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ORIGINAL ARTICLE

Detection of complex genomic signatures associated with risk in plasma cell disorders

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Plasma cell disorders (PCD) range from benign to highly malignant disease. The ability to detect risk-stratifying aberrations based on cytogenetic and molecular genetic assays plays an increasing role in therapeutic decision making. In this study, 58 patients were chosen for screening by comparative genomic hybridisation microarray (aCGH) to identify the new high-risk prognostic markers of chromothripsis and chromoanasynthesis. All patients had an unequivocal clinical diagnosis of a plasma cell disorder (plasma cell myeloma (PCM)(n = 51) or monoclonal gammopathy of undetermined significance (MGUS)(n = 7)) and an abnormal FISH result. There were a total of 17 complex genomic events identified across 9 patient samples, which were selected for further investigation by high definition single nucleotide polymorphism (HD-SNP) microarray. Each event was analysed and characterised for chromothripsis, chromoanasynthesis or a complex stepwise chromosomal event. We describe an effective method to identify the new high-risk prognostic markers of chromothripsis and chromoanasynthesis in plasma cell disorders.

Keywords Plasma cell disorders, myeloma, microarray, chromothripisis, chromoanasynthesis

Introduction

Monoclonal plasma cell disorder (PCD) is a spectrum of disorders that include monoclonal gammopathy of undetermined significance (MGUS), smouldering multiple myeloma (SMM) and symptomatic myeloma (PCM) (1,2). Heterogeneous clinical and biological features characterise PCD. Genomic abnormalities detected at diagnosis provide important prognostic information and are among the most important factors in predicting initial response to chemotherapy, remission duration and overall survival. Genetic risk stratification can assist in guiding specific chemotherapeutic interventions, such as the use of Bortezomib and high dose therapy (HDT) or novel agents, for patients categorised into high-risk groups (2–4).

Current genetic risk stratification guidelines have been established by the International Myeloma Working Group (IMWG), which also incorporates the Mayo Clinic's stratification approach—stratification for myeloma and risk adapted therapy (mSMART—see methods section) and is regularly reviewed (2,3,5). These guidelines have been used to provide a risk estimate based on genomic data from traditional karyotype and fluorescent in-situ hybridization (FISH) results. However, the incorporation of microarray findings may provide a more accurate disease classification for the treatment of these patients.

The use of microarray technology in the evaluation of haematological malignancies has rapidly gained popularity in response to the need for significantly greater molecular resolution of the whole genome to aid in diagnostic, prognostic and individualised patient treatment (6–8). In many cases it has begun to replace the need for conventional karyotyping and the use of extensive FISH panels for haematological malignancies (9,10). The value of aCGH in a clinical setting compared to traditional karyotype and multiple FISH analysis has been previously reported (6,10–13).

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In this study we screened a series of PCD patient samples by interrogating their molecular genetic profile utilizing aCGH. Further interrogation of complex genomic signatures was performed on a subgroup using a HD-SNP microarray platform. The aim was to characterise these complex findings, which may have significant clinical relevance especially with respect to treatment failure and or recurrence risk.

The use of aCGH has revealed new and emerging genetic risk factors that had hitherto remained undiagnosed, such as chromosome 1 aberrations, 12p deletions, 5q gains and evidence of the recently described phenomenon of chromothripsis and chromoanasynthesis (7,14–17). Chromothripsis is a phenomenon whereby a localised chromosome, chromosome arm or segment is shattered and repaired in a one-off catastrophic event that occurs at one time point rather than being acquired over many cell cycles such as a "step-wise" event. This event results in significant DNA rearrangements of which its genomic signature using microarray based analysis appears as an oscillation between two to three copy number states with loss of heterozygosity (LOH) (7,18–22). Chromoanasynthesis also appears to be acquired in a one-off event, however it is characterised by gained or amplified segments that retain heterozygosity (23,24).

Whilst these phenomena have been described as having a strong association with high-risk disease in myeloma and other haematological diseases, there has been no definitive mechanism by which to characterise these changes identified predominantly with the use of microarray technology (7,14,23,25). From a clinical diagnostic perspective, we sought to further investigate complex genomic events identified using CGH array analysis by HD-SNP microarray analysis to better characterise these multifaceted alterations.

Materials and methods

Patients and specimen ascertainment

58 samples from patients with a clinical diagnosis of PCD (based on the WHO criteria) were positively selected for microarray studies according to an abnormal interphase FISH (iFISH) result and DNA availability. The samples were comprised of a mix of diagnostic or relapsed bone marrow cells. A comprehensive iFISH panel analysis was performed on all samples and a diagnostic report issued. DNA was then extracted and a microarray analysis was performed.

The patient cohort characteristics are shown in Table 1 and the specific PCD classification is provided.

Table 1 Patient characteristics

Basic characteristics of patients in this study		
Patients (n = 58)		
Median age at diagnosis, years (range)	68	(43-93)
Female sex, N (%)	17	29%
WHO diagnosis		
PCM	51	88%
MGUS	7	10%

Abbreviations: WHO: World Health Organisation; PCM: Plasma cell myeloma; SMM: Smouldering multiple myeloma; MGUS: Monoclonal gammopathy of uncertain significance.

Enrichment of CD138 positive cells

Enrichment of CD138+ plasma cells was performed on all patient samples before testing using the EasySep methodologies as previously published (6).

Interphase FISH studies

i-FISH was performed using a break-apart probe for 14q32(*IGH*) and dual fusion probes for 4p16(*FGFR3*) / 14q32(*IGH*), 11q13(*MYEOV*) / 14q32(*IGH*) and 14q32(*IGH*) / 16q23(*MAF*) (Cytocell, UK) according to the UK Haemato-Oncology Best Practice Guidelines. Measurements of uncertainty were set at the levels recommended by the European Myeloma Network (10% for break-apart and dual fusion probes and 20% for locus specific probes) (26).

DNA extraction & quality assessment

DNA was initially extracted using either a QIAsymphony (Qiagen, USA) robot or a QIAcube (Qiagen, USA) robot using the relevant protocols. Clean up of the DNA was performed using a Zymo DNA Clean & Concentrator™ kit (no. 04004) (Zymo Research, USA) according to the manufacturer's instructions. DNA quality was assessed using a NanoDrop 2000 spectrophotometer.

Microarray analysis

Whole genome microarray analysis was performed using an oligonucleotide array (8x60k oligonucleotide array, CCMC design) (BlueGnome, UK). Labelling, hybridization and scanning were performed as per the Agilent Technologies user manuals. For comparison, sex-matched reference DNA supplied by Agilent Technologies was used (Agilent Technologies, USA). Analysis was performed using BlueFuse Multi v2.5 software (BlueGnome, UK).

Results were visualised using the BlueFuse Multi v2.5 software program (BlueGnome, UK). The nucleotide positions listed in BlueFuse are based on the UCSC Genome Browser's February 2009 human reference sequence (hg19; NCBI Build 37).

Data was analysed using a 3-probe calling criteria for Log2 values of >0.3 and <-0.3 and a smoothing of 2. Additional criteria for low mosaic calls of ≥ 10-probes with a Log2 value of 0.10 for gains and -0.10 for losses, was also applied.

Accurate alignment of genomic data for oligonucleotide microarray data where large amounts of the genome were lost or gained was impeded by the software's fundamental

Table 2 Cytogenetic Risk Classification

High Risk	Standard Risk
t(14;16)(q32;q23)	All others including;
t(14;20)(q32;q11)	Hyperdiploidy
del (17)(p13)—TP53 *	(≥47 chromosomes with trisomies
t(4;14)(p16;q32) *	of odd numbered chromosomes)
Non-Hyperdiploid	t(11;14)(q13;q32)
Gain 1q21	t(6;14)(p21;q32)
Deletion 1p	

* Patients' risk may be reduced with the choice of therapy.