

COLON

APPLICATIONS OF 1H-NMR METABOLOMIC PROFILING APPROACH ON CANCER DISEASE.

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Nuclear Magnetic Resonance (NMR) spectroscopy is the only non-destructive technique which can identify and quantify complex mixtures of metabolites on small sample volumes and after an easy sample preparation protocol. For this reason the evaluation of the human serum metabolome is used to monitor the changes in the metabolism for example during cancer progression and after drug treatments. At National Cancer Institute in Naples there is a 600-MHz NMR spectrometer with cryoprobe, equipped with an automation system able to acquire spectra in automation on twenty-four samples. This instrument is, therefore, very useful to apply the metabolomic profiling approach on different biological fluids such sera but also urine, saliva and others. Our group is performing studies on the evaluation of metabolomic profiling on liquid biopsy samples collected in melanoma and colorectal cancer (CRC) patients at different stages of the disease and/or at different time points before and during treatments. In detail, we evaluated the serum metabolome on metastatic colorectal patients subjected to first line bevacizumab plus chemotherapy and on metastatic melanoma patients subjected to different immunotherapy treatments. Through this approach, we identified the metabolites that present statistically significant different levels in the patients groups compared to healthy donors, and those that increase or decrease during the melanoma and CRC progression. Moreover, we were able to group in separate clusters the patients with different outcome in terms of overall survival and to identify a set of metabolites that, either before or during treatments, can discriminate patients with favorable than those with worst outcome. Overall, the obtained results suggest that metabolomic profiling by NMR is a potent and affordable method to select the patients, to predict outcome for cancer treatment, and, hence, to improve the early detection and prognosis definition of cancer disease.

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THE EXTRACELLULAR MATRIX PROTEIN EMILIN2 AS A REGULATOR OF THE MYELOID RESPONSE IN A MODEL OF INFLAMMATION-INDUCED COLON CARCINOGENESIS.

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EMILIN2 is an extracellular matrix molecule exerting pleiotropic effects in the tumor microenvironment overall functioning as a tumor suppressive molecule. EMILIN2 affects tumor cell viability and proliferation by activating apoptosis and functioning as a negative regulator of the Wnt/ β -catenin axis. Interestingly EMILIN2 expression is down-modulated in a number of tumors including breast and colorectal cancer (CRC). Given its involvement in the regulation of Wnt signaling, a crucial pathway in colon carcinogenesis, and its altered expression in CRC, we took advantage of the EMILIN2 null mouse model to assess its role in CRC development, subjecting the mice to the inflammation-related AOM/DSS protocol. EMILIN2 KO mice developed a significantly higher number of tumors compared to wt mice. Tumors from EMILIN2 KO mice were more undifferentiated and at an advanced stage compared to the tumors from control mice. Surprisingly, tumors from EMILIN2 KO mice did not display any changes in the activation of the Wnt/ β -catenin pathway compared to the controls. Instead, the severe tumorigenesis in EMILIN2 KO mice resulted strongly related to colitis induction and to the inflammatory response due to the DSS administration. Moreover, tumors from EMILIN2 KO mice were characterized by a higher number of macrophages and granulocytes than those from WT mice. Similar alterations in the KO model were found during the acute phase of inflammation: mice subjected to DSS treatment alone developed a more severe colitis than WT mice. Accordingly, the infiltration of myeloid cells within the intestinal mucosa was altered and the serum level of a number of cytokines, including IL-1 β , INF- γ , TNF- α and IL-10, was affected. Preliminary in vitro analyses showed that EMILIN2 may modulate monocytes and macrophages migration, while not affecting their adhesion and viability. Our results let us suggest that EMILIN2 may affect colon carcinogenesis impinging on the inflammatory microenvironment associated with the tumor.

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NGS TECHNOLOGY FOR PREDICTING RESPONSE TO NEOADJUVANT CHEMORADIATION IN LOCALLY ADVANCED RECTAL CANCER.

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In locally advanced rectal cancer neoadjuvant chemoradiation before radical surgery reduces local recurrence rate, and is standard treatment. However, patients undergoing this multimodality treatment are exposed to perioperative morbidity risk including long-term bowel, bladder, and sexual dysfunction, and permanent colostomy. Pathological complete response is obtained in up to one-third of rectal cancers treated by neoadjuvant chemoradiation, and is associated with favourable long-term oncologic outcome. Based on this, organ-sparing strategies are being explored in patients with clinical complete response. On the other hand, patients with poor pathological response have a high risk of local and distant recurrence and receive no benefit from neoadjuvant treatment. At present, methods for predicting response to chemoradiation are not available. Starting in 2014 we have established a prospective rectal cancer biorepository in which tumor tissue biopsies, are collected at 3 different time points: 1- before chemoradiation; 2- during chemoradiation (after 5 RT sessions); 3- after chemoradiation completion; (Study # CRO-2013-21). To date 90 patients have been enrolled in the study. Based on the hypothesis that lack of response may depend on specific gene alterations, we propose to analyze our biorepository tissue samples utilizing ACC Genomics platform in order to identify predictive fingerprints. Next Generation Sequencing (NGS) technology coupled with the unique longitudinal tissue samples availability warrant for a high probability of success. Discovered genetic markers will be prospectively validated within an already existing ACC rectal cancer network. The successful development of this project may translate into personalized treatment strategies by the identification of patients who might safely enter an organ preserving program, while sparing chemoradiation related toxicity to patients displaying resistant genetic profile. Moreover, the proposed study have the potential of gaining more insight into the molecular mechanisms of chemo- and radiation resistance.

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THE BIOLOGICAL CLOCK IMPACTS METABOLIC ACTIVITY IN COLON CANCER PROGRESSION.

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The circadian circuitry is an internal timing system that allows organisms to adapt biological processes to the geophysical time and is operated by biological clocks ticking through a set of genes and proteins hardwiring transcriptional and translational regulatory feedback loops. These drive the oscillatory expression of target genes and regulate cellular processes, including metabolism and the cell cycle, involved in tumour development and progression. A tight link between the biological clock and metabolism was previously postulated. Besides dramatic metabolic alterations, cancer cells show severe changes in the biological clock with likely consequences in tumour progression and treatment response. Increasing efforts have been made to elucidate the connection between the circadian circuitry, tumour progression and cancer-associated metabolic alterations, yet a more detailed knowledge of this interplay is still missing. We investigated distinctive time-related transcriptomic and metabolic features in a cellular model of colon cancer progression and we showed that a disrupted clock leads to altered temporal profiles of gene expression, metabolic reprogramming and changes in drug response. Based on different oscillation profiles of the metabolic pathways (glycolysis and oxidative phosphorylation), we identified a set of candidate genes that mediate clock-driven metabolic reprogramming in carcinogenesis. A consequent disruption of the core-clock gene *Bmal1* led to time-dependent metabolic alterations in colon cancer cell lines, as well as changes in treatment response. The results provide novel evidence regarding the complex interplay between the circadian clock and metabolic alterations in carcinogenesis and pave the way to a possible optimization of therapy and timing of treatment. Our data further reinforces the existence of a reciprocal interplay between the metabolic genes and the circadian clock, and identifies novel connections between both systems that may play a pivotal role in colon cancer progression and response to therapy.

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CANCER CELL-SELECTIVE TRANSCRIPTOME ANALYSIS REVEALS NEW COLORECTAL CANCER MOLECULAR SUBTYPES WITH IMPROVED BIOLOGICAL RESOLUTION AND SUPERIOR PREDICTIVE AND PROGNOSTIC PERFORMANCE.

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Abstract Molecular classification of colorectal cancer (CRC) based on gene expression profiling of tumor samples is known to be heavily affected by transcripts of stromal origin. As a consequence, current CRC transcriptional subtypes reflect an admixture of cancer cell-intrinsic traits and tumor microenvironment features. Whether selective analysis of the cancer cell transcriptome could improve CRC subtyping, remains an open issue. In patient-derived xenografts (PDXs), human transcripts only originate from cancer cells, because stromal transcripts are of mouse origin. We therefore assessed cancer-cell intrinsic transcriptional features of CRC by generating human-specific expression profiles of 515 PDXs from 244 CRC patients, and performing unsupervised class discovery. We identified five "CRC intrinsic subtypes" (CRIS A-E) only partially overlapping with the current ones, and robustly enriched for distinct molecular, functional and phenotypic traits: (i) CRIS-A: mucinous, glycolytic, CIMP, and enriched for microsatellite instability or mutations in KRAS; (ii) CRIS-B: marked TGF- β pathway activity, epithelial-mesenchymal transition, poor prognosis and resistant to standard chemotherapy; (iii) CIN, CRIS-C: elevated EGFR signaling and sensitivity to EGFR-targeted treatments; (iv) CRIS-D: MSS, WNT activation, and IGF2 overexpression and amplification; (v) CRIS-E: MSS, Paneth cell-like phenotype and higher frequency of TP53 mutation. CRIS subtypes successfully categorized independent sets of CRC cell lines, primary and metastatic tumors, for a total of over 3000 samples profiled by microarray- and RNAseq-based platforms. The new subtypes displayed unprecedented predictive and prognostic performances, independent from known markers including stromal signatures, whose integration with CRIS further enhanced prognostic significance.

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COMBINED LOW DENSITIES OF FOXP3+ AND CD3+ TUMOR-INFILTRATING LYMPHOCYTES IDENTIFY STAGE II COLORECTAL CANCER AT VERY HIGH RISK OF PROGRESSION.

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BACKGROUND:

A prognostic immunoscore, based upon intra- and peri-tumoral densities of CD3+ and CD8+ cells, is under development for colorectal cancer (CRC) prognostication. FoxP3+ tumor-infiltrating lymphocytes (TILs) have been also shown to impact on CRC prognosis, but are not included in the immunoscore. We aimed to compare the prognostic significance of CD3+ and FoxP3+ TILs densities in stage II/III CRC.

METHODS:

Whole-tissue sections from 413 pT3-pT4 non-metastatic CRC were analyzed for FoxP3+ and CD3+ TILs densities at the tumor invasive front. Prognostic values of TILs were assessed by recursive partitioning (CART®) and multivariate analysis of patient demographics and pathologic variables, including tumor microsatellite status (MS-status).

RESULTS:

CART® analysis recognized an effect of TILs on the recurrence risk only within the decisional tree of stage II CRC, in which low FoxP3+TIL densities ranked first (OR 5.95; 95%CI, 2.52-14.0; $p < 0.001$), low CD3+TIL densities further stratifying the risk (OR, 4.51; 95%CI, 1.61-12.6; $p < 0.001$) for patients with low FoxP3+TILs only. TILs interacted with tumor stage ($p = 0.03$ for FoxP3+; $p = 0.003$ for CD3+), and with MS-instability ($p = 0.05$ for FoxP3+) at multivariate analysis. In stage II MS-stable (MSS) CRC, individual low densities of FoxP3+TIL were associated with worse outcome than that associated with low densities of CD3+TIL (HR, 6.60; 95%CI, 2.66-16.3 vs. HR, 4.94; 95%CI, 1.83-13.3, at Cox multivariate), and their concomitant low densities were associated with the highest risk of progression (HR 7.53; 95%CI, 3.51-16.1; $p < 0.001$).

CONCLUSIONS:

Combined assessment of FoxP3+ and CD3+ TIL populations at the invasive front coupled with MS-status accurately depicts the outcome of patients with stage II CRC. However, nodal status outplays enhanced prognostication reached by parallel assessment of un-related TIL sub-populations, thus pointing to events that counteract the protective role of TILs since nodal invasion. Studies from randomized controlled trials are warranted to implement TIL evaluation for clinical use.

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EXTENSIVE CHARACTERIZATION OF BRAF MUTATED METASTATIC COLORECTAL CANCER: RESULTS FROM THE “BRAFF, BECOOL” PLATFORM.

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BACKGROUND:

BRAFV600E mutation assessment is routinely tested in mCRC. The unfavourable outcome of mutated patients is well established and their specific clinical features have been repeatedly defined and reported.

PATIENTS/METHODS:

Main eligibility criteria included: mCRC and BRAF V600E mutation as assessed on primary tumour or any metastatic site. A prognostic score was developed by an internal cross-validation procedure: the whole population was splitted in a training (67%) and in a testing (33%) sample; this process was repeated 10 times. Primary endpoint was overall survival (OS).

RESULTS:

a total of 395 mCRC patients with a BRAFV600E mutation were included. At MV analysis, independent predictive factors of OS were ECOG performance status Ca199; LDH; neutrophil/lymphocyte ratio; tumor grading; liver metastases; lung mets; lymphnode mets. Two different scoring systems were built: a «complete» score (0-18), selecting all significant covariates; a «simplified» score (0-11), selecting only significant clinico-pathological covariates, excluding laboratory values. With “complete” score, proportion of patients with low (0-4), intermediate (5-8) and high (9-18) score was 39%, 46% and 15%, respectively. Median OS was 27.6, 18.7 (Hazard Ratio interm. vs low 1.89, 95%CI 1.25 – 2.86, p=0.003) and 6.6 months (HR high vs low 4.95, 95%CI 2.89 – 8.47, p<0.0001), respectively. Analysis adjusted for type of first-line treatment produced similar results. Median progression-free survival was 11.1, 8.6 (HR interm. vs low 1.36, 95%CI 0.94 – 1.97, p=0.11) and 4.1 months (HR high vs low 3.50, 95%CI 1.98 – 6.20, p<0.0001). Similar results were obtained with the simplified score.

CONCLUSIONS:

These results should be replicated in an independent cohort, but the internal cross-validation makes this model robust enough to justify the effort of a confirmatory study. Strong and reliable prognostic factors in new molecular subgroups of mCRC will be determinant for stratifying clinical trials and for adjusting translational analyses.