ANALYSIS OF LOCUS-SPECIFIC LINE-1 AND ALU ELEMENT DNA METHYLATION REVEALS NOVEL EARLY EPIGENETIC CHANGES IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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BACKGROUND: Retrotransposons, such as LINE-1 (L1) and Alu elements, comprise more than 25% of the human genome. Their ability to retrotranspose throughout the genome is normally suppressed by epigenetic mechanisms. However, this repression is frequently lost in solid tumours through internal and external stimuli, and consequently somatic retrotransposition can be an initiating event in carcinogenesis. The epigenome in chronic lymphocytic leukaemia (CLL) is shaped by the maturation stage of the cell of origin, and its evolution during disease progression is correlated with the acquisition of genetic abnormalities associated with poor patient prognosis. Early work has demonstrated that L1 and Alu hypomethylation are associated with the acquisition of 17p deletions in CLL, but to date there has been no comprehensive or locus-specific analysis of retrotransposon DNA methylation.

AIMS: To develop an approach to enable locus-specific analysis of L1 and Alu subfamily DNA methylation using the Illumina Infinium 450K microarray platform (H450K) and apply this to study aberrant methylation of L1 and Alu elements in CLL.

METHODS: H450K probes mapping to retrotransposons were identified using RepeatMasker. The probeset was applied to a publicly-available dataset from a study of 138 CLL patients and 13 healthy individuals available from the International Cancer Genome Consortium. Leading hits were further analysed in Gene Expression Omnibus (GEO) datasets from 1,169 healthy individuals, 764 acute lymphoblastic leukaemia (ALL) patients, 174 acute myeloid leukaemia (AML) patients, and 31 diffuse large B-cell and Burkitt's lymphoma patients, and also prospective samples from 82 future CLL cases (<18 years from diagnosis) and 82 age-matched controls within the Melbourne Collaborative Cohort Study.

RESULTS: We identified 9,549 probes mapping to 117 L1 subfamilies, and 12,806 mapping to 37 Alu subfamilies. In normal B-cells from healthy individuals, DNA methylation at these sites was routinely high (mean β: 0.75), with greater variation observed in older subfamilies (L1M and AluJ) in comparison to the youngest (L1H/L1PA and AluY), especially at CpGs within 200 bases of TSS. We identified 10,782 CpG sites within L1 and Alusequences that were differentially methylated between CLL patients and healthy individuals (Pfdr<0.05), of which 55 were

hypomethylated in >90% of CLL patients but never in healthy individuals. Hypomethylation of Alu elements was associated with evolutionary age, with older subfamilies (AluJ) displaying greater changes than younger ones (AluY). Hypomethylation of 17 leading hits was highly confined to CLL, never observed in healthy individuals and infrequently in ALL, AML and lymphoma. In prospective samples, methylation at each of the 17 loci, located across the genome, was highly correlated within individual patients. In contrast to diagnosed CLL patients, hypomethylation at the loci was observed in only 9 future CLL cases (11%). Notably, however, this was more commonly observed in samples taken <7 years before diagnosis (7 of 24, 29%) than in those taken more than 7 years before diagnosis (2 of 58, 3%).

CONCLUSIONS: We have identified locus-specific hypomethylation events of L1 and Alu elements that are highly frequent and specific to CLL, and which are present prior to diagnosis for some patients. Further work is required to establish how these epigenetic changes correspond to modulation of global DNA methylation patterns in leukaemogenesis.