Analysis of global methylation status under the microenvironment in glioblastoma cells

Introduction Glioblastoma multiforme is the most aggressive brain tumour and it has reported that cellular subpopulation of tumours display several characteristics of stem cells highly expressing stem cell specific markers such as SOX2. Glioblastoma stem cells (GSCs) are at the root of tumour recurrence and are regarded as a potential target for anticancer therapy. Microenvironment within the tumour might be important to regulate and/or maintain its properties via epigenetic modifications including DNA methylation. In this study, we explored whether different culture conditions lead to alter the status of methylation in glioblastoma cells, U87-MG.

Material and methods Cells were maintained in normal culture condition and spheres were cultivated in serum-free on low-attachment plate. Genomic DNA was converted using bisulfite conversion kit (Zymo EZ DNA methylation kit). Converted genomic DNA was used for infinium methylation assay. Images were captured by Illumina’s iScan Control software. After correction and filtering raw data, differentially expressed methylation list was determined using |delta_mean| ≥ 0.2 (the difference of methylation signal, avg beta of Case – avg beta of Control) and p-value<0.05 of independent t-test in which the null hypothesis was that no difference exists among 2 groups. All data analysis and visualisation of differentially expressed genes was conducted using R 3.0.2 (www.r-project.org).

Results and discussions We observed that expression of SOX2 at the mRNA level consistently increased in cells cultured without serum on the low-attachment plate compared with physiological culture condition. Genome-wide methylation analysis shows that ninety eight genes were significantly hypomethylated (forty three genes, 43.88%) or hyper-methylated (fifty five genes, 56.12%) under the condition without serum on the low-attachment plate compared with physiological culture condition. Furthermore, these results might give a macroscopic insight into differential methylation status in structure gene such as 5’ untranslated region (UTR), transcription start site (TSS), and 3’UTR under the low-attachment condition without serum. In addition, methylation status of three miRNAs under the low-attachment condition without serum was altered and their functional roles require further exploration.

Conclusion Global methylation status was distinctly changed in glioblastoma spheres under the serum-free and low-attachment condition (Fund no: NRF-2017R1A1A05000839).

The other 98%: Making sense of non-coding variation

Introduction Despite the ever-increasing availability of whole genome sequencing datasets, our ability to understand functional impact of genetic variation remains largely restricted to the coding portion of the genome. Although a selection of methods exist to estimate variant damage potential, these methods are heavily biased towards coding variation and have limited ability to detect potentially functional variants in non-coding regions. Furthermore, these methods either ignore tissue context of the variant or combine epigenetic annotations from multiple tissues, often dominated by blood cell lines, and are therefore poorly suited for predicting functional impact of variants in regulatory elements that act in a tissue-specific manner.

Material and methods In order to address these limitations, we have developed a machine learning classifier aimed at prioritising non-coding variants while taking into account relevant tissue context. We defined ’functional impact’ as the propensity of a variant to cause allele-specific chromatin accessibility. DNase Hypersensitive Site (DHS) variants found to display preferential accessibility, as well as a matched set of negative variants, were annotated with a range of features including a set of core epigenetic marks from the ROADMAP Epigenomics project matched to the tissue of origin of the positive variant set. We then combined several machine learning algorithms to train an ensemble classifier. On a balanced test set (equal number of positive and negative variants) our model achieved area under curve (AUC) of ~75%. On a realistic dataset, where the negative variants outnumbered the positive ones 100:1, we achieved AUC of 90%.

Results and discussions To demonstrate the importance of tissue context when estimating functional impact of non-coding variants, we compared the classifier’s performance on the same set of variants annotated with epigenetic information from either a closely matched or an unrelated tissue. We observed a notable drop in performance when using epigenetic context from a mismatched tissue. We show that our method drastically outperforms existing damage estimation tools in its ability to predict allele-specific chromatin accessibility. Finally, we demonstrate the utility of our method by successfully prioritising experimentally validated regulatory variants among the large number of variants within the same linkage disequilibrium block.

Conclusion The above method will aid in interpretation of whole genome sequencing datasets generated in cancer and rare disease studies.

Acknowledgements

References

Introduction Breast carcinoma (BC) encompasses a heterogeneous group of tumours with a great variability at the molecular and morphological levels and clinical outcome.

Material and methods In this retrospective study we investigated the potential prognostic role of 9 candidate biomarkers in a cohort of 305 breast cancer (BC) patients, both lymph node negative (151) and lymph node positive (154). The analysed genes belonged to the RAS pathway (RAF1, ERBB2, PIK3B, AKT1, AKT3, BUT NOT AKT1 AND AKT2, CONFLICTS A LONGER SURVIVAL RATE TO LESS AGGRESSIVE BREAST CANCERS

Acknowledgements

References
AKT2, AKT3), RB pathway (RB1 and CDK2) and cellular differentiation (K8). The expression profiles were investigated by real-time qPCR in formalin-fixed and paraffin-embedded tissues, and correlated to immunohistochemical-based molecular classes, namely luminal A, luminal B, Her2 + and TN. The study was approved by the Ethical Committee of the University of Trieste.

Results and discussions In our cohort lymph node involvement resulted to be related to the contribution of several genes at the primary tumour tissue level. Some of those genes resulted to be more expressed in LN negative BC, such as PIK3B, RB1 and AKT3, while some others were more expressed in LN positive BC, such as HER2 and AKT1. Our results show higher expression levels of PIK3B and AKT3 in less aggressive BC and higher expression levels of AKT1 in more aggressive BC highlighting the complex regulation of that pathway in BC. Shorter cancer specific survival was recorded in patients expressing higher levels of AKT1 and AKT2. Furthermore, better cancer specific survival was recorded in luminal A BC patients expressing higher levels of AKT3 (p=0.005 in LN- and p=0.01 in LN+).

Conclusion By comparing gene expression in lymph node negative and lymph node positive breast cancers, we found that AKT3 is an independent favourable prognostic factor for luminal A BC patients. Our results showed that a high expression level of AKT3, but not AKT1 and AKT2 was associated to better outcome and longer cancer specific patients’ survival in those patients who display the luminal A molecular class irrespective of lymph node involvement.

CASE-CASE GWAS TO IDENTIFY GERMLINE METASTASIS RISK VARIANTS IN SPORADIC COLORECTAL CARCINOMAS

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Introduction Colorectal cancer (CRC) is the third most frequently diagnosed cancer and a leading cause of cancer mortality worldwide. Majority of mortality is due to metastasis to distal organs such as liver or lung. Stage IV CRC patients by definition have distant metastasis. Up to 50% of stage III (with node involvement) and 25% of early stage I/II CRC patients succumb to distal metastasis. Stage III as well as high risk stage II CRC patients are offered adjuvant therapy after surgery but not all patients benefit from the chemotherapy. In this study, we wish to look for genes and pathways that contribute to metastasis in CRC to better understand the mechanisms and to search for potential prognostic markers and therapeutic targets.

Material and methods Samples from 2500 sporadic CRC patients age 50 years or more with no family history of CRC and with known cancer staging and metastasis status were subjected to whole genome microarray analysis using Affymetrix SNP6 array. Metastasis-positive status of the patients is confirmed based on distal organ involvement attributable to primary CRC, either from histopathological report or computed tomography/positron emission tomography (CT/PET) scan. Metastasis-negative status is confirmed with at least 5 years of follow-up with no distal organ involvement. DNA was extracted from fresh frozen mucosa collected at least 5 cm away from the tumours. Samples with genotyping call rate less than 95% were excluded and population stratification was examined based on principal component analysis. At the single nucleotide polymorphism (SNP) level, SNP with call rate <99% and minor allele frequency <0.01 were excluded. Whole genome Correlation/Trend test was performed by comparing patients with stages I/II that did not metastasize to patients from stage IV as well as stages I, II that succumbed to metastasis.

Results and discussions Preliminary data of 622 patients shows two potential regions associated with metastasis, one implicating a gene in the Wnt signalling pathway while the other a gene in the Hippo pathway. Dysregulation of both pathways have been previously reported to play important roles in CRC tumour initiation, progression and metastasis. Analysis from a larger discovery panel to increase the genetic power of the study will be presented while an independent replication panel for validation is being recruited.

Conclusion Initial screen implicated two genes in the Wnt and Hippo pathways associated with risk of metastasis in sporadic CRC.

PO-350 PROMO: AN INTERACTIVE TOOL FOR ANALYSING LARGE MULTI-OOMIC CANCER DATASETS

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Introduction Modern genomic datasets may include thousands of samples, each measured by several high-throughput technologies and described by extensive clinical information. Analysis and visualisation of such large multi-label multi-omic datasets pose significant challenges not easily met by existing bioinformatics tools. PROMO (Profiler of Multi-Omics data) is an interactive tool, designed to meet these challenges.

Material and methods PROMO provides various data exploratory methods, enables applying clustering analysis on both samples and features and utilising various popular useful statistical tests including survival analysis and enrichment analyses of subject clinical parameters. Special multi-omic integrative features include joint multi-omic sample clustering and identification of inter-omic feature correlation.

Results and discussions We will describe PROMO’s main capabilities and show how it can be used for analysing TCGA/GDC’s Breast Cancer datasets for tumour subtype detection and biomarker identification, as done for Luminal-A subtypes in Netanely et al. Breast Cancer Research 18:74 (2016).

Conclusion PROMO provides researchers with an extensive array of tools for quick analysis of large multi-omic cancer datasets.

PROMO is freely available for download at http://acgt.cs.tau.ac.il/promo