Thi-Qar Medical Journal (TQMJ):Vol.(21),No.(1),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

Increased Nuclear NDRG1 Expression in Prostate Cancer and Positively Associated with Increasing Gleason Grade and Stage, While Decreased Membranous and Cytoplasmic NDRG1 Expression in Prostate Cancer

Dhafer A. Alghezi
Paul Whitley
Mark Beresford
Rebecca Bowen
John Mitchard and Andrew D. Chalmers

Summary

Background: Prostate cancer has few prognostic biomarkers, and differential diagnosis and prognosis of aggressive versus non-aggressive tumors can be challenging clinically.

Aims: This study aims to investigate the hypothesis that NDRG1 might be a potential biomarker for prostate tumor and could distinguish between aggressive tumors requiring radical intervention and those that have a good prognosis. It is thought to be a potential biomarker that can predict the clinical progression and prognosis of different kinds of tumors. However, its role in prostate cancer remains unclear.

Materials and methods: NDRG1 expression has been evaluated by immunohistochemistry using a tissue microarray cohort with 96 cases including normal, adjacent normal and malignant prostate tissues.

Results: This study showed nuclear, cytoplasmic and membranous NDRG1 immunostaining in both normal and malignant prostate tissues. Reduction of membranous and increased nucleocytoplasmic NDRG1 staining is shown in prostate cancer compared to normal prostate and is positively associated with increasing primary Gleason grade, but not with clinical stage. Nuclear NDRG1 staining is increased significantly in prostate cancer and is positively associated with increasing primary Gleason grade and clinical stage. In contrast, cytoplasmic NDRG1 staining is decreased significantly in prostate cancer compared to normal prostate, but not associated with other clinical features.
Conclusion: This preliminary data suggests that NDRG1 may have a role in cancer development and/or aggressiveness and warrants further investigation to understand its function and establish if it could be a potential diagnostic biomarker for prostate cancer.

Keywords: Prostate cancer, NDRG1, Immunohistochemistry

Introduction

Prostate cancer (PCa) is an abnormal growth that usually begins in the prostate glands. It is a heterogeneous disease and represents the second most frequent malignancy and is the second highest cause of death in males after lung cancer (1,2). Adenocarcinoma is a most common type of PCa which is found in more than 90% of Pca patients, and it originates from the glandular regions of the prostate gland (3,4). A few diagnostic and prognostic biomarkers have been identified for PCa, including prostate-specific antigen (PSA) (5). However, there is a need for more specific and/or sensitive biomarkers in PCa diagnosis and especially for measuring PCa prognosis. The major goal of this study was to identify proteins that are differentially expressed between normal and malignant prostate tissues and/or between different Gleason grades and clinical stages. This is important to improve our understanding of the molecular basis of PCa formation and progression and potentially help in the development of future biomarkers.

NDRG1 is an intracellular protein that is thought to be a tumor suppressor that plays a role in inhibiting cell proliferation and invasion (6). NDRG1 expression and/or localisation has been studied in several kinds of malignancy, including PCa. Of particular importance for this study, altered expression levels and localisation of NDRG1 has been suggested to play a role in PCa progression and differentiation.

NDRG1 expression and/or localisation have been studied in both normal and malignant tissues. Cytoplasmic NDRG1 expression was increased significantly in a range of cancer types, including colorectal (7), hepatocellular (8,9), thyroid (10) and prostate tumors (11) compared to normal or benign tissues. In contrast, a study showed reduced cytoplasmic NDRG1 significantly in PCa tissues and PCa cell lines compared to BPH and NP cell lines, respectively (6). In terms of NDRG1 membranous expression, a previous study showed decreased membranous NDRG1 expression in PCa compared to NP tissues (12). NDRG1 expression has been reported in the nucleus of NP tissues (6) and in a small number of PCa cases (11 of 148 cases) (12). However, to our knowledge, if there is an association between levels of nuclear NDRG1 expression in normal vs. malignant tissues, it has not been investigated before. Therefore, there is evidence to suggest that altered cytoplasmic/membranous, but not nuclear, NDRG1 expression is linked to PCa.

The published evidence on NDRG1 expression in different Gleason grades is also complicated. For example, there was no significant association reported between NDRG1 expression and Gleason grade (11,13). In contrast, other publications showed that there was an association between NDRG1 staining and cancer grade, including PCa. For example, a previous PCa study in 2010 found increased cytoplasmic NDRG1 expression significantly associated with increasing Gleason grades (12), whereas, Bandyopadhyay *et al.* reported decreased cytoplasmic NDRG1 expression significantly with increasing Gleason grades (14). Membranous NDRG1 staining was also detected in PCa and was negatively associated with increasing Gleason grade (12). There was no previous study that studied nuclear NDRG1 expression in normal vs. malignant prostate tissues. A study on renal cell carcinoma tissues showed that nuclear NDRG1 staining was negatively associated with increasing grades (15). Therefore, there is conflicting evidence to suggest that changes in nuclear, membranous and cytoplasmic NDRG staining are linked to the Gleason grade of

PCa. On balance, there seems to be slightly more evidence to support a reduction in higher grades.

Several published studies describe the association between NDRG1 staining and cancer stage, including PCa. For example, PCa patients with advanced stage had low NDRG1 staining compared to those with localised PCa (14). In contrast, two other studies showed membranous and cytoplasmic NDRG1 expression not associated significantly with the clinical stage of PCa. (11,13). Therefore, there is greater evidence to suggest that alterations in NDRG1 expression are not linked to PCa stages.

Given the evidence, described above, to suggest a link between NDRG1 expression/localisation and PCa it was decided to examine nuclear, membranous and cytoplasmic NDRG1 staining and localisation in normal and malignant prostate tissues, including recurrence and non-recurrence. The hypothesis used predicted that membranous and cytoplasmic NDRG1 staining will be decreased in PCa compared to NP tissues. Membranous and cytoplasmic NDRG1 staining will be negatively associated with Gleason grade, but not stage. These hypotheses are based on the direction of association that was supported by of the most convincing of the studies, but as the evidence is contradictory other possible hypotheses could also have been proposed. There is little evidence regarding the nuclear expression of NDRG1 and PCa, so no hypothesis was proposed, but its expression was examined to see if an association was observed.

Materials and methods

This retrospective study was covered by the National Health Service (NHS) ethical and research approval (REC reference: 13/WS/0153; IRAS project ID: 112241). In this study, a TMA cohort (PR1921) consists of 96 cases, 80 of them were PCa, whereas, the rest were normal or normal tissues that were adjacent to the PCa, termed adjacent normal (8 cases for each). Each case was represented with two core tissue biopsies to form a total of 192 cores. The clinical data of the patients are shown in Table 1.

Table 1: Clinical data of prostate sample in TMA cohort.

Clinical data		TMA cohort %
Number of samples	Normal	16
	Malignant	80
Age range	Normal	21-68
	Malignant	20-85
Primary Gleason grade	3	13 (16.25%)
	4	46 (57.5%)
	5	18 (23. 75)
	ND	3 (2.5%)
T category	T1-T2	51 (63.8%)
	T3-T4	28 (35%)
	N/A	1 (1.2%)
N category	N0	65 (81.2%)
	N1	14 (17.5%)
	ND	1(1.3%)
M category	M0	64 (80%)
	M1	15 (18.7%)
	ND	1 (1.3%)

Immunohistochemistry

Immunohistochemistry (IHC) staining was carried out using anti-NDRG1 rabbit monoclonal (Abcam, catalogue number Ab124689). 5um thick sections of prostate tissues were baked overnight at 37°C. Before IHC, deparaffinization and rehydration through graded ethanol series of decreasing ethanol concentration (100%, 95% & 70% respectively) for a minute each concentration were necessary to remove the paraffin from tissues and to rehydrate tissue samples, respectively. Tissues were then permeabilized with 0.5% Triton X-100 in phosphate buffer saline (PBS), subjected to heat-induced epitope retrieval in a citrate buffer, pH 6 with 0.05% Tween 20 for 30 minutes at 90°C, and allowed to cool to room temperature for 20 minutes. Subsequently, the sections were incubated in 3% H_2O_2 (Dako peroxidase) at room temperature for 10 minutes, followed by rinsing gently three times with Phosphate buffer saline (PBS) for 5 minutes each. After blocking for 30 minutes in 10% normal goat serum and 0.5% BSA in PBS, samples were treated with anti-ABCG2 antibody, dilution1:250 (Dako, Ely, UK) overnight at 4°C.

On the next day, immuno-detection was performed using the EnVision+ Kit (K400611-2 and K401011-2, Dako, Ely, UK) following the manufacturer's instructions with DAB exposure for 5 minutes. The sections were counterstained with Vector Hematoxylin solution (H3401, Vector Laboratories, Peterborough, UK) at room temperature for a minute to stain the nucleus of cells. Slides then were rinsed thoroughly with the running tap water for 3 minutes. To differentiate the hematoxylin stain, the slides were then soaked three times in 70% ethanol with 1% HCl. The slides were also immersed for a minute in an alkaline solution that was prepared by adding 1% ammonium hydroxide to 70% ethanol to restore the bluing stain of Haematoxylin. At this point, the staining steps were finished. After that, the slides were washed with two changes of different ethanol concentrations 95% and 100% for a minute. Slides were then washed twice with Histoclear for 2 minutes each. The next day, the slides were ready to examine under a light microscope (Nikon Eclipse E800) equipped with a Nikon digital camera (DS-U1 CCD). The procedure of IHC was carried out according to (15).

For assessment of IHC staining, the whole sections were examined under a 20x objective to determine the nuclear, cytoplasmic and membranous expression of NDRG1 staining in prostate tissues. The localization of NDRG1 was scored as Pattern 1: predominantly in the cell membrane. Pattern2: predominate in nucleocytoplasmic. Pattern 3: not detectable expression (13,16). The nuclear and cytoplasmic NDRG1 staining was scored using a semi-quantitative scoring system as the following: the percentage of positive cells was scored as: (0: 0; 1: 1-25%; 2: 26-50%; 3:51-75%; and 4: 76-100%) and the intensity was graded as (0: negative, 1: weak, 2: moderate; and 3: strong). The final score represents the sum of the proportion and intensity scores, which ranged from 0 to 7 (17).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com, including mean, standard error and standard deviation values as well as the other statistical analysis such as a frequency distribution test and histogram. Statistical analysis was carried out either using unpaired T-test or one-way A-NOVA with Tukey's multiple comparisons tests. Results were considered significant if the P. value was ≤ 0.05.

Results

A) Immunohistochemical localisation and expression of NDRG1 in the normal and malignant prostate tissues.

The IHC result showed membranous NDRG1 staining in the TMA prostate tissue samples with variable levels of staining, ranging from strong (Figure 1 A, red arrowhead), moderate (Figure 1 B& C, red arrowheads), weak (Figure 1 F, red arrowhead) and negative (Figure 1, G). Cytoplasmic NDRG1 staining was also observed in normal and malignant prostate tissues and the intensity of signal varied widely, ranging from strong (Figure 1 E, black arrowhead), moderate (Figure 1 C, D & F, black arrowheads), weak (Figure 1 A, B, black arrowheads) and negative (Figure 1 G). In addition to membranous and cytoplasmic staining, normal and malignant prostate tissue had nuclear NDRG1 staining with variable levels of staining, ranging from strong (Figure 1 D, arrow), moderate (Figure 1 B, arrow), weak (Figure 1, F, arrow) and negative (Figure 1, G, arrow). A negative control (no primary antibody) showed no significant background staining in prostate tissue (Figure 1, H, arrow).

B) Association between NDRG1 immunostaining and histopathological parameters of prostate cancer in the TMA cohort

Having carried out IHC staining, the membranous, cytoplasmic and nuclear localisation of NDRG1 staining was then quantified and compared to the histopathological and clinical data available for the TMA cohort. Having scored the localisation, the amount of staining was then quantified and the potential association between NDRG1 results and histopathological parameters of PCa analysed.

1) NDRG1 localisation

Three different NDRG1localisations were observed in the TMA prostate tissues using IHC, including predominant membranous, predominant nucleocytoplasmic and negative. The statistical analysis looked first at the NDRG1 localisation in normal vs. malignant prostate tissues. Quantification of the IHC staining showed a significant difference between NDRG1 localisation in normal vs malignant prostate tissues ($p= <0.0001$). Decreased membranous and increased nucleocytoplasmic NDRG1 staining was observed in PCa tissue compared to those with a normal nature (Figure 2, A). Membranous NDRG1 staining was predominantly localised in 69% of PCa compared to 35% of NP tissues (Figure 2, A). In contrast, nucleocytoplasmic NDRG1 localisation was found in 61% of PCa compared to 22% of NP. In addition, NDRGI localisation showed significantly associated with primary Gleason grade ($p=0.0021$). The frequency distribution tests showed membranous NDRG1 localisation negatively associated with increasing primary Gleason grade, whereas, nucleocytoplasmic localisation was positively associated with primary Gleason grade (Figure 2, B).

In addition, NDRGI localisation showed significantly associated with primary Gleason grade ($p=0.0021$). The frequency distribution tests showed membranous NDRG1 localisation negatively associated with increasing primary Gleason grade, whereas, nucleocytoplasmic localisation was positively associated with primary Gleason grade (Figure 2, B).

The membranous NDRG1 localisation was found in 80% of primary Gleason grade 3 and a quarter of Gleason grade 5 tissues. In contrast, three-quarters of tissues with a Gleason grade 5 had nucleocytoplasmic

NDRG1 localisation, whereas, NDRG1 was found to be predominantly nucleocytoplasmic localised in 20% of PCa with a Gleason grade 3 (Figure 2 B). In contrast, there was no significant difference between NDRG1 localisation and clinical stage (TNM) (Figure 2 C, D&E).

2. NDRG1 staining

Having quantified the localisation, the amount of nuclear and cytoplasmic NDRG1 staining was quantified and then compared between normal vs. malignant prostate, different primary Gleason grades, clinical stages, using the clinical data available for the TMA cohort. The range of nuclear and cytoplasmic NDRG1 scores varied between 0-7 (Figure 3).

The first analysis looked at the staining of NDRG1 in the TMA prostate tissues. Quantification of the IHC showed nuclear NDRG1 staining increased significantly in PCa compared to NP tissues (p=0.0002) (Figure 3 A & Table 2). In contrast, a significant reduction for cytoplasmic NDRG1 staining was observed in PCa compared to NP tissues (p=<0.0001) (Figure 3 B & Table 2). There was a significant difference in nuclear NDRG1 scores among different primary Gleason grades (p= 0.0226) (Figure 3, C & Table 2). Analysis of the IHC, using Tukey's multiple comparisons tests showed nuclear NDRG1 staining significantly decreased in PCa tissues with a primary Gleason grade 3 compared to those with a grade 4 (p= 0.0395) or a grade 5 (p= 0.0271) (Figure 3, C & Table 2), but not between a Gleason grade 4 and 5 (p= 0.8083) (Figure 3 C & Table 2). Cytoplasmic NDRG1 staining was observed to a trend toward lower in primary Gleason Grade 5, but the results were not significant (p= 0.1742) (Figure 3, C & Table 2). In addition, nuclear NDRG1 staining was increased significantly in advanced PCa stage compared to localised PCa (T3-4 vs T1-2) and N (N1 vs N0) (p= 0.0214 & 0.0039, respectively) (Figure 4A &B & Table 2). However, there was no significant association between nuclear NDRG1 staining and PCa metastasis (p= 0.1627) (Figure 4 C & Table 2). Cytoplasmic NDRG1 staining was not associated with a clinical stage TNM (Table 2).

In summary, membranous NDRG1 localisation was lower in PCa compared to NP tissues, whereas nucleocytoplasmic localisation was higher. Reduction of membranous and increased nucleocytoplasmic NDRG1 staining was positively associated with increasing primary Gleason grade, but not with clinical stage. In addition, nuclear NDRG1 staining was increased in PCa and was positively associated with increasing primary Gleason grade and clinical stage. In contrast, cytoplasmic NDRG1 staining was decreased significantly in PCa compared to NP, but not associated with other PCa clinical features.

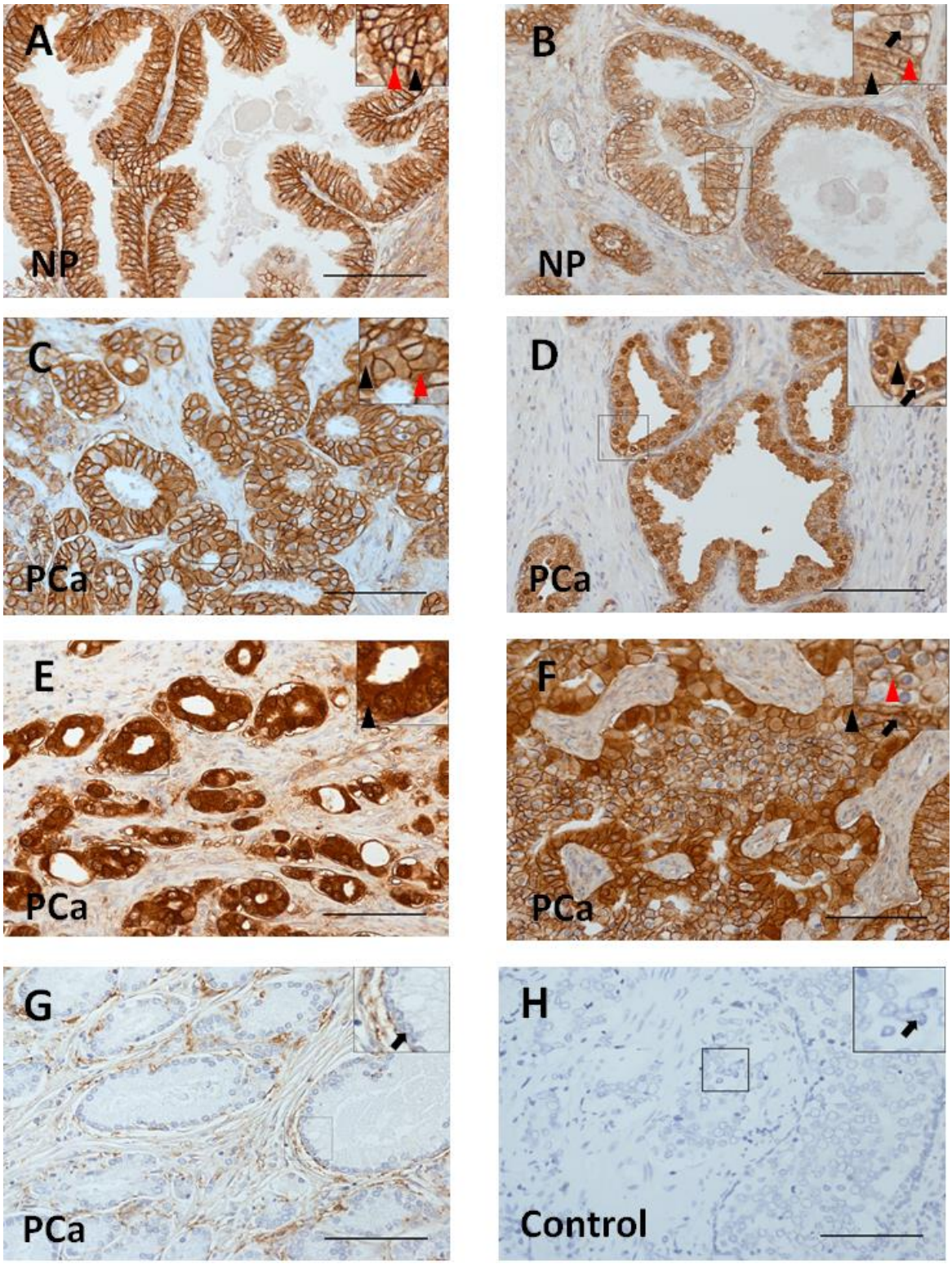

Figure 1: NDRG1 staining in samples from the TMA cohort. NDRG1 staining was found heterogeneously in both normal and malignant tissues of the prostate. (A) Predominant membranous (Red arrowhead) and cytoplasmic (Black arrowhead) NDRG1 staining in NP. (B) Moderate nuclear (Black arrow), membranous (Red arrowhead) and weak cytoplasmic (Black arrowhead) NDRG1 staining in NP. (C) Moderate membranous (red arrowhead) and cytoplasmic (Black arrowhead) NDRG1 staining in PCa. (D)Strong nuclear (Black arrow) with moderate cytoplasmic (Black arrowhead) NDRG1 staining in PCa. (E) Strong cytoplasmic (Black arrowhead) NDRG1 staining in PCa. (F) Weak nuclear (Black arrow), membranous (Red arrowhead) and moderate cytoplasmic (Black arrowhead) NDRG1 staining in PCa. (G) Negative staining for NDRG1 (Black arrow) in PCa. (H) The negative control (no primary antibody) showed no staining (Black arrow) in prostate tissue. Scale bars=100µm.

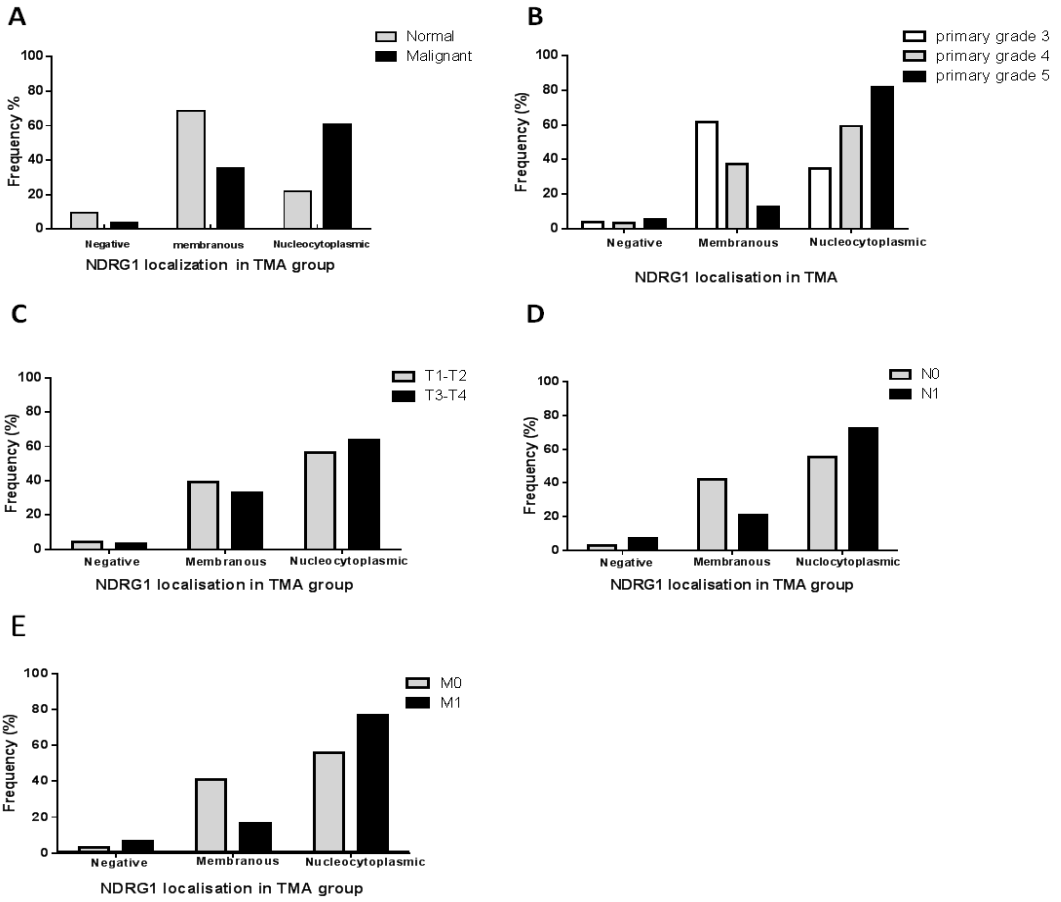

Figure 2: Localisation of NDRG1 in TMA prostate samples. Immunohistochemical staining of NDRG1 localisation was quantified in the TMA group using the localisation 1 score. (A) Predominant membranous NDRG1 staining was higher in NP compared to PCa, whereas, predominant nucleocytoplasmic NDRG1 staining was higher in PCa compared to NP tissues (P= <0.0001). (B) Reduced membranous and increased nucleocytoplasmic NDRG1 localisation was positively associated with increasing primary Gleason grade (P=0.0021). (C) There was no clear difference between NDRG1 localisation and stage T (P=0.3770). (D) There was no clear difference between NDRG1 localisation and stage N (P=8161). (E) There was no clear difference between NDRG1 localisation and stage M (P=0.1392). The mean of five random fields was taken per prostate sample. Statistical significance was determined with Chi-square and frequency tests for each set of conditions. NP (n=16), PCa (n=80), grade 3 (n=13), grade 4 (n=46), grade 5 (n=18), M0 (n= 64) and M1 (n=15).

Figure 3: Quantification of nuclear and cytoplasmic NDRG1 staining in both normal and malignant TMA prostate tissues. IHC staining of NDRG1 was quantified in the TMA cohort using the proportion and intensity 1 score for nuclear and cytoplasmic IHC staining. (A) Nuclear NDRG1 staining was significantly increased in PCa compared to NP (p= 0.0002). (B) Cytoplasmic NDRG1 staining was significantly decreased in PCa compared to NP (P= < 0.0001). (C) NDRG1 nuclear staining showed a significant difference among primary Gleason grades (p= 0.0226) and the multicomparisonTukey's tests showed nuclear NDRG1 staining lower in primary Gleason grade 3 compared to grade 4 (p= 0.0395) or grade 5 (p= 0.0271). (B) Cytoplasmic NDRG1 staining was not associated significantly with primary Gleason score groups (p= 0.01742). Unpaired or one-way ANOVA tests were conducted to determine the statistical difference for each set of conditions. NP (n=16), PCa (n=80), grade 3 (n=13), grade 4 (n=46), grade 5 (n=18).

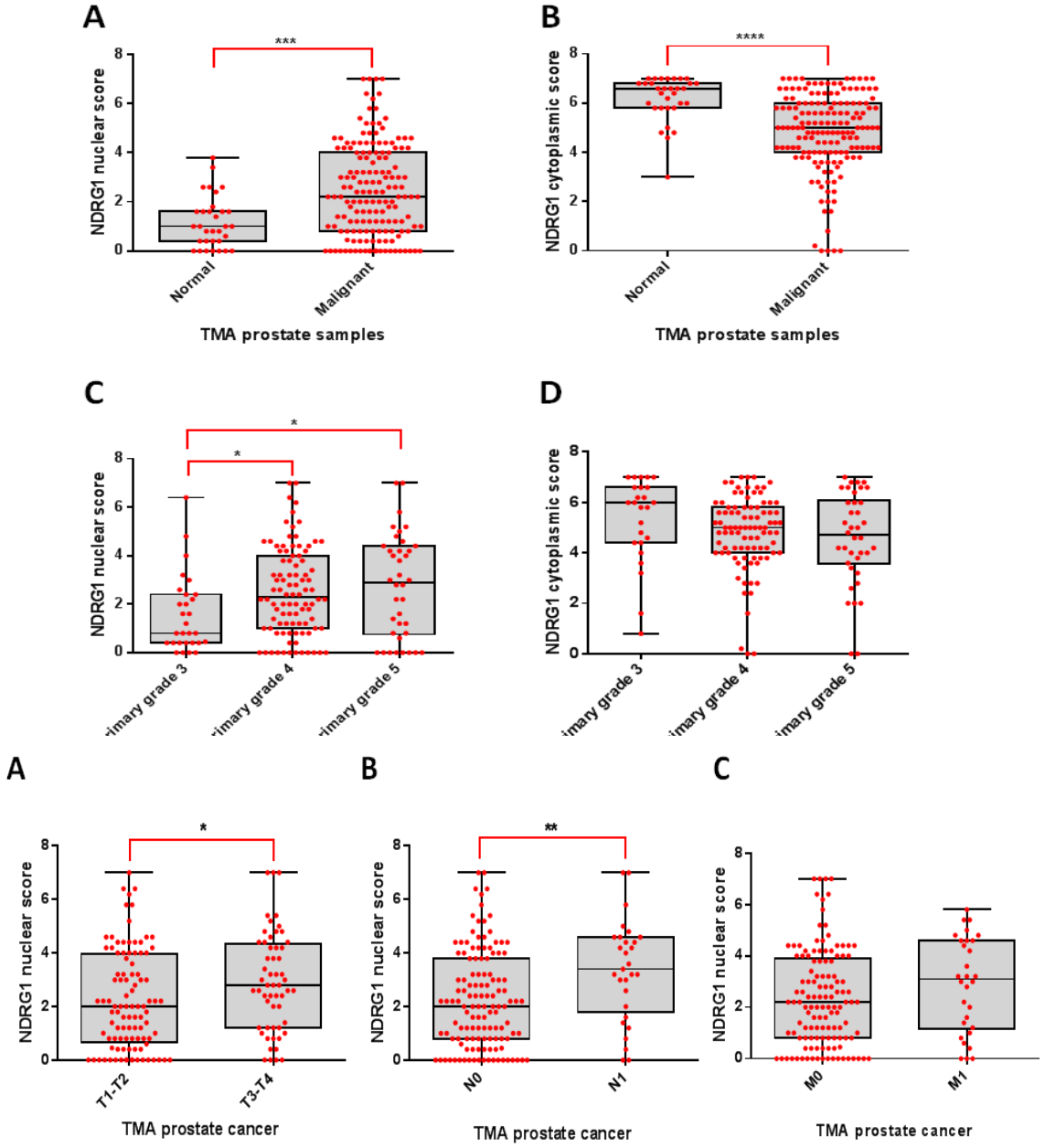

Figure 4. 1 Quantification of nuclear NDRG1 staining in PCa stages. Nuclear NDRG1 staining was quantified in the TMA group using the proportion and intensity 1 score. (A) Nuclear NDRG1 staining was significantly increased in the advanced stages (T3-4) compared to localised PCa (T1-2) (p= 0.0214). (B) Nuclear NDRG1 staining was significantly associated with lymph node status (p= 0.0039). (C) Nuclear NDRG1 was not associated significantly with metastasis (P= 0.1627). Unpaired tests were conducted to determine the statistical difference for each set of conditions. T1-2 (n=51), T3-4 (n=28), N0 (n= 65), N1 (n= 14), M0 (n= 64) and M1 (n=15).

Table 2: Nuclear and cytoplasmic NDRG1 staining results with clinical data

Comparison	Nuclear NDRG1 staining			Cytoplasmic NDRG1 staining		
	Results		p. value	Results		p. value
Normal vs malignant	Higher in malignant		0.0002	Lower in malignant		<0.0001
Primary Gleason grade 3.4.&5	Higher in high Gleason grade	Anova test	0.0226	No statistically significant difference	Anova test	0.1742
		Grade 4 vs. Grade 3	0.0395		Grade 4 vs. Grade 3	0.30004
		Grade 5 vs. Grade 3	0.0271		Grade 5 vs. Grade 3	0.1544
		Grade 5 vs. Grade 4	0.8083		Grade 5 vs. Grade 4	0.7377
Stage (T)	Higher in PCa with high stage (T3-T4)		0.0214	No statistically significant difference		0.1452
Stage (M)	No statistically significant difference		0.1627	No statistically significant difference		0.1313
Stage (N)	Higher in PCa with lymph node invasion (N1)		0.0039	No statistically significant difference		0.9412

Discussion:

There are several studies focused on either NDRG1 localisation or staining in normal and malignant prostate. However, these studies come with contradictory results. IHC results in both cohorts showed that decreased membranous localisation of NDRG1 was observed in PCa compared to NP and was positively associated with increasing primary Gleason grade, but not with stage and replace. In contrast, nucleocytoplasmic localisation was increased significantly in PCa and was positively associated with increasing primary Gleason grade, but not with stage and relapse. The association between NDRG1 localisation and Gleason grade in the Bath cohort was not significant, which maybe because of the small sample size. However, this data is consistent with the previous findings (11,12,13), suggesting that the change in NDRG1 localisation might play a role in PCa formation and differentiation.

In addition, this study is the first to quantify nuclear NDRG1 staining and shows an association between nuclear NDRG1 staining in normal vs malignant, different primary Gleason grades, clinical stages and relapse. The IHC data from the TMA cohort shows that nuclear NDRG1 staining was increased significantly in PCa compared to NP tissues and was positively associated with increasing primary Gleason grade and clinical stage. However, the nuclear NDRG1 staining in the Bath cohort was not significant, perhaps due to the small sample size in this cohort. However, our data was supported by Song *et al.* who reported that nuclear NDRG1 expression was increased in colorectal carcinoma compared to normal tissues (12) as well by Hosoya *et al.* who reported increased nuclear NDRG1 was positively associated with grade and stage of renal cell carcinoma (16), suggesting that increased nuclear NDRG1 expression plays an important role in PCa formation and progression and could be a predictive marker for a poor prognosis.

Cytoplasmic NDRG1 staining was decreased in malignant prostate tissues compared to NP tissues from the TMA cohort, but not the Bath cohort. This result was largely agreed with a previous finding (6), suggesting that reduction cytoplasmic NDRG1 staining might play a role in PCa formation. However, this data was contradictory with (11,12). Cytoplasmic NDRG1 staining was not associated significantly with primary Gleason grade, stage and relapse. This data is consistent with a previous prostate report (11), but not with the other data (11,14). This difference between the literature could be because of the sample size, the use of different antibodies and/or the use of different methods. Taken together, a reduction in cytoplasmic NDRG1 level, in addition to the increase in nuclear NDRG1 described above, could play a role in PCa formation and progression.

What role might NDRG1 play in cancer formation? Previous studies have been found either an oncogenic or tumor suppressor role of NDRG1 in cancer. However, the majority of the studies have reported a tumor suppressor role, including for PCa (12,18). There are many different possible mechanisms to explain this role in cancer. First, NDRG1 upregulates frequently rearranged in advanced T cell protein (FRAT1) which plays an important role in preventing binding of Glycogen synthase kinase 3 beta (GSK3B) with the distraction complex, that subsequently prevents phosphorylation of β-catenin and then prevents nuclear translocation of phosphorylated β-catenin (18). This study also found that blocking nuclear β-catenin translocation may occur through NDRG1's role to inactivate P21- activated kinase 4 (PAK4) protein, which plays a transporter role for β-catenin. Therefore, loss of cytoplasmic NDRG1 could increase Wnt signalling (19), which is consistent with

the β-catenin data described above. In addition, a previous study found that increased NDRG1 in pancreatic cancer cell lines inhibits PI3K and Ras signalling pathways through increasing PTEN and SMAD4 which inhibit tumor progression (20), providing another mechanism to explain its tumor suppressor role.

In addition to a tumor suppressor role, previous literature has reported an oncogenic role for NDRG1. Increased NDRG1 may promote the growth of tumors through its role in promoting metastasis and angiogenesis, as well as protecting cells from apoptosis (21). A study done by Ai *et al* reported that increased NDRG1 expression may promote oesophagal squamous cell carcinoma progression through modulating the Wnt signalling pathway by affecting transducin-like enhancer of split 2 (TLE2) and β-catenin (22). This is confirmed by our data which found a moderate negative correlation between nuclear staining of β-catenin and nuclear NDRG1 (R=-0.56) (data are not shown), suggesting that increased nuclear NDRG1 reduces nuclear β-catenin levels. In colorectal carcinoma, NDRG1 translocation from the membrane to the nucleus of cells may play a role in lymph node metastasis by regulation of E-Cadherin expression (12). This is confirmed by our data which found increased nuclear NDRG1 significantly associated with lymph node metastasis in the TMA cohort.

Taken together, this study suggests that decreased membranous and cytoplasmic NDRG1 levels in PCa cells may downregulate PTEN and activate PI3K signalling pathways which lead to increased proliferation and inhibit apoptosis. Whereas increased nuclear localisation of NDRG1 may lead to a decrease in nuclear β-catenin transcriptional activity, however, this is complicated as a reduction in cytoplasmic may have the opposite effect. The change of NDRG1 localisation and/or increased nuclear NDRG1 level could be a poor indicator for PCa prognosis and it could be a useful prognostic biomarker for PCa. Further studies are needed to confirm the staining patterns of NDRG1 using either IHC with a second independent antibody or RNAscope to detect the mRNA level of NRGG1 in prostate tissue tissues. In addition, It would be very interesting to be to use a large cohort with clinical data about a risk of relapse to confirm if there is an association between NDRG1 staining and biochemical relapse. Future research is also needed to determine if there is an association between NDRG1 localisation and PTEN level in PCa. Finally, it would also be interesting to investigate NDRG1 functional roles in PCa using an *in vitro* cell culture approach.

Acknowledgements The authors are grateful to the University of Thi-Qar for PhD scholarship funding of Dhafer .A. Alghezi. They are grateful to the Department of Cellular Pathology at the Royal United Hospital, Bath, for specimen handling and curation. The authors would like to thank Professor Robert Kelsh at the University of Bath for providing use of imaging facilities.

References

1. Lang, S.H.; Frame, F.M. & Collins, A.T. Prostate cancer stem cells. *The Journal of pathology*, 2009, 217(2), pp. 299-306.
2. Kirby, R.S. *Prostate cancer.* 7th ed.; Abingdon: Abingdon: Health Press, 2012.
3. Bagnall, P. Diagnosis and treatment of prostate cancer. *Nursing times*, 2014, 110(9), p. 12.
4. Dunn, M.W. & Kazer, M.W. Prostate Cancer Overview. *Seminars in Oncology Nursing*, 2011, 27(4), pp. 241-250.
5. Qu, M.; Ren, S.C. & Sun, Y.H. Current early diagnostic biomarkers of prostate cancer. *Asian journal of andrology*, 2014, 16(4), pp. 549-54.
6. Li, Y., Pan, P., Qiao, P. & Liu, R. Downregulation of N-myc downstream regulated gene 1 caused by the methylation of CpG islands of NDRG1 promoter promotes proliferation and invasion of prostate cancer cells. *International journal of oncology*, 2015., 47(3), pp. 1001-8.
7. Koshiji, M., Kumamoto, K., Morimura, K., Utsumi, Y., Aizawa, M., Hoshino, M., Ohki, S., Takenoshita, S., Costa, M., Commes, T., Piquemal, D., Harris, C.C. & Tchou-Wong, K.M. Correlation of N-myc downstream-regulated gene 1 expression with clinical outcomes of colorectal cancer patients of different race/ethnicity. *World journal of gastroenterology*, 2007,13(20), pp. 2803-10.
8. Angst, E., Sibold, S., Tiffon, C., Weimann, R., Gloor, B., Candinas, D. & Stroka, D. Cellular differentiation determines the expression of the hypoxia- inducible protein NDRG1 in pancreatic cancer. *British Journal of Cancer*, 2006, 95(3), p. 307.
9. Sibold, S., Roh, V., Keogh, A., Studer, P., Tiffon, C., Angst, E., Vorburger, S.A., Weimann, R., Candinas, D. & Stroka, D. Hypoxia increases cytoplasmic expression of NDRG1, but is insufficient for its membrane localization in human hepatocellular carcinoma. *FEBS letters*, 2007, 581(5), pp. 989-94.
10. Gerhard, R., Nonogaki, S., Fregnani, J.H., Soares, F.A. & Nagai, M.A. NDRG1 protein overexpression in malignant thyroid neoplasms. *Clinics (Sao Paulo, Brazil)*, 2010, 65(8), pp. 757-62.
11. Symes, A.J., Eilertsen, M., Millar, M., Nariculam, J., Freeman, A., Notara, M., Feneley, M.R., Patel, H.R., Masters, J.R. & Ahmed, Ax. Quantitative analysis of BTF3, HINT1, NDRG1 and ODC1 protein over-expression in human prostate cancer tissue. *PLoS One*, 2013, 8(12), p. e84295.
12. Song, Y., Oda, Y., Hori, M., Kuroiwa, K., Ono, M., Hosoi, F., Basaki, Y., Tokunaga, S., Kuwano, M., Naito, S. & Tsuneyoshi, M. N-myc downstream regulated gene-1/Cap43 may play an important role in malignant progression of prostate cancer, in its close association with E-cadherin. *Human Pathology*, 2010, 41(2), pp. 214-222.
13. Caruso, R.P., Levinson, B., Melamed, J., Wieczorek, R., Taneja, S., Polsky, D., Chang, C., Zeleniuch-Jacquotte, A., Salnikow, K., Yee, H., Costa, M. & Osman, I.

Altered N-myc downstream-regulated gene 1 protein expression in African-American compared with caucasian prostate cancer patients. *Clinical cancer research : an official journal of the American Association for Cancer Research,* 2014, 10(1 Pt 1), pp. 222-7.

14. Bandyopadhyay, S., Pai, S.K., Gross, S.C., Hirota, S., Hosobe, S., Miura, K., Saito, K., Commes, T., Hayashi, S., Watabe, M. & Watabe, K. The Drg-1 gene suppresses tumor metastasis in prostate cancer. *Cancer research,* 2003, 63(8), pp. 1731-6.

15. Alghezi, D., Whitley, P., Beresford, M., Bowen, R., Mitchard, J. and Chalmers, A. Decreased ABCG2 expression in Prostate cancer and negatively asssociated with poorly differentiated grade and biochemical recurrence. *Thi-Qar Medical Journal*,. 2020, 20 (*2*), pp.243-256.

16. Hosoya, N., Sakumoto, M., Nakamura, Y., Narisawa, T., Bilim, V., Motoyama, T., Tomita, Y. & Kondo, T. Proteomics identified nuclear N- myc downstream- regulated gene 1 as a prognostic tissue biomarker candidate in renal cell carcinoma. *BBA - Proteins and Proteomics,* 2013, 1834(12), pp. 2630-2639.

17. Dalley, A.J.; Pitty, L.P.; Major, A.G.; Abdulmajeed, A.A. & Farah, C.S. Expression of ABCG 2 and B mi- 1 in oral potentially malignant lesions and oral squamous cell carcinoma. *Cancer Medicine,* 2014, 3(2), pp. 273-283.

18. Bae, D.-H., Jansson, P.J., Huang, M.L., Kovacevic, Z., Kalinowski, D., Lee, C.S., Sahni, S. & Richardson, D.R. The role of NDRG1 in the pathology and potential treatment of human cancers. *J Clin Pathol,* 2013, 66(11), p. 911

19. Jin, R., Liu, W., Menezes, S., Yue, F., Zheng, M., Kovacevic, Z. & Richardson, D.R. The metastasis suppressor NDRG1 modulates the phosphorylation and nuclear translocation of β- catenin through mechanisms involving FRAT1 and PAK4. *Journal of cell science,* 2014, 127(Pt 14), p. 3116

20. Kovacevic, Z., Chikhani, S., Lui, G.Y., Sivagurunathan, S. & Richardson, D.R. The iron-regulated metastasis suppressor NDRG1 targets NEDD4L, PTEN, and SMAD4 and inhibits the PI3K and Ras signaling pathways. *Antioxidants & redox signaling,* 2013, 18(8), pp. 874-87.

21. Wei, W., Bracher-Manecke, J.C., Zhao, X., Davies, N.H., Zhou, L., Ai, R., Oliver, L., Vallette, F. & Hendricks, D.T. Oncogenic but non-essential role of N-myc downstream regulated gene 1 in the progression of esophageal squamous cell carcinoma. *Cancer biology & therapy,* 2013, 14(2), pp. 164-74.

22. Ai, R., Sun, Y., Guo, Z., Wei, W., Zhou, L., Liu, F., Hendricks, D.T., Xu, Y. & Zhao, X. NDRG1 overexpression promotes the progression of esophageal squamous cell carcinoma through modulating Wnt signaling pathway. *Cancer biology & therapy,* 2016, 17(9), pp. 943-54.

Thi-Qar Medical Journal (TQMJ):Vol.(21),No.(1),2021
Web Site: https://jmed.utq.edu.iq Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218, ISSN (Online):1992-9218

زيادة التصبيغ المناعي النووي لمعلم NDRG1 في سرطان البروستاتا وارتباطه بشكل إيجابي بزيادة الدرجة والمرحلة ، بينما انخفض التصبيغ المناعي الغشائي والهيولي لمعلم NDRG1في سرطان البروستاتا

ظافر عبد الله فرحان الغزي [١،٢،*]، بول ويتلي[٣]، مارك بيريسفورد [٤]، ربيكا بوين[٤]، جون ميتشارد [٥]، أندرو تشالمرز [٣]

١) قسم الأحياء المجهرية الطبية والمناعة، كلية الطب، جامعة ذي قار، ذي قار، العراق.
٢) وحدة بحوث السرطان في كلية الطب جامعة ذي قار، ذي قار العراق
٣) قسم الأحياء والكيمياء الحيوية، جامعة باث، باث، المملكة المتحدة.
٤) قسم الأورام، مستشفى رويال يونايتد، باث، المملكة المتحدة.
٥) قسم علم الأمراض الخلوية، مستشفى رويال يونايتد، باث، المملكة المتحدة.
*) ايميل الباحث المراسل: Dr.daf79@utq.edu.iq; Dr.daf79@gmail.com

الخلاصة

المقدمة: يحتوي سرطان البروستاتا على عدد قليل من المؤشرات الحيوية التنبؤية، ويمكن أن يكون التشخيص والتشخيص التفريقي للسرطان العدواني مقابل غير العدواني تحديًا سريريًا.

الهدف من الدراسة: تهدف هذه الدراسة إلى التحقيق في الفرضية القائلة بأن NDRG1 قد يكون علامة بيولوجية محتملة لورم البروستاتا ويمكنه التمييز بين الأورام العدوانية التي تتطلب تدخلًا جذريًا وتلك التي لديها تشخيص جيد. يُعتقد أنه علامة بيولوجية محتملة يمكنها التنبؤ بالتقدم السريري والتشخيص لأنواع مختلفة من الأورام. ومع ذلك، لا يزال دوره في سرطان البروستاتا غير واضح.

المواد وطرق العمل: تم تقييم التعبير المناعي لـ NDRG1 بطريقة التصبيغ المناعي النسيجي الكيميائي باستخدام ٩٦عينه نسيجه طبيعية وسرطانيه.

النتائج: أظهرت الدراسة ان هناك تلطيخ NDRG1 النووي وهيولي والغشائي في كل من أنسجة البروستاتا الطبيعية والخبيثة. وظهر انخفاض في تلطيخ NDRG1 الغشائي وزيادة تلطيخ NDRG1 في سرطان البروستاتا مقارنة بالبروستات الطبيعي وكان مرتبطًا بشكل إيجابي بزيادة درجة جليسون الأولية، ولكن ليس مع المرحلة السريرية. تمت زيادة تلطيخ NDRG1 النووي بشكل ملحوظ في سرطان البروستاتا وكان مرتبطًا بشكل إيجابي بزيادة درجة جليسون الأولية والمرحلة السريرية. في المقابل، انخفض تلطيخ NDRG1 الهيولي بشكل ملحوظ في سرطان البروستاتا مقارنة بالبروستاتا الطبيعي، ولكن لم يرتبط بالسمات السريرية الأخرى.

الاستنتاج: تشير هذه البيانات الأولية إلى أن NDRG1 قد يكون له دور في تطور السرطان و / أو العدوانية ويتطلب مزيدًا من التحقيق لفهم وظيفته وتحديد ما إذا كان يمكن أن يكون علامة بيولوجية تشخيصية محتملة لسرطان البروستاتا.

الكلمات المفتاحية: سرطان البروستات، NDRG1 ، التصبيغ المناعي النسيجي الكيميائي