



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):** Metà periodo

**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)

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

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  $\geq$  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.

During the first part of this project, I started to test whether the qPCR assays work using RNA extracted from healthy donor plasma samples.

Small RNA isolation (extracellular vesicle RNA and total RNA) from 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).

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.

Next step will be the detection of miRNA, snoRNA sdrRNA and tRFs in plasma (Evs and total RNA) from metastatic prostate cancer patients and evaluate their diagnostic, prognostic, predictive, and monitoring potential.

## REFERENCES

- [1] Fabris L, Ceder Y, Chinnaiyan AM, Jenster GW, Sorensen KD, Tomlins S, Visakorpi T, Calin GA. The Potential of MicroRNAs as Prostate Cancer Biomarkers. *Eur Urol.* 2016 Aug;70(2):312-22
- [2] Martens-Uzunova ES, Olvedy M, Jenster G. Beyond microRNA--novel RNAs derived from small non-coding RNA and their implication in cancer. *Cancer Lett.* 2013 Nov 1;340(2):201-11
- [3] Martens-Uzunova ES, Jalava SE, Dits NF, van Leenders GJ, Møller S, Trapman J, Bangma CH, Litman T, Visakorpi T, Jenster G. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. *Oncogene.* 2012 Feb 23;31(8):978-91
- [4] Larne O, Martens-Uzunova E, Hagman Z, Edsjö A, Lippolis G, den Berg MS, Bjartell A, Jenster G, Ceder Y. miQ--a novel microRNA based diagnostic and prognostic tool for prostate cancer. *Int J Cancer.* 2013 Jun 15;132(12):2867-75.
- [5] Bijnsdorp IV, van Royen ME, Verhaegh GW, Martens-Uzunova ES. The Non-Coding Transcriptome of Prostate Cancer: Implications for Clinical Practice. *Mol Diagn Ther.* 2017 Aug;21(4):385-400. doi: 10.1007/s40291-017-0271-2.
- [6] Martens-Uzunova ES, Hoogstrate Y, Kalsbeek A, Pigmans B, Vredenburg-van den Berg M, Dits N, Nielsen SJ, Baker A, Visakorpi T, Bangma C, Jenster G. C/D-box snoRNA-derived RNA production is associated with

malignant transformation and metastatic progression in prostate cancer. Oncotarget. 2015 Jul 10;6(19):17430-44.

[7] Olvedy M, Scaravilli M, Hoogstrate Y, Visakorpi T, Jenster G, Martens-Uzunova ES. A comprehensive repertoire of tRNA-derived fragments in prostate cancer. Oncotarget. 2016 Apr 26;7(17):24766-77. doi: 10.18632/oncotarget.8293.

[8] Blondal T, Jensby Nielsen S, Baker A, Andreasen D, Mouritzen P, Wrang Teilum M, et al. Assessing sample and miRNA profile quality in serum and plasma or other biofluids. Methods. 2013 Jan;59(1):S1-6. doi: 10.1016/j.ymeth.2012.09.015.

La Società Italiana di Farmacologia dichiara che i dati personali comunicati dal Socio sono trattati in conformità alle disposizioni del D. Lgs. 196/2003 ed alla normativa comunitaria secondo quanto indicato specificamente nell'informativa privacy reperibile sul sito internet della Società all'indirizzo [https://sif-website.s3.amazonaws.com/uploads/attachment/file/240/Informativa\\_Privacy\\_SIF\\_Generica.pdf](https://sif-website.s3.amazonaws.com/uploads/attachment/file/240/Informativa_Privacy_SIF_Generica.pdf) che il Socio, con la sottoscrizione del presente Contratto, dichiara di aver compiutamente visionato, compreso e accettato.

Data 12/10/2018

Firma



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