SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

NOME E COGNOME: Stefania Crucitta

UNIVERSITÀ: Università di Pisa

DIPARTIMENTO (in caso di borsa per soggiorno all'estero specificare l'ente presso cui si è svolta la ricerca): Department of Urology, Erasmus MC, Rotterdam, The Netherland

TUTOR (in caso di borsa per soggiorno all'estero specificare il tutor dell'ente presso cui si è svolta la ricerca): Dr. Elena Martens-Uzunova

TIPOLOGIA DI BORSA RICEVUTA: Borsa per soggiorno all'estero

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): Finale

TITOLO DELLA RELAZIONE: Detection of small RNAs associated with prostate cancer in liquid biopsies

RELAZIONE:

MiRNAs are the focus of much attention because of their function as modulators of gene expression and their strong biomarker properties [1]. The Erasmus MC (Guido Jenster, Elena Martens-Uzunova) has an established expertise in the analysis of miRNAs and other small non-coding RNAs (sncRNAs) [2]. Recently, miRNA expression during PCa progression was analyzed in 102 PCa tissue samples by microarrays and sncRNA sequencing [3]. Differential expression analysis identified and independently validated a diagnostic classifier (from 54 miRNAs) that detects PCa with 95% accuracy. The diagnostic and prognostic performance of this miRNA subset was further validated in independent patient cohorts. The combination of the 4 best performing miRNAs called miRNA index quote (miQ) predicts PCa with high accuracy ranging from 0.77 to 0.95 in 5 different cohorts. miQ is a robust marker successfully discriminating PCa from non-PCa even in specimens containing <25% tumor cells. Importantly, miQ is an independent predictor of aggressiveness which outperforms PSA, and has clear potential to be used as a clinical tool for PCa diagnosis and as a prognostic marker of disease progression [4].

Over the recent years, deep sequencing technologies targeting the miRNA transcriptome revealed the existence of many different RNA fragments derived from small noncoding RNA (sncRNAs) species other than miRNA. Although initially discarded as RNA turnover artifacts, accumulating evidence suggests that smaller RNAs derived from small nucleolar RNA (snoRNA) and transfer RNA (tRNA) are not just random

degradation products but rather stable entities, which have functional activity in the normal cell and are deregulated in cancer [5].

It was demonstrated that the small RNA transcriptome of PCa is dominated by sncRNAs other than miRNAs. Many of these sncRNAs originate from snoRNAs and tRNAs and are referred to as snoRNA-derived RNAs (sdRNAs) and tRNA-derived fragments (tRFs), respectively. Detailed analysis of deep-sequencing data on PCa specimens from radical prostatectomies shows that sdRNAs and tRFs are upregulated in malignant tissue compared with normal prostate or benign prostate hyperplasia tissue and are candidate diagnostic biomarkers. Validation qPCR results show that snoRNA and sdRNA expression is significantly increased in cancerous compared to normal adjacent prostate further supporting these sncRNAs as novel diagnostic markers. Furthermore, the expression of 2 snoRNAs and derived from them sdRNAs is further increased already at the time of radical prostatectomy in a specific subset of PCa patients that developed aggressive metastatic cancer years after surgery [6]. Besides snoRNA and sdRNA, the expression levels of two tRFs with opposing expression patterns (tRF-544, derived from tRNAPheGAA and tRF-315, derived from tRNALysCTT) are associated with high grade, recurrent disease. The calculated expression ratio tRF-315/tRF-544 in two separate cohorts (from EMC, n=50 and from the university of Tampere, Finland, n=104) significantly discriminates high from low grade PCa (Gleason score < 7 vs. Gleason score ≥ 7). Moreover, high expression ratio is significantly associated with poorer progression-free survival and shorter period to disease relapse PSA relapse after radical prostatectomy) [7].

The aim of this project is to investigate the expression levels of panels of small RNA markers for PCa using plasma samples from men with various stages of disease.

Methods

Blood samples were collected with two different tubes (*Cell Safe* and *EDTA*) and centrifuged at 1600 g for 10 min at RT. Plasma samples were then centrifuged at 12000 g for 10 min at 4°C to remove cellular debris. Plasma was stored at - 80 °C until analysis.

Small RNA isolation (extracellular vesicle RNA and total RNA) from 200 µl of plasma samples was tested with different kit. RNA extracted was reverse transcribed using TaqMan cDNA miRNA Synthesis kit (Applied Biosystems, Waltham, Massachusetts, USA). Quantitative real-time PCR (qPCR) was performed on an Applied Biosystems ABI 7900 thermocycler (Applied Biosystems, Waltham, Massachusetts, USA) and on StepOne Plus (Thermofisher Scientific).

Results

During the first part of this project, I started to test whether the qPCR assays work using RNA extracted from healthy donor plasma samples. Firstly, I tested the hemolysis ratio as previously described [8]. Blondal et al. (2013) suggested that the ratio of red blood cell-enriched miR-451a to miR-23a, the latter microRNA being unaffected by hemolysis, can be used as a surrogate indicator of hemolysis. The delta Cq

(miR-23a-miR-451) of <5, 5–8 and >8 are, respectively, indicative of samples at low, moderate or severe risk of hemolysis [8]. All the EVs RNA samples were under the threshold of 5, suggesting the lower risk of erythrocyte contamination. Instead, 6 of the total RNA samples were over the threshold of 8, suggesting the high risk of hemolysis.

Then, I analyzed the expression of snoRNA, tRFs, miQ and miRNAs. The qPCR analysis of the expression of snoRNA and tRFs resulted differently between samples isolated with both methods. The qPCR analysis of the expression of miQ miRNAs was similar between samples. Interestingly, there was no amplification of miR183 in EVs RNA samples compared to total RNA samples. The qPCR analysis of the expression of miRNA21, miR214 and miR378 were similar between samples. The use of two different blood collection tubes (Cell safe and EDTA) didn't interfere with the qPCR analysis.

During the last part of this experience, I tested the hemolysis ratio on EVs RNA isolated from 11 cancer patients. All the samples were over the threshold of 5, suggesting severe risk of hemolysis and erythrocyte contamination. Then, I analyzed the expression of miQ and miRNAs The qPCR analysis of the expression of miQ miRNAs resulted differently between samples. The expression of miRNA 375 and miRNA 214 was present only in one sample, instead, miRNA 21 was expressed in all the samples analysed. The measured levels of miRNAs in plasma from patients varied in the presence of hemolysis, and since hemolysis and other factors affected miRNA expression, it is important to consider these confounders while developing miRNA-based assays. In conclusion, this methodologic study demonstrates the feasibility of small RNA isolation method in plasma samples and detection by qPCR. Further studies will be direct to understand how to avoid erythrocyte contamination.

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Data 18/12/2018 _____ Firma Stefano Cucurachi _____

Da inviare a: Società Italiana di Farmacologia – e-mail: sif.soci@segr.it; sifcese@comm2000.it