

New Doctorial Cancer Research

Epigenetic Heterogeneity in Prostate Cancer: Impact of Genetic and Epigenetic Alterations on Diagnosis and Prognosis



Rui Manuel Ferreira Henrique

Rui Manuel Ferreira Henrique

Institute of Biomedical Sciences Abel Salazar, University of Porto

Ph.D. Dissertation date: July 27, 2006

Supervisor: Carlos Lopes and Manuel R. Teixeira

The main theme of this PhD thesis was the heterogeneity of prostate cancer at the genetic and epigenetic levels and its impact on disease detection and assessment. We focused our research efforts on (i) the investigation and development of new prostate cancer markers, (ii) the systematic quantitative evaluation of epigenetic alterations at key cancer-related genes in high-grade prostatic intraepithelial neoplasia (HGPIN), providing relevant clues for an epigenetic progression model of prostate cancer, and (iii) the characterization of the functional consequences of aberrant promoter methylation in gene expression.

A quantitative promoter methylation profile of cancerous and noncancerous prostate tissues identified two new target genes, APC and RAR β 2, which, in addition to GSTP1, provide sensitive and specific detection of prostate cancer in tissue specimens. Moreover, an association between promoter methylation and clinicopathological parameters of tumor aggressiveness was found. Indeed, APC, GSTP1, and Cyclin D2 promoter methylation correlate with higher Gleason score, whereas APC, Cyclin D2, GSTP1, MT1G, RAR β 2, and RASSF1A promoter methylation are associated with more advanced disease stage. Interestingly, prostate tumors with pathological features of low aggressiveness display very low or null levels of methylation at APC, GSTP1, and RAR β 2 gene promoters.

Because APC, GSTP1, and RAR β 2 gene promoters were differentially methylated in prostate cancer, HGPIN, and benign prostate tissue, these three markers were used to assess the epigenetic similarity among HGPIN lesions and prostate carcinoma. We found that HGPIN lesions are epigenetically heterogeneous and that some of them are clonally related with their index adenocarcinoma. Moreover, in a putative histopathological prostate cancer progression model, the acquisition of promoter methylation at RAR β 2 and APC precedes the same alteration at GSTP1. This latter alteration, which is more tumor specific, might identify HGPIN lesions more prone to develop into invasive adenocarcinoma.

The consequences of promoter methylation in gene expression among prostate tissues and cancer cell lines produce dissimilar results according to the target genes analyzed and the methodology used to assess methylation and expression. Using a conventional methylation-specific PCR (MSP) assay, we found a lower

frequency of CRBP1 promoter methylation in prostate carcinoma and HGPIN than with the use of the more sensitive quantitative MSP. Moreover, no correlation was established between CRBP1 promoter methylation and protein expression assessed by immunohistochemistry. On the other hand, 14-3-3 σ promoter methylation is an almost universal event in prostate adenocarcinoma, HGPIN, benign prostatic hyperplasia (BPH), and prostate cancer cell lines. Interestingly, there was no qualitative difference in 14-3-3 σ mRNA expression among prostate cancer cell lines with very different levels of promoter methylation. Moreover, 14-3-3 σ promoter methylation levels did not significantly differ among prostate carcinoma and BPH, which have been reported to have loss of expression and preserved expression, respectively, and thus additional mechanisms might be necessary to achieve effective 14-3-3 σ silencing. Because similar findings were apparent concerning MT1G promoter methylation, although this was a significantly less frequent event in prostate tissues, quantitative methylation and expression assays were performed to investigate Cyclin D2 epigenetic alteration and gene silencing in prostate cancer. We found that Cyclin D2 aberrant promoter methylation and expression are inversely correlated in prostate tissues, and also that exposure of highly-methylated cancer cell lines to a demethylating agent restores gene expression, implying that this epigenetic alteration is, indeed, an effective mechanism of gene silencing. Finally, owing to the significant differences in promoter methylation and expression found among morphologically normal prostate tissue, HGPIN, and prostate carcinoma, we concluded that epigenetic silencing of Cyclin D2 is associated with the development of the malignant phenotype in prostate carcinogenesis.

In conclusion, we established a quantitative epigenetic signature of prostate cancer, which provided novel and promising markers for disease detection and assessment. Moreover, the characterization of the epigenetic heterogeneity of prostate cancer and its precursor lesions set the basis for a better understanding of the timing and sequence of acquisition of epigenetic alterations in prostate carcinogenesis. Finally, the investigation of the complex interactions between gene expression and epigenetic alterations revealed the exciting possibilities, but also the potential caveats, associated with the use of modulators of the epigenome in prostate cancer treatment.

Comment by Jorge Soares

Epigenetics is a term coined from the Greek to identify the “upon” genetics modifications of the genome. In recent years there has been an increasing interest in the epigenetics of cancer, being notorious that our understanding of cancer biology was rather limited if only the classical approach to disclose the intimate gene alterations would focus our attention.

The transcriptional silencing of tumor suppressor genes by promoter CpG island hypermethylation can undoubtedly contribute to carcinogenesis. Therefore,

new classes of molecular markers based on human cancer-related epigenetic alterations have been investigated, namely, global DNA hypomethylation, gene hypomethylation, and promoter gene hypermethylation, as well as loss of imprinting leading to silencing of a specific allele.

In his PhD thesis work, Dr. Rui Henrique focused on the quantitative promoter methylation of a set of regulatory genes implicated in prostate tumorigenesis. The purpose was to investigate putative markers that might recognize early cancer stages, thus far improving the accuracy of the disease detection and, at the same time, contributing to establish the most appropriate strategies for the patients management.

The most relevant findings of Dr. Rui Henrique are (i) APC, RAR β 2 are novel target genes that, in addition to promoter hypermethylation of GSTP1 gene, proved to be sensitive and specific indicators of prostate cancer in biopsy tissue samples; (ii) higher Gleason score and pathological stage are correlated with the quantitative hypermethylation profile of certain sets of genes, allowing to design epigenetic signatures for prostate cancer aggressiveness; (iii) the acquisition of promoter methylation at glutathione-S-transferase P1 gene occurs later than at APC and RAR β 2 genes, which can potentially identify lesions more prone to evolve for an invasive phenotype.

An interesting component of Henrique's investigation was the comparison between prostate cancer and its preneoplastic counterpart, the so-called high-grade prostate intraepithelial neoplasia (HGPIN) lesions. Among this group of epigenetically heterogeneous lesions, some of them were proved to be clonally related to their index adenocarcinoma.

These findings not only shed light on our understanding of the prostate cancer mechanisms but also hypothesize that new markers can be incorporated in our armamentarium to support the clinical decision of performing radical prostatectomy in patients found to have HGPIN lesions in sextant biopsies.

The interactions between gene expression and epigenetic changes are still to be fully clarified, but seem to constitute one of the most exciting fields to be investigated in translational medicine. They anticipate future directions for a more comprehensive approach to misdirected epigenetics of cancer as well as for the manipulation of the methylation cell status as promising targets to fight certain forms of cancer.

This PhD thesis collects a series of excellent papers that aimed to answer some of the aforementioned questions. The results obtained by Dr. Rui Henrique provide interesting clues to our understanding of prostate carcinogenesis. They also demonstrate the great promise of DNA methylation markers as new tools in prostate cancer diagnosis, namely, overcoming the lack of sensitivity of the classical PSA serum test and showing a potential benefit to monitoring tumor severity and patient management.