CHAPTER ONE
INTRODUCTION

1.1 Introduction:
Prostate cancer, also known as carcinoma of the prostate, is the development of cancer in the prostate. Prostate is a gland in the male reproductive system. Most prostate cancers are slow growing; however, some grow relatively quickly. The cancer cells may spread from the prostate to other parts of the body, particularly the bones and lymph nodes. It may initially cause no symptoms. In later stages it can lead to difficulty urinating, blood in the urine, or pain in the pelvis, back or when urinating (Galani, 2015).
Prostate cancer is the sixth most common cancer in the world and accounts for 9.7% of cancers in men. It is the leading cause of new cancer in men and is second only to lung cancer as a cause of cancer related deaths in men (Baig, et al. 2012).
Prostate cancer is the most common cancer in Sudanese men. The age standardized rate is 10.3 and mortality is 8.7 per 100,000 populations. It ranked second among all cancers in both sexes after breast cancer (Elamin, et al.2015).
The risk factors of the prostate cancer include: age, ethnicity, family history, genetic susceptibility, diet, hormonal factors (Crawford, 2003).
Method of diagnosis of prostate cancer is prostate specific antigen (PSA), digital rectal examination (DREs), transrectal ultrasound (TRUS), magnetic resonance imaging (MRI), biopsy, computed tomography scan (CT), and bone scan (James, 2014, Galani, 2015).
Prostate cancer treatment include surgical treatment, radiation therapy, cryosurgery, hormone therapy, proton beam therapy, and chemotherapy (Chen and Zhao, 2013).
The P63 gene encodes six protein isoforms. The transactivating isoforms has similar actions with p53, while the N-isoforms inhibit transcription activation by p53 and Trans activating isoforms. P63 is expressed in stratified epithelia and basal cells of the prostate and salivary glands. In mammary epithelium p63 has been shown to express only in the myoepithelial layer (Stefanou, et al. 2004).

Previous study demonstrated that p63 is not expressed in prostate carcinomas. This finding supports the hypothesis that prostatic carcinomas have a secretory phenotype. Because they have shown that p63 is expressed in virtually all basal cells, p63 immunohistochemistry may be a valuable tool for the differential diagnosis of benign versus malignant prostatic lesions. In addition, because of the universal expression of p63 by basal cells, p63 immunohistochemistry may be a useful adjunct to morphological analysis in the prostate surgical pathology setting (Signoretti, et al. 2000).
1.2 Objective:

1.2.1 General objective:
To detect p63 expression in prostate tumors by immunohistochemistry and its correlation with histopathological diagnosis and cancer grade.
CHAPTER TWO
LITERATURE REVIEW

2.1 Scientific background:
Prostate cancer and benign prostatic hyperplasia are two major prostate diseases that increase with aging. The incidence rate of both diseases are currently showing tendency to increase. Prostate cancer may behave in many different ways in different men. It may be relatively slow growing, but it may also be more aggressive in it is behavior with tendency to metastasize or spread to the lymph nodes, bones, or other part of the body (Suzuki, 2009, Galani, 2015).

2.2 Histology of the prostate:
The prostate is a walnut-sized gland at the base of the bladder. Urine from bladder travels through urethra to the penis. The urethra goes right through the center of prostate. This portion of urethra is known as prostatic urethra. The ducts from prostate empty into the prostatic urethra. The ejaculatory ducts also drain into prostatic urethra at this point (Cramer, 2007). The histology of prostate is that of a branched duct gland. Two cell layers, a luminal secretory columnar cell and an underlying basal cell, line each gland or duct. The lumens of normal prostatic glands and ducts contain multilaminated eosinophilic concretions, termed corpora amylacea, that become more common in older men. Calculi are larger than those corpora with a predilection for ducts that traverse the length of surgical capsule, separating the transition and peripheral zones (Hricak and Scardino, 2009).

2.3 Disorders of prostate:
2.3.1 Benign disorders:
2.3.1.1 Benign Prostatic Hyperplasia (Nodular Hyperplasia):
Benign prostatic hyperplasia (BPH) is an extremely common abnormality. BPH is characterized by proliferation of both stromal and epithelial elements, with
resultant enlargement of the gland and in some cases, urinary obstruction, it is clear that excessive androgen-dependent growth of stromal and glandular elements has a central role. BPH does not occur in males castrated before the onset of puberty or in men with genetic diseases that block androgen activity (Kumar, et al. 2013).

2.3.1.2 Prostatitis:
Prostatitis is inflammation of the prostate. Often this inflammation may be caused by bacterial or fungal infection, but may be caused by other factors. There is no direct evidence that prostatitis is a precursor to prostate cancer or that it leads to prostate cancer. However, some investigators feel that some cancers, including prostate cancer, could be triggered by inflammation associated with infection (Cramer, 2007).

2.3.1.3 Adenosis (Atypical Adenomatous Hyperplasia) of Prostate:
Adenosis is one of the most common pseudo neoplastic lesions in the prostate that may be confused with adenocarcinoma because of its cytologic and architectural features. The two most important distinguishing features that favour adenosis are the lack of significant cytological atypia and the presence of basal cells (Baydar, 2015).

2.3.2 Malignant disorder:
2.3.2.1 Carcinoma of the prostate:
Carcinoma of the prostate is a common cancer of older men between 65 and 75 years age. Carcinoma of the prostate arises most commonly in the outer, peripheral gland and may be palpable by rectal examination, although currently many are non palpable (Kumar, et al. 2013).

2.3.2.2 Squamous cell carcinoma:
It usually occurs in the seventh decade of age, with symptoms of urinary obstruction (due to bladder outlet obstacle) or bone pain due to osseous metastases (Munoz, et al. 2007).
2.3.2.3 Transitional cell carcinoma (TCC):
Transitional cell carcinoma (TCC) is a multifocal disease that can develop anywhere in the entire urinary tract, including the prostatic urethra (PU). The mucosa lining the PU and the prostatic ducts have the same transitional cell lining as the bladder; therefore, TCC can originate from or invade the prostate (Kirkali and Canda, 2006).

2.3.2.4 Prostatic Intra Epithelial Neoplasia (PIN):
PIN consists of pre-existing prostatic ducts and acini lined by cytologically atypical cells and is subdivided into low grade and high grade PIN (LGPIN and HGPIN) (Montironi, et al. 2011).

2.4 Epidemiology of prostate cancer:
Prostate cancer is the sixth most common cancer in the world, the second most common cancer in men, and the most common cancer in men in Europe, North America, and parts of Africa. The number of new cases estimated was 513,000 patients in 2000, while the number of new cases estimated was 1.1 million people in 2012. The cancer will be known as the most common cancer in men in future. This cancer includes 15% of all new cancer cases in men. Approximately 70% of all new cases of cancer occur in developed countries. The lowest incidence of the disease is seen in Asian countries, and included 14% of all cases in 2008, especially in Tianjin, China (1.9/100,000 person-years). The highest incidence occurred in North America and Scandinavia, especially in African-American people (137/100,000 person-years) (Pakzad, et al. 2015).

Prostate cancer is the most common cancer in Sudanese men. The age-standardized rate is 10.3 and mortality is 8.7 per 100,000 populations. Recently, prostate cancer was the most common cancer among male patients treated at the NCI-UG. It ranked first among cancer male patients (n = 268) treated in the NCI, central Sudan (2006–2009). The disease was found equally distributed
among different tribes and most cases (85.4%) presented with stage III and IV. The mean age of patients was 72.2 ±9.25 (Elamin, et al. 2015).

2.5 Risk factors of prostate cancer:

2.5.1 Age:
Prostate cancer is predominantly a disease of elderly men; more than 75% of new prostate cancers are diagnosed in men older than 65 years (Fisher, 2008).

2.5.2 Family history and genetic susceptibility:
The risk of developing prostate cancer doubles for men who have a father or brother affected by prostate cancer, and risk increases further when multiple first-degree relatives are affected (Crawford, 2003).

2.5.3 Diet:
Higher meat consumption has been associated with a higher risk in some studies. Lower blood levels of vitamin D may increase the risk of developing prostate cancer and there is a little role for dietary fruits and vegetables in prostate cancer occurrence (Mustafa, et al. 2016).

2.5.4 Hormonal and other factors:
The growth and differentiation of the prostate is under androgen control. androgen ablation either surgically or with luteinizing hormone–releasing hormone agonists is an effective strategy in the treatment of advanced prostate cancer. Other factors like high body mass index (BMI) and bone mass may be associated with prostate cancer (Crawford, 2003).

2.6 Diagnosis and treatment of prostate cancer:

2.6.1 Diagnosis of prostate cancer:
Prostate cancer is diagnosed by number of tests:

2.6.1.1 Prostate specific antigen (PSA) test:
PSA levels between 4 and 10 ng/mL regarded as abnormal. However, PSA value of 4 ng/mL was reported in both men with prostatic adenocarcinoma and benign
hyperplasia. Therefore, the cutoff point for total PSA was lowered to 0.2–2.1ng/mL for screening Sudanese men for prostate cancer (Elamin, et al. 2015).

2.6.1.2 Digital rectal examination (DRE):
A digital rectal examination (DRE) are performed by a clinician physically examining the prostate via the rectum for any bumps, enlargements, or suspicious hard areas (James, 2014).

2.6.1.3 Prostate biopsy:
Biopsy gun inserts and removes special hollow-core needles (usually three to six on each side of the prostate) in less than a second. Tissue samples are then examined to determine whether cancer cells are present, and to evaluate the microscopic features (Gleason score) of any cancer found (Mustafa, et al. 2016).

2.6.1.3.1 Gleason score (GS):
The GS is the sum of the primary and secondary patterns with a range of 2 to 10. Biopsies are graded from 1–5 and then an aggregate score incorporating the principal and major secondary score is produced (eg, 3 + 4 = 7). Scores conventionally tend to be grouped into the following broader risk categories: 1–5: low-grade prostate cancer, 6–7: intermediate-grade cancer (most prostate cancers fall into this group), 8–10: high-grade cancer. However, some studies have shown that the prognosis of GS 7 cancers varies considerably (Stark, et al. 2009 , James, 2014).

2.6.1.4 A computerized tomography (CT) scan:
It is used with the introduction of high-speed multidetector helical scanners, it is now possible to acquire a CT study with high spatial resolution in a very short time (Hricak and Scardino, 2009).
2.6.1.5 Magnetic resonance imaging (MRI) scan:
Prostate MRI has better soft tissue resolution than ultrasounds. Currently (MRI) is used to identify targets for prostate biopsy using fusion MRI with ultrasound or MRI-guidance alone (Mustafa, et al. 2016).

2.6.1.6 Bone scan:
A bone scan show whether any cancer cells have spread from prostate to bone. By using small amount of a safe radioactive dye via arm vein, two to three hours later, a scan is used to find if prostate cancer cells have spread to bone (Galani, 2015).

2.6.1.7 Trans Rectal Ultrasound (TRUS):
TRUS provides imaging of the prostate and seminal vesicles using a 7.5-MHz biplane intra-rectal probe measuring 2.5 cm in diameter (image the outline of the prostate, identify cysts, abscesses and calcifications within the prostate, and be used to determine prostate volume) (Borley and Feneley, 2009).

2.6.2 Treatment of prostate cancer:
2.6.2.1 Surgery:
Surgery is mainly suggested for high-risk locally advanced prostate carcinoma. Radical prostatectomy and pelvic lymphadenectomy (PLDN) are mostly applicable surgery types in prostate cancer (Chen and Zhao, 2013).

2.6.2.2 Radiation therapy:
External-beam radiotherapy (EBRT), and brachytherapy are widely used treatment strategies for prostate cancer. Brachytherapy consists of transperineal implantation of small radioactive pellets into the prostate gland with ultrasound or MRI guidance (Pomerantz, et al. 2008, Chen and Zhao, 2013).

2.6.2.3 Cryosurgery:
In this strategy, the supercooled liquid is sprayed on the diseased tissue by using liquid nitrogen as the cooling solution to destroy abnormal and diseased tissue (Galani, 2015).
2.6.2.4 Hormonal therapy:
Androgens are regarded as the fuel for hungry prostate tumor. Androgen deprivation therapy (ADT) with either medical or surgical approach is regarded as the initial treatment for metastatic prostate cancer (Chen and Zhao, 2013).

2.6.2.5 Chemotherapy:
The use of chemotherapy in patients with hormone refractory prostate cancer (HRPC) has shown significant improvements in pain and quality of life, as well as decreases in PSA level. The common chemotherapeutic drugs used as the treatments of advanced prostate cancer include mitoxantrone, doxorubicin, vinblastine, paclitaxel, docetaxel, and some others (Chen and Zhao, 2013).

2.7 P63 and its relation with prostate cancer:
p63 is essential for differentiation of prostatic basal cells, and basal cells are essential in maintaining normal differentiation of luminal cells and integrity of prostatic ducts. However, basal cells (therefore p63) are not required for development and regeneration of prostate. P63 isoforms are functionally distinct in regard to cell fate commitment, particularly in epidermal differentiation. The differentiation of epidermis appears to be regulated by the balance between isoforms containing and lacking the transactivation domain. (Kurita, et al. 2004).
The p63 transcription factor belongs to a family that includes two structurally related proteins, p53 and p73. Whereas p53 plays a well-established role in tumor suppression, p63 and p73 play unique roles in morphogenesis. In particular, p63/2 mice have major defects in their limb and craniofacial development, as well as a striking absence of stratified epithelia. This phenotype could be explained by either inability of the p63/2 ectoderm to develop into epithelial lineages, or by lack of stem cell character necessary to sustain epithelial morphogenesis and renewal (Pellegrini, et al. 2001).
Analysis reveals p63 expression in the epithelial cells of stratified epithelium, including skin, oesophagus, exocervix, tonsil, bladder and the basal cells in glandular organs, including breast, bronchi and prostate (Gary, et al. 2006).

Immunohistochemical analysis of p63 expression in benign and malignant human prostate tissue specimens were performed. Strong p63 reactivity was observed in benign prostate sections stained with monoclonal 4A4 antibody against p63. Nuclear staining in prostate tissue was present in basal cells of the epithelium of benign areas within the pathogenic prostate glands. No expression of P63 was observed in malignant areas of the prostate cancer specimens (Rathore, 2010).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials:
Archived tissue blocks obtained from samples of prostate tumors were used in this study.

3.2 Methods:

3.2.1 Study design:
This is a hospital based analytical case control study aimed to detect p63 tumor marker in prostate tumor using immunohistochemical method.

3.2.2 Study sample:
Forty tissue blocks were obtained from prostate tumors samples, twenty samples were previously diagnosed as malignant prostate tumors and twenty samples were diagnosed as benign tumors. Patient's identification data were obtained from patient's files.

3.2.3 Study area:
This study was held in Radiation and Isotope Center at Khartoum (RICK) and Omdurman teaching hospital in 2016.

3.2.4 Immunohistochemical staining:
Immunohistochemical staining was carried out using new indirect dextran polymer immune peroxidase technique. The sections of 3µm thickness were obtained from formalin fixed paraffin embedded tissue by using a rotary microtome. Following deparaffinization in Xylene, slides were rehydrated through a graded series of alcohol (100%, 90%, 70%, and 50%) and were placed in distilled water. The antigens were retrieved using water bath (PT LINK) with Tris EDTA buffer (pH 9) for thirty minutes at 95°C, and then washed in phosphate buffer saline (pH 7.4) for five minutes. Then sections were circulated by Dako pen, endogenous peroxidase activity was blocked with 3% hydrogen peroxide for ten
minutes, the slides then treated with anti p63 primary antibody for 20 minutes at room temperature in a humidity chamber. Then washed in phosphate buffer saline (pH 7.4) for 3 minutes. Then sections were incubated in dextran polymer –Horse Reddish Peroxidase (HRP) secondary antibody for 15 minutes, then washed in three changes of phosphate buffer saline (pH 7.4), after that incubated in 3, 3 diaminobenzidine tetrahydrochloride (DAB) substrate solution for 5 minutes, then washed in running water. Then counter stained in Mayer‘s haematoxylin stain for one minute and washed in water. After that dehydrated, cleared and mounted in DPX mounting media.

3.2.5 Result interpretation:
All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining. Detection of more than 5 cells with brown nucleus per one field considered as positive result.

3.2.6 Data analysis:
Data analysis was done using SPSS 20 computer program. Frequencies, mean and chi-square test values were calculated.

3.2.7 Ethical consideration:
Sample collected after taking ethical acceptance from hospital administration.
CHAPTER FOUR

RESULT

The study includes forty samples, 20 (50%) samples were benign (benign prostatic hyperplasia) and 20 (50%) samples were malignant (prostatic adenocarcinoma) as indicated in table (4.1).
The age of study population range between 50 and 90 with mean age of 71 years. Majority of patients were more than 65 years representative 28 (70%) and the remaining 12 (30%) were younger than 65 years as indicated in table (4.2).
The grade of malignant samples include grade I in 3 (15%) samples, grade II in 3 (15%) samples, grade III in 4 (20%) samples, grade IV in 7 (35%) samples, and grade V in 3 (15%) samples as indicated in table (4.3).
P63 positive expression was found in (11/20) in malignant samples, and (9/20) showed negative expression, while benign samples showed (15/20) positive samples, and (5/20) negative samples. This result showed insignificant association (P.value =0.185) as indicated in table (4.4).
Table (4.1): Distribution of histopathological diagnosis among study population:

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>20</td>
<td>50%</td>
</tr>
<tr>
<td>Prostatic adenocarcinoma</td>
<td>20</td>
<td>50%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.2): Distribution of age group among the study population:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>More than 65 years</td>
<td>28</td>
<td>70%</td>
</tr>
<tr>
<td>Less than 65 years</td>
<td>12</td>
<td>30%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.3): Distribution of malignant tumor grade:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td>Grade II</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td>Grade III</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td>Grade IV</td>
<td>7</td>
<td>35%</td>
</tr>
<tr>
<td>Grade V</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.4): Relation between histopathological diagnosis and P63 expression:

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>P63</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive N (%)</td>
<td>Negative N (%)</td>
</tr>
<tr>
<td>Malignant</td>
<td>11(27.5%)</td>
<td>9(22.5%)</td>
</tr>
<tr>
<td>Benign</td>
<td>15(37.5%)</td>
<td>5(12.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>26(65.0%)</td>
<td>14(35.0%)</td>
</tr>
</tbody>
</table>
Microphotograph (4.1): Benign prostatic hyperplasia showed nuclear positive expression of p63 (40X).
Microphotograph (4.2): Prostatic adenocarcinoma showed negative expression of p63 (40X).
CHAPTER FIVE
DISCUSSION

In this study forty samples were investigated by immunohistochemical stain. Concerning the age group of study population, most patients were aggregating in more than 65 years group, indicating that men more than 65 years are more affected with prostate cancer. This result agree with Galani, (2015), who reported that prostate cancer is predominantly a disease of older men (aged 65–79 years). Also agree with Elamin, et al. (2015), who reported that the mean age of patients was 72.2 ± 9.25.

All study populations were diagnosed with prostatic adenocarcinoma and benign prostatic hyperplasia this agree with Albasri, et al. (2014), in which their result showed that Adenocarcinoma was the commonest histological subtype seen malignant lesions, and In the benign group, BPH was the most common lesion.

The malignant tumor grade revealed that more frequent grade is grade IV (Gleason 7) due to late diagnosis of cancer, this result is compatible with Albasri, et al. (2014), who reported that Gleason grading of the prostate showed that moderately differentiated carcinomas (Gleason score of 5-7) comprised the largest group, also compatible with Arshad and Ahmad, (2013), who reported that Gleason score 7 was the commonest score in their study.

The expression of P63 revealed that there was no significant association between marker expression in benign and malignant prostate tumors and this may be due to shedding of secretory cells leaving basal cells.

This study disagreed with Signoretti, et al.(2000), who reported that p63 is a reliable prostate basal cell marker and that the Np63 isotype is the most abundantly represented in normal prostate basal (PrEC) cells. Because p63 protein is consistently undetectable in prostate cancers, we propose that p63 expression may
be used in the differential diagnosis between benign and malignant lesions of the prostate. Also disagreed with Baig, et al.(2012), in which their study concluded that prostatic adenocarcinomas were p63 negative and most of the benign ambiguous lesions of prostate were p63 positive. Hence, p63 is a reliable basal marker and can be used in morphologically difficult cases when the differential diagnosis is adenocarcinoma of prostate.
CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6.1 Conclusion:
From this study we conclude that:

- The age of prostate cancer patients in our study is commonly more than 65 years.
- Most histological type of prostate cancer is prostatic adenocarcinoma.
- Grade of prostate cancer found mostly is grade IV.
- There no association between p63 and histopathological diagnosis of prostate tumor.

6.2 Recommendation:
From this study we recommended that:

- Further study should be done on expression of P63 in Prostate tumors tissues with large sample size.
REFERENCES:


Appendix 1:
Materials and instruments for processing and staining of the specimens include:

- Disposable gloves.
- Rotary microtome.
- Positively charged slides (thermo).
- Cover glasses.
- Dry oven.
- Water path (PT LINK).
- Coplin jars.
- Humidity chamber.
- Ethanol (100%, 90%, 70%, 50%).
- Xylene.
- Mayer's haematoxylin (Haematoxylin, DW, potassium or ammonium alum, sodium iodate, citric acid and chloral hydrate).
- Tris EDTA buffer (pH 9).
- Phosphate buffer saline (pH 7.4).
- Peroxidase blocker (0.3% hydrogen peroxide in methanol).
- Primary antibody (anti-human P63).
- Secondary antibody (dextran polymer conjugated secondary antibody+horse reddish peroxidase).
- DAB (3,3 diaminobenzaldehydetetrahydrochloride) substrate solution.
- DPX.
Monoclonal Mouse Anti-Human p
def3 Protein
Clone DAK-p
def3
Kode M7317

Tilgængelighed

Til in vitro-diagnostik brug.
Monoclonal Mouse Anti-Human p
def3 Protein, Clone DAK-p
def3, er beregnet til brug ved immunhistochemi. Antikörper til p
def3-protein, en basal regulator af epithelforskelingsforskere (1), kan være nyttige ved identifikation af prostaatanderkrævende som en hjælp ved identifikationen af bærende prostaatadkrævende og prostaatadkrævende (2, 3). Antikörper til p
def3 kan også være nyttige som en hjælp ved identifikationen af bryskerkrævende i og bryskerkrævende (4), til at identificere plasmadekrokrævende fra lungedanekrævende (5, 6), og til at identificere ærlige celler fra bryskerkrævende (7). Den adskilte funktion af et eller en af forskerne eller fælles af forskerne, der ikke kunner aftevne, er en af de længere vigtige testudtæt af en kvantitativ patologi.

Synonymer for antigen

p
def3-proteinet tilhører p
def3-genfamilien, der også omfatter p
def3, p
def3-genet kodirer flere isoforer, isoforer, der indholder et potent amino-længere trykortindhængende (TPA
def3-isoforer) og isoforer, som måler det område (p
def3-isoforer) (8, 9). Men de 
def3-isoforer kan transduceres p
def3-længere, f.eks. l, o og p
def3-til 
def3- og inducere apoptose og cellelystopt (10). p
def3 er ikke et tumorsupressor (11). TPA
def3-isoforerne virker på en dominant-negativ måde ved at korrurere om p
def3-længere og indirekte fremme cellelyst ved at modvirke aktiviteten af apoptosen og cellelystopt ved TPA
def3-isoforer og p
def3 (1, 10, 11). p
def3 er en molekule for trykstabilisering af tumorceller, men også af p
def3-suppressoreffekter i mere invasive tumorceller, herunder enkelte celler, der ikke kan flygte fra cellernes lyse og metabolisere (10). Manglende p
def3 er dog ikke en absolutt antikaske for invasive celler, og selv om p
def3 eksemplificeres i et fæltes af bryskerkrævende, findes der sjældnere tilfælde af nuclear p
def3-ekspression (9).

Ofte har tumorceller endnu overproduceret af både TPA
def3- og TPA
def3-isoforerne, hvor TPA
def3 er expresseret på en multivariat niveau. Nogle tumorceller og plasmadekrokrævende i hovedet har nemt viser overproduktion af p
def3-protein i forbindelse med en beskedet korrektion af p
def3-geneforekomsterne, men de vigtigste p
def3-isoforer er TPA
def3-isoforer. I nasanatomygogene kancerer og anslagte plasmadekrokrævende er TPA
def3-isoforer er en af de vigtigste isoforer (9).

De prædominante lokaliserer af p
def3-protein er i basale celler i normale epitel i specifikke organer, som den spædbørn, prostatita, uddrag, uddrag og vajina, og i basale celler i indre organer og strukturen i hud. Prostatita, uddrag, uddrag og vajina, og i basale celler i indre organer.

Medfølgende reagens

Monoklonalt murine anlæg, havet i fylde, der som celforbrugerstabiliser (indholdende fuldt bovind serum), der er lav ydelse for 0,05 mM, TPA
def3, 4 mM, pH 7,2, og indeholder 0,15% Tween 20, Tween 20, Tween 20. Phi: Dako, 2 g/20 ml, 2 g/20 ml, 2 g/20 ml. Typer: Dako, 2 g/20 ml, 2 g/20 ml, 2 g/20 ml.

Immungren

Syntetisk p
def3-antikaske fra det kæmpe DNA-bindende domæne af human p
def3-protein.

Specificitet

I Western-blot analyse visueraliserer antistoffer bånd, der varierer i de forventede melkylvaage, og i halo i halo i halo til ekspresionsprofilerne for de forskellige isoforer (TPA
def3- og TPA
def3-isoforer) af p
def3 i HCC1106 plasmadekrokrævende og kolonkrævende.

Forhåndserg

1. Maksimalt udadvendt personale.
2. Produktet indeholder natriumazid (NaAzid), et kemikalie, der er meget giftigt i sin rene form. Selvom koncentrationen i produktet ikke er klassificeret som farlig, kan natriumazid reagere med al uddrag og dannere meget explosive ophobninger af metaller. Efter ophobning skyldes med regelrige værner ved for at hindre ophobning af metaller i stoffet.
3. Om med alle produkter, der er afdelt fra biologiske kilder, bør der tagtlig tage de indvendige håndteringsteknikker.
5. Udbredt ophobning skal bortskaffes i henhold til lokal, national og EU-retnings linjer.

Ophøring

Ophøres ved 28°C. Må ikke anvendes efter udførsel dateren på flasken. Hvis reagensen opbevares ofte ikke iかけてs, skal disse reagenser verificeres af brugeren. Der er ingen synlige tegn på, at produktet kan være ustabilt. Det bør derfor ikke bruges af patienter, der har kartelte antistoffer til p
def3. Kontakt teknisk support hos Dako, hvis der opstår eventuelle problemer.
**Lynvejledning**

<table>
<thead>
<tr>
<th>Trin</th>
<th>Formålen</th>
<th>Kommentarer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiksering</td>
<td>EnVision FLEX™, High pH (kode K8004)</td>
<td>20 min. HIER, 3-4 x ved hjælp af PT Link og PT Link Rinse Station</td>
</tr>
<tr>
<td>Forynding</td>
<td>1:50</td>
<td>20 min. inkubation</td>
</tr>
<tr>
<td>Foryndingsbuffer</td>
<td>Dako Antikub dilueret (kode S8989)</td>
<td>Foryndes umiddelbart før brug</td>
</tr>
<tr>
<td>Negativ kontrol</td>
<td>Dako Negative Control, Mouse IgG2a (kode K0943)</td>
<td>30 min. inkubation</td>
</tr>
<tr>
<td>Visualisering</td>
<td>EnVision™ FLEX, High pH (kode K5000/K8010)</td>
<td>20 min. inkubation, 2-5 min. DAB++ inkubation</td>
</tr>
<tr>
<td>Kontrastfarve</td>
<td>EnVision™ FLEX Hematoxylin (kode K8008/K8018)</td>
<td>5 min. inkubation</td>
</tr>
<tr>
<td>Kontrolløv</td>
<td>Torsol, prosta</td>
<td>Nuklear farvning</td>
</tr>
<tr>
<td>Objektlip</td>
<td>FLEX INC Microscope Slides (kode K8200)</td>
<td>Anbefales for at vælge de højere hænger bedre fast på objektlispensen.</td>
</tr>
<tr>
<td>Monitoring</td>
<td>Ikke-vandlig permanent monterings påkrævet</td>
<td>Efter farvning skal sejltørre dehydreres, tørres og monteres ved hjælp af et permanent monteringsmedie.</td>
</tr>
<tr>
<td>Instrumenter</td>
<td>Autostainer Link 48 og Autostainer Plus</td>
<td>Anvend instrumentspændiske flaske (kode SK200-SK203 og kode SK202)</td>
</tr>
</tbody>
</table>

*Brugen skal altid følge tildelingsetiketten for at få de detsiljerede anvisninger i farvningproceduren og håndtering af produkter.

**Klarlæggelse af preparater**

Paraffin: Antisefel kan anvendes til markering af formalinfikserede, paraffininddelede vævsnit.

Vævssprederne skal skues i snit med en tykkelse på ca. 4 μm.

Forbehandling: Det er nødvendigt at forbehandle formalinfikserede, paraffininddelede vævsnit med vandeminduret epipon-retrival (HER). Det er vigtigt at anvide PT Link og PT Link Rinse Station.


Forsætning: Anvend EnVision™ FLEX Wash Buffer (2x) (kode K8007), der har sugetemperatur. Låt dem ligge i Wash Buffer 1-5 minutter.

Vævssnitene med rørre udrenere under behandlingen eller under den efterfølgende immunhistochemiske farvningprocedur. Det anbefales at anvende FLEX INC Microscope Slides (kode K8200), så vævssnitten hænger bedre fast på objektlispensen. Efter farvning skal sejltørre dehydreres, tørres og monteres ved hjælp af et permanent monteringsmedium.

**Farvingsprocedure**


Negativ kontrol: Det er anbefalet at bruge Dako Negative Control, Mouse IgG2a (kode K0943), som negativ kontrolsmogen, for at fastslå dens sugetemperatur.

Visualisering: Farvningseffekten er EnVision™ FLEX, High pH (kode K8000/K8010) ved brug af et 2-5 minutters inkubation ved sugetemperatur.

**Fortolkning af farvning**

Det cellulære farvningstørre er nutrænt. Cytoplasmatiske farvning er også blevet rapporteret i normalt væv (12).
Resultatendegn

<table>
<thead>
<tr>
<th>Værvtype (antil analyserede)</th>
<th>Positive værvæ Marker</th>
<th>Værvtype (antil analyserede)</th>
<th>Positive værvæ Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryst (3)</td>
<td>3/3 Emmerceller (30%), ctasatorns</td>
<td>Ovare (3)</td>
<td>G3</td>
</tr>
<tr>
<td>Kongstørre (10%)</td>
<td>3/3 Emmerceller (10%), proplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bryst (3)</td>
<td>3/3 Basalceller (90%), nuklear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach (3)</td>
<td>1/3 Plasencell (30%), ctasatorns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon (3)</td>
<td>1/3 Basalceller (100%), nuklear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrium (3)</td>
<td>2/3 Uteruskep (100%), proplasm</td>
<td></td>
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</tr>
<tr>
<td>Oesophagus (3)</td>
<td>3/3 Epitelyt (100%), nuklear</td>
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</tr>
<tr>
<td>Appendix (3)</td>
<td>1/3 Basalceller (90%), nuklear</td>
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<td></td>
</tr>
<tr>
<td>Tyre (3)</td>
<td>1/3 Nucliek (100%), nuklear</td>
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<tr>
<td>Liver (3)</td>
<td>1/3 Basalceller (100%), nuklear</td>
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</tr>
<tr>
<td>Lymphocytes (3)</td>
<td>2/3 T-lymph (100%), nuklear</td>
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</tr>
<tr>
<td>Lymphocytes (3)</td>
<td>2/3 Uterus (100%), nuklear</td>
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</tr>
<tr>
<td>Lymphocytes (3)</td>
<td>2/3 Eptelyst (100%), nuklear</td>
<td></td>
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<tr>
<td>Liver (3)</td>
<td>1/3 Basalceller (100%), nuklear</td>
<td></td>
<td></td>
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</tbody>
</table>


Referencer

13. Dako in-house documentation D10309
14. Dako in-house documentation D14567

Symbolerklargning

<table>
<thead>
<tr>
<th>REF</th>
<th>Kædestegn</th>
<th>°C</th>
<th>Temperaturregulering</th>
<th>Avisendelses inden</th>
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<tbody>
<tr>
<td>IHD</td>
<td>In-situ diagnostic med miske</td>
<td>LOT</td>
<td>Biologisk</td>
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</table>