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EACR 2023 Congress Abstracts

Contents

Proffered Papers

10-minute talks awarded for the highest scored abstracts, embedded in the scientific symposia sessions. These presentations are not accompanied by a poster.

Posters in the Spotlight

Tuesday 13 June, 17:30 - 18:30, Poster and Exhibition Hall
Wednesday 14 June, 17:15 - 18:15, Poster and Exhibition Hall

Dedicated sessions taking place in the spotlight area within the Poster and Exhibition Hall. Poster presenters with high scoring abstracts will give short presentations of up to 10 minutes. Their posters will also be available to view during the Poster Discussion Sessions.

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We explored the utility of cutting-edge computational tools to address the needle-in-a-haystack problem of identifying tumor-associated TCRs in LS. Our candidates are undergoing experimental validation in a T cell antigen specificity assay using a panel of MHC class II neoantigens. This study lays the groundwork for the development of a non-invasive cell-free monitoring of cancer development in LS patients.

EACR23-1157
Protocol optimization for cfRNA-based liquid biopsies

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Introduction
During the last decade, liquid biopsies have become increasingly important diagnostic tools in early-stage cancer due to their high sensitivity and minimal invasiveness. Cell-free RNA (cfRNA) is informative of both the circulating signature of the tumour and the systemic response of the host against it. Because of this, cfRNA is an invaluable tool for cancer early detection, when the number of tumour cells is limited. Unfortunately, since the field of cfRNA-based liquid biopsies is still young, several technical limitations remain, limiting the reproducibility and applicability of this approach.
The goal of this project is to study all the steps involved in the processing of plasma samples to determine their cfRNA profiles and define a standard, reliable and robust protocol for cfRNA analysis.
Material and Methods
We extracted blood from three donors, each in three different blood collection tubes. From each tube, we performed two different plasma isolation protocols. Then we tested two different commercial RNA isolation methods, three different DNAse treatment protocols and two different commercial library preparation kits.
After sequencing, we processed the data using an open-source bioinformatic pipeline and computed the correlation between the gene expression and performed multiple statistical tests between the samples to identify which of the processing steps affected the most the quality of the samples or changed the most their cfRNA profile.
Results and Discussions
Our results show very high variability between all the different methodologies. We observed that the blood collection tube and the RNA extraction and library preparation kits cause the highest variation in the cfRNA profiles, causing the greatest effect on the quality of the sample. However, we found that other steps, such as the blood plasma isolation and DNAse treatment, don't affect the samples as drastically as we initially thought.
Conclusion
Our results show that possible variations of the protocol have a high effect on the final cfRNA profile, masking the biological variability and biassing the results, highlighting the need for a defined and thoroughly tested protocol. Here we propose the optimal combination of methods for robust and reproducible cfRNA studies. Although there is still work to be done in order to obtain an optimised standard protocol, we have identified the most critical steps in the procedure, focusing on those that are crucial to generate the most accurate results.

EACR23-1167
MicroRNA-based signature in liquid biopsy for early detection of lung cancer

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Introduction
Lung cancer is the deadliest cancer worldwide. Programs screening has been applied in high-risk subjects (smokers; 50-75 yrs) and has proven to reduce lung cancer deaths in high-income countries. Biomarkers detection in body fluids may guide more precise selection of high-risk subjects but this approach has not been currently employed for lung cancer screening. **Aims:** To identify a miRNA-based signature for early detection of lung cancer in liquid biopsies.
Material and Methods
We analyzed plasma and sputum samples from high-risk subjects (n=54), and patients (n=60) diagnosed with non-small cell lung cancer (NSCLC), no metastatic. Samples were subjected to RNA isolation followed by miRNA expression using Human v3 miRNA panel (NanoString technologies). Counts were normalized by housekeeping candidates, and differentially expressed miRNAs were filtered out by Rosalind software according to fold-change ($\geq\pm1.3$), p-value (≤0.01), and ROC Curve (AUC>0.70).
Results and Discussions
Overall, high-risk subjects were stratified in LungRADS1 (n=14) and LungRADS2 (n=40), and cancer patients were diagnosed with adenocarcinoma (n=28), squamous cell carcinoma (n=27) and others (n=5). No differences were observed related to age, sex, and tobacco exposure in high-risk and cancer groups ($p>0.05$). For plasma, we observed seven differentially expressed miRNAs able to distinguish cancer from non-cancer samples, and three of them presented with high accuracy (AUC = 0.70 – 0.74). We observed a significantly different miRNA expression profile in plasma samples between early stage and locally advanced disease (fold-change $\geq\pm1.5$; p-value ≤0.01). For sputum, we observed four differentially expressed miRNAs able to distinguish cancer from non-cancer samples, and one of them presented with high accuracy (AUC = 0.70).
Conclusion
We identified fluid-specific miRNA signatures in liquid biopsies potentially to be employed in lung cancer screening programs to better guide selection of high-risk subjects for early detection of lung cancer.

EACR23-1195
Correlation between genetic and

proteomic expression of predictive biomarkers of response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer identified by DIA mass spectrometry

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Introduction

Neoadjuvant chemoradiotherapy (nCRT) has become common in treating locally advanced rectal cancer (LARC) to improve local control and survival rates, but the response to treatment varies. To identify who may benefit most from nCRT, previous research using DIA-MS identified 915 differentially expressed proteins between responder and non-responder groups. The current study aimed to evaluate the correlation between genetic and proteomic expression levels of candidate markers of response.

Material and Methods

We used the STRING analysis tool to understand better the interaction between differentially expressed proteins (DEPs). After the analysis was performed the transcription-level tool ROCplotter was used to shortlist potential biomarkers. The study included 42 patients with locally advanced rectal cancer (LARC), categorized as responders or non-responders, and selected promising biomarkers with an AUC> 0.7, ROC p-value <0.05, and Mann Whitney p-value <0.05. *In silico* analysis was used to determine candidates for validation in a cohort of 45 patients treated with neoadjuvant chemoradiotherapy. Gene expression analysis was performed on candidates with the best predictive potential using qRT-PCR and TaqMan Gene Expression Assay. Statistical analysis was performed using Mann Whitney t-test and ROC curve analysis (p-value <0.05).

Results and Discussions

After performing STRING analysis, the data showed that the responder group had several protein-rich groups with high levels of interactions, particularly in pre-mRNA processing. There was a strong correlation between proteins involved in information RNA processing and genes whose protein products participate in translation. The PPI enrichment p-value was less than 1.0e-16. Out of 915 differentially expressed proteins (DEPs), ROCplotter analysis identified 23 promising biomarkers that met all three criteria. Out of 23 DEPs, we selected the top four genes (CRKL, HAS1, COPB1, and MGLL) to validate in our cohort of samples. However, after statistical analysis, there was no correlation between gene expression and protein expression in the analyzed cohort of samples ($p>0.05$).

Conclusion

Changes in the expression profile of analyzed genes may be regulated on the post-transcriptional level. Translation control is essential in differentiating responses to neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer.

EACR23-1196
Metabolic Profiling of Extracellular Vesicles from Ascitic Fluids of Ovarian Cancer Patients

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Introduction

The accumulation of ascitic fluid (AF) in the peritoneal cavity is a hallmark of Ovarian Cancer (OvC), the most lethal gynecological malignancy. AF is enriched in cells and their secreted products, which contain cues that promote tumor growth and dissemination. One of such products, extracellular vesicles (EVs), acts as versatile cell-cell communication mediators and have been described to be involved in the pathogenesis of cancer, namely promoting metastasis and chemoresistance. As such, EVs have emerged as potential non-invasivecirculating biomarkers in liquid biopsies for the early detection and prognosis of diseases. Yet EVs remain largely under-studied regarding their metabolic content. Here we present a platform for the isolation and metabolomic characterization of AF-derived EVs from OvC patients which can be applied to identify novel disease biomarkers and therapeutic targets.

Material and Methods

EVs were isolated from 14 OvC patient biofluids, either AF (11) or peritoneal washes (3), using an ultracentrifugation-based protocol, and further