

## **Role of Pomegranate in Regulation of Genes and Mechanisms of Prostate Cancer Metastasis: A Review**

**Divya Sharad Kasarle**

Scientific Assistant, DSK Nutrition Research Centre, pvt. Ltd,  
Mumbai, 400092, Maharashtra  
*dkasarle@gmail.com*

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**Abstract:** *The most common cancer occurring in men after skincancer is the prostate cancer. Though most of the men suffering with prostate cancer do not die even after five years of being diagnosed, the disease can bring out drastic changes in their life as it progresses further. Stubborn prostate cancer with aggressive tumours can metastasize to the other parts of the body and in later stages they may stop responding to the therapeutic drugs. Pomegranate fruit (Punicagranatum, also known as Anar) has been used since ancient times for its wide range of health benefits. Pomegranate juice (abbreviated as PJ) is rich in polyphenols that show potential anti-cancer properties as revealed from various research studies. This review explains the importance of Pomegranate components (Luteolin, Punicalagins and Ellagic acid) in regulating the expression of genes involved in prostate cancer and its potential role in controlling the pathways associated with prostate cancer metastasis and tumourigenesis. Further, the studies have demonstrated that pomegranate juice components interfere with the biochemical and cellular processes involved in tumour progression and angiogenesis. Pomegranate has proved its potential health benefits hence it has been titled as the “Jewel of fruits”*

**Keywords:** *Prostate Cancer, genes, metastasis, Pomegranate, anti-cancer.*

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### **1. INTRODUCTION**

Prostate cancer is a type of carcinoma (mostly adenocarcinoma) in prostate gland (walnut shaped gland) of male reproductive system. Mostly prostate cancers develop from the cells that have the ability to produce prostate fluid, which is an important fluid involved in nourishment of sperm cells in semen [1]. It has been found that some abnormal cells of the epithelial lining in the prostate gland grow and divide more rapidly because of mutations in the genetic material, hence forming aggressive tumorous. Common distant sites of prostate adenocarcinoma are bone tissue, lymph nodes, lungs, liver and brain. [3] Family history, old age and obesity mainly contribute to the risk of prostate cancer [2].

In Phyto-medicine system, Pomegranate has been extensively used as a source of prevention and treatment of prostate cancer. Pomegranate juice has potential phytonutrients which have the ability to alter the expression of genes and signalling molecules involved in cell cycle control, angiogenesis, cancer progression and metastasis [3].

Dose dependent consumption of pomegranate juice can counteract with free radicals formed due to oxidative stress and genotoxicity and reduce the levels of Prostate Specific Antigen (PSA, which is elevated in men with prostate cancer) [3].

### **2. MATERIALS AND METHODS**

Literature survey was initiated in July 2015 using “Pomegranate” and “Prostate Cancer” as key words in PubMed, PubChem, Mayo Clinic, Genetics Home Reference, Natural News and National Centre for Biotechnology Information (NCBI) websites, via computerized search to obtain both research and review articles. An extensive review of the literature was carried out and the data was collected. Google search engine was used to refer journal articles, news articles, and images, relevant pages from books, recent case studies, advertisements and magazine reviews to update information for this paper. All the abstracts and full text articles in pdf format were downloaded, extensively studied and analysed. Images of cellular pathways, mechanisms of metastasis, and ethno medicinal properties of pomegranate, cancer nutrition and health charts were examined in detail and PowerPoint presentations were made for understanding the theory behind the data. Cancer Info- graphic highlights, research videos, research updates, cancer facts and figures, global atlas of cancer, clinical and statistical data

pertaining to prostate cancer was obtained from American Cancer Society website. The statistical data which was available in the form of graphs and pie charts was screened thoroughly for obtaining latest information in order to include it in this review. Using the above information, computerized images and chemical structures were constructed using in-built tools in the computer such as Paint Brush and Snipping tools. Various computer applications such as Photoshop, Pixlr Autodesk, collage maker, and Windows photo viewer were also used for refining the images and converting them in JPEG format for obtaining clarity of the images. All the images in this review are self-generated by the author.

### 3. REVIEW OF LITERATURE

As per the estimates of American Cancer Society, about one man in seven have chances to be diagnosed with Prostate cancer and about one man in thirty eight will die because of prostate cancer. [1] Prostate cancer generally occurs in men with the age of 60 and above (rarely below 40). Many signalling pathways are deregulated either because of overexpression or under expression of genes, during the prostate cancer progression. [1]

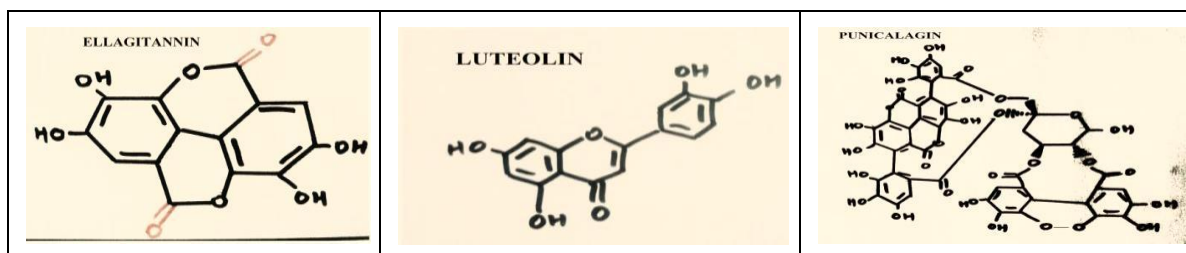
#### 3.1. Metastasis of Prostate Cancer

Metastasis is a process in which a cancer cell or a tumour cell leaves the site of primary tumour (in this case the prostate gland), travels to the distant tissues or organs via blood circulation, survives in the foreign environment and gets settled in the distant organ or tissue to develop a secondary tumour. It leads to tumour progression and tumour induced angiogenesis by formation of new blood vessels [4]. Metastasis of prostate cancer involves five major steps:

- Migration and invasion of tumour cells and metastasis promoted by tumour infiltrating B and T lymphocytes in prostate. [4]
- Neoplastic cells undergo metaplasia and rapid cell division and are released into the blood circulation in the form of single cells or clumps. [4]
- These cells manage to survive in circulation and are free to circulate and travel to nearby and distant organs or tissues or lymph nodes. [4]
- Arrest and diapedesis of these cells takes place in the capillaries of the distant organs or tissues. [4]
- Lymphatic or blood vessel walls are penetrated and lead to the growth of the promulgated tumour cells. [4]

#### 3.2. Effective Components of Pomegranate Juice

Pomegranate juice is rich in natural modulators of gene expression with high antioxidant, anti-atherogenic, anti-hypertensive, anti-tumour or anti-cancerous and anti-inflammatory capacity. Pomegranate juice contains components such as luteolin, Ellagitannins, punicalagins, anthocyanins, hydrolysable tannins and sugars. [8] Ellagitannins are hydrolysed to ellagic acid in our body. They have ability to disrupt mitochondrial membrane potential [18]. Luteolin, ellagic acid and punicalagins together inhibit the growth of hormone dependent prostate cancer cells by promoting dehydration of androgen receptor, thereby reducing the levels of androgen. [9] They can also inhibit hormone refractory cells. [9] They can also increase the levels of Protein Kinase C and lead to anti-proliferation and apoptosis. Anthocyanins show antioxidant activity by scavenging reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. (Aaron Goertz et.al.) [18] *Table 1. Chemical structures of pomegranate polyphenols: Ellagitannin, Luteolin and Punicalagin [18]*



#### 3.3. Mechanisms which can be Controlled by Pomegranate Juice

##### a. Cell Cycle Arrest:

Pomegranate juice has the ability to arrest cell cycle. (Gunter Seelinger et.al.) [27]

**Table2.** *Effect of PJ on cell cycle regulatory molecules*

Cell cycle regulatory molecules	Role	Effect of PJ
Cyclin D, Cyclin E , cdk 2, 4, 6	G1/S Phase transition	Down-regulated [27]
P 21 and P 27	cdk inhibitors	Up-regulated [27]

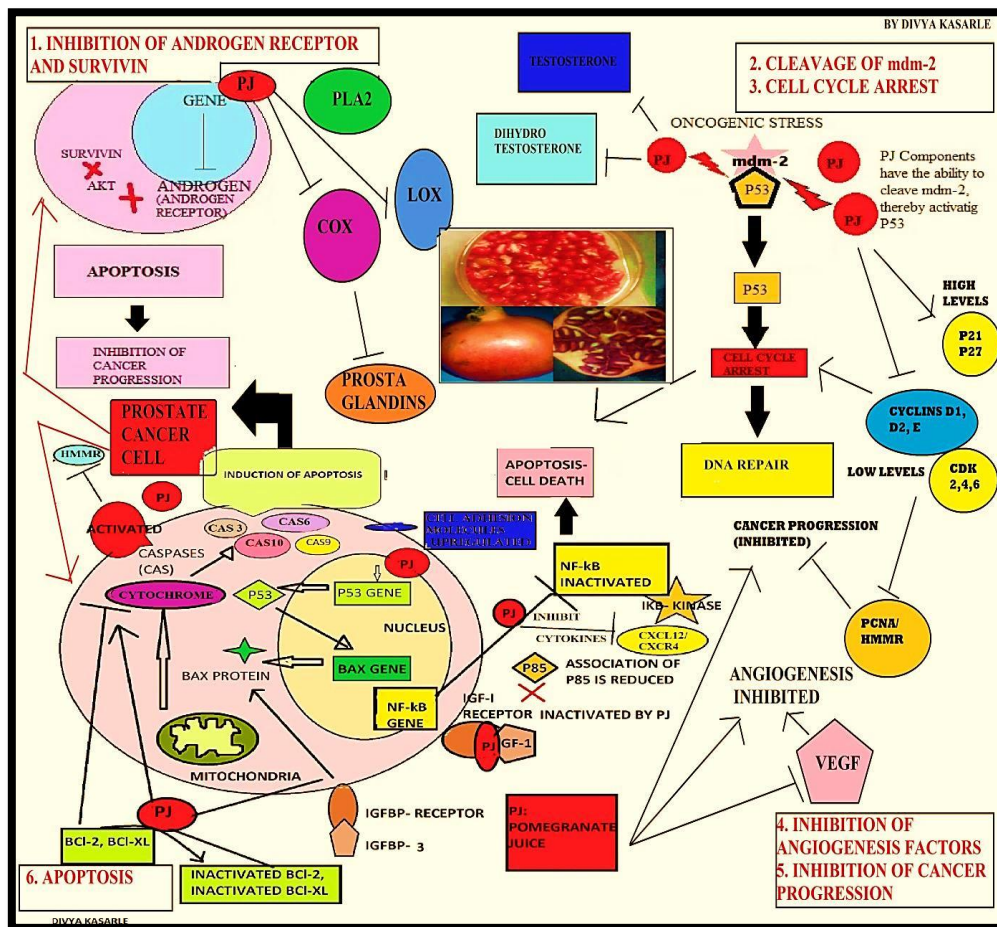
- b. Cell proliferation and migration:** Cell proliferation molecules such as PCNA are inhibited by pomegranate juice. Cell migration allows the tumour cells to migrate to distant sites. Cell migration molecules HMMR, Collagen A1, Anillin and Nexillin help them to migrate to various parts of the body. Hence these molecules are down regulated by pomegranate phytonutrients. [27]
- c. Angiogenesis:** Hypoxic conditions have very low oxygen levels, thus stimulating angiogenesis. Hypoxia inducible factor 1 (HIF1) and vascular endothelial growth factor (VEGF) get activated in hypoxic conditions. (Jianming Lu et.al.) [25] Pomegranate Ellagic acidinhibit HIF1 and VEGFR2 Kinase activity, thereby regulating STAT3 mediated signalling. (Eleonara Turrini et.al) [15], (Maryam R. Sartippour et.al. and Seeram et.al.) [26] Pomegranate juice components inhibit angiogenesis by interfering with cell proliferation, migration, tube formation and sprout formation. [26]

**3.4. Genes and Pathways Controlled by Pomegranate**

- a. P53 Pathway:** P53 is a tumour suppressor which regulates the cell cycle when it is in its activated state. It is synthesized by TP53 gene. The role of P53 is to enhance apoptosis, autophagy, cell cycle arrest and repair. (Zaynab S. Abdul-Gany et.al.) [24] However, due to oncogenic or genotoxic stress, the p53 gene is inactivated because oncogene product recruits mdm2 molecule which binds to p53. Hence in such conditions, pomegranate juice can activate the inactivated p53 (Zaynab S. Abdul-Gany et.al.) [24] by cleaving mdm2-p53 complex, thereby releasing activated p53 to carry out its main function i.e. DNA Repair. (Aaron Goertz et.al.) [18], [22], [23], [24]
- b. Apoptotic pathway:** Apoptosis (cell death) requires proper functioning of cysteine proteases and death receptors. [32] Cysteine proteases such as Caspases 3,8,9 and 10 are activated by components in pomegranate juice. [23], [27], [28], [29], [30]. In particular, caspase-3 is an effector caspase which plays an important role in death pathways. [33] The ratio of Bax/Bcl-2 is an important factor for determining the chances of a cell to undergo apoptosis. [33] An increase in Bax relative to Bcl-2 activates capsase 3 and thus releases cytochromes capable of inducing apoptosis mediated by mitochondria [33]. Bax (apoptotic activator) and Bak (Homolog of Bcl2 but functions differently) are up- regulated [27] whereas Bcl2 (anti apoptotic factor) and Bcl XL (B cell lymphoma extra large) are down regulated by components of pomegranate juice [27]
- c. Androgen biosynthesis pathway:** Androgen and androgen receptors are important regulators for progression of prostate cancer and are involved in prostate cancer metastasis. [11], [12], [13] Some studies have revealed that pomegranate juice can control the levels of androgen. [14] Ellagic acid and punicalagins reduce the expression of genes which are responsible for synthesizing androgens. These genes are HSD3B2 (3beta-hydroxysteroid dehydrogenase type 2), AKR1C3 (aldo-ketoreductase family 1 member C3), 5-  $\alpha$ - reductase type I gene and 3-  $\beta$ - hydroxyl-dehydrogenase type II gene. [14], [15]
- d. Survivin Pathway:** Survivinprotein level is high in case of prostate cancer. (EleonaraTurrini et.al) [15] This protein is responsible for inhibiting apoptosis and promoting cell survival, thus helping in prostate cancer progression. (EleonaraTurrini et.al) [15] Pomegranate extract reduces the levels of survivin protein by blocking the enzyme which synthesizes survivin protein and inhibits Akt phosphorylation [16].
- e. Steroid Biosynthesis Pathway:**Two types of variations are found in SRD5A2 gene which are associated with Prostate cancer i.e. Val89Leu (V89L) and Ala49Thr (A49T). [7] These mutations have shown to increase the activity of genes which synthesize testosterone and dihydro-testosterone hormones which play an important role in male sexual characteristics. [5], [6], [7]Pomegranate polyphenols can also reduce the levels of testosterone and dihydro-testosterone excessively generated through steroid biosynthesis, (Lei Wang et.al. and Manuela Martins-Green et.al.) [17] which in turn can reduce the levels of PSA.
- f. Canonical Signalling Pathway:** This pathway is an inflammatory signalling pathway in primary prostate cancer. Interleukins, P450 and CCL5 are down regulated by pomegranate juice

components such as punicalagins. [18] Some studies revealed that Chemokines, which are known as inflammatory cytokines CXCL12/CXCR4 were inhibited by luteolin, Ellagic acid and punicic acid present in pomegranate juice. They also activated c-jun N-terminal kinase (JNK), p 38 and extracellular regulated kinase (Lei Wang et.al. and Manuela Martins-Green et.al.) [17], (Aaron Goertz et.al.) [18], [20], [21], [27] NF-k B activation is important for protecting the cells from Tumour Necrosis Factor (TNF) induced apoptosis. (Aaron Goertz et.al.) [18] It also increases the expression of genes synthesizing inflammatory cytokines. (Aaron Goertz et.al.) [18]Luteolin in pomegranate juice has been shown to increase the expression of Ikb kinase, which inhibited NF-k B. [19]

- g. Cell adhesion pathway:** Degradation of the Ikb kinase can result in the nuclear translocation of NF-kB inflammatory molecule (P. Pushpangadan et. al.) [31] . Cell adhesion molecules include ICAM, VCAM, E-cadherin etc. They bind to extra cellular matrix. Cell adhesion molecules such as E-cadherin, and ICAM-1 are upregulated by pomegranate juice. (Gunter Seelinger et.al.) [27]
- h. Arachidonic Acid Pathway:** Arachidonic acid is membrane based molecule and is catalysed by cyclooxygenases (COX-1 and COX-2), resulting in the formation of prostaglandinG2 (PGG2) which undergoes a reduction reaction to form PGH2.(P. Pushpangadan et.al.) [31]COX mediates production of Prostaglandins, which are central controllers of inflammation. PLA<sub>2</sub>, COX and LOX (Phospholipase 2, cyclooxygenase and lipoxygenase respectively) are down regulated by Ellagic acid in pomegranate juice. [17]
- i. IGF-1 pathway:** The IGF system (IGF-I, IGF-II, IGF-binding protein, and IGF-IR) performs an important role in the growth of various cancer cells including prostate cancer cells. (Ashok Kumar Pandurangan et.al.) [33] Some studies revealed the following effects of pomegranate juice components in regulating the IGF-1 pathway: Reduction of the IGF-1-induced tyrosine phosphorylation of IGF-1R and the minimized association of p85 with IGF-1R. (Ashok Kumar Pandurangan et. al.) [33]



**Figure1.** Summary of the role of pomegranate juice in regulating the genes and pathways involved in prostate cancer metastasis and tumour progression: The above figure shows cascade of reactions occurring in a cancer cell and the inhibitory action of pomegranate juice (abbreviated as PJ).

#### **4. RESULTS AND DISCUSSIONS**

Various studies both in vitro and in vivo demonstrated the ability of pomegranate and its healing potential to contrast various biochemical processes involved in cancer progression and metastasis. Chemoprevention and delay of cancer progression can be achieved by naturally derived compounds such as pomegranate polyphenols. Different components of pomegranate juice and extract function differently by simultaneously attacking various mechanisms involved in cancer metastasis. This is an advantage of pomegranate as it has proven its efficacy in tackling complexities of prostate cancer and various other types of cancers by deregulating multiple signalling pathways involved in cancer metastasis.

- **Clinical Studies:** Studies of potential anti-cancer properties of pomegranate in vitro and in vivo were carried out by using standardized laboratory procedures by researchers worldwide, which include molecular assays (DNA extraction, Inter simple sequence repeat studies, RAPD-PCR analysis and electrophoresis), cytogenetic investigations, genotoxicity evaluation and study of chromosomal aberrations, Sperm cells studies, biochemical and statistical assays. [26], [27] Cell cultures, animal models were majorly used by researchers. Very few clinical trials on human volunteers have been reported.
  - i. **Cell cultures:** In a study carried out by Sartippour et.al. And colleagues, incubation of prostate cancer cells and endothelial cells of umbilical vein in vitro, with standardized Ellagic acid inhibited cell proliferation under low oxygen levels. [26]
  - ii. **Animal models:** Biological test animals such as albino mice, nude mice and Sprague Dawley rats were used as animal models by researchers to assess and examine the potential health benefits and risks associated with pomegranate juice and pomegranate extracts. [34], [35], [36] Some studies revealed that pre-treatment of male rats with pomegranate juice and extract decreased the genotoxicity induced by chemical mutagens and carcinogens (Abdou H.S., Salah et.al.) [34]. Significant anti-proliferative properties of pomegranate were revealed in one of the studies involving PC-3 invasion of cell, through Matrigel and growth inhibition of PC-3 xenografts in athymic nude mice (Albrecht M et.al) [35], (Lansky EP et.al.) [36].
  - iii. **Human volunteers:** The first clinical trial of pomegranate in patients with prostate cancer was conducted by Allan. J. Pantuck et.al. and his colleagues in Los Angeles. They conducted a study over a period of 13 months which included phase II clinical trials on patients suffering from recurrent prostate cancer. They reported that the prolongation of PSA doubling time was statistically significant due to the action of pomegranate juice and extract. (Allan. J. Pantuck et.al.) [37] In another clinical study, men with increased levels of PSA consumed 8 ounces of pomegranate juice daily and were subjected to clinical trials. The study revealed that PSA levels declined in 35%-50% of the patients suffering from recurrent prostate cancer. [38] Pomegranate juice or supplements might interfere with ACE (Angiotensin converting enzyme) inhibitors and other blood pressure drugs. [39]

Cancer prevention studies over a large group of prostate cancer free people are also currently being carried out by researchers at American Cancer Society, for improving the lifesaving technology and research. They are collecting survey data and bio specimens in order to study and monitor their health profiles [1]

#### **5. CONCLUSION**

It is very difficult to prevent most cases of prostate cancer since the exact cause of prostate cancer is not yet known and the risk factors such as family history and age cannot be controlled. However the risk can be lowered by managing body weight, hygiene, physical activity and diet. Though it is safe to consume, people taking some blood pressure medications may contact their health adviser prior to consuming it. Pomegranate juice can be used as a complementary therapy along with the medical treatment because there is neither any evidence nor approvals of FDA that pomegranate can alone cure cancer. However there are evidences that it can control the possible mechanisms involved in cancer progression. Very few studies pertaining to the anti-cancer property of pomegranate have been conducted so far and hardly any clinical trials have been conducted on people. However there is growing laboratory research on anti-cancer activity of pomegranate which is promising. Still efficient clinical research in this subject is needed to understand the pharmacokinetics, pharmacogenomics,

bioavailability and mode of action of its active constituents and to standardize the treatment procedures in order to fully exploit its therapeutic potential.

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#### **AUTHOR'S BIOGRAPHY**



**Divya Sharad Kasarle**, She has done Master of Science with specialization in genetics, in Department of Genetics, Osmania University, Hyderabad, India. She has been former Project Fellow (Cytogenetics) at Institute of Genetics and Hospital for Genetic Diseases, Hyderabad. She is currently working as a Scientific Assistant in DSK Nutrition Research Centre, pvt. Ltd, Mumbai, Maharashtra, India.