Importance of miR-20a Expression in Prostate Cancer Tissue

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Abstract. Background: MicroRNAs (miRNAs), which are endogenously expressed regulatory noncoding RNAs, have an altered expression in tumor tissues. MiRNAs regulate cancer-related processes such as cell growth and tissue differentiation, and therefore, might function as oncogenes or tumor-suppressor genes. The aim of our study was to assess the expression of mir-20a, let-7a, miR-15a and miR-16 in prostate cancer (PCa) and benign prostatic hyperplasia (BPH) tissue and to investigate the relation between the expression of miRNAs and the clinicopathological features of PCa. Patients and Methods: The study group comprised 138 patients: 85 patients with BPH and 53 patients with PCa. The total RNA was isolated from the tissue specimen core and miRNA expressions were quantified using a real-time RT-PCR method (TaqMan MicroRNA Assays). U6snRNA was used for the normalization of the miRNA expression. Results: miR-20a expression was significantly higher in the group of patients with a Gleason score of 7-10 in comparison with the group of patients with a Gleason score of 0-6 (p=0.0082). We found no statistical differences in the miRNA expressions (mir-20a, let-7a, miR-15a and miR-16) in the PCa tissue samples in comparison with the BPH tissue samples. Conclusion: Our result shows that the more dedifferentiated PCa cells have a higher expression of miR-20a and this supports the oncogenic role of miR-20a in PCa carcinogenesis. The evaluation of miRNA expression could yield new information about PCa pathogenesis.

Prostate cancer (PCa) is the most commonly diagnosed cancer, and the second leading cause of cancer-related deaths of men in Western countries (1). Nevertheless, with regard to incidence, the molecular basis of PCa is insufficiently characterized. There is an increasing interest in the role of the new regulatory molecules, microRNAs (miRNAs), in cell processes, which could contribute to a better understanding of cancer pathology.

miRNAs are endogenously expressed, small non-coding RNAs, which regulate gene expression by the inhibition of the translation and/or decreasing of the stability of target mRNAs. Briefly, the miRNA expression in eukaryotic cells is realized in several steps. The miRNA gene is transcribed in the nucleus from the genomic DNA by RNA polymerase Pol II to produce a long transcript called a primary microRNA (pri-miRNA). U6snRNA was used for the normalization of the miRNA expression. Results: miR-20a expression was significantly higher in the group of patients with a Gleason score of 7-10 in comparison with the group of patients with a Gleason score of 0-6 (p=0.0082). We found no statistical differences in the miRNA expressions (mir-20a, let-7a, miR-15a and miR-16) in the PCa tissue samples in comparison with the BPH tissue samples. Conclusion: Our result shows that the more dedifferentiated PCa cells have a higher expression of miR-20a and this supports the oncogenic role of miR-20a in PCa carcinogenesis. The evaluation of miRNA expression could yield new information about PCa pathogenesis.

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Key Words: Prostate cancer, miRNA, miR-20a, let-7a, miR-15a, miR-16.
E. elegans and its role in timing of stem cell division and differentiation was identified (9). let-7 is a member of the let-7 family and so far this family includes 10 mature sequences in humans (10). The let-7 family is often present in multiple copies in the genome. A letter is used to indicate isoforms with slightly different sequences. A subsequent number indicates the same sequences arising from different genomic locations (e.g. let-7a-1). In general, the function of let-7 is to promote the differentiation of cells.

The next most often studied group of miRNAs is the mir-17-92 cluster which contains miR-20a. Today there is evidence that the mir-17-92 cluster is involved in many types of human cancer. The amplification of the region containing this cluster was described in lymphoma (11, 12) and lung cancer (13). Loss of heterozygosity of these genes was recorded in breast cancer (14), hepatocellular carcinoma (15) and nasopharyngeal carcinoma (16-18). These results show that members of the mir-17-92 cluster could play a role both as oncogenes and tumor-suppressor genes.

A group of miRNAs with an antioncogenic role in cancerogenesis is the cluster miR-15 and miR-16. The role of these miRNAs was first identified in chronic lymphocytic leukemia (CLL). This cluster is located in the CLL frequently deleted or translocated region 13q14.3. Both miRNAs negatively regulate BCL2 at the post-transcriptional level (19).

Identification of deregulated miRNAs in cancer tissue can focus interest on these miRNAs and can help to determine their place in the cascade of carcinogenesis and finally they may become a promising targets for therapy.

The aim of our study was to asses the expression of let-7a, mir-20a, miR-15a and miR-16 in PCa tissue and benign prostatic hyperplasia (BPH) and investigate the relationship between expression of these miRNAs and clinicopathological features of PCa. We chose these miRNAs on the basis of previously published studies, based mainly on high throughput assays (20-23). This is the first direct study of these miRNAs in biopsied patient samples.

### Patients and Methods

**Patients.** Our study group consisted of 138 patients who underwent a prostate biopsy between May 2006 and September 2008 in one of two Departments of Urology (the University Teaching Hospital in Pilsen and the University Teaching Hospital of the First Medical Faculty of Charles University in Prague). All the patients exhibited an elevated serum total prostate-specific antigen (tPSA) level and/or abnormal digital rectal examination. A previous biopsy was not an exclusion but had to have been performed at least three months prior to the study. The median age was 66.5 years (range 48-85 years). According to the histological verification, our group of patients was divided into 85 patients with BPH and 53 patients with PCa. The numbers of patients in compared groups are shown in Table I. The value of the Gleason score and TNM classification was not available for a small number of patients. Approval was obtained from the Institutional Ethics Committee and written informed consent from each patient. Patients who had had any transurethral manipulation, or radiotherapy, or who were on hormonal therapy, or had an indwelling catheter or acute urinary infection before the biopsy were excluded from the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>miR-20a</th>
<th>let-7a</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>25% Median 75%</td>
<td>25% Median 75%</td>
</tr>
<tr>
<td>BPH</td>
<td>85</td>
<td>1.5476 3.8637  8.8766</td>
<td>9.5137 17.2677 39.3966</td>
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<tr>
<td>PCs</td>
<td>53</td>
<td>1.2746 3.5554  7.2100</td>
<td>8.0000 17.4550 30.9100</td>
</tr>
<tr>
<td>Organ-confined cancer</td>
<td>41</td>
<td>1.2746 3.5554  7.02100</td>
<td>7.7275 12.8171 30.9100</td>
</tr>
<tr>
<td>Advanced and metastatic</td>
<td>11</td>
<td>1.0353 3.6808  7.4643</td>
<td>15.4550 20.8215 83.2859</td>
</tr>
<tr>
<td>Gleason score 0-6</td>
<td>27</td>
<td>1.1892 1.8661  3.8637</td>
<td>6.9644 12.5533 27.8576</td>
</tr>
<tr>
<td>Gleason score 7-10</td>
<td>20</td>
<td>3.5222 6.2467 15.0999</td>
<td>13.0431 18.0551 73.2024</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>miR-15a</th>
<th>miR-16</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>25% Median 75%</td>
<td>25% Median 75%</td>
</tr>
<tr>
<td>BPH</td>
<td>85</td>
<td>0.9013 1.6021  3.4343</td>
<td>9.3827 19.6983 48.5029</td>
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<tr>
<td>PCs</td>
<td>53</td>
<td>0.7738 1.6818  3.3636</td>
<td>6.1903 14.7230 42.2243</td>
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<td>0.7220 1.6586  3.3173</td>
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<td>Advanced and metastatic</td>
<td>11</td>
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<td>18.3792 20.8215 16.8507</td>
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<td>20</td>
<td>1.4315 2.9332  3.8025</td>
<td>8.7442 22.1075 90.5084</td>
</tr>
</tbody>
</table>
Results

We investigated differences in the expression of mir-R-20a, let-7a, miR-15a and miR-16 in the BPH and PCa. All the measured values of expression and the P-values are summarized in Table I. We found no statistically significant differences in the expression of these miRNAs in BPH tissue in comparison with PCa tissue. Furthermore, we compared the expression in tumor tissue between different TNM stages of the disease. We compared the group of patients with organ-confined cancer (TNM 1c+2a+2b+2c) with those with a higher extent of the disease (advanced and metastatic; TNM 3a+3b+3c+1cM+2cM+3aM). We recorded no statistically significant differences in the expression of mir-R-20a, let-7a, miR-15a and miR-16. Finally, we analyzed the differences in the expression of mir-R-20a, let-7a, miR-15a and miR-16 in two groups divided according to the degree of differentiation of PCa tissue, i.e. those with a Gleason score of 0-6 and those with a Gleason score of 7-10. We observed that the expression of miR-20a was statistically significantly higher in the group with a Gleason score of 7-10 than in the group with a Gleason score of 0-6 (p=0.0082) (Figure 1). There were no differences in the expression of let-7a, miR-15a and miR-16 between these groups.

Discussion

In the past few years, research has revealed the role of miRNAs in the regulation of cell processes such as proliferation and apoptosis, as well as in the pathology of these processes. Despite the fact that the sequence of particular steps of the carcinogenesis of PCa is not known, it is evident that there is a deregulation of apoptosis and proliferation. We assessed the miRNAs which participate in these processes. We observed a higher expression of mir-R-20a in the group with a Gleason score of 7-10 than in the group with a Gleason score of 0-6. The Gleason score is an important variable describing the behavior of PCa and has been correlated with pathologic stage, metastasis and outcome (28). miR-20a is one of the members of the mir-17-92 cluster.

Sylvestre et al. described an overexpression of miR-20a in the human prostate cancer cell line PC3 using PCR (21). Volinia et al. recorded an up-regulation of miR-20a in PCa tissue using a microarray assay (22). The identified function of miR-20a is the modulation of the translation of the E2F2 and E2F3 mRNAs via binding sites in their 3'-untranslated region (21). This supports the oncogenic behavior of miR-20a. The same authors also observed antiapoptotic activity. miR-20a overexpression reduced apoptosis in the PC3 cell line (21). Our result supplements the previous findings and shows that the more dedifferentiated cancer cells (Gleason score 7-10) have a higher expression of oncogenic miR-20a.
It was reported that the other investigated miRNAs (let-7a, miR-15a and miR-16) have an antioncogenic function (3). The results of recent studies show that the RAS oncogene could be regulated at least by some members of the let-7 family (29). There is evidence that LIN28 plays an important role in the biogenesis of mature let-7 miRNA as a negative post-transcriptional regulator. High LIN28 levels prevent the processing of pri-let-7 by the microprocessor complex and also prevent pre-let-7 from turning into mature let-7 by dicer (10). This example also demonstrates that the assessment of pri-miRNA or pre-miRNA does not always corresponds with the level of mature miRNA. The miR-15a and miR-16 cluster is located in cancer frequently deleted or translocated region 13q14.3. Among the targets of miR-15a and miR-16, the antiapoptotic protein BCL2 was identified, which is overexpressed in many malignancies. Bonci et al. reported that in cancer cells of advanced prostate tumors, the miR-15a and miR-16 levels was significantly decreased (23). We did not observe differences in expression of miR-20a, let-7a, miR-15a and miR-16 between BPH and PCa. It should be noted that the behavior of BPH does not necessarily correspond with that of normal prostatic tissue; it was not possible for us to obtain normal prostate tissue.

In conclusion, our result extends the findings of previous studies and shows that the more dedifferentiated prostate cancer cells have a higher expression of miR-20a, supporting the oncogenic role of miR-20a in carcinogenesis of PCa.

Acknowledgements
This study was supported by grant IGA NR/8918-3 from the Ministry of Health of the Czech Republic and by Czech government research project MSM 0021620819.

References


Received March 21, 2010
Revised June 12, 2010
Accepted June 18, 2010