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ORIGINAL ARTICLE

Reduction of QM protein expression correlates with tumor grade in prostatic adenocarcinoma

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The QM protein is a transcription cofactor inhibiting the activity of AP-1 transcription factors and is also a ribosomal protein participating in protein synthesis. While protein synthesis is known to be increased in many cancers, inhibition of AP-1 activity presumably suppresses development and growth of sex-hormone-regulated tumor cells. The present study is the first report on immunohistochemical data of QM in human prostatic tissues. Paraffin sections of human prostate cancer samples were immunohistochemically stained for QM. The staining scores were analyzed with the clinicopathologic data of the patients. QM protein expression was found in all normal prostate glands adjacent to prostate cancer and in various intraepithelial neoplasia (PIN). In prostate cancer, the staining intensity and stained areas were decreased, compared to the normal glands and PIN lesions; in high-grade tumors only some patches of tumor cells showed positivity. Intense (3+) staining was mostly observed in the Gleason grade three areas (48%) compared to grade 4 and 5 areas (22%), although both low and high-grade tumors showed similar percentages of weakly stained areas. Moreover, staining in prostatic adenocarcinoma was often topographically patchy and varied from negative or weak (1+) to intense (3+). There was an inverse correlation from normal to low-grade tumors and then to high-grade tumors. However, in high-grade tumors, the positive areas were mostly confined to peripheral aspects of tumors and were particularly strong in foci of perineural invasion. This preliminary study suggests that decreased QM expression may be associated with early development of prostate cancer, but later a high level of QM may facilitate progression of the tumors to a more aggressive phenotype.
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Introduction

The QM gene was originally cloned by subtractive hybridization between the tumorigenic Wilms tumor cell line and its nontumorigenic derivative which contained a t(X;11) translocation chromosome.[1,2] This gene was found to be expressed at a much higher level in the nontumorigenic microcell hybrid and thus was originally considered to be a tumor repressor gene for Wilms tumor. However, it was later found out that this gene was located at X chromosome (Xq28) and had no homologous sequence on 11p, which indicates that it may not be directly relevant to Wilms tumors.[3] Although little is known about its expression pattern in human tissues, the QM gene is known to be expressed, at least at RNA level, in many adult tissues of the mouse, including cardiac and striated muscle, brain, testis, ovary, pregnant and nonpregnant uterus, liver, placenta, calvarium, whole rib, skin, lung, kidney and total embryo.[4] However, QM expression was found to be significantly reduced in adult kidney and cardiac muscle when compared to embryos. These data suggest that the level of the QM mRNA is altered in a developmental fashion and may be inversely correlated with differentiation. In addition, an abundance of QM mRNA found in testis also suggests that this gene escapes X inactivation in male germ cells and may be needed for protection of germ cells developing into cancer. Studies on a diverse array of eukaryotes show that the QM gene is highly conserved. The rate of sequence divergence of the various homologs was found to be slow, in the order of 1% change every 22 million years. The conservation suggests a critical role of QM in eukaryotic cells.[5,6]

The main physiological role of QM protein is still largely unknown, but limited literature suggests several

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functions of the QM protein: first, the QM protein may be a transcription factor or cofactor. Both human QM and chicken homolog of QM/Jif-1 was reported to interact with the transcription factor c-Jun and thus inhibited c-Jun to activate genes containing AP-1 binding sites.[7,8] Although some investigators report that such interaction rarely occurs *in vivo*,[9] others further show that the interaction with c-Jun occurs via binding to the leucine zipper region of c-Jun, which is controlled by a protein kinase C phosphorylation event via zinc domain.[10] C-Jun and c-Fos are transcription factor subunits that bind as a homo or hetero-dimer complex to the AP-1 binding site in the promoter regions of many genes, especially those regulated by sex hormones.[11,12] Moreover, it has been shown that presenilin 1 can inhibit c-Jun function by promoting the translocation of QM from the cytoplasm to the nucleus.[13] Since both c-Jun and c-Fos are proto-oncogenes, the inhibition of AP-1 activity by QM by intervening with c-Jun/c-Fos dimerization suggests a possibility that QM may be a tumor suppressor or growth inhibitor. Second, QM is known as 60S ribosomal protein L10 (RPL10) and is likely to be involved in the late steps of the 60S ribosomal subunit assembly.[14] Although in yeast lack of QM proves lethal.[15,16] QM seems to be dispensable in certain cells with high protein synthetic rate, since its expression is not detectable in some cells such as hematopoietic cells and bone marrow cells in adult mice.[17] Third, QM has been shown to interact with several Src family kinases such as c-Yes, and thus may participate in signal transduction of these kinases in many intracellular functions, including cell stability, division, proliferation, migration and differentiation.[18] QM protein may regulate cell proliferation, differentiation and apoptosis through one or all of these functions, that is, regulation of transcription, protein synthesis, signal transduction and/or other unknown mechanisms.

Two opposite patterns of correlation between QM expression levels and cell proliferative potential have been reported. Studies with bromodeoxyuridine labeled mouse embryos showed a striking inverse correlation between QM expression and cellular proliferation within developing cartilage, in which the zones showing higher proliferative capacities manifest lower levels of QM.[19] This inverse correlation dovetails with the aforementioned consideration that QM may function as a tumor suppressor or growth inhibitor. On the other hand, growth arrest of fibroblasts in the cell cycle by serum starvation concomitantly reduces the level of QM expression, and the expression of QM increases dramatically upon serum stimulation.[20,21] Several reports have also indicated that QM expression decreases as differentiation proceeds, which in general correlates with a lower proliferative capacity.[22] An 80% of reduction in QM expression has been observed during adipocyte differentiation.[23] All these data suggest that a higher level of QM may be associated with increased cell proliferation, which is understandable if the main function of QM is to participate in protein synthesis, since more rapidly proliferating cells need a high protein synthetic rate. However, the role of QM in prostate cancer and any other cancers is still unknown.

Materials and methods

Sample selection

In all, 70 consecutive radical prostatectomy specimens containing prostatic adenocarcinoma were retrieved from the tissue repository of the department of pathology at Wayne State University, between the years of 2000 and 2001. The clinicopathologic features recorded were patient age, race, pathologic stage, grade, percentage of the gland involvement, and tumor volume. The tumors were staged according to the 2002 AJCC/TNM and graded according to Gleason grading system.

Immunohistochemical evaluation of QM protein expression in prostatic tissues

Immunohistochemical staining was performed on 5-μm thick, formalin-fixed, paraffin-embedded tissue sections using an avidin-biotin complex (ABC) method described previously.[24] Sections were deparaffinized and blocked with 3% peroxide for 10 min. Antigens were retrieved by heating in a microwave oven in a 50 mM citrate buffer, pH 6.0, after boiling for 5 min. After being blocked with 6% normal goat serum, the sections were incubated with the primary antibody for 3 h, followed by 1.5 h incubation with a second antibody conjugated with biotin (Vector Laboratories Inc., Burlingame, CA). The sections were then incubated with peroxidase-conjugated avidin (K355, Dako, Corporation, Carpinteria, CA) for 30 min, followed by color development with diaminobenzidine and peroxide. All procedures were carried out at room temperature. The primary antibodies used were rabbit polyclonal C-17 and N-17 from Santa Cruz Biotechnol-

Table 2 Clinico-pathologic features not correlating with QM immunoreactivity

	Tumor vol. >3.20	*Tumor vol. <3.20*	*Tumor confined*	*Not confined*
QM (+)	11/30 (37%)	22/40 (55%)	15/33 (45%)	18/37 (49%)
QM (−)	19/30 (63%)	18/40 (45%)	18/33 (55%)	19/37 (51%)
P-value	$P=0.2391$		$P=0.88$	

Table 1 Clinico-pathologic features correlating with QM immunoreactivity

	PGI>6	*PGI<6*	*Gleason grade 3 pattern*[a]	*Gleason grade 4 & 5 patterns*[b]
QM (+)	14/39 (36%)	20/31 (64%)	30/63 (48%)	6/27 (22%)
QM (−)	25/39 (64%)	11/31 (36%)	33/63 (52%)	21/27 (78%)
P-value	$P=0.03$		$P=0.04$	

[a]Gleason grade 3 pattern: detected in cases with 3+3, 3+4, 4+3 (Gleason score 6 and 7).
[b]Gleason grade 4 and 5 pattern: detected in cases with 3+4, 4+3, 4+4, 4+5 (Gleason score 7 and above).

ogy Inc., CA, at 1:80 dilution. To control for signal specificity, serial sections were made from five samples and were subjected to the same staining procedure, with a normal rabbit IgG to replace the respective primary antibody. This control staining did not give rise to a signal, demonstrating the specificity of the signal given by the primary antibodies.

Slides were scored semiquantitatively based on staining intensity and distribution. Staining was evaluated for each Gleason grade pattern when more than one pattern

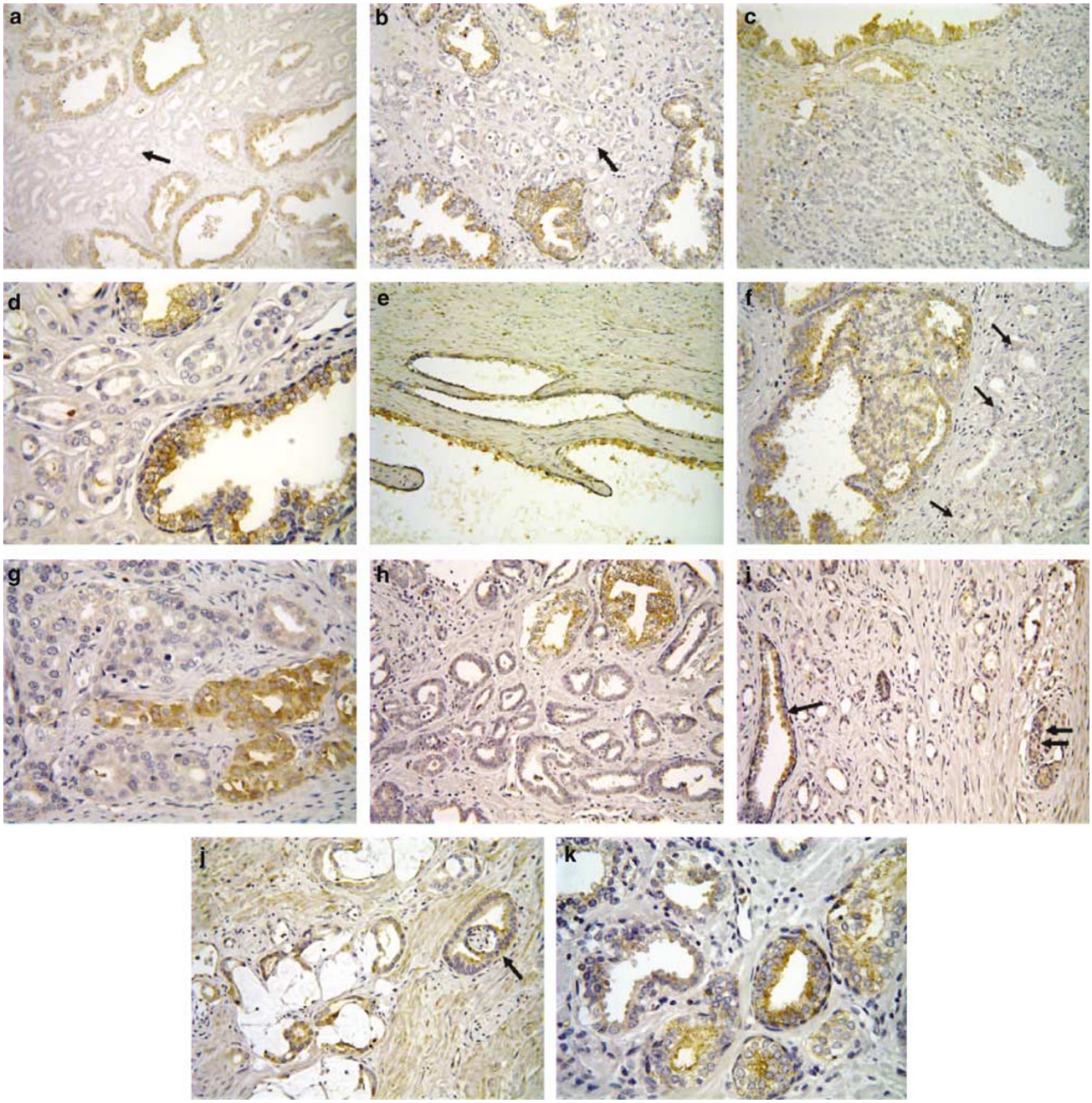

Figure 1 Immuhistochemical staining of QM in pancreatic lesions. (**a** and **b**) Photos taken from two different tumors showing that non-neoplastic glands as well as HGPIN areas show cytoplasmic granular and intense (3+) immunoreaction, while tumor cells are negative (arrow). (**c**) Beneath the non-neoplastic gland with positive immunoreaction for QM protein is the high-grade prostatic adenocarcinoma; Gleason score 8 (4+4). (**d**) Close-up view of the negative prostatic adenocarcinoma Gleason pattern 3, and neighboring benign prostatic gland with 3+ immunoreaction for QM protein. (**e**) Atrophic glands with attenuated epithelium only partially show positive immunoreaction depending on the amount of cytoplasm of secretory cells. (**f**) Intense positive immunoreaction in the gland with high-grade prostatic adenocarcinoma and carcinoma *in situ* and negative immunoreaction in the neighboring prostatic adenocarcinoma (arrow). (**g**) QM expression was often topographically patchy in the neoplastic glands. (**h**) Comparison between the cytoplasmic granular and intense (3+) immunoreaction of the non-neoplastic prostatic glands and weak to moderate staining of neoplastic glands. (**i**) Regional positivity is mostly confined to peripheral and especially perineural areas (single arrow indicates benign prostatic gland, double arrow indicates perineural area). (**j**) Regional positivity is mostly confined to peripheral and perineural areas (arrow indicates perineural invasion). (**k**) Luminal weak staining compared to cytoplasmic granular and intense (3+) immunoreaction of the non-neoplastic prostatic glands.

was present. Non-neoplastic areas showed diffuse and strong (3+) immunoreaction and thus were used as an internal control. By comparing with that seen in the adjacent non-neoplastic areas, intensities were classified into strong (3+), moderate (2+) or weak (1+). Distribution was scored as diffuse (>50% tumor cells staining), regional (10–50% tumor cells staining) and focal (<10% tumor cells staining) or negative. In all, 10% groupings were used for the percentage of cells that stained positive.

Statistics

Comparisons of patient and tumor characteristics were performed using χ^2 test with yate's correction. $P<0.05$ was considered statistically significant.

Results

Clinico-pathologic findings

The series included 70 patients, composed of 36 African American and 34 Caucasian patients. The median age of the patients was 58 years, with a range of 41–72 years. The clinicopathologic features that correlated with QM immunoreactivity is shown in Tables 1 and 2. Regardless of grade and stage, tumor volume and percentage of the tumor involvement of the prostate gland were determined. Tumor volume higher than 3.20 was noted in 30 cases (ranging from 3.22 to 15.03) and lower than 3.20 was noted in 40 cases (ranging from 0.9 to 3.19). Prostate gland involvement more than 6% was seen in 39 cases (ranging from 6.4 to 23.17), whereas less than 6% was seen in 31 cases (ranging from 0.5 to 5.87). Of the 70 patients, 33 had organ confined and 37 had advanced disease, which included extraprostatic extension ($n=29$), seminal vesicle involvement ($n=8$) and/or lymph node metastasis ($n=2$). The Gleason score was 6 (3+3) in 43 cases, 7 in 20 cases (10 of them were 3+4 and 10 of them were 4+3), and >7 in seven cases (one of them was 5+3, one of them was 5+4, and five of them were 4+4).

Expression of QM protein in prostatic lesions

In normal prostate glands, QM expression was cytoplasmic and granular in the secretory cell cytoplasm, but not in the basal cell layer. Strong immunohistochemical staining (3+) was observed in non-neoplastic prostatic glands. Normal tissue was therefore used as internal control with staining intensity in tumor cells assigned by comparison. Moreover, in cancer tissue or the adjacent normal tissue, endothelial cells and smooth muscle cells of various blood vessels and capillaries showed strong positive staining (3+). These cells also served as internal positive control for the cases that did not contain benign lesions or positive tumor patches. Tumor staining of equal intensity was considered 3+. The epithelium in high-grade prostatic intraepithelial neoplasia (HGPIN) also demonstrated consistent strong positive immunoreactivity (Figure 1a–d). In various PIN lesions, the staining was mainly in the apical layers of cells and rarely in the basal layer that was known to be more proliferating; thus, the level of QM protein seemed to show a reverse correlation to proliferative capacity. The attenuated epithelium of atrophic glands only partially showed granular and intense (3+) cytoplasmic QM staining, and in foci with extremely thinned epithelium, a total lack of detectable positive immunoreaction was noted (Figure 1e).

Positive staining with intense (3+) granular cytoplasmic immunoreaction was mostly observed in the Gleason grade 3 areas (48%) compared to grade 4 and 5 areas (22%) (Table 1), although both lower (Gleason grade 3) and higher (Gleason grade 4 and 5) grades showed a similar percentage of weakly stained areas, which ranged from 10 to 70%, with a mean value of 30%. Comparison of Gleason score 6 with 7, $\geqslant 7$, and 7, regarding QM staining, as well as comparison of Gleason score 6 with 7 (3+4), and 7 (4+3) are shown in Tables 3 and 4, respectively. When major and minor grades were combined, comparison was not statistically significant, but correlated significantly with Gleason grade 3 pattern compared to higher grade patterns of 4 and 5 (Table 1). Staining in prostatic adenocarcinoma was often topographically patchy and varied from negative or weak (1+) to intense (3+) staining, showing a trend of decreased expression in the percentage of stained areas compared to non-neoplastic glands (Figure 1f–h). Thus, there seemed to be an inverse correlation between the staining intensity/areas and the tumor grades. However, in high-grade tumors, especially in cases with only regional positivity (10–50% tumor staining), the positive areas were mostly confined to peripheral aspects of tumors and were particularly strong in foci of perineural invasion (Figure 1i and j).

The staining was localized to the secretory cell cytoplasm, no matter whether it was in non-neoplastic glands, PINs or positive patches of cancer cells. In some non-neoplastic glands or glandular lesions, the staining

Table 4 Comparison of Gleason score 6 with 7 (3+4), and 7 (4+3)

	Gleason score 6 (3+3)	*Gleason score 7 (3+4)*	*Gleason score 6 (3+3)*	*Gleason score 7 (4+3)*
QM (+)	20/43	5/10	20/43	6/10
QM (−)	23/43	5/10	23/43	4/10
P-value	$P=0.84$		$P=0.67$	

Table 3 Comparison of Gleason score 6 with 7, =or >7, and >7, regarding QM immunoexpression

	Gleason score 6 (3+3)	*Gleason score 7 (3+4) (4+3)*	*Gleason score 6 (3+3)*	*Gleason score =or>7 (4+3) (4+4) (4+5) (5+3)*	*Gleason score 6 (3+3)*	*Gleason score >7 (4+4) (4+5) (5+3)*
QM (+)	20/43	10/20	20/43	13/27	20/43	3/7
QM (−)	23/43	10/20	23/43	14/27	23/43	4/7
P-value	$P=0.79$		$P=0.89$		$P=0.85$	

was mainly localized to the apical cytoplasm (Figure 1k). Some intraluminal contents were also positive. These observations raised a question as to whether QM might also be a secretory protein. The staining manifested a diffuse pattern in non-neoplastic glands and high-grade PIN lesions, whereas staining in those neoplastic areas was often topographically patchy. It is unclear whether this difference in staining patterns between cancer and noncancer tissues reflects a difference in QM function.

Discussion

The present study is the first report on the immunohistochemical data of the QM protein in a human tissue and a human cancer. In this study, we found that QM protein was highly expressed in non-neoplastic prostatic glands but was downregulated in prostatic adenocarcinoma. Positive expression of QM protein was observed in 48% of the Gleason grade 3 areas which is composed of well formed glandular structures with open lumina. The percentage of the positive areas dropped to 22% in higher-grade areas, which is characterized by ill-defined glandular formations with closed lumina or solid growth pattern for grade 4, and infiltration of scattered neoplastic cells for grade 5 pattern. These observations show an inverse correlation between QM levels and the differentiation status and are consistent with some early *in vitro* data suggesting QM protein as a putative tumor suppressor gene.[8,25] Thus, downregulation of QM may be associated with the development of prostate cancer in humans and may be used as a marker for early stage of prostate cancer.

In this study, the expression of QM protein in prostate tumor cells did not correlate with the extra-prostatic extension or high tumor volume, but correlated with the low-grade prostatic adenocarcinoma (Gleason grade 3), and with the low percentages of the prostatic gland involvement. The cases with tumor involvement $<6\%$ of the prostatic gland showed higher percentage of staining. One interesting finding that seemingly opposes the above observations is the presence of positive immunoreactivity in peripheral aspects of tumors, and particularly in those foci of perineural invasions. Perineural invasion is a known mechanism by which prostate cancer cells penetrate the prostatic capsule and spread.[26] Therefore, it is possible that QM protein may have dual effects on prostatic carcinogenesis; that is, its downregulation may facilitate early phases of the cancer formation but its expression may later drive progression of the tumors to a more aggressive tumor phenotype. A recent study using different cell clones derived from LNCaP prostate cancer cell line showed that the levels of QM (RPL10) and several other ribosomal proteins (RPL32 and RPL16) were higher in the androgen-independent cells than in the androgen-dependent cells, indicating that these proteins were increased during progression to the androgen-independent phenotype.[27] Keeping with this idea, it is likely that the tumors with characteristics of perineural invasion may contain androgen-independent clones of prostate cancer cells. These data, together with our observation, suggest that QM may promote prostate cancer progression to more aggressive phenotype and this role may also be related to its function as a ribosomal protein in protein synthesis. On the other hand, the possible inhibition of early carcinogenic processes is more likely to be related to its known effect as AP-1 inhibitor. These possibilities certainly warrant more detailed studies in the future.

In summary, the present study reports, for the first time, data describing the expression of the immunohistochemically detected QM protein in human prostatic tissue and cancer. The decreased QM protein levels in the lineage from nonneoplastic glands, HGPIN, low-grade prostatic adenocarcinomas to high-grade tumors suggest that the QM protein may have an inhibitory effect on the early stage of the cancer development, which support the proposed role of QM protein as a tumor suppressor. However, a high QM protein level may be needed later for the progression of prostate cancer to more aggressive phenotype. More studies are needed to elucidate the role of QM protein in different stages of prostate cancer development and progression.

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