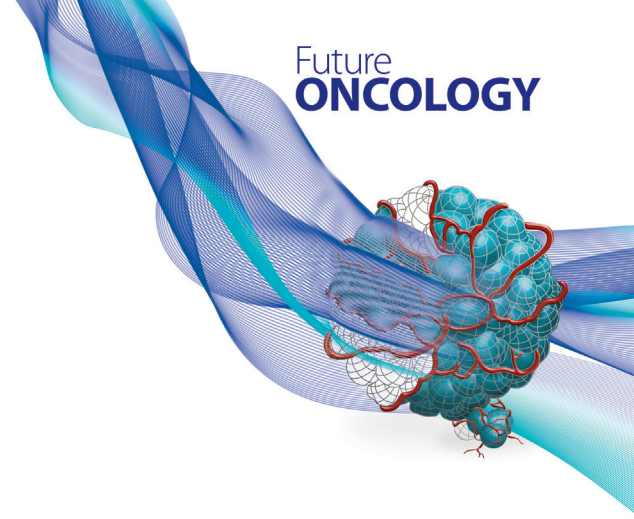
Future ONCOLOGY



COMMENTARY

Harnessing novel modalities: field carcinogenesis detection for personalizing prostate cancer management

"Partial wave spectroscopic microscopy represents a sea change in biomedical optics and may provide an approach to bring 'precision medicine' to the management of early-stage malignancies, especially prostate cancer as a platform to tackle the issue of cancer overdiagnosis."

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Prostate cancer

Despite being the most common solid organ cancer in American males (one in every six men), the mortality rate from prostate cancer (PCa) ranks only fifth in USA (one in every 35 men) [1]. This discordance is due to the indolent nature of most PCa, presenting one of the most vexing issues of PCa patient management – that is, 'overdiagnosis'. Unfortunately, with the increasing use of prostate-specific antigen (PSA) as the first-line clinical biomarker, it is estimated that 40–50% of PCa patients are overdiagnosed and treated without benefit [2], thus providing the impetus for the US Preventive Services Task to recently recommend against PSA screening in all men [2,3]. We discuss, herein, results from our recent investigations of a novel approach to ameliorate the PCa overdiagnosis conundrum by predicting disease aggressiveness on the index surveillance biopsy [4].

Attempts to predict PCa aggressiveness have relied upon the usual pathological parameters, including cancer grade (Gleason score), cancer stage, number of cores involved with cancer (% cancer/core), PSA density, among others. In clinical practice, the Gleason score approaches status as the prognostic 'gold standard'. It is determined by the summation of the scores for histological grade of the most common and the next most common or most aggressive clones, underscoring the heterogeneity that is the hallmark of the disease. Gleason scores of 1–4 equate to well differentiated and usually indolent PCa, while scores of 8–10 correspond to poorly differentiated and biologically aggressive disease. However, most men with PCa have an intermediate Gleason score of 6–7 with ambiguous clinical connotations [2]. The dilemma is that while most patients will have innocuous disease making any therapy unnecessary, a minority (~10%) will progress to potentially fatal disease, and delaying treatment may risk losing the window of opportunity for cure. Current guidelines eschew definitive therapy in favor of active surveillance (close monitoring with serial prostate biopsies) [5]. Unfortunately, due in large part to patient and physician concerns over silent progression to potentially fatal cancer, only one in ten eligible men actually undergo active surveillance [6]. Hence, one of the major clinical challenges in PCa is to develop accurate biomarkers for prognostication of Gleason 6–7 disease.

KEYWORDS
• biomarker • miRNAs
• nanocytology • overdiagnosis
• prostate cancer • PWS

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Concept of field carcinogenesis

One emerging approach for risk stratification is through exploiting the concept of field carcinogenesis: the idea that a focal neoplastic lesion originates in a fertile mutational background and fosters transformation through the accumulation of stochastic genetic and epigenetic events. These molecular changes are accompanied by alterations in high order chromatin that are below the diffraction limit of light and hence appear pathologically normal. However, if subjected to super-resolution approaches (e.g., electron microscopy), striking nano-architectural alterations are identified [7,8]. Indeed, using a novel optical technology, partial wave spectroscopic microscopy (PWS), our group has demonstrated profound changes in field carcinogenesis in colon, pancreas, esophagus, ovarian and lung malignancies [9–12]. While less explored in prostate, there is compelling evidence of field effect in this multifocal malignancy as indicated by genetic similarities in the multiple independent neoplastic clones [13,14]. Furthermore, a plethora of molecular markers (gene expression, methylation and mitochondrial alterations, among others) have been postulated as forerunners of PCa tumorigenesis and observed to be dysregulated in microscopically normal epithelium from patients harboring prostate cancer (i.e., field carcinogenesis) [15–17]. Some of these have already been applied in clinical practice via the clinical laboratory improvement amendments (CLIA)-based laboratory developed tests (LTDs) such as ConfirmMDx (epigenetic field defects) and the Prostate Core Mitotic Test (detecting mitochondrial DNA) [18].

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Other currently available US FDA approved biomarker tests for PCa prognostication include the (Prostate Health Index [PHI] blood test) and the PCa antigen 3 (PCA3) urine test. Several tissue-based tests have also been recently CLIA-certified, including Oncotype DX® (proliferation pathway), Prolaris (cell progression genes), Prostarix (metabolic pathway) and ProMArk (genetic modulations accessed by immunofluorescent imaging). Since most PCa possess multiple foci, it is believed that a combination of markers would be more predictive, for example, Mi-Prostate Score (combination of T2-ERG fusion rearrangement recurrency + PSA levels + PCA3 tests) and 4K score (combination of total PSA, free/unbound PSA, intact PSA and kallikrein-related peptide 2 [hK2]) [18]. The overall PCa biomarker field is rapidly evolving, and, thus, it is difficult to forecast which tests will have traction in the clinical arena.

MiRNAs: potential molecular assessment of PCa field carcinogenesis

Recently, miRNAs have garnered attention as important epigenetic modulators of gene expression. A number of groups, including our own, have shown that miRNA may be altered in field carcinogenesis in organs such as the colon [19]; however, there are only minimal previous reports regarding PCa. As a proof of concept, we performed TaqMan Low-Density Array (TLDA) Cards (Applied BioSystems) on PCa samples from patients with Gleason 6 PCa undergoing active surveillance in a case–control design. We noted that in future progressors (median follow-up ~3 years, there were profound alterations in miRNA from sections of predominantly normal epithelial/stromal cells **(Figure 1)**. There were significant miRNA alterations in progressors versus nonprogressors with both upregulation (*miR-26b*, *29c*, *132*, *329*, *363*, *517*, *519*, *520* and *Let-7a*) and downregulation (*miR-155*, *194*, *301b*, *361*, *455* and *140*). While preliminary, this demonstrates that molecular signatures may be able to serve as accurate prognostic markers. One concern, however, is that the known molecular heterogeneity of PCa may also occur in field carcinogenesis, thus possibly impugning the robustness of these approaches.

PWS: potential optical assessment of PCa field carcinogenesis

Assessing high order chromatin or other global markers of neoplastic transformation could conceptually be more attractive, since these changes are believed to be one of the final common denominators in carcinogenesis. One factor that has impeded implementation of this strategy in clinical practice has been practicality. For instance, although transmission electron microscopy has been the standard and highly accurate technique, it is both expensive and labor intensive, and thus, not translatable into clinical practice. Our group, therefore, developed PWS to probe the nanoarchitecture with unprecedented accuracy and convenience. This novel technique exploits photons traveling in a single dimension, allowing interrogation of the nanoscale landscape (20–200 nm). This approach provides statistical insight via assessment of fluctuations in refractive index termed disorder strength (L_d). Elevation in L_d appears to be a ubiquitous event in early

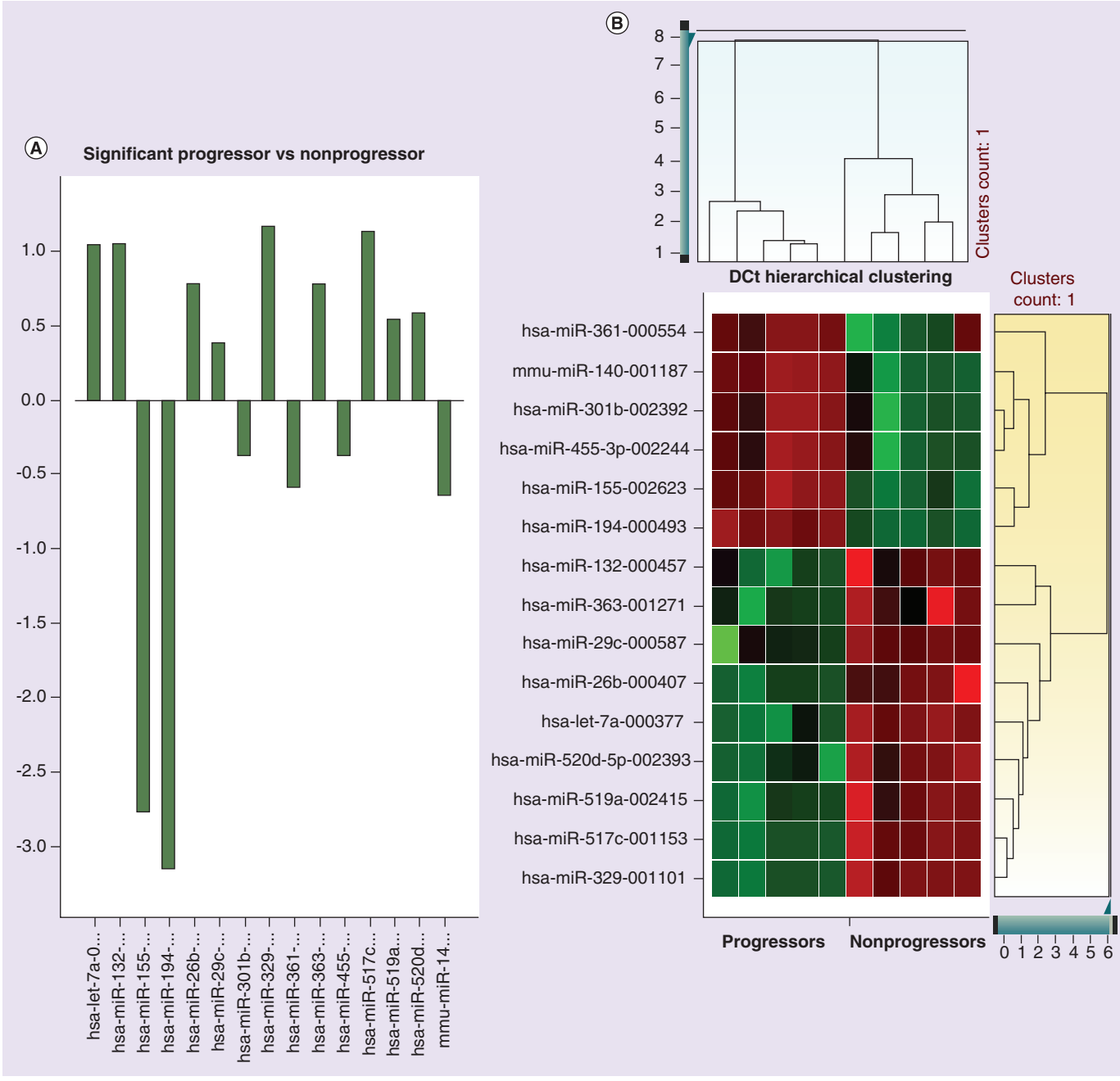

Figure 1. miRNAs as prognostic biomarkers of aggressive prostate cancer. (A) Differentially expressed miRNAs in transrectal biopsies from patients who were future progressors versus nonprogressors to prostate cancer. Fold change was calculated as log10 of relative quantitation. **(B)** Hierarchical clustering of DCt values of differentially regulated miRNAs in progressors versus nonprogressors.

carcinogenesis and may reflect the transcriptional activation that is critical for neoplastic transformation [20]. Conceptually, L_d represents 'clumpiness' of the chromatin, suggesting increasing heterogeneity (e.g., nucleosome unwinding and DNA looping, among others) and hence transcriptional activation [8]. We have extensively validated L_d as a highly sensitive noninvasive biomarker in the normal epithelium accurately predicting risk of colon, pancreas, esophagus, ovarian and lung carcinogenesis [9–12]. In a recent publication, we applied this technology to PCa clinical prognostication in order to mitigate the harms of overdiagnosis [4].

With a case control design, we assessed PWS readings from 38 Gleason patients prospectively

enrolled in an active surveillance program at a single institution (NorthShore University HealthSystem). We were able to adapt PWS to histological slides (previous studies used cytological brushings) to interrogate ~30 regions of normal-appearing epithelium per patient slide (encompassing ~150 epithelial cells). Importantly, we observed that patients who subsequently progressed pathologically demonstrated neoplastic transformations with a profound increase in nanoarchitectural disorder indicated by an increased mean L_d, compared with future nonprogressors (1.30 ± 0.0614 vs 1 ± 0.065, respectively, p = 0.002). There were no variations in the brightfield readings between the progressors and nonprogressors, providing further evidence that the observed changes are not occurring at molecular/histological levels, but at genetic/epigenetic levels. With both high sensitivity (88%) and specificity (72%), L_d differentiated progressors from nonprogressors with very good accuracy (area under the receiver operator characteristic curve of 0.81) [4]. While our previously reported PWS-based studies were for prediction of risk of presence of neoplasia, this was our first successful attempt to perform nanocytology to prognosticate.

Conclusion & future perspective

This performance estimates should be considered a baseline, with future improvement likely by separately assessing nuclei versus cytoplasm and also epithelium versus stromal to develop a more precise marker of disorder. Furthermore, prostate field carcinogenesis may not represent a stand-alone approach, but one to be combined with other tissue (e.g., miRNAs), blood (e.g., free PSA) and urine (e.g., PCA3) biomarkers to further enhance prognostication. Overall, the assessment of biomarkers of field carcinogenesis is particularly apropos for PCa, given the innate heterogeneity resulting from multiple independent tumor clones. This complements the Gleason scoring system which also focuses on this tumor heterogeneity. Intriguingly, our studies present miRNAs as potential field markers for consequential prostate tumorigenesis. From a perspective of practicality and robustness (i.e., avoidance of overfitting), the ability to distill nanoarchitectural disorders into a single biomarker (L_d) has considerable appeal, making this approach potentially superior to other modalities. PWS represents a sea change in biomedical optics and may provide an approach to bring 'precision medicine' to the management of early-stage malignancies, especially PCa as a platform to tackle the issue of cancer overdiagnosis.

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