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Research Highlight

# *In vivo* exploration of the functional activity of the non-coding 8q24 prostate cancer risk locus

Dolores J. Lamb[1, 2], Mounia Tannour-Louet[1]

[1]*Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030, USA*
[2]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA*

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Genome-wide association studies (GWAS) have successfully identified a substantial number of risk variants for prostate cancer [1–10]. In total, more than 20 single nucleotide polymorphisms (SNPs) that predispose to prostate malignancy have been found scattered throughout the genome in coding and non-coding regions. The 8q24 chromosomal region is an intriguing locus as it harbors five independent susceptibility variants (rs10086908; rs16901979; rs620861; rs6983267; rs1447295) that span a 440-kb genomic interval devoid of genes or protein-coding transcripts. Thus, it is thought that the effects of the 8q24 variation on the predisposition to cancer may be mediated by *cis*-regulatory mechanisms that control the expression patterns of a neighboring gene important for cancer tumorigenesis and/or progression.

Interestingly, the proto-oncogene *MYC* lies immediately downstream of the 8q24 variants. Since this key transcription factor, which controls cell proliferation, is commonly amplified or over-expressed in various cancers including prostate cancer, Wasserman and colleagues [11] investigated in a recent issue of *Genome Research* whether any of the 8q24 associations are mediated through long-range *cis*-regulatory effects on *MYC* expression.

Using a Tn7-transposon-mediated random insertion, the authors [11] introduced a β-galactosidase reporter into overlapping bacterial artificial chromosomes (BAC) that encompassed the 8q24 non-coding prostate cancer risk variants. The modified BAC clones were then used as enhancer-trapping systems in which any long-range enhancer contained within the BAC can drive the spatio-temporal expression of β-galactosidase. BAC clones were injected into fertilized mouse oocytes to create transgenic mice. *LacZ* expression was assayed at multiple points of prostate organogenesis (postnatal days 0 and 8) and maturation (postnatal day 21). The overlapping design of the BAC clones helped to define the minimum region of interest, as identical expression patterns of *lacZ* expression between distinct BACs have to be shared by the common genomic interval. Prostate enhancer activity contained within a 59-kb interval harboring the variant rs6983267 was identified. Transgenic mice harboring a *lacZ* reporter plasmid driven by either the G (risk) allele or T (non-risk) allele of the 8q24 variant rs6983267 were subsequently generated. Unlike the non-risk allele, the risk allele displayed *in vivo* prostate enhancer activity resembling the pattern previously observed in mice with the whole BAC containing the risk allele. Importantly, the expression pattern of the rs6983267-containing enhancer correlates with endogenous expression of *MYC* in the prostate. The authors concluded that the rs6983267-containing enhancer may increase the risk of prostate cancer through its role in allelic-

Correspondence to: Prof. Dolores J. Lamb, PhD and Dr Mounia Tannour-Louet, PhD, Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030, USA.
Fax: +1-713-798-5577
Email: dlamb@bcm.tmc.edu
or mlouet@bcm.tmc.edu

specific control of MYC expression in the prostate.

These findings complement the available published studies that show the influence of non-coding variants on the control of the expression of genes that may predispose to cancer. For instance, the 10q11.2 prostate cancer risk SNP rs10993994, which is located 57 bp centromeric of the first exon of the *MSMB* gene and which encodes β-microseminoprotein (prostatic secretory protein 94), affects *MSMB* gene expression [12]. An intronic variant at 18q21.1 also affects *SMAD7* expression and the risk for colorectal cancer [13].

In previous studies, the 8q24 SNP rs6983267 was independently found to be associated with colorectal cancer. In both colorectal and prostate cancer cell lines, the two allelic variants of rs6983267 showed differential binding of TCF4 to an enhancer element that physically interacts with the *MYC* promoter, which is 300-kb telomeric to rs6983267 [14–17]. Despite this physical interaction, no association was observed between the rs6983267 genotype and *MYC* gene expression in normal prostate cells or prostate cancer cells [18]. Since Wasserman and colleagues assessed the *in vivo* activity of this enhancer during organogenesis and not in adult prostate tissue, these observations may imply that the rs6983267 variant-containing enhancer potentially exerts its influence long before tumorigenesis occurs.

When studying the enhancer properties of the risk allele of rs6983267, investigations in prostate cancer cell lines are conflicting. Enhancer activity has been seen in LNCaP but not in PC3, possibly due to the lack of androgen receptor expression [13]. Sotelo *et al*. [19] noted that the rs6983267-containing enhancer was unable to drive luciferase expression in LNCaP and PC3 unless cells were co-transfected with TCF4 and β-catenin expression vectors [19]. Under such conditions, the enhancer activity was observed in LNCaP but with the rs6983267-T non-risk variant driving stronger expression than the rs6983267-G risk allele. Further studies are needed to clarify these observations.

Of note, this study [11] highlights the need for functional follow-ups to GWAS studies in order to provide a molecular explanation about the association of the identified non-coding SNP with the disease risk. This stresses the importance of conducting *in vivo* investigations in whole-complex systems, such as mouse models. The technique used by Wasserman and colleagues is appealing for scanning non-coding variants on a megabase scale for their potential *cis*-regulatory effects. However, assaying enhancers in their natural genomic context has its limitations, as described by Wasserman and colleagues: the *lacZ* reporter used may be difficult to assess in tissues that have high endogenous expression of β-galactosidase, such as in the intestine in colorectal cancer studies. By contrast, the use of other reporter systems, such as GFP or luciferase, allows for *in vivo* real-time monitoring of prostate cancer growth through bioluminescence imaging or fluorescence microscopy. These non-invasive alternatives may also allow the application of additional stressors to the animals with additional risk factors that promote and/or contribute to prostate cancer.

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