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Mavridis, Konstantinos

MicroRNA 224: a KLK-targeting miRNA with deregulated expression and promising prognostic value in prostate cancer

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Hsa-miR-224-5p (miR-224) is a tumor-related miRNA, which targets the expression of several Kallikrein (KLK) genes. The present study aims to analyze miR-224 expression in prostate cancer (CaP) cancer and to evaluate its prognostic significance. Snap - frozen tissue samples were obtained from a well-characterized cohort of CaP and benign prostatic hyperplasia (BPH) patients. MiR-224 expression levels were measured using qPCR after the isolation, polyadenylation and reverse transcription of total RNA via a poly(T) adapter. MiR-224 was found to be downregulated in CaP compared to BPH patients ($p < 0.001$). The expression levels of miR-224 were also gradually decreased in advanced pathological stage and high Gleason score CaP tumors (all p values < 0.05). MiR-224 expression was associated with improved biochemical progression-free survival of CaP patients (HR=0.314, $p = 0.013$). Our data reveal that the kallikrein-targeting miR-224 can be viewed as a potential prognostic biomarker for prostate cancer patients.

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Meinander, Kristian

Hydrocarbon isosteres of disulfide bridges in peptides that stimulate the proteolytic activity of KLK3

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Stimulation of the enzymatic activity of kallikrein-related peptidase 3 (KLK3, also known as prostate specific antigen, PSA) may be beneficial for patients with prostate cancer due to the antiangiogenic activity of KLK3. So far, only a few peptides have been reported to stimulate KLK3. The most potent ones are denoted C4 and B2, both of which increase the proteolytic activity of KLK3 several-fold at micromolar concentrations. However, no small molecule compounds with comparable activity have been reported. The *in vivo* use of natural peptides is highly challenging. With this in mind we set out to construct synthetic protocols for replacing both internal and terminal disulfide bridges with more stable hydrocarbon isosteres. We have synthesized several pseudopeptide analogs based on both the C4 and B2 peptides. All of these pseudopeptides show a significant stimulating effect on KLK3, the most potent ones showing a more than two-fold activation at a concentration of 20 µg/ml (13-14 µM).

Scorilas, Andreas

*1- Downregulation of the *KLK2*- & *KLK4*-targeting *miR-378a* predicts the short-term relapse of prostate cancer patients*

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The deregulation of several miRNAs in prostate cancer (PCa) provides a novel pool of candidate tumor markers. Using *in silico* approaches, we have identified hsa-miR-378a-3p (miR-378a) as a potential post-transcriptional regulator of *KLK2* and *KLK4*. The aim of the study is the evaluation of the clinical significance of miR-378a for the prediction of PCa patients' outcome. Prostate tissue specimens obtained from 73 PCa and 64 BPH patients. Following extraction, total RNA was polyadenylated and reverse transcribed using a poly(T) adapter. A SYBR-Green based qPCR assay was applied thereafter for the quantification of miR-378a levels. Although miR-378a levels were not significantly altered between PCa and BPH, a statistically strong downregulation of miR-378a was observed in higher Gleason score ($p=0.018$) and larger diameter ($r_s=-0.402$; $p=0.034$) tumors, as well as in patients with elevated PSA serum levels ($r_s=-0.365$; $p<0.001$). Moreover, Kaplan-Meier survival curves pointed out the significantly shorter disease-free survival (DFS) of the PCa patients with reduced miR-378a levels ($p=0.034$). Finally, in high-recurrence risk patients, the reduced miR-378a levels revealed to be an independent predictor of poor DFS ($p=0.030$). Our data highlight that miR-378a loss increases the risk for short-term relapse of the PCa patients.

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*2- *KLK8* mRNA expression analysis in breast cancer: downregulation in cancerous tissues and in metastatic disease*

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KLK8, like other members of the *KLK* family, may prove to be a promising clinical tool for breast cancer (BC). The objective of this study was the quantification of *KLK8* mRNA expression in BC tissues, and the initial determination of its clinical utility as a potential BC biomarker. Therefore, total RNA was isolated from 50 breast tumors and their matched normal adjacent tissues, cDNA was prepared and *KLK8* mRNA expression analysis was performed by comparative Ct method. The mRNA levels of *KLK8* were found to be significantly downregulated in BC specimens compared to non-cancerous counterparts ($p=0.002$). Furthermore, patients with metastatic BC showed significantly lower *KLK8* expression compared to those with organ confined tumors ($p=0.031$). Moreover, *KLK8* mRNA levels were found to be significantly increased in HER2-positive compared to HER2-negative BC ($p=0.017$). Additionally, a negative correlation was observed between *KLK8* and PgR expression levels ($r_s=-0.352$; $p=0.048$). On the contrary, no significant relationship was observed between *KLK8* mRNA levels and histological tumor grade, TNM stage, or ER status. Nevertheless, our preliminary analysis, which will be extended in a larger patients' cohort, proposes a possible tumor suppressor role of *KLK8* in BC and demonstrate its biomarker potential for this malignancy.