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AN INSIGHT INTO BREAST CANCER METASTASIS TO BONE

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ABSTRACT

Breast cancer is the second leading fatal cancer in women following lung cancer. Out of three, two metastasize to bone. There are various factors (growth factors, genetic factors & some signaling pathways) responsible for metastasis, proliferation and colonization of cancerous cells to the bone. Once cancerous cells colonize to bone, they start a vicious cycle, which includes secretion of the osteolytic factors and tumor growth. The process of metastasis is a multistep process. Also, there are different types of breast cancer which includes Ductal carcinoma, lobular carcinoma, invasive ductal carcinoma and invasive lobular carcinoma. There are various therapeutics available against breast cancer, which focus on blocking osteoclast differentiation to prevent bone resorption. Various bioactive active compounds are reported to possess anticancer properties, which include Vitamin D, lysine, green tea extract, kampferol etc. They can act as nutritive therapy against cancer. This review deals with the risk factors and classification of breast cancer, mechanism of metastasis, current therapeutics available and their side effects.

KEYWORDS: *Breast cancer, metastasis, growth factors, signaling pathways,bone.*

INTRODUCTION

Cancer is a disease with many possible causes which includes genetic factors, life style, tobacco consumption, diet & physical activity (1). Normal body cells grow and divide to repair injury or to replace worn out cells and damaged cells (2). Then at the end they die. This is known as cell cycle. In cancer, the cells break this cycle and proliferate abnormally and uncontrollably. There are various reasons for the cells to break the cell cycle and proliferate abnormally. Some of them include genetic mutations, exposure to certain chemicals, and exposure to UV rays etc. Once the cells start to divide abnormally they form tumors. These tumor cells receive nutrients and growth factors from nearby normal cells and grow continuously. Some cells get detached from the tumor and travel to some distant organ through the blood circulation and colonize there. These cells then grow and form secondary tumors and this process of colonizing and growing in distant organs is known as metastasis

BREAST CANCER

The human body is comprised of trillions of cells. Normally during the fetal life the cells grow and divide frequently to make the new born grow (3). This is a regulated process. But as the person becomes an adult the cells mostly divide to repair injury or replace dying cells (3). Cancer is a disorder of uncontrolled growth of cells, morphological transformation, dysregulation of apoptosis and enhanced invasive activity (4). These cells divide randomly which is not required by the body (5). Cancer can start in any part of body like lungs, breast, liver etc (3). This review deals with breast cancer. Breast tissue consists of two types of cell i.e. lobular cells and ductal cells (6). Carcinoma may start from milk duct or from the lobules. Carcinomas of these cells are known as ductal carcinoma or lobular carcinoma (6). Cancer is a heterogeneous disease that is present in various forms (4).

RISK FACTOR

A risk factor is anything that affects the chance of getting a disease. Different cancers have different risk factors. For example, exposing skin to strong sunlight is a risk factor for skin cancer. Smoking is a risk factor for cancers of the lung, mouth, larynx (voice box), bladder, kidney, and several other

organs (7). Breast cancer risk factors are mentioned below:

GENDER

Simply being a female is an important risk factor for developing breast cancer. Males also develop breast cancer, but it is about 100 times more common among females than in males. This is likely because males have less of the female hormones estrogen and progesterone, which promote breast cancer cell growth (7, 8)

AGE

The risk of developing breast cancer increases as one gets older. About 1 out of 8 invasive breast cancers are found in women younger than 45, while about 2 of 3 invasive breast cancers are found in women aged 55 or older (7,8)

GENETIC RISK FACTORS

About 5% to 10% of breast cancer cases are thought to be hereditary, resulting directly from gene defects (called *mutations*) inherited from a parent. The following gene mutations have been implicated in breast cancer-

BRCA1 and BRCA2

The most common cause of hereditary breast cancer is an inherited mutation in the *BRCA1* and *BRCA2* genes. In normal cells, these genes help prevent cancer by making proteins that keep the cells from growing abnormally. If a mutated copy of either gene is inherited from a parent, a high risk of developing breast cancer during a lifetime. The encountered risk may be as high as 80% for members of some families with *BRCA* mutations. These cancers tend to occur in younger women and more often affect both breasts than cancers in women who are not born with one of these gene mutations. Women with these inherited mutations also have an increased risk for developing other cancers, particularly ovarian cancer.(7)

AR: ANDROGEN RECEPTOR

AR gene provides instructions for making a protein called an androgen receptor. Androgens are hormones that are important for normal male sexual development before birth and during puberty. Androgen receptors bind to the androgens and respond appropriately to these hormones. The resulting androgen receptor complex then binds to DNA and regulates the activity of androgen responsive genes. By turning the genes on or off as necessary, the androgen receptor helps direct the development of male sexual characteristics. In one region of the AR gene a DNA segment known as CAG is repeated multiple times. Some studies have suggested that a long CAG repeat region is associated with an increased risk of breast cancer in women (9)

ATM: ATAXIA TELANGIECTASIA

The ATM gene provides instructions for making a protein that is located primarily in the nucleus of cell, where it helps control the rate at which cells grow and divide. People with mutation in one copy of the gene is associate with an increased risk of developing cancer (10)

BRIP1: BRCA1 INTERACTING PROTEIN C-TERMINAL HELLICASE 1

The BRIP1 gene provides instructions for making a protein that is involved in repairing damaged DNA. BRIP1 protein interacts with the protein produced from BRCA1 gene. These two proteins work together to mend broken strands of DNA, which prevents cells from accumulating genetic damage that can trigger them to divide uncontrollably. BRIP1 and BRCA1 help control the rate of cell growth and division, these proteins are described as tumor suppressors (11).

CHEK 2 GENE: CHECK POINT KINASE 2

The CHEK 2 gene provides instructions for making a protein called check point kinase 2 (CHK2). This proteins act as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing too rapidly or in an uncontrolled way (12)

DIRAS3: DIRAS FAMILY, GTP-BINDING RAS-LIKE 3.

The *DIRAS3* gene is a member of a large family of genes known as Ras genes. Genes in this family provide instructions for making proteins that control cell growth and maturation. The DIRAS3 protein differs from other proteins in the Ras family in that it suppresses the growth of cells, whereas other Ras family proteins encourage cell growth. Genes that suppress cell growth and division are known as tumor suppressor genes. The proteins made from these genes keep cells from growing and dividing too fast or in an uncontrolled way.

ERBB2: "V-ERB-B2 AVIAN ERYTHROBLASTIC LEUKEMIA VIRAL ONCOGENE HOMOLOG 2."

The *ERBB2* gene provides instructions for making a protein called the ErbB2 growth factor receptor. This receptor is located on the surface of cells,

where it associates with similar receptors to form a complex. Growth factors bind to these similar receptors (ErbB3, for example) and trigger the receptor complex to relay signals inside the cell.

NBN: "NIBRIN."

The *NBN* gene provides instructions for making a protein called nibrin. This protein is involved in several critical cellular functions, including the repair of damaged DNA. Mutation in this gene is associated with increased risk of Breast Cancer.

TP53: "TUMOR PROTEIN P53."

The *TP53* gene provides instructions for making a protein called tumor protein p53. This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing too fast or in an uncontrolled way.

RAD50: "RAD50 HOMOLOG

The *RAD50* gene provides instructions for making a protein that is involved in several critical cellular functions, including the repair of damaged DNA.

FAMILY HISTORY OF BREAST CANCER

Breast cancer risk is higher among females whose close blood relatives suffer from this type of cancer. Having one first-degree relative (mother, sister, or daughter) with breast cancer approximately doubles a female's risk. Having 2 first-degree relatives increases the risk about 3-fold (7).

PERSONAL HISTORY OF BREAST CANCER

A female with cancer in one breast has a 3- to 4-fold increased risk of developing a tumor in the other breast or in another part of the same breast. This is different from a recurrence (return) of the first cancer (7).

RACE AND ETHNICITY

Overall, white females are slightly more likely to develop breast cancer than are African-American females, but African-American females are more likely to die of this cancer. However, in women under 45 years of age, breast cancer is more common in African- American women. Asian, Hispanic, and Native-American women have a lower risk of developing and dying from breast cancer (7).This can be attributed to their environment in which they live (13).

DENSE BREAST TISSUE

Breasts are made up of fatty tissue, fibrous tissue, and glandular tissue. Dense breast tissue (as seen on a mammogram) is characterized by glandular and fibrous tissue and less fatty tissue. Unfortunately, dense breast tissue can also make mammograms less accurate. A number of factors can affect breast density, such as age, menopausal status, the use of drugs (such as menopausal hormone therapy), pregnancy, and genetics (7). These factors effects in different ways-

AGE AND MENOPAUSE

During menopause, hormonal changes in the body cause the breast tissue to become less dense. So, in general, younger premenopausal women have denser breast than older post menopausal women.

PREGNANCY

Breast density decreases somewhat with each pregnancy. So, more children a women has given birth to, the less dense her breast tend to be.

3.7.3 Genetic factors- Having dense breast appears to run in family and is likely related to some genetic factors.

BREAST CANCER CLASSIFICATION

Breast cancer is classified into different group based on their invasive property and their origin area in the breast:

DUCTAL CARCINOMA INSITU (DCIS)

DCIS also known as intraductal carcinoma. It is most common type of invasive non-breast cancer. In this the cancerous cells are inside the ducts and have not invaded the walls of the ducts.

LOBULAR CARCINOMA INSITU (LCIS)

In this the cancerous cells resides inside the lobules. This is also a non invasive breast cancer.

INVASIVE (INFILTRATING) DUCTAL CARCINOMA (IDC)

IDC starts in the milk ducts of breast, invade the wall of the duct and reaches fatty tissue of the breast. After it reaches the fatty tissues it can metastasize to other organs through lymphatic system and blood stream.

INVASIVE (INFILTRATING) LOBULAR CARCINOMA (ILC)

ILC starts in lobules and it can metastasize to other parts of the body.

LESS COMMON TYPE OF BREAST CANCER

INFLAMMATORY BREAST CANCER (IBC)

It accounts for about 1% to 3% of all breast

cancers. In this type of cancer the skin of breast looks red, thick and pitted. Usually no lumps are observed. In early stages it can be confused with a infection called mastitis.

TRIPLE NEGATIVE BREAST CANCER

These are usually invasive ductal carcinomas where cells lack estrogen and progesterone receptors. They also lack HER2(Human epidermal growth factor receptor 2) protein on their surface. These types of cancers tend to grow more rapidly than other type of breast cancers because these cells lack receptors so they neither respond to hormone therapy nor drugs which target HER2.

PAGET DISEASE OF NIPPLE

This type of breast cancer starts in ducts and spreads to the skin of nipple and areola. The skin of nipple and areola appears crusted and scaly with areas of bleeding and oozing.

PHYLLODES TUMOR

These are very rare kind of tumors, which develop into connective tissue of breast. These are usually benign, but can be malignant.

METASTASIS TO DIFFERENT ORGANS

Cancer begins when cells in a particular part of body start growing uncontrollably (3). This is known as abnormal growth of cells (3). In these cells the DNA is damaged and yet the cell does not undergo apoptosis (3). Instead they keep on dividing and generate cells thus acquiring the same mutation (3). They can also get detached from the primary site and enter into the blood circulation or lymph nodes with this they get transported to the distant organ known as secondary site. After reaching distant organ the tumor cells go for extravasation and establish a microenvironment (15). Then they proliferate and metastasize and form secondary tumors (3).These secondary tumors cells have subtle differences not just in their phenotype but also in the responses to the therapy (16). This is known as metastasis. Common sites for metastasis are bone, liver, brain and lungs. The cancerous cells that detached from the primary site may lay dormant for a long period of time before going for metastasis (6). These cancer cells release growth factors which helps them to thrive and proliferate in other organs to form secondary tumors (17). It is also reported that the cancerous tissue contains a sub-population of cells which possess different properties like different gene expression which help these cells for angiogenesis, invasiveness and metastasis (17). According to one theory, it is postulated that the cancerous cells undergo clonal selection and only those cells are allowed to detach from the primary site which possess these properties (4). The success of these metastatic cells depends on the ability of these cells to survive in the new microenvironment (17). Skeleton is the most common site for metastasis (18) because continuous and dynamic turnover of the bone matrix (due to bone remodeling) provides a fertile ground for tumor cells to utilize the available resources such as growth factors, cytokines and receptors for their homing and proliferation (19).

BONE METASTASIS

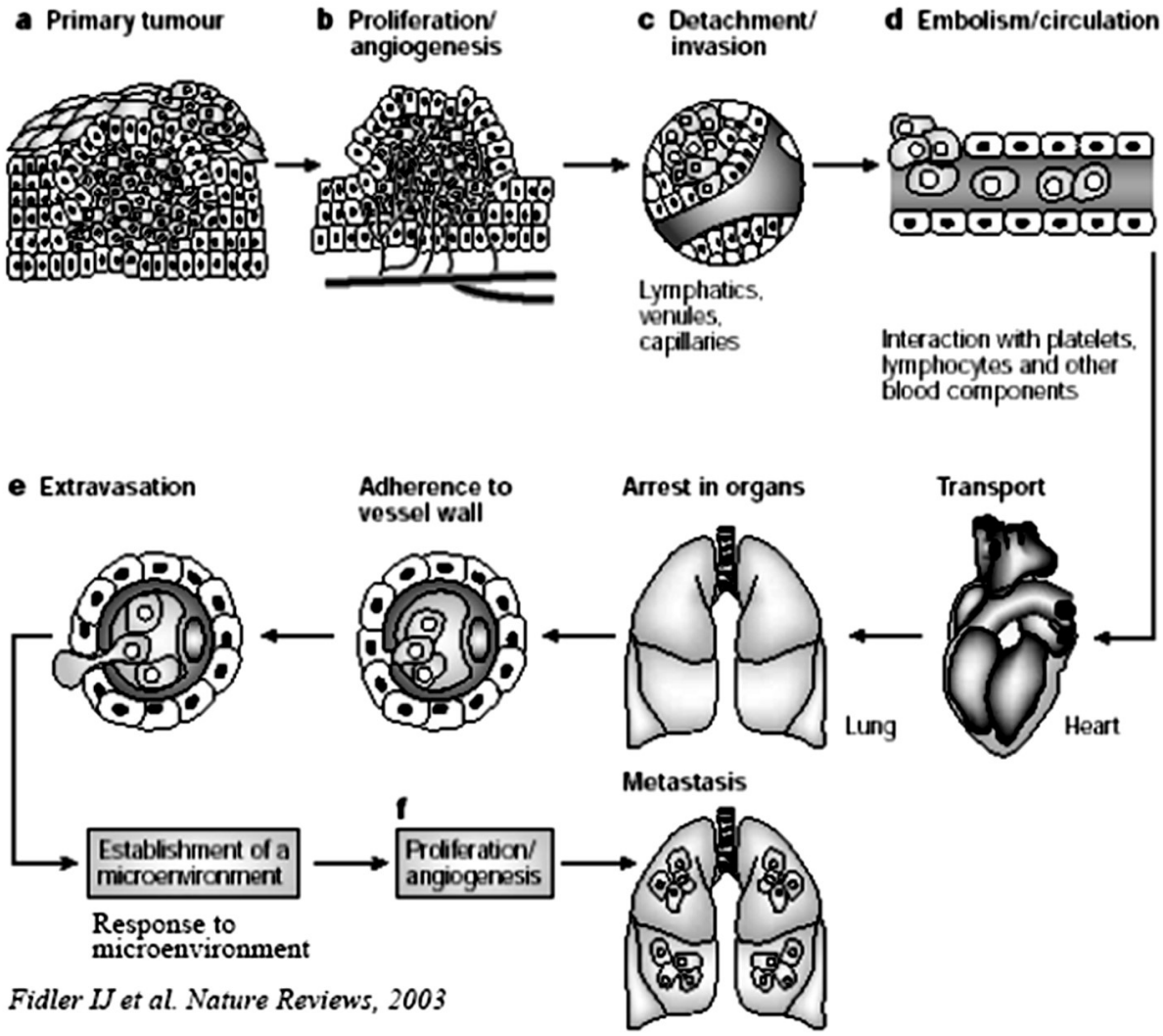

Figure 1
Figure showing steps in metastasis (20).

Metastatic cancer means the cancer started in one part of body and spreads to the distal part (3). Two out of three breast and prostate cancer metastasize to bone (3). Sir Stephen Paget enunciated a "soil and seed" hypothesis which states that certain tumor cell will selectively colonize a distant organ because of the presence of a favorable environment for their growth (19). Skeletal localization of tumor cells is because of its biological and molecular characteristics (19). Metastasis occurs commonly to the spine, pelvis, femur, humerus , ribs and skull. It can press on the spinal cord and this can lead to nerve damage and paralysis if untreated (3). Secondly, a high turnover of the bone matrix and availability of the growth factors like endothelin 1 (ET-1), fibroblast growth factor (FGF), transforming growth factor (TGF), IL6 and IL8 in the bone microenvironment provide a fertile ground for the initial growth and proliferation of the cancerous cell. Kong and coworkers hypothesized that there are specific genes that lead to the emergence of metastatic cell in primary tumor, but there are more specific set of genes responsible for the cellular activities, which help these cells in bone metastasis (19). These specific genes mainly encode cell surface and secretary proteins which help in the multistep process of metastasis, which includes colonization, angiogenesis and proliferation in the bone microenvironment (19). These genes include MMP 1 (Matrix metallo protease), CKC chemokine receptor , IL 11, Connective tissue growth factor and osteopontin. They are expressed in various combinations and enhance the potential of the cancerous cell to metastasize to the bone (19). Individual expression of these genes reduces the ability of cancerous cell for skeletal tropism (19). A metastatic cell tends to colonize the highly vascularized areas of skeleton and they disrupt not only bone physiology but also haematopoeisis and the immune system (5). Bones are made up of three types of cells, osteoclasts (bone resorpting cell), osteoblasts (bone forming

cells) and stromal cells (21). Correspondingly to this 3 types of cancers are detected in bone:

- Osteolytic
- Osteoblastic
- Mixed (21)

Majority of patients which shows metastasis from breast are lytic or mixed type of cancers (21), whereas metastasis from prostate cancer is predominantly osteoblastic (19). Mixed appearance indicates the osteoblastic and osteolytic processes (21). It is also reported that some tumors are initially osteoblastic and later they become osteolytic (16). Bone matrix is a large store house of various growth factors and cytokines which are released at the time of bone remodeling (19,5). These factors includes Insulin like growth factors (IGF), Transforming growth factor (TGFβ) (19), bone morphogenic protein (BMP), platelet derived growth factors (PDGF). These growth factors promote the colonization and proliferation of the tumor cells and start a vicious cycle, in which the tumor cells enhance the osteoclastic bone resorption with the help of these growth factors (15). These osteoclasts resorb bone, releasing more growth factors which includes TGFβ, parathyroid hormone related peptide (PTHrP), IL1, IL6, IL8, IL11 and TNFα (15). These factors up regulate the expression of RANKL (receptor activator of NF kappa B ligand) on osteoblast and stromal cells. RANKL promotes the expression of a surface glycoprotein extracellular matrix metalloproteinase inducer (EMMPRIN) which further induces MMP expression (22). This in turn promotes osteogenesis (4) and starts forming osteolytic lesions.

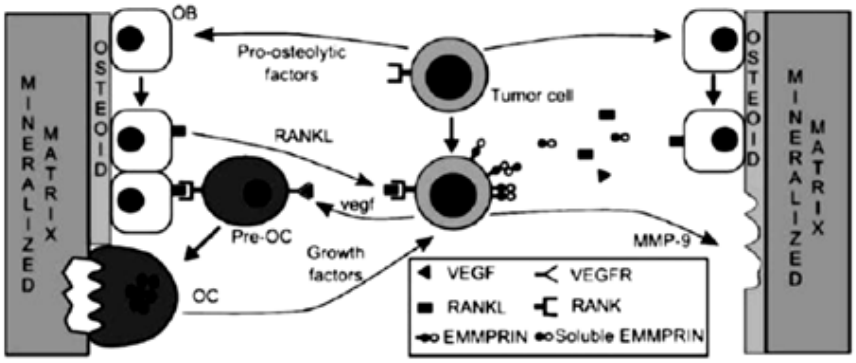

Figure 2

Schematic representation of the role played by EMMPRIN in the mechanism inducing osteolytic metastases. Molecular interactions among osteoblasts, osteoclasts, and tumor cells are shown. Tumor cells localized in the bone marrow produce factors (i.e., pro-osteolytic factors) that stimulate osteoclast formation by increasing the expression of RANKL on osteoblasts. RANKL binds to its receptor RANK and stimulates EMMPRIN expression in tumor cells. This in turn promotes the release of MMP-9 and VEGF. MMP-9 contributes to the digestion of organic matrix, whereas VEGF stimulates osteoclastogenesis. These two events favor tumor cells proliferation providing growth factors to tumor cells, thus contributing to the creation of the vicious cycle (Pre-OC, preosteoclast; OC, osteoclast; OB, osteoblast).

GROWTH FACTORS SECRETED BY TUMOR CELLS AND THEIR ROLE IN PATHOGENESIS OF OSTEOLYTIC LESIONS (19)

Factors	Role in pathogenesis
PTHrP (Parathyroid Hormone related peptide)	Up regulates RANKL expression and decreases OPG (osteoprotegerin) expression.
RANKL (receptor activator of NFkB ligand)	Stimulates osteoclastogenesis by binding directly to RANK.
IL6	Increases osteoclastogenesis via gp 130 signal transduction pathway, also enhances the effect of PTHrP.
IL1	Increases osteoclastogenesis (RANKL dependent and independent pathway), also promotes osteoclast activation and survival.
TNFα	Increases osteoclastogenesis and osteoclast activation via gp 130 signal transduction pathway as well as RANKL primed pathway.
IL8	Increases osteoclastogenesis by direct stimulation of CXCR1 receptors on the osteoclast

	precursor.
IL11	Increases osteoclastogenesis via gp 130 signal transduction pathway.
M-CSF	Up regulates RANKL expression on stromal cells: characteristic role for attracting osteoclast to resorptive sites and prolongs survival of the mature osteoclast by inhibiting apoptosis.
TGFβ	Inhibits osteoclast formation but can also directly stimulate osteoclast formation. (In absence of RANKL)
Prostaglandin	Up regulates RANKL expression and enhances the effect of soluble RANKL.
VEGF	Induces angiogenesis and promotes osteoclastogenesis.
MMP (Matrix metallo Protease)	Assist osteoclast mediated bone resorption.

MULTISTEP PROCESS OF METASTASIS

Breast cancer metastasis to bone is a complex, multistep event in which bidirectional interaction of a tumor cell with the cellular elements occurs in different environments:

1. Primary neoplasm
2. In circulation
3. Bone microenvironment

These metastatic cells must possess capacity to enter into blood circulation invade the bone marrow stroma cell, create its own blood supply and reach to the endosteal bone surface. The cells which survive this process enter the wide channeled sinusoids of bone cavity and start metastasis (16). Abundant sinusoids (small blood vessel or open pore capillary) and sluggish blood supply enhance the molecular interaction (majorly includes integrins) between tumor cells and endothelium which is important for their initial colonization in bone (16).

OSTEOLYTIC TUMORS

In these tumors the bone undergoes resorption. There are various growth factors which are secreted by tumor cells (19) as well as bone cells which together promote osteoclastogenesis. There are also pathways (Wnt signaling pathway, MAP Kinase pathway) involves which further enhances the metastatic process. In presence of macrophage colony stimulating factor (M-CSF) (4)(19) leads to expression of RANKL which then combines with the receptor RANK and leads to the activation of various signaling cascades (19,18) like NFκB, P38, Mitogen activated protein kinase (MAP), JUN amino terminal kinase which further helps in formation, maturation and survival of osteoclast leading to bone resorption (19). Monocytes are activated to form osteoclast via osteoblast (4). RANK receptor, a member of TNFα family, which is present on osteoblast and stromal cells have the ability to support differentiation and proliferation of cancerous cells. Even osteoclast differentiation and activation is mediated by RANKL (5).

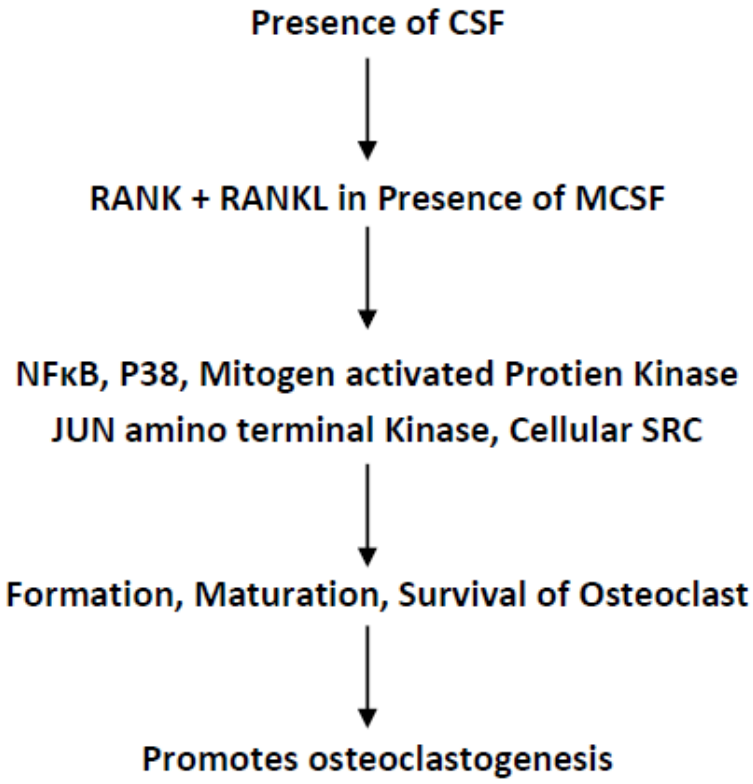

Figure 3
Flowchart showing osteoclastogenesis in osteolytic tumors via RANK and RANKL interaction leads to further degradation of bone.

Other factors that promote osteoclastic lesions-

FACTORS SECRETED BY TUMOR CELLS

Many factors secreted by the tumor cells help in the proliferation, differentiation and survival. These factors also help tumor cells in angiogenesis and help to survive at distant sites (4). There are various important factors that are secreted by tumor cells which include IL11, IL8, TNFα, PTHrP (parathyroid hormone related peptide) & CSF (4) (19). Key factors among these are PTHrP and CSF (16).

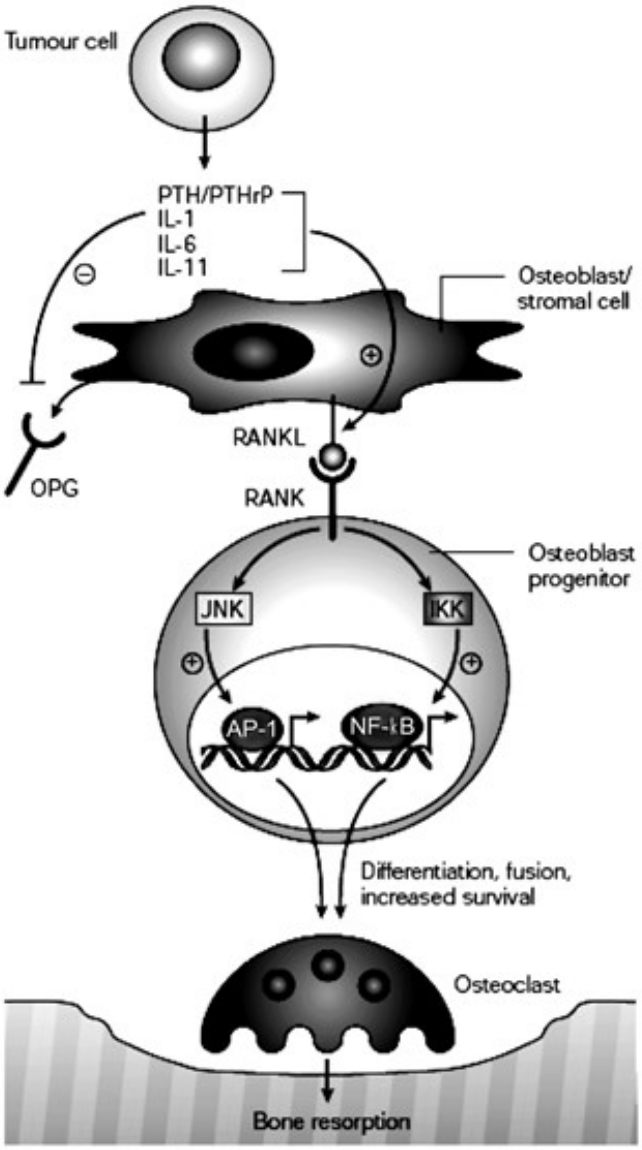

Figure 4
Figure showing RANK-RANKL interaction which further activates NFκB and JUN pathway in osteolytic bone metastasis.

PTHRP (PARATHYROID HORMONE RELATED PEPTIDE)

PTHrP is a analogue of parathyroid hormone (6). Increase in its expression in cancer cells enhances osteolytic lesions (5). It acts on the PTH receptors and increase bone resorption. Its expression increases when the tumor cells are present at the metastatic sites (16,23). PTHrP stimulates the activity of osteoclast by stimulating production of cytokine RANKL (16,6). RANKL expression is also increased when the tumor cells interact with the bone marrow stromal cells (16). PTHrP substitute for PTH in tissues that have their common receptors and thus participates in haemostasis and suppressing PTH (6). PTHrP also activates transient T Cell which further enhance osteoclastogenesis (4). Instead of combating with the cancer cells, T cells and macrophages may assist these cells in their survival and dissemination by mitigating the immune response. These cancer cells escape from the immune surveillance when they don't have any specific surface marker which differentiates them from the normal cells and sometimes after recognition by T cells also, T cell is unable to kill them.

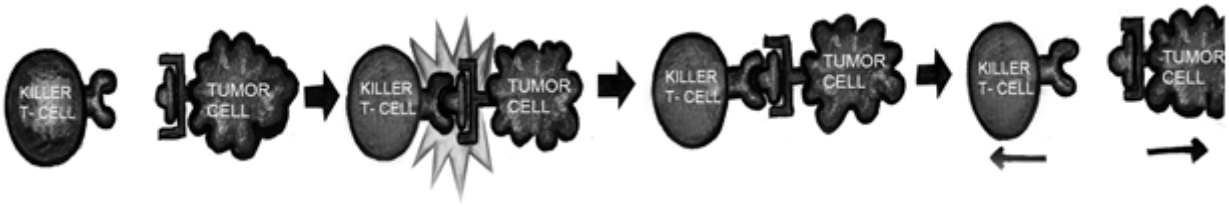

Figure 5
Immune response to cancer cells.

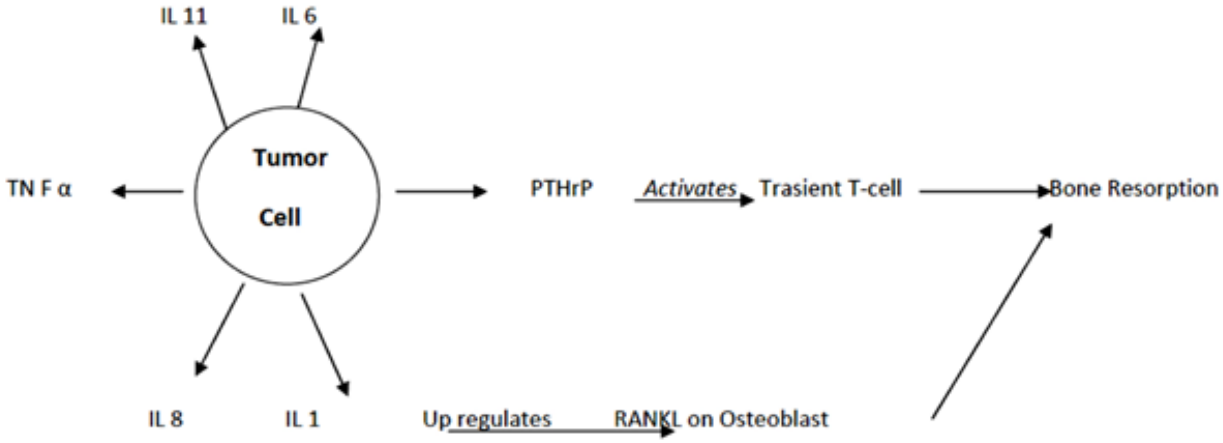

Figure 6
Diagram showing various factors released by tumor cells which leads to bone resorption.

TGFB (TRANSFORMING GROWTH FACTOR)
TGFβ is secreted by osteoclasts (24) and cancerous cells. It activates expression of PTHrP through both smad dependent and smad independent pathway which in turn induce osteoclastogenesis (5,25). Several MMP released from the bone matrix activate TGFβ which in turn enhance pre osteolytic factors through PTHrP (5,25). TGFβ also acts on other cells and make them sensitized for the growth factors secreted by the cancerous cells.

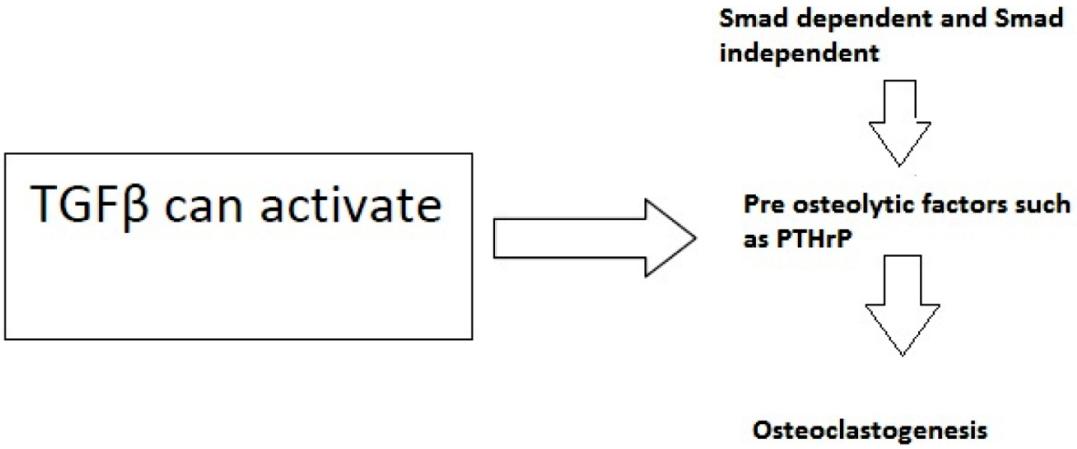

Figure 7
Flowchart showing activation of smad dependent and independent pathway by TGFβ which leads to osteoclastogenesis.

SMAD DEPENDENT PATHWAY
Smad proteins are important intercellular proteins which show their activity through TGFβ receptors and ultimately regulate gene expression (26)

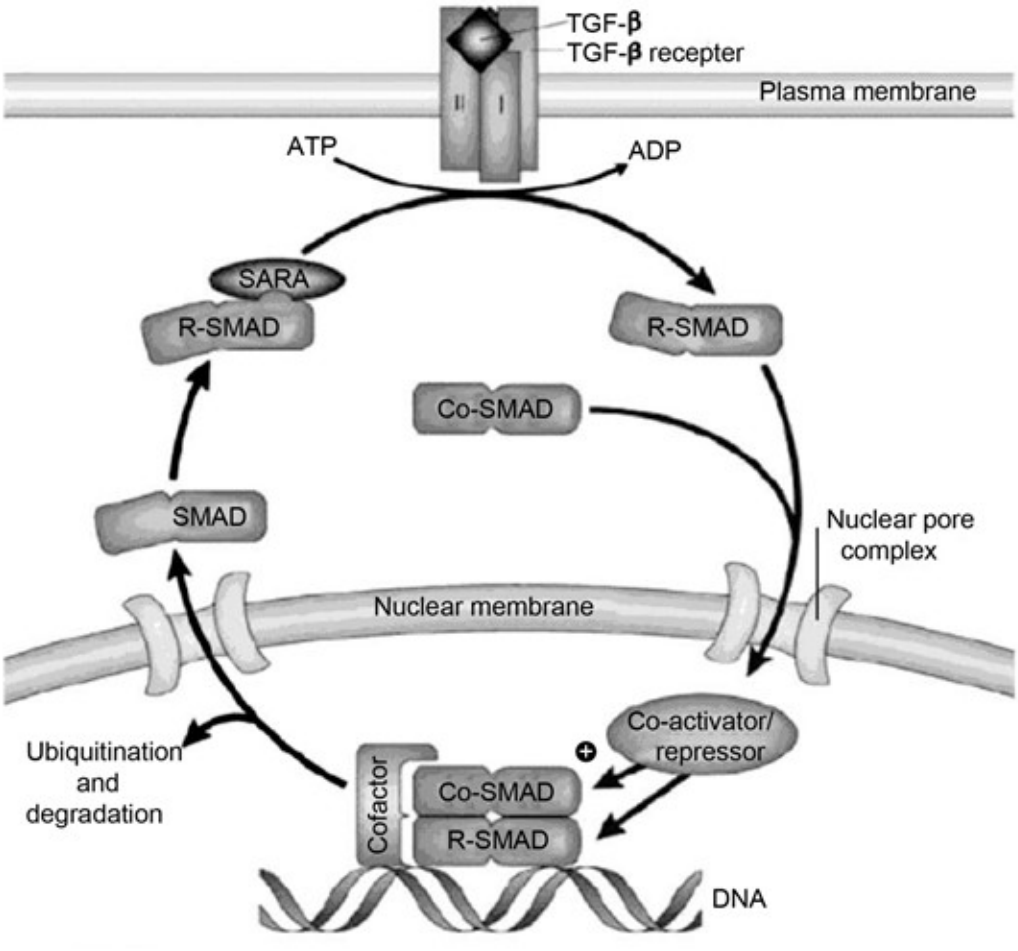

Figure 8
Smad dependent pathway.

SMAD INDEPENDENT PATHWAY

There are other non-smad proteins which participates in TGFβ signaling. The proteins which participate in this pathway are GTPase Ras, extracellular signal related kinases (ERK), p38 mitogen activated protein kinase (p38 MAPK) and c-Jun N-terminal kinases (JNKs). This pathway can directly activate ERK pathway without participation of smad proteins. Which in turn activate preosteolytic factors (26).

RUNX 2 (RUNT RELATED TRANSCRIPTION FACTOR-2)

Runx2 is a transcription factor produced by the metastatic cells and helps in enhacement of osteolytic lesions (5). It is considered to be the key regulator of osteoblast differentiation. It downregulates the osteoblast proliferation to promote bone turn over (5). RUNX 2 responds to TGFβ stimulation by increase in expression of Indian Hedge Hog, which further stimulates levels of PTHrP resulting in increased osteolysis (5). RUNX 2 also enhances the chances of survival of breast cancer cells in bone microenvironment (5). It also enhance osteolysis by increasing expression of RANKL through PTHrP (5).

Figure 9
Flowchart showing osteolysis through TGFβ via Indian Hedge Hog protein.

CATHEPSIN K

It is expressed by osteoclasts on the cell surface adjacent to bone (21). It is also produced by bone marrow stromal cells and macrophages (18). It is a key molecule which is responsible for the osteoclastic breakdown of collagen, it also enhances production of pro-inflammatory cytokines (IL6,IL11,IL8) and promote angiogenesis (18). Substrate for Cathepsin K is SPARC (secreted protein acidic and rich in cystein) and proteolytic degradation of which produces factors such as VEGF, PDGF, FGF-2 which further promote angiogenesis (18).

MMP (MATRIX METALLO PROTEASE)

MMP are proteases which contribute to the cancer initiation by promotion of angiogenesis, activating the stimulating growth factors and inhibiting the inactivation growth factors (27). These are divided into four groups- collagenases, stromelysins, gelatinases and membrane type MMP (27). Activation of MMP is associated with the cleavage of 10 kD amino terminal domain of MMP (27). These enzymes helps in further invasion of cancerous cells by degrading the component of ECM (extra cellular matrix) like collagen (27).

MMP may promote angiogenesis by two different mechanisms-

By degrading barriers- MMP degrade the basement membrane that surrounds capillaries, followed by invasion of the surrounding stroma by the underlying endothelial cells in the direction of the angiogenic signal. By liberating factors- MMP liberates the growth factors which promotes or maintain the angiogenic phenotype.

COX (CYCLO-OXYGENASES)

There are two types of cox enzyme COX-1 and COX-2. COX-1 is constitutively expressed whereas the expression of COX-2 is limited to kidneys, bone etc (5). In breast cancer cells the levels of COX-2 are found to be elevated (5). This increase in COX-2 leads to the secretion of PGE2 (prostaglandin) which is associated with inflammation, cell growth and metastasis (5). PGE2 then binds with its receptor EP4 and stimulates monocytes to form mature osteoclasts and increases production of RANKL leading to bone resorption (5). Secondly, cell-cell interaction between the breast cancer cell and osteoblast also lead to overexpression of cox-2 by activating NFκB/ mitogen activated protein (MAP) pathways (5).

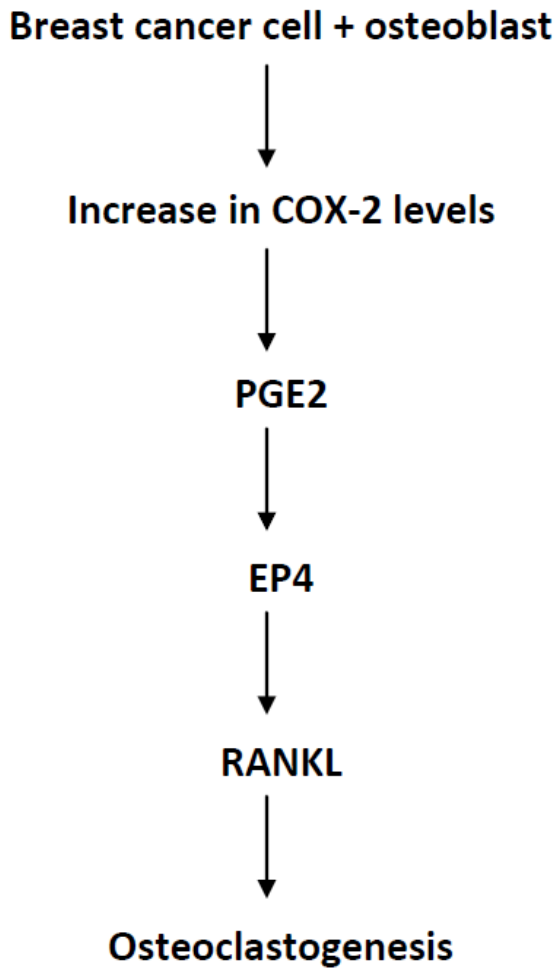

Figure 10
Initiation of osteoclastogenesis by tumor cells by releasing different factors.

INHIBITORS OF OSTEOCLASTOGENESIS

OSTEOPROTEGERIN (OPG)

OPG is a secreted TNF receptor and acts as decoy receptor for RANKL (19). It prevents the interaction of RANK and RANKL by sequestering RANKL (19). OPG forms a complex with RANKL to prevent its interaction with RANK (28). Hence OPG decreases osteoclastogenesis and enhance osteoclast apoptosis (19). So, the ratio of RANKL to OPG determines the extent of bone degradation. It also inhibits mineral deposition in osteoid (5).

Figure 11
Figure showing inhibition of RANK and RANKL by OPG

OTHER FACTORS

ESTROGEN

Estrogen is a key factor which effects bone remodeling (5) and bone turn over. It suppresses the production of RANKL by enhancing the production of OPG, which is a decoy inhibitor of RANKL (5). It also promotes apoptosis of osteoclast and inhibit their differentiation (5) and slows down the bone resorption.

INTEGRINS

Integrins are the cell surface molecules which play a key role in metastasis (17). Alteration in integrin expression and function has been reported in breast cancer and this leads to the dissociation of cancer cells from primary tumor and metastasis site (17). These cancer cells appear to travel as a part of the fibrin clot so they can reach at the distal site. Integrin αvβ3 has been shown to be up regulated in metastatic tumors.

GENES INVOLVED

These genes can be grouped into two categories-

PROTO-ONCOGENES

Mutant alleles of these genes are called as oncogenes. Since mutation in a single allele of a proto-oncogene can lead to cellular transformation, such mutations are considered dominant.

TUMOR SUPRESSOR GENES

Both alleles of a tumor suppressor gene must be altered for transformation to occur. They encode proteins which prevents the formation of tumors. These can be again divided into promoters and Caretakers.

PROMOTERS

These are traditional tumor suppressor genes. They act as breaks on the cell cycle. Mutation in these genes makes the cells to proliferate uncontrollably.

CARETAKERS

Caretaker genes are responsible for processes that ensure the integrity of the genome, such as those involved in DNA repair (29) There are various genes which are thought to be involved in breast cancer they include erbB2, c-myc, ras and tumor repressor genes P53 and Rb (27). Some of the oncogenes may contribute to carcinogenesis by regulating the expression of MMP's (27). Other genes which are responsible for metastasis include CXCR4, CTGF, IL11 and OPN [11]. In this CXCR4 is responsible for homing, CTGF for angiogenesis and IL11, OPN for osteolysis. This breast cancer then metastasizes to bone with the help of various gene expression and growth factors. There are various therapeutics available but all have their own side effects.

1Retinoblastoma (RB) is a rare childhood tumor of the eye .Most cases (60-70%) are sporadic (as opposed to inherited), occur unilaterally (affecting one eye), and present in children 1-4 years of age. The remaining 30-40% of patients have a hereditary form of retinoblastoma and thus have inherited a germline cancer predisposing mutation. In families with the inherited form of retinoblastoma, the disease shows an autosomal dominant inheritance pattern (29).

Genes encoding Cdk inhibitors are tumor suppressor genes. Mutational inactivation of CDK inhibitors also drives the cell cycle by unregulated activation of cyclins and CDKs. One such inhibitor, encoded by the **p16** gene, is a common target of deletion or mutational inactivation in human tumors.

p53: a key tumor suppressorp53, located on chromosome 17p13.1, is the single most common target for genetic alteration in human tumors. In fact, more than 50% of human tumors contain mutations in this gene. Thus it is among the most important "brakes" on tumor formation. Homozygous loss of the p53 gene is found in virtually every type of cancer, including carcinomas of the breast, colon, and lung – the three leading causes of cancer deaths.

THERAPEUTICS

There are various therapies available against bone metastasis which generally focus on blocking osteoclast differentiation or stimulating their apoptosis to prevent bone resorption (16).

SURGERY

In this the tumor is removed surgically. The surgeon will try to remove the tumor as much as possible. The tissue removed from the patient will often be examined by a pathologist for signs of tumor cells near the edge of the incision. This is to ensure that all detectable cancer cells have been removed. If no cancer cells are visible in the tissue surrounding the excised tissue, the specimen may be said to have 'clean margins'. This refers to the

fact that all visible tumor cells have been removed. (30)

BISPHOSPHONATES

These are class of compounds that are used to inhibit tumor induced osteolysis. It also inhibits angiogenesis and induce osteoclast to go for apoptosis by binding to hydroxyapatite of the bone matrix and are ingested by the osteoclast which then undergo apoptosis (5). It also helps in increasing bone density (5).

HORMONE BLOCKING THERAPY

Block the estrogen receptor, so that tumor will not receive growth factors to proliferate. Also they can block the production of estrogen with aromatase inhibitor, Ex- anastrozole (31). These aromatase inhibitor drugs block the activity of the enzyme aromatase, which is responsible for the formation for estrogen (32). When aromatase is blocked there will be no production of estrogen, ultimately tumor will not proliferate because of absence of estrogen.

CHEMOTHERAPY

Certain combinations of chemicals are given for ex- cyclophosphamide and doxorubicin. It is given in estrogen negative tumor. These chemicals target the tumor cells.

RADIATION

A measured quantity of radiation is targeted on the area to destroy the fast growing tumor cells.

ANTIBODY RELATED THERAPIES

14.6.1 Antibody against RANKL

Denosumab is an antibody against RANKL and shown to prevent osteoclast differentiation (5).

ANTIBODY AGAINST PTHRP

It is reported that cancer metastasis can be blocked by directing monoclonal antibody against PTHrP (16).

SIDE EFFECTS

Side effects can be defined as unwanted effects of treatment. (33)

SIDE EFFECTS OF SURGERY

Cure by surgical removal depends on the size, location and stage of the disease. Sometimes, the whole organ affected by tumor is removed (30). Which brings about
weight shift in the body (34)

SIDE EFFECTS OF BISPHOSPHONATES INCLUDES

- Stomach upset
- Inflammation and erosion of oesophagus
- Flu like symptoms
- Osteonecrosis of jaw
- Heart failure
- Coronary artery disease
- Fluctuations in blood calcium levels

SIDE EFFECTS OF HORMONE BLOCKING THERAPY

This therapy works best for hormone receptor positive cells. (33) side effects include:

- Hot flushes and sweat
- Vaginal dryness or discharge
- Feeling sick
- Painful joints
- Tiredness (33)

SIDE EFFECTS OF CHEMOTHERAPY

Chemotherapy works by destroying fast growing tumor cells as well as fast replicating normal cells like hair follicles. Chemo drugs can also damage cells of-

- Heart
- Kidney
- Bladdar
- Lungs (35)

It can also cause-

- Nausea
- Vomoting
- Sore mouth
- Diarrhea
- Allergic and dermatologic reactions (36)

SIDE EFFECTS OF RADIATION

- Reddening and soreness of skin
- Discomfort and swelling of breast
- Tiredness
- Firmer breast tissue
- Shrinking of breast tissue
- Small red marks
- Darker skin

NUTRITIVE THERAPY

There are wide range of compounds which are found to be effective against cancer. Some of them are –

VITAMIN D

Vitamin D and its analogues are found to decrease the levels of PTHrP which in turn decrease the process of osteoclastogenesis (37). Vit D is also

found to exert anti proliferative and pro apoptotic effects on the target tissue (23). It is accompanied by arresting cell cycle in G0 or G1 phase by regulating the expression of key regulators of cell cycle such as increases cyclin dependent kinase (cdk) inhibitors (38). It can also suppress the growth of cancer by modulating bone environment.

LYSINE

Lysine interferes with the activation of plasminogen into plasmin, by binding on the plasminogen active site (39). Lysine with ascorbic acid and proline enable proper synthesis and hydroxylation of collagen fibres (39). Lysine also enhances ECM stability as it is a key component for synthesis and hydroxylation of collagen fibres which is major component of ECM (39).

GREEN TEA EXTRACT

Green tea extract contributes to control cancer cell growth, metastasis, angiogenesis and other aspects of cancer progression (39)

KAMPFEROL

It is a flavonoid extracted from the medicinal plant *Polygonam Tinctorium* and is found to have stimulatory effect on differentiation and mineralization of the pre osteoblastic cell line (40). When it is combined with ipriflavone it enhances the calcium deposition in bone (40). Other compounds which are extracted from this plant have antiviral and antimicrobial activities (40).

CONCLUSION

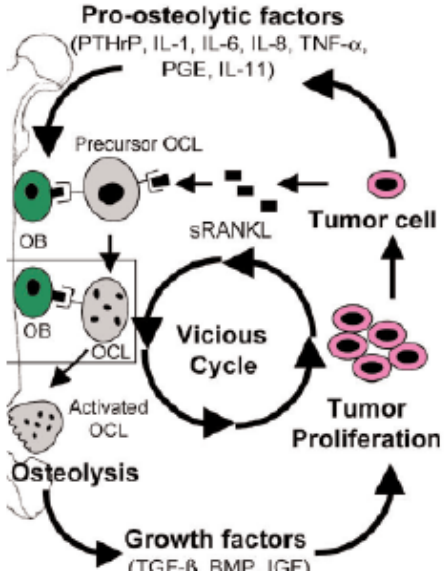

Figure 12
Figure showing vicious cycle

Vicious cycle in osteolytic bone metastasis. The pro-osteolytic factors secreted by the tumor cells (PTHrP, IL-1, IL-8, IL-11, soluble RANKL, TNF-α,and PGE) promote osteolysis by stimulating osteoclast formation and maturation. The growth factors secreted following osteolysis (BMP, IGF, and TGF-β) are stimulatory for tumor growth, which results in increased tumor burden and eventually more osteolysis. The inset delineates the regulation of osteoclast formation and activation. RANKL on the osteoblast/stromal cells interacts with the RANK on the osteoclast precursors in the presence of M-CSF to stimulate their differentiation into mature osteoclasts. BMP, bone morphogenetic protein; IGF, insulin-like growth factor; M-CSF, macrophage colony-stimulating factor; OB, osteoblast; OCL, osteoclast; PG, prostaglandin; PTHrP, parathyroid hormone related peptide; IL, interleukin; RANKL, receptor activator of nuclear factor-κB ligand; TGF, transforming growth factor; TNF, tumor necrosis factor.

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