Human telomerase and α-methylacyl-coenzyme A racemase in prostatic carcinoma. A comparative immunohistochemical study

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Abstract

Human telomerase detected by in situ hybridization has been demonstrated to be a useful tool for the diagnosis of malignancy and has also been tested by reverse transcriptase-polymerase chain reaction in several tumors such as hepatic cell carcinoma, melanoma, colonic carcinoma, gastric carcinoma, biliary carcinoma, breast carcinoma, mesothelioma, lung carcinoma, female tract carcinoma, and prostatic carcinoma. A monoclonal antibody (clone Tel-24) that allows for the detection of human telomerase reverse transcriptase (hTERT) in paraffin blocks of archival material has recently been developed. Carcinomas of cervix, endometrium, and breast have been studied by this method, but its value in prostatic carcinoma has not been explored; for that reason, we studied benign and malignant prostatic lesions by immunohistochemistry using paraffin embedded tissue. The aim of the study was to define the sensitivity and specificity of hTERT in prostate cancer, in comparison with α-methylacyl-coenzyme A racemase (AMACR) (P504-S). Fifty-five specimens of diverse prostatic lesions were selected for study (43 needle biopsies and 12 transurethral resections); there were 61 malignancies (47 infiltrating carcinomas and 14 high-grade prostatic intraepithelial neoplasias [PIN]) and 29 benign lesions (10 basal cell hyperplasias, 12 nodular hyperplasias, 4 chronic prostatitis, and 3 atrophic glands). Signal for hTERT nucleolar was detected in 31 of 47 infiltrating adenocarcinomas, in 11 of 14 PIN, and in none of 27 benign lesions (sensitivity, 71%; specificity, 100%). Diffuse cytoplasmatic positivity for AMACR was found in 37 of 41 infiltrating adenocarcinomas, in 7 of 7 PIN, and in 6 of 22 benign lesions (sensitivity, 91%; specificity, 72%). These results indicate that hTERT is highly specific of malignancy, with no false-positive cases; however, it had lower sensitivity than AMACR.

Keywords: Telomerase; hTERT; Prostate carcinoma; Immunohistochemistry; Racemase; AMACR; P504-S

1. Introduction

Telomeres are small extensions located at the end of the chromosome arms, and they are only found in eukaryotic cells. These structures play a crucial role in cellular replication because they defend DNA from degradation, recombination, and fusion of the terminal regions that occur in fragmented chromosomes [1-3]. The human somatic cells normally loose 50 to 200 sequences of telomeric nucleotides in each cell division [2-4]. That telomeric reduction has been proposed to be the "mitotic clock" that regulates the total number of mitoses programed for every cell and eventually signals cellular senility [3-5]. This mechanism avoids cumulative genetic changes and chromosomal instability that can lead to malignant transformation [3,6].

In cultures of "immortal" cells, a short but constant telomere has been identified, its length is not shortened in each division like in normal cells, suggesting that telomeric stability allows the cell to proliferate indefinitely [7]. It is presumed that the telomere length is maintained by the enzyme telomerase that exists in most neoplastic cells and probably contributes to the continuous proliferation [3]. For
Table 1
Prostatic lesions studied by IHC

<table>
<thead>
<tr>
<th>Procedure</th>
<th>hTERT (%)</th>
<th>AMACR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinomas</td>
<td>66% (31/47)</td>
<td>90% (37/41)</td>
</tr>
<tr>
<td>Gleason 6</td>
<td>50% (6/12)</td>
<td>85.7% (6/7)</td>
</tr>
<tr>
<td>Gleason 7</td>
<td>60% (6/10)</td>
<td>100% (9/9)</td>
</tr>
<tr>
<td>Gleason 8</td>
<td>37.5% (3/8)</td>
<td>75% (6/8)</td>
</tr>
<tr>
<td>Gleason 9</td>
<td>100% (12/12)</td>
<td>100% (12/12)</td>
</tr>
<tr>
<td>Gleason 10</td>
<td>100% (4/4)</td>
<td>80% (4/5)</td>
</tr>
<tr>
<td>PIN</td>
<td>78.5% (11/14)</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>Benign lesions</td>
<td>0% (0/27)</td>
<td>27% (6/22)</td>
</tr>
<tr>
<td>Prostaticis</td>
<td>0/4</td>
<td>50% (1/2)</td>
</tr>
<tr>
<td>Nodular hyperplasia</td>
<td>0/12</td>
<td>18% (2/11)</td>
</tr>
<tr>
<td>Basal cell hyperplasia</td>
<td>0/10</td>
<td>40% (2/5)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>0/3</td>
<td>33% (1/3)</td>
</tr>
</tbody>
</table>

The human telomerase complex is composed of 3 subunits: the RNA component of human telomerase (hTR), the catalytic component human telomerase reverse transcriptase (hTERT), and the protein associated to telomerase (TP1) [9].

Expression of hTERT is constant in cells with telomerase activity, and it has been considered as the most important marker for such activity. High levels of hTERT expression have been identified in malignant neoplasias and cancer cell lines but never in normal tissues and nonneoplastic cell lines. It was reported in 1994 that the activity of telomerase could be detected in human tumors [10,11]. Since then, several techniques including polymerase chain reaction and in situ hybridization have been developed to detect hTERT in neoplastic tissue; however, these techniques are costly and require tissue homogenates. More recently, immunohistochemistry (IHC) methods in paraffin-embedded material have been developed to evaluate telomerase activity in several malignancies. The signal of hTERT and hTR is localized in the nucleus of the neoplastic cells [12,13].

To differentiate prostate carcinoma from cancer mimickers, several IHC studies have been used: detection of high-molecular-weight keratin in the basal cells of benign lesions and β-methylacyl-coenzyme A racemase (AMACR) in the cytoplasm of cancer cells [14,15]. However, the studies that have tested telomerase in prostate cancer by IHC are limited and have used polyclonal antibodies [16].

The purpose of the present work was to explore the expression of a monoclonal antibody for hTERT (clone Tel-24) compared with AMACR in malignant and benign conditions of the prostate using archival paraffin embedded tissue.

### 2. Material and methods

Fifty-five specimens of prostatic tissue with diverse neoplastic and nonneoplastic lesions were selected from the surgical pathology files of our institution. The number of slides per case ranged from 1 to 5, but only 1 slide was selected for the study of IHC. Of these, 43 were transrectal needle biopsies and 12, transurethral resections. A total of 90 different lesions were identified in 55 slides: 47 infiltrating adenocarcinomas, 14 prostatic intraepithelial neoplasias (PIN), 10 basal cell hyperplasias, 12 nodular hyperplasias, 4 chronic prostatitis, and 3 atrophic glands.

Immunohistochemical reactions using the method of streptavidin-biotin-peroxidase-diaminobenzidine-for hTERT (1:100, monoclonal mouse, clone Tel-24, BIO SB Inc, Santa Barbara, Calif) and for AMACR, P504-S, 1:200, monoclonal rabbit, BIO SB). The tissue sections were deparaffinized and subjected to antigen retrieval using a pressure cooker with a retrieving solution (ImmunoDNARetrivecit ratir, BIO SB). The IHC expression was detected using a high-sensitivity system (ImmunDector HRP/DAB, BIO SB).

Because of the retrospective character of the study, several lesions seen in hematoxylin and eosin-stained sections were lost in deeper sections used for IHC; for that reason, 1 malignant and 3 benign lesions were eliminated for hTERT and 13 malignant and 8 benign lesions for AMACR.

The reactivity for hTERT was identified in the nucleolus, as well as the signal for AMACR in the cytoplasm. A

[Fig. 1. Human telomerase reverse transcriptase in infiltrating adenocarcinoma of the prostate, the signal is strongly positive in the nucleoli.]

![Fig. 1](image)

[Fig. 2. Values of sensitivity (Se), specificity (Sp), PPV, and PPV of hTERT vs AMACR in prostatic carcinoma.]

![Fig. 2](image)
positive reaction was considered if the signal was strong even if it was focal or if it was visible in more than 50% of cells even if it was weak. Nuclear reactivity was considered as negative for both antibodies.

3. Results

Reaction for hTERT was identified in 31 (66%) of 47 infiltrating adenocarcinomas, 11 (78.5%) of 14 PIN, and in none of 27 benign lesions (Table 1). The signal was positive in 1 to 4 nucleoli per cell (Fig. 1). These nucleoli sometimes covered 80% of the nuclear surface and were localized at the periphery. A direct relationship trend of Gleason grade, signal intensity, and percentage of positive cases was found, but it was non-significant by statistical analysis. Occasionally, benign lesions had prominent nucleoli; however, the reaction for hTERT was negative in those cases.

Sensitivity of hTERT for the diagnosis of cancer was 70%; specificity, 100%; predictive positive value (PPV), 100%; and predictive negative value (PNV), 60% (Fig. 2).

Cytoplasmic reactivity for AMACR was found in 37 (90%) of 41 infiltrating adenocarcinomas, in 7 (100%) of 7 PIN, and in 6 (27%) of 22 benign lesions (Table 1). In PIN and benign lesions, the signal predominated in the apical portion of the cytoplasm. On the other hand, in invasive adenocarcinoma, the reactivity was diffusely seen in the cytoplasm, and the signal showed a higher intensity (Fig. 3).

The sensitivity for the detection of carcinoma was 91%; the specificity, 72%; PPV, 39%; and PNV, 80% (Fig. 2).

4. Discussion

Immunohistochemistry techniques to detect telomerase in paraffin-embedded tissue have been recently used in several malignant neoplasms [9,13]. In the specific case of adenocarcinoma of the prostate, there is only 1 publication, by Lezkowski et al [9], who used a polyclonal antibody of whole mouse serum against the catalytic subunit of telomerase. In that work, the signal was seen in the nucleus in 64% to 100% of the cancer cells; however, they also found reactivity in skeletal muscle and basal cells of benign lesions, including atrophy. In the present work, using monoclonal antibody for hTERT, the only signal considered positive was nucleolar, whereas all cytoplasmic or nuclear reactivity were regarded as negative. Our results are in agreement with recent studies by deletion analysis, demonstrating that hTERT and hTTR are localized in the nucleolus, the assembling site of other ribonucleoproteins [12].

In prostate carcinoma, Lezkowski et al [9] showed a direct relationship between telomerase activity and tumor grade; similarly, in cancer of the uterine cervix, the activity of hTERT increased progressively in metaplasia, dysplasia, invasive cancer, and metastasis [13]. These findings suggest that the activity of telomerase increases in direct relationship to neoplastic transformation. In the present series, in spite of stronger signal intensity in Gleason score 8 and 9, no significant difference with lower scores could be demonstrated.

Other works have shown that the level of telomerase detected by reverse transcriptase–polymerase chain reaction correlates with overexpression of C-MYC but has no relationship with the level of prostatic specific antigen or with the histological grade [8].

Regarding the expression of AMACR, the results of the present work are in agreement with other authors; it was constantly expressed in carcinoma, but it was also expressed in 27.2% of the benign lesions, similar to the 21% reported by Beach et al [14]. In benign cells, the signal was observed mainly in the membrane and apical portion of the cytoplasm, while it stained diffusely the cytoplasm of malignant cells. The sensitivity of AMACR of 90% shown in this work is similar to 94% reported by Jiang et al [15]; these authors referred a “high specificity,” but the percentage was not specified.

In comparison with racemase, hTERT has lower sensitivity but higher specificity, and it was never seen in benign lesions even those with prominent nucleoli; this feature renders the immunohistochemical study for hTERT specially useful in suspicious lesions found in needle biopsies and has advantage over AMACR because it does not render false-positive cases. However, both antibodies may have a role in differentiating prostatic cancer from cancer mimics. In the present work, the combination of diffuse cytoplasmic signal for AMACR and nucleolar for hTERT was virtually diagnostic of carcinoma; for that reason we suggest the combined use of both antibodies.

References


