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Large-scale evaluation of SLC18A2 in prostate cancer reveals diagnostic and prognostic biomarker potential at three molecular levels

Christa Haldrup[a], Anne-Sofie Lynnerup[a], Tine Maj Storebjerg[a], Søren Vang[a], Peter Wild[b], Tapio Visakorpi[c], Christian Arsov[d], Wolfgang A. Schulz[d], Johan Lindberg[e], Henrik Grönberg[e], Lars Egevad[f], Michael Borre[g], Torben Falck Ørntoft[a], Søren Høyer[h], Karina Dalsgaard Sørensen[a,*]

[a]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark
[b]Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland
[c]Prostate Cancer Research Center, Institute of Biosciences and Medical Technology (BioMediTech), University of Tampere, and Fimlab Laboratories, Tampere University Hospital, Tampere, Finland
[d]Department of Urology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany
[e]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden
[f]Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden
[g]Department of Urology, Aarhus University Hospital, Aarhus, Denmark
[h]Institute of Pathology, Aarhus University Hospital, Aarhus, Denmark

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ABSTRACT

Limitations of current diagnostic and prognostic tools for prostate cancer (PC) have led to over-diagnosis and over-treatment. Here, we investigate the biomarker potential of the *SLC18A2* (*VMAT2*) gene for PC at three molecular levels. Thus, *SLC18A2* promoter methylation was analyzed in 767 malignant and 78 benign radical prostatectomy (RP) samples using methylation-specific qPCR and Illumina 450K methylation microarray data. SLC18A2 transcript levels were assessed in 412 malignant and 45 benign RP samples using RNAseq data. SLC18A2 protein was evaluated by immunohistochemistry in 502 malignant and 305 benign RP samples. Cancer-specificity of molecular changes was tested using Mann–Whitney U tests and/or receiver operating characteristic (ROC) analyses. Log rank, uni- and multivariate Cox regression tests were used for survival analyses. We found that *SLC18A2* promoter hypermethylation was highly cancer-specific (area under the curve (AUC): 0.923–0.976) and associated with biochemical recurrence (BCR) after RP in univariate analyses. SLC18A2 transcript levels were reduced in PC and had independent prognostic value for BCR after RP (multivariate HR 0.13, $P < 0.05$). Likewise, SLC18A2 protein was

Abbreviations: AN, Adjacent normal; BPH, Benign prostatic hyperplasia; CRPC, Castration-resistant prostate cancer; PIN, Prostate intraepithelial neoplasia; MPC, Metastatic prostate cancer; RP, Radical prostatectomy; BCR, Biochemical recurrence; HR, Hazard ratio; FF, Fresh-frozen; FFPE, Formalin-fixed paraffin-embedded; ROC analysis, Receiver operating characteristic analysis; PSA, Serum prostate-specific antigen; TCGA, The Cancer Genome Atlas; TMA, Tissue microarray; qMSP, Quantitative methylation-specific PCR; LMD, Laser capture microdissection.

* *Corresponding author*. Aarhus University Hospital, Department of Molecular Medicine, Palle Juul-Jensens Boulevard 99, DK-8200 Aarhus N, Denmark. Tel.: +45 78455316; fax: +45 86782108.
E-mail address: kdso@clin.au.dk (K.D. Sørensen).

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down-regulated in PC (AUC 0.898) and had independent prognostic value for BCR (multivariate HR 0.51, $P < 0.05$). Reduced SLC18A2 protein expression was also associated with poor overall survival in univariate analysis (HR 0.29, $P < 0.05$).

Our results highlight *SLC18A2* as a new promising methylation marker candidate for PC diagnosis. Furthermore, SLC18A2 expression (RNA and protein) showed promising prognostic potential beyond routine clinicopathological variables. Thus, novel *SLC18A2*-based molecular tests could have useful future implications for PC detection and identification of high-risk patients.

1. Introduction

Prostate cancer is the most frequent male cancer in the developed world (Jemal et al., 2011). Whereas most prostate cancers are relatively slow growing and tumors localized to the prostate can be cured by radical prostatectomy (RP) or radiation therapy, metastatic prostate cancer is incurable. Thus, early detection and treatment is essential. Many prostate cancers, however, may not cause clinical symptoms within the patient's lifetime and as treatment is associated with severe side-effects, only men with aggressive prostate cancer should be treated (Canfield et al., 2014; Popiolek et al., 2013). Prostate cancer is definitively diagnosed by histopathological evaluation of prostate biopsies, but the initial indicator is often increased serum prostate specific antigen (PSA). Serum PSA has relatively high sensitivity, but rather low specificity for prostate cancer. Its use has therefore led to a large increase in prostate cancer diagnoses and treatments. Due to the high prevalence of indolent prostate cancers and lack of accurate prognostic tools to guide treatment decisions, a significant number of men may be over-treated (Canfield et al., 2014). Novel molecular markers may aid in early detection and in determining the aggressiveness of prostate cancer, but are generally not used in current clinical practice.

Prostate cancer is characterized by aberrant DNA hypermethylation of promoter-associated CpG islands at a large number of genes (Baylin et al., 2001; Kim et al., 2011). A subset of these genes show reduced mRNA expression upon DNA methylation, and hence are potential drivers of cancer development (Sproul et al., 2012) and often considered candidate tumor suppressors (Yang and Park, 2012). Candidate DNA methylation markers for prostate cancer have been reported to be hypermethylated in 70–100% of prostate cancer samples (Ahmed, 2010; Haldrup et al., 2013; Kristensen et al., 2014; Park, 2015), and are thus highly attractive for diagnostic applications. In addition, a few candidate DNA methylation markers have demonstrated promising prognostic value for prediction of PSA recurrence after RP (Banez et al., 2010; Haldrup et al., 2013; Kristensen et al., 2014; Schatz et al., 2010; Strand et al., 2014; Weiss et al., 2009).

The solute carrier family 18 member 2 (*SLC18A2*) gene encodes the transmembrane protein vesicular monoamine transporter 2 (SLC18A2/VMAT2). SLC18A2 protein localization and function is well characterized in neuronal cells where it facilitates uptake of monoamines into cytoplasmic large dense core and synaptic vesicles (Lawal and Krantz, 2013). SLC18A2 is highly expressed in monoamine secreting neuroendocrine tumors (Graff et al., 2001; Jakobsen et al., 2001; Saveanu et al., 2011; Uccella et al., 2006). In contrast, we previously reported a reduction in SLC18A2 immunoreactivity levels in 506 prostate adenocarcinoma samples when compared to 70 benign prostate tissue samples (Sorensen et al., 2009). In addition, in our previous study, reduced SLC18A2 protein levels had independent prognostic value for PSA recurrence after RP in multivariate Cox regression analysis including standard clinicopathological parameters, but this was not validated in an independent cohort (Sorensen et al., 2009). Notably, our previous results indicated hypermethylation of the *SLC18A2* promoter-associated CpG island and reduced SLC18A2 mRNA expression in prostate cancer, but was based only on small patient sample sets ($n < 21$) (Sorensen et al., 2009).

We here aimed to evaluate the biomarker potential of *SLC18A2* promoter methylation and RNA expression for PC, and to independently validate our previous finding that SLC18A2 protein immunohistochemistry has prognostic potential (Sorensen et al., 2009). To this end we analyzed hundreds of patient samples for *SLC18A2* promoter methylation, SLC18A2 mRNA and protein levels. We found that the *SLC18A2* promoter was frequently hypermethylated in prostate cancer in three distinct datasets and, furthermore, was significantly associated with PSA recurrence in two independent RP cohorts in univariate, but not in multivariate analysis corrected for routine clinicopathological factors. Moreover, SLC18A2 mRNA levels were reduced in prostate cancer, and low mRNA levels had prognostic value for PSA recurrence after RP independent of standard clinicopathological parameters. This is the first report to demonstrate a significant association between *SLC18A2* methylation and transcript levels and PC prognosis. Finally, we also confirmed that SLC18A2 protein was down-regulated in prostate cancer by immunohistochemical analysis of a new tissue microarray (TMA) representing 506 RP patients. Importantly, in this large independent RP cohort, loss of SLC18A2 protein had independent prognostic value for PSA recurrence in multivariate Cox regression analysis, thus successfully validating our previous results (Sorensen et al., 2009). Notably, loss of SLC18A2 protein was also associated with poor overall survival. Hence, the present work for the first time demonstrate a significant association between loss of SLC18A2 protein and overall survival after RP.

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