Aim. To detect expression of EMT-related genes in prostate tumor samples and analyze a possible correlation between the gene expression level and clinical characteristics of prostate cancer in different groups. Methods. Expression of 19 genes was analyzed in 37 frozen samples of prostate cancer tissues at different tumor stages and Gleason scores, 37 paired conventionally normal prostate tissues and 20 samples of prostate adenomas, using quantitative PCR. Results. We have found that nine genes were expressed differently in benign and malignant prostate tumors, namely AR (isoform 1), AR (isoform 2), PTEN, VIM, MMP9, KRT18, PCA3, HOTAIR and SCHLAP1. When different tumor stages were compared, we could identify six differentially expressed genes: KRT18, MMP9, VIM, PCA3, HOTAIR and SCHLAP1; when samples of tumors with different Gleason score were compared, we found that eight genes were expressed differently: AR (isoform 1), CDH1, KRT18, MMP9, OCLN, PCA3, HOTAIR and SCHLAP1. The data had a high level of heterogeneity potentially due to various molecular subtypes of prostate cancer, i.e. a luminal subtype with a high expression of CDH1, OCLN, AR (isoform), KRT18, NX3-1 and PSA; the stem-like subtype with the high expression of mesenchymal markers CDH2, FN1 and VIM and low expression of the epithelial markers. It is noteworthy that lncRNAs were specifically expressed in these two molecular subtypes. Conclusions. EMT-related genes were differentially expressed in benign and malignant prostate tumors. High heterogeneity of expression levels, especially in adenocarcinoma groups, might suggest the existence of at least two different molecular subtypes, luminal and stem-like. Further experiments are necessary for specification of the molecular subtypes of prostate adenocarcinoma.

Keywords: prostate tumors, EMT, relative gene expression, molecular subtypes, lncRNA.

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Introduction

Prostate cancer is one of the most commonly diagnosed cancers among men in the world. It is highly heterogeneous and complicated cancer type, when taking into account a wide variety of multiple genetic and demographic factors that affect cell transformation, as well as the different origins of cancerous cells [1, 2]. One of the critical molecular process for tumor progression is epithelial-to-mesenchymal cell transition (EMT), i.e. when epithelial cells are losing their characteristics and acquiring properties of the mesenchymal cells [3, 4].

It is known already, that many genes are involved in the EMT. There are well characterized changes in gene expression pattern for both, epithelial and mesenchymal cells [5]. We have selected several such genes, to study their expression and to analyze whether such genes may serve as biomarkers and/or classifiers of various subtypes of prostate cancer. Well known tumor suppressor genes, that are involved in EMT in prostate cancer, are \textit{NKX3-1}, \textit{PTEN} and \textit{CDH1}. Unaltered activity and expression of \textit{NKX3-1} and \textit{PTEN} are essential for a normal prostate functioning [6]. E-cadherin, encoded by the \textit{CDH1} gene, is one of the main markers of epithelial cells. Loss of CDH1 expression has been implicated in progression and metastasizing [7]. Another protein, playing a critical role in maintaining the barrier properties of a tight junction in epithelial cells is occludin (\textit{OCLN} gene). Occluding has anti-metastasizing [8].

The opposite function in prostate cancer show genes as, for example, N-cadherin, encoded by the \textit{CDH2} gene, fibronectin (\textit{FN1}) and vimentin (\textit{VIM}). These proteins are is a markers of mesenchymal cells, and their expression corresponds to more aggressive tumor phenotype [9].

Metalloproteinases accompany the malignant cell transformation and metastasizing [5, 10]. In particular, \textit{MMP9} expression is associated with invasiveness and metastatic properties, infiltration of the tumor supporting cells and angiogenesis. \textit{MMP2} expression increased with growth of a TNM grade and angiogenesis.

The androgen receptor (\textit{AR}) and its isoforms are steroid receptor and function as transcription factors. There is a cross-talk between AR signaling and the EMT. It means that deviations in a structure and function of AR can induce the EMT upon tumor progression [11].

\textit{PSA} is one of the most known prostate cancer markers, encoded by the prostate-specific gene kallikrein 3 (\textit{KLK3}). \textit{PSA} is a normal prostate antigen, but its expression is increasing dramatically in prostate carcinogenesis. Nevertheless, inflammation, infection, trauma and benign prostatic hyperplasia (BPH) are also the causes of the elevated level of serum PSA. Therefore, PSA-based screening for prostate cancer is plagued by false positives, resulting in a positive predictive value of only 25 to 40 % [12].

\textit{KRT18} is expressed in normal prostate luminal cells. \textit{KRT18} was overexpressed in a number of epithelial human cancers [13, 14]. In certain cases this marker is used to assess the differentiation stage of tumor tissues. \textit{MKI67} encodes a nuclear protein Ki67, a marker of proliferation. The association with the clinical outcomes of prostate cancer was described for Ki67, together with another marker, \textit{MIB-1}. [15].
Upon cancer initiation and progression the obvious differences between normal and tumor cell was detected not only for proliferation and differentiation, but for apoptosis as well. \(\text{CASP3}\) gene encodes caspase 3 protein, which is the most studied of the effector caspases. Expression of \(\text{CASP3}\) was increased upon tumor progression in breast and prostate carcinomas [16, 17]. XIAP (X-linked inhibitor of apoptosis protein) is a member of an IAP protein family, which consists of eight different proteins that were originally described as inhibitors of apoptosis. Some of them can bind and neutralize caspasas. In prostate cancer, levels of \(\text{XIAP}\) are significantly higher, than in prostatic intraepithelial neoplasia [18].

The EMT might be regulated by IncRNA regions [19–21]. There are the prostate specific IncRNA region, encoding \(\text{PCA3}\). Prostate cancer antigen 3 (PCA3) is highly expressed in prostate cancer tissues, compared to normal prostate s [19]. There are accumulating evidences, that PCA3 is also involved in AR signaling. High expression of the another gene, encoded by IncRNA region, \(\text{HOTAIR}\), is associated with metastasizing and poor prognosis in many tumor types [20]. Yet another gene, encoded by IncRNA region, \(\text{SCHLAP1}\), is overexpressed in prostate cancer. \(\text{SCHLAP1}\) levels may predict poor patient outcomes, including metastasizing and cancer specific mortality [21].

In total, we selected 19 genes associated with the EMT and studied their expression in both, benign and malignant prostate tumors.

The aim of our study is:

To detect relative expression of EMT-related genes in prostate tumor samples and analyze a possible correlation between gene expression level and clinical characteristics of prostate cancer in different groups.

Materials and Methods

A collection of prostate tissues. Samples of cancer tissue and CNT (at an opposite side of tumor) were frozen in a liquid nitrogen immediately after surgical resection at the National Cancer Institute (Kyiv, Ukraine). Benign prostate tumors (prostate adenoma samples) were collected at the Institute of Urology (Kyiv, Ukraine) after radical prostatectomy and frozen, as described above. The samples were collected in accordance with the Declaration of Helsinki and the guidelines issued by the Ethic Committee of the Institute of Urology of National Academy of Medical Sciences of Ukraine and of the National Cancer Institute of National Academy of Sciences of Ukraine (NASU), and an Ethic Committee of the Institute of Molecular biology and genetics of NASU. Experimental studies were conducted on 37 prostate adenocarcinoma samples of different Gleason score and stages; 37 paired CNT samples; 21 samples of benign prostate tumors (adenomas). Tumor samples were characterized, according to an International System of Classification of Tumors, based on the tumor-node-metastasis (TNM) and the World Health Organization (WHO) criteria. Clinical and pathological characteristics of prostate cancer samples are presented on Table 1.

Total RNA isolation and cDNA synthesis. 50–70 mg of frozen prostate tissues were disrupted to powder in liquid nitrogen. Total RNA was extracted by TRI-reagent (SIGMA) according to the manufacturer’s protocol. Total
RNA concentration was analyzed by spectrophotometer (NanoDrop Technologies Inc. USA). The total RNA samples quality was determined in a 1% agarose gel by band intensity of 28S and 18S rRNA (28S/18S ratio). cDNA was synthesized from 1 µg of the total RNA previously treated with RNase free DNase I (Thermo Fisher Scientific, USA) using RevertAid H Minus M-MuLV Reverse Transcriptase (Thermo Fisher Scientific, USA) according to the manufacturer’s protocol.

Quantitative PCR. Relative gene expression (RE) levels of 19 genes were detected by QPCR, using Maxima SYBR Green Master mix (Thermo Fisher Scientific, USA) on Bio-Rad CFX96 Real-Time PCR Detection System (USA) under the following conditions: 95 °C – 10 minutes, following 40 cycles of 95 °C – 15 seconds, 60 °C – 30 seconds, elongation 72 °C – 30 seconds. Primers for genes were selected from qPrimerDepot (https://primerdepot.nci.nih.gov/).

Table 1. Clinical and pathological characteristics (CPC) of prostate cancer samples.

| Sample N | Age | TNM   | Stage | GL | PSA ng/ml | Sample N | Age | TNM   | Stage | GL | PSA ng/ml |
|----------|-----|-------|-------|----|-----------|----------|-----|-------|-------|----|-----------|
| 1        | 54  | T2cNxM0 | II    | <7 | 27,3      | 20       | 52  | T2bN0M0 | III   | 7  | 24,6      |
| 2        | 56  | T2cNxM0 | II    | <7 | 25,2      | 21       | 60  | T3bN0M0 | III   | >7 | 12,1      |
| 3        | 55  | T2pN0M0 | II    | <7 | 5,0       | 22       | 53  | T2bNxM0 | II    | >7 | 6,9       |
| 4        | 67  | T2cN0M0 | II    | <7 | 29,1      | 23       | 63  | T3bNxM0 | III   | >7 | 20,9      |
| 5        | 66  | T2bNxM0 | II    | <7 | 6,5       | 24       | 56  | T3bN0M0 | III   | >7 | 84,2      |
| 6        | 71  | T2bN0M0 | II    | <7 | 12,8      | 25       | 48  | T3bNxM0 | III   | >7 | 51,0      |
| 7        | 57  | T2aN0M0 | II    | <7 | 9,3       | 26       | 65  | T2bNxM0 | II    | >7 | 33,0      |
| 8        | 67  | T2aNxM0 | II    | <7 | 18,6      | 27       | 61  | T2bNxM0 | III   | >7 | 0,5       |
| 9        | 63  | T2aN0M0 | II    | <7 | 13,3      | 28       | 76  | T3aN0M1 | IV    | >7 | 37,8      |
| 10       | 54  | T2aN0M0 | II    | <7 | 6,0       | 29       | 54  | T3bNxM0 | III   | >7 | 106,0     |
| 11       | 74  | T3bNxM0 | III   | <7 | 23,6      | 30       | 58  | T2cN1M0 | IV    | >7 | 17,0      |
| 12       | 68  | T1cNxM0 | I     | 7  | 8,2       | 31       | 58  | T3aN0M0 | III   | >7 | 25,1      |
| 13       | 68  | T2cNxM0 | II    | 7  | 19,3      | 32       | 63  | T2bN0M0 | II    | >7 | 20,3      |
| 14       | 64  | T2cNxM0 | II    | 7  | 19,8      | 33       | 62  | T2cN0M1 | IV    | >7 | 22,6      |
| 15       | 77  | T2aNxM0 | II    | 7  | 11,7      | 34       | 67  | T3bNxM0 | III   | >7 | 16,0      |
| 16       | 69  | T2cNxM0 | II    | 7  | 13,9      | 35       | 63  | T3bNxM0 | III   | >7 | 86,3      |
| 17       | 54  | T2aNxM0 | II    | 7  | 7,1       | 36       | 66  | T2cN1M0 | III   | >7 | 2,3       |
| 18       | 62  | T2aNxM0 | II    | 7  | 5,6       | 37       | 65  | T2cN0M0 | II    | >7 | 9,7       |
| 19       | 69  | T2cN0M0 | II    | 7  | 14,3      | 38       | 66  | T2cN0M0 | II    | >7 | 22,6      |

Note: TNM - Classification of Malignant Tumors, based on the tumor-node-metastasis, GL – Gleason score, PSA – prostate specific antigen concentration in blood.
Four reference genes *TBP, HPRT, ALAS1* and *TUBA1B* were used for gene expression normalization [22]. Two main models for RE levels calculation were used. There were Livak method $2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$ methods – representing relative quantities and fold changes accordingly [23].

**Statistical analysis.** The Kolmogorov-Smirnov and Lilliefors tests were used for assessing normality of distribution. Kruskal-Wallis test was used for determine differences in multiple comparison between experimental groups. Wilcoxon Matched Pairs test was performed for dependent sampling of RE prostate adenocarcinoma and paired conventional normal tissues tissues. The Benjamini-Hochberg procedure with false discovery rate 0.10 was used to correct p value under multiple comparisons detection [24].

Dunn-Bonferroni post hoc test was used to determine RE differences between pairs of prostate samples groups. Spearman’s rank correlation test was used to determine correlations between gene expression levels and CPC of prostate tumors, RE and correlations between the investigated genes.

**Results**

RE of 19 genes were assessed to monitor differences between prostate adenocarcinoma samples (T), conventionally normal prostate tissues (N) and benign prostate tumors (adenomas) (A) (Table 2A, 2B). According to a statistical analysis, RE of genes in an adenoma group did not show the Gaussian distribution; therefore, nonparametric statistical tests and methods were used.

We found 9 genes with statistically significant differences ($p < 0.05$) in RE between 3 investigated groups by the Kruskal-Wallis test with FDR correction: *AR* (1 isof), *AR* (2 isof), *PTEN, VIM, MMP9, KRT18, PCA3, HOTAIR* and *SCHLAP1*. Gene expression profiles in all groups are shown on Figure 1. We found that values were heterogeneous in each group, especially in a carcinoma group for the majority of the investigated genes.

Following Dunn-Bonferroni post hoc method for multiple comparisons, we found differences in RE for the 15 pairs of groups for these genes (Table 2B). Thus, RE of *AR* (1 isof), *AR* (2 isof) and *PTEN* are the highest in the adenoma group and are significantly decreased in the adenocarcinoma group for both *AR* transcripts and *PTEN* ($p = 0.021$, $p = 0.047$, $p = 0.015$, respectively). Similar significant changes were observed for the adenoma group and normal prostate tissues for *AR* (1 isof) and *PTEN* ($p = 0.045$; $p = 0.047$). RE of *VIM* was the highest also in adenomas and showed the significant differences, compared with group of normal prostate tissues ($p = 0.007$), whereas no differences with adenocarcinoma group were found. The opposite situation was observed in RE of *MMP9, PCA3* and *SCHLAP1* in adenomas. They showed the lowest levels, and this was significantly different, compared with RE in adenocarcinomas – ($p = 0.0001$, $p = 0.001$, $p = 0.013$, correspondingly) and in normal tissues – for *MMP9* ($p = 0.015$) and *PCA3* ($p = 0.006$). RE of *KRT18* and *HOTAIR* demonstrated similar trend of changes. The highest levels of RE was observed in the adenocarcinoma group, and this was significantly different with both normal tissue groups ($p = 0.018$, $p = 0.047$, correspondingly) and the adenoma group ($p = 0.001$, $p = 0.0001$), which demonstrated the lowest RE. Only the
Table 2. Descriptive statistics of the data on RE in the prostate adenocarcinoma (T), conventionally normal tissue (N) and adenoma (A) sample groups (2A) and RE differences between pairs of groups (2B)

| Gene   | Sample group | Median | Minimum | Maximum | 25th percentile | 75th percentile | p < 0.05* |
|--------|--------------|--------|---------|---------|-----------------|-----------------|-----------|
| AR (1 isof) | T            | 1,377  | 0,413   | 3,353   | 0,911           | 2,228           | 0,017     |
|        | N            | 1,428  | 0,447   | 6,834   | 1,069           | 2,119           |           |
|        | A            | 2,129  | 0,879   | 4,936   | 1,623           | 2,672           |           |
| AR (2 isof) | T            | 0,020  | 0,000   | 0,069   | 0,012           | 0,033           |           |
|        | N            | 0,022  | 0,000   | 0,083   | 0,015           | 0,028           |           |
|        | A            | 0,029  | 0,014   | 0,051   | 0,023           | 0,038           |           |
| CASP3  | T            | 0,318  | 0,040   | 0,869   | 0,170           | 0,419           |           |
|        | N            | 0,288  | 0,058   | 0,685   | 0,194           | 0,402           |           |
|        | A            | 0,303  | 0,152   | 1,088   | 0,207           | 0,587           |           |
| CDH1   | T            | 3,237  | 0,053   | 10,097  | 1,572           | 4,676           |           |
|        | N            | 3,707  | 0,888   | 8,253   | 2,562           | 4,942           |           |
|        | A            | 2,752  | 0,004   | 8,941   | 2,001           | 3,661           |           |
| CDH2   | T            | 0,103  | 0,001   | 1,361   | 0,056           | 0,146           |           |
|        | N            | 0,125  | 0,023   | 1,101   | 0,073           | 0,255           |           |
|        | A            | 0,083  | 0,020   | 0,482   | 0,060           | 0,178           |           |
| FN1    | T            | 4,840  | 1,401   | 41,496  | 3,557           | 8,230           |           |
|        | N            | 4,706  | 1,677   | 13,307  | 2,987           | 6,879           |           |
|        | A            | 4,051  | 1,779   | 8,593   | 3,017           | 5,234           |           |
| KRT18  | T            | 21,740 | 1,798   | 84,078  | 16,570          | 31,828          | 0,0004    |
|        | N            | 15,868 | 4,798   | 46,191  | 9,987           | 19,905          |           |
|        | A            | 12,631 | 0,692   | 31,273  | 6,102           | 17,625          |           |
| MKI67  | T            | 0,127  | 0,014   | 1,220   | 0,049           | 0,212           |           |
|        | N            | 0,098  | 0,000   | 0,303   | 0,061           | 0,141           |           |
|        | A            | 0,102  | 0,039   | 0,267   | 0,068           | 0,152           |           |
| MMP2   | T            | 12,894 | 1,852   | 33,504  | 6,501           | 17,772          |           |
|        | N            | 9,950  | 1,855   | 29,948  | 6,825           | 12,982          |           |
|        | A            | 10,628 | 5,126   | 25,315  | 7,863           | 13,108          |           |
| MMP9   | T            | 0,918  | 0,028   | 27,489  | 0,345           | 2,466           | 0,001     |
|        | N            | 0,674  | 0,052   | 8,441   | 0,243           | 1,385           |           |
|        | A            | 0,196  | 0,011   | 1,046   | 0,122           | 0,424           |           |
| NKX3-1 | T            | 0,314  | 0,028   | 6,543   | 0,110           | 0,571           |           |
|        | N            | 0,410  | 0,024   | 5,112   | 0,242           | 0,828           |           |
|        | A            | 0,344  | 0,032   | 1,640   | 0,139           | 0,543           |           |
| OCLN   | T            | 0,568  | 0,059   | 3,790   | 0,336           | 1,214           |           |
|        | N            | 0,524  | 0,117   | 2,484   | 0,391           | 0,900           |           |
|        | A            | 0,396  | 0,227   | 1,574   | 0,332           | 0,593           |           |
| PSA    | T            | 351,254| 6,947   | 796,138 | 62,441          | 547,561         | 590,290   |
|        | N            | 355,608| 4,584   | 1114,739| 198,460         | 590,290         |           |
|        | A            | 323,470| 1,203   | 768,968 | 78,968          | 520,212         |           |
Expression of epithelial-mesenchymal transition-related genes in prostate tumours

Table 2A (breakover)

| Gene  | Sample group | Median   | Minimum | Maximum | 25th percentile | 75th percentile | p<0.05* |
|-------|--------------|----------|---------|---------|-----------------|-----------------|---------|
| PTEN  | T            | 8,762    | 2,278   | 25,232  | 6,354           | 12,322          | 0.015   |
|       | N            | 9,262    | 2,958   | 29,824  | 6,185           | 12,558          |         |
|       | A            | 16,989   | 5,875   | 90,619  | 8,964           | 22,065          |         |
| VIM   | T            | 11,352   | 3,929   | 24,109  | 8,120           | 15,202          | 0.007   |
|       | N            | 9,253    | 9,09    | 34,038  | 6,056           | 12,017          |         |
|       | A            | 12,947   | 7,836   | 31,417  | 10,163          | 15,474          |         |
| XIAP  | T            | 0.349    | 0.078   | 0.995   | 0.215           | 0.479           |         |
|       | N            | 0.344    | 0.044   | 0.925   | 0.219           | 0.488           |         |
|       | A            | 0.301    | 0.182   | 0.896   | 0.235           | 0.386           |         |
| PCA3# | T            | 0.647    | 0.007   | 56,165  | 0.074           | 19,017          | 0.001   |
|       | N            | 0.318    | 0.000   | 27,228  | 0.088           | 1,970           |         |
|       | A            | 0.044    | 0.001   | 7,342   | 0.023           | 0.132           |         |
| HOTAIR# | T         | 0.015    | 0.000   | 0.079   | 0.004           | 0.037           | 0.0004  |
|       | N            | 0.004    | 0.000   | 0.057   | 0.001           | 0.011           |         |
|       | A            | 0.001    | 0.000   | 0.015   | 0.000           | 0.003           |         |
| SCHLAP1# | T        | 0.029    | 0.000   | 2.350   | 0.015           | 0.307           | 0.017   |
|       | N            | 0.032    | 0.000   | 1.148   | 0.013           | 0.079           |         |
|       | A            | 0.008    | 0.000   | 0.117   | 0.002           | 0.018           |         |

2B.

| Genes   | Pairs with differences | p-value ** |
|---------|------------------------|------------|
| AR(1isof) | T/A               | 0.021      |
|          | N/A                | 0.045      |
| AR (2isof) | T/A              | 0.047      |
| KRT18   | T/A                | 0.001      |
|          | T/N                | 0.018      |
| MMP9    | T/A                | 0.000      |
|          | N/A                | 0.015      |
| PTEN    | T/A                | 0.015      |
|          | N/A                | 0.047      |
| VIM     | N/A                | 0.007      |
| PCA3#   | T/A                | 0.001      |
|          | N/A                | 0.006      |
| HOTAIR# | T/A                | 0.000      |
| SCHLAP1# | T/A               | 0.013      |

Notes: * – Kruskal-Wallis test data significant with FDR=0.1;
** – Dunn-Bonferroni post hoc method for multiple comparisons
# – lncRNA
**Fig. 1.** RE profiles in prostate adenocarcinomas, conventionally normal tissues and adenomas: **A**) RE profile of *AR* (1 isof), *AR* (2 isof) and *MMP9* genes; **B**) RE profile of *PTEN*, *VIM*, *KRT18*; **C**) RE profile of *PCA3*, *HOTAIR*, *SCHLAP1*.
KRT18 and HOTAIR genes showed RE differences between the adenocarcinoma and normal prostate tissue groups, when analyzed by the Dunn-Bonferroni post hoc method.

The other way to find the differences between adenocarcinoma and normal tissue groups is to compare paired tumor-normal tissue samples (from one patient). We have performed Wilcoxon Matched Pairs test with FDR correction (FDR = 0,1) (Table 3). 6 genes showed the statistically significant differences in RE between prostate adenocarcinoma samples and paired conventionally normal tissues: KRT18, MKI67, MMP2, MMP9, VIM, HOTAIR.

These differences may be dependent on the statistical analysis, i.e. on an algorithm of the method and its sensitivity. Further analysis is necessary, for sure.

A stage of a cancer disease is one of the most important CPCs. We grouped all samples of prostate cancer and corresponding normal tissues into two groups: 1–2 stages (22 samples) and 3–4 stages (15 samples). After analysis, we found 6 genes with statistically significant differences in RE in these experimental groups (Table 4A, 4B).

6 out of 19 genes demonstrated significant differences in RE. All these genes showed similar trends in previously analyzed three groups. Among these genes there were 3 coding genes (KRT18, MMP9 and VIM) and 3 genes, encoded by IncRNA regions (PCA3, HOTAIR, SCHLAP1). It was found 11 pairs with significant RE differences.

Almost all genes showed significant RE differences in the adenoma group, compared with the adenocarcinoma group or normal prostate tissue at various stages, when the Dunn-Bonferroni post hoc method was used. Surprisingly, no differences between the adenocarcinoma and the normal tissue groups was revealed, at the same stage.

Among CPC, a Gleason score is an important parameter for description of prostate cancer differentiation, aggressiveness and also for prognosis. Three grouping parameters of Gleason score were used to divide the prostate adenocarcinoma group (T) and respective conventionally normal prostate tissues (N) into 3 groups: Gleason score < 7 (GL < 7) (11 samples), Gleason score = 7 (GL = 7) (9 samples), Gleason score > 7 (GL > 7) (17 samples). Moreover, we also used the adenomas group (A) for comparison.

In total, we had 7 sample groups for RE analysis (Table 5A and Table 5B). Ten of 19 genes showed the significant differences in RE, according to the Kruskal-Wallis test. The Dunn-Bonferroni post hoc method for multiple comparisons has confirmed the significant differences only for 8 out of 10 genes. Noteworthy, the levels of RE of CDH1 in prostate adenocarcinoma with different Gleason score showed a high grade of heterogeneity. The highest expression was observed in the adenocarcinoma group (GL = 7), which had significant differences with the adenocarcinoma (GL > 7) group (the lowest expression in carcinomas)

### Table 3. RE differences between prostate adenocarcinoma samples and paired normal tissues (dependent sampling)

| Gene   | p-value & | Gene   | p-value & |
|--------|-----------|--------|-----------|
| KRT18  | 0.0004    | MMP9   | 0.0136    |
| MKI67  | 0.0168    | VIM    | 0.0097    |
| MMP2   | 0.0106    | HOTAIR | 0.0065    |

Note: & – Wilcoxon Matched Pairs test significant with FDR=0,1
**Table 4.** Descriptive statistics of the data on RE with significant differences in T, N, A sample groups at the various stages (st) (4A) and differences in RE between the pairs of prostate groups at different stages (4B).

| Gene   | Sample group | Median   | Minimum | Maximum | 25th percentile | 75th percentile | p<0.05* |
|--------|--------------|----------|---------|---------|----------------|----------------|---------|
| KRT18  | T (1-2 st)   | 19,699   | 1,798   | 53,528  | 14,052         | 29,296         | 0,0014  |
|        | T (3-4 st)   | 27,333   | 4,517   | 84,078  | 19,084         | 44,961         |         |
|        | N (1-2 st)   | 15,612   | 4,798   | 23,940  | 9,817          | 17,485         |         |
|        | N (3-4 st)   | 18,203   | 5,534   | 46,191  | 10,064         | 34,570         |         |
|        | A            | 12,631   | 0,692   | 31,273  | 6,102          | 17,625         |         |
| MMP9   | T (1-2 st)   | 1,020    | 0,072   | 6,867   | 0,215          | 2,677          | 0,0033  |
|        | T (3-4 st)   | 0,833    | 0,028   | 27,489  | 0,399          | 2,466          |         |
|        | N (1-2 st)   | 0,565    | 0,061   | 2,257   | 0,170          | 1,351          |         |
|        | N (3-4 st)   | 0,864    | 0,052   | 8,441   | 0,263          | 3,042          |         |
|        | A            | 0,196    | 0,011   | 1,046   | 0,122          | 0,424          |         |
| VIM    | T (1-2 st)   | 11,254   | 3,929   | 22,227  | 6,375          | 14,506         | 0,0164  |
|        | T (3-4 st)   | 13,299   | 4,126   | 24,109  | 8,521          | 16,545         |         |
|        | N (1-2 st)   | 8,965    | 0,909   | 20,286  | 5,072          | 10,537         |         |
|        | N (3-4 st)   | 10,613   | 4,212   | 34,038  | 6,859          | 12,958         |         |
|        | A            | 12,947   | 7,836   | 31,417  | 10,163         | 15,474         |         |
| PCA3   | T (1-2 st)   | 0,510    | 0,012   | 56,165  | 0,074          | 10,750         | 0,0043  |
|        | T (3-4 st)   | 0,793    | 0,007   | 40,224  | 0,073          | 22,755         |         |
|        | N (1-2 st)   | 0,775    | 0,021   | 14,769  | 0,159          | 1,970          |         |
|        | N (3-4 st)   | 0,256    | 0,000   | 27,228  | 0,041          | 22,031         |         |
|        | A            | 0,044    | 0,001   | 7,342   | 0,023          | 0,132          |         |
| HOTAIR | T (1-2 st)   | 0,013    | 0,000   | 0,076   | 0,000          | 0,035          | 0,0014  |
|        | T (3-4 st)   | 0,004    | 0,000   | 0,079   | 0,002          | 0,025          |         |
|        | N (1-2 st)   | 0,003    | 0,000   | 0,051   | 0,001          | 0,008          |         |
|        | N (3-4 st)   | 0,003    | 0,000   | 0,057   | 0,001          | 0,011          |         |
|        | A            | 0,000    | 0,000   | 0,015   | 0,0001         | 0,001          |         |
| SCHLAP1| T (1-2 st)   | 0,018    | 0,000   | 2,274   | 0,005          | 0,029          | 0,008   |
|        | T (3-4 st)   | 0,297    | 0,000   | 2,350   | 0,013          | 0,847          |         |
|        | N (1-2 st)   | 0,019    | 0,000   | 0,110   | 0,004          | 0,042          |         |
|        | N (3-4 st)   | 0,038    | 0,000   | 1,148   | 0,014          | 0,350          |         |
|        | A            | 0,005    | 0,000   | 0,117   | 0,001          | 0,017          |         |
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| Genes   | Pairs with differences | p-value ** |
|---------|------------------------|------------|
| KRT18   | T (3-4 st)/A           | 0.0015     |
|         | T (3-4 st)/N (1-2 st)  | 0.0269     |
| MMP9    | T (1-2 st)/A           | 0.0115     |
|         | T (3-4 st)/A           | 0.0056     |
| VIM     | N (1-2 st)/A           | 0.012      |

| Genes   | Pairs with differences | p-value ** |
|---------|------------------------|------------|
| PCA3    | T (1-2 st)/A           | 0.0265     |
|         | T (3-4 st)/A           | 0.0134     |
|         | N (1-2 st)/A           | 0.0123     |
| HOTAIR  | T (1-2 st)/A           | 0.0009     |
|         | T (3-4 st)/A           | 0.0164     |
| SCHLAP1 | T (3-4 st)/A           | 0.0052     |

Notes: * – Kruskal-Wallis significant with FDR=0.1; ** – Dunn-Bonferroni post hoc method for multiple comparisons

(p = 0.041). These differences were indistinguishable in the total cancer group. The directions of the RE changes in the normal tissue group was similar to the adenocarcinoma group. However, there were no statistically significant differences in comparison with the adenocarcinoma or with the adenoma group. RE levels of AR (1 isof) changed similarly in the adenocarcinoma and the normal tissue groups. The significant changes were observed only between the adenocarcinoma (GL < 7) and the adenomas groups (p = 0.028. RE of OCLN in the adenocarcinoma group RE with GL = 7 gene expression level had maximal value whereas adenoma group and adenocarcinoma group with GL < 7 had minimal values and statistically significant differences with adenocarcinoma GL = 7 (p = 0.033, p = 0.049 correspondently). A similar pattern of changes in RE was observed for the MMP9 and HOTAIR genes. The lowest values of RE were registered in the adenoma group; the differences were statically significant with the adenocarcinoma groups, where the highest expression of these genes were observed (MMP9 p = 0.0001, p = 0.043, HOTAIR p = 0.0033, p = 0.005, respectively). KRT18 and SCHLAP1 were expressed at the highest levels in the adenocarcinoma group GL > 7, and this differs significantly in comparison with the adenoma group (p = 0.018, p = 0.024 correspondently).

Significant RE changes showed PCA3, when the adenoma group and the adenocarcinoma group GL = 7 (and normal tissues group GL = 7 as well) were compared (p = 0.027, p = 0.023, respectively).

Although the Kruskal-Wallis test showed differences of RE levels between 7 groups for the CASP3 and XIAP genes, another paired test, namely the multiple comparisons Dunn-Bonferroni post hoc method, did not confirm this data.

We would like to draw attention again to the fact that RE values for almost all genes in groups of conventionally normal tissues with different Gleason score had the same directions of expression change as the coincident adenocarcinomas groups, although the Dunn-Bonferroni post hoc method did not show any significant differences. Probably, these alterations are the result of a cancer-normal tissue cross-talk in organism.
Table 5. Descriptive statistics of the data on RE with significant differences in the T, N, A sample groups with the various Gleason score (GL<7, GL=7, GL>7) (5A) and RE differences between pairs with different GL (5B)

| Gene   | Group | Median | Minimum | Maximum | 25th percentile | 75th percentile | p-value * |
|--------|-------|--------|---------|---------|-----------------|-----------------|-----------|
| AR (1 isof) | T GL<7 | 1,148  | 0,413   | 2,789   | 0,574           | 1,626           | 0,008     |
|         | T GL=7 | 1,992  | 0,787   | 2,765   | 1,661           | 2,539           |           |
|         | T GL>7 | 1,323  | 0,440   | 3,353   | 0,911           | 2,228           |           |
|         | N GL<7 | 1,357  | 0,447   | 6,834   | 0,783           | 1,430           |           |
|         | N GL=7 | 1,961  | 1,145   | 3,227   | 1,354           | 2,629           |           |
|         | N GL>7 | 1,720  | 0,537   | 4,153   | 0,967           | 2,098           |           |
|         | A      | 2,129  | 0,879   | 4,936   | 1,623           | 2,672           |           |
| CASP3   | T GL<7 | 0,249  | 0,040   | 0,532   | 0,121           | 0,352           | 0,046     |
|         | T GL=7 | 0,414  | 0,170   | 0,579   | 0,365           | 0,450           |           |
|         | T GL>7 | 0,290  | 0,074   | 0,869   | 0,148           | 0,394           |           |
|         | N GL<7 | 0,265  | 0,075   | 0,647   | 0,166           | 0,310           |           |
|         | N GL=7 | 0,399  | 0,294   | 0,572   | 0,334           | 0,486           |           |
|         | N GL>7 | 0,234  | 0,058   | 0,685   | 0,149           | 0,320           |           |
|         | A      | 0,303  | 0,152   | 1,088   | 0,207           | 0,587           |           |
| CDH1    | T GL<7 | 3,035  | 0,896   | 8,601   | 1,521           | 3,954           | 0,010     |
|         | T GL=7 | 5,301  | 0,053   | 10,097  | 5,062           | 5,934           |           |
|         | T GL>7 | 2,815  | 0,161   | 7,247   | 1,462           | 3,764           |           |
|         | N GL<7 | 3,707  | 1,858   | 5,466   | 2,864           | 4,531           |           |
|         | N GL=7 | 4,942  | 2,562   | 5,705   | 3,519           | 5,118           |           |
|         | N GL>7 | 2,931  | 0,888   | 8,253   | 1,935           | 4,724           |           |
|         | A      | 2,752  | 0,004   | 8,941   | 2,001           | 3,661           |           |
| KRT18   | T GL<7 | 19,122 | 4,854   | 53,528  | 13,718          | 49,804          | 0,012     |
|         | T GL=7 | 21,912 | 1,798   | 31,828  | 16,570          | 29,296          |           |
|         | T GL>7 | 23,485 | 4,517   | 84,078  | 17,656          | 39,360          |           |
|         | N GL<7 | 15,571 | 4,798   | 46,191  | 8,058           | 19,663          |           |
|         | N GL=7 | 15,652 | 8,424   | 23,940  | 10,366          | 17,847          |           |
|         | N GL>7 | 16,505 | 5,534   | 42,552  | 10,064          | 23,924          |           |
|         | A      | 12,631 | 0,692   | 31,273  | 6,102           | 17,625          |           |
| MMP9    | T GL<7 | 1,795  | 0,642   | 6,867   | 1,122           | 2,897           | 0,001     |
|         | T GL=7 | 0,345  | 0,072   | 5,503   | 0,100           | 0,918           |           |
|         | T GL>7 | 0,810  | 0,028   | 27,489  | 0,313           | 2,466           |           |
|         | N GL<7 | 0,855  | 0,067   | 3,042   | 0,319           | 1,891           |           |
|         | N GL=7 | 0,389  | 0,061   | 2,257   | 0,243           | 0,946           |           |
|         | N GL>7 | 0,674  | 0,052   | 8,441   | 0,170           | 2,168           |           |
|         | A      | 0,196  | 0,011   | 1,046   | 0,122           | 0,424           |           |
### Table 5A (breakover)

| Gene | Group | Median | Minimum | Maximum | 25th percentile | 75th percentile | p-value * |
|------|-------|--------|---------|---------|-----------------|----------------|----------|
|      |       |        |         |         |                 |                |          |
| **OCLN** |       |        |         |         |                 |                |          |
| T GL<7 | 0.361 | 0.137 | 1.431 | 0.205 | 0.571 | 0.007 |
| T GL=7 | 1.080 | 0.525 | 3.790 | 0.649 | 1.846 |          |
| T GL>7 | 0.565 | 0.059 | 3.491 | 0.318 | 1.157 |          |
| N GL<7 | 0.411 | 0.248 | 0.893 | 0.310 | 0.478 |          |
| N GL=7 | 0.636 | 0.512 | 1.376 | 0.537 | 0.949 |          |
| N GL>7 | 0.565 | 0.117 | 2.484 | 0.391 | 1.061 |          |
| A     | 0.396 | 0.227 | 1.574 | 0.332 | 0.593 |          |
| **XIAP** |       |        |         |         |                 |                | 0.019    |
| T GL<7 | 0.299 | 0.078 | 0.570 | 0.191 | 0.400 |          |
| T GL=7 | 0.479 | 0.330 | 0.995 | 0.349 | 0.623 |          |
| T GL>7 | 0.332 | 0.088 | 0.633 | 0.164 | 0.407 |          |
| N GL<7 | 0.261 | 0.060 | 0.925 | 0.191 | 0.392 |          |
| N GL=7 | 0.522 | 0.251 | 0.677 | 0.420 | 0.597 |          |
| N GL>7 | 0.287 | 0.044 | 0.568 | 0.160 | 0.459 |          |
| A     | 0.301 | 0.182 | 0.896 | 0.235 | 0.386 |          |
| **PCA3** |       |        |         |         |                 |                | 0.006    |
| T GL<7 | 0.647 | 0.017 | 22.755 | 0.046 | 9.739 |          |
| T GL=7 | 8.480 | 0.012 | 56.165 | 0.355 | 26.476 |          |
| T GL>7 | 0.374 | 0.007 | 52.746 | 0.081 | 19.972 |          |
| N GL<7 | 0.240 | 0.021 | 24.781 | 0.049 | 0.369 |          |
| N GL=7 | 1.574 | 0.088 | 14.769 | 1.182 | 3.060 |          |
| N GL>7 | 0.289 | 0.000 | 27.228 | 0.041 | 1.649 |          |
| A     | 0.044 | 0.001 | 7.342  | 0.023 | 0.132 |          |
| **HOTAIR** |      |        |         |         |                 |                | 0.004    |
| T GL<7 | 0.017 | 0.003 | 0.056 | 0.007 | 0.043 |          |
| T GL=7 | 0.008 | 0.000 | 0.047 | 0.002 | 0.030 |          |
| T GL>7 | 0.013 | 0.002 | 0.079 | 0.004 | 0.048 |          |
| N GL<7 | 0.003 | 0.0004 | 0.027 | 0.001 | 0.004 |          |
| N GL=7 | 0.003 | 0.0003 | 0.051 | 0.001 | 0.018 |          |
| N GL>7 | 0.008 | 0.0004 | 0.057 | 0.003 | 0.011 |          |
| A     | 0.001 | 0.0002 | 0.015 | 0.000 | 0.003 |          |
| **SCHLAP1** |     |        |         |         |                 |                | 0.052    |
| T GL<7 | 0.020 | 0.002 | 2.350 | 0.010 | 0.037 |          |
| T GL=7 | 0.018 | 0.001 | 1.120 | 0.013 | 0.242 |          |
| T GL>7 | 0.270 | 0.000 | 1.651 | 0.018 | 0.452 |          |
| N GL<7 | 0.042 | 0.004 | 0.989 | 0.022 | 0.079 |          |
| N GL=7 | 0.017 | 0.0003 | 0.110 | 0.014 | 0.024 |          |
| N GL>7 | 0.038 | 0.002 | 1.148 | 0.013 | 0.204 |          |
| A     | 0.008 | 0.0003 | 0.117 | 0.002 | 0.040 |          |
When a 2^-ddCt model was used to calculate RE in adenocarcinoma groups (2^-dCt gene expression in each tumor sample was normalized to the paired normal tissue sample), no differences between the groups of adenocarcinoma and normal prostate tissue were shown for all of 19 genes. This result confirms our assumption about the cross-talk between tumors and normal tissue. Importantly, the 2^-ddCt model is used to characterize only a range of the fold changes, but not the quantity of the mRNA of a certain gene.

### Correlations between clinical and pathological characteristics and RE levels

The Spearman’s rank correlation (r^s) test produced a number of correlations between CPC (Gleason score, Age and PSA level) and the gene expression (Table 6 A). Thus, only RE of the CDH2 correlated with the Gleason score (r^s = 0.338, p < 0.05). RE of the SCHLAP1 correlated with the age and the PSA levels. PSA level was shown positive correlation (r^s = 0.436, p < 0.05), but Age group had reverse correlation with SCHLAP1 RE (r^s = -0.376, p < 0.05). The greatest number of correlations with RE was obtained for the Stage characteristic. There were six negative correlation of Stage with CDH1 (r^s = -0.385, p < 0.05), AR (1 iso), AR (2 iso) (r^s = -0.381, r^s = -0.390, accordingly p < 0.05), OCLN (r^s = -0.360, p < 0.05), NKX3-1 (r^s = -0.353, p < 0.05) and XIAP (r^s = -0.352, p < 0.05).

### Correlations between gene RE levels.

Correlation indexes between investigated genes (Table 6B) had more strong significance compared to CPC. The strongest indexes (r^s |0.603–0.817|) with very high significance (p < 0.0001) had 10 correlations. The highest value r^s = 0.817 was for NKX3-1 with CDH1, r^s = 0.754 had CASP3 with XIAP, and the highest inverse r^s = -0.724 had KRT18 with FN1. The greatest number of maximal correlations was for OCLN (3 ones) with AR (1 iso), NKX3-1, XIAP.

Moreover, we revealed 44 correlations between investigated genes (r^s |0.402–0.652|) with significance p < 0.01 and 23 correlations (r^s |0.338–0.422|) with significance p < 0.05. Large quantity of correlations between genes testify to close interrelation of molecular networks and carcinogenic processes, in which investigated genes participate.

### Discussion

The EMT is an important process in carcinogenesis. It is known that increase in expression
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Table 6. Spearman Rank Order Correlations (r<sup>s</sup>) of CPC with genes RE (6A) and between gene RE r<sup>s</sup> (6B)

**6A.**

| CPC    | CDH1 | CDH2 | AR (1 isof) | AR (2 isof) | FN1 | VIM | OCLN | MMP2 | MMP9 |
|--------|------|------|-------------|-------------|-----|-----|------|------|------|
| GL     | −0.171 | 0.338 | 0.023       | 0.033       | 0.072 | −0.076 | 0.127 | −0.057 | −0.135 |
| Stage  | −0.385 | 0.181 | −0.381      | −0.390      | 0.184 | 0.247 | −0.360 | 0.151 | 0.140 |
| PSA ng/ml | −0.233 | −0.026 | −0.147      | −0.097      | 0.065 | 0.087 | −0.011 | 0.080 | −0.069 |
| Age    | −0.001 | 0.066 | −0.154      | −0.069      | 0.213 | −0.224 | −0.071 | −0.058 | −0.029 |

| CPC    | NKX3-1 | PCA3 | PSA | KRT18 | MKI67 | CASP3 | XIAP | PTEN | HOTAIR | SCHLAP1 |
|--------|--------|------|-----|-------|-------|-------|------|------|--------|---------|
| GL     | −0.052 | 0.013 | 0.055 | 0.039 | −0.016 | 0.049 | 0.026 | 0.102 | 0.101 | 0.189 |
| Stage  | −0.353 | −0.128 | −0.305 | −0.052 | −0.276 | −0.126 | −0.352 | 0.092 | 0.223 | −0.006 |
| PSA ng/ml | −0.263 | 0.127 | 0.072 | 0.107 | 0.029 | −0.046 | −0.046 | −0.270 | 0.003 | 0.436 |
| Age    | 0.085  | −0.151 | −0.300 | −0.208 | 0.053 | 0.145 | 0.154 | −0.007 | 0.177 | −0.376 |

**6B.**

| Genes  | CDH1 | CDH2 | AR (1 isof) | AR (2 isof) | FN1 | VIM | OCLN | MMP2 | MMP9 |
|--------|------|------|-------------|-------------|-----|-----|------|------|------|
| CDH1   | 1,000 |      |             |             |     |     |      |      |      |
| CDH2   | 0.027 | 1,000|             |             |     |     |      |      |      |
| AR (1 isof) | 0.439 | 0.272 | 1,000       |             |     |     |      |      |      |
| AR (2 isof) | 0.374 | 0.155 | 0.665       | 1,000       |     |     |      |      |      |
| FN1    | −0.271 | 0.447 | 0.069       | 0.099       | 1.000 |      |      |      |      |
| VIM    | −0.422 | −0.033 | −0.235      | −0.444      | 0.214 | 1.000 |      |      |      |
| OCLN   | 0.585 | 0.057 | 0.609       | 0.523       | −0.077 | −0.243 | 1.000 |      |      |
| MMP2   | −0.429 | 0.226 | −0.053      | −0.185      | 0.298 | 0.450 | −0.377 | 1.000 |      |
| MMP9   | −0.178 | −0.095 | −0.278      | −0.175      | −0.001 | 0.371 | −0.205 | 0.354 | 1.000 |
| NKX3-1 | 0.817 | 0.167 | 0.540       | 0.538       | −0.199 | −0.514 | 0.603 | −0.453 | −0.304 |
| PCA3   | 0.483 | −0.429 | 0.151       | 0.380       | −0.224 | −0.299 | 0.512 | −0.651 | −0.269 |
| PSA    | 0.528 | −0.363 | 0.346       | 0.304       | −0.560 | −0.316 | 0.479 | −0.446 | −0.354 |
| KRT18  | 0.402 | −0.529 | −0.064      | 0.103       | −0.724 | −0.238 | 0.363 | −0.447 | 0.017 |
| MKI67  | 0.227 | 0.110 | 0.356       | 0.430       | −0.127 | −0.275 | 0.467 | 0.013 | −0.016 |
| CASP3  | 0.250 | 0.568 | 0.492       | 0.389       | 0.485 | −0.166 | 0.443 | 0.012 | −0.330 |
| XIAP   | 0.381 | 0.408 | 0.572       | 0.524       | 0.238 | −0.283 | 0.654 | −0.197 | −0.308 |
| PTEN   | 0.015 | 0.317 | 0.232       | 0.012       | 0.263 | 0.287 | −0.004 | 0.497 | 0.231 |
| HOTAIR | −0.420 | 0.552 | −0.131      | 0.062       | 0.561 | 0.090 | −0.428 | 0.206 | 0.122 |
| SCHLAP1| −0.025 | −0.263 | −0.007      | 0.127       | −0.223 | −0.012 | 0.356 | −0.099 | −0.192 |
of mesenchymal markers and decrease of epithelial marker expression is a feature of tumor progression, invasiveness and metastasizing, and this indicates, as a rule, the aggressiveness of tumor and poor disease prognosis [25].

Crucial changes in expression of the EMT-related genes have been shown on different solid tumors and the cell line models. It is difficult, to investigate EMT on biopsies, because there are many types of cells in each tissue sample, that influence on the registered gene expression. Every tissue sample contains also normal epithelial cells, different types of immune cells, stromal components (fibroblasts, endothelial cells) etc. Therefore, an important task is to determine the gene expression pattern in the specific cell types, to understand the nature of changes.

Our results have shown that changes in RE changes of different genes are often heterogeneous, especially in prostate adenocarcinoma group. Probably, the ordinary grouping did not reflect the sample heterogeneity and also the stage, type, different factors of carcinogenesis and molecular characteristic of the types and subtypes of prostate cancer [2]. Differences in RE of more, than 10–20 folds in one group suggest that there are samples with both, low and high expression levels, as also unaltered one. To understand the nature of these variations, it is necessary to determine the causal carcinogenic factors and molecular processes.

We accomplish k-Means clustering of prostate adenocarcinomas RE for all 19 genes to search characteristic RE profiles for prostate cancer subtyping. It is possible to divide this adenocarcinoma group from 2 to 4 clusters, but the best number of clusters is two (using all 19 investigated genes as an example) (Figure 2). Cluster 1 has 20 adenocarcinoma samples (54 %). Cluster 2 has 17 adenocarcinoma samples (46 %).

All genes in two clusters could be sorted into three groups, according to their RE levels: 1) high RE in cluster 1 (these genes are potential markers of cluster 1); 2) high RE in cluster 2 (these genes are potential markers of cluster 2); 3) without changes in both clusters.

| Genes     | NKX3-1 | PCA3 | PSA  | KRT18 | MKI67 | CASP3 | XIAP | PTEN | HOTAIR | SCHLAP1 |
|-----------|--------|------|------|-------|-------|-------|------|------|--------|---------|
| NKX3-1    | 1,000  |      |      |       |       |       |      |      |        |         |
| PCA3      | 0,389  |      |      |       |       |       |      |      |        |         |
| PSA       | 0,483  | 0,660| 1,000|       |       |       |      |      |        |         |
| KRT18     | 0,338  | 0,522| 0,694| 1,000 |       |       |      |      |        |         |
| MKI67     | 0,468  | 0,092| 0,285| 0,201 | 1,000 |       |      |      |        |         |
| CASP3     | 0,444  |      |      |       |       |      |      |      |        |         |
| XIAP      | 0,575  | 0,214| 0,030|       | -0,151| 0,392 |      |      |        | 0,754   |
| PTEN      | 0,024  |      |      |       |       |       |      | 0,118| 0,114  | 1,000   |
| HOTAIR    | -0,253 | -0,310| -0,532| -0,652|       | -0,171| -0,003| -0,031| 0,029  | 1,000   |
| SCHLAP1   | -0,019 | 0,376| 0,575| 0,613 |       | 0,186 | -0,016| -0,071| -0,334 | -0,422  |

Note: \( p < 0.0001 \) (bold type), \( p < 0.01 \) (bold+italic type), \( p < 0.05 \) (italic type) with FDR=0.1
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Clusterization data well agree with Spearman correlations.

Genes in cluster 1 that are highly expressed, are epithelial markers (CDH1, OCLN.) and prostate luminal cell markers (AR(1 isof), KRT18, NKX3-1, PSA). Strong expression of these genes is typical for the luminal subtype of tumors [26, 27]. In addition, cluster 1 showed high RE of two IncRNA (PCA3 and SCHLAP1). PCA3 is very high specific IncRNA in prostate cancer cells [28]. SCHLAP1 is associated with metastatic progression in prostate cancer [29].

Moreover, high RE of AR (2 isof) in cluster 1, which is tissue specific variant (named AR45) and extrinsic for normal prostate, is very important for modulation of AR function especially in the presence of adrenal androgens [30].

The opposite situation of RE levels is in cluster 2. Here the low expression of epithelial cells markers was observed, and high expression of mesenchymal markers (CDH2, FN1, VIM). This is typical for the basal subtype of prostate cancer, on the one hand [27, 31]. On the other hand, it is also characteristic for cancer stem cells [31]. Besides, the high RE of HOTAIR is also detected, and HOTAIR is the specific IncRNA of cancer stem-like cell subpopulation [30].

We suppose, that prostate cancer samples from different clusters could be a subjects of different oncogenic pathways, carcinogenic mechanisms and, as a result, they could have different drug sensitivity and prognosis [27].

Prostate cancers were sub-grouped in from 3 to 7 subtypes, according to as transcriptome changes [27, 31], based on genetic, epigenetic and transcriptome alterations of hundreds of genes [32]. Therefore, to propose a molecular signature of cancer subtypes, we shall investigate and characterize the specific changes of RE levels further.

Conclusions

Investigation of RE of the 19 EMT-related genes in benign and malignant prostate tumors has shown the 9 differentially expressed genes, namely AR(1 isof), AR(2 isof), PTEN, VIM, MMP9, KRT18, PCA3, HOTAIR, and SCHLAP1. Interestingly, RE patterns were similar in the conventionally normal tissue group and the corresponding adenocarcinoma groups. This
means that these conventionally normal tissues contained tumor cells and could not serve as an adequate control.

RE values of all investigated genes showed high levels of heterogeneity, especially in the prostate adenocarcinoma group. Presence of at least two different molecular subtypes of prostate adenocarcinoma may explain a high dispersion in RE levels of the EMT-related genes. The first is a luminal subtype with high expression of epithelial and luminal markers and two lncRNA (PCA3 and SCHLAP1), and the second is a stem-like subtype with low expression of luminal and high expression of mesenchymal markers and high expression of lncRNA HOTAIR. The further experiments are needed to confirm these findings.

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даних експресії, особливо в групах аденокарциноми, може бути свідченням принаймні двох різних молекулярних підтипів: люмінального і стовбурового (базальному). Нами продемонстровано, що умовно-нормальні тканини передміхірувої залози не є адеквітним контролем. Для уточнення молекулярних підтипів аденокарциноми передміхірувої залози необхідні додаткові дослідження.

**Ключові слова:** пухлини передміхірувої залози, ЕМП, відносна експресія генів, молекулярні підтипи, некодуючі РНК.

**Експресія генів, пов’язаних з епітеліально-мезенхімальним перехідом у пухлинах передміхірувої залози**

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**Мета:** встановити відносну експресію у ЕМП-пов’язаних генах у зразках пухлин передміхірувої залози та проаналізувати можливу кореляцію та зв’язок між рівнем експресії генів у різних групах пухлин та клінічними характеристиками раку передміхірувої залози.

**Методи:** Відносні рівні експресії 19 генів у 37 заморожених зразках тканин раку передміхірувої залози з різними показниками Глісона та стадіями пухлин, 37 парних умовно-нормальних зразків тканини передміхірувої залози та 20 зразків аденоми проаналізовано кількісною ПЛР (QPCR).

**Результати:** Було виявлено 9 диференційно експресованих генів у доброкачествених і злоякісних пухлинах простати: (AR (1 isof), AR (2 isof), PTEN, VIM, MMP9, KRT18, PCA3, HOTAIR, SCHLAP1). На різних стадіях раку виявлено 6 диференційно експресованих генів (KRT18, MMP9, VIM, PCA3, HOTAIR, SCHLAP1), а за різними оцінками Глісона виявлено 8 диференційно експресованих генів (AR (1 isof), CDH1, KRT18, MMP9, OCLN, PCA3, HOTAIR, SCHLAP1). Спостерігався значний рівень дисперсії даних. Це можна пояснити наявністю різних молекулярних підтипів раку передміхірувої залози: люмінальний підтип (висока експресія CDH1, OCLN, AR (1 isof), KRT18, NXX3-1, PSA) і стовбуровий (базальній) підтип (висока експресія мезенхімальних маркерів CDH2, FN1, VIM і низька експресія епітеліальних маркерів). Досліджені некодуючі РНК були специфічно експресовани в двох молекулярних підтипах.

**Висновки:** пов’язані з ЕМП гени диференційно експресуються в доброкачествених та злоякісних пухлинах передміхірувої залози. Висока дисперсія
пов рака предстательной железы: люминального подтипа (высокая экспрессия CDH1, OCLN, AR (1 изоф), KRT18, NKX3-1, PSA) и стволового (базального) подтипа (высокая экспрессия мезенхимальных маркеров CDH2, FN1, VIM и низкая экспрессия эпителиальных маркеров). Исследованные некодирующие РНК специфически экспрессировались в двух молекулярных подтипах. Выводы: Гены, связанные с ЭМП, были дифференциально экспрессированы в доброкачественных и злокачественных опухолях предстательной железы. Высокая дисперсия данных экспрессии, особенно в группе аденокарцином, может свидетельствовать, по меньшей мере, о двух разных молекулярных подтипах: люминальном и базальном. Мы продемонстрировали, что условно-нормальные ткани простаты не являются адекватным контролем. Для уточнения молекулярных подтипов аденокарциномы предстательной железы необходимы дополнительные исследования.

Ключевые слова: опухоли предстательной железы, ЭМП, относительная экспрессия генов, молекулярные подтипы, некодирующие РНК.

Received 25.08.2017