_000093 Collagen, type V, alpha 1
ILMN_1656057 PLAU 0.0305 NM_002658 Plasminogen activator, urokinase
ILMN_1761540 SEMA3F 0.0305 NM_004186 Sema domain, immunoglobulin domain (ig), short basic domain, secreted, (semaphin) 3F
ILMN_1753143 DKFzp761L1918 0.0305 NM_033103 Homo sapiens rhophilin-like protein mRNA, complete cds.
ILMN_2148944 ADY4 0.0305 NM_139247 Adenylate cyclase 4
ILMN_1812618 ARAP3 0.0305 NM_022481 ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3
ILMN_1681081 AGPAT2 0.0359 NM_006412 1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)
ILMN_1743275 SH3RF3 0.0359 NM_001099289 SH3 domain containing ring finger 3
ILMN_1656951 APCDD1 0.0359 NM_153000 Adenomatosis polyposis coli down-regulated 1
ILMN_1719756 ZAP70 0.0359 NM_207519 NM_001079 Zeta-chain (TCR) associated protein kinase 70kDa
ILMN_1768732 SPAG16 0.0437 NM_00125436 Sperm associated antigen 16

Figure 2 Heatmap plot of the most significant DNA methylation and gene expression probes distinguishing prednisolone good responders (PGR) from poor responders (PPR) after LIMMA analysis (BH adjusted p-value < 0.05). DNA methylation probes distinguished PGR from PPR while gene expression probes did not.
tumour function remains to be determined. Confirmation of this stability is crucial in order to identify molecular responses to treatment within the xenograft that could be extrapolated back to patients.

Here, we have determined the stability of genome-wide DNA methylation and gene expression profiles between primary tumour cells and matching xenograft tumour cells from a small number of paediatric ALL cases with differential response to prednisolone. A high correlation in both DNA methylation and gene expression profiles was observed in all cases, confirming the stability of these molecular features of primary tumours in the mouse system. Differences in DNA methylation and gene expression between primary and xenograft samples were negligible in magnitude (Additional file 3: Figure S1) and comprised of a small fraction of probes for each array platform. The differentially methylated genes include MYOD1, GPR6 and SLC27A6 (Table 1). Many genes associated with minor expression differences were part of the globin gene family and genes involved in oxygen transport and include HBB, AHSP, HBD, HBA2 (Table 2). This is likely to have arisen by the differences in cellular composition as the primary tumour samples contained a milieu of haematopoietic cells, including human erythrocytes that were absent in the xenograft samples that comprised of mononuclear cells derived from the murine spleen. Given the high degree of correlation and clustering of matching primary and xenograft samples after unsupervised hierarchical clustering of the most varied probes for DNA methylation and gene expression, the xenografts described in this study are an accurate reflection of their corresponding primary tumours.

While a number of candidate genes whose DNA methylation and/or gene expression status were associated with prednisolone response, given the small sample numbers and inherent genetic heterogeneity of the tumours, the significance of these genes remains unclear. Using hierarchical clustering, the most significant probes for DNA methylation discriminated prednisolone response while the gene expression probes did not (Figure 2), reflecting the more variable nature of gene expression compared to DNA methylation [21,22]. Using SEQUENOM, we were able to replicate DNA methylation changes at four genes associated with prednisolone response indicative of a potential DNA methylation biomarker. Taking methylation and expression status together, 20 genes were differentially regulated between good and poor responders to prednisolone (Table 3). While the genes were found to be part of apoptotic and cell signalling pathways, their significance remains unclear given the small numbers in each group. PAWR demonstrated significant overexpression and hypomethylation across PPRs compared to PGRs. This is a WTI interacting protein that also functions as a transcriptional repressor with pro-apoptotic functions and tumour resistance [23]. While the down regulation of PAWR confers poor prognosis in a range of conditions.
of solid tumours [24,25], its role in haematological malignancy is less clear, with expression detectable in a range of leukaemias [26]. Our results warrant further investigation of PAWR to determine a potential role in prednisolone response and responses to other novel drug regimens in an expanded xenograft cohort.

Another gene with potential interest is POU4F1, which appears to be differentially regulated according to Table 3:

| Threshold                                      | Gene_symbol.x | Gene_description.x                                      | Expression | Methylation | methDiff | expDiff |
|------------------------------------------------|---------------|----------------------------------------------------------|------------|-------------|----------|---------|
| Upregulated and less methylated in PPR         | H2AFY2        | H2A histone family, member Y2                            | 0.1173     | 0.1782      | -0.2204  | 4.0116  |
| (>-2-fold expression, < -0.2 methylation)     | H2AFY2        | H2A histone family, member Y2                            | 0.1173     | 0.3973      | -0.3007  | 4.0116  |
|                                                | MTX2          | Metatin 2                                                | 0.0190     | 0.3673      | -0.4061  | 3.9695  |
|                                                | PAWR          | PRKC, apoptosis, WT1, regulator                          | 0.0000     | 0.3154      | -0.4185  | 3.6350  |
|                                                | PAWR          | PRKC, apoptosis, WT1, regulator                          | 0.0000     | 0.3973      | -0.3026  | 3.6350  |
|                                                | MYO3A         | Myosin IIIA                                             | 0.0305     | 0.2944      | -0.4156  | 3.4893  |
|                                                | MYO3A         | Myosin IIIA                                             | 0.0305     | 0.3154      | -0.4061  | 3.4893  |
|                                                | MTX2          | Metatin 2                                                | 0.0119     | 0.3673      | -0.4061  | 3.1908  |
|                                                | BX537570      |                                                       | 0.2492     | 0.0333      | -0.3571  | 3.1574  |
|                                                | BX537570      |                                                       | 0.2492     | 0.1707      | -0.4761  | 3.1574  |
|                                                | CTSC          | Cathepsin C                                             | 0.0624     | 0.1782      | -0.4239  | 2.6472  |
|                                                | CTSC          | Cathepsin C                                             | 0.0624     | 0.2267      | -0.2834  | 2.6472  |
|                                                | CTSC          | Cathepsin C                                             | 0.0766     | 0.1782      | -0.2834  | 2.5574  |
|                                                | CTSC          | Cathepsin C                                             | 0.0766     | 0.2267      | -0.4239  | 2.5574  |
|                                                | MOSC1         | Nerve growth factor receptor (TNFRSF16) associated protein 1 | 0.0578 | 0.5189 | -0.2051 | 2.5035 |
|                                                | NGFRAP1       | Myristoylated alanine-rich protein kinase C substrate     | 0.5467     | 0.4154      | -0.2603  | 2.3418  |
|                                                | MPO           | Myeloperoxidase                                          | 0.5020     | 0.4097      | -0.2226  | 2.3130  |
|                                                | CTSC          | Cathepsin C                                             | 0.0578     | 0.1782      | -0.2834  | 2.1699  |
|                                                | CTSC          | Cathepsin C                                             | 0.0578     | 0.2267      | -0.4239  | 2.1699  |
|                                                | CCR7          | Chemokine (C-C motif) receptor 7                         | 0.2918     | 0.4434      | -0.2264  | 2.0570  |
|                                                | PLS3          | Plastin 3 (T isoform)                                    | 0.6662     | 0.3610      | -0.2919  | 2.0256  |
| Downregulated and more methylated in PPR       | POU4F1        | POU class 4 homeobox 1                                   | 0.1004     | 0.4404      | 0.3017   | -5.3928 |
| (<-2-fold expression, > 0.2 methylation)       | CYB5R2        | Cytochrome b5 reductase 2                                | 0.1734     | 0.5066      | 0.2699   | -4.3486 |
|                                                | TMED6         | Transmembrane emp24 protein transport domain containing 6 | 0.0504 | 0.5271 | 0.2317 | -4.1584 |
|                                                | CRMP1         | Collapsin response mediator protein 1                    | 0.3007     | 0.4431      | 0.3668   | -3.8640 |
|                                                | CRMP1         | Collapsin response mediator protein 1                    | 0.3007     | 0.3996      | 0.2607   | -3.8640 |
|                                                | IRX3          | Iroquois homeobox 3                                      | 0.5401     | 0.4957      | 0.2710   | -2.9003 |
|                                                | LDOC1         | Leucine zipper, down-regulated in cancer 1               | 0.2608     | 0.6055      | 0.2578   | -2.4859 |
|                                                | DSC3          | Desmocollin 3                                            | 0.5401     | 0.4585      | 0.3377   | -2.2248 |
|                                                | DSC3          | Desmocollin 3                                            | 0.5401     | 0.4402      | 0.3561   | -2.2248 |
|                                                | ANXA5         | Annexin A5                                               | 0.5923     | 0.3699      | 0.2228   | -2.0708 |
|                                                | ANXA5         | Annexin A5                                               | 0.5923     | 0.3727      | 0.2386   | -2.0708 |
to prednisolone response (Table 3). However in our analysis, statistical significance was not achieved with the modest sample size of our panel. POLI4F1 has been shown to have a role in regulating the expression of B-cell markers in t(8;21) positive acute myeloid leukaemia [27-29]. Its role in B-cell ALL response to prednisolone remains unclear and could be a potential gene target for further characterisation in an expanded B-cell ALL xenograft panel.

While our study did not identify statistically significant genes associated with prednisolone response, we present here a first pass analysis using low-resolution microarray platforms to interrogate DNA methylation and gene expression across our model system. We demonstrate that our B-cell ALL xenograft panel recapitulates the DNA methylation and gene expression profiles of the primary tumour and will facilitate future genome-wide interrogation of gene expression and DNA methylation using next generation sequencing methodology.

Conclusions

Patient-derived tumour xenograft models offer superior utility as preclinical models over cell line systems with their ability to recapitulate the milieu and microenvironment of the primary tumour. However, the extent of gene expression and epigenetic stability within the xenograft has remained unclear at least in the haematological setting. We have demonstrated that the gene expression and DNA methylation profiles of cells taken from the spleens of engrafted mice are highly correlated to the original primary tumour. Given the similarity to the primary tumour, our study confirms the opportunity to investigate gene expression and DNA methylation biomarkers in response to novel treatment strategies.

Availability of supporting data

The data sets supporting the results of this article are included within the article and its additional files. All microarray data presented in this paper have been deposited into
Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) with the identifiers GSE57581 and GSE57491.

Additional files

Additional file 1: Table S1. SEQUENOM EpiTYPER primers used in this study.

Additional file 2: Table S2. Differentially methylated probes between primary and xenograft tumours.

Additional file 3: Figure S1. Heatmap plot of the most significant DNA methylation (A) and gene expression (B) probes differentiating primary to xenograft tumours. While the samples clustered accordingly, the magnitude of DNA methylation and gene expression differences across these probes were minimal.

Additional file 4: Table S3. DAVID ontology list of functional pathways of genes found to be associated with prednisolone response.

Additional file 5: Figure S2. SEQUENOM Validation of 17 probes identified as significantly differentially methylated between primary and xenograft tumours. The green side column depicts PGR samples, while red depicts PPR samples. DNA methylation of these probes were able to separate tumours on prednisolone response, with 4 (depicted in Figure 4) giving the most discriminatory power.

Competing interests

The authors declare no actual or perceived competing interests.

Authors’ contributions

NCW, VAB, JMC, RS and RBL designed the study; NCW, VAB and JM performed the experiments; NCW, VAB, JM, MP-B and JN analysed data; NCW, VAB, RS and RBL interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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