Prognostic value of the hypoxia signature on neuroblastoma

Fardin P[(1)], Rosasco L[(2,3)], Barla A[(2)], Mosci S[(2)], Versteeg R(4), Verri A[(2)], Varesio L[(1)]

[(1)] Laboratory of Molecular Biology, G. Gaslini Institute, Genoa, Italy
[(2)] DISI, University of Genoa, Genoa, Italy
[(3)] Brain & Cognitive Sciences Department McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02139
[(4)] Department of Human Genetics, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands

Motivation

Neuroblastoma is the most common extracranial solid tumor in childhood and shows notable heterogeneity with regard to both histology and clinical behavior. Hypoxia, a local decrease of oxygen tension, is a critical determinant of tumor progression inducing angiogenesis, matrix degradation and inhibiting apoptosis and cell differentiation. Therefore, hypoxia is related to poor prognosis in human cancer and it has a profound impact on neuroblastoma aggressive behavior. Clues to the prognosis of cancer are reflected at the time of surgical removal in the pattern of gene expression in the primary tumor. The ultimate goal is to identify specific gene expression signatures that define subsets of tumors and that will ultimately allow to predict the clinical course. The present system for cancer classification groups together tumors with important differences in clinical behavior. As expected, there is an enormous variation in gene expression patterns among tumors that would be grouped together. Unsupervised analysis of the gene expression pattern has led to the first tentative definition of gene expression signature that adds independent prognostic information to that provided by a risk assessment based on clinico-pathologic factors exclusively. The challenge is to devise methods suitable for deriving from in vitro controlled systems biologicallyinspired signatures relevant for clinical applications. Our approach is based on the current knowledge of the tumor biology which is instrumental to select the features that are critical for the tumor growth and spreading. Hence, such features represent potential candidates possibly including relevant biomarkers for diagnosis or prognosis. These features were defined in molecular terms using gene expression profiling in a process that led to gene expression signatures specific for various biological characteristics of the tumor. Our aim was to define the hypoxia signature from in vitro controlled system and to test its prognostic value on the gene expression profiles of a large patients cohorts with known outcome.

Methods

9 neuroblatoma (NB) cell lines were cultured under normoxic and hypoxic conditions and the gene expression profile was assessed by Affymetrix GeneChip U133 plus 2.0. We used unsupervised and supervised statistical learning algorithms tailored to model small samples of high-dimensional data and we enhanced their performance by incorporating prior information specific to the problem obtained from the Gene Ontology. A database consisting of 88 gene expression profiles of neuroblastoma patients was used for the survival analysis (Affymetrix GeneChip U133 Plus 2.0). The predictive power of our hypoxia-associated signatures was compared with the commonly accepted risk factors by Kaplan-Meier curves and log-rank test for overall survival and event-free survival. We assessed the correlation of neuroblastoma major risk factors with overall survival in our patient group by univariate and multivariate Cox proportional-hazards regression.

Results

The clustering analysis on the expression profile of NB cell lines revealed that hypoxia was not the major factor characterizing the biological model and that the cell lines segregated according to their N-myc status. To assess the more subtle role of hypoxia in the cell lines gene expression we performed a supervised analysis which allowed us to define the hypoxia signature. In order to do so, we used robust multivariate machine learning tools. To evaluate the role of N-myc and the cross talk between N-myc and hypoxia, we performed the same analysis on the cell lines with or without N-myc overexpression. We obtained three different hypoxia signatures and we tested their potential prognostic value on the patients database. When all the patients are considered in the survival analysis, the signatures resulted in good curves and good separ-

ation of groups of patients according to the prognosis, but they were as good as the existing stratification factors. On the other hand, the signatures had better prediction power when applied to those subgroups of patients, as intermediate risk neuroblastoma patients, that the other stratification risks fail to classify. Based on these results the hypoxia signature can be considered as novel prognostic indicator that can predict the survival intermediate risk neuroblastoma patients and could be of great support for clinical applications in cancer prognosis.

Contact : paolofardin@ospedale-gaslini.ge.it