

# Prostate cancer cells produce exosomes modulating metastasis to the bones

## Abstract

Prostate cancer metastasis to the bone is a frightening cause of death world-wide. Patients do not live a functional and active life before they eventually die. The major reason for prostate cancer metastasis is the use of poor bio markers such as Prostate-specific antigen (PSA) and histopathological grading to diagnose prostate cancer incorrectly, indicating that a patient who has a stage four prostate has a benign tumor. New and effective markers for prostate cancer are urgently needed. The goal of this review is to examine modulation of prostate cancer (PCa) exosome interaction with prostate cancer micro-environment as a major support system to prevent incurable bone cancer. Conventional prostate cancer therapy is weakened by exchange of growth factors between cancer cells and its surroundings, leading to increase tumor growth and metastasis to other parts of the body. The surrounding of tumor includes prostate cancer exosomes, which can be release under suitable condition. Basically, the interactions between prostate cancer exosomes, growth factors and cell micro-environment support uncontrollable metastasis of prostate cancer to the bone. PCa exosome modification may be the solution to the increasing patient death caused by prostate cancer metastasis to the bone. PCa exosome is in the fore-front of research as a biomarker for early prostate cancer detection.

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## Mini review

In 2017, 1,688,780 new cancer cases and 600,920 cancer deaths are projected to occur in the United States.<sup>1</sup>, this corresponds to about 1,650 deaths per day.<sup>1</sup>. One common cause of this daily death is prostate cancer metastasis to bone.<sup>2,3</sup> Bone metastases occur in most tumor types but are most prevalent in cancers of the breast, prostate, and lung.<sup>4</sup> These bone lesions can cause deadly skeletal conditions, including spinal cord or nerve root dislocation, bone surgery, hypercalcemia, pathologic fractures, and extreme bone pain requiring immediate radiotherapy.<sup>5</sup> all of which can significantly lower the patient's quality of life.<sup>6-9</sup> and may negatively affect patient survival.<sup>10,11</sup> Current research focus on reduction of pain, prevention of abnormal skeletal conditions, and maintenance of quality of life to managing patients with metastatic bone cancer.<sup>12</sup> However, these efforts have failed because diagnosis most of bone cancer lead to incurable bone cancer.<sup>8</sup>. This review examines modulation of prostate cancer (PCa) exosome interaction with prostate cancer micro-environment as a major support system to prevent incurable bone cancer.

Treatment of metastatic prostate cancer has proven to be very challenging as a result of sustained activity of the androgen receptor,<sup>13-15</sup> aided by prostate cancer exosome activities. Although first stage of PCa can be treated successfully, one in three of the cases of PCa progress to a more advance form, which often leads to patient death.<sup>16</sup> or abnormal bone structure in 80% of the patients.<sup>17</sup> Prostate tumors can be inactive or very aggressive, often metastasizing to the bone and other organs, thereby causing significant morbidity and mortality.<sup>18</sup> Bone cancer present a 5-year survival rate of just 30% against 100% for restricted disease.<sup>17</sup>

The major challenge that leads to prostate cancer metastasis to the tissues such as the bone, is the failure to detect prostate cancer at the early stage. A serious clinical challenge in prostate cancer is the inability of current diagnostic tests, including prostate-specific

antigen (PSA) screening and histopathological grading, to distinguish between malignant and benign tumors.<sup>19</sup> A timely diagnosis of PCa is helpful in reducing death from prostate cancer metastasis.<sup>20</sup> A protein mainly secreted by prostate cells, prostate specific antigen (PSA), has been used as a blood-based biomarker for prostate cancer for more than 50years.

PSA is present in normal prostatic secretions and its levels are often elevated in prostate cancer patients.<sup>21</sup>. Serum PSA levels have been utilized as a prostate cancer biomarker for over 21years and PSA screening has transform the clinical management of the disease.<sup>22</sup> However, PSA has intrinsic limitations, including lack of specificity, leading to over-diagnosis and over-treatment of prostate cancer. Even though PSA is a high-end tool, it lacks specificity and is therefore not considered an excellent biomarker.<sup>23</sup> Thus, new and specific markers for prostate cancer are highly needed.

Consequently, concerted efforts are being made on searching for alternative prostate cancer biomarkers, particularly those that can predict the aggressiveness of the disease and drive better treatment decisions. PCa exosome is in the fore-front of research as a biomarker for prostate cancer detection. A good understanding of PCa exosome as a biomarker will eliminate unnecessary prostate biopsies, and support the urologist in recommending best treatment practices.

Exosomes are found naturally in blood, urine, cerebrospinal fluid, breast milk, saliva, ascetic fluid, and amniotic fluid in very high numbers.<sup>24-27</sup> Exosomes are tiny membrane vesicles with a double layered lipid released by most living cells.<sup>28-30</sup> The release of exosome is potentially impacted by the sorting of the cargo inside the exosome. The mechanisms underlying the sorting of cargo into the intraluminal vesicles (ILVs) have not been explained in detail. Both endosomal sorting complex required for transport (ESCRT)-dependent and independent signals have been suggested to determine the sorting of exosomes.<sup>31</sup>

There are other important factors that impact exosome release

in cancer; accumulation of intracellular  $\text{Ca}^{2+}$  resulting in increased exosome secretion,<sup>32</sup> oncogenes and tumor suppressors regulating exosome secretion (p53-regulated protein tumor suppressor-activated pathway 6 (TSAP6) induces exosome secretion under stressed conditions),<sup>33–35</sup> low micro environmental pH increasing exosome secretion and uptake by recipient cells.<sup>36</sup>

Furthermore, enzymes control the secretion of exosomes. Heparanase is an enzyme with elevated level in cancer. Overexpression of heparanase promotes exosome secretion.<sup>37</sup> Interestingly, exosomes from normal mammary epithelial cells inhibit exosome secretion by breast cancer cells, implicating a feedback control to maintain dynamic equilibrium.<sup>38</sup> Similarly, treating prostate cancer cells with exosome from normal prostate cells has potential therapeutic effect.

Conventional prostate cancer therapy is weakened by exchange of growth factors between cancer cells and its surroundings, leading to increased tumor growth and metastasis to other parts of the body.<sup>39</sup> Over the last 30 years, considerable amount of research has been done in order to understand the exosome mediated cell–cell communication mechanisms.<sup>40</sup> These efforts have revealed various new facets of material transport across biological membranes and have verified, to a great extent, the role of exosomes in disease development.<sup>24,41</sup>

Metastatic and therapy resistant cancers sustain on robust biological interaction networks arising from gene–gene, gene–microRNA (miRNA), protein–protein, parallel signaling as well as intracellular, intercellular and distant cell interactions.<sup>42,43</sup> Cancer–derived exosomes facilitate the cancer cells and their microenvironment to form a site that support tumor growth.<sup>44–46</sup> Moreover, exosome concentration in the plasma of prostate cancer patients measured by nanoparticle tracking analysis (NTA), was more than that in the plasma of non–cancerous individuals.<sup>47</sup> The spread of oncogenes by exosomes and microvesicles secreted by tumor cells have also been reported.<sup>48</sup> If release of prostate cancer exosomes continues in this trend, there will be uncontrollable prostate cancer metastasis to the bone.

Another factor that determines the impact of exosome is absorption of exosomes by the surrounding cells. The uptake of exosomes occurs through a well–defined process and is controlled by transmembrane proteins.<sup>49</sup> Studies suggest that the tetraspanin–integrin complex help in specificity, permitting the adhesion of exosomes to the right cells.<sup>50,51</sup> Furthermore, a pro–inflammatory environment may potentiate the expression of receptor molecules such as ICAM–1 (Intercellular Adhesion Molecule 1) on the membrane surface, with high tendency for exosomes attach to the target cells.<sup>52</sup> Although the specific mechanism that drive exosomes absorption is unknown, the activity of the T–cell receptor/CD3 complex and the chemokine receptor CXCR4 on exosomes of T–cells propose a juxtracrine pathway through receptor–ligand linkage.<sup>53</sup>

Alternatively, exosomes can merge with the cell membrane of target cells, which leads to a release of their content into the cytoplasm.<sup>52,54</sup> Cellular absorption of exosomes can occur by phagocytosis in an actin–cytoskeleton and phosphatidylinositol 3–kinase –associated manner.<sup>52</sup> Further studies are needed to understand how exosomes are directed to PCa cells and whether exosome protein results in organotropism associated with metastatic disease.

It is important to investigate the link between exosome protein and the metastatic potential of cancer after treatment, as indicated by unfolding findings. Exosomes facilitated transport of proteins between tumor cells can result into chemo resistance, which means high metastatic potential. Exosomes from docetaxel–resistant prostate

cancer cell lines can confer chemo resistance to non–resistant prostate cancer cell lines through exosomes facilitated transport of drug transporter, MDR–1.<sup>55</sup> These observations represent a new possibility of how cancerous cells may modulate the immune system, becoming chemo resistant with the help of exosomes.

This chemo–resistant property may be impacted through biochemical change of connective tissue via exosome protein activities. Exosomes from prostate cancer cell lines contain TGF– $\beta$ 1 protein, which is transported to receiver cells in a functional form. A *Study* have showed that TGF– $\beta$ 1 expressing exosomes can initiate the development of fibroblasts to myofibroblasts.<sup>56</sup> Myofibroblasts release considerable matrix remodeling proteins within the tumor microenvironment and contribute to tumor angiogenesis.<sup>57</sup> Cancer exosome–induced stimulation of fibroblasts could favor tumor angiogenesis. In addition, cancer cells transfer membrane–bound EGFR to endothelial cells via exosomes.<sup>56</sup> This transfer triggers the autocrine VEGF/VEGFR–2 pathway in endothelial cells and further enhance tumor angiogenesis (development of new blood vessels to support the tumor). Cancer cells also stimulate immune cells to enhance tumor invasion, tumor angiogenesis and distribution.<sup>58</sup>

Exosomes derived from cancer cells activate the immune cells capable of weakening the immune systems' fight against cancer. Tumor–derived exosomes switch on myeloid–derived suppressor cells (MDSC).<sup>59</sup> MDSCs cause immunosuppression in cancer by downregulating the T cell feedback.<sup>60</sup> Tumor–derived exosomes from various tumor cell lines enhance interleukin–6 (IL–6) production in MDSCs, through the activation of the Toll–like receptor 2 via the membrane–linked heat shock protein.<sup>59</sup> IL–6 release triggered autocrine phosphorylation of Stat3 inside MDSCs, which enhance their immunosuppressive effect on immune system.

Moreover, studies have revealed that tumor–derived exosomes express Fas ligand.<sup>61</sup> Fas containing exosomes can impact immunosuppression via apoptosis of tumor–reactive CD8+ T lymphocytes.<sup>62,63</sup> The dead tumor–reactive CD8+ T lymphocytes cannot attack the cancer cells in this state. Likewise, cancer cells release exosomes that increase the level of regulatory T (Treg) cells.<sup>62,64</sup> Tregs cause immunosuppression in the tumor microenvironment by hindering the function of anti–tumorigenic T cells.<sup>65</sup> and antigen presenting dendritic cells.<sup>66–68</sup>

Additionally, given that exosome secretion is triggered by an intracellular increase of calcium ( $\text{Ca}^{2+}$ ),<sup>32</sup> combination therapy involving intracellular calcium inhibition will be effective against cancer. Immunotherapeutic autologous dendritic cell–derived exosomes,<sup>69,70</sup> and intracellular calcium inhibition drugs may be effective in preventing cancer metastasis to the bone.

Furthermore, exosomes produced by melanoma cells function as vesicles to transfer the receptor tyrosine kinase (RTK) from melanoma cells to the bone marrow–derived progenitor cells, thereby enhancing the metastatic process.<sup>71</sup> Exosome protein concentration was highest in melanoma patients with the most advanced form of the disease; additionally, late–stage individuals harboring protein–deficient exosomes had a survival advantage compared to those with protein–rich exosomes.<sup>72</sup>

Regardless of the protein content of exosome, cells are known to deliver proteins between the intracellular organelles via membrane vesicles containing compatible receptors to ensure traffic specificity; the results accumulated over the last ten years have demonstrated that a heterogeneous group of vesicles are also released from the

cell surface and used as intercellular signalosomes in information exchange, even over a long distance.<sup>73</sup> This information exchange is facilitated by molecules such as protein, RNAs,<sup>74</sup> breakdown products of signaling pathways, viruses.<sup>75</sup> and, even recently, miRNAs.<sup>76</sup> during transportation involving exosomes. RNAs behave in a unique way, different from other exosome cargo.<sup>77</sup> Several miRNA expression profiles have been reported for prostate cancer, showing altered expression levels in prostate cancer tissue as compared to normal tissue.<sup>78–83</sup> Several cellular processes like proliferation, differentiation, and apoptosis are shown to be regulated by miRNAs<sup>84</sup> and miRNAs are found abnormally expressed in many types of cancer.<sup>79,81, 85–87</sup>

Notably, miRNAs are currently being investigated as prognostic and diagnostic tools for prostate and other types of cancer.<sup>88,89</sup> Studies on blood-based exosome miRNAs as biomarkers for prostate cancer are emerging, however most of these are at initiation stage, vary in method, and lack characterization in the form in which the extracellular miRNAs are found.<sup>90</sup> This makes exosome RNA a potential focus in studies related to prevention of prostate cancer metastasis to the bone.

Besides RNA's, intracellular calcium ion level can also be used to detect PCa condition, majorly because calcium ions (Ca<sup>2+</sup>) act as second messenger to regulate gene transcription, cell proliferation, migration and death of PCa cells.<sup>91</sup> The extracellular calcium-sensing receptor (CaSR) plays a major role in the maintenance of a physiological serum ionized calcium (Ca<sup>2+</sup>) concentration by regulating the circulating levels of parathyroid hormone.<sup>92</sup> The heterodimeric G-protein coupled CaSR is the most important receptor on the cell surface for detecting extracellular calcium. In normal tissue, CaSR is responsible for the physiological regulation of calcium homeostasis in several organs such as kidney, breast, gastrointestinal tract, bones and parathyroid glands.<sup>93</sup> The binding of calcium on CaSR activates a variety of signaling pathways that are important for PCa cell survival. Although CaSR may also have a tumor suppressing capacity in e.g. gastric and colon cancer, it has been demonstrated to be involved in bone metastasis of several tumor entities, such as renal cell carcinoma, prostate carcinoma and breast cancer.<sup>94</sup>

Furthermore, Ca<sup>2+</sup> is a chemopreventive agent for colon cancer.<sup>95,96</sup> It acts at the molecular level to inhibit or delay carcinogenesis in the colon, however, the mechanism is not fully understood. A study found that CaSR is expressed in human colon epithelium.<sup>97,98</sup> Compared with normal colon crypt epithelial cells, a loss of CaSR expression is observed in differentiated carcinomas, whereas little or no CaSR expression is found in undifferentiated and invasive carcinomas.<sup>98</sup> Thus, the expression of CaSR may be linked with either abnormal differentiation or malignant progression of PCa, or even both.

## Conclusion

This review highlights exosomes as a substitute biomarker for traditional diagnostic test such as prostate specific antigen (PSA) testing and digital rectal examination (DRE). PSA is not dependable as a PCa biomarker and levels can be high because of other disease conditions.<sup>99</sup> Therefore, more effective bio-markers are needed for PCa prognosis, possibly preventing metastasis to the bone. PCa exosome modification may be the solution to the increasing patient death caused prostate cancer metastasis to the bone. This revision considers prostate cancer exosomes study as a compelling study for decreasing the metastasis of prostate cancer to the bones.

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## Conflicts of interests

The authors declare that there are no conflicts of interest.

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