Profiling Three-Dimensional Nuclear Telomeric Architecture of Myelodysplastic Syndrome and Acute Myeloid Leukemia Defines Patient Subgroups

Gadji Macoura1, 2, Awe Julius Adebayo1, 3, Rodriguez Prerana1, Kumar Rajat4, Houston Donald S.4, Ludger Klewes1, Tandakha Ndiaye Dièye2, Rego Eduardo Magalhães5, Falcão Passetto Roberto5, de Oliveira Fábio Morato5, Mai Sabine1

1- The University of Manitoba, Manitoba Institute of Cell Biology (MICB), Cancer Care Manitoba (CCMB), The Genomic Centre for Cancer Research and Diagnosis (GCCRD), The University of Manitoba, 675 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 0V9
2- The Cheikh Anta Diop University of Dakar, Laboratory of Haematology and Immunology, National Centre of Blood Transfusion of Dakar, BP 5002, Dakar Fann, Senegal
3- School of Life Sciences, Skövde University, Box 408, 541 28, Skövde, Sweden
4- Section of Hematoloy/Oncology, Department of Internal Medicine, University of Manitoba, 675 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 0V9
5- Department of Internal Medicine – Division of Hematology, School of Medicine of Ribeirão Preto, University of São Paulo – Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil; National Institute of Science and Technology in Stem Cell and Cell Therapy, Ribeirão Preto, Brazil.

Running title: Nuclear Telomeric Architecture Profiling in MDS and AML

Keywords: Nuclear Architecture, MDS, AML, Anemia, Telomeres, Genomic Instability

Competing interest statement: The authors have no conflict of interest to disclosure

Corresponding author: Dr Sabine Mai
Manitoba Institute of Cell Biology, 675 McDermot Ave, Winnipeg Manitoba, Canada R3E 0V9 Email: smai@cc.umanitoba.ca
Statement of translational relevance

We describe three-dimensional (3D) nuclear telomere dysfunction in Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML). Blindly performed profiling of MDS and AML allows for the categorization of both MDS and AML patients and patient subgroups. This is the first time that such a clear distinction in between MDS and AML becomes reality based on specific quantitative 3D nuclear telomeric parameters that include telomeric sizes, telomere numbers/nucleus, the presence of telomeric aggregates, nuclear volumes, and the telomere distribution in the nucleus (1-3). Our 3D nuclear telomere marker is proposed as a new structural molecular biomarker for MDS and AML with possible future implications on clinical management of patients.

Summary:

Purpose

Myelodysplastic Syndromes (MDS) are a group of disorders characterized by cytopenias, with a propensity for evolution into Acute Myeloid Leukemias (AML). This transformation is driven by genomic instability but mechanisms remain unknown. Telomere dysfunction might generate genomic instability leading to cytopenias and disease progression.

Experimental Design

We undertook a pilot study of 94 patients with MDS (56 patients) and AML (38 patients). The MDS cohort consisted of RCMD (32 cases), RA (12 cases), RAEB1 (8 cases), RAEB2 (1 case), RARS (2 cases), and MDS with isolated del(5q) (1 case). The AML cohort was composed of AML-M4 (12 cases), AML-M2 (10 cases), AML-M5 (5 cases), AML-M0 (5 cases), AML-M1 (2 cases), AML-M4eo (1 case), and AML with multidysplasia related changes (1 case). Three-dimensional quantitative fluorescent in situ hybridization of telomeres was performed on nuclei from bone marrow samples and analyzed using TeloView™.

Results
We defined 3D nuclear telomeric profiles based on telomere numbers, telomeric aggregates, telomere signal intensities, nuclear volumes, and nuclear telomere distribution. Using these parameters, we blindly subdivided the MDS patients into 9 subgroups and the AML patients into 6 subgroups. Each of the parameters showed significant differences between MDS and AML. Combining all parameters revealed significant differences between all subgroups. 3D telomeric profiles are linked to the evolution of telomere dysfunction defining a model of progression from MDS to AML.

**Conclusions**

Our results show distinct 3D telomeric profiles specific to patients with MDS and AML that help subgroup patients based on the severity of telomere dysfunction highlighted in the profiles.

**Introduction**

Myelodysplastic syndromes (MDS) are composed of a heterogeneous group of clonal disorders affecting hematopoietic progenitors that display genomic instability (4). MDS is characterized by dysplasia and ineffective haematopoiesis (5,6). MDS represents one of the five major categories of myeloid neoplasms according to the classification of hematological cancers by the World Health Organization (WHO) (5,6,7). Myeloid neoplasms display a fundamental characteristic feature that is stem-cell-derived clonal myelopoiesis with altered proliferation and differentiation (6). MDS, *de novo* or secondary to ionizing radiation, toxins or chemotherapeutic drug exposure, generally follows a course of progressive cytopenias with varying degrees of anemia, leucopenia, and thrombocytopenia. This renders the patients dependent on red cell transfusions or susceptible to infection or hemorrhage (5,7). Pathologically, the two main characteristics of MDS are excessive intramedullary apoptosis of hematopoietic cells accompanied by excessive proliferation in the marrow (8,9). This excessive apoptosis is largely cytokine-mediated, with a number of pro-inflammatory cytokines being over-expressed in the marrows such as TNFα, TGFβ, and IL1β. This phenomenon is related to the non-specific haploinsufficiency of *RP14* (10-13). However, the cellular and molecular mechanisms of the cytopenias are not well understood.

Importantly, 1/3 of cases of MDS are characterized by progression to acute myeloid leukemia (AML/MDS), by the accumulation of genetic abnormalities and blockage of cell differentiation
and accumulation of blast cells. The pathology of MDS and AML/MDS are cytologically
distinguishable since the cell types show very different morphologies including their dysplastic
features. The evolution of MDS to AML/MDS is characterized by genomic instability (14, 15).
Acute myeloid leukemia (AML) is a clonal expansion of myeloid blasts in bone marrow, blood or
other tissues, and is distinguished from MDS by the increased numbers of blast cells (more than
20% of blast cells in the marrow). MDS and AML are sustained by genomic instability, which is
a hallmark of all cancers (14, 16). Although chromosomal aberrations are common in both MDS
and AML, a substantial proportion of cases have normal karyotypes (15).

One major cause of genomic instability and cellular apoptosis is telomere dysfunction. However,
few studies have addressed the impact of telomeres in MDS and AML/MDS (14, 17). Telomeres
are the ends of chromosomes capped by a protective protein complex, termed shelterin (18).
Proper telomere capping preserves chromosomal integrity and prevents terminal end-to-end
fusions. Telomere loss or dysfunction results in breakage-bridge-fusion cycles, aneuploidy and
ongoing chromosomal rearrangements (15). Telomeres also are thought to contribute to
chromosome positioning within the nucleus (19). The 3D nuclear organization of telomeres
allows for a distinction between normal and tumour cells: nuclei of the latter tend to be
disorganized, and commonly contain telomeric aggregates (20). Alterations in telomere
architecture and telomeric dysfunction are associated with the onset of genomic instability (2, 3,
21). Our previous work showed altered 3D telomeric organization in different types of cancer (3,
15, 20, 21, 22, 23, 24, 25, 26). However, despite several studies demonstrating the role of
telomere dysfunction in the occurrence of hematopoietic malignancies, little is known about their
possible role(s) in the evolution of MDS and transition to AML/MDS (27). Data describing the
average telomere length and individual telomere length in MDS and AML showed that the
transition from MDS to AML was characterized by increased telomere shortening and
chromosomal abnormalities (14, 17, 28, 29, 30, 31). However, the underlying mechanism(s)
remain unknown. To decipher cellular and molecular mechanisms in MDS and AML, we
examined telomere dysfunction and alterations in the 3D nuclear telomere architecture.

3- Materials and Methods

3.1- Patients
This study received approval by the Research Ethics Board on human studies at the University of Manitoba (H2010:277), and in Brazil (1735/2011). Patients were enrolled at regular basis during their clinical follow-up after informed consent and before any treatment. The patient population included in this study was composed of 7 patients from Cancer Care Manitoba (CCMB) and 87 patients from Brazil. In this latter country, all patients' samples were sent to the Hematology Service of the University Hospital, School of Medicine of Ribeirão Preto, University of São Paulo. The diagnosis of MDS and AML was based on morphology, immunophenotypic analysis and cytogenetics. The patients enrolled for this investigation have no previous diagnosis of other hematological disease. The diagnosis and clinical management of our cohort patients were made according to their clinical presentation based on clinical features, pathological, immunophenotypic, cytological, and cytogenetic diagnoses as recommended by the WHO.

3.2- Clinical surrogates, FAB and WHO classifications

Clinical data were collected from a total of 94 patients from Brazil and Manitoba following their enrolment in this study between 2006-2011 (Brazil) and 2010-2011 (Manitoba). Only patients with suspicion of MDS or AML were included in this cohort before any treatment. According to WHO criteria based on clinical, pathological and cytogenetic diagnoses, our cohort was composed of 56 cases of MDS and 38 cases of AML. The MDS cohort consisted of Refractory Cytopenia with Multilineage Dysplasia (RCMD = 32 cases), Refractory Anaemia (RA = 12 cases), Refractory Anaemia with Excess of Blasts 1 and 2 (RAEB-1 = 8; RAEB-2 = 1 case), Refractory Anaemia with Ring Sideroblasts (RARS = 2 cases), and MDS associated with isolated del(5q) (1 case). The AML cohort was composed of AML-M4 (12 cases), AML-M2 (10 cases), AML-M5 (5 cases), AML-M0 (5 cases), AML-M1 (2 cases), AML-M4eo (1 case), and AML with multidysplasia related changes (1 case) (Tables S1 and S2). The mean age of the patients was 58 years (range 17 to 88 years), and the median age 62 (range 17 to 88 years) (Suppl. data Tables S1 and S2).

3.3- Sampling

Bone marrow-aspiration was performed from each patient following standard clinical procedure and sent to the haematological laboratory, and to the cytogenetic laboratory for clinical and pathological diagnosis, and to our research laboratory for the analysis of the 3D nuclear telomeric
architecture. Cytological diagnosis and the cytogenetic determination of chromosomal abnormalities were assessed according to standard protocols (supplemental data Tables S1, S2). Samples from Brazil were 3D-fixed prior to shipment to the Manitoba Institute of Cell Biology (MICB), where samples from CCMB were 3D directly fixed. 3D fixation was performed according to our standard methods (21, 26).

3.4- Three-dimensional (3D) Quantitative Fluorescent In Situ Hybridization (Q-FISH)

The QFISH procedure was performed as described previously (21, 26). Briefly, after incubation in 3.7% formaldehyde/ 1xPBS for 20 minutes, slides were immersed in 20% glycerol/ 1xPBS for 1 hour. Then, the nuclei were treated by four repeated cycles of freeze-thaw in glycerol/ liquid nitrogen. The next step was equilibration of the slides in 0.1 HCL solution and fixation in 70% formamide/ 2xSCC for at least 1 hour. Immediately after this fixation, 8μl of PNA telomeric probe (Dako, Glostrup, Denmark) were applied to the slides, which were sealed and placed into a Hybrite™ (Vysis, Abbott Diagnostics, Des Plains, IL). Denaturation was performed at 80°C for 3 minutes for both cells and probe, followed by their hybridization at 30°C for 2 hours. The slides were then washed twice for 15 minutes in 70% formamide/ 10mM Tris pH 7.4 followed by washing in 1x PBS at room temperature for 1 minute while shaking and in 0.1x SSC at 55°C for 5 minutes while shaking. Furthermore, the slides were washed in 2x SSC/ 0.05% Tween 20 twice for 5 minutes each at room temperature while shaking. Thereafter, the slides were counter-stained with 4',6-diamino-2-phenylindole (DAPI) (0.1μg/ml) and cover slipped with ProLong GOLD antifade reagent (Invitrogen, Molecular probes, USA) prior to image acquisition.

3.5- Image acquisition and 3D image analysis using TeloView™ system

We performed 3D image analysis on 30 interphase nuclei per slide using an AxioImager Z1 microscope (Carl Zeiss Canada Ltd., Toronto, Canada.) and an AxioCam HRm charge-coupled device (Carl Zeiss Canada Ltd.). A 63-x oil objective lens (Carl Zeiss Canada Ltd.) was used at acquisition times of 600 milliseconds (ms) for Cy3 (telomeres) and 7 ms for DAPI (nuclei). Sixty z-stacks were acquired at a sampling distance of $x,y: 102$ nm and $z: 200$ nm for each slice of the stack. AxioVision 4.8 software (Carl Zeiss Canada Ltd.) was used for 3D image acquisition, and a constrained iterative algorithm (1, 32) was used for deconvolution. Deconvolved images were...
converted into TIFF files and exported for 3D-analysis using the TeloView™ software program (1, 32) (Figure 1 A and B).

3.6- Data analysis

TeloView™ (1, 21, 26) computes five parameters on each sample:

1- the number of signals, i.e. the number of telomeres
2- the signal intensity, i.e. the telomere length
3- the number of telomere aggregates, which means clusters of telomeres that are found in close proximity that cannot be further resolved as separate entities by TeloView™ at an optical resolution limit of 200 nm
4- the a/c ratios determined by representing the nuclear space occupied by the telomeres as an ovoid, with two main axes, "a" and "b", that are equal in length, and a third axis, "c", that has a different length (1). This distribution of telomeres in the three-dimensional space of the nucleus varies with cell cycle; as the specific stages of the cell cycle (G0/G1, S, and G2) phases have characteristic a/c ratios, one can determine where they reside in the cell cycle (1). The a/c ratio is a means of defining progression through cell cycle in interphase cells (26).
5- the distance of each telomere from the nuclear centre versus the periphery

These parameters in a 3D nucleus define all 3D telomere features we measured.

Three types of histograms were produced: i) line graphs showing the distribution in the intensity of the acquired telomere fluorescent signals; ii) histograms of the distribution of the number of aggregates per cell; and iii) histograms of the distribution of the number of acquired signals per cell. The percentage of cells having telomeric aggregates, and the mean number of signals, and mean number of aggregates per cell was calculated. The histogram data from different samples were combined into a single chart for comparison.

3.7- Statistical considerations

Fifteen subgroups were defined based on their 3D telomeric profiles. The telomeric parameters (number, length, telomere aggregates, nuclear volumes, and a/c ratio) were compared between these fifteen subgroups using a nested factorial analysis of variance. Multiple pairwise comparisons using a least square mean tests followed a significant omnibus subgroup effect.
Distribution of telomere intensities in MDS and AML clinical categories was compared between various patient subgroups using chi-square contingency analysis. Cell parameters averages were analyzed between subgroups with nested factorial analysis of variance taking both patient and cellular variations into account. Significance level was set at alpha = 0.05. Classification of the different clinical subgroups of patients according to the number of telomeres (signals), telomere lengths (intensities), and the number of telomeric aggregates were determined by stepwise discriminate analyses.

4- Results

4.1- 3D Nuclear profiling using the number of telomeres, nuclear distribution of telomeres, signal intensities, and telomeres aggregates.

All samples were analyzed in a blinded fashion. We first analyzed the 3D telomere profiles (Figure 1C), i.e. the total number of telomeres versus their lengths and aggregates in each cell of each sample using TeloView™ (1, 21, 26). Subsequently, the 3D telomere profile was defined by the distribution pattern of telomeres per sample according to their intensity (length and aggregate formation). This profile allows one to identify in each patient cell population those nuclei with short, intermediate and long telomeres. Patients showing the same profiles were classified in the same subgroup. According to the 3D telomere profiles, we were blindly able to classify all the patients into fifteen different subgroups without knowing their clinical diagnosis (Figures 2, 3; supplemental data Figure S1, Table S5).

All patients of a subgroup display similar 3D telomeric profiles. Comparison with clinical diagnosis (see section 4.3) after the classification according to telomere profile showed that all MDS patients were classified in the 9 first subgroups (Figure 2, subgroups from A to F; and from I to K; supplemental data Figure S1, Table S5), whereas all but 2 of the AML patients were classified in the last 6 subgroups (Figure 3, subgroups G, H, L, M, N and O; supplemental data Figure S1, Table S5). Statistical analyses showed significant differences between the MDS-subgroups and the AML subgroups (p < 0.001). Each of the quantitative telomere parameters exhibited significant differences between MDS and AML. Furthermore, statistical analyses combining all 3D telomere parameters revealed significant differences between all subgroups (p < 0.05) (Supplemental data Table S5).
Studying the dynamic variation of the number of telomeres versus the telomere intensities and the number of telomeres aggregates, we suggest a chronological evolution between these different subgroups (Figure 2A to Figure 3O, supplemental data Figure S1, Table S5). In fact, these profiles appear linked to the evolution of increased telomere dysfunction in these subgroups. We suggest that the evolution of MDS progresses from early low to later high level of telomere dysfunction, i.e. from early stage of MDS to more severe stages followed by the transformation to AML (Figure 2A to Figure 3O) (Figure 4). Future studies into this process are warranted.

4.2- 3D telomere profiles versus chromosomal abnormalities in the fifteen subgroups

To evaluate the chromosomal abnormalities in the fifteen blindly examined subgroups, the code was removed and patients were placed into their respective disease groups. We compared the cytogenetic data in the fifteen subgroups (Table 1), and we found patients with distinct cytogenetic aberrations, patients sharing identical chromosomal abnormalities in all groups (Table 1; Figure 4) as well as patients with normal karyotypes. Overall, the cytogenetic features of each patient and group did not indicate cumulative acquisitions of chromosomal abnormalities from one subgroup to the following one.

We conclude that the cytogenetic analyses of the samples did not discriminate the patient subgroups (Figure 4). This is in contrast to their definition based on the 3D nuclear telomeric profiles (Table 1; Figure 4).

4.3- 3D Telomere parameters in MDS and AML

We analyzed the telomere parameters between patients with clinical diagnoses of MDS and AML (Table 2). Number of signals per nucleus, telomere aggregates per nucleus, total telomere intensity, and average intensity of signals were all higher in the AML cases than in the MDS cases ($p < 0.0001$ for all comparisons except the average intensity of signal for which $p < 0.05$).

We conclude that telomere parameters are highly accurate to distinguish MDS to AML. This reinforces our model of applying the 3D telomeric profiles for the classification of both MDS and AML (Figures 2 and 3; supplemental data Figure S1, Table S5).

4.4- 3D Telomere parameters versus cytogenetic risk factor classifications of AML
The WHO classification of AML takes into account the cytogenetic and molecular data to predict the clinical outcome of AML-patients following treatment. Eight cases of our AML-patients fall into the “Favourable” category, 12 into “Intermediate”, and 5 into “Unfavourable”, 9 into “AML not otherwise specified”, and 4 lacked karyotype information. We compared the 3D-telomeric profiles of each WHO group of patients to the other groups and vice-versa. Comparing the telomere parameters (section 4.4) between the “Favorable”, “Intermediate” and “Unfavorable” categories, only the number of signals per nucleus displays a difference, with the “Unfavourable” cases having on average 35% more signals than the “Favourable” cases ($p = 0.03$). All other comparative pairing analyses using these parameters were not significant (Supplemental data Table S3). However, according to 3D telomere profiles, the 6- AML subgroups are different from WHO classes. We conclude that future validation with larger patient cohorts is needed to establish our 3D telomeric profiling as an additional tool for clinical management in AML. Notwithstanding, these results reflect a relative cytological homogeneity of the categories that might be linked to the increase number of blast cells. Indeed, AML is characterized by increasing number of blasts due to the hiatus of maturation that avoids the differentiation of precursor cells. The number of blasts in AML can vary from 20 to 70 and to finally reach 100%. At this latter level, maybe all the blasts cell display the same 3D telomere profile paving the way of a possible link between number of blast and telomere dysfunction. Indeed, telomere dysfunction is a continuum with increased genomic changes as is the continuing increased number of blasts. We conclude that the cytogenetic categorisations from WHO classification of AML did not reflect the genomic instability in AML as observed using the 3D telomeric profiles (Figures 2 and 3; Table 2; and Supplemental data Figure S1, Table S5).

4.5- 3D Telomere parameters compared to IPSS and WPSS in MDS-patients

Using the defined indexes such as IPSS (International Prognostic Scoring System) and WPSS (WHO Prognostic Scoring System) (33, 34) to establish the prognostic of the follow-up of MDS-patients, we classified our patients and compared these indexes with aforementioned telomere parameters. Using IPSS, our MDS-patients fall into 3 groups: IPSS < 2 (47 cases); IPSS > 2 with age < 60 years (3 cases); and IPSS > 2 with age > 60 years (6 cases). Comparative statistical analyses using the different telomere parameters between these 3 groups show no significant differences ($p > 0.05$) (Suppl. data Table S4a).
Furthermore, all our MDS-patients fall in 4 groups using WHO Prognostic Scoring System (WPSS): WPSS = 0 (43 cases); WPSS = 1 (12 cases); WPSS = 2 (1 case); and WPSS = 3 (1 case). Comparative statistical analyses using the different aforementioned telomere parameters between these 4 groups show no significant differences, except the average intensity of all the telomere signals between both groups in the WPSS = 0 and WPSS = 1 ($p = 0.03$) (Suppl. data Table S4b). We found that the prognostic indexes such as IPSS, and WPSS did not discriminate MDS patients while this was achieved using their 3D nuclear telomeric profiles. These indexes although taking into account the cytogenetic abnormalities, appear less representative of the disease when compared to the 3D telomeric profiles (Suppl. data Table S4; Figures 2 and 3; Table 1).

4.6- 3D telomeres and Cell Cycle Distribution in MDS and AML

We next investigated nuclear volumes, the distribution of the telomeres per nuclear volume and the $a/c$ ratio. These three parameters allow for the characterization of cell cycle distribution, similar to Ki67 (26), cell size and overall distribution of telomeres within the 3D nuclear space. The nuclear volume is significantly higher in AML than MDS ($p < 0.0001$), which likely might be attributable to the increased number of blast cells in AML. This early process of transformation of MDS to AML is detected by the variation in the nuclear volume. Furthermore, this latter is significantly different between WHO AML classes (all $p < 0.05$). This reinforces the value of the increased numbers of blast cells in the prognostic course of AML that our tool can detect very early.

We evaluated whether there was a difference in cell cycle between our patients with MDS and AML. While the $a/c$ ratio in AML patients was somewhat higher, this did not achieve significance (Table 2). In line with the $a/c$ ratio, telomere distribution per nuclear volume did not show any significant difference between MDS and AML. This might be related to a similar cycling state of cells in both diseases, where cells are in a proliferative state.

Finally, in MDS subgroups defined by the IPSS or the WPSS, no significant difference was found in any of the three aforementioned parameters (Suppl. Data Table S4). This is in line with the fact that the IPSS and the WPSS do not reflect the cell cycle of MDS, nor its proliferative index.
5- Discussion

We analyzed the 3D nuclear telomeric architecture, and determined the telomere numbers, the presence of telomere aggregates, telomere signal intensities, nuclear volumes, and nuclear telomere distribution in MDS and AML. Using these parameters, we determined 3D telomeric profiles, and we were able to blindly subdivide the MDS patients into 9 subgroups and the AML patients into 6 subgroups. WHO classifications distinguish also 8 classes of MDS and 4 major classes (regrouping many entities in one class) of AML (5, 6, 7).

Analyzing the evolution of telomere dysfunction in MDS and AML allows us to propose a model of telomere dysfunction in the progression from MDS to AML. Our results show distinct telomeric profiles specific to patients with MDS and AML, and imply for the first time a chronological and evolutionary process of telomere dysfunction in both diseases. Our data are consistent with the hypothesis that telomere dysfunction generates genomic instability, leading to worsening cytopenias and to the transformation of MDS to AML. 3D telomere profiles seem to be a potential biomarker that, in the future, may improve the clinical management of these patients.

3D telomere profiles are indicative of apoptosis and proliferation in MDS

In all subtypes of MDS, high rates of apoptosis mediated by inflammatory cytokines in the bone marrow contribute to the ineffective hematopoiesis and peripheral cytopenias (11, 13, 35). However, apoptosis is not inherited uniformly by all the cell lineages in the marrow, since the cells most sensitive to apoptosis are the ones undergoing premature death in the marrow while those most resistant to it are the cells that make it into the blood (35, 36). This assertion is strongly supported by the fact that granulocytes from normal healthy donors are more sensitive to apoptosis than the clonal granulocytes in MDS (36). The initial clonal expansion and the selective apoptosis of three different cell lineages in the marrow remain to be understood. Haploinsufficiency of both RPS14 and RBM22 genes may contribute to this phenomenon in the pathogenesis of the 5q- syndrome (6), but the mechanisms in other subtypes of MDS remain to be elucidated.

One major causal phenomenon producing apoptosis and genomic instability is telomere dysfunction (15). Telomere dysfunction is tissue- and cell type specific, and may explain the occurrence of both proliferation and apoptosis in specific cell lineages. In MDS, cytopenias affect
three cell lineages (erythroid, granulocytic and megakaryocytic lineages). Taking into account that the granulocytic cell lineage is more resistant to apoptosis, the fact that anaemia is most often the first manifestation of marrow failure in MDS might be explained by apoptosis due to telomere dysfunction in this specific cell lineage. This also might explain the abnormalities in the megakaryocytic lineage. In the end, telomere dysfunction might prevent maturation in the granulocytic lineage, which resists apoptosis, and maintains this cell lineage at the level of blast cells. The result of this phenomenon is the increase of blast cells and the transformation of MDS to AML. Telomere dysfunction might generate proliferation in one cell lineage (cells in which genomic instability gives a proliferative advantage), and apoptosis in another cell lineage (cells in which genomic instability leads to cell death) at the same time. It may thus explain the clonal expansion and the selective apoptosis in the bone marrow. A suitable assessment of telomere dynamics in MDS and AML might explain the pathogenesis of both diseases (28, 29). Taking these findings into account, a chronological and evolutionary process of telomere dysfunction in both diseases might be linked to worsening cytopenias and to the transformation of MDS to AML.

**3D telomere profiles define MDS and AML subgroups**

Using TeloView™ to evaluate telomere dynamics in every cell, we show for the first time different 3D telomeric profiles specific to subgroups of MDS and AML (Figures 2 and 3; supplemental data Figure S1). These profiles follow an apparently sequential progression, consistent with a working model of telomere changes as drivers of evolution of MDS to AML (Figure 4). It will therefore be important to investigate with larger patient cohorts whether these telomere profiles can serve as biomarker of MDS and AML.

While we have not examined a longitudinal profile of single patients, our data set describes the existence of MDS- and AML-specific 3D nuclear telomere dysfunction. Using our 3D nuclear parameters blindly, we were able to subgroup the patients into MDS and AML and to subdivide these disease groups further. Future analyses will have to provide evidence on a patient-by-patient basis as to whether 3D nuclear telomere dysfunction can serve as reliable biomarker in predicting disease progression. Indeed, our previous work has demonstrated that one key difference between normal and cancer cells is the presence of telomere aggregates (20). We found telomere aggregates in all the samples in both diseases, but with a significant difference
between MDS and AML (Table 2; \( p < 0.0001 \)). This might allow for the detection of the early stages of MDS in patients presenting with anemia since changes in nuclear architecture precede the development of chromosomal abnormalities (20, 37). It was shown that telomere shortening in MDS is independent of the presence of cytogenetic abnormality (29).

Nuclear telomere profiling seems to be more accurate to reflect genomic instability underlying MDS and AML than FAB classification of both diseases. Further analyses according to the WHO classification are in line with that, despite the consideration of cytogenetic and molecular abnormalities in MDS and AML patients. However, all these clinical classes of AML show genomic instability that we profiled accurately. 3D nuclear telomeric profiles are the direct reflection of chromosomal abnormalities, rather than clinical response to treatment grouping (15, 26). Telomere-associated genomic instability might, in the future, be a better indicator of the clinical outcome of MDS/AML. This hypothesis, of course, will need to be validated in a prospective clinical trial.

**Comparison of 3D telomeres profiles with the IPSS and WPSS in MDS**

Analyses of MDS patients in parallel to the available prognostic markers IPSS and WPSS led to the conclusion that both indexes did not discriminate MDS patients while this was achieved using their 3D nuclear telomeric profiles. Indeed, IPSS, which is based on percent marrow blasts, type of cytogenetic abnormalities, and degree and number of cytopenias, and WPSS, which adds to IPSS number of blood transfusions, both predict survival and evolution to acute leukemia characterized by increased genomic instability. We propose that monitoring 3D telomere dysfunction might be a very powerful marker to measure this transformation. Furthermore, it may be a better indicator of therapeutic response since an optimal response will lead to a normal cellular biology, including elimination of abnormal telomeric aggregates and the increase of normal 3D telomeric profiles.

**3D telomeres profiles as a potential biomarker of genomic instability in MDS and AML**

MDS and AML both undergo a dynamic process of genomic instability, which may be monitored by the 3D analyses of telomeres. Combinatorial analyses of these 3D nuclear telomere parameters versus different classification of MDS and AML strengthen the validity of our telomeric profiles for the classification of MDS vs. AML and describe patient subgroups in both. Notwithstanding, due to the difficulties to obtain 3D preserved patient samples at different time points of the disease and to have a longitudinal follow-up of the patients due to slow evolution of MDS in...
several cases, we are beginning to investigate an animal model of MDS/AML to follow disease progression and 3D telomere dysfunction during this process (38). C57BL/6-Tg(Vav1-NUP98/HOXD13)G2Apla/J hemizygote mice develop myelodysplastic syndromes with peripheral blood cytopenia and dysplasia and normocellular to hypercellular bone marrow. By 14 months of age a subset of hemizygotes succumbs to malignant acute myeloid leukemia or severe anemia and leucopenia. This mutant mouse strain may be useful in studies of myelodysplastic syndromes and leukemia and to validate our 3D nuclear telomeric profiling of both diseases.

6- Conclusion

Our results demonstrate significant differences in the 3D nuclear telomeric organization between MDS and AML in terms of telomere numbers, telomeres aggregates, telomere length, nuclear volume, and telomere distribution, suggesting that progressive telomere dysfunction may contribute to the evolution of MDS and AML.

Acknowledgements

The authors are grateful to Dr William Foulkes (McGill) for careful review of the manuscript, to Mary Chuang for statistical analyses, and to Shubha Mathur for help with image analysis. The authors acknowledge the patients who make this study feasible. This study was supported by post-doctoral fellowship from Manitoba Health Research Council (MHRC) to MG, and an operating grant from the Canadian Institute of Health Research (CIHR) to SM.

Funding

Manitoba Health Research Council (MHRC) (MG), and the Canadian Institutes of Health Research (CIHR) (SM) supported this work.

Contributions of each author

MG: set-up the project, oversaw the project, defined the experimental design, performed the different manipulations, analysed the data, and wrote the first draft of the manuscript.

JAA: as a master student trainee under the supervision of MG, participated actively to the different manipulations of the study.
RP: as a summer student trainee under the supervision of MG, participated actively to the
different manipulations of the study for two months
RK and DH: did the clinical management and follow-up of the patients in CCMB, corrected and
approved the manuscript
LK: participated to the sampling from MICB/CCMB
TND: participated to the experimental design
REM: reviewed and commented the clinical diagnosis of the samples from Brazil
De OFM, FPR: provided samples from Brazil and all the clinical data of their patients, corrected
and approved the manuscript
MS: Supervisor of MG, JAA and RP, supervised the whole project, reviewed and revised the
manuscript.
References


Figures and Legends

Table 1: Patients subgroups according to 3D telomeric profiles and their cytogenetic data.

Table 2: Telomere parameters according to clinical diagnosis.

Figure 1: A) 2D and B) 3D view of QFISH of bone-marrow cell nucleus (blue), and telomeres (red); C) Graph distribution of number of telomeres according to their intensity (length of telomeres) for one sample; this 3D telomere distribution is one part of the 3D telomeric profile. (Red bars) separate the 3 cell populations with short, intermediate and long telomeres, respectively.

Figure 2: MDS graphs of Panel A (composed by subgroups A, B and C; see supplemental data Figure S1, Table S5) that might evolve to Panel B (composed by subgroups D, E and F; see supplemental data Figure S1, Table S5) or to Panel C (composed by subgroups I, J and K; see supplemental data Figure S1, Table S5)

Figure 3: AML Graphs of Panel D (composed by subgroups G and H; see supplemental data Figure S1, Table S5); Panel E (composed by subgroups L and M; see supplemental data Figure S1, Table S5) and Panel F (composed by subgroups N and O; see supplemental data Figure S1, Table S5). MDS Panel B (Figure 2) might evolve to AML Panel D (see supplemental data figure S1, Table S5); MDS Panel C (figure 2) might evolve to AML Panels E or F (see supplemental data Figure S1, Table S5).

Figure 4: Proposed modal of chronological evolution of 3D telomere profiles of patients from MDS to AML
Table 1: Patients subgroups according to 3D telomeric profiles and their cytogenetic data.

<table>
<thead>
<tr>
<th>Telomere profile subgroups</th>
<th>Patients</th>
<th>Cytogenetic results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Figure 2A</strong></td>
<td>P8</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P34</td>
<td>47,XX,-1[10] + 46,XX[12]</td>
</tr>
<tr>
<td></td>
<td>P32</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2B</strong></td>
<td>P40</td>
<td>46,XY,del(13q)del(4q) + 46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P37</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P46</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P39</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P44</td>
<td>46,XX,del(2q23) + 46,XX[17]</td>
</tr>
<tr>
<td><strong>Figure 2C</strong></td>
<td>P11</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P52</td>
<td>44,XX,-1[10] + 46,XX[14]</td>
</tr>
<tr>
<td></td>
<td>P12</td>
<td>47,XY,-9[10] + 46,XX[14]</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>46,XX,del(20)(q12) + 46,XX[13]</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>46,XX,del(20)(q12) + 46,XX[13]</td>
</tr>
<tr>
<td></td>
<td>P20</td>
<td>47,XX,-1[2] + 46,XX[18]</td>
</tr>
<tr>
<td></td>
<td>P10</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P19</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P93</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P55</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2D</strong></td>
<td>P33</td>
<td>46,XX,del(1q22) + 46,XX[13]</td>
</tr>
<tr>
<td></td>
<td>P5</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td></td>
<td>P45</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td></td>
<td>P50</td>
<td>47,XX,-1<a href="q10">7</a> + 46,XX[16]</td>
</tr>
<tr>
<td><strong>Figure 2E</strong></td>
<td>P35</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P42</td>
<td>46,XX,del(5)(1q) + 46,XX[11]</td>
</tr>
<tr>
<td></td>
<td>P22</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P15</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>47,XY,-1(1q22) + 46,XX[13]</td>
</tr>
<tr>
<td></td>
<td>P16</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td></td>
<td>P18</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2F</strong></td>
<td>P49</td>
<td>47,XY,-1[10] + 46,XY[14]</td>
</tr>
<tr>
<td></td>
<td>P17</td>
<td>46,XX,del(5)(1q) + 46,XX[15]</td>
</tr>
<tr>
<td></td>
<td>P54</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P56</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td><strong>Figure 2G</strong></td>
<td>P74</td>
<td>46,XY(13q21-22+)(q21-22)+46,XY[12]</td>
</tr>
<tr>
<td></td>
<td>P71</td>
<td>46,XY(13q21-22+)(q21-22)+46,XY[13]</td>
</tr>
<tr>
<td></td>
<td>P85</td>
<td>46,XY(13q21-22+)(q21-22)+46,XY[9]</td>
</tr>
<tr>
<td></td>
<td>P75</td>
<td>45,XY+46,XY[10]</td>
</tr>
<tr>
<td><strong>Figure 2H</strong></td>
<td>P68</td>
<td>No metaphase available</td>
</tr>
<tr>
<td></td>
<td>P76</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2I</strong></td>
<td>P23</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P21</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P26</td>
<td>46,XY,del(7)(p15) + 46,XX[14]</td>
</tr>
<tr>
<td></td>
<td>P27</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P29</td>
<td>46,XX,del(20)(q12)+46,XX[6]</td>
</tr>
<tr>
<td></td>
<td>P30</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P38</td>
<td>47,XY,-1(1q22)+46,XX[12]</td>
</tr>
<tr>
<td></td>
<td>P41</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P43</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P9</td>
<td>45,XY+46,XY[12]</td>
</tr>
<tr>
<td></td>
<td>P6</td>
<td>45,XY+46,XY[12]</td>
</tr>
<tr>
<td></td>
<td>P7</td>
<td>46,XX,del(20)(q12)+46,XY[16]</td>
</tr>
<tr>
<td></td>
<td>P13</td>
<td>46,XX,del(7)(p15)+46,XY[12]</td>
</tr>
<tr>
<td></td>
<td>P31</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P14</td>
<td>47,XY,+8[20]</td>
</tr>
<tr>
<td></td>
<td>P36</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P94</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P55</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td><strong>Figure 2J</strong></td>
<td>P1</td>
<td>46,XY[20]</td>
</tr>
<tr>
<td><strong>Figure 2K</strong></td>
<td>P92</td>
<td>Very complex karyotype with about 10 abnormalities</td>
</tr>
<tr>
<td><strong>Figure 2L</strong></td>
<td>P94</td>
<td>47,XY,+8[12]+46,XY[13]</td>
</tr>
<tr>
<td></td>
<td>P98</td>
<td>46,XY[14]</td>
</tr>
<tr>
<td></td>
<td>P87</td>
<td>46,XX[14]</td>
</tr>
<tr>
<td></td>
<td>P79</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2M</strong></td>
<td>P40</td>
<td>45,XY,-7[5]+46,XY[12]</td>
</tr>
<tr>
<td></td>
<td>P91</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2N</strong></td>
<td>P66</td>
<td>45,XX,inv(16)(p13q22)[8]+46,XY[10]</td>
</tr>
<tr>
<td></td>
<td>P76</td>
<td>47,XY,+4[10]+46,XY[10]</td>
</tr>
<tr>
<td></td>
<td>P81</td>
<td>45,XX,inv(16)(p13q22)[12]+46,XY[10]</td>
</tr>
<tr>
<td><strong>Figure 2O</strong></td>
<td>P64</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P57</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td></td>
<td>P58</td>
<td>46,XX[20]</td>
</tr>
<tr>
<td><strong>Figure 2P</strong></td>
<td>P70</td>
<td>46,XX[18]</td>
</tr>
<tr>
<td></td>
<td>P73</td>
<td>45,XX,del(1q22)[10]+46,XY[10]</td>
</tr>
</tbody>
</table>
| **Table 2**: Telomere profile subgroups and their patients' characteristics.**

**Table 3**: Cytogenetic results for each patient subgroup.
Table 2: 3D Telomere parameters according to clinical diagnosis.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Total number of signals</th>
<th>Total number of aggregates</th>
<th>Total intensity</th>
<th>Average intensity of all signals</th>
<th>a/c ratio</th>
<th>Nuclear volume</th>
<th>Telomere per K volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>Std Dev</td>
<td>mean</td>
<td>Std Dev</td>
<td>mean</td>
<td>Std Dev</td>
<td>mean</td>
</tr>
<tr>
<td>MDS</td>
<td>20.10</td>
<td>8.17</td>
<td>1.81</td>
<td>1.56</td>
<td>307567</td>
<td>137676</td>
<td>15740</td>
</tr>
<tr>
<td>AML</td>
<td>31.10</td>
<td>13.09</td>
<td>3.32</td>
<td>2.46</td>
<td>523641</td>
<td>245868</td>
<td>17504</td>
</tr>
</tbody>
</table>

$p_{value}$ between MDS and AML

- $p < 0.0001$
- $p < 0.0001$
- $p < 0.0001$
- $p = 0.02$
- $p < 0.0001$
- $p < 0.0001$
- $p = 0.48$

Std Dev: standard deviation
Figure 1

A

B

C

Downloaded from clincancerres.aacrjournals.org on September 22, 2017. © 2012 American Association for Cancer Research.
Figure 2
Figure 4

Disease progression

Cytogenetic data

Evolution of telomere dysfunction
Clinical Cancer Research

Profiling Three-Dimensional Nuclear Telomeric Architecture of Myelodysplastic Syndrome and Acute Myeloid Leukemia defines patient subgroups

Macoura Gadji, Julius Adebayo Awe, Prerana Rodriguez, et al.

Clin Cancer Res  Published OnlineFirst April 26, 2012.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-0087

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2012/04/26/1078-0432.CCR-12-0087.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.