The novel therapeutic implications of emerging biotechnologies in diagnostics and treatment of prostate cancer

Muhammad Naveed*, Komal Imran', Ayesha Mushtaq', Sana Tehreem', Sami Ullah Khan

*Department of Biochemistry and Biotechnology, University of Gujrat, Pakistan
Department of Biotechnology, University of Gujrat, Sialkot Sub-campus, Pakistan
Department of Botany, Women University AJ & K Bagh, Pakistan

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Abstract

The definitive goal of present study is to summarize the role of biotechnology in developing new therapeutic strategies for metastasized prostate cancer. Globally prostate cancer is considered as the second deadliest tumor of men due to limitations of standard therapies. Prostate cancer is highly prevalent in more developed countries such as America and Australia as compared to less developed countries. Traditional chemotherapeutic drugs and independent metastasized prostate cancer treatments poised the enthusiasm for advanced approaches such as immunotherapy, combined gene therapy and Nano biotechnology. These advanced initiatives promised better understandings of the genetic basis biochemical pathways of prostate cancer and make cancers therapies more personalized and predictive. Here we have provided a timely overview about current and near term applications of emerging biotechnologies as a perspective of future search regarding to in vitro diagnostics, in vivo diagnosis and in vivo therapeutics. This review outlines the importance of early prostate cancer diagnosis by tackling the problems of tumor microenvironment interactions with the hope of advancing new concepts in the era of treatment. Furthermore, study explained recent trends of prostate cancer incidence, mortality and survival rate among developed and Asian countries that would urge scientists for the development of newer molecular markers and imaging technologies for its management.

*Corresponding Author: Muhammad Naveed  naveed.quaidian@gmail.com
Introduction
Prostate cancer is recognized as the highest prevalent and most common form of malignancy in the western men with a slower rate of progression(Boring,1992). Mostly males die with this disease not from this. It is a non-cutaneous tumor of elderly males especially of western America. According to a survey in 2012 more than 241,740 cases were diagnosed and about 28000 patients were expired due to this disease(Siegel, 2012). In Asian countries the incidence of this disease is very lower but development of this disease is seen in them after their immigration to west. Hereditary and environmental components are the intrinsic features of this cancer. Prostate cancer is characterized as androgen related cancer and its prevalence through growth, proliferation, differentiation, secretion; survival depends upon AR signaling(Takayama et al., 2007). But in the case of androgen independent prostate cancer, tumor remains active after the surgical excision of androgen. Prostate cancer is second death causing tumors due to the limitation in therapeutic therapies when the tumor has been metastasized (Martel, 2003).Primary screening tests for prostate cancer such as serum prostate antigen testing (PSA) have many limitations; unable to judge aggressiveness of cancer and often lead to under treatment or overtreatment of patients(Caram et al., 2016).PSA levels unable to differentiate among metastasized cancer patients tends to poor diagnosis(Hussain et al., 2006).

For localized prostate cancer surgical excision of androgen and radiotherapy are effective treatment options but new therapeutic strategies are required for metastatic prostate cancer. Increasing efforts are required for better understandings of mechanisms and pathways of resistance to cure metastatic resistant prostate cancer (Aragon-Ching 2013, Naveed et al., 2016a).

It seems like a combination of treatment methods will be an effector against metastatic prostate cancer (Stevens, 1996).The advanced and novel techniques are the combined radiotherapy and gene therapy for this purpose (Kaliberov, 2005).

This review explains the current approaches; population based prostate cancer registries, challenges and future directions for monitoring treatment response in cancer patients. The aim of this review is to highlight basic mechanisms of prostate cancer relevant to its pathogenesis, the role of biotechnology in the development of new approaches for diagnostics and treatment of metastasized and localized prostate cancer.

Epidemiology
A database named Globscan contains data about the rate of incidence, mortality and prevalence of different types of cancers geographically per year(Ferlay, 2004). In this review, we have compared the data of year 2002, 2012 and 2016 for a sharp outlook on the epidemiologic features of prostate cancer geographically.

Occurrence and mortality rate in 2002
It is reported that about 679000 men were identified with prostate cancer in 2002 and age standardized rate is 25.3 cases/100 000 population, 76% of this population was belonged to developed countries (Ferlay, 2004). In established countries it is 19% of all spotted cancers while in less established countries it is 5% indicating 6-fold difference in rate compared to established (56.2 cases/100 000 population) and less established countries (9.6 cases/100 000 population).

In established countries it is 19% of all spotted cancers while in less established countries it is 5% indicating 6-fold difference in rate compared to established (56.2 cases/100 000 population) and less established countries (9.6 cases/100 000 population).

The highest rate of cancer has been seen in USA and lowest in Bangladesh. Morality rate of men throughout the world was estimated 221000 in 2002 indicating 8.2 deaths/100 000 populations (Kamangar, 2006). 60% of these men belonged to established countries (13.5 deaths/100 000 population) and 5.2 deaths/100 000 population in less established countries(Sim, 2005). Mortality rate is two and half times higher in established countries. Prostate cancer caused 1% of all male deaths and 7% of whole male deaths in 2002 (Organization 2004). In established countries it is seventh most common reason of death in males (Table 1).
Table 1. Age standardize occurrence and mortality rate for prostate cancer in certain regions (per 100,000 population), 2002.

<table>
<thead>
<tr>
<th>Country</th>
<th>Occurrence</th>
<th>ASR(W)</th>
<th>Mortality</th>
<th>ASR(W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>679023</td>
<td>25.3</td>
<td>221002</td>
<td>8.2</td>
</tr>
<tr>
<td>More Developed</td>
<td>513464</td>
<td>56.2</td>
<td>130382</td>
<td>13.5</td>
</tr>
<tr>
<td>Less developed</td>
<td>165347</td>
<td>9.4</td>
<td>90514</td>
<td>5.2</td>
</tr>
<tr>
<td>America</td>
<td>239930</td>
<td>124.8</td>
<td>1133</td>
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</tr>
<tr>
<td>United Kingdom</td>
<td>27463</td>
<td>56.7</td>
<td>9834</td>
<td>18.4</td>
</tr>
<tr>
<td>Australia</td>
<td>10807</td>
<td>76.0</td>
<td>2646</td>
<td>17.9</td>
</tr>
<tr>
<td>Pakistan</td>
<td>2308</td>
<td>5.6</td>
<td>1436</td>
<td>3.5</td>
</tr>
<tr>
<td>India</td>
<td>16786</td>
<td>4.6</td>
<td>10867</td>
<td>3.0</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>115</td>
<td>0.3</td>
<td>71</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Incidence and mortality rate in 2012

It is estimated that about 1.1 Million men were detected with prostate cancer worldwide in 2012 and 70% of these cases (759,000) were from developed regions (Forman, 2013). The rate of prostate cancer is higher in New Zealand, Northern America and Australia (ASR 111.6 and 97.2 for each 100,000, respectively), and in Western and Northern Europe. Rate of occurrence is higher in these countries due to practice of PSA testing and regular biopsy (Robinson et al., 2005). But the rate of occurrence has been lowered for less developed countries such as in Asian population such as 10.5 and 4.5 in eastern and south central Asia. It is reported 307,000 deaths in 2012 due to prostate cancer makes it foremost cause of death in men with 6.6% of the total men deaths (Curado et al., 2007). As the in the PSA testing, there is less effect of mortality as compared to occurrence therefore less distinction has been seen in mortality rates universal such as 165,000 in established countries while 142,000 in less established countries. Morality rate is higher in black population 29 cases/100,000 while very low in central South Asia 2.9/100,000 and intermediate in America (Table 2).

Table 2. Age standardize occurrence and mortality rate for prostate cancer in certain regions (per 100,000 population), 2012.

<table>
<thead>
<tr>
<th>Country</th>
<th>Incidence</th>
<th>ASR(W)</th>
<th>Mortality</th>
<th>ASR(W)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1094916</td>
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<td>307481</td>
<td>7.8</td>
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<tr>
<td>More Developed</td>
<td>741966</td>
<td>68.0</td>
<td>142014</td>
<td>10.0</td>
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<tr>
<td>Less developed</td>
<td>352950</td>
<td>14.5</td>
<td>165467</td>
<td>6.6</td>
</tr>
<tr>
<td>America</td>
<td>233159</td>
<td>98.2</td>
<td>30383</td>
<td>9.8</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>45406</td>
<td>73.2</td>
<td>10595</td>
<td>13.1</td>
</tr>
<tr>
<td>Australia</td>
<td>21966</td>
<td>115.2</td>
<td>3333</td>
<td>12.9</td>
</tr>
<tr>
<td>Pakistan</td>
<td>3041</td>
<td>5.3</td>
<td>2356</td>
<td>3.9</td>
</tr>
<tr>
<td>India</td>
<td>19095</td>
<td>4.3</td>
<td>12231</td>
<td>2.7</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>923</td>
<td>1.7</td>
<td>717</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Incidence and mortality rate 2013-17

Since 2012 more than 1.1 million people have been diagnosed with prostate cancer however this rate has been increasing day by day across the world with every passing year. The International Agency for Research on Cancer published a study that this burden is expected to grow to 1.7 million newer cases and 49,9000 deaths by 2030.
American society estimated 180,890 newer prostate cancer cases in America with an incident rate of 129.7 and 26,120 deaths with a rate of 20.4 per 100,000 people in 2016 (Siegel et al., 2016). AIHW also estimated 18,138 newer cases in Australia with 3,398 deaths of suffering patients with a rate of 25.2 and death rate 12.8 in year 2016. Cancer Search UK estimated 46,700 new cases in UK by 2014 with an incidence rate of 26.0 and 11,287 deaths with a mortality rate of 13.0. The Indian Express published a report in 2016 concluding incidence rate of prostate cancer is 9-10/100,000 which is higher as compared to other parts of Asia but lower than UK, America and other Europe. World Health Rankings published data for worldwide age justified death rates per 100,000 populations in 2014 tabulated in Table 3.

Table 3. Age standardize mortality rate and ranking of certain regions for prostate cancer (per 100,000 population), 2014.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guyana</td>
<td>108.33</td>
</tr>
<tr>
<td>2</td>
<td>Zimbabwe</td>
<td>69.94</td>
</tr>
<tr>
<td>3</td>
<td>Barbados</td>
<td>64.73</td>
</tr>
<tr>
<td>4</td>
<td>Tob</td>
<td>62.20</td>
</tr>
<tr>
<td>5</td>
<td>Jamaica</td>
<td>52.12</td>
</tr>
<tr>
<td>11</td>
<td>Nigeria</td>
<td>41.48</td>
</tr>
<tr>
<td>19</td>
<td>Uruguay</td>
<td>31.71</td>
</tr>
<tr>
<td>28</td>
<td>Sweden</td>
<td>27.22</td>
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<tr>
<td>32</td>
<td>Central Africa</td>
<td>26.92</td>
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<tr>
<td>34</td>
<td>Norway</td>
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</tr>
<tr>
<td>38</td>
<td>Turkey</td>
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<td>40</td>
<td>Chile</td>
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<tr>
<td>56</td>
<td>United Kingdom</td>
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</tr>
<tr>
<td>58</td>
<td>Netherlands</td>
<td>20.23</td>
</tr>
<tr>
<td>60</td>
<td>Australia</td>
<td>20.14</td>
</tr>
<tr>
<td>70</td>
<td>France</td>
<td>18.21</td>
</tr>
<tr>
<td>81</td>
<td>New Zealand</td>
<td>17.02</td>
</tr>
<tr>
<td>93</td>
<td>Germany</td>
<td>15.98</td>
</tr>
<tr>
<td>97</td>
<td>Spain</td>
<td>15.16</td>
</tr>
<tr>
<td>99</td>
<td>United states</td>
<td>14.81</td>
</tr>
<tr>
<td>100</td>
<td>Canada</td>
<td>14.80</td>
</tr>
<tr>
<td>113</td>
<td>Italy</td>
<td>11.53</td>
</tr>
<tr>
<td>122</td>
<td>Kuwait</td>
<td>10.39</td>
</tr>
<tr>
<td>126</td>
<td>Syria</td>
<td>9.82</td>
</tr>
<tr>
<td>132</td>
<td>Iran</td>
<td>8.96</td>
</tr>
<tr>
<td>141</td>
<td>Qatar</td>
<td>7.25</td>
</tr>
<tr>
<td>142</td>
<td>Japan</td>
<td>6.90</td>
</tr>
<tr>
<td>144</td>
<td>Iraq</td>
<td>6.54</td>
</tr>
<tr>
<td>148</td>
<td>Saudi Arabia</td>
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<tr>
<td>153</td>
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<td>159</td>
<td>India</td>
<td>3.27</td>
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<tr>
<td>160</td>
<td>China</td>
<td>3.19</td>
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<tr>
<td>165</td>
<td>Sri Lanka</td>
<td>2.22</td>
</tr>
<tr>
<td>170</td>
<td>Bangladesh</td>
<td>1.38</td>
</tr>
<tr>
<td>172</td>
<td>Bhutan</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Screening and diagnosis of prostate cancer

Screening

Specific kinds of tests are performed to diagnose disease such as cancer in asymptomatic individuals at early stages in the process of screening (Wolf et al., 2010). The chief goal of prostate cancer screening is to enhance life span off cells and to reduce symptomatic metastasized prostate cancer. There are three main categorize for screen-detected cancer patients. First those whose cancer will outcome in death without early analysis and treatment, second those who have good consequences in the nonappearance of screening and third those for whom early diagnosis and treatment recover survival. Early screening of prostate cancer is done by testing the amount of prostate specific antigen and digital rectal exam (DRE) but neither of these assessments is 100% accurate. These tests can be false negative giving false sense of security to a person having cancer and can be false positive leading some men to prostate biopsy when they don’t have prostate cancer. Additional matter is that even if the screening finds a cancer the
doctors will not be able to tell whether it is truly dangerous or not (Andriole et al., 2010). Doctors end up struggling whether a patient requires treatment or it might be able to follow without being treated.

Table 5. Treatment progress of prostate cancer.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Surveillance</td>
<td>To stop over treatment, accept side effects in men.</td>
</tr>
<tr>
<td>Radical Prostatectomy</td>
<td>To cure disease completely.</td>
</tr>
<tr>
<td>Radiation Therapy</td>
<td>To precisely target localized prostate cancerous cells.</td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>To treat benign prostate cancer through freezing cancerous cells.</td>
</tr>
<tr>
<td>Hormonal agents and prostate cancer therapy</td>
<td>To slow down and stop the cancer growth</td>
</tr>
<tr>
<td>Chemotherapeutics</td>
<td>To increase life expectancy.</td>
</tr>
<tr>
<td>Anti-apoptotic chemical agents</td>
<td>To induce apoptosis for cancer regression.</td>
</tr>
<tr>
<td>Tumor stromal fibroblastic microenvironment therapy</td>
<td>To reduce the invasiveness or ultimately block the spreading of tumor cells</td>
</tr>
<tr>
<td>Immune microenvironment prostate cancer therapy</td>
<td>To metastasized prostate tumor by killing cancerous cells.</td>
</tr>
<tr>
<td>Angiogenesis prostate cancer therapy</td>
<td>To block tumor development and metastasis</td>
</tr>
<tr>
<td>Gene Therapy</td>
<td>To treat metastasized resistant prostate cancer by targeting cancer progression molecular events.</td>
</tr>
<tr>
<td>miRNAs as therapeutic agents</td>
<td>To act as tumor suppressor agents by inhibiting metastasized prostate cancer.</td>
</tr>
<tr>
<td>Nanotechnology</td>
<td>To increase chances of successful treatment by reducing rate of toxicity to the surrounding cells.</td>
</tr>
</tbody>
</table>

Primary screening tests

Prostate specific antigen (PSA) blood test

Prostate specific antigen (PSA) is released by the cells in prostate gland. PSA is initiate in semen and present in small volume in blood. Most healthy men have level of PSA 4ng/ml while in prostate cancer this level goes up. PSA more than 10 showing that prostate cancer presence is more than 50%. But if a man has PSA level lower than 4 it is not a guarantee that a man may develop cancer(Changet al., 2010). PSA is used as a molecular indicator for detection and monitoring for prostate cancer. There is a distinction between two terms known as pretreatment and post treatment related with PSA dynamics. Pretreatment PSA levels rely on both malignant and nonmalignant events in the untreated prostate patient. The advantage of PSA screening and primary treatment ranges from 0-1 prostate malignancy deaths avoided per 1000 men screened (Carter et al., 1992).The correlation between carcinogenesis and rise in PSA is aforementioned in (Fig. 1).

Early studies reported that PSA screening. Tests for early diagnosis and screening of prostate cancer with their specifications are illustrated in Table 4.

PSA as a molecular indicator of a disease

Special PSA tests

PSA occur in two major forms; one is involved with proteins whereas other is freely moved in blood (unattached). Percent Free PSA determines the ratio of unattached PSA comparing to total PSA.

Percent free PSA

Men with prostate cancer have lower levels of unattached PSA from normal ones. Percent free PSA guides a doctor either a patient will go for a prostate biopsy means lower fPSA indicates more likelihood of having prostate cancer (Carter et al., 1992).
PSA velocity
This test measures how fast PSA increases over time. Usually PSA level raises gradually with the progression of age but individuals that are on the risk of developing cancer may rough increase PSA level (Perrin, 2006). It is not preferably recommended as a screening test for prostate cancer.

PSA density
PSA density test is used for males that have larger prostate gland. Size of prostate gland is measured by doctors’ transectal ultrasound and then divides PSA number by prostate size. Developed PSA density specifies a great likelihood of prostate cancer but it is not as useful as percent free PSA test.

Digital rectal exam (DRE)
Doctor supplements a gloved lubricated finger into the rectum to feel bumps and stiff areas on prostate that might cause cancer. This exam is rough but not painful and needs short time. It can screen prostate patients which have normal PSA level.

Formal diagnosis
Formal diagnosis of prostate cancer involves needle biopsy (Society, 2015). The sample is obtained from prostate and then examined in labs for the diagnosis of prostate cancer. A core needle biopsy is done by urologist for diagnosis of prostate cancer.
Transectal ultrasound (TRUS)
A minor probe about the width of finger is greased and placed in rectum, probe give sound waves entering in prostate and generate echoes then echoes are picked up by probe and computer transforms into black white image. TRUS is not used as screening test but it can guide biopsy needle to right place, detect size of prostate gland and PSA density.

Prostate biopsy
In biopsy a body tissue is removed and use as a sample to looked under a microscope.

Core needle biopsy is mostly used for prostate cancer diagnosis by an urologist or a surgeon. A thin hollow needle is rapidly inserted through the wall of rectum into prostate and drawn out having a small cylinder of prostate tissue (Loeb et al., 2011). Most urologists take 12 samples then biopsy samples are sent to lab and pathologist diagnose the disease in a sample. Pathologists give a Gleason score to your sample if cancer is found ranging from 2 to 10. Higher value of Gleason score indicates the higher level of growing and spreading cancer.

Imaging techniques
Bone scan: A radioactive material is injected in the body and absorbed by diseased bone cells and a radionuclide bone cell shows whether a cancer has been metastasized to bones or not(Association, 2011).Metastasis at the early stages is not detected by this scan. Bone scan is not ordered except there is an elevated PSA and high Gleason score.

Computer tomography
A detailed cross sectional image of a body is taken with rotating x ray beam from many angels to detect metastases of cancer towards other organs and abnormally enlarged lymph nodes(Carter et al., 2006). CT scan is not well-ordered unless there is a higher PSA level and high Gleason score.

CT scan can be often assist in visualizing the cancer if it has spread to nearby lymph nodes or if cancer came back after treatment whether it is localized or spread to other organs in pelvis.

Magnetic resonance imaging
MRI is like a CT scan with only one difference that magnetic fields are used as an alternative of x-rays. MRI is not effective in diagnosis of microscopic sized cancers but is a better choice when end rectal coil (probe) is used; it might be uncomfortable so medicine can be used before scan. MRI is a preferable choice to get a clear picture of prostate when cancer has been spread outside the prostate to nearby structures.
Advancement in diagnosis field
Restrictions for PSA as an indicative and molecular marker led to the discovery of new therapeutic markers by using current biotechnological methods such as genomic study, DNA microarray robotic systems to allow follow up of PCa (Grouse et al., 2001).

Serum Markers
Serum markers provide the non-invasive exposure, sorting and follow up of PCa. Antibody microarray is a satisfactory method for the detection of serum markers leading to diagnosis of differential expression of proteins in normal and diseased person.

In prostate cancer patient's expression of five proteins named as immunoglobulin M, Von Will brand Factor, immunoglobulin G, alpha 1 chymotrypsin and vilin is found high from normal men (Miller et al., 2003).

This approach can be combined with genomics for the identification of post transcriptional modification (Unwin et al., 1999).

Tissue markers
DNA microarray generated a gene expression profile shows that EZH2 gene is a tissue marker are highly expressed in hormone refractory metastasized prostate cancer (Tavtigian et al., 2001). It was demonstrated that deregulated expression of this tissue marker was involved in cancer progression. RT PCR is used for detection of specific markers of prostate tissues in peripheral blood, bone marrow and lymph nodes to detect whether it is a localized tumor or metastasized (Gardner et al., 2002; Naveed et al., 2015).

Nanotechnology as prostate cancer diagnostics
As many cancers are asymptomatic such as prostate cancer so detection of tumor cells is very vital with the timing of detection (Ferrari, 2005). Previously diagnosis techniques (PSA testing) is either non specific or only elevated in advanced stages so various emerging technologies (nanotechnology) promising the early detection of these dangerous disease (Cuenca et al., 2006).

Nanoparticles are used for his purpose which gives specific and sensitive detection of cancerous cells. Quantum dots (QDs) comprise of a core system made up of transition metal with a unique physical property of tumor detection (Smithet al., 2006). Each QDs emit a light of a specific color and many QDs can be used in a single experiment to identify different targets depending on core system and size and QDs emit light ranges from visible to infrared spectrum.

When the functional ligands are involved with the QD shell then the tumor detection is expected (Stroh et al., 2005). Prostate specific membrane antigens (PSMA) are known to be over expressed in prostate cancer cell line so we can conjugate prostate specific membrane antigen (PSMA) antibody to QDs for the detection of prostate cancer cells.

Gold nanoparticles can also be used for tumor detection but depending upon the thickness of gold layers they can absorb and emit light (Loo et al., 2004). A very hopeful nanoparticle practice which is recently undergoing clinical trials with the super paramagnetic monocrystalline iron oxide in humans (Shenet al., 1993). Nano medicines have an excessive role in early cancer recognition, and monitoring of prostate cancer treatment. Schematic pattern of cancer diagnosis is explained in (Fig.2).

Treatment of prostate cancer
Active surveillance
Active surveillance is a method in which treatment is late in patients who are at lower risk of developing cancer, lower risk of rapid progression of disease, agree on close monitoring with this approach (Mohler, 2010). The goal of this approach is to stop over treatment, accept side effects in men who does not require treatment with an ability to cure disease when a true time comes for treatment. Patients are continuously monitored and lifestyle is changed. Patient is candidate of active surveillance if disease is at its initial stage indicating: PSA has not changed much over the time when it is 10 or less or prostate cancer is at T1 or at T2 stage and Gleason score is equal or less than 6.
Any patient who selects active surveillance approach must have the mindset and mental forte to accept the continued presence of tumor cells within body. Approximately 20% men in UCSF’s trial have been engage with this type of treatment. Progress in the treatment options of prostate cancer with their effects is explained in Table 5.

**Radical prostatectomy (Surgery)**
When prostate cancer is limited to prostate an approach to remove prostate gland plus surrounding tissues is adopted with a goal of curing disease completely (D’Amico et al., 1998). Surgery requires two to four hours under general anesthesia. A urinary catheter is placed in penis to channel urine right from the bladder to outside the body. It consists of three main types such as Retro pubic the prostate gland is removed by an incision between the scrotum and anus in the skin and Laparoscopic is a retro pubic prostatectomy, but is performed through five very small (less than 1.0 cm) openings using probes.

Patients who have PSA below 10 or Gleason score 6 or less and confined prostate cancer can be cured 90% over a five or ten-year period. Radical prostatectomy can be an effective treatment for intermediate and even high risk patients but sometimes additional therapy is also required. The most significant side effects of this approach are erectile dysfunction and urine incontinence. Skilled and experience surgeons can reduce the effects and severity of these conditions.

**Radiation therapy**
Radiation Therapy has been used in the last 100 years for the treatment of prostate cancer by eliminating cancer from prostate gland and by avoiding spread of disease (Wilt et al., 2012). There are three diverse types as: External beam radiation therapy (EBRT) by X-rays, EBRT with proton beams and Brachytherapy.

**External Beam Radiation Therapy by X–rays**
First of all, imaging techniques are utilized to locate prostate gland and other surrounding structures by marking them externally or internally. X-Rays Radiation is focused on marked regions from external to the body. Patients are treated five days per week that continue to the period of 7-8 weeks. This approach will be more effective by combining EBRT with brachytherapy. The advances in techniques directed to the development of Three-Dimensional Conformal Radiation Therapy (3D Conformal). In this approach a sophisticated computer program is used for precise target selection from four to nine different directions (Bill-Axelson et al., 2005). It reduces the possibility of affecting other tissues with increasing dose of radiations for better treatment outcomes. The advanced method of 3D Conformal is Intensity Modulated Radiation Therapy (IMRT) in which shape and intensity of different radiations are varied to further minimize damage to other tissues. An even more innovative form of IMRT is Image Guided Radiation Therapy (IGRT) in which duplicate imaging of the prostate is led to alter the directions of the beams for variations in location of the organ. EBRT can induce frequent urination, diarrhea, burning sensation during urination and blood in urine. These symptoms are lessening and disappeared over time and relief is achieved with medication (Gerber et al., 1996).

**External Beam Radiation Therapy with Proton Beams**
This approach uses charged particles to treat cancer cells or 3D conformal beam method combined with X-Ray therapy (Welch and Albertsen, 2009). It is claimed that this technique can reduce damage to surrounding organs but it is not proved. Studies have shown that it is current treatment for localized prostate cancer.

**Brachytherapy**
Both forms of brachytherapy use ionizing radiations to terminate cancer cells. In an endless seed implant minor radioactive pellets are implanted into a prostate (Yamoah, Stone et al., 2011).
It contains radioactive isotopes such as palladium-103, iodine-125 and Cesium 131 and left always in body for continued radiations in a specific duration. While in Temporary Brachytherapy high energy radioactive material iridium-192 is committed to a wire then placed into hollow needles for short periods and withdrawn from prostate. Computer programs are used to determine where and how long radioactive source is present in prostate. Short term side effects are seen after this treatment such as discolored urine or urinary problems and perineal pain. A slight percentage will experience urine incontinence. The success of both external beam radiation therapy and brachytherapy is specified by the extent of decline of the PSA and the lowermost level of PSA that is mentioned as base. It may take one to four years after radiation therapy to approach a base.

**Cryosurgery**
This approach is utilized for the treatment of benign prostate cancer through freezing cancerous cells. Liquid nitrogen containing probes is being inserted into the prostate gland for their manipulation under ultrasound waves. Prostate tissue is destroyed due to this process in localized prostate cancer patients. Maximum effectiveness can be obtained by freezing the entire prostate gland with impacts on side nerve bundles. But this strategy can be ended with urinary incontinence. Better outcomes can be achieved through improvements in practice of cryosurgery and its technology.

**Hormonal agents and prostate cancer therapy**
Prostate cancer is initially being treated with Hormonal therapy. Hormones such as androgens and androgen receptors known as AR are responsible for the carcinogenesis as well as for regular prostate growth. Androgen receptors are expressed in castration resistant prostate cancers tissues (CRPC) and show response to lower concentration of androgens and alteration in AR hormones either by amplification and truncation may causes differential ligand and antagonist specificity and affinity. As previous studies show that there is a low level of testosterone (the main male hormone) in prostate cancer cells (Gururajan et al., 2012).

In this treatment, there is a reduction in androgen level may slow down and stop the cancer growth because some cancers stages form their own testosterone.

Two types of methodologies are used that are known as old and advanced therapies that include orchiectomy or estrogen patches and luteinizing hormone releasing hormone (LHRH) respectively. In orchiectomy, the testes are removed surgically that are the main source of androgens in the men. This surgical removal of testes is an effective technique but it is permanent. So the use of estrogen compounds another approach such as diethylstilbestrol (DES) that reduces the level of testosterone. By using this estrogen compounds there are a number of side effects such as breast enlargement, increase body weight that leads to increase the risk of heart attacks or heart stocks. To overcome these side effects estrogen patches are developed for prostate cancer patients.

Now-a-days hormonal therapy is the amalgamation of two main medications. First medication is the luteinizing hormone-releasing hormone (LHRH) agonist or analogue. This technique causes hormonal control system to be modified and shut down the testosterone production from testes. The medications include the time release preparations of LHRH medications that is inserted under the skin or injected into the muscle for three to four months. For example there are some common LHRH agonists available named as Zoldex, Trelstar and Lupron.

LHRH sometimes cause some “flare” or increase in testosterone level that is over by a newer medication, Degarelix approved by FDA recently, it is GnRH receptors antagonists. They work by directly blocking the receptors that initiates the production of testosterone. During the hormonal therapy time, the level of testosterone in the serum should be checked, approximately 20-50 ng/dl or lower than this value. The second medication is non-steroidal anti androgen that ultimately block the ability of using the androgens by the androgen receptors that are still producing by adrenal glands e.g. Eulexin, Nilandron.
Now days, this combination of medications are used efficiently such as total androgen blockade (TAB) or combined androgen blockade (CAB).

This advanced approach inhibits the enzymes important for conversion of other androgens or other hormones to dihydrotestosterone (DHT), which is required for the stimulation of both cancerous and normal cell growth (Roach and Thomas 2012).

**Side effects related to hormonal therapy**

There are certain side effects that are related to the hormonal therapy that is mentioned above that includes the erectile dysfunction and decrease in the sexual desire or both. Hot flashes are another side effect that disappears gradually with time. Breast tissue growth in the form of breast tenderness is also a major side effect. Osteoporosis is a direct effect of lowered level of testosterone in which the thinning of bones occurs. A medication known as bisphosphates is the effective remedy of the osteoporosis if there is highly less bone density than an oral dose of Fosamax. Relatively less frequent side effects are the nausea and diarrhea as well as elevated blood pressure or abnormal liver function that is caused by the heavy dose of anti-androgens.

**Chemotherapeutics to treat prostate cancer**

A novel cytotoxic chemotherapeutic is the Cabazitaxel is act as a semisynthetic taxane that have activity in vitro to overcome the effect of multidrug-resistant prostate cancer cell lines (Mitaet al., 2009). Who failed docetaxel-based chemotherapy, these patients undergoes a preclinical observation that leads to randomized trials with CRPC.

If the patients had the PSA progression, they were eligible for study new lesions on bone scan. The patients that are in the phase3 trail are injected with cabazitaxel for every 3 weeks.

The life expectancy increases 15 months as compared to 12 months in the individuals that use other medication such as mitoxantrone (de Bono et al., 2010).

**Anti-apoptotic chemical agents and prostate cancer**

In some cases the regression of cancer still required an active apoptotic process.

This is a unique feature that the androgen receptors (AR) blocking have the ability to induce apoptosis. In this instant a therapy for prostate cancers demand reversal of the apoptotic avoidance processes(July et al., 2002). Androgens related or non-related advanced therapies are developed that by pass the apoptotic resistance pathways that are responsible for drug resistance. Several anti-sense oligo nucleotides targeting the numerous anti apoptotic genes such as clusterin, MDM2, Bcl-2, bcl-X, c-RAF and insulin like growth factors binding proteins. The expression of clusterin is upregulated and increases with Gleason score and after the blockade of AR (Miyake et al., 2000). It is actually an investigational anti-sense chemical known as custirsen that down regulates the high expression of clusterin and gradually elevates the level of apoptotic level of prostate cancer cells (Chiet et al., 2008). In the patients with CRPC, the elevated level of docetaxel or prednisone or OGX-011/ is deal in combination with medication of docetaxel or prednisone in prostate cells (Chiet et al., 2010).

**Side effects of chemotherapy**

There are numerous side effects including the fatigue and nausea after that the dosage of chemotherapeutic drug lowers to minimal level. The risk of lower blood pressure and lower blood count is the main side effects that lead to weight loss and ultimately hair loss.

**Tumor stromal fibroblastic microenvironments in prostate cancer**

This is a unique area for the treatment of cancerous patients of prostate tissue. Regular prostate development and neoplastic prostate progression is regulated by tumor-stroma interactions demonstrating the role of stroma physical structures in prostate carcinogenesis and progression (Sung and Chung, 2002). Atissue recombination technique had been utilized to check the response of AR-negative and AR-positive mice demonstrated that UGM
isolated from AR-negative testicular feminized mice were unable to trigger prostate cyto differentiation, morphogenesis and AR-positive wild-type mice give positive signals for differentiation and growth. Results of this study on mice showing androgens biochemical signaling from the stroma physical structures are essential for the differentiation of normal prostate epithelium cells (Chung, 1991). The critical role of prostate stromal fibroblasts promotes the cancer progression by using the cell recombination models (Camps et al., 1990).

Particularly, the progression and developmental stages of prostate tumor from androgen-independent to androgen-dependent state resulted through cellular interactions between organ specific stromal physical structures including bone stromal or prostate cells and prostate cancer cells in mice in lab (Chung, 1994). The study emphasized signaling between tumor cells and stromal micro environment in development of cell lines of prostate cells (Thalmann et al., 2010).

Stromal cells has ability to surround the cancerous cells that are directed to modulate the migration, invasiveness, cell development and metastasis resulting in the establishment of tumor linked fibroblast (CAF) that have functionally distinct morphology compared to normal stromal cells that shows the reciprocal cellular interactions between CAF or stromal fibroblast and cancerous cells (Rhee et al., 2001).

Human prostate tumor cells (LNCaP) when co-cultured with micro carrier beads that are already inserted in bone or human boneprostate stromal cells and prostate gland leads to the stable and non-randomized genotypic/phenotypic modifications in both tumor/stromal cells and LNCP human cells derived from these growth environment gained the ability to metastasized that ultimately increases the level of chemokines such as CCL5, CXCL5 or transcription factors like HIF-1α and brain derived neurotropic factor (BDNF). Several studies shows that a number of soluble stromal inducers such as TGF-β, IL-6 interacting with the receptors of prostate cancer cells, IGF-1, and HGF/SF helps to migrate the cancerous cells to other parts of the body. So these are the target for therapies to reduce the invasiveness or ultimately block the spreading of tumor cells (Chunget al., 2005).

**Immune microenvironment as a target for prostate cancer therapy**

Destruction of immune system of the body is also a hallmark of cancer and act as a critical regulator of cancer progression. In the previous study, it is hypothesized that the killing of cancerous cells in the presence of immune therapy is highly adaptive cure of prostate tumor.

The spreading of tumors from localized state to metastasis state where low-grade metastasis induces interaction of host defense system with the cancerous cells. In vitro analysis use mice lacking B and T cells known as SCID or mice lacking B, T and NK cells known as non-SCID and mice which lack T cells known as at hemic immune deficient mice (Andreuet al., 2010). The result shows that the immune-deficient mice have high level of growth that facilitating the preclinical target study of cancer therapies. Recent studies have uncover the fact that in majority of cancers are infiltrated by the immune cells further provoke tumor development (Ammirante et al., 2010).

**Angiogenesis and prostate cancer therapy**

Angiogenesis is a regulator of dissemination and growth of prostate cancer cells. In this process a complex that interacts with the vascular growth factor VEGF, integrin and matrix metalloproteinase causes its initiation. In cancer, inhibition of this protein complex can ultimately block tumor development and metastasis. Previous literature evidenced that VEGF level within CRPC cells act as a prognostic biomarker and prevents cancerous cells survival in patients (Stadler et al., 2004). Bevacizumab is an antibody that blocks the interaction of VEGF-A to the VEGF-R and inhibits the angiogenesis of the cancerous cells also a potential target for prostate cancer therapy (Ferrara, 2005).
Gene therapy

Gene Therapy is an important approach for the managing of genes, which are involved in development, and resistance of disease. In the last few years’ advancements in fields of molecular biology, cell biology, and gene delivery systems either viral or non-viral, therapeutic agents and tissue specific DNA promoters directed to the development of in gene therapy approach (Shirakawa et al., 2000). This development is a new critical step in treatment of metastasized resistant prostate cancer patients (Naveed et al., 2016a). Current available strategies are citoreductive approaches. Prostate gland is an appropriate tissue for gene therapy due to unique antigens present in it such as PSA, PSMA, human glandular kalikrein 2 (Mabjeesh et al., 2002). Clinical trials directed that gene therapy is a save option for the treatment of prostate cancer but constant gene therapeutics still need to be verified.

Recent studies proved that androgen independent prostate cancer cells apoptosis has been examined by gene therapy (Steiner et al., 2002). Gene therapy allows targeting specifically the molecular events involved in prostate cancer progression. Specific genes are over expressed or less expressed at the level of DNA and proteins at RNA level are new therapeutic targets for gene therapy (Naveed et al., 2016b). As an example PAR-4 gene which induces apoptosis of cancer cells is used as therapeutic target for cancer (Ast, 2003).

The great challenge is to contact and eradicate metastatic cells in treatment of advanced prostate cancer (Sadeghi and Hitt, 2005). Therefore, effective approaches are required to target primary and distant tumor cells. One of the known approaches is the progress of precise gene promoter regulatory sequences with expression at reserved sites (Naveed et al., 2014). Efforts have taken to develop a prostate specific tissue promoter such as osteocalcin promoter that will target osseous metastases (lethal form of disease) (Kubo et al., 2003). A new approach is a development of specific chimeric constructs with heterologously expressed TRLP protein leading to poor survival of prostate cancer cells by inducing apoptosis (Zhanget al., 2003).

The progress of improved delivery systems is essential for the effective gene therapy strategies (Gardner et al., 2002). The vectors used to transfer genes in the cells have both benefits and drawbacks such as adenovirus can transfer large genetic construct with high productivity regardless of cell cycle consideration without genotoxicity. But this vector has temporary expression of genetic construct and most persons have native immunity which limits systematic administration. Oncolytic herpes viral vectors and lenti virus vectors with tissue specific promoters are also used for this purpose. Such vectors have also been designed that will definitely and precisely target prostate cancer cells with the combination of abilities of viral genomes and cancer cells factors.

Another question is which kind of DNA is transferred during gene therapy, its answer lies in objective of researcher. For example, herpes simplex virus encodes thymidine kinase which can convert clinically permitted pro drugs into a powerful intracellular toxin interfering DNA replication in suicide gene therapy.

This strategy will kill a cell that is cancerous and limits the cell ability to infect the surrounding cells. Another concept arose by targeting hypoxia response sorting of prostate cells elimination of tumor cells can be achieved in case of advanced prostate cancer patients (Anastasiadis et al., 2003).

Gene therapy can also be combined with the straight therapies such as chemotherapy and radiations. Clinical trials have been initiated for chemo-gene-therapy and radio-gene-therapy. RNA interference (RNAi) approach can be used as an innovative radiation/chemotherapy sensitizing agent to make cancerous cells more sensitive by DNA dependent protein kinase catalytic subunit (DNA-PK) and targeting signaling or repair proteins (ATM, ATR) (Collis, Swartz et al., 2003).

A research group demonstrated sKDR gene expression can be targeted by employing radiation inducible promoter with recombinant adenovirus
AdVEGF-sKDR will effectively inhibit prostate cancer growth is the example of combination of gene therapy and radiotherapy (Kaliberov et al., 2005).

**miRNAs as therapeutic agents for prostate cancer therapy**

MicroRNAs are single-stranded and small non-coding RNAs comprising of 18–22 nucleotides which cause either gene silencing by decreasing mRNA solidity or by inhibition of translation (Chenet et al., 2008). MiRNA plays important role in many progressions such as cell differentiation, cell development, cell proliferation, cell cycle control, apoptosis and DNA damage metabolism (Bartel, 2004).

Chromosomal rearrangements influence the expression of miRNA. MiRNA's expression profiling in human cancers has rising importance due to its applications in analysis, staging and treatment of various cancer types. miRNAs are being observed as new molecular biomarkers for finding and estimation of treatment and diagnosis (Calin and Croce, 2006). In cancer patients the level of miRNAs is examined to testify whether these will be useful as a therapeutic agent in cancers or not.

Many MicroRNAs act as tumor suppressor agents and by increasing their expression levels we can inhibit metastasized prostate cancer. Aberrant expression of these MicroRNAs is examined in many cancer patients (Chen, 2005). Recently a study predicts MicroRNA 124 has ability to act tumor suppressor agent in prostate cancer. AR signaling pathway is vital in prostate cancer and it is found that MicroRNA 124 correlates with AR signaling pathway with its antitumor activity (Chuet et al., 2015).

A negative feedback twist is existed between AR expression and MicroRNA 124 and AR expression can be suppressed by MicroRNA 124. So if we over express this MicroRNA (biomarker) in prostate cancer patients, it will inhibit carcinogenesis and metastases resistance by provoking a positive step towards treatment. The major breakthrough and advances in biomarker development for prostate cancer is mentioned in (Fig. 3).

Some problems have been raised during the utilization of miRNA as: There is no recognized endogenous (‘housekeeping’) miRNA control to regulate miRNA levels in body fluids, the process responsible for the release of miRNAs into body fluids and its functional role is not well understood and there have been variations in the analysis results of some miRNAs in numerous cancers. In future high advance technologies can solve this problem (Kim and Kim, 2013).

**Nanotechnology in the rapeutics**

Nanotechnology has become the slogan of the science community subsequently last two decades (LaRocque et al., 2009). One subset of nanotechnology is Nano medicines which has unique properties of small nano particles for precise treatment of cancerous cells leaving the vigorous cells undamaged.

These particles of smaller size than the conventional therapeutic agents providing an ease to reach places which are inaccessible in past. Two main problems can be solved in treatment of cancer by nano medicines, first early detection of cancer with greater chances of successful treatment and second reduce rate of toxicity to the surrounding cells (Kwon, 2003). Maximum of the chemotherapeutic agents are poorly soluble in water and expressed by toxic diluters. Recent study demonstrated that this challenge can be prevented by integrating anticancer drugs in hydrophobic nano carriers such as Doxil and Abraxane (Sparreboom et al., 2005). Abraxane has eliminated the allergic reaction reaction related with this solvent while Doxil has shown significant anti-cancer value (Gradishar et al., 2005).

A novel strategy is required for the delivery of nanomedicines to specific targets where these agents have greatest effect on cancerous cells (Gao et al., 2005). For this purpose, tumor specific ligands are used. Scientists have been successful in creating nanoparticle with the use of copolymers. Copolymer is filled with chemotherapeutic agents and layered with polyethylene glycol to escape immune system. These copolymers attached with PSMA in prostate cancer and reduce tumor progression. Each component of this nanomedicine is approved by FDA (Farokhzad et al., 2006).
Extensive research has been carried on Gold nano shells with near infrared light for their therapeutic role in the handling of cancer (Hirsch et al., 2003). In this approach nanoshells are targeted at tumor sites and exposed with an 820-nm, 4 W/cm² light pulses allowing change in temperature for the destruction of tumor cells leaving surrounding healthy cells intact (El-Sayedet al., 2006). A next step is to binds these shells with ligands for the creation of tumor seeking particle that can target oncogenes in solid tumors (Sengupta et al., 2005).

Further research has been directed to integrating various compounds in an only one particle (Sengupta et al., 2005). Theory relates that various compounds are present in a single particle they may have synergetic effect on tumor cells in a precise direction by their release as an immediate succession (Jain, 2001). For this purpose, chemotherapeutic agent such as doxorubicin is combined with anti-angiogenesis agent such as combretastatin and introduced at a tumor site; anti-angiogenesis lipid layer will be degraded after entering in a tumor cell and causing release of a drug by vascular collapse.

This approach proved effective in mouse replicas and similar efficiency is expected for humans.

Recently scientist is steeping toward a new dimension in nanoparticle tumor treatment arena. Concept trials have been conducted on drug carrying phages as a preliminary proof (Yacoby et al., 2007). Filamentous phages have been proven effective delivery system for substances such as antibiotics.

Almost 10000 drug molecules can be loaded per phage protein coat by genetic engineering. As this system is promising for the distribution of antibiotics, the researchers are trying to check its efficacy for nanomedicines (Baret et al., 2008). The researchers are also changing the phage coat in demand to decrease the host-immune response, optimize pharmacokinetics and increase tumor selectivity.

Conclusion

In this review we have summarized epidemiology of prostate cancer over the world as a threat of most prevalent disease of specific age standardized men and methods utilized for in vivo diagnostics ortherapeutics & in vitro diagnostics of prostate cancer. Earlier methods have their own series of unwanted effects that are hazardous for overall health of cancer patients.

The recent developments and breakthroughs in diagnostic markers, chemotherapeutic and immunotherapeutic agents (cabazitaxel [chemotherapy] and sipuleucel-T [immunotherapy]), targeted gene therapy and nanotechnology brought a hope for remedy that is operative and harmless. Although there is still sufficient work to be done but some very promising new advanced treatment approaches is in the works.

Primary detection of prostate cancer is a very key aspect in cancer treatment and recently significant improvements have been seen largely due to advances in the top-down and bottom-up Nano biotechnology. But still future studies and clinical practices are required to eliminate sufferings and deaths from cancers. So for this purpose researchers are giving their best efforts to develop new imaging techniques to identify tumor cells, new diagnostic markers in blood, moving tumor cells and bone marrow, to determine treatment efficacy of individual chemotherapeutic agents, immunotherapeutic agents and radiation therapy.

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