MOLECULAR EVALUATION OF CELL-OF-ORIGIN (COO) IN DIFFUSE LARGE B- CELL LYMPHOMAS

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Background/Aims. Diffuse large B-cell lymphoma (DLBCL) is one of the most frequent and aggressive B-lymphomas. It represents an heterogeneous group in regard to morphological features, genetic alterations and clinical behaviour [1]. Two distinct molecular subtypes, called Activated B- Cell -like (ABC) and Germinal Center B-cell -like (GCB), have been defined using gene expression profiling (GEP) on the basis of Cell-Of-Origin (COO). This classification acquires a prognostic and therapeutic value and therefore it is important for patient stratification [2]. In standard practice, COO is defined by the immunohistochemistry (IHC)-based Hans algorithm carried-out on formalin-fixed paraffin-embedded (FFPE) tissue [3]. Recent technological improvements allow to apply GEP directly on RNA extracted from FFPE tissue. Aim of this study is to validate the GEP-approach comparing data obtained with the IHC method.

Materials and Methods. A cohort of 83 DLBCL patients was collected for this study. Archival FFPE samples were obtained from the Department of Pathology-AUO Policlinico di Modena. IHC stainings were performed under an automated standardized protocol (Ventana Benchmarks; Ventana, Tucson, USA) using antibodies against CD10, BCL6 and MUM1. The IHC profiling was obtained in accordance with the Hans algorithm. mRNA from representative FFPE sections was extracted with an automatic method (Maxell, Promega) and quantified with Expose (Trinean). 50- 500 ngr of total RNA were used for GEP in a NanoString platform (Nanostring Tecnologies, Seattle, USA) with application of the Lymph2Cx panel that includes a 20 gene-code set, as described by Scott et al. [4]. Data analysis was performed following the manufacturer's protocol.

Results. Application of Hans algorithm on IHC sections allowed to identify 46 ABC (46/83, 55%) and 37 GBC (37/83, 44%) whereas 1 case was Unclassified (1/83,1%). Nanostring Lymph2Cx algorithm identified 26 ABC (26/83, 31%), 43 GCB (43/83, 52%) and 13 Unclassified DLBCL (13/83, 16%). Among the 83 cases, only one failed to meet the required RNA quality for the test. Comparison between IHC- and Lymph2Cx-results showed 57 concordant cases (57/69, 83%) and 12 discordant cases (12/69, 17%), after exclusion of the Failed and molecularly Unclassified samples. In particular, 24 cases were defined as ABC and 33 as GBC by both IHC and Lymph2Cx. Among the 12 discordant results, 10 cases were identified as ABC by IHC examination whereas they were defined as GCB by Nanostring analysis. Moreover, Hans algorithm identified 11 ABC and 2 GBC among the 13 Nanostring Unclassified DLBCL.

Conclusions. In summary, our study demonstrates a good degree of correlation between the IHC- based approach and GEP by Nanostring Platform. From a technical point of view, Lymph2Cx allows to assign the DLBCL subtype using low amounts of RNA, standardized protocols and rapid turn-around-time. Nanostring platform and Lymph2Cx panel could be useful tools for DLBCL patients' stratification.

References

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TRANSCRIPTOMICS LANDSCAPE OF NECROPTOSIS GENES IS ASSOCIATED WITH DENDRITIC CELLS INFILTRATION: A PAN-CANCER STUDY OF 5,451 PRIMARY SOLID TUMORS

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Objective. Necroptosis (NPC) is a form of programmed cell death that culminates with the rupture of the cell membrane followed by the releasing of cellular elements. Evidence showed that tumors with high expression of NCP-related genes are associated with high cytotoxic CD8+ T-cell infiltrates, mediated by signaling from Dendritic (DC) and CD4+ T-cells. This study shows a pan-cancer view of the relationship between NCP and immune infiltration and their prognostic relevance across 24 cancer types from The Cancer Genome Atlas (TCGA).

Materials and methods. Gene expression RNA-seq data from 5,451 primary solid tumors were considered, excluding cases with treatments before surgery and with residual tumor. A deconvolution algorithm was used to estimate the level of tumor-infiltrating immune cells in each RNA- seq sample, considering the populations: B-cells, CD4 T-cells, CD8 T-cells, Macrophages and DC. For each immune population, the relative infiltration score was dichotomized at low and high infiltration using the 25th and 75h percentiles, respectively. Logistic regression and likelihood ratio test were applied to 163 genes belonging to Necroptosis pathway from KEGG database to test whether they are significantly associated to the infiltration of a specific immune population. FDR-adjusted p-values <0.05 were considered statistically significant. The prognostic relevance of the NCP genes significantly correlated with the infiltration was evaluated by Cox regression and log-rank test.

Results. DC and CD4+ T-cells showed the highest number of cancer types (8) reporting more than half genes of NCP pathway significantly correlated with their infiltration. CD8+ T-cell infiltration correlated with >50% of NCP genes in 5 of these 8 cancer types: Kidney-Renal, Breast, Prostate, Pancreatic and Thyroid tumors. DC also showed the highest number of NCP genes (69) correlated with their infiltration in more than half of the analyzed cancer types, including the main genes involved in NCP execution: RIPK1, RIPK3, MLKL and CFLAR. 60 and 58 of these genes showed a prognostic relevance ($p<0.05$) for overall and disease-free survival in at least one cancer type, respectively.

Conclusions. NCP has a relevant role in eliciting immune response against tumor through DC- mediated immunity in specific cancer types. In the new incoming era of immunotherapy, immune profiling of tumor from high throughput-derived transcriptomics and genomics data, holds a great potential in order do define specific biomarkers for prognostic or predictive purposes.