10P Emerging role of Telomeric repeat-containing RNA TERRA in hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the most frequent primary tumor of the liver and the third cause of cancer-related deaths. The identification of candidate molecular targets and biomarkers in HCC clinical practice are needed. The signatures of aberrant long non-coding RNAs (lncRNAs) expression in HCC tissues, their extracellular release and stability had led to their exploration as diagnostic and prognostic tools as well as potential therapeutic targets for HCC. Telomeric-Repeat Containing RNA (TERRA) consists of 100nt-9Kb subtelomeric-derived transcripts able to base-pair with TERC RNA, acting as telomerase allosteric inhibitor. Little is known on the role of lncRNA TERRA in HCC.

Methods: By qPCR we measured TERRA expression in tumor and peritumoral (PT) tissues of HCC patients, as well as in plasma and in HCC cells. HCC patients (n.25) did not receive any treatment before surgical resection. By nickel-based isolation method (NBI) we isolated from the conditioned medium (CM) of HA22T/VGH cells the extracellular vesicles (EVs), subsequently analyzed by qNANO instrument.

Results: Global TERRA expression was significantly downregulated in HCC vs PT tissues ($p=0.025$) and ROC analysis revealed a significant ability to distinguish HCC from PT ($p=0.03$). Extracellular TERRA transcripts were significantly higher in plasma of HCC patients compared with healthy subjects and logistic regression model strongly evidenced the potential diagnostic ability of circulating TERRA (AUC=0.76; 95% CI=0.624-0.873; $p=0.0004$). HA22T/VGH cells expressed TERRA, but most of the transcripts are released into the CM, also encapsulated in EVs (mean diameter=185nm, concentration=$163x10^6$/ml). Treatment of HCC cells with the multi-kinase inhibitor (KI) sorafenib significantly increased TERRA expression ($p=0.001$) and decreased ($p=0.01$) its release in EVs (mean diameter=252nm, concentration=$209x10^6$/ml).

Conclusions: Our results provide evidence on TERRA dysregulation in tissues and liquid biopsy of HCC patients, thus focusing on a novel potential non-invasive biomarker of diagnosis and downstream target of the KI. TERRA detected in the EVs of HCC cells open a new field of cancer research to comprehend its role at the extracellular level.

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11P Convoluted role of H19 long non-coding RNA in regulating ICAM-1 and PVR in breast cancer patients

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Background: Breast cancer (BC) is one of the major biomedical research priorities worldwide. Luminal B and triple negative BC patients represents the worst prognosis and highest incidence of recurrence. Among those patients, metastasis is the missing piece in the puzzle. Consequently, extensive research is urgently required to identify specific molecular targets responsible for luminal B and TNBC induced risk of metastasis. ICAM-1 (known as CD54) and PVR (known as CD155) are transmembrane glycoproteins that have been emerged as novel molecular targets that are markedly up-regulated in BC patients. Our group is recently focusing on unraveling the role of Long non-coding RNAs in regulating vital molecular targets in BC patients. LncRNAs regulating ICAM-1 and PVR is still a virgin field. Therefore, the aim of this study is to identify a lncRNA that could dually target ICAM-1 and PVR simultaneously.

Methods: Forty BC patients were recruited. Bioinformatic analysis was performed to identify a lncRNA that could directly or indirectly target ICAM-1 and PVR. MDA-MB-231 and MCF-7 cells were cultured. Cells were transfected using Scrambled and H19 siRNAs by lipofection Technique. Total RNA was extracted using biozol from tissues and cell lines. Reverse transcription and qRT-PCR were performed. MTT, scratch and colony forming assays were performed. Student t-test was performed for statistical analysis.

Results: In silico, H19 lncRNA was found to indirectly target ICAM-1 and PVR through STAT3 axis. H19, ICAM-1 and PVR were found to be upregulated in BC patients. Upon patients stratification, H19 and PVR were found to be specifically upregulated in luminal B BC patients while ICAM-1 was found to be specifically upregulated in TNBC patients. Upon Knocking down of H19, transfection effeciency was evaluated and H19 siRNAs resulted in paradoxical impacts on ICAM-1 and PVR. Yet, H19 siRNAs resulted in a repression of cellular viability, colonogenicity and migration capacity.

Conclusions: H19 inhibitory impact on the metastatic mediator ICAM-1 overrides its inductory effect on PVR in BC cells. Therefore, this study highlights H19 as an oncogenic lncRNA in BC and a novel molecular target for resistant luminal B and TNBC patients.

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12P DNMT1/3-targeting microRNAs are over-expressed in the exosomes of estrogen-resistant breast cancer cells

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Background: The hormonal therapy is among the most effective treatment of the hormone-dependent breast cancer however its efficiency is limited by the acquired resistance to the hormonal drugs. Previously we have shown the exosomes involvement in the transferring of the hormonal resistance from the resistant to the sensitive cells, and here the study of the features of the exosomes of the resistant cells was performed.

Methods: Experiments were performed on the MCF-7 breast cancer cells, MCF-7/T resistant subline developed under long-term tamoxifen treatment, and MCF-7/exoT resistant subline developed under resistant exosomes treatment. Exosomes were prepared from the conditioned medium by the differential ultracentrifugation, and exosome imaging was carried out by transmission electron microscope. The analysis of exosomal microRNAs was performed by HiSeq2500 and at least 5 million reads per samples were obtained. Library preparation was carried out with NEBNext® Small RNA Library Prep Set for Illumina® (E7330S). More than 2500 miRNAs were identified in the exosomal samples. DNA methylation was evaluated by the RRBS (Reduced Representation Bisulfite Sequencing) method.

Results: The analysis of the exosomal microRNAs revealed 471 microRNAs over-expressed in the exosomes of the resistant cells. Among them, three DNMT1/3-targeting microRNAs - miR-148b-3p, miR-193a and miR-383, were identified. The subsequent analysis of the cellular proteins revealed the decreased expression of DNMT1/ DNMT3 both in the primary-resistant MCF-7/T cells and in the exosome-treated MCF-7/exoT cells. The suppression of DNMT1/3 correlated with the hypomethylation of particular CpG islands in DNA of the resistant cells.

Conclusions: Taken together, the results obtained demonstrate the important role of the DNA (de)methylation in the exosome-mediated transferring and maintaining of the hormone resistance in the breast cancer cells.

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13P The role of long non-coding RNA RAMS11 in promoting colorectal cell development and metastasis

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Background: Over the past decades, accumulating research evidences revealed that abnormal expressions of long non-coding RNAs (lncRNAs) are associated with tumour initiation, progression, metastasis, and resistance to cancer therapies. Therefore, lncRNAs are considered to be potential biomarkers for many cancer types. In the current study, we examined the expression and molecular mechanisms of a newly identified lncRNA called RNA associated with metastasis 11 (RAMS11) and its association with the development of colorectal cancer (CRC).

Methods: Quantitative RT-PCR was used to determine the expression of RAMS11 in 4 CRC cell lines (DLD-1, HT-29, HCT-116, and SW480) and normal colon cells CCD-112-CoN. To evaluate the biological and physiological functions of RAMS11 in CRC cells, CCK-8 cell proliferation assay, colony formation assay, and wound healing migration assay were performed after RAMS11 knockdown. The expressions of autophagy/ apoptosis/mTOR/EMT pathway proteins were determined by Western blotting to evaluate the molecular mechanisms of RAMS11 in CRC cells.

Results: We found that RAMS11 was significantly upregulated in CRC cell lines compared to the normal cells. The knockdown of RAMS11 reduced CRC cells proliferation, and migration through mTOR dependent induction of autophagy, promotion of apoptosis, and inhibition of epithelial-mesenchymal transition (EMT) process.