Hypomethylation of DNA in Pathological Conditions of the Human Prostate

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ABSTRACT

The overall 5-methylcytosine content of DNA from normal, benign hyperplastic, and neoplastic human prostate tissue was analyzed. DNA methylation levels were significantly lower in prostates with benign prostatic hyperplasia and metastatic tumors. In contrast, nonmetastatic prostate tumor DNA had a 5-methylcytosine content essentially the same as in normal tissue. The results suggest a correlation between hypomethylation and metastatic capacity.

INTRODUCTION

It has been shown that a variety of changes in the genome may lead to tumor formation. These alterations may be relatively small, taking the form of point mutations (1), or be as large as chromosomal translocations (2). In addition, there is a growing body of evidence that DNA methylation plays a significant role in carcinogenesis (3-9). The methylated bases are almost exclusively 5-methylcytosine and occur mainly in the dinucleotide CpG (10), where approximately 4% of the total cytosine residues may be methylated in normal differentiated human cells.

The methylation of cytosine residues need not be static, but is a dynamic process with evidence suggesting that it is an important mechanism for the regulation of gene expression (3, 4, 10, 11). The information currently available suggests that there is a strong correlation between hypomethylation and carcinogenesis (5, 7-9). This study has therefore concentrated on the status of DNA methylation in cancer of the prostate, since this is an extremely common tumor occurring in men and which has been estimated to account for approximately 30,000 deaths annually in the United States alone (12). Furthermore, the prostate may provide a useful model for the study of DNA methylation in pathological conditions, which may be either a nonmalignant enlargement of the gland (known as BPH) (3) or a malignancy. The malignant conditions may present as either nonmetastatic (local) tumors or metastatic (disseminated) types. Generally, all of these pathological conditions require surgical treatment, although only those patients with metastatic tumors have a relatively poor prognosis.

A number of tumor markers have been evaluated for their ability to predict the clinical course of prostatic cancer, but none has proven to be consistently useful for the individual patient (13).

In this study the methylation status of prostate-derived DNA was examined in an attempt to establish whether there is a correlation between the degree of methylation and the development of pathological conditions of the prostate. The results obtained suggest a statistically significant correlation between hypomethylation and the development of both BPH and metastatic tumors. Hypomethylation was not observed in nonmetastatic tumors, despite their identification as malignancies by histological techniques.

MATERIALS AND METHODS

Purification of DNA. DNA was isolated as described elsewhere (14) from fresh surgical or autopsy samples obtained from Tygerberg Hospital, South Africa.

Analysis of DNA for 5-Methylcytosine Content. DNA was hydrolyzed in 88% formic acid at 180°C for 30 min. The sample was dried, and resultant free bases were analyzed and quantified by high-performance liquid chromatography (15). The percentage of 5-methylcytosine was calculated according to the following formula.

\[
\% \text{ of m}^5\text{C} = \frac{m^5\text{C} \times 100}{m^5\text{C} + \text{cytosine}}
\]

RESULTS

The 5-methylcytosine content of the prostatic DNA samples is presented according to groups in Table 1. These results show clearly that the mean values for methylation in both BPH and metastatic tumors are significantly lower in comparison to normal prostate samples. Approximately one-half (43%) of the BPH samples and all of the metastatic cancer samples had a 5-methylcytosine content of less than 3.6%, whereas none of the normals or nonmetastatic tumors was within this range. There was also a statistically significant difference in DNA hypomethylation between BPH and Ca(met) as well as in a comparison between normal, BPH, and Ca(met) (one-way analysis of variance). This table also shows that there is a statistically significant difference among the mean ages of the groups sampled in comparison to the normal DNA, but there are no significant differences among BPH, non-Ca(met), and Ca(met) groups.

The distribution of methylation obtained in the samples was analyzed. The majority (83%) of normal samples have a 5-methylcytosine content greater than 3.8%, and none of the normals tested had a m^5C content of <3.6%. In contrast, few of the BPH samples fell within the majority normal range and were evenly distributed between 3.61 to 3.8% and <3.6%. The sample with the highest value for BPH was regarded as being in the lower range of normal. The metastatic tumor samples were all markedly hypomethylated, and all m^5C values were <3.6%. None of these samples overlapped with values obtained from normal DNA, and there was a small overlap only with BPH samples. The nonmetastatic (local) tumor DNA, on the other hand, had an apparently normal m^5C content and did not overlap at all with either BPH or Ca(met) samples.

Since there are considerable differences in prostatic DNA methylation between the two groups of cancer patients, they have been examined more closely. The results of this investigation show that the development of metastatic tumors as opposed to local tumors is not age dependent nor does it appear related to tumor grade (Table 2).

We found a considerable difference in the m^5C content between the lowest value of non-Ca(met) and highest value of Ca(met) samples (0.23%). No relationship between age and methylation status of tumor DNA from cancer patients was detectable. In addition, no correlation between an increase in...
DNA HYPMETHYLATION IN PATHOLOGICAL HUMAN PROSTATE

Table 1 5-Methylcytosine content of prostatic DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group age (yr)</th>
<th>% of mC</th>
<th>% of individuals with mC content &lt;3.6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>32.8 ± 11.3 (6)</td>
<td>4.01 ± 0.11</td>
<td>0</td>
</tr>
<tr>
<td>BPH</td>
<td>75.7 ± 8.3 (7)</td>
<td>3.62 ± 0.14, &lt;0.01</td>
<td>43</td>
</tr>
<tr>
<td>Non-Ca(met)</td>
<td>77.0 ± 6.1 (4)</td>
<td>4.07 ± 0.28 (NS)</td>
<td>0</td>
</tr>
<tr>
<td>Ca(met)</td>
<td>71.1 ± 2.4 (6)</td>
<td>3.29 ± 0.24, &lt;0.001e</td>
<td>&lt;0.01e, &lt;0.001f</td>
</tr>
</tbody>
</table>

* Mean ± SD.
* Numbers in parentheses, number in group.
* Significance of difference from normal (Student’s t test).
* NS, not significant.
* Significance of difference from BPH (Student’s t test).
* Significance of difference comparing normal, BPH, and Ca(met) by one-way analysis of variance.

Table 2 mC content of prostatic tumor DNA and patient condition

<table>
<thead>
<tr>
<th>Age of patient (yr)</th>
<th>% of mC</th>
<th>Tumor grade*</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>4.34</td>
<td>None (1 m postoperative)</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>4.25</td>
<td>None (3 m postoperative)</td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>3.89</td>
<td>None (6 m postoperative)</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>3.81</td>
<td>None (6 yr postoperative)</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>3.58</td>
<td>At presentation (survived 41 mo)</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>3.48</td>
<td>At presentation (alive, 7 mo)</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>3.44</td>
<td>At presentation (survived 49 mo)</td>
<td></td>
</tr>
<tr>
<td>76*</td>
<td>3.21</td>
<td>At presentation (survived 6 mo)</td>
<td></td>
</tr>
<tr>
<td>73</td>
<td>3.14</td>
<td>At presentation (survived 7 mo)</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>3.80</td>
<td>At presentation (NO)</td>
<td></td>
</tr>
</tbody>
</table>

* Tumor grading: 1, well-differentiated tumor; 2, intermediate-grade tumor; 3, poorly differentiated tumor; NO, not obtainable.
* Patient had primary tumor which was surgically removed. Later (57 mo), a further tumor was found in the prostate; this is the sample analyzed in this study.

DISCUSSION

In this study the extent of DNA methylation in the human prostate was measured in conditions of carcinogenesis. The results suggest a correlation between hypomethylation and worsening pathological condition from normal [i.e., hypomethylation proceeds in the following order: normal → BPH → cancer (metastatic)]. A possible exception is the condition of nonmetastatic (or local) tumors, where the degree of methylation found is essentially the same as for normal prostate. Because of the small sample size of nonmetastatic tumors, we cannot exclude the possibility that some such tumors may be hypomethylated. The samples for DNA extraction were chosen in such a way that grossly necrotic tissue was avoided as was tissue which appeared normal. Where there was doubt, the tumor sample was reduced in size to reduce the possibility of contamination from surrounding healthy tissue. It is possible that the various populations of cells in the normal prostate may have different degrees of DNA methylation; therefore the results shown are at best an average from all the cell types within the given sample. Clearly if one or more of the populations became either under- or overrepresented, this bias would be reflected in the overall methylation status of the DNA. At present, however, it is not feasible to analyze the methylation status of various cell types from intact tissue samples of the adult human prostate.

It has been reported by a number of studies that hypomethylation is observed in tumor DNA (7–9); however, few of these studies have investigated the change in methylation from the same tissue under normal conditions (16). No such study has previously been done on the prostate as far as can be ascertained.

Results similar to those reported in this study have shown a progressive decline in the degree of DNA methylation from normal through benign to primary and finally secondary malignancies (7, 17).

From the information available in this study it is not possible to ascertain whether a change in methylation is a common factor between aging and carcinogenesis. Although our results show that there is a statistically significant difference in the degree of methylation between the normal prostate and either BPH or metastatic carcinomas of the prostate, there is also a significant difference in the mean age of the individuals in the normal group compared to the pathological states. We were not able to obtain normal prostate samples in the same age range to investigate whether age is a determining factor; however, we are inclined to believe that it is not necessarily important. In support of this, we would like to point out that the degree of methylation of prostatic DNA in nonmetastatic tumors is the same as normal prostatic DNA, although there is a considerable age difference in the group. In addition, there is a considerable difference in methylation between the metastatic tumor DNA and BPH DNA, although little difference is observed between mean group age. Finally, no age-related methylation decline was observed within any given group. Although we did not observe any correlation with age and methylation change, we cannot rule out the possibility that methylation changes in certain genes may not be age related and be involved in carcinogenesis (16), since our technique cannot detect specific alterations in methylation patterns.

It is clear that some tumor DNAs are not hypomethylated, since in our study only metastatic tumor DNA was. We need therefore to differentiate between two types of tumor. (a) A situation may arise whereby a decrease in DNA methylation [leading to alterations in gene expression (4, 8, 10, 11, 16)] leads initially to a hyperplastic state (BPH) and with further hypomethylation to the formation of a tumor(s). (b) Changes in methylation per se may not lead to tumor formation, but other factors such as point mutations (1) may be responsible in these cases for tumor formation.

It is therefore not possible to use the methylation of DNA as a diagnostic aid; however, knowledge of the degree of methylation of any given tumor DNA might be a useful indicator of prognosis, since the results presented here show that extensive hypomethylation correlates with poor survival in prostatic carcinomas.

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REFERENCES

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