NEXTFRONTIERS TOCURECANCER2022
DE 22 A 25/06 - 100% ONLINE

A.C.Camargo Cancer Center
Especializado em Vida

Identification of epigenetic alterations in pediatric cancer patients conceived by in vitro fertilization

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Introduction

- Pediatric cancer, the leading cause of death due to disease among children and adolescents, currently has a poorly understood multifactorial etiology.

- There is a hypothesis that environmental factors acting during embryogenesis can disrupt epigenetic signaling, resulting in diseases after birth, including cancer.

- Key steps of the epigenetic reprogramming occur during embryogenesis and in vitro fertilization (IVF) can cause aberrant epigenetic modifications, which in turn could trigger diseases during lifespan, including cancer.

- There is a controversial association between IVF and an increased risk of pediatric cancer.

Cohort and Methods

We investigated the peripheral blood methylome of 11 patients born through IVF who developed pediatric cancer (IVF/cancer), and compared them with methylome data obtained from 12 healthy children without familial history of cancer, and 4 healthy dizygotic twins from the IVF/cancer patients.

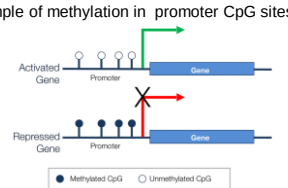
STUDY COHORT

- IVF/cancer group
  - 11 patients born through IVF who developed childhood cancer
- Controls
  - 16 children without cancer (4 twins from the IVF/cancer group included)

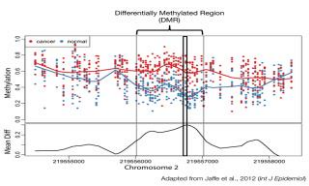
DNA METHYLATION ANALYSIS

Infinium® MethylationEPIC (Illumina)
~850K methylation sites across the genome

Example of methylation in promoter CpG sites



The analysis was done using the R software with the Chip Analysis Methylation Pipeline (ChAMP)



The objective was the investigation of differentially methylated positions (DMP; CpG sites) or regions (DMR) in IVF/cancer individuals probably originated due to the IVF procedure.

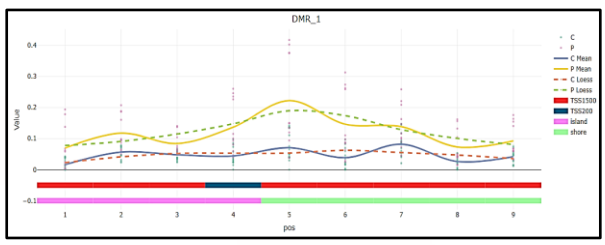
Results

- After adjusted P-value using Bonferroni correction, DMPs were not identified.

- Several DMRs (minimum of 7 probes and adjusted P-value of <0.05) were detected.

Analysis A – 11 IVF/cancer patients (P) x 12 healthy controls (C)	25 DMRs
Analysis B – 4 IVF/cancer patients (P) x respective 4 dizygotic twins	22 DMRs

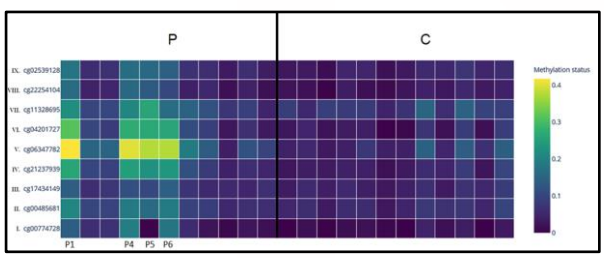
- The most significant DMR, detected in all three analysis, was located at the promoter region of the *LHX6* at 9q33.2.



DMR located at the *LHX6* gene

- This 9q33.2 DMR was found to be hypermethylated in patients, presenting at least 9 CpG sites in each analysis (minimum methylation difference - Δβ of 0.07).

- Four patients (P1, P4, P5, and P6) presented higher methylation levels compared to the group. A separate analysis was performed for the nine DMPs of this *LHX6* DMR, considering only these four patients and the control group.



Heatmap showing the DNAm level of nine DMPs located in the original DMR

- Eight of the nine investigated CpGs (cg00485681, cg17434149, cg21237939, cg06347782, cg04201727, cg11328695, cg22254104, and cg02539128) exhibited significantly higher methylation levels in patients P1, P4, P5, and P6 if compared to controls

Conclusion

The most statistically significant DMR found in this study maps to the promoter region of the transcriptional factor *LHX6*, which is involved in embryogenesis and head development. This gene is a potential tumor suppressor gene in glioma, pancreatic, head and neck, breast, lung, and cervical cancer. *LHX6* promoter hypermethylation has already been related to transcriptional silencing, and it is described as either hypermethylated or partially methylated in cervical, head and neck, pancreatic, lung and liver cancers. Likewise, the DMR we found is hypermethylated in the promoter region.

In conclusion, methylation differences were detected between IVF patients and controls. This study is ongoing and new analysis will be performed to investigate if these differences are related to cancer or IVF. Further steps for this work includes the analysis of germline variants in cancer predisposition genes in these patients through whole-exome or cancer panel.

Contact

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Funding and support

CAPES CNPq FAPESP instituto de biociências Centro de Pesquisa sobre o Genoma Humano e Células-Tronco genoma itaci