

INVESTIGATION OF MIR-205 EXPRESSION AND ITS METHYLATION STATUS IN PROSTATE CANCER

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In prostate cancer (PCa), abnormal expression of several microRNAs (miRNAs) has been reported. Increasing evidence shows that aberrant epigenetic regulation of miRNAs is a contributing factor to their altered expression in cancer. In this study, we investigated whether expression of miR-205 in PCa is related to the DNA methylation status of its promoter and locus region.

PCR analysis of miR-205 expression was performed in PCa cell-lines and clinical specimens. CpG methylation analysis of the miR-205 promoter and miR-205 locus was performed in PCa cell-lines and clinical specimens via pyrosequencing. The effect on promoter and locus methylation status in cells treated with demethylating agents including 5-aza-2'-deoxycytidine (decitabine), knockdown of DNA methyltransferase 1 (DNMT1) and knockdown of enhancer of zeste homolog 2 (EZH2) was also examined. Finally, the biological significance of miR-205 in PCa cells was assessed using *in vitro* bioassays.

miR-205 was significantly down-regulated across PCa cell-lines. This correlated inversely with the methylation status of the miR-205 promoter and miR-205 locus, which is hypermethylated across this panel of cell-lines in both regions. Interestingly, a trend towards an inverse correlation was evident between miR-205 methylation, for both regions, and miR-205 expression levels in clinical specimens. Moreover, in PC3 cells, miR-205 expression was subsequently elevated by treatment with demethylating agents suggesting its expression is regulated by methylation. miR-205 promoter and locus methylation status, following treatment with 5-aza-2 deoxycytidine in PC3 cells, showed no change in methylation levels in either region. Finally, over-expression of miR-205 in PC3 cells inhibited growth and clonogenic potential, as well as inducing apoptosis.

Preliminary findings provide evidence that miR-205 is abnormally expressed in PCa and appears to have a tumour suppressor role in PCa. This study investigated the role of DNA methylation in regulating miR-205 expression. It is evident that DNA methylation of the regions analysed may not be responsible for regulating miR-205 expression; thus, other regions within the promoter and locus regions, which have not been identified, may be more important. Furthermore, histone modifications may have a role, alongside DNA methylation, in regulating miR-205 expression. Future work entails investigating the biological and prognostic value of miR-205 in PCa.