P **01** - 016

INTERPLAY BETWEEN GABA METABOLISM AND EPIGENETIC REGULATION IN THE CONTROL OF GLIOMA CELL PROPERTIES

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Cell populations with differing proliferative, stem-like and tumorigenic states co-exist in most tumors and especially malignant gliomas. Whether metabolic variations can drive this heterogeneity by controlling dynamic changes in cell states is unknown. Metabolite profiling of human adult glioblastoma stem-like cells upon loss of their tumorigenicity revealed a switch in the catabolism of the GABA neurotransmitter toward enhanced production and secretion of its by-product GHB (4 hydroxybutyrate). This switch was driven by succinic semialdehyde dehydrogenase (SSADH) downregulation. Enhancing GHB levels via SSADH downregulation or GHB supplementation triggered cell conversion into a less aggressive phenotypic state. GHB affected adult glioblastoma cells with varying molecular profiles, along with cells from pediatric pontine gliomas. In all cell types, GHB acted by inhibiting α-ketoglutarate-dependent Ten–eleven Translocations (TET) activity, resulting in decreased levels of the 5-hydroxymethylcytosine epigenetic mark. In patients, low SSADH expression was correlated with high GHB/α-ketoglutarate ratios, and distinguished weakly proliferative/ differentiated glioblastoma territories from proliferative/non-differentiated territories. Our findings support an active participation of metabolic variations in the genesis of tumor heterogeneity.

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P **01** - 017

INTRATUMOR MORPHOLOGICAL HETEROGENEITY IN BREAST CANCER AS A MODEL FOR THE STUDY OF CANCER INVASION

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Keywords: breast cancer, tumor heterogeneity, cancer invasion, epithelial-mesenchymal transition

Background: Different architectural arrangements of tumor cells within the primary tumor named as histological or morphological structures probably reflect specific cancer invasion patterns. In this study, we focused on the intratumor morphological heterogeneity in breast cancer (BC) and analyzed whether morphologically distinct structures (tubular, alveolar, solid, trabecular and discrete) demonstrate invasive characteristics.

Material and methods: Fifty BC patients (T1-4N0-3M0-1) were included in this study. Laser microdissection-assisted gene expression microarrays were applied to perform transcriptome profiling of morphological structures. Immunohistochemistry analysis was used for validation of proteins differentially expressed in morphological structures.

Results: Each type of morphological structures demonstrated specific gene expression profiles and signaling pathways. Most importantly, morphological structures differed from each in the number of expressed genes of the epithelial and mesenchymal phenotypes and the association with cancer invasion pathways. Tubular, alveolar, and solid structures were characterized by the same proportion of epithelial and mesenchymal genes; however, solid patterns of tumor cells demonstrated protrusions of collectively invading cells with loss or overexpression of certain proteins involved in the realization of cancer invasion program. Trabecular and discrete groups of tumor cells showed a dramatic decrease in the expression of epithelial genes, a significant increase in the number of mesenchymal markers and, surprisingly, the highest proliferative activity (as determined by Ki-67 immunostaining). Discrete groups of tumor cells, as well as solid structures, were characterized by the significant regulation of cytoskeletal dynamics by the Rho family of GTPases. Signaling pathways, which are linked with the activity of matrix metalloproteases, transendothelial migration, and cancer invasion in general, were significantly associated with discrete groups of tumor cells and tended to be related to trabecular structures.

Conclusions: Different morphological structures in BC represent transcriptionally distinct tumor cell subpopulations with varied degree of epithelial-mesenchymal transition and invasion. Overall, intratumor morphological heterogeneity in BC represents a promising model for the study of cancer invasion.

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P **01** - 018

SINGLE CELL GENOMIC ANALYSIS PIPELINE USING DEPARRAY TECHNOLOGY: AN APPLICATION TO STUDY CIRCULATING TUMOR CELL SUB-POPULATIONS

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Keywords: single-cell, genomics, DEParray, Tumor cells heterogeneity, CTC

High-throughput Genomics analysis at single cell level resolution is a major revolution to understand molecular mechanisms occurring in heterogeneous tissues. These approaches allow us to decipher genetics portrait of cell subpopulations including rare cells present in physiological or pathological tissues (as stem cells or other underrepresented cells) or in blood (as rare CTC). Different technologies coupling unique cell sorting (C1 single cell autoprep system from fluidigm, FACS, ICELL8 from Wafergen, chromium single cell from 10X genomics etc…) with DNA/RNA amplification followed by PCR or New Generation Sequencing analysis permit to reach genetics/transcriptomics and epigenetics knowledge at single cell level. However none of these technologies permit to associate cell phenotypic features such as size, shape, presence or absence of one or more target surface proteins and genetics features. The technology called DEPArray technology (Silicon Biosystems) allows purification of multiple different types of cells (by group of cells or at single cell level) from a single sample identified by combinations of multiple intracellular and extracellular markers, as well as with the use of morphological features such as circularity or size. This technology permits the isolation of rare cells subpopulations coming from fresh culture, or Circulating Tumour Cells (CTC) (Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. Carter L et al, Nat Med. 2016 Nov) but also from FFPE tissues (Digital Sorting of Pure Cell Populations Enables Unambiguous Genetic Analysis of Heterogeneous Formalin-Fixed Paraffin-Embedded Tumors by Next Generation Sequencing. Bolognesi C et al, Sci Rep. 2016 Feb).

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