



**Karolinska
Institutet**

Institutionen för medicin,

Karolinska Universitetssjukhuset Huddinge

Prognostic and biological implications of epigenetic changes in leukemia

AKADEMISK AVHANDLING

som för avläggande av medicine doktorexamen vid Karolinska
Institutet offentligen försvaras i föreläsningssalen R64,
Karolinska Universitetssjukhuset, Huddinge

Fredagen den 20:e januari 2012, kl 09.30

av

Stefan Deneberg

Specialistläkare

Huvudhandledare:

Docent Sören Lehmann
Karolinska Institutet
Institutionen för medicin

Bihandledare:

Professor Christer Paul
Karolinska Institutet
Institutionen för medicin

Fakultetsopponent:

Dr Lars Bullinger
University of Ulm
Department of Internal Medicine III

Betygsnämnd:

Professor Dick Stockelberg
Sahlgrenska Universitetssjukhuset
Enheten för hematologi

Professor Dan Grander
Karolinska Institutet
Institutionen för onkologi och patologi
Cancer Centrum Karolinska

Docent Fredrik Öberg
Uppsala Universitet
Institutionen för genetik och patologi
Rudbeckslaboratoriet

Stockholm 2012

ABSTRACT

The field of epigenetic research in hematology and oncology is rapidly expanding. Even so, reliable data linking epigenetic changes to clinical outcomes are scarce.

We conducted two retrospective studies in AML. The first (paper 1) was performed in 107 AML patients without a previous history of MDS where we approximated the global DNA 5-methylcytosine content with a methylation sensitive restriction enzyme assay, the promoter DNA methylation status of three known tumor suppressor genes, CDKN2B (p15), HIC1 and CDH1, and in a subset of 20 patients genome-wide promoter methylation by the Illumina HumanMethylation27 array.

Promoter methylation of CDKN2B was common (66%), and associated with better overall and disease free survival in uni- and multivariate analysis. Average genome wide promoter methylation levels were also associated with overall and disease free survival and correlated inversely with global 5-methylcytosine content, which in turn associated with response to induction therapy.

The second study (paper 2) was restricted to cytogenetically normal de-novo AML cases. In a test group of 58 samples we investigated genome wide promoter methylation by the Illumina HumanMethylation27 array and correlated the methylation patterns with the mutational status of NPM1, FLT3, CEBPA, IDH1, IDH2, DNMT3A and clinical parameters.

We found increased promoter methylation in NPM1 and IDH mutated samples with specific methylation patterns for these two mutations. Compared with a control group of normal myeloid progenitor cells from 9 donors the most differentially methylated genes in AML were those that in previous studies were targeted by Polycomb group proteins in embryonic tissue. Furthermore, we found that the methylation levels of the Polycomb targeted genes were associated with overall and progression free survival. The prognostic association was confirmed in a validation cohort of 60 patients and retained significance in multivariate analysis.

The third study of this thesis (paper 3) was designed to search for the second tumor suppressor gene commonly thought to reside on chromosome 11q21-23 in CLL, based on the finding of two microdeletions in a previous study.

Through DNA methylation screening we found a 48% prevalence of aberrant promoter methylation of the shared two-directional promoter of BTG4 / microRNA-34b/c. Functional studies with stress incubation of primary CLL samples as well as the HG3 cell line showed an selective up-regulation of miR-34b/c transcripts in unmethylated cells, but no induction of BTG4 regardless of methylation status. Chromatin immunoprecipitation experiments showed the presence of repressive chromatin marks in both CLL and normal lymphocytes, which may explain our observation that the basal expression levels of miR-34b/c were low both in normal lymphocytes and CLL cells regardless of methylation status, compatible with a "epigenetic switch" from conditional to permanent silencing in methylated samples.

We conclude that DNA methylation patterns are associated with mutational status and clinical outcomes in AML. Furthermore we believe that miR-34b/c may function as a tumor suppressor gene in CLL, incapacitated by an epigenetic switch mechanism in approximately 50% of CLL samples.