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PLATFORM FOR THE DEVELOPMENT AND IMPLEMENTATION OF MOLECULAR CLASSIFIERS FOR PEDIATRIC CANCER

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The **aim of the present project** was to address the pressing clinical need to translate more efficiently the advances in research and innovation to paediatric cancer patients, in terms of new and optimized tools for supporting clinical decisions, improving treatment options and optimizing patient management.

Over the last decade, important genetic studies have radically changed the understanding of the biology underlying the paediatric tumours, revealing a considerably more complex landscape than previously thought, very different from cancer in adults. These genetic findings have provided a more objective knowledge of the clinical behaviour of these tumours and have provided opportunities to identify molecular markers for more precise classification, prediction of prognosis and therapy response of paediatric tumours. A paradigmatic example is the recent classification of medulloblastoma, the most common malignant brain tumour in the paediatric age. Four principal subgroups of medulloblastoma have been described, which are characterized by distinct epigenetic and genetic profiles as well as differing clinical courses. These subgroups have become increasingly important for the management of patients, helping to define more accurately the treatment and the clinical outcome. The assignment of patients to these molecular subgroups is important, but complex to accomplish. Genomic approaches are needed, which inevitably increase cost and complexity, requiring trained personnel, equipment and resources. Many centres worldwide that treat patients with brain tumours are still unable to classify medulloblastomas into the principal genetically-defined consensus categories WNT, SHH and non-WNT/non-SHH, currently included in the WHO classification of CNS tumours 2021. Consequently, a significant number of patients cannot benefit from the clinical advances associated with methylation-based medulloblastoma classification. Our research group has recently developed an epigenetic classifier based on the methylation profile of a six-cytosine signature that allows for classification of medulloblastoma into the clinically relevant subgroups of WNT, SHH and non-WNT/non-SHH, with an accuracy (99%) equivalent to genome-wide DNA methylation microarray and gene-signature profiling methods (Gómez, Soledad et al. "A Novel Method for Rapid Molecular Subgrouping of Medulloblastoma." Clinical cancer research: an official journal of the American Association for Cancer Research vol. 24,6 (2018): 1355-1363. doi:10.1158/1078-0432.CCR-17-2243). The six-cytosine classifier represents a simplified approach for accurate, rapid, and cost-effective classification of single medulloblastoma DNA samples.

The objective of this project was to provide tools (classifiers and digital platform) and new technical approaches to ease the application of our epigenetic classifier to the clinical practice, enabling centres to classify accurately patients with medulloblastoma. By using the experience that we acquired with medulloblastoma, we also aimed to develop robust molecular classifiers for other paediatric tumours with a clinical need for disease classification.

2. Results

Epigenetic Genotyping Application (EpiGe-App)

In the present project, we have developed a decision support system (DSS) to enable accurate classification of medulloblastoma tumours into the molecular subgroups WNT, SHH, or non- WNT/non-SHH, using a clinically applicable quantitative PCR (qPCR)-based approach. The DSS was developed using an epigenetic classifier based on the methylation profile of a six-cytosine signature, previously developed by our group (Gómez, Soledad et al. "A Novel Method for Rapid Molecular Subgrouping of Medulloblastoma." Clinical cancer research: an official journal of the American Association for Cancer Research vol. 24,6 (2018): 1355-1363. doi:10.1158/1078-0432.CCR-17-2243). To increase the applicability of the approach, we built an interactive, user-friendly web application (EpiGe-App - https://www.epige.irsjd.org/) that enables the automated interpretation of qPCR methylation data, the designation of the methylation status of cytosines, prediction of the molecular subgroup, and reporting of the methylation class of the medulloblastoma. For the study, we analysed 4,740 samples comprising DNA methylation microarray data (3,096 medulloblastoma, 1,613 non-medulloblastoma tumours and 31 normal tissues), and 65 qPCR data of tumours (55 medulloblastoma and 10 non-medulloblastoma samples) and blood samples (6 healthy individuals). The study has been published in iScience Cell press (Gómez-González, Soledad et al. "EpiGe: A machine-learning strategy for rapid classification of medulloblastoma using PCR-based methyl-genotyping." iScience vol. 26,9 107598. 12 Aug. 2023, doi:10.1016/j.isci.2023.107598).

New approaches for the analysis of the epigenetic classifier

Our aim was to analyse our panel of epigenetic markers in liquid biopsy. With this aim, we collected more than 200 samples of cerebrospinal fluid (CSF) and 75 plasma/serum

obtained from 62 patients with a medulloblastoma tumour. The primary tumours of patients had been previously classified by our group using Illumina Infinium HumanMethylation 450 BeadChip or Illumina Methylation EPIC BeadChip 850K arrays, as well as our panel of six epigenetic markers (Gómez, Soledad et al. "A Novel Method for Rapid Molecular Subgrouping of Medulloblastoma." Clinical cancer research: an official journal of the American Association for Cancer Research vol. 24,6 (2018): 1355-1363. doi:10.1158/1078-0432.CCR-17-2243). We designed specific probes to enable us to discriminate between single base-pair changes cytosine (methylated) and thymine (unmethylated) in circulating tumour DNA using Digital PCR technology. Ongoing work: We are currently testing the specificity/sensitivity of the digital PCR probes to detect methylation changes.

Development of new epigenetic classifiers

Our classification strategy for medulloblastoma may prove to be useful also in the context of other paediatric tumours. We are currently using the workflow developed for medulloblastoma to develop a similar classification approach for ependymoma, a rare type of paediatric brain tumor. Our preliminary results show that we are able to classify 9 of the 10 molecular subgroups described for ependymoma using a reduced set of cytosines with subgroup specific differential methylation patterns. The tenth molecular subgroup is an aggressive and minority molecular subgroup. Methylation data of this subgroup is scarce. (Ghasemi, David R et al. "MYCN amplification drives an aggressive form of spinal ependymoma." Acta neuropathologica vol. 138,6 (2019): 1075-1089. doi:10.1007/s00401-019-02056-2). We have recently established a collaboration with the research group that has recently described this subgroup of tumours. This has enabled us to enlarge the dataset of our ependymoma cohort. The analysis of the methylation data is currently ongoing, aimed at identifying potential subgroup-specific epigenetic markers, with robust classification capacity. We have generated two predictive models, based on Extreme Gradient Boosting, a scalable distributed gradient-boosted decision tree machine learning library. The cross-validation strategy that was performed was a 3-fold CV rep 5 during the model training with 75% of samples, and it was tested on the remaining 25% of samples. The identified set of epigenetic markers are being validated in a set of independent ependymoma samples. Our next objective is to develop a DSS to enable accurate classification using a qPCR approach. The DSS will be integrated in the classification platform EpiGe-App (https://www.epige.irsjd.org/) for automated analysis and classification of these

tumours. The results of the study will be reported in a scientific article, and the possibility of a patent will be considered.

3. Impact on health

This project has contributed to develop an appropriate, easy to use decision-support system for clinical purposes in the field of paediatric cancer. The advances made by this project have been rapidly translated to clinical practice for supporting clinical decisions, improving treatment options and optimizing patient management, and will thus impact significantly on the health and outcome of patients. The PECA Platform has provided a web-app (EpiGe-App - https://www.epige.irsjd.org/), whereby all centres that have qPCR technology are able to classify their tumour samples in a robust, rapid, cost-effective and user friendly manner. The workflow developed in this project can be applied to other tumours, including non-paediatric, to different (epi)-genetic data and can be employed for multiple biomarkers. The knowledge acquired in this project will facilitate the development of other tools/platforms/services that may improve the quality and sustainability of healthcare systems, through quicker and better clinical decisions and timely delivery of effective personalized treatment, with reduction of delays and associated costs. This multifaceted project involving integrated computational analyses and experimental validation has contributed to a more comprehensive understanding of the molecular pathogenesis of medulloblastoma and, potentially, of other paediatric cancers.

4. Publications

- Gómez-González, Soledad et al. "EpiGe: A machine-learning strategy for rapid classification of medulloblastoma using PCR-based methyl-genotyping." iScience vol. 26,9 107598. 12 Aug. 2023, doi:10.1016/j.isci.2023.107598

-Gene-Olaciregui, Nagore et al. "Clinical and Molecular Evolution of an ALK-Driven Infant-Type Hemispheric Glioma Treated Sequentially With Second- and Third-Generation Anaplastic Lymphoma Kinase Inhibitors." JCO precision oncology vol. 7 (2023): e2200547. doi:10.1200/PO.22.00547

-Esperanza-Cebollada, Elena et al. “A miRNA signature related to stemness identifies high-risk patients in paediatric acute myeloid leukaemia.” British Journal of Haematology vol. 202,1 (2023): 96-110. doi:10.1111/bjh.18746

-Under internal revision: “EpiGe-App: A web-based tool for the automated classification of medulloblastoma subgroups directly from quantitative PCR experiments”