IN THE SPOTLIGHT

miRSNP-Based Approach Identifies a miRNA That Regulates Prostate-Specific Antigen in an Allele-Specific Manner

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Summary: A recent study identified genetic variations within the miRNA response elements of miRNA target genes (miRSNP) that can affect the base paring between miRNAs and their targets, and hence alter miRNA–target interactions. The authors also undertook functional validation studies and were able to demonstrate that overexpression of *miR-3162-5p* resulted in a 20% decrease in expression of the *KLK3* rs1058205 SNP T-allele. *Cancer Discov; 5(4); 351–2. ©2015 AACR.*

See related article by Stegeman et al., p. 368 (4).

The promise of genome-wide association studies (GWAS) is to uncover SNPs that are related to human disease. There is a spectrum of potential clinical applications through which GWAS can help to improve patient care, including disease risk prediction and screening, disease classification, and drug development and toxicity (1). In the past few years, a wave of GWAS has identified thousands of genome wide–significant associations with more than 500 human complex traits, including susceptibility to a wide variety of diseases, such as cancer (2).

After a period of initial enthusiasm, however, it became obvious that there are a number of challenges that we need to face to be able to obtain complete descriptions of the susceptibility architecture of biomedical traits of interest and to translate the information gathered into improvements in clinical management. These include the need for systematic, well-powered, genome-wide surveys with a rigorous study design. Another interesting observation was that studies have identified many variants with each affecting multiple traits, suggesting that pleiotropic effects on human complex traits may be widespread (3). Moreover, there is still a gap between GWAS findings and the identification of "causal" variants.

miRNAs are small noncoding RNAs that are predicted to regulate up to two thirds of all human genes. They are reported to contribute to a number of critical biologic processes, including cell proliferation, differentiation, and development. They have also been documented to be involved in the pathogenesis of a number of human malignancies. miRNAs function via base pairing with complete or partially complementary sequences within the 3′-untranslated region of their target mRNAs. This results in downregulation of the target protein through either mRNA cleavage, destabilization of the mRNA through shortening of its poly(A) tail, or blocking protein translation.

In the current issue of *Cancer Discovery*, Stegeman and colleagues (4) highlighted a new dimension in GWAS, which is the identification of genetic variations within the miRNA response elements of miRNA target genes (called miRSNPs) that can affect the base paring between miRNAs and their targets and hence can either enhance or abolish miRNA–target interactions. The authors investigated the role of 2,169 putative miRSNPs within prostate cancer–related genes by evaluating the genetic association of these miRSNPs in a large cohort of approximately 50,000 individuals recruited through 23 studies involved in the PRACTICAL consortium. This is the first large-scale study conducted on miRSNPs in prostate cancer, which led to the identification of 22 miRSNPs to be associated with prostate cancer risk. As expected, these miRSNPs are mostly close to the previously identified prostate cancer risk regions identified by genome-wide association studies; however, this study has narrowed down these regions of putative causal SNPs, leading to the identification of candidate genes *PHC3*, *GMEB2*, *PDK1*, *ARL3*, *MCAT*, *TTLL12*, and *TMEM17*, whose function in cancer pathogenesis needs to be evaluated in future studies. The authors also reported two miRSNPs, rs1010 in *VAMP8* and rs311497 in *GMEB2*, to be associated with aggressive prostate cancer.

In addition to the importance of SNPs of the coding genome, more attention has recently been directed to SNPs in introns and noncoding regions. Recent studies highlighted the presence of disease-associated SNPs found within the miRNA genes or located in their precursor molecules (pre-miRNA) and their adjacent upstream/downstream regions. A publicly available database and search engine has recently been developed (http://202.38.126.151/hmdd/mirsnp/search/) for the identification of putative miRNA-related SNPs from GWAS and to provide a prediction for subsequent functional impact of these SNPs on the efficiency of related miRNA–target interactions (5).

The study by Stegeman and colleagues (4) highlights the need to investigate the functional significance of SNP and opens the door for the discovery of new classes of biomarkers

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that can enhance our ability for diagnosis or assessment of prognosis for many diseases, including cancer. The results also bring PSA, the well-known prostate cancer biomarker, back to the stage as a player in prostate cancer pathogenesis (6). It is interesting to note that the PSA [encoded by the human kallikrein-like peptidase 3 gene (*KLK3*)] has been highlighted using this approach. Moreover, the study suggests functional/causal associations between risk-related miRSNPs and the pathogenesis of prostate cancer. The authors undertook functional validation studies and were able to demonstrate that overexpression of *miR-3162-5p* resulted in a 20% decrease in luciferase signal for the *KLK3* rs1058205 SNP T-allele vector construct, whereas no significant change was observed for the C allele, suggesting that *miR-3162-5p* has specific affinity for the T allele. Further validating these results, they observed a 36% reduction in cellular KLK3 protein expression following *miR-3162-5p* overexpression in the LNCaP cell line (TT rs1058205 SNP), demonstrating that decreased KLK3 expression induced by *miR-3162-5p* targeting of the T allele represents a mechanism by which the rs1058205 T allele may be associated with increased prostate cancer risk. These observations are further supported by the association of *KLK3* rs1058205 with serum PSA levels.

The genetic factors influencing PSA expression may have implications in prostate cancer risk prediction, and interpretation of PSA screening might therefore need correction for these risk-associated functional KLK3 genetic variants. The authors also provided proof-of-concept by demonstrating the functional role of an additional miRSNP associated with aggressive disease. It would be interesting to establish the functional impact of all 22 miRSNPs identified in this study on miRNA binding. It would also be worth studying the combination of miRSNPs and the affected miRNAs for their potential as a diagnostic and prognostic biomarker for prostate cancer.

The study also bridges an important gap in our understanding of the risk for prostate cancer. The number of SNPs that were identified in previous studies accounts for only a small proportion (around 35% of the familial risk), suggesting that additional SNPs remain to be identified (7). The results of the study can also have important therapeutic implications. miRNAs represent attractive therapeutic targets because of their small size, easier transfection, and less toxicity. The miRNA–target interactions can be of therapeutic benefit.

Although the differential expression of miRNAs and their potential roles as biomarkers in cancer have been documented in the literature (8), the mechanisms leading to miRNA dysregulation remained largely to be elucidated. The current study by Stegeman and colleagues (4) highlights a new control mechanism for miRNAs in cancer. Although the expression level of miRNA itself may not be significantly changed, alteration in the miRNA response elements of the target could produce the same effect and should be investigated.

Finally, the results highlight the role of the miRNA–Kallikrein axis of interaction and its potential involvement in prostate cancer pathogenesis (9). They also emphasize the recently emerging concept of the presence of an miRNA network of interactions involving PSA and other kallikreins in addition to non-kallikrein genes (10). An important consideration in this regard is that miRNA–gene interactions should not be viewed as one-on-one interactions but rather as the overall effect of a group of miRNAs on a "biological process."

It is now clear that there is a higher level of complexity in the pathogenesis of cancer and that integrated genomics is gradually becoming an essential tool for a better understanding of cancer pathogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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CANCER DISCOVERY

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