MonocarboxylateTransporter 2 (MCT2) as Putative Biomarker in Prostate Cancer

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BACKGROUND. Monocarboxylate transporter 2 (MCT2) is a transmembrane protein involved in the transport of monocarboxylates such as pyruvate and lactate. In a previous study we described overexpression of MCT2 in prostate carcinoma raising the hypothesis of using MCT2 as a possible biomarker in prostate cancer.

With the present study we aimed to compare the pattern of expression of MCT2 and alpha-methylacyl-CoA racemase (AMACR), in prostate carcinoma, PIN lesions, non-neoplastic prostate tissue, and normal prostate and compare their sensitivity and specificity. Also, we wanted to evaluate the value of using MCT2 in combination with AMACR and the negative markers 34βE12 or p63 to detect prostate cancer.

METHODS. A total of 349 cases, including prostate carcinoma, non-neoplastic prostate tissue and PIN lesions, from radical prostatectomies were examined by immunohistochemistry for AMACR, MCT2, p63, and 34βE12, using tissue microarrays (TMAs). Normal prostate from radical cystoprostatectomy was also studied.

RESULTS. Our study revealed that MCT2, similarly to AMACR, was consistently expressed in prostate cancer regardless of the Gleason score. In combination with AMACR and p63 or 34βE12, MCT2 helped to improve the diagnosis of prostate carcinoma. Also, overexpression of MCT2 as well as AMACR in PIN lesions may indicate the involvement of these two proteins in prostate cancer initiation.

CONCLUSIONS. We provided evidence for the presence of MCT2 in prostate cancer, selectively labeling malignant glands. Importantly, assessment of MCT2 together with AMACR, along with the negative markers, highly increases the accuracy in prostate cancer diagnosis. *Prostate* © 2012 Wiley Periodicals, Inc.

KEY WORDS: alpha-methylacyl-CoA racemase; cancer biomarkers; prostate cancer diagnosis

INTRODUCTION

Worldwide, prostate cancer is the second most common malignancy in men after lung cancer [1]. Diagnosis of prostate cancer glands can sometimes present a diagnostic challenge for pathologists, since, prostate carcinoma can mimic benign prostate glands [2] and the architectural or cytologic clues for the diagnosis of carcinoma may not always be seen in small foci of suspicious glands. Also, diagnosis of prostate cancer can be difficult in needle biopsies or in minimal residual cancer of radical prostatectomies, presenting one of the major challenges in surgical pathology. Underdiagnosis of a small focus of prostatic adenocarcinoma or overdiagnosis of a benign lesion mimicking cancer is not uncommon and can cause unfortunate consequences for patients and is a liability for pathologists. Therefore, it would be of great importance and usefulness to identify molecular markers with high sensitivity and specificity for prostate carcinoma.

Monocarboxylate transporters (MCTs) are transmembrane proteins which facilitate the membrane transport of important monocarboxylates, such as pyruvate and lactate. In glycolytic tumors, they promote the efflux of lactic acid, being important players in the maintenance of the tumor intracellular pH [3,4]. In a first study we assessed the immunoexpression of MCTs 1, 2, 4 and their protein chaperones in a well characterized prostate carcinoma series [5]. This study revealed a significant increase of MCT2 expression in tumor cells with a predominance of the strong score, which means that we could distinguish between MCT2 expression in tumor tissue from the expression of this protein in non-neoplastic tissue.

In the same study [5], we noted that MCT2 staining was very similar to AMACR (alpha-methylacyl-CoA racemase), an enzyme currently used in prostate cancer diagnosis, which is a peroxisomal and mitochondrial enzyme that was found to be up-regulated in prostate cancer [6–8]. AMACR plays an important role in bile acid biosynthesis and β-oxidation of branched-chain fatty acids and mediates the interconversion of (R) and (S)-2-methyl-branched-chain fatty acyl-CoAs [9,10], however, the biological significance of its expression in tumorigenesis is still not elucidated.

Some studies suggested that the use of AMACR as a positive marker alone may be misleading since expression of AMACR might be seen in benign glands, and non-malignant lesions [11]. Therefore, other studies report the use of AMACR as a positive marker along with the basal cell-specific negative markers 34βE12 and p63, which are absent in the vast majority of prostate carcinomas, to enhance the diagnostic accuracy and reduce the chance of misdiagnosis [12]. 34βE12 is a high-molecular-weight cytokeratin that is expressed in the cytoplasm of basal cells rather than in luminal or secretory cells [13]; p63 has selective expression in the basal cell compartment of various epithelial tissues and has high sensitivity in identifying the nuclei of basal cells in benign prostatic lesions [14–16].

This study aims to compare the sensitivity and specificity of MCT2 and AMACR in recognizing prostate cancer, by analyzing the immunohistochemical expression of both markers in a large series of prostate samples, including normal prostate, adjacent non-neoplastic tissue, PIN lesions and tumor tissues, and assess their clinico-pathological value. Also, we aimed to measure the sensitivity and specificity of combining MCT2 and AMACR as positive markers with the negative markers p63 and 34βE12, using tissue microarrays (TMAs), which recapitulate the small problematic foci of glandular proliferation that are generally encountered in prostatic biopsy specimens.

MATERIALS AND METHODS

Case Selection and Tissue Microarray Construction

Prostate samples were obtained from 349 patients with prostate carcinoma (including adjacent non-neoplastic tissues, PIN lesions, and primary tumors), with a median age of 64 years (range 46–74) selected from a cohort of patients who underwent radical prostatectomy in Centro Hospitalar do Porto—Portugal as a primary therapy (no preceding hormonal or radiation therapy) for clinically localized prostate cancer between 1993 and 2010. Benign prostate tissue was obtained from cystoprostatectomy specimens.

TMAs were constructed as previously reported [5]. Tumors were staged using the 2010 pTNM AJCC classification [17], which includes extra-prostatic extension and graded using the Gleason grading system 2005 [18].

Although there is no universal method of sampling prostate cancer tissue for immunohistochemistry slides, using either standard slides or TMAs, the histological features of the sampled areas that we sampled were representative of the final Gleason score for the case.

Immunohistochemistry

MCT2, AMACR, p63, and 34βE12 detection. Immunohistochemistry for MCT2, AMACR, p63, and 34βE12 was performed according to avidin–biotin–peroxidase complex principle with the primary

antibody for MCT2 (sc-14926, Santa Cruz Biotechnology, Santa Cruz, CA), AMACR (504R-16, Cell Marque), p63 (MS-1084-P, Neomarkers), and 34βE12 (334M-8, Cell Marque) diluted 1:200, 1:50, 1:100, and 1:100, respectively. Negative controls were performed by omitting of the primary antibody. Normal kidney was used as positive control for MCT2, AMACR, and p63. Human skin was used as 34βE12 positive control.

Tissue sections were counterstained with hematoxylin and permanently mounted.

Immunohistochemical Evaluation

All samples were scored for AMACR and MCT2 protein expression intensity. Protein expression was scored as negative (score = 0), weak (score = 1), moderate (score = 2), or strong (score = 3). Moderate or strong staining intensity was considered positive (score = 2 or 3) as previously described for AMACR [19].

Each case positive for AMACR and MCT2 was also evaluated for the percentage of glands/cells that stained for AMACR and MCT2 and scored as: <5%, 5–50%, and >50%.

Positive immunohistochemical staining for 34βE12 and p63 was defined as nuclear reactivity for p63 and cytoplasmic positivity for 34βE12 [12,20].

Criteria for interpretation of the antibody combination were as follows: cases were considered true-positive when 34βE12/p63 and AMACR, all of the three antibodies stained as for a malignant lesion, that is, 34βE12 and p63 stains absent and AMACR present. Using the combination with p63/AMACR/MCT2 or 34βE12/AMACR/MCT2 true-positive was considered when p63 and 34βE12 stains were absent but AMACR and/or MCT2 stains were present [20].

Each reaction was observed by two experienced pathologist (J.R.V. and C.L.), without prior knowledge of associated clinical or pathology staining information. Discordant results were discussed in a double-head microscope (J.R.V. and C.L.).

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL). All comparisons were examined for statistical significance using Pearson's chi-square (χ^2) test, being the threshold for significance $P < 0.05$.

RESULTS

Prostate samples were organized into TMAs, including 349 neoplastic tissues, 40 PIN lesions, 203 non-neoplastic, and 13 normal prostate cases
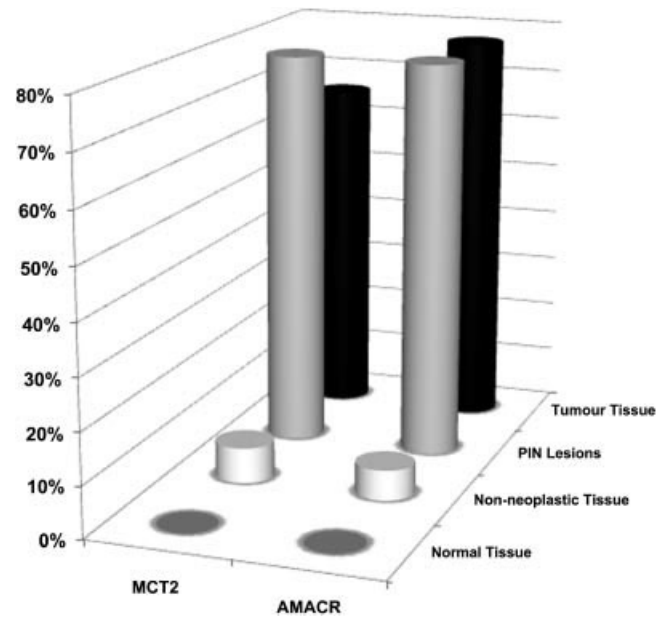

Fig. I. Frequency of MCT2 and AMACR expressions in normal prostate tissue, non-neoplastic tissue, PIN lesions, and tumor tissue samples.

from cystoprostatectomy were analyzed for MCT2, AMACR, 34βE12, and p63 immunohistochemical expression.

Figure 1 summarizes MCT2 and AMACR expressions in normal prostate, adjacent non-neoplastic tumor tissue, PIN lesions and primary tumor tissues. For the expression of both markers, AMACR and MCT2, we can observe a significant increase in tumor tissues and PIN lesions compared to normal prostate and non-neoplastic tissue samples ($P < 0.001$). Normal prostate samples were both negative for MCT2 and AMACR expressions. From 203 non-neoplastic samples, 14 stained for MCT2 (6.9%) and 12 for AMACR (5.9%). Both proteins showed overexpression in PIN lesions (77.5% for both). From the 349 malignant cases, 230 (65.9%) stained for MCT2 and 270 (77.4%) for AMACR.

Figure 2 shows representative immunohistochemical reactions for MCT2, AMACR, p63, and 34βE12 in normal prostate tissue (Fig. 2A, D, G, and J), PIN lesions (Fig. 2B, E, H and K, respectively), and tumor (Fig. 2C, F, I and L, respectively). The staining observed for MCT2 was very similar to that observed for AMACR. In most cases, adenocarcinomas showed moderate to strong staining in the majority of tumor cells for both proteins. Expression was uniformly cytoplasmic with a typical fine granular pattern. Nuclear staining of p63 and cytoplasmic staining for 34βE12 were confined to basal cells in normal prostate tissue (Fig. 2G and J, respectively).
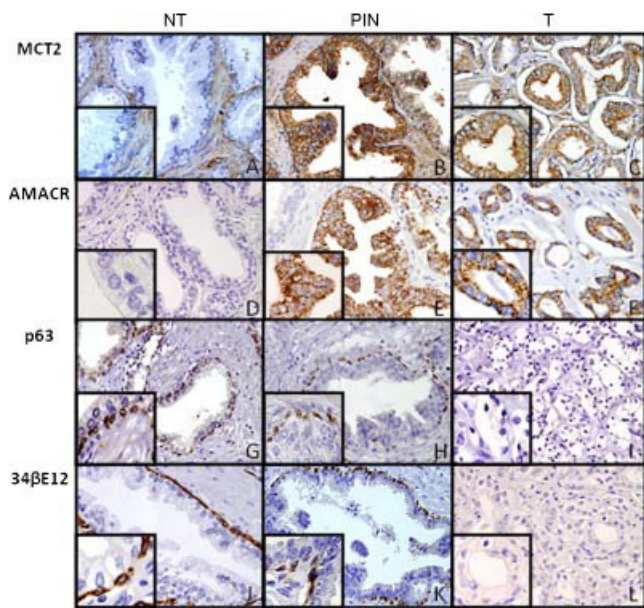
Fig. 2. Immunohistochemical expression of MCT2 (**A**–**C**), AMACR (**D**–**F**), p63 (**G**–**I**), and 34βEl2 (**J**–**L**) in normal prostate tissue (NT), PIN lesions (PIN), and prostate tumor tissues (T). Main pictures are at 200× magnification and insets are at 400×.

To assess if the staining extension pattern of MCT2 and AMACR in tumor tissues is associated with Gleason score, we compared the extension distribution of MCT2 and AMACR positivity in all Gleason grades (Tables I and II, respectively). We noted that

regardless Gleason score, the diffuse overexpression (>50% of tumor stained) of AMACR and MCT2 predominated and was observed in 65.5% and 57.4% of the cases, respectively.

Assessment of associations between MCT2 and AMACR expressions and the clinico-pathological data revealed no significant associations with Gleason score, pathological stage, patients' age, or preoperative serum specific antigen, perineural invasion, or biochemical recurrence (data not shown) as it was already observed for MCT2 using a different scoring methodology [5].

Specificity and sensitivity to detect tumor were calculated for the markers individually (Table III), as well as within the triple combinations (Table IV). From the 349 malignant samples stained, observing the different antibodies individually, 349 (100%) did not stain for 34βE12 or p63, 230 (65.9%), and 270 (77.4%) were positive for MCT2 and AMACR, respectively (Table III). From 203 benign prostatic TMA samples stained, 189 (93.1%) stained for 34βE12 and 191 (96.7%) stained for p63. For AMACR and MCT2, 191 (94.1%) cases and 189 (93.1%) benign samples were negative for AMACR and MCT2, respectively (Table III).

From the 349 malignant prostatic samples classified as malignant lesions, 270 (77.4%) stained negatively for 34βE12 and p63 and positively for AMACR (Table IV). Using the criteria of one negative marker (34βE12) plus two positive markers (AMACR/MCT2)

TABLE I. Extension of MCT2 Expression in Prostate Cancer Relative to Gleason Score

Gleason score	No. of cases	Positive cases	Extension of the staining		
			<5%+	5% to 50%+	>50%+
≤5	3	2 (0.8%)	0	0	2
6	83	55 (23.9%)	18	18	19
7	246	162 (70.4%)	23	35	104
≥8	17	11 (4.9%)	2	2	7
Total	349	230 (100%)	43 (18.7%)	55 (23.9%)	132 (57.4%)

TABLE II. Extension of AMACR Expression in Prostate Cancer Relative to Gleason Score

Gleason score	No. of cases	Positive cases	Extension of the staining		
			<5%+	5% to 50%+	>50%+
≤5	3	3 (1.1%)	0	1	2
6	83	62 (23%)	3	16	43
7	246	196 (72.6%)	21	52	123
≥8	17	9 (3.3%)	0	0	9
Total	349	270 (100%)	24 (8.9%)	69 (25.6%)	177 (65.5%)

TABLE III. Sensitivity and Specificity for the Antibodies Evaluated Separately

	34βE12	p63	AMACR	MCT2	Total
True positive (malignant Cases)	349	349	270	230	349
True negative (benign cases)	189	191	191	189	203
Sensitivity (%)	100	100	77.4	65.9	—
Specificity (%)	93.1	94.1	94.1	93.1	—
Positive predictive value (%)	96.1	96.7	95.7	94.3	—
Negative predictive value (%)	100	100	70.7	61.4	—

to identify a malignant lesion we have 327 of 349 malignant cases (87.7%) staining like a malignant lesion. The same results were obtained when using p63 as negative marker, that is, 87.7% of malignant cases staining like a true malignant case.

We also calculated the specificity, positive predictive value, and negative predictive value of the three combinations. We observed that all have a specificity and positive predictive value of 95.9% and 96.5% but with the use of two negative markers and only one positive marker we obtain a negative predictive value of 69.3% whereas with both AMACR and MCT2 as positive markers and only one negative marker, p63 or 34βE12, we can obtain a negative predictive value of 89.6% or 89.7%, respectively (Table IV).

DISCUSSION

With the major effort to early detect prostate cancer by men mass screening, there have been an increasing number of small foci of cancer encountered in prostate specimens. Inconclusive images on standard H&E staining are occasionally encountered, implying but not confirming the presence of malignancy. Such findings are often described as ''atypical foci'' and in most cases dictate a second biopsy [21–23].

It has been shown that using AMACR as a positive marker in association with the traditional basal cell-specific 34βE12 and/or p63 as negative markers can help to confirm the diagnosis when small atypical glands are identified by routine H&E staining [12,20].

It becomes more evident that it is crucial to use a combination of positive and negative markers for immunohistochemical analysis to assist in the diagnosis of prostate cancer.

In the present study we analyzed the pattern of expression of MCT2 and AMACR in a large number of prostate cancer and benign prostate tissues to compare the sensitivity and specificity of MCT2 to detect prostate cancer when compared to AMACR, an already established prostate cancer biomarker. Immunohistochemistry revealed that like AMACR, MCT2 is overexpressed in the majority of prostate cancer cases with diverse pathologic characteristics. This overexpression occurs in virtually all Gleason grades with a predominancy of diffuse overexpression, with more than 50% of tumor stained in positive cases, meaning that the positivity of MCT2 as well as AMACR is independent of the Gleason score, which is in accordance with the data reported for AMACR [19].

Expression of MCT2 was comparable with AMACR, allowing prostate cancer diagnosis in a minimal amount of tissue, giving few false-negative/positive data.

When we observe the results of immunohistochemical staining for the antibodies evaluated individually, we noted that 34βE12 and p63 were the most sensitive and specific markers to distinguish prostate cancer, with 100% sensitivity for both and 93.1% and 94.1% of specificity for 34βE12 and p63, respectively. However, they are negative markers and there are many limitations of using negative markers alone for the

TABLE IV. Sensitivity and Specificity for the Antibodies Evaluated in Triple Combinations

	34βE12−/p63−/ AMACR+	34βE12−/MCT2 and/ or AMACR+	p63−/MCT2 and/ or AMACR+	Total
True positive (malignant cases)	270	327	327	349
True negative (benign cases)	179	189	191	203
Sensitivity (%)	77.4	87.7	87.7	—
Specificity (%)	88.2	93.1	94.1	—
Positive predictive value (%)	91.8	95.9	96.5	—
Negative predictive value (%)	69.3	89.6	89.7	—

diagnosis of carcinoma, such as the fact that basal cells could be patchy or discontinuous in some benign lesions and lead to misdiagnosis. Consequently, negative staining for p63 or 34βE12 in a few glands suggestive of cancer is not proof of their malignancy since benign glands might not show uniform positivity with these markers.

Analyzing the results of immunohistochemical staining for the antibodies evaluated in the triple combination, we observed that the use of two positive markers with one negative marker instead of one positive marker with two negative markers improves the sensitivity to detect prostate cancer as well as the negative predictive value, which was 69.3% using the 34βE12/p63/AMACR combination and 89.6% or 89.7% when using 34βE12/MCT2 and/or AMACR or p63/MCT2 and/or AMACR. This result means that with these combinations of markers, we decrease the possibility of diagnosing benign prostate tissue as prostate cancer.

The observation that similarly to AMACR, MCT2 also stains strongly in PIN lesions, if on one hand decreases the specificity of these proteins, on the other hand indicates that these two proteins may be involved in tumor initiation. However, further studies are needed to clarify the role of both markers on prostate cancer initiation/progression.

CONCLUSIONS

Our study points to the consistent overexpression of MCT2 in prostate cancer, which is comparable to AMACR, an already established biomarker in prostate cancer. Importantly, assessment of MCT2 together with AMACR, along with the negative markers p63 and 34βE12, highly increases the accuracy in prostate cancer diagnosis.

ACKNOWLEDGMENTS

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