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IMPACT OF CANCER AND THERAPY ON EPIGENETIC LANDSCAPES OF NORMAL CELLS: DESCRIBING A NOVEL ENDPOINT IN SURVIVORSHIP

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INTRODUCTION

- With the advent of modern multi-drug chemotherapy regimens, several aggressive hematologic malignancies now have excellent relapse-free survival characteristics.
- However, long-term follow-up studies show increased incidences of secondary malignancies[1,2], lifestyle diseases[3] and other surrogate features of accelerated aging.

The context

- Aging is a complex, pervasive, and progressive phenomenon common to all biological systems. Over the past few decades, a new unifying theory of aging has been validated in multiple studies viz. the "**Information Theory of Aging**" - proposes that aging in eukaryotes is due to the loss of transcriptional networks and epigenetic information over time[4].
- In a recent study done in mice[5], induced double-strand breaks caused a strong aging phenotype without any other long-term genotoxicity. However significant epigenetic dysregulation with aberrant DNA methylation (DNAm) consistent with age acceleration (EAA) was noted.
- However, the extent of such changes in humans remain largely unknown.

The concern

- This raises the specter that standard cytotoxic chemotherapy, which also acts directly or indirectly via DNA-damage, may impact the epigenetic landscape i.e. DNA methylation, resulting in an accelerated aging phenotype in keeping with the prevailing **Information theory of aging** and should be studied in the context of survivorship.

AIM

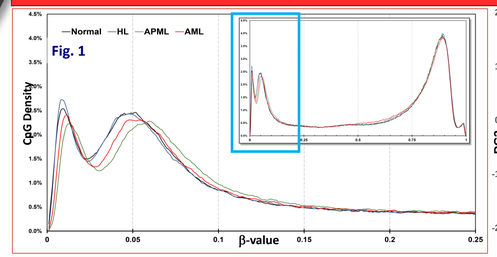
- To study epigenetic dysregulation in long-term cancer survivors treated with standard cytotoxic cancer therapy in comparison to age-matched normal controls.

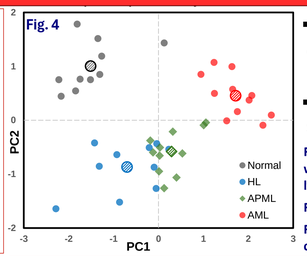
METHODS

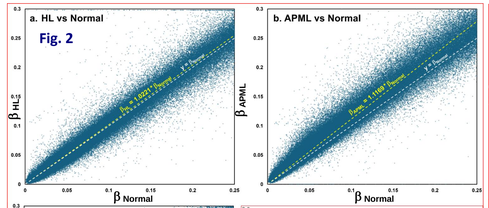
- In this observational cohort study, we conducted an analysis of whole blood methylation profiles in survivors of Hodgkin's lymphoma (HL), Acute Pro-Myelocytic Leukemia (APML) and Acute Myeloid Leukemia (AML). Age-matched, normal individuals were tested as controls.
  - **n**=40 (10 subjects of 30-50 years of age in each group including controls.)
  - In CR1, disease-free more than 5 years from diagnosis.
- Whole-genome methylation patterns were assessed using Illumina's **Infinium Methylation EPIC v2.0 BeadChips**[6] . The methylation data represent the current methylation profiles of nucleated myeloid/lymphoid cells and therefore, the progenitor cells that underwent DNA-damage abuse years ago during treatment with various cytotoxic agents.

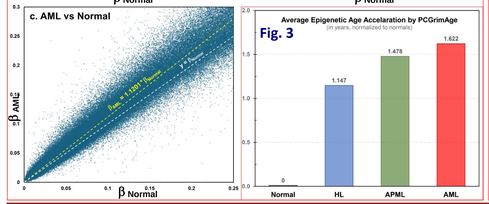
RESULTS

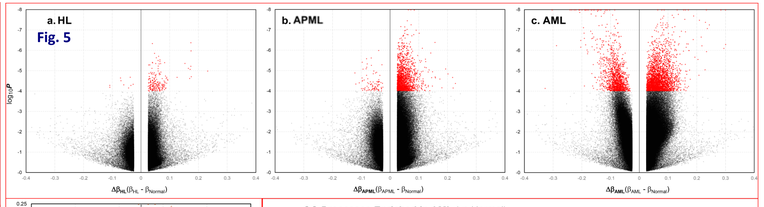
- We demonstrate that in comparison to healthy age-matched control subjects, DNAm levels were slightly but significantly higher in the blood of cancer survivors (Fig.1) and these changes primarily at CpGs that are normally unmethylated (β<0.1, Fig. 1).
- On an average, we observed **2.21%, 11.69% and 12.01%** higher methylation in the lowest quartile of the 935K CpG sites on the Methylation EPIC v2 BeadChip array in HL, APL, and AML survivors respectively (Fig.2).
- When normalized against healthy controls, HL, APL, and AML survivors showed an **EAA of 13.8, 17.8 and 19.5** months, respectively as measured by PCGrimAge[7] (Fig. 3).
- PCA analysis of the most significantly differentially methylated CpGs (DMCs) showed a clear separation between subjects of the four cohorts (Fig. 4).

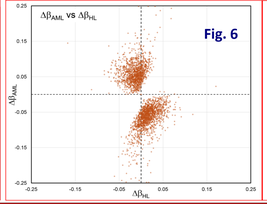


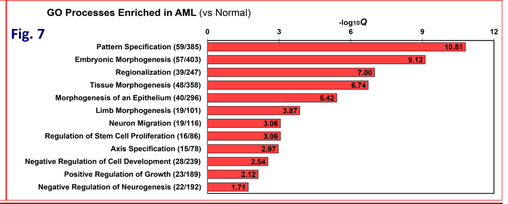












- Differential Methylation analysis showed that HL, APL, and AML cohorts had **211**, **2085 and 4,248** significant DMCs (***p***<0.0001), respectively (Figs. 5 and 6).
- Gene Ontology (GO) and pathway analysis of these is shown in Fig.7.

Figure 1: β-values at the lowest quartile of the 935K CpGs on the MethylationEPIC v2 array in controls, HL, APL, and AML subjects. The whole β-value plot is shown in the inset.

Figure 2 : Scatter plots of methylation levels (β-values) of the CpGs in the lowest quartile in HL, APML, and AML subjects (y-axis) in comparison to the controls (x-axis).

Figure 3: Epigenetic Age-Accelaration as measured by PCGrimAge for subjects in each cohort.

Figure 4: PCA analysis on β-values of the top 1000 DMCs for each of the four cohorts.

Figure 5 : Volcano plots showing the DMCs in HL(a), APML(a) and AML(c) when compared to normal. x and y-axes indicate Δβ (disease-normal) and $\log_{10}P$. CpGs with $p < 0.0001$ are shown in red.

Figure 6: Plot of Δβ in AML vs Normal (y-axis) against Δβ HL vs normal (x-axis).

Figure 7: Gene Ontology Pathway analysis of processes enriched in AML in comparison to normal controls.

CONCLUSIONS

- Significant changes in DNA methylation were noted in the cancer survivor cohorts in comparison to age-matched controls.
- Our findings suggest that hematological malignancies and their chemotherapies cause varying long-term epigenetic changes in normal hematopoietic progenitors resulting in age acceleration.
- Epigenetic clocks and methylation profiling seem to be novel, robust, and quantitative tools for evaluating cancer survivorship endpoints.
- Our observations underscore the need for further research and refinement of treatment algorithms to minimize long-term effects in line with these novel end-points.

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