P33 The role of TET1 and hydroxymethylation in high-risk prostate cancer

Eur Urol Suppl 2019;18(8):e3097

Smeets E.[1], Spans L.[1], Moris L.[1], Handle F.[1], Helsen C.[1], Gevaert T.[2], Joniau S.[3], Claessens F.[1]

[1]KU Leuven, Department of Cellular and Molecular Medicine, Leuven, Belgium, [2]KU Leuven, Department of Development and Regeneration, Leuven, Belgium, [3]UZ Leuven, Department of Urology, Leuven, Belgium

Introduction & Objectives: The Ten-eleven translocation (TET) proteins catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Hydroxymethylation is not only part of the DNA demethylation pathway, but is also a stable mark on itself. Aberrant methylation patterns contribute to cancer formation by influencing gene expression, but the role of 5hmC is largely unknown. Therefore, there is a need for a better understanding of the function of DNA hydroxymethylation in cancer development. In this work, we investigated the role of TET1 and genomic 5hmC in a high-risk prostate cancer (HRPC) cohort of which exome sequencing data are available.

Materials & Methods: Prostate biopsies of patients with HRPC were collected via the PEARL consortium (UZ Leuven) after radical prostatectomy. Biopsies were used for immunohistochemical staining, DNA was extracted for copy number analysis and RNA for qRT-PCR. Subsequent in vitro and in vivo experiments have been carried out. Prostate cancer cell lines (PC3, LNCaP) have been used to evaluate the effect of modulating TET1 levels on proliferation and migration. We developed an inducible prostate-specific Tet1 knock out mouse model for investigating the effect of Tet1 in vivo in the murine prostate. Ethical approval has been obtained for both the usage of patient data and working with laboratory animals.

Results: Immunohistochemical analysis of tumor versus non-tumor prostate biopsies from HRPC patients showed a strong reduction of genome wide 5hmC and milder reduction of 5mC levels in prostate cancer tissue compared to controls. Copy number analysis showed a loss of TET1 in 6 out of 39 samples. Strikingly, the mRNA levels of TET1 were decreased in almost all tumor samples. In prostate cancer cells, TET1 overexpression affects tumor properties such as migration and proliferation. In order to create a more physiological setting, in vivo experiments using prostate-specific Tet1KO mice are being carried out at the moment. We are studying the effect of the Tet1KO on gene expression and the relation to changes in 5mC and 5hmC patterns.

Conclusions: In conclusion, our findings indicate that there is a drop in (hydroxy)methylation levels in HRPC which correlates with decreased TET1 expression and in some cases by a copy number loss of TET1. Using in vitro and in vivo models, we are currently investigating the effect on gene expression and cancer-related genes. This will unravel the regulation of gene expression by hydroxymethylation, which is a crucial factor in the understanding of prostate cancer development and progression.