A TRANSCRIPTOMIC PROFILE PREDICTS CLINICAL OUTCOME IN STAGE III COLORECTAL CANCER PATIENTS TREATED WITH ADJUVANT CHEMOTHERAPY

1)Nobili S. 2)D'aurizio R. 3)Perrone G. 4)Magi A. 5)Lapucci A. 6)Tassi R. 7)Napoli C. 8)Picariello L. 9)Landini I. 10)Brugia M. 11)Mazzei T. 12)Tonelli F. 13)Mini E.

University of Florence

Introduction

5-year overall survival of stage III colorectal cancer (CRC) patients (pts) treated with standard adjuvant chemotherapy (a fluoropyrimidine - FP with or without oxaliplatin - OHP) is still unsatisfactory and highly variable (42-88%). Although single molecular biomarkers and molecular signatures predictive of adjuvant chemotherapy outcome have been identified in CRC, none of them has been validated.

We hypothesized that differences in gene expression may be responsible for the variability of prognosis of stage III CRC pts treated with adjuvant chemotherapy.

The goal of this study was to identify molecular biomarkers predictive of response to FP-based adjuvant chemotherapy in stage III CRC pts.

Materials and Methods

From a large case series of CRC pts who received standard adjuvant chemotherapy (5-fluorouracil/capecitabine with or without OHP) we selected two groups with favorable (no evidence of disease recurrence within 5 years from chemotherapy, n=12) or unfavorable (evidence of disease recurrence within 3 years from chemotherapy, n=12) prognosis, according to stringent eligibility criteria. We analyzed fresh frozen primary CRC explants according to an IRB-approved protocol. Whole transcriptomic sequencing was performed by Ion Proton System (Ion Torrent Thermo Fisher Scientific). After quality control, produced reads were aligned to all transcripts to measure the gene expression levels in each sample. To identify differentially expressed genes between the two groups a statistical analysis was performed using DESeq2 package of R Bioconductor repository. Oncomine database was used to classify genes. A validation of the accuracy of the RNA sequencing methodology was performed by randomly analyzing a number of genes by RT-PCR in two groups of pts.

Results

On average 16190 (min/max: 6711/17790) expressed genes were detected using RNA sequencing. The correlation of gene expression between the two groups of CRC patients by Pearson correlation coefficient, ranged from 0.90 to 0.94. Although correlation analysis showed that the overall transcriptomic profiles were correlated among CRC pts, 108 genes resulted statistically differentially expressed (p value <0.01, fold discovery rate <0.01) between the two groups: 104 genes were upregulated and 4 downregulated in the unfavorable prognosis group compared with the favorable prognosis group. Magnitude of fold changes was within -2.5 to +3.5. Among these,

42 genes belonging to the olfactory signaling pathways, were not further considered. Among 66 remaining genes, 19 were pseudogenes, 7 uncharacterized non-coding RNA, 4 were involved in the immune response (e.g. IFNs) and one was a miRNA (MIR548I1). All these genes were upregulated. Further 9 genes were cancer-related (6 upregulated (e.g. CETN1 involved in cell adhesion) and 3 downregulated (e.g. ROR2 involved in Wnt pathway). The remaining genes (n=26), most of which involved in key cellular processes (e.g. RNA processing: UPF3A upregulated; apoptosis: TMEM150B downregulated) have not yet been associated with cancer and/or cancer prognosis. Fifteen out of 66 differentially expressed genes have been selected for validation by RT-PCR. In 6 out 15 genes, findings obtained by RNA-seq have been confirmed ($p<0,001$); in 8 out 9 remaining genes a trend according to the RNA-seq results was observed.

Conclusions

Stage III CRC pts with favorable and unfavorable prognosis following adjuvant chemotherapy differ at a transcriptomic level. These findings, after a proper validation in larger case series (currently ongoing), may have important implications for FP-based adjuvant chemotherapy.

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