



A microRNA molecular signature of aggressive prostate cancer

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To date, prostate cancer (PCa) remains a medical challenge, being one of the most prevalent causes of cancer deaths in men worldwide (1). A major innovation in the management of PCa was demonstrated by the measurement of prostate specific antigen (PSA) in the serum of PCa patients in the mid-1980s; however, it is well known that measurement of PSA levels is associated with over-diagnosis and over-treatment. Although over-treatment may be reduced by improved risk stratification where very low or low risk PCa can be monitored by active surveillance and intermediate or high risk PCa can be subjected to treatment, many urologists and patients are reluctant to delay treatment due to the absence of a reliable indicator of aggressive disease and thus, there is a possibility of missing treatment of aggressive PCa patients (2,3). A single threshold PSA test is unable to distinguish between high and low risk PCa (4), and prostate biopsy is often unreliable for the prediction of cancer grade, as only a small fraction of the prostate is sampled during a biopsy for staging (5). Several promising blood based and urinary biomarkers such as the prostate health index (PHI), 4K score and PCA3 for tumour aggressiveness have been identified and recommended to reduce the number of unnecessary biopsies in PSA tested men (6,7). However, in order to appreciate the clinical value of these biomarkers, additional unbiased prospective studies are still required. It is anticipated that the availability of unique molecular signatures and novel biomarkers would lead to an improvement in the management of patients with aggressive PCa, and microRNAs (miRNAs) are pioneers in this area.

The prevailing understanding was that the genome consists of regions with little coding material of importance; however, recent advances have shown that these regions are not so

barren after all. A part of the so called “non-coding” genome in fact encodes for critical gene regulators called miRNAs that are present in stable forms in the circulation and thus, can play an important role as diagnostic and prognostic biomarkers for several diseases (8). Mature miRNAs were initially detected in the cell free fractions of blood such as serum and plasma (8), and subsequently found in other body fluids and tumour tissues (9). We have recently reviewed the diagnostic and prognostic value of miRNAs, along with several detection methodologies which provides important insights into the use of miRNAs as non-invasive cancer biomarkers (9).

Knowledge about uptake, packaging and release of miRNAs is crucial to determine their regulatory functions, and double lipid membrane vesicles, called exosomes, have been found to play a crucial role in this regard (10). The usefulness of exosome miRNAs has been evaluated by several studies including a report by Li *et al.* showing elevated levels of exosomal miR-141 in metastatic PCa patients in comparison to benign prostatic hyperplasia (BPH) patients and healthy controls (11). Similarly, Huang *et al.* showed that the plasma exosomal level of miR-1290 and miR-375 was associated with poor survival of castration resistant PCa patients (12). Apart from serum and plasma, Samsonov *et al.* indicated upregulation of miR-21, miR-141 and miR-574 in urinary exosomes isolated from PCa patients and healthy controls using a lectin-based agglutination method (13). Therefore, exosomal miRNAs may be utilized as non-invasive molecular signatures specific to patients with an increased risk of developing aggressive PCa, but it is difficult to differentiate intermediate grades from aggressive forms due to the heterogeneity of PCa.

To address this issue, in a recent study published in

Proceedings of the National Academy of Sciences (PNAS) (14), Alhasan and colleagues developed a high-throughput, spherical nucleic acid (SNA)- and microarray-based miRNA expression profiling platform, called the Scano-miR bioassay, to determine the exosomal miRNA expression. Authors used the Scano-miR bioassay in a discovery set of 16 serum samples from patients with varying grades of PCa, i.e., ≥ 8 Gleason Score for high or very high risk and Gleason Score =6 for very low or low risk PCa, and healthy individuals. Furthermore, a molecular signature score was calculated, as done by Zeng *et al.* previously (15), to ensure diagnostic reliability upon combining the differentially expressed miRNAs in a blind study. In this way, a molecular signature consisting of five miRNAs (miR-200c, miR-605, miR-135a*, miR-433 and miR-106a) was identified capable of differentiating indolent and aggressive forms of PCa with 89% accuracy after validation in a second cohort by quantitative real time PCR (qRT-PCR) (14). This unique molecular signature may assist in stratifying patients who may benefit from therapy from those who may only require close monitoring through active surveillance.

In the above study, the serum expression of miR-200c was highly elevated in very high risk PCa patients suggesting its role in predicting metastasis, and similar results have been obtained in studies focussing on colorectal and gastric cancer (16,17), suggesting the role of this miRNA as a general marker of metastasis. In another study, miR-200c has been found to be part of a five biomarker panel for the detection of metastatic castration resistant PCa supporting the findings of Alhasan and colleagues (18). Dysregulation of miR-106a has been reported in lung and gastric cancer (19) and has been previously linked to PCa. miR-106a was also one of the previously reported participant of several biomarker panels for high risk PCa (20), but the other miRNAs have not been previously associated with PCa.

PCa is very heterogeneous in nature where cancer specific survival rate is higher in patients with a low risk of disease progression compared to those with aggressive disease. The investigation carried out by Alhasan and colleagues lead to the discovery of a novel miRNA signature capable of differentiating indolent from aggressive forms of PCa at a higher rate than typical Gleason scoring of biopsy samples, representing a simple diagnostic tool without the need for surgical intervention. The Scano-miR bioassay does not rely on enzymatic amplification of a specific target as many of the current miRNA detection methods do, and therefore, allows multiplexing and detection of multiple miRNAs at the femtomolar levels in single samples. In

addition, pathway analysis was also performed to identify targets of the miRNA panel and some of the targets were found to be known drivers of tumorigenesis.

So far, specific miRNA expression patterns have been proposed for PCa subclasses, but further studies are required and several questions need to be addressed to aid the establishment of miRNA signatures for cancer diagnosis and prognosis. Some of these necessitate an understanding of the relationship between circulating and tumour derived miRNAs, their mechanism of uptake and release, their response to inflammation and modifications, and finally their role in tumorigenesis and metastasis. Although several new techniques have improved the specificity and sensitivity of miRNA detection, the lack of referenced procedures for sample preparation, RNA extraction, endogenous control selection, sample size calculation, etc. makes it difficult to compare results between independent studies. The major challenge is to overcome these hurdles and identify reliable miRNA biomarkers for the stratification of cancer patients. The development of digital PCR would remove the dependence on a miRNA normalization control which is not yet established for qRT-PCR, where an exogenous control is used mostly. Besides, digital counting technologies such as next-generation sequencing (NGS) and the NanoString nCounter miRNA Expression Assay may be used for miRNA profiling. NGS allows the discovery of new miRNAs and confirmation of already known miRNAs as opposed to microarrays and qRT-PCR, and overcomes the limitations of background signal, microarray panel difference and cross-hybridization issues associated with the use of microarrays. The NanoString nCounter system is another new hybridization-based technology which directly detects miRNAs of interest using target specific, colour-coded probes without the need for reverse transcription or amplification by qRT-PCR (20). All these advancements in the area of biomarker research and inclusion of other criteria such patient age, PSA level, clinical tumour stage, Gleason Score, etc. for risk assessment from a PCa perspective followed by multivariate analyses, will not only help in accurate cancer detection, but will also facilitate the development of novel strategies for cancer therapy.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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