Hypoxia Classifier is a New and Accurate Outcome Predictor for Neuroblastoma

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Motivation
Neuroblastoma is the most common pediatric solid tumor of the sympathetic nervous system deriving from ganglionic lineage precursors. It is diagnosed during infancy and shows notable heterogeneity with regard to both histology and clinical behavior. Hypoxia, a condition of low oxygen tension occurring in poorly vascularized areas, has a profound effects on tumor cell growth, susceptibility to apoptosis, and resistance to radio- and chemotherapy. We developed a classifier to predict the outcome of neuroblastoma patients based on the expression profile of a hypoxia gene signature and we studied the relationship between tumor hypoxia and outcome.

Methods
Gene expression profiles of 182 neuroblastoma tumors were used to develop and validate an Artificial Neural Network classifier to predict patients’ outcome (alive or dead at 5 years after diagnosis). Multi Layer Perceptron (MLP), a feedforward ANN, has been selected as model to develop the classifier. The training, testing and validation steps have been performed using WEKA which implements the MLP model. The cohort was divided in two groups of 100 and 82 patients. The expression data of 100 tumors was used in a leave-one-out framework to construct the classifier and the expression data of the remaining 82 tumors constituted the external validation dataset. The classifier was trained and tested on the expression values of the 62 probesets constituting NB-hypo signature, a gene signature previously identified as an independent risk factor in neuroblastoma. Additional gene expression analysis was performed by gene set enrichment analysis (GSEA) and tumor hypoxia was assessed by immunohistochemistry.

Results
The NB-hypo classifier predicted the outcome of the validation cohort with an accuracy of 87% and was able to correctly predict all localized and stage 4S tumors. In particular, NB-hypo classifier identified all the poor outcome patients in the low-intermediate risk classes. Gene set enrichment analysis revealed that NB-hypo classifier is very accurate in predicting the hypoxic status of the tumor even in the limited number of cases in which the latter does not correlate with the outcome. The hypoxic status of tumors was
further validated by CAIX and VEGF determination by immunohistochemistry on tumor slices. We developed a new and robust classifier capable of predicting neuroblastoma patient’s outcome with very low error rate that decreases to negligible level when applied to localized or 4S stage tumors. The NB-hypo classifier is a ready to use product, easily shareable among institutions that can be of immediate application in the clinical setting. We provide evidence that the poor outcome is related to the hypoxic state of the tumor suggesting that patients identified as poor outcome by NB-hypo classifier may benefit from therapeutic protocols targeting tissue hypoxia.

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