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Transcriptional Levels of *PRUNE-1* are Correlated to Prognostic Parameters in Epithelial Tumors

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Abstract

Background: The human orthologue of Drosophila PRUNE protein (*PRUNE-1*) regulates cell motility through its phosphodiesterase activity and interaction with protein partners, acting as a negative regulator of Nm23-H1, a tumor suppressor protein. Here, we aimed to understand the molecular alterations in epithelial tumors through quantification of *PRUNE-1* transcriptional levels in patients with Prostate Cancer (PCa) and Breast Cancer (BC).

Methods: mRNA expression was detected by RT-qPCR in peripheral blood from 31 PCa, 30 Benign Prostatic Hyperplasia BPH, 48 Breast Cancer, and 41 Benign Breast Disease patients.

Results: Transcriptional levels of h-PRUNE in PCa were associated to high grade of Gleason score. Regression analyses had shown correlation between Prostate Specific Antigen, Gleason and *PRUNE-1* mRNA expression. In BC, transcripts were significantly higher in HER-2 overexpression group compared to luminal patients.

Conclusion: Up-regulation profile of *PRUNE-1* may indicate convergent pathways for progression and aggressive PCa and BC. Our results pointed *PRUNE-1* as a putative gene for identify metastasis.

Keywords: Epithelial tumors; *PRUNE-1*; Transcription; Metastasis

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Introduction

The *PRUNE-1* (or *h-PRUNE/DRES17*) is the human homologue of the *Drosophila*gene that encodes a member of the phosphodiesteraseprotein family (PDE). The PDE activity catalyzes the hydrolysis of adenosine and guanosine-3'-5'-cyclic monophosphate (cAMP and cGMP) to their inactive forms 5'-monophosphates. These cyclic nucleotide second messengers play a central role in signal transduction and physiological responses. Binding to Nm23-H1, a tumor suppressor protein, *PRUNE-1* acts as a negative regulator, and with GSK-3β cooperatively promotes the disassembly of focal adhesions [1].

The search for new putative targets in Prostate Cancer (PCa) and Breast Cancer (BC), the most common malignant tumors after non-melanoma skin cancers, will help elucidate neoplastic biology, particularly metastatic phenotype. In this brief report, we quantified, thought qPCR, the transcriptional levels of *PRUNE-1* in peripheral blood from patients with PCa; benign prostatic hyperplasia (BPH); BC and benign breast disease (BBD) to better define the clinical and pathological roles of this gene in epithelial tumors progression.

Patients and Methods

This study was conducted at the Laboratory of Nanobiotechnology of the Federal University of Uberlândia (UFU), approved by UFU Research Ethics Committee (005/2001 and 176/2008). Blood samples from 150 patients were grouped into four classes: 31 PCa; 30 BPH; 48 BC and 41 BBD. Informed consent was obtained from all participants.

The average age of patients was 64 years old (range 51-82 years) for PCa group and 68 years old (range 50-82 years) for BPH group. According to TNM classification, 18 (70%) and 8 (30%) prostate tumors were T1/T2 and T3/T4, respectively. For Gleason score 40% presented Gleason ≥7 and 60%

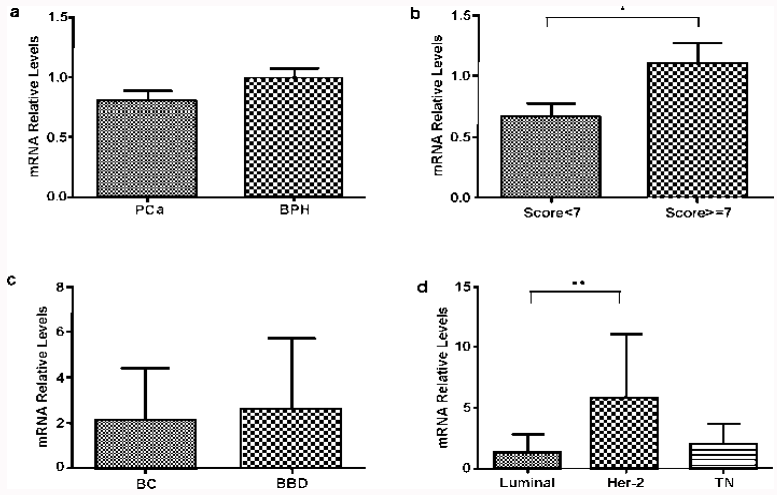

Figure 1: Transcriptional quantification of *PRUNE-1* in epithelial tumors: Prostate Cancer (PCa) and Breast Cancer (BC). (a) No difference between PCa and Benign Prostatic Hyperplasia (BPH)samples (b)The transcripts were significantly different in patients with Gleason score ≥7. *p=0.029 (c) No difference between BC and Benign Breast Disease (BBD) samples (d) Higher levels of *PRUNE-1* were detected in HER-2 over expression group **p=0.048. TN: triple-negative breast cancer.

Gleson <7. The average levels of serum total PSA for PCa was 7.5 (range from 1.9 to 13.44) and for BPH patients was 11.95 (range 2.92 to 18.16).

Considering BC patients, the average age was 55 years old (range 31–89 years) and for BBD was 48 years old (range 18–79 years). Patients with BC were classified according to histological grading (Nottingham system) as grade I (GI) in 3 (9%), GII in 25 (73%), and GIII in 6 (18%). For hormone receptors 34 (85%) were positive for Estrogen Receptor (ER) and 31 (80%) for Progesterone Receptor (PR). HER-2 status was considered as positive (score 3^+), including 19 cases (51%) and negative (score $0–1^+$) in 18 BC patients (49%). In conclusive scores 2^+ were excluded from statistical analyses.

Total RNA was extracted using TrizolLs Reagent (Invitrogen) following the manufacturer's recommendations. Reverse transcription and amplification reactions to validate RNA quality were conducted as described elsewhere [2]. QPCR reactions were prepared to a final volume of 10 µL, which was composed by 2 µL of cDNA, 5.0 µl of Master Mix SYBR® Green reagent (Applied Biosystems), and 5 pmol of primers. All reactions were conducted in an ABI 7300 Real Time PCR Systems (Applied Biosystems) and analyzed according to delta threshold cycle value (ΔCt) method. Pairs of primers were designed for *PRUNE-1* amplification: forward 5'-CAGCCCTTCTGCATGGAAC-3', and reverse 5'-TTTCTCTTGGGTAGGTCTGG-3' and Beta-2-microglobulin transcripts were used for normalization as previously described [2]. Mann-Whitney and regression analyses were carried out to verify differences and correlations between groups. T-student test was performed to verify significance of regression test. Statistical significance was considered when $p < 0.05$ and carried out using GraphPad Prism 5 (GraphPad Soft-ware, La Jolla, CA, USA) and Bioestat software.

Results

The distribution of *PRUNE-1* mRNA in the peripheral blood was not different among PCa and BPH groups. Patients with Gleason ≥7 presented1.7 fold higher transcriptional (Figure 1a and b) ($p = 0.029$) and it was not found any significant association to other clinicopathological features. Positive correlation between Gleason (X axis) and *PRUNE-1* (Y axis) mRNA expression was verified through univariate regression ($y = 6.0610 + 0.5754x$ and $R^2 = 0.0772$). Multivariate regression demonstrated a negative correlation to PSA(X1) ($y = - 0.3773 - 0.0321 X1 + 0.2173 X2$ and $R^2 = 0.0815$).

In BC, there were no significant difference in *PRUNE-1* expression between tumor and benign samples. Transcripts amplified from peripheral blood did not also demonstrate significant differences according to clinicopathological data. Considering intrinsic subtypes, transcriptional levels were significantly associated to HER-2 over expression group ($p = 0.048$) comparing to luminal tumors (Figure 1c and d).

Discussion

Over expression of *PRUNE-1* in breast, colorectal, and gastric cancers is correlated with lymph node and distant metastases, once increased levels of this gene may function as a negative regulator of tumor suppressor activities of nm23-H1. In fact, amplification of 1q21-q22 had already been described as a common molecular event in metastatic lesions associated with poor outcome [3].

The survival of patients with PCa is related to several factors, including extent of tumor, histologic grade, patient's age, and PSA levels. In accordance with Gleason system, prostate cancers with high grade (poorly differentiated) tend to be aggressive and they are likely to grow and spread quicker. Better prognostic is related to lower score and this is one of the most important prognostic factors for PCa treatment [4]. We found that prostate cancer showed increased levels of h-*PRUNE* in higher Gleason score, suggesting that it plays an important role in cancer development, thus serving as a progression marker.

Over expression of *PRUNE*-1 in BC has also been associated to cell motility, lymph node involvement and metastasis formation [5]. Comparing the expression levels of the mRNA marker with the clinicopathological features we found an up-regulation in HER-2 subtypes comparing to luminal one. The HER2-2 oncogene is amplified and/or overexpressed in approximately 20% of breast cancers. This marker predicts for trastuzumab adjuvant therapy and is a strong prognostic factor for relapse and poor overall survival [6]. Therefore, the correlation between HER-2 subtype and *PRUNE-1*

up-regulation may indicate convergent pathways for progression and metastatic BC. Our work demonstrated an association between high levels of *h-PRUNE RNA* and aggressiveness of BC and PCa. The identification of molecules that are capable of in hibith – PRUNE signaling pathway are of great relevance for the development of new drugs to treat cancer metastasis. Further studies with larger cohorts including patients undergoing different outcomes are truly necessary to better understand the real clinical relevance of *h-PRUNE* pathway.

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