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Awards of Best Oral Presentations

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MCT1 PROMOTES CANCER CELL MIGRATION, INVASION AND METASTASIS

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Lactic acid, the end-product of glycolysis, is emerging as a pleiotropic tumor growth-promoting factor. Here, we decided to assess its function in tumor metastasis. Transmembrane lactic acid transport is mediated by passive lactate-proton symporters of the monocarboxylate transporter (MCT) family. Among them, MCT1 has been shown to contribute to lactate-fueled cancer cell respiration and angiogenesis, and its pharmacological inhibition is currently under clinical evaluation. We and others previously showed that MCT1 promotes cancer cell migration. However whether and how MCT1 contributes to the metastatic process has not yet been thoroughly investigated. In this study, we report that MCT1 is upregulated in highly invasive SiHa-F3 human cervix adenocarcinoma cells, compared to wild-type SiHa cells. MCT1 silencing or inhibition impaired lactic acid efflux from wild-type SiHa cells as well as their migration and invasion towards serum *in vitro*, without affecting cell viability. To further investigate the contribution of MCT1 to metastatic progression, we used spontaneously metastatic 4T1 mouse mammary carcinoma cells. Compared to controls, 4T1 cells silenced for MCT1 (shMCT1) displayed decreased lactic acid export, migration and invasion, but clonogenic activity on soft agar and primary tumor growth rate were conserved. Interestingly, silencing MCT1 was sufficient to block spontaneous 4T1 cell metastasis to the lungs following subcutaneous primary tumor implantation in syngeneic mice. Altogether, our results suggest that MCT1 is an attractive therapeutic target for metastasis prevention. We are currently investigating the relative impact of proton *versus* lactate transport by MCT1 on the metastatic process.

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BREAST CANCER PATIENT PROFILING BASED ON CYCLING HYPOXIA: DOWNSCALING OF A MICROARRAY-BASED PROGNOSTIC GENE SIGNATURE TO A ROBUST QPCR ASSAY

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Local and temporal heterogeneities in tumor O_2 distribution are nowadays described as a hallmark of tumors. Since this environment called 'cycling hypoxia' is quite hostile (even for tumor cells), we recently postulated that the associated transcriptome could represent a prognostic biomarker of cancer progression/aggressiveness. Human cancer cell lines covering a large diversity of tissues and genetic alterations were thus submitted to cycling hypoxia (or maintained under normoxic conditions) and corresponding mRNA samples were analysed by hybridization using Human Gene 1.0 ST Affymetrix microarrays. A cycling hypoxia (CycHyp) signature was derived from this work and the performance of this ~100-gene signature was evaluated using publicly available GEO data sets. A shorter version of the CycHyp signature (~10-gene) was also designed in order to exploit RT-qPCR to interrogate human residual tumor samples obtained from the Belgian virtual tumourbank.

GEO data sets allowed us to collect information on the survival of 2,150 patients with primary breast cancer. The CycHyp prognostic potential was first established in patients independently of the receptor status of the tumors. The discriminating capacity of the CycHyp signature was further increased in the ER^+ $HER2^-$ patient populations including those with a node negative status. Furthermore, the CycHyp prognostic signature outperformed major prognostic metagenes including Oncotype DX and Mammaprint ($P<0.001$) as well as random gene signatures ($P<0.001$).

To evaluate the potential of the signature on *de novo* breast tumor materials (> 50 patient samples from UCL and ULg biobanks), we also developed and validated a methodology (i) to extract mRNA from both frozen and FFPE samples using automated Maxwell RSC16 instrument, (ii) to reverse transcribe fragmented mRNA into exploitable cDNA using specifically designed primers and (iii) to quantitatively analyse transcript expression by optimizing the location of Taqman probe/primers in the most indicative gene regions (through a careful dissection of Affymetrix transcript cluster-based data). This strategy led us to document that the 10-gene version of the CycHyp signature could also discriminate between high and low risk breast cancer patients (HR > 3).

In conclusion, our findings indicate that the CycHyp signature represents a new generation of prognostic biomarker reflecting a generic environmental condition in tumors. When applied to breast cancer, the ~100-gene CycHyp signature has a powerful prognostic value independently of molecular risk factors and importantly maintains its potential of decision-making tool when restricted to the qPCR-based evaluation of the expression of the 10 most representative genes.

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METABOLOMIC ANALYZES OF THE TUMOR MICROENVIRONMENT AFTER NEOADJUVANT RADIOTHERAPY IMPLICATED IN SURGERY-RELATED METASTATIC SPREADING

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Purpose: Neoadjuvant radiotherapy (RT) is used in many cases of cancer and aims at improving tumor local control and patient overall survival. RT schedule and the timing of surgery are mostly empirical based on clinical experiences. However in the case of Locally advanced rectal cancer, the timing of surgery following neoadjuvant RT appears crucial for patient overall survival. Therefore, we hypothesized that radiotherapy may influence the tumor phenotype as well as the tumor microenvironment and consequently metastases formation. We developed a pre-clinical model of neoadjuvant RT to study the impact of different RT schedules on TME and metastatic dissemination and tried to predict metastatic profile with metabolic profile of the tumor at the time of surgery.

Materials and methods: To mimick neoadjuvant RT used in clinic, we subcutaneously injected human mammary cells (MDA-MB-231) into the flank of SCID mice. Once tumors reached 400mm³, we locally irradiated the primary tumor with different neoadjuvant RT schedules (5x2Gy and 2x5Gy) inspired from clinical practice but adapted to mice. We surgically removed carefully tumors 4 or 11 days after the end of RT and kept the mice alive during 6 weeks for metastatic growth. Then we sacrificed the mice and looked for lung metastases highlighted by human Ki-67 immunohistochemical staining. Tumor extracts were analyzed by Nuclear Magnetic Resonance (NMR). Data were analyzed with powerful statistical tool (supervised and multivariate analyses).

Results: The occurrence of lung metastases is totally different according to the radiotherapy schedule and the time of surgery. After 2x5Gy, the size and the number of lung metastases were smaller when surgery was performed at 11 days after the end of RT, compared to 4 days. Inversely, in the 5x2Gy schedule, applying surgery at 4 days protected the mice against lung metastases compared to surgery at 11 days (Leroi et al., 2015). Tumor volumes are the same in all groups and cannot be incriminated in the difference of lung metastases. These results suggest that **the timing of surgery and RT schedules are both important factors that influence the formation of metastases.** In order to identify metabolic profile predicting treatment benefit, we performed NMR analysis on primary tumors collected at the time of surgery. We observed a correlation between metabolic profile and metastatic profile for individuals in the different groups. Intriguingly, we observed a decrease in some metabolites (i.e. glutamate, taurine, glycine, betaine) following RT and an increase in lipid signal in irradiated tumors.

Conclusion: We developed a powerful *in vivo* model of neoadjuvant radiotherapy allowing us to demonstrate the impact of neoadjuvant RT schedule and the timing of surgery on metastatic profile. Moreover, NMR analyses and discriminant analyses showed an impact of neoadjuvant radiotherapy on tumor microenvironment which could be correlated to the metastatic profile.

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GENOME-SCALE IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF LONG NON-CODING RNAS AFFECTED BY COLORECTAL CANCER HETEROGENEITY

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For the last decade, large-scale efforts to harness the power of high-throughput molecular profiling strategies have emerged. In particular, gene expression profile (GEP) studies have revealed themselves to be a powerful tool in the classification process of cancers as they have allowed precise distinction between cases and subsequent differential treatment. However, their focus has been turned on mRNAs arising from well annotated protein-coding genes (PCGs). This said, irrefutable evidence is starting to emerge showing that the genome clearly produces functional non-coding RNAs such as miRNAs and, in particular, long non-coding RNAs (lncRNAs). Colorectal cancers (CRCs) are known for their overlapping molecular heterogeneity making the development of reliable ways to classify CRCs a complex task which requires additional levels of information. With this in mind, recent studies have shown that a tumour's molecular features -such as DNA methylation patterns, chromosome stability and oncogene mutational status- and prognosis are reflected by its PCGs expression profile which have been used to better sub-classify colorectal tumours. Yet, the contribution of lncRNAs to CRC heterogeneity is poorly understood and the role played by lncRNAs in CRC requires further investigation. In this study, we used a publically available GEP dataset consisting of 566 CRC samples to explore the prognostic value of lncRNAs and their association with molecular features and/or CRC subtypes. Moreover, for clinically relevant lncRNAs we generated hypothesis on their potential targets and functional roles and in CRC. Finally we confirmed the validity of our hypothesis with *in vitro* experimentation for a candidate lncRNA. We found 33 lncRNAs to be significantly associated to relapse free survival (RFS) rates ($p<0.001$), 173 were differentially expressed (Fold Change < 0.67, Fold Change > 1.5; FDR < 0.05) according to key clinical molecular features and another 263 lncRNAs were differentially expressed in at least one CRC subtype compared to all others. All in all, we have generated a list of 212 unique clinically relevant lncRNAs which our results suggest are involved in many biological processes such as epithelial-to-mesenchymal transition (EMT), angiogenesis, cell cycle regulation, and signal transduction. Furthermore we show for a lncRNA named lnc-BLID-5 that its hypothesized role in EMT is, in part, due to its regulation of the GJA1 gene, a gap junction protein involved in EMT as cells depleted for lnc-BLID-5 suffer from a downregulation of the GJA1 gene. In conclusion, we show the extent to which lncRNAs are affected by molecular features commonly used to assess different tumour biologies and CRC heterogeneity in general, and provide a priority list of lncRNAs for further investigation in CRC to which we add confidence through the experimental confirmation of our functional prediction for a selected lncRNA candidate.

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WHOLE TRANSCRIPTOME SEQUENCING OF LIVER METASTASES IDENTIFIES PATHWAYS ASSOCIATED WITH NON-ANGIOGENIC REPLACEMENT GROWTH

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Introduction: Patients with liver metastases (LMs) are often treated with anti-angiogenic therapy. Cancer cells are more or less sensitive to this type of treatment depending on the histological growth pattern (HGP) adopted by the metastases. Two major HGPs have been described: A. a replacement (R-)HGP in which cancer cells replace normal hepatocytes and respect the liver architecture; and B. a desmoplastic (D-)HGP in which the LM is surrounded by a desmoplastic rim. We recently revealed that a R-HGP is associated with resistance to anti-angiogenic therapy and in this study we seek to characterize replacement growth in search of druggable targets and predictive biomarkers for anti-angiogenic therapy.

Materials and Methods. Tissue samples from 19 patients with LMs (7 R- and 12 D-GPs) were harvested and the tumor interface (i.e. cancer cells adhering to normal liver) was selectively sampled. RNA was extracted, reverse transcribed into cDNA and subjected to a stranded library preparation protocol using Illumina's TruSeq chemistry. Paired-end sequencing was performed using 2 lanes of an Illumina HiSeq1500 system. Raw reads were quality controlled and contaminating reads were removed. Mapping was done using TopHat2 with Bowtie2 against the human reference genome (GRCh37). Read counting was performed using HTSeq and ambiguous reads (MAPQ-score<40) were ignored. Differential expression between R- and D-HGPs was assessed using Limma, EdgeR and DESeq2. Significant genes (P-value<5%) identified by 2/3 algorithms were considered relevant. Pathway analysis was performed using Expression2Kinases (E2K) on genes overexpressed in the R-HGP only.

Results: On average, 51.569.726 reads per sample were obtained resulting into 93.962.743 alignments. Of these, 67% mapped to exons, 5% to 5'UTRs, 23% to 3'UTRs and 3% to introns. After read counting, we identified 7.534 annotated genes with at least 1 read count per million reads mapped in at least 3 samples. Comparing R- and D-HGPs, we identified 366 genes that were differentially expressed by at least 2/3 algorithms, of which 153 were overexpressed in the R-HGP. E2K identified 9 transcription factors (i.e. STAT3, GATA1, HINFP, NFE2, SP1, RELA, SP3, TCF4, KLF11) with target genes significantly enriched in the list of genes overexpressed in the R-HGP. These transcription factors were connected by a network of 124 nodes and 1.388 edges, including 25 druggable kinases. Pathway enrichment analysis of the protein-protein interaction network revealed Notch-, Wnt-, INFgamma-, IL6- and ERK-signalling as potential drivers of the R-HGP expression profile.

Discussion: Our study demonstrates the feasibility of using RNA-sequencing coupled with systems biology to identify pathways responsible for replacement growth of liver metastases. The true meaning of our results can only be revealed by functional validation studies, but co-culture experiments already showed a role for IL6/STAT3-signalling in mediating cross-talk between hepatocytes and colon cancer cells. Our results provide the basis for continued research into the distinct biology of the histological growth patterns of liver metastases.

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ZEB2 STEPS OUT FROM THE DARK SIDE OF TUMOR METASTASIS BY CONTROLLING NATURAL KILLER CELL MEDIATED CANCER IMMUNOSURVEILLANCE

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Induced expression of the ZEB2 in various epithelial cancer cell lines results in the repression of a wide range of cell adhesion genes, allowing these cells to become motile and upon xenotransplantation invade into surrounding tissue and subsequently spread and metastasize. Based on these results, blocking ZEB2 functions or preventing its expression is many times suggested as a potential new therapy for solid tumors.
Here we show that ZEB2 not only orchestrate cancer cell invasion, but as well plays pivotal roles in cancer immunosurveillance. Zeb2 expression increases during differentiation of Natural Killer cells. Targeted deletion of *Zeb2* in NK cells impaired their maturation, survival and trafficking, and compromised *in vivo* anti--tumor response. As preclinical studies have suggested that cancer cells can only spread and grow by avoiding detection and destruction by the immune system and therefore it is one of the hallmarks of cancer. Therefore, we believe that non--cancer cell--specific therapies that focus on downregulating ZEB2 expression/function, or the effects on their downstream targets could result in adverse consequences by targeting the anti--tumor immunity as well.