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An Integrative Model of Prostate Cancer Interaction with the Bone Microenvironment

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Abstract

Despite advanced efforts in early diagnosis, aggressive surgical treatment, and use of targeted chemotherapies, the prognosis for many cancers is still dismal. This emphasizes the necessity to develop new strategies for understanding tumor growth and metastasis. Here we use a systems approach that combines mathematical modeling and numerical simulation to develop a predictive computational model for prostate cancer and its subversion of the bone microenvironment. This model simulates metastatic prostate cancer evolution, progressing from normal bone and hormone levels to quantifiable diseased states. The simulations clearly demonstrate phenomena similar to those found clinically in prostate cancer patients. In addition, the major prediction of this model is the existence of low and high osteogenic states that are markedly different from one another. The existence and potential realization of these steady states appear to be mediated by the Wnt signaling pathway and by the effects of PSA on TGF- β , which encourages the bone microenvironment to evolve. The model is used to explore several potential therapeutic strategies, with some potential drug targets showing more promise than others: in particular, completely blocking Wnt and greatly increasing DKK-1 had significant positive effects, while blocking RANKL did not improve the outcome.

Keywords

Mathematical model; Prostate cancer; Bone metastasis

1 Introduction

Prostate cancer (PCa) is the most common malignancy and a leading cause of cancer-related death in American men [44]. As PCa progresses, it has a predisposition to metastasize to bone as opposed to other sites resulting approximately 90% of men with advanced PCa having bone micrometastases [8]. The difference between survival rates of localized prostate cancer and cancer that has metastasized throughout the body is marked: the former has a

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nearly 100% five-year survival rate, while the latter is associated with survival rates of 28% [43]. PCa bone metastases are somewhat unique compared to metastases from most other cancers. Specifically, most cancers induce osteolytic (i.e., bone resorptive) lesions, whereas PCa induces osteoblastic (bone productive) lesions [34]. The mechanisms that account for bone being a favored site for PCa growth and the ultimate induction of osteoblastic lesions are not well defined. Understanding these mechanisms could lead to the identification of new therapeutic targets. Modeling metastasis and attempting to interfere with the pathology *in silico* can be vital to understanding the disease pathology and has the potential to guide drug development efforts.

Prostate cancer metastases in the bone microenvironment overwrite the natural, tightly controlled processes of bone remodeling to better suit the growth of the tumor. Primarily, two specialized cell types orchestrate the formation and degradation of bone: osteoblasts, which produce bone matrix and aid its mineralization [29], and osteoclasts, a unique cell type that dissolves bone mineral [47]. Osteoblasts can be induced to express receptor activator of nuclear factor kappa-B ligand (RANKL) in response to bone resorption-stimulating factors such as parathyroid hormone (PTH) [13]. Osteoblasts also express a negative regulator of bone resorption, osteoprotegerin (OPG), which inhibits the interaction between RANK and RANKL by acting as a decoy receptor of RANKL [45]. Studies of diseases associated with defects in bone formation have demonstrated the crucial importance of the Wnt signaling pathway [18] in promoting bone formation and inhibiting bone resorption. Canonical Wnt signaling promotes differentiation of uncommitted osteoblast precursors (OBu) into active osteoblasts (OBa), which in turn increases bone formation [18, 30]. Wnt signaling also induces the up-regulation of OPG expression and down-regulation of RANKL expression in osteoblasts, resulting in the inhibition of bone resorption [30]. Mesenchymal cells secrete Dickkopf-related protein (DKK-1), a Wnt signaling pathway inhibitor that antagonizes canonical Wnt signaling by blocking its interaction with low-density lipoprotein receptor-related proteins [24].

Calcium, as a nutrient, is most commonly associated with the formation and metabolism of bone. Osteoblasts uptake blood calcium (Ca^{2+}) in order to produce bone [4]. However, as Ca^{2+} is reduced in the bloodstream, the parathyroid glands secrete parathyroid hormone (PTH) [36]. Osteoblast precursors are stimulated to produce RANKL by the PTH, which also inhibits osteoblasts from producing OPG. RANKL encourages osteoclast precursors (OCp) to differentiate into active osteoclasts (OCa), which resorb bone and release both Ca^{2+} and TGF- β in its latent form, LTGF- β [16]. Increasing calcium levels signal the parathyroids to stop producing PTH [7]. In time, LTGF- β is transformed into its active form, which encourages osteoclast apoptosis as well as the differentiation of uncommitted osteoblast precursors into osteoblast precursor cells. TGF- β also inhibits the differentiation of osteoblast precursors (OBp) into active osteoblasts [17]. This way, equilibrium is maintained by the body in order to preserve calcium and bone homeostasis.

When prostate cancer metastasizes to bone, the natural equilibrium is entirely disrupted. The main reason is that prostate cancer cells produce Wnt in far larger quantities than usually present in the body. This can be somewhat mitigated by the fact that prostate cancer cells that have not fully acclimated (PCe) to the bone microenvironment also produce DKK-1

[23]. All prostate cancer cells produce modified parathyroid hormone (PTHrP) [3]; however, prostate cancer cells that have adapted to the bone microenvironment (PCI) also produce prostate specific antigen (PSA), which inhibits PTHrP [12]. Overexpression of Wnt results in increases in OBa and their predecessors, which rapidly produce RANKL [6, 54]. This causes bone formation while simultaneously stimulating OCa production which leads to rapid bone resorption. This results in large bone turnover, releasing LTGF- β [33]. When LTGF- β is activated, it encourages the proliferation of PCI [21]. This, in turn, increases the concentration of Wnt, creating a bidirectional crosstalk in which the growing tumor remodels bone to sustain additional cancer growth; this is the so-called “vicious cycle” of prostate cancer metastasis [11].

2 Model Development

In order to quantify the influence of critical pathways that mediate crosstalk between PCa and the bone microenvironment, we will track temporal changes of the following cell and tissue types listed in Table 1.

Additionally, we will model the following important signaling proteins and chemokines that mediate bone remodeling and PCa growth listed in Table 2.

A schematic diagram of the model is given in Figure 1, and the model equations and their explanations are provided in the subsections below.

2.1 Bone Cell Remodeling Equations

Our mathematical model of the bone microenvironment is based on that of [49]; however, we have posed several modifications. In particular, one new feature of our model is that we incorporate not only the effects of TGF- β on bone cell differentiation, but also the effects of Wnt and DKK-1. TGF- β is an important regulator of osteoblast and osteoclast activity during bone growth and remodeling [20]. There are also molecular links between Wnt signaling and bone development and remodeling [30]. Wnt not only promotes OBU differentiation into OBp as TGF- β does, but also encourages OBp to differentiate into OBa, in direct contrast to TGF- β [18]. DKK1 regulates Wnt signaling and inhibits osteoblastogenesis [24, 30]. Equations 1–5 below describe the temporal changes in the evolution of the bone cells and the total bone volume, as mediated by this signaling network.

2.1.1 Osteoblast Precursor Cells (OBp)

$$\frac{dOBp}{dt} = \underbrace{\delta_{OBu} \pi_{OBp} OBu}_{\text{Generation of OBp by OBu}} - \underbrace{OBp \left(\delta_{OBp1} \frac{K_{TGF\beta r1}}{TGF\beta + K_{TGF\beta r1}} + \delta_{OBp2} \frac{WNT}{WNT + K_{WNT a2}} \right)}_{\text{Differentiation of OBp into OBa}} \quad (1)$$

$$\pi_{OBp} = \frac{TGF\beta}{TGF\beta + K_{TGF\beta a1}} + \frac{WNT}{WNT + K_{WNT a1}} - \frac{TGF\beta}{TGF\beta + K_{TGF\beta a1}} \frac{WNT}{WNT + K_{WNT a1}} \quad (2)$$

In equation (1), we assume that bone marrow stem cells (BMSCs, or in terms of the model, *OBu* for uncommitted cells) are in constant supply, and that, on average, every *OBu* mitotic event produces one *OBp*. This assumption is based on the fact that the osteoblastic lineage derives from an abundant pool of stem cells and is in line with other mathematical models of bone formation [38, 49]. The first term ensures that differentiation of *OBu* into *OBp* relies on sources of *OBu*, *TGF-β*, and *Wnt*. Specifically, active *TGF-β* and *Wnt* stimulate *OBu* to differentiate into osteoblast precursor cells (*OBp*). The generation of osteoblast precursors is simultaneously controlled by two ligands, therefore we use the functional form derived in [49] in equation (2) to describe these enhanced effects. We choose to subtract the product of the activation expressions, as we assume that there are no synergistic interactions between *Wnt* and *TGF-β* in activating *OBu* differentiation. The second term describes the differentiation of *OBp* into *OBa*. Here, active *TGF-β* inhibits osteoblast precursor cells from differentiating into active osteoblasts (*OBa*), while *Wnt* stimulates this differentiation.

The parameters δ_{OBu} , δ_{OBp1} , and δ_{OBp2} are the differentiation rates of bone marrow stem cells and osteoblast precursors; respectively. The parameters $K_{TGF\beta_{a1}}$, $K_{TGF\beta_{r1}}$, $K_{WNT_{a1}}$, and $K_{WNT_{a2}}$ represent activation (or repression) constants for *TGF-β* and *Wnt* respectively, which are the ligand concentrations that induce a half-maximal cellular response.

2.1.2 Active Osteoblasts (OBa)

$$\begin{aligned} \frac{dOBa}{dt} = & \underbrace{OBp \left(\delta_{OBp1} \frac{K_{TGF\beta_{r1}}}{TGF\beta + K_{TGF\beta_{r1}}} + \delta_{OBp2} \frac{WNT}{WNT + K_{WNT_{a2}}} \right)}_{\text{Differentiation of } OBp \text{ into } OBa} \\ & - \underbrace{\alpha_{OBa} OBa}_{\text{Apoptosis of } OBa} \end{aligned} \quad (3)$$

Equation (3), for temporal changes in activated osteoblasts, describes the only source of active osteoblasts (*OBa*) as the differentiation of *OBp* into *OBa*, and so the first term is identically equal to the additive inverse of the differentiation term in equation (1). The apoptotic rate of osteoblasts, α_{OBa} , is linearly proportional to the amount of osteoblasts in the system, and is not altered by the hormonal environment.

2.1.3 Active Osteoclasts (OCa)

$$\begin{aligned} \frac{dOCa}{dt} = & \underbrace{\delta_{OCp} \frac{RANKL}{RANKL + K_{RANKL_{a1}}} OCp}_{\text{Differentiation of } OCp \text{ into } OCa} \\ & - \underbrace{\left(\alpha_{OCa1} + \alpha_{OCa2} \frac{TGF\beta}{TGF\beta + K_{TGF\beta_{a2}}} \right) OCa}_{\text{Apoptosis of } OCa} \end{aligned} \quad (4)$$

In equation (4), we consider the osteoclast precursor (OCp) population to be constant in the system, as an analogue to OBU, and set differentiation to have a maximum rate δ_{OCp} of osteoclasts (OCa) generated per day. We modify this maximum with a dependency on RANKL, which signals differentiation of OCp to OCa, where $K_{RANKL_{a1}}$ is the concentration of RANKL that induces a half-maximal response. We also take the apoptosis of OCa to be linearly dependent on the amount of OCa in the system. However, the proportionality is presumed to vary, starting with a base rate α_{OCa1} and then increasing as TGF- β increases. Here, α_{OCa2} is the maximum TGF- β induced apoptosis rate and $K_{TGF\beta_{a2}}$ is the half-maximal concentration of TGF- β . This contrasts slightly with [49], as they assume that all osteoclast destruction is mediated by TGF- β , and we incorporate this additional term as a simplification of other factors that induce OCa apoptosis that we do not explicitly track, e.g. IL-6 [53].

2.1.4 Total Bone Volume ($BONE_t$)

$$\frac{dBONE_t}{dt} = \underbrace{BONE_p}_{\text{Production of bone}} - \underbrace{BONE_r}_{\text{Resorption of bone}} = \underbrace{K_{form} \frac{Ca^{2+}}{Ca^{2+} + K_{Ca^{2+}}}}_{\text{Production of bone}} OBa - \underbrace{K_{res} OCa}_{\text{Resorption of bone}} \quad (5)$$

Bone volume (Equation (5)) is changed by only two factors: bone synthesis by osteoblasts and bone resorption by osteoclasts. Here, $BONE_t$ is considered to be the normalized volume of total bone material. The first term represents bone production by osteoblasts, which is usually assumed to be directly proportional to the amount of osteoblasts in the system [38, 49]. However, bone formation requires calcium, so we augment this term to depend on calcium concentration in low calcium conditions, while allowing bone synthesis to remain relatively independent of calcium levels otherwise. The second term denotes bone resorption by osteoclasts, which is simply considered to be directly proportional to the number of osteoclasts in the system. The parameters K_{form} and K_{res} are the relative bone resorption and formation rates respectively, and $K_{Ca^{2+}}$ is the concentration of calcium at which bone formation rate is halved.

In summary, Equations (1) – (5) describe the ways in which uncommitted stem cells are induced by factors such as Wnt and TGF- β to commit to osteoblast precursor cells. These cells can be induced by Wnt to become active osteoblasts that secrete mineral ($BONE_p$) and increase the total bone volume ($BONE_t$). RANKL induces osteoclast precursors to become active osteoclasts that induce resorption ($BONE_r$) of bone and decreases the total bone volume. Active TGF- β induces active osteoclasts to become apoptotic and promotes the differentiation of osteoclast precursors into active osteoclasts through enhancing RANKL production. In the next section we develop the model equation of the proteins and chemokines that regulate bone remodeling.

2.2 Bone Regulation Signaling Molecule Equations

Bone is a dynamic tissue that undergoes continual adaption. These tightly coordinated events require the synchronized activities of multiple cellular participants. Below we develop equations for several key molecular players that regulate the cell types involved.

2.2.1 Calcium (Ca^{2+})

$$\frac{d\text{Ca}^{2+}}{dt} = \underbrace{i}_{\text{Absorption from dietary intake}} - \underbrace{\frac{i\text{Ca}^{2+}}{S_{\text{Ca}^{2+}} + \frac{PTHrP}{S_{PTH}}\text{Ca}^{2+}}}_{\text{Calcium excretion}} + \underbrace{Q \left(K_{res} OCa - \frac{\text{Ca}^{2+}}{\text{Ca}^{2+} + K_{\text{Ca}^{2+}}} K_{from OBa} \right)}_{\text{Release of calcium due to bone remodeling}}$$

(6)

The inclusion of blood calcium ions (Ca^{2+}), as described in Equation (6), is a novel addition to the model. Calcium acts as a limiting resource for bone growth, without which the model can predict drastic inflation of bone. The parameter i represents absorption from dietary intake, while the second term describes calcium excretion. Here, S_{PTH} and $S_{\text{Ca}^{2+}}$ represent normal steady state levels of parathyroid hormone and calcium in the system. Dietary intake is considered roughly equal to urinary excretion under normal conditions, and even when the body is trying to recuperate calcium, excretion is still proportional to the uptake of calcium. This assumption is used for fundamental reasons: the body's purpose in excreting calcium is retaining an isotonic molarity in the bloodstream [35]. Ergo, if intake increases, excretion must increase to compensate. A linear relationship is used because in adults without cancer, there is a constant isotonic maintenance of approximately the same calcium molarity in blood and no net bone growth; therefore, any intake must have an equal removal. Therefore, the excretion term simplifies to i when calcium is at steady state to keep with this assumption. As calcium decreases in the system, excretion slows to a trickle, which is only further aided by PTHrP, which is scaled by the steady state PTH value to retain appropriate units. The last term changes the calcium in the system based on the creation and resorption of bone. The conversion factor Q denotes how much calcium is released with one percent destruction of bone volume; it has units of $\frac{M\text{Ca}^{2+}}{\% \text{Bone}}$, and it is derived from several different analyses of bone geometry and structure. The parameter Q can be taken to be a constant in this model only because bone volume remains relatively unchanged: the linearity of bone volume change to blood calcium variation breaks down as volume is significantly altered.

2.2.2 Latent Transforming Growth Factor Beta (LTGF β)

$$\frac{dLTGF\beta}{dt} = \underbrace{\mu K_{res} OCa}_{\text{Release from resorbed bone}} - \underbrace{\lambda_{LTGF\beta} LTGF\beta}_{\text{Degradation}} - \underbrace{\left(\beta_{LTGF\beta_1} + \beta_{LTGF\beta_2} \frac{PSA}{PSA + K_{PSA_{a1}}} \right) LTGF\beta}_{\text{Activation into } TGF\beta} \quad (7)$$

The inclusion of latent TGF- β is another new feature of our model. TGF- β is produced by most tissues, including bone, as a complex that is biologically inert [5]. The release of active protein from this latent form is critical for TGF- β to initiate effects on target cells [5]. Therefore, the inclusion of the latent complex and the mechanisms responsible for active release are important for understanding TGF- β actions. Consistent with the known physiology, in equation (7), latent TGF- β is released when bone is resorbed and it is constantly degraded at rate $\lambda_{LTGF\beta}$. The parameter μ , in the first term, converts the amount of bone resorbed into the amount of LTGF- β released. In the third term we see that in the absence of PSA, latent TGF- β is activated at rate $\beta_{LTGF\beta_1}$. In the presence of PSA, there is enhanced activation of latent TGF- β into active TGF- β at maximum rate $\beta_{LTGF\beta_2}$. The parameter $K_{PSA_{a1}}$ is the half-saturation constant of the PSA-mediated response of latent TGF- β .

2.2.3 Active Transforming Growth Factor Beta (TGF β)

$$\frac{dTGF\beta}{dt} = \underbrace{\left(\beta_{LTGF\beta_1} + \beta_{LTGF\beta_2} \frac{PSA}{PSA + K_{PSA_{a1}}} \right) LTGF\beta}_{\text{Activation by } TGF\beta} - \underbrace{\lambda_{TGF\beta} TGF\beta}_{\text{Degradation}} \quad (8)$$

In equation (8), latent TGF- β (inactive) is cleaved by PSA and other proteins to make active TGF- β , which is then constantly degraded at rate $\lambda_{TGF\beta}$. We recognize that TGF- β is also produced by prostate cancer cells in the presence of PSA, but we assume this relative amount is small enough to be neglected in comparison to that released by bone resorption.

2.2.4 Total Parathyroid Hormone (PTH_{total})

$$PTH = \frac{\beta_{PTH} S_{Ca^{2+}}}{\lambda_{PTH} Ca^{2+}} \quad (9)$$

$$PTHrP = \frac{\beta_{PTHrP} PC}{(\lambda_{PTHrP_1} + \lambda_{PTHrP_2} PSA)} \quad (10)$$

PTH is not only an important endocrine regulator of calcium, it also differentially regulates expression of RANKL and OPG [25]. In this model, we track both PTH, which is naturally present in the bone, and PTHrP, which is produced by prostate cancer cells and is a key mediator for communication and interactions between prostate cancer and the bone microenvironment [32]. The steady state PTH and PTHrP equations (10) are derived based on [38] under the following assumptions: 1) binding reactions leading to upregulation and down-regulation of both PTH and PTHrP are much faster than the cell responses, and therefore the quasi-steady approximation is valid, 2) PTH is produced naturally in the bone at a constant rate, 3) natural degradation of both PTH and PTHrP is proportional to their respective concentrations, and 4) PTH and PTHrP binding to its receptors on osteoblast precursor cells and active osteoblasts is the same. To these general bone regulation assumptions we add the following PCa-specific assumptions for PTHrP: 1) PTHrP is only produced by PC and 2) PTHrP is degraded by PSA or in other words, the PTHrP degradation rate increases as PSA levels increase. Here, $PC = PCe + PCl$, representing the total amount of PCa cells in the bone. The parameters β_{PTH} and β_{PTHrP} are the production rates of PTH and PTHrP, respectively, and λ_{PTH} and λ_{PTHrP1} are their natural degradation rates. The rate of PSA-dependent degradation of PTHrP is given by λ_{PTHrP2} . Adding equations (9) and (10), we get the total amount of PTH protein at steady state given in equation (11).

$$PTH_{total} = \frac{\beta_{PTH} S_{Ca^{2+}}}{\lambda_{PTH} Ca^{2+}} + \frac{\beta_{PTHrP} PC}{(\lambda_{PTHrP1} + \lambda_{PTHrP2} PSA)} \quad (11)$$

2.2.5 Osteoprotegerin (OPG)

$$OPG = \frac{\beta_{OPG} \frac{K_{PTH_{total}r1}}{K_{PTH_{total}r1} + PTH_{total}} OBa + P_{OPG,d}(t)}{\frac{\beta_{OPG}}{OPG_{max}} \frac{K_{PTH_{total}r1}}{K_{PTH_{total}r1} + PTH_{total}} OBa + \lambda_{OPG}} \quad (12)$$

The steady state OPG equation (12) is derived directly from [38]. Consistent with both experimental data and the mathematical model in [38], we assume that OPG is only expressed on OBa cells and OPG is downregulated by the PTH proteins. Recall, $PTH_{total} = PTH + PTHrP$. The critically important difference here is that OPG is upregulated by PSA indirectly via PTHrP decrease. The parameter β_{OPG} is the OBa-dependent production rate of OPG, OPG_{max} is the carrying capacity for OPG in the system, and λ_{OPG} is the constant degradation rate. The half-saturation constant for the effect of PTH proteins on OPG production is given by $K_{PTH_{total}r1}$. The function $P_{OPG,d}(t)$ represents external dosing; it is included here for completeness, however, it will be set to zero in the simulations unless otherwise noted.

2.2.6 Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)

$$RANKL = \frac{P_{R_L,d}(t) + \beta_{RANKL} OBp}{(1 + K_{A,OPG} OPG + K_{A,RANK} R) \left(\frac{\beta_{RANKL}}{\rho PTH_{total} + K_{PTH_{total\alpha 1}}} + \lambda_{RANKL} \right)} \quad (13)$$

The steady state RANKL equation (13) is derived directly from [49], where its production is controlled by the total amount of PTH protein. RANKL binds to both OPG (at rate $K_{A,OPG}$) and RANK (at rate $K_{A,RANK}$). The parameter ρ is the maximum amount of RANKL that a given osteoblast precursor can produce, and R denotes the amount of RANK available on OCp cells. Again, the difference here is that production is also indirectly under the control of PSA through its role in PTHrP degradation. The parameters β_{RANKL} and λ_{RANKL} are the OBp-dependent production rate of RANKL and RANKL degradation rate; respectively. The half-saturation constant for the effect of the PTH proteins on RANKL production is given by $K_{PTH_{total\alpha 1}}$ and $P_{R_L,d}(t)$ represents external dosing.

Equations (6) – (13) describe proteins and chemokines that regulate bone remodeling. Completely new features of our model include the incorporation of calcium as a key mediator of bone production, the consideration of the important role of latent TGF- β in producing the active form of the protein, and the inclusion of prostate cancer-derived PTHrP and its role in osteoblastic progression. In the next section we develop equations for prostate cancer subversion of the bone microenvironment.

2.3 Prostate Cancer Cell Equations (PCe)

The spread of cancer to bone has several complex steps. Our model begins after the processes of localized tissue invasion and vessel intravasation/extravasation into the bone. Once in the bone, prostate cancer cells must acclimate to the bone microenvironment and avoid attacks from the immune system. So, while adjusting to their new surroundings these cells undergo phenotypic changes that lead to the new, bone-conditioned tumor cells being somewhat different from the primary tumor cells that arrived there. We distinguish between these two types of prostate cancer cells by calling those newly arrived cells early prostate cancer cells (PCe) and terming those that have been conditioned to the bone microenvironment late prostate cancer cells (PCl). The equations for each type of cancer cell is provided below.

2.3.1 Early Prostate Cancer Cells (PCe)

$$\begin{aligned} \frac{dPCe}{dt} = & \underbrace{\sigma_{PCe}}_{\text{Constant source from primary site}} + \underbrace{\mu_{PCe} \left(1 - \frac{PC}{PC_{max}} \right)}_{\text{Logistic Growth}} PCe \\ & - \underbrace{\nu_{PC} PCe}_{\text{Transition to PCl}} - \underbrace{\alpha_{PCe} PCe}_{\text{Apoptosis of PCe}} \end{aligned} \quad (14)$$

In equation (14), we assume that all cancer cells are initially introduced into the bone as PCe at a constant rate σ_{PCe} from the primary tumor site. Once in the bone microenvironment, these cells proliferate logistically at rate μ_{PCe} and carrying capacity PC_{max} . As PCe cells become accustomed to the bone, they begin to undergo phenotypic changes to become PCI cells at rate ν_{PC} or they become apoptotic at rate α_{PCe} . Here, $PC = PCe + PCI$, representing the total population of PC cells.

2.3.2 Late Prostate Cancer Cells (PCI)

$$\frac{dPCI}{dt} = \underbrace{\nu_{PC} PCe}_{\text{Transition to PCI}} + \underbrace{\left(\mu_{PCI} + \mu_{PCI} \pi_{ligands}^{PCI} \right) \left(1 - \frac{PC}{PC_{max}} \right) PCI}_{\text{Logistic Growth}} - \underbrace{\alpha_{PCI} PCI}_{\text{Apoptosis of PCI}} \quad (15)$$

$$\pi_{ligands}^{PCaL} = \frac{TGF\beta}{TGF\beta + K_{TGF\beta a3}} + \frac{WNT}{WNT + K_{WNT a3}} - \frac{TGF\beta}{TGF\beta + K_{TGF\beta a3}} \frac{WNT}{WNT + K_{WNT a3}} \quad (16)$$

Late prostate cancer cells (PCI) evolve directly from PCe cells (first term in Equation (14)), proliferate logistically, and become apoptotic at rate α_{PCI} . Because the proliferation of PCI cells is simultaneously controlled by both Wnt and TGF- β , we used the functional form similar to that described in Equation (2) to describe these effects. The parameter μ_{PCI} is the proliferation rate of PCI cells in the absence of Wnt and TGF- β , whereas μ_{PCI} represents the maximum ligand-dependent proliferation rate. The parameters $K_{TGF\beta a3}$ and $K_{WNT a2}$ represent the half-maximal concentrations of TGF- β and Wnt respectively, which are the ligand concentrations inducing a half-maximal cell response.

PCa growth in bone is regulated by a variety of signaling molecules. In the next section we derive equations for the roles of Wnt, DKK-1, and PSA in metastatic progression in the bone.

2.4 Prostate Cancer Signaling Molecule Equations

Dysregulation of Wnt signalling can lead to several types of cancer, including prostate cancer [31]. Further, prostate cancer-produced Wnts have been shown to induce osteoblastic activity in PCa bone metastases [15].

2.4.1 Wnt Proteins (WNT)

$$\frac{dWNT}{dt} = \underbrace{\beta_{WNT} \frac{K_{DKK1}}{K_{DKK1} + DKK1} PC}_{\text{Production of Wnt by PC}} - \underbrace{\lambda_{WNT} WNT}_{\text{Natural Degradation}} \quad (17)$$

Wnt is naturally present in small concentrations in the body to regulate tissue generation. However, assume in equation (17) that its concentration is sufficiently low in adult males

that we may consider natural Wnt production to be negligible. The first term describes Wnt production by all prostate cancer cells ($PC=PCe+PCl$) in order to alter the bone microenvironment with maximum production rate, β_{WNT} . DKK-1 is an antagonist to Wnt production, with half-maximal concentration K_{DKK1} . In the second term, we also include a natural degradation rate for Wnt, λ_{WNT} .

2.4.2 Dickkopf Wnt Signaling Pathway Inhibitor-1 (DKK1)

$$\frac{dDKK1}{dt} = \underbrace{\beta_{DKK1} \frac{TGF\beta}{K_{TGF\beta r1} + TGF\beta} OBp}_{\text{Production of DKK1 by OBp}} + \underbrace{\beta'_{DKK1} PCe}_{\text{Production of DKK1 by PCe}} - \underbrace{\lambda_{DKK1} DKK1}_{\text{Natural Degradation}} \quad (18)$$

DKK-1 evolution in the prostate cancer system is primarily derived from production by OBp, which is activated by TGF- β at a maximum rate β_{DKK1} and half-maximal response $K_{TGF\beta r1}$ [55]. DKK-1 is also produced, albeit in significantly smaller amounts, by PCe at rate β'_{DKK1} . The third term is the natural degradation of DKK-1 at rate λ_{DKK1} .

2.4.3 PSA

$$\frac{dPSA}{dt} = \underbrace{\beta_{PSA} PCl}_{\text{Production of PSA by PCl}} - \underbrace{\lambda_{PSA} PSA}_{\text{Natural Degradation}} \quad (19)$$

Measurement of PSA is an important procedure in the metastatic work-up of prostate cancer patients [52]. In equation (19), we neglect natural bodily sources of PSA and focus exclusively on cancer cell synthesis, specifically late stage cancer cell synthesis, and assume that it occurs at rate β_{PSA} . We also include natural degradation rate λ_{PSA} .

Equations (14) – (19) describe the progression of prostate cancer cells once they enter the bone. In summary, early prostate cancer cells, PCe, arrive in the bone and eventually transform into late cancer cells, PCl. All cancer cells produce Wnt and its overexpression induces differentiation of OBU to OBp to Oba and also induces PCl proliferation. Our goal is to use the full model to determine prostate cancer growth patterns in the bone and to investigate the blockage of key molecular pathways. Before embarking on this task, we first describe the homeostatic steady state analysis and parameter estimation for the model.

3 Parameter Estimation

Below, we introduce the parameter values associated with the mathematical model. Initially, we focus on the parameters for the bone remodeling model in the absence of cancer and then proceed to discuss parameters associated with modulation of the bone in response to tumor growth.

3.1 Steady State Calculations and Parameter Estimation for Bone Remodeling

Many of the parameters for the bone remodeling model are available in the existing literature. We have used a variety of sources to parameterize this model. A complete list of parameters values taken from the literature and their sources for the bone remodeling model can be found in Table 3 below.

The following sections are calibrations of our new model's parameters and steady states in the absence of prostate cancer cells. In this situation, we expect the bone cells and chemical regulators to all approach normal, homeostatic levels.

3.1.1 Steady State Equations For Latent and Active TGF- β —The only completely new equations in the bone model without cancer cells are the ones for calcium, equation (6), and latent TGF- β , equation (7). Therefore, we determine the normal steady state levels of calcium, latent TGF- β , and active TGF- β to ensure homeostasis below. All other steady state analysis can be found in [49].

We define the calcium steady state to equal regular hormonal levels without cancer, $S_{Ca^{2+}} = 1.25 \times 10^{-3} M$, and we know the bone resorption rate K_{res} from Table 3. We can now solve for bone formation rate K_{form} by considering equation (5) and ensuring that total bone volume remain at steady state as follows:

$$\begin{aligned} \frac{dBONE_t}{dt} &= \frac{Ca^{2+}}{Ca^{2+} + K_{Ca^{2+}}} K_{form} OBa - K_{res} OCa \\ 0 &= \frac{S_{Ca^{2+}}}{S_{Ca^{2+}} + K_{Ca^{2+}}} K_{form} OBa - K_{res} OCa \\ \therefore K_{form} &= \frac{K_{res} OCa (S_{Ca^{2+}} + K_{Ca^{2+}})}{OBa S_{Ca^{2+}}} \end{aligned} \quad (20)$$

When there are no prostate cancer cells in the bone and when PSA levels are negligible, equations (7) and (8) give the following steady state levels of latent and active TGF- β :

$$\begin{aligned} LTGF\beta &= \frac{\mu K_{res} OCa}{\lambda_{LTGF\beta} + \beta_{LTGF\beta_1}} \\ TGF\beta &= \frac{\beta_{LTGF\beta_1} LTGF\beta}{\lambda_{TGF\beta}} \end{aligned} \quad (21)$$

3.1.2 Calculation of Bone Cell Differentiation Rates—Now that we have steady state relations for all of the bone-related proteins, we can use them to derive relations for the differentiation rates of OBU, OBp, and OCp cells in the absence of cytokines produced by prostate cancer cells (e.g. Wnt, PSA, PTHrP, and DKK-1). This allows us to estimate the values of these parameters that will ensure homeostasis when not exposed to cancer cells.

Solving (1) for the differentiation rate of the uncommitted cells we find:

$$\begin{aligned}
\frac{dOB_p}{dt} &= \delta_{OB_u} OB_u \pi_{OB_p}^{ligands} - \delta_{OB_p1} OB_p \frac{K_{TGF\beta_{r1}}}{TGF_{\beta} + K_{TGF\beta_{r1}}} \\
0 &= \delta_{OB_u} OB_u \pi_{OB_p}^{ligands} - \delta_{OB_p1} OB_p \frac{K_{TGF\beta_{r1}}}{TGF_{\beta} + K_{TGF\beta_{r1}}} \\
\therefore \delta_{OB_u} &= \frac{\delta_{OB_p1} OB_p \frac{K_{TGF\beta_{r1}}}{TGF_{\beta} + K_{TGF\beta_{r1}}}}{OB_u \pi_{OB_p}^{ligands}}
\end{aligned} \tag{22}$$

Similarly, from equation (3) and (4) for the osteoblast and osteoclast precursor cells; respectively, we obtain:

$$\begin{aligned}
\delta_{OB_p1} &= \frac{\alpha_{OB_a} OB_a}{OB_p \frac{K_{TGF\beta_{r1}}}{TGF_{\beta} + K_{TGF\beta_{r1}}}} \\
\delta_{OC_p} &= \frac{\left(\alpha_{OC_{a1}} + \alpha_{OC_{a2}} \frac{TGF_{\beta}}{TGF_{\beta} + K_{TGF\beta_{a2}}} \right) OC_a}{\left(\frac{RANKL}{RANKL + K_{RANKL_{a1}}} \right) OC_p}
\end{aligned} \tag{23}$$

By substituting known parameters and steady values for all cells and proteins, we are able to determine values for these differentiation rates that ensure that the cell populations achieve the correct homeostatic steady state values, which we use as initial conditions. The exact values for bone cell steady states can be found in Table 1. Our newly calculated parameter values are given in Table 4. The differences between our values and those computed by [49] can be attributed to our more detailed model of TGF- β dynamics, which were not previously considered.

3.1.3 Conversion Between Bone Volume and Calcium—In the following calculation of the conversion of bone marrow volume to calcium, we make several simplifying assumptions. We consider the skeleton to be composed of cylindrical bones (estimated by dimensions involved in calculating clavicle size) and we assume that bones are entirely marrow wrapped in cortical bone of constant thickness. We assume that all bone growth occurs radially outward, with no bones growing longer. We take all the calcium in the bone to be sequestered in the form of fully carbonated hydroxyapatite: $Ca_5(PO_4)_3CO_3$. For every mole of hydroxyapatite, there are five moles of Ca^{2+} ion bound to the matrix. The amount of calcium in moles is directly proportional to the moles of apatite, which can be converted into the mass of apatite via the division by apatite's molar mass (545g/mol). Bone apatite mass is 0.65 times cortical bone mass. Therefore, to convert from the volume bone in cm^3 to moles of calcium, we multiply the former by $\frac{65}{109} \text{ mol/g} \times 1.85 \text{ g/cm}^3$, the density of cortical bone. Blood volume is about .07 L/kg body mass. Using 70kg for an adult male weight, volume comes to 4.9 Liters. Ergo, each cubic centimeter of cortical bone dissolved into the bloodstream results in approximately 0.22 M increase in blood calcium concentration.

We derive an expression for Q , the amount of calcium released with 1% destruction of bone volume, by multiplying the $0.22M/cm^3$ by cortical bone volume in cubic centimeters divided by bone marrow volume in percent, and we obtain a conversion factor that is technically a function of bone volume. However, with the addition of bone volume calcium dependence, bone volume tends to remain relatively constant. Ergo, we substitute 100% for the dependence on bone marrow volume and obtain $Q = 14.9398 M Ca^{2+}/\% Bone$.

3.2 Parameter Estimation for Cancer Dynamics

In order to validate our approach that models both early (newly arrived in bone; PCe) and late (conditioned to the bone microenvironment; PCI) growth events, MC3T3 cells, a murine pre-osteoblast cell line, were grown in osteoblast differentiation media. After differentiation into osteoblasts, the media was refreshed to complete media and the cells were incubated for 24 hours at which time conditioned media was collected. Then the human prostate cancer cell lines, PC-3 and Du145, were plated and incubated in either normal complete medium with the addition of 10% FBS (to model the early stage) or a mixture of 50% complete medium and 50% MC3T3-conditioned medium with the addition of 10% FBS to the mixture (to model the late bone metastatic stage). After 24 hours of cell growth, cells were harvested into single cell suspensions. To determine the proliferative rate, cell suspensions were stained with the nuclear stain bromodeoxyuridine (BrdU) and subjected to flow cytometry to determine the percent of proliferating cells. To determine apoptotic fraction, cells were stained with an antibody to poly(ADP) ribose polymerase (PARP) a marker for apoptosis, then subjected to flow cytometry to determine the PARP positive percent of the population.

The results of these experiments are given in Table 5 and validate the differences in the growth potential for early and late prostate cancer cells proposed in our model. We also use this data to compute the percentage differences in proliferation and apoptosis between the two cells types in order to estimate parameters for PCa growth in humans.

Our mathematical model describes in vivo prostate cancer growth in humans, therefore parameters associated with PCa growth must be adjusted accordingly. Although difficult to accurately measure in humans, prostate cancer is known to be slow-growing, displaying tumor volume doubling times of months or years [22]. The doubling time for radical prostatectomy specimens of human prostate cancer, as opposed to the cell lines described above, has been reported to range from 0.6 to 3.6 months [51]. It has also been reported that serum PSA levels are proportional to the volume of prostate cancer and that the PSA doubling time in untreated patients should reflect the tumor volume doubling time [42]. In [37], data from 250 prostate cancer patients is reported to exhibit a median PSA doubling time 45 days (range 4.7–1108 days). We selected 30 days as representative doubling time within these ranges (changing this value does not change the qualitative features of our results) and computed the PCe proliferation rate (μ_{PCe}) using this baseline. However, because this growth rate is associated with tumors in an androgen rich environment, which is not the initial microenvironment they encounter in the bone, we scale this rate based on the assumption that androgen induces a 4-fold increase in proliferation.

The ligand-independent PCI proliferation rate (μ_{PCI1}) is assumed to be the sum of μ_{PCE} and the percent difference in average proliferation for PCI and PCE from the in vitro data. The ligand-dependent PCI proliferation rate is again computed based on the assumption of a 4-fold increase in proliferation due to ligand stimulation. Although the in vitro data show that, on average, a very small percentage of cells are apoptotic per day, we estimate the early prostate cancer cell death rate (a_{PCE}) by assuming that 10% of cells are apoptotic per day during early metastatic tumor growth in humans due to the initial incompatibility of the bone microenvironment. Similar to the derivation of μ_{PCI1} , the late prostate cancer cell apoptosis rate (a_{PC}) is assumed to be the sum of a_{PCE} and the percent difference in the average apoptosis for PCI and PCE from the in vitro data. Finally, although the PCE to PCI transition rate (ν_{PC}) is not known experimentally we assume that 1% transition per day. A full list of the parameters for the model equations that describe the dynamics of tumor growth (equations 14–19) are given in Table 6.

4 Results

Our model formulation and parameter choices ensure the bone cell populations and bone volume start at and remain in their respective homeostatic steady states, before the introduction of prostate cancer cells (results not shown). When cancer cells are introduced, several hormonal changes disturb this equilibrium, causing the system to create an environment more hospitable for the cancer. For our baseline parameters, when prostate cancer cells are present, we see in Figure 2 that the system approaches a steady state associated with high osteogenesis (characterized by a 25-fold increase in OBa in Figure 2a) and high tumor burden (Figure 2b). Though initially PCE is the dominant tumor cell type, the high osteogenic state is ultimately characterized by a significantly higher ratio of PCI to PCE (from approximately 1:106 at the low osteogenic state to approximately 10:1 at the high osteogenic state).

Figure 2 also shows how the chemical mediators of cancer and bone growth evolve with time. PTH increases to more than 60 times its pre-cancer value, and the RANKL/OPG ratio, an important effector of bone resorption, increases from the pre-cancer level 0.1233 to 20.5526 at the high osteogenic state in the model (Figure 2e). This imbalance stimulates osteoclastogenesis, which is increased by a factor of about 6 at steady state (Figure 2a). TGF- β increases over time (Figure 2f), which is indicative of elevated bone turnover. This is substantiated by Figure 3, which shows that bone production and resorption both rose substantially. The pre-cancer value of bone formation is set to be equal to the pre-cancer value of bone resorption, resulting in homeostasis as shown in equations (5) and (20). The slope of the bone volume plot in Figure 2c, when cancer is present, is exactly equal to the amount of bone created minus the amount of bone destroyed. This rapid bone turnover ultimately results in net bone creation (a 0.6% increase over the entire body as shown in Figure 2c), which requires calcium, depleting bloodstream reserves (Figure 2d).

Our results agree with those in [49] on several key points. Specifically, when cancer cells are present, RANKL levels and bone cell numbers are increased, while OPG levels are decreased. The temporal evolution of bone volume is different between this model and that in [49]. Here we see that volume increases over time while [49] predicts a net decrease in

bone. This difference is consistent with the fact that [49] describes a bone-resorbing cancer, whereas this model focuses on prostate cancer, which is known to produce bone [48].

An interesting feature of the new model is the potential for the system to approach a steady state associated with low osteogenesis (characterized by only a 5-fold increase in OBa) and a lower tumor burden (See Figure 2a). Our numerical analysis of the system suggests that a saddle-node bifurcation occurs when $\beta_{WNT} = 0$ and that the addition of any Wnt at all to the system renders the low osteogenic state unstable. For the parameters of cancer progression without intervention (baseline parameters), we see that while PCe is the dominant cancer cell type and DKK-1 is almost completely suppressing Wnt, the system attempts to approach the low osteogenic steady state (Figure 2). However, as PCl begins to take over and Wnt levels rise, the system is forced to the stable steady state associated with high osteogenesis. This suggests that Wnt is a major driving force in bone remodeling in prostate cancer and DKK-1 may be an important roadblock in cancer development.

To analyze the mechanisms that contribute to the existence of the low osteogenic state, we examine the significant variables associated with prostate cancer metastasis under various conditions: unimpeded bone and cancer growth (baseline), Wnt inhibition ($\beta_{WNT} = 0$), PSA inhibition ($\beta_{PSA} = 0$), and blockage of PSA's activation of TGF- β ($\beta_{LTGF\beta_2} = 0$). These results are displayed in Figures 4 through 6, where we observe that all the variables plotted present distinct steady states characterized by the low osteogenic state differing by a magnitude of at least 2, in most cases, from the high osteogenic state. However, we also observe that the existence and/or stability of the low and high osteogenic states with respect to β_{WNT} and $\beta_{LTGF\beta_2}$ for PCs (Figure 5) and PSA (Figure 6a) is different than the other system variables. In Figure 4 we see that for OBa cells, Wnt inhibition results in the existence and stability of the smaller, low osteogenic steady state, while blockage of PSA catalyzed TGF- β activation ($\beta_{LTGF\beta_2}$) eliminates the existence of this steady state and the approach to the high osteogenic state is delayed. Most of the variables react similarly to OBa when Wnt production and PSA activation of TGF- β are prevented. We shall refer to this behavior, where a variable presents two osteogenic states such that the lower occurs around $t = 2000$ days and depends on PSA activation of TGF- β , while the latter depends on the presence of Wnt and occurs around $t = 5000$ days, as the *canonical behavior*.

Further inspection of the model simulations reveals that not all variables exhibit this canonical behavior. Both PCl and PSA deviate from the canonical behavior in that they do not transiently approach their low osteogenic state when Wnt is present in the system. Instead, they exceed it without pause, only approaching the low osteogenic state when Wnt production is zero. This occurs because by the time PCl and PSA begin to increase, the rest of the system is already near the high osteogenic state. Similar to canonical behavior, setting $\beta_{LTGF\beta_2}$ to zero does indeed instigate a delay in reaching the high osteogenic state for both PCl and PSA, and inhibiting PSA production has a similar effect to inhibiting PSA mediated TGF- β activation for PCl. However, while reducing $\beta_{LTGF\beta_2}$ does delay the approach to the high osteogenic state for PCe, inhibiting Wnt results in a high osteogenic state that is characterized by an increase in the number of PCe cells. This is most likely caused by decreased competition with PCl and helps emphasize that Wnt is critical for full transition of the disease from the low osteogenic state to the high osteogenic state.

For variables that demonstrate canonical behavior, the high osteogenic state diverges from the low osteogenic state because of the presence of Wnt in the system. As shown in Figure 7, there is a single stable state when Wnt is not produced, but as the Wnt production rate increases from zero, the low osteogenic state becomes unstable and a stable high osteogenic state emerges.

However, these data imply that there is a causative agent that encourages cellular growth and microenvironment remodeling before the evolution of significant amounts of Wnt, and when this agent is blocked, the microenvironment should effectively stabilize until Wnt increases. In our model, PSA appears to be this agent, as blocking it eliminates the transient approach to the low osteogenic state and results in a significant delay in reaching the stable high osteogenic state. This temporal profile is the result of slow temporal increases in Wnt as PC cells increase; however, it is not able to have its full protumorigenic effect in the presence of DKK-1 until the shift to the high osteogenic state occurs around 8000 days (Figure 4a).

We conjecture that PSA accelerates osteogenesis in the model via the catalysis of latent TGF- β into its active form. We test this hypothesis through bifurcation analysis, sampling the low and high osteogenic states at different values of $\beta_{LTGF\beta_2}$, the maximum rate of PSA mediated activation of TGF- β . The bifurcation diagram shown in Figure 8 reveals two important details about PSA's activation of TGF- β . Firstly, when PSA is entirely inhibited from activating TGF- β , the low osteogenic state is essentially identical to the cancer-free steady state. This implies that the additional activation of TGF- β is critical for the establishment of a low osteogenic state. Secondly, the analysis reveals that this additional activation has little effect on the high osteogenic state, indicating that while this activation is important for accelerating the growth of cancer, it has little impact on final tumor burden. This implies that there is a requirement for TGF- β levels to rise before the high osteogenic state can be established, which is consistent with the results found in [10] detailing that TGF- β inhibition can help ameliorate metastasis.

Taken together, the model predictions agree with experimental observations on many counts, both quantitatively and qualitatively. We therefore suggest that it is able to characterize the progression of prostate cancer metastasis and its impact on the bone microenvironment.

Discussion

In this paper, we have developed a mathematical model of metastatic tumor growth in an effort to explain the dynamic interplay between prostate cancer and bone. The model modifies and extends several previously published mathematical investigations into tumor growth in the bone microenvironment. Important new features of this model include a greatly altered bone microenvironment, driven by increases in both PSA and Wnt. Interestingly, this new model admits solutions with multiple identifiable states: a high osteogenic state and a low osteogenic state. The low osteogenic state is characterized by lower tumor burden, a less altered bone environment, and a high amount of DKK-1 relative to Wnt. Numerical simulations suggest that the low osteogenic state emerges because excess PSA increases the activation of TGF- β , and bone remodeling is stalled at that state until sufficient amounts of Wnt are produced. This increase in Wnt provokes the system to

advance into a high osteogenic state, which is characterized by high tumor burden, a highly modified bone microenvironment, and larger amounts of Wnt relative to DKK-1.

In order to improve patient outcomes for aggressive cancers, an increasing amount of research is now being aimed at understanding the molecular biology of the tumor growth and metastasis in an attempt to selectively target pathways involved in accelerated tumor growth. As shown in Figure 5, blocking Wnt at the tumor site has the potential to be an effective anticancer measure, locking the system into the low osteogenic state. When PSA is first high enough to become diagnostically relevant, Wnt is still extremely low in the system. Hence, the system has yet to switch from the low osteogenic state to the high osteogenic state. As Wnt production is decreased, the high osteogenic state begins to approach the low osteogenic state, and the high osteogenic state becomes identical to the low osteogenic state when Wnt production is completely blocked. Figure 5 also shows that blocking Wnt results in a larger proportion of the total prostate cancer cell population being in the PCe cell state and the tumor burden is decreased overall. This eases the burden on other avenues of treatment, improving patient prognosis. Fully inhibiting DKK-1 degradation has the same effect as blocking Wnt entirely, again producing the same effect on cancer that occurs in Figure 5: the low osteogenic state is now the ultimate state of the system, as there is insufficient Wnt to overcome DKK-1. Increasing DKK-1 production drastically eventually causes the same effect shown in Figure 5, but DKK1- production must be increased to clinically infeasible levels that to achieve this.

The final cellular and chemical profile for our post-cancer model is consistent with several of the results obtained in [39]. As shown in Figure 3, the final value for the RANKL/OPG ratio falls within 19.62 ± 6.52 , in agreement with [39] for late stage prostate cancer metastasis. They also observe an increase in TGF- β and propose that it is the result of increased bone turnover. As shown in Figure 2, TGF- β is indeed significantly increased in our model and the hypothesis of increased bone turnover in [39] is supported by Figure 3. We also note that the low osteogenic state agrees with the general results in [39], with an increased RANKL/OPG ratio (Figure 3B), TGF- β (Figure 2F), and bone turnover (Figure 3A). Importantly, all three of these values fall between the pre-cancer and high osteogenic state values, emphasizing the transient nature of the low osteogenic state. Although our results agree with those in [39] on many counts, there is one noteworthy point for further discussion. Specifically, in [39], they observed relatively constant calcium levels while the model presented here predicts vastly decreased calcium levels. However, a case study in [46] shows that calcium levels can decrease due to cancer metastasis.

We also explored the possibility of RANKL being an effective potential inhibitor of cancer cell proliferation, as suggested in [2]. We tested two distinct possibilities: one where RANKL is completely blocked from the start, and the other where RANKL is completely blocked at diagnostically significant levels of PSA. As shown in Figure 9, however, both of these not only failed to prevent PC proliferation, they also exacerbated the progression of PCe to PCl and ultimately accelerated the progression of the cancer overall.

The major prediction of this model is the existence of low and high osteogenic states that are markedly different from one another. Measuring cell counts directly over the course of

disease would be the most precise method of determining whether this prediction holds; however, this is not particularly viable, as it is impractical to collect bone samples over the entire course of the disease. Instead, the most viable method of testing this model would be to attempt to block Wnt production in metastatic individuals in a doubleblind clinical trial. Should this model hold, blocking Wnt production should decrease tumor burden significantly in the experimental group. However, a more directly measurable result of this is the effect on TGF- β , whose levels are more easily measurable in a laboratory environment [26]. If the model predictions are correct, TGF- β levels should drop upon treatment with Wnt inhibitory drugs, as shown in Figure 6. It is also noteworthy that our model's prediction of a low osteogenic state could provide a partial explanation for the survivability of prostate cancer before it reaches Stage 4, as metastasis is limited at the low osteogenic state. The abrupt decrease in survivability upon reaching Stage 4 can then be explained by a shift to the high osteogenic state. If this model is validated, new treatment options can be developed with a focus on anti-Wnt protocols. Although the significance of Wnt and RANKL as contributors to tumor subversion of the bone microenvironment is unquestionable, we still do not fully understand how therapies targeted against these proteins will work *in vivo*. Continued modeling efforts in this direction have the potential to shed light on such important issues.

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Figure 1.

Schematic diagram illustrating the proposed interactions and feedback loops for the mathematical model. PCe = PCa early and PCl = PCa late. OBU = undifferentiated bone cells, OBP and OCP = osteoblast and osteoclast precursors; respectively. OBA and OCA = active osteoblasts and osteoclasts; respectively. LTGF β = latent TGF- β , BONEt = total bone, BONEp = bone production, and BONEr = bone resorption.

Figure 2.

PC growth and modification of the bone microenvironment. Temporal dynamics of osteoblasts and osteoclasts (a), prostate cancer cells (b); bone volume (c); calcium levels in the bloodstream (d); chemical mediators of osteogenesis, PTH, RANKL, and OPG (e); active and latent TGF- β (f); and WNT, DKK1, and PSA (g).

Figure 3.
Plot of a) Bone Turnover and b) RANKL/OPG ratio in response to prostate cancer subversion of the bone microenvironment.

Figure 4.

Behavior of a) OBa, b) OBp, c) OCa under baseline conditions, blocking WNT, blocking PSA, and blocking PSA's activation of TGF- β .

Figure 5.

Behavior of a) PCe and b) PCI under baseline conditions, blocking WNT, blocking PSA, and when blocking PSA's activation of TGF- β .

Figure 6. Behavior of a) PSA, b) RANKL, and c) TGF β under baseline conditions, blocking WNT, blocking PSA, and blocking PSA's activation of TGF β .

Figure 7.

Bifurcation diagram showing the steady state active osteoblasts (OBa) with respect to WNT production rate (β_{WNT}). The dot represents the baseline value used in simulations.

Figure 8.

Bifurcation diagram of steady state active osteoblasts (OBa) with respect to maximum rate of PSA mediated activation of TGF- β ($\beta_{LTGF\beta_2}$). The dot represents the baseline value used in simulations.

Figure 9.

The effects of RANKL interventions on a) proliferation of PCl and b) proliferation of PCe. The No RANKL curves represent RANKL blockage from time $t = 0$, and Block RANKL represents RANKL blockage from the time when PSA is diagnostically significant, at $0.118 \text{ } \mu\text{M}$ (derived from the standard 4 ng/mL used as a benchmark in PSA testing [50].)

Table 1

Cell Types and Initial Conditions

Cell Type	Description	Initial Values	Units
OBp	Osteoblast precursor cells	1.2005084×10^9	# of cells
OBa	Active Osteoblasts	1.0001628×10^9	# of cells
OCa	Active Osteoclasts	1.674764×10^8	# of cells
BONE _t	Normalized Bone Volume	100	%
PCe	Early PCa cells	0	# of cells
PCl	Late PCa cells	0	# of cells

Table 2**Bone Regulatory Proteins**

Signaling Molecule	Description	Units
RANKL	Receptor activator of NF- κ B ligand	pM
OPG	Osteoprotegerin	pM
Ca ²⁺	Bloodstream calcium	M
PTHrP	Parathyroid hormone-related protein	pM
TGF β	Active TGF- β	pM
LTGF β	Latent TGF- β	pM
WNT	Wnt signaling protein	pM
DKK1	Dickkopf-related protein	pM
PSA	Prostate Specific Antigen	pM

Table 3

Parameters Based on Literature for the Bone Remodeling Model

Variable	Description	Value	textbfUnits	Reference
OBu	Uncommitted osteoblasts	5.12×10^6	# of cells	[49]
OCp	Osteoclast precursor cells	2.00×10^9	# of cells	[49]
α_{OBa}	OBa apoptosis rate	0.30	per day	[49]
α_{OCa1}	OCa apoptosis rate ($-TGF_{\beta}$) ¹	0.30	per day	
α_{OCa2}	OCa apoptosis rate ($+TGF_{\beta}$)	1.2	per day	[49]
$\beta_{LTGF\beta_1}$	$LTGF_{\beta}$ transition rate ($-PSA$) ²	6.00	per day	[1]
$\beta_{LTGF\beta_2}$	$LTGF_{\beta}$ transition rate ($+PSA$) ²	6.00	per day	[1]
β_{RANKL}	RANKL production rate	2.15×10^{-7}	pM per cell per day	[49]
β_{DKK1}	Natural DKK1 production rate	4.35×10^{-7}	pM per cell per day	Calculated
β_{PTH}	PTH production rate	9.74×10^2	pM per day	[49]
β_{PTHrP}	PTHrP production rate	1.28×10^{-7}	pM per cell per day	[9]
β_{OPG}	OPG production rate	2.06×10^{-6}	pM per cell per day	[49]
i	Calcium intake rate	1.00×10^{-3}	M per day	[19, 28, 41] ³
$S_{Ca^{2+}}$	Normal calcium levels	1.25×10^{-3}	M	[40]
OPG_{max}	Carrying capacity for OPG	7.98×10^2	pM	[49]
ρ	Maximum RANKL on OBp	1.92×10^{-6}	pM per cell	[49]
$K_{TGF\beta_{a1}}$	TGF_{β} -OBu activation constant	4.28×10^{-4}	pM	[49]
$K_{TGF\beta_{r1}}$	TGF_{β} -OBp repression constant	2.49×10^{-4}	pM	[49]
$K_{TGF\beta_{a2}}$	TGF_{β} -OCa activation constant	4.28×10^{-4}	pM	[49]
$K_{WNT_{a1}}$	Wnt-OBu activation constant ⁴	4.28×10^{-4}	pM	
$K_{WNT_{a2}}$	Wnt-OBp activation constant	4.28×10^{-4}	pM	[9]
$K_{RANKL_{a1}}$	RANKL-OCp activation constant	47.9	pM	[49]
$K_{PTH_{total_{r1}}}$	PTH_{total} - OPG repression constant	2.21×10^{-1}	pM	[49]
$K_{A,OPG}$	Rate of RANKL binding to OPG	5.68×10^{-2}	per pM	[49]
$K_{A,RANK}$	Rate of RANKL binding to RANK	7.19×10^{-2}	per pM	[49]
$K_{PTH_{total_{a1}}}$	PTH-RANKL activation constant	2.09×10^2	pM	[49]
K_{DKK1}	DKK-1 repression constant ⁴	4.28×10^{-4}	pM	
K_{res}	Bone resorption rate	1.28×10^{-10}	% per cell per day	[49]
μ	TGF_{β} stored in the bone	1.00	pM per %	[49]
$\lambda_{LTGF\beta}$	Latent TGF_{β} degradation rate	11.09	per day	[27]
$\lambda_{TGF\beta}$	TGF_{β} degradation rate	499.1	per day	[27]
λ_{PTH}	PTHrP degradation rate ($-PSA$)	3.84×10^2	per day	[49]
λ_{PTHrP_1}	PTHrP degradation rate ($-PSA$)	86.0	per day	[9]
λ_{PTHrP_2}	PTHrP degradation rate ($+PSA$)	60	per pM per day	[9]

Variable	Description	Value	Units	Reference
λ_{OPG}	OPG degradation rate	4.16	per day	[49]
λ_{RANKL}	RANKL degradation rate	4.16	per day	[49]

¹ Chosen to be the same as α_{OBa}

² Estimated based on values given in [1], adjusted for human bone.

³ These three together help establish an effective range for calcium with lower and upper bounds.

⁴ Chosen to be the same as

Table 4

Calculated Values for the Bone Remodeling Model

Variable	Description	Calculated Value	Units
δ_{OBu}	OBu differentiation rate	1.73×10^3	per day
δ_{OBp1}	OBp differentiation rate	2.65×10^{-1}	per day
δ_{OCp}	OCp differentiation rate	3.20×10^{-1}	per day
K_{form}	Bone formation rate	2.26×10^{-11}	% per cell per day
Q	Calcium released with 1% destruction of bone volume	14.9398	$\frac{M\ Ca^{2+}}{\%Bone}$

In Vitro Prostate Cancer Cell Growth Parameters

BrdU-positive percentage	Proliferation rate per day	Apoptotic cells percentage	Apoptosis rate per day
DUI45-CM	PCa late	DUI45-CM	PCa late
DUI45-con	PCa early	DUI45-con	PCa early
PC3-CM	PCa late	PC3-CM	PCa late
PC3-con	PCa early	PC3-con	PCa early

Table 6

Parameters for the Model With Cancer Dynamics

Variable	Description	Value	Units	Reference
α_{PCe}	PCe apoptosis rate ¹	2.34×10^{-3}	per day	
α_{PCI}	PCI apoptosis rate ¹	4.27×10^{-3}	per day	
σ_{PCe}	PCe translocation rate ²	100	cells per day	
μ_{PCe}	PCe proliferation rate ¹	4.67×10^{-3}	per day	
μ_{PCI1}	PCI proliferation rate (–Ligands) ¹	4.39×10^{-3}	per day	
μ_{PCI2}	PCI proliferation rate (+Ligands) ¹	1.76×10^{-2}	per day	
$\beta_{LTGF\beta_2}$	LTGF β transition rate (+PSA) ³	6.00	per day	
β_{WNT}	Wnt production rate	6.39×10^{-9}	pM per cell per day	[9]
β'_{DKK1}	DKK1 production rate ⁴	6.39×10^{-9}	pM per cell per day	
β_{PSA}	PSA production rate	6.39×10^{-9}	pM per cell per day	[9]
PC_{max}	Carrying capacity for PC ⁵	2.6×10^{12}	# of cells	
ν_{PC}	PCe transition rate	0.01	per day	
K_{PSAa1}	PSA-LTGF β activation constant ⁴	4.28×10^{-4}	pM	
$K_{TGF\beta a3}$	TGF β -PCI activation constant ⁴	4.28×10^{-4}	pM	
K_{WNTa3}	Wnt-PCI activation constant ⁴	4.28×10^{-4}	pM	
λ_{WNT}	Wnt degradation rate	2	per day	[9]
λ_{DKK1}	DKK1 degradation rate ⁴	2	per day	
λ_{PSA}	PSA degradation rate	4.0	per day	[9]

¹Estimated as described in Section 3.2.²Based on 5 liters blood per person and 1 disseminated tumor cell (DTC) per 5 ml, we can estimate 1000 DTC per human marrow. Assuming a 10% apoptosis rate and steady state DTC, we arrive at 100 cells translocating to the bone per day³Chosen to be the same as $\beta_{LTGF\beta_1}$ so that the overexpression of PSA can at most double the maximum transition rate⁴Chosen to be the same as the value used for WNT⁵Total bone marrow volume is estimated as 2.6L ([49] Table 2). Potentially all of this could be replaced by PC cells, resulting in a volume of 2.6L of cells. Assuming 1 cc = 10^9 cells, then 2.6 L = $2600 \text{ cc} \times 10^9 = 2.6 \times 10^{12}$ cells.