-Review-

Extraterritorial osteoclast traits of primary cancer cells

Henning M. Schramm

Society for Cancer Research, Institute Hiscia, 4144 Arlesheim/Switzerland. E-mail:h.schramm@vfk.ch

Received: 2011-07-07 **Accepted:** 2011-08-28 **Published:** 2011-10-20
DOI:10.5430/jst.v1n2p65 **URL:** http://dx.doi.org/10.5430/jst.v1n2p65

Abstract
Cancer cells seem to originate in the epithelium however the majority of their phenotypic functions and traits do not resemble an epithelian but a preosteoclast/osteoclast cell. This applies not only to bone metastases but also the primary tumor, where these characteristics are exhibited extraterritorially. The most remarkable common features between osteoclasts and cancer cells include, among many others, matrix-resolving properties, hormone and neuronal dependence, coupling with mesenchymal cells, migrating and transmigrating properties, neurogenetic activities, trafficking to the bone, immune deviation, sensitivity to antirheumatics, bisphosphonates, polyphenols and steroids, constitutive activation of NFkappaB, and the same cytokine and chemokine signalling pathways. Below we present the corresponding findings reported in scientific publications and discuss various aspects of this congruency between cancer cells and preosteoclasts/osteoclasts.

Key words:
Cancer cells; Osteoclasts; Mesenchymal cells; Phenotypic properties; Signaling pathways

INTRODUCTION
Cancer cells start to proliferate within epithelial cells, and they seem to originate here with their cytokeratin scaffold. However, many of their traits are more similar to the cells of macrophage/monocyte lineage than to epithelial cells. To explain this peculiar feature of cancer cells, Pawelek and others proposed that cancer cells coopt macrophage traits through macrophage-tumor cell fusions. [1] In our previous studies we investigated why no primary carcinoma had been detected in the auditory inner ear in various large clinical studies on inner-ear tumors. As the development of preosteoclast lineage is inhibited in the auditory inner ear, we raised the question of whether these cells are required for "malignant transformation of epithelial cells". [2] In order to examine this question in more detail, we here compare the traits of cancer cells with those of preosteoclast lineage.

Both cell types, macrophages and preosteoclasts, originate from the same progenitor cell; it is therefore not surprising that the macrophages already share many traits with cancer cells. However, some important traits are not shared, such as coupling with mesenchymal cells or sensitivity to antirheumatics, bisphosphonates, polyphenols and other substances and the common receptor for osteocalcin - not expressed by macrophages.

Cancer cells and preosteoclasts differ in one decisive respect: that the former may proliferate limitlessly whereas the preosteoclast cells fuse with each other to form multinucleated giant cells. Assuming as a hypothesis that both cells may originate from a common progenitor cell, the question arises whether a common prerequisite may underlie these two

different processes. Before we discuss this question, we will compare the various features of cancer cells with those of preosteoclasts, and focus our review on aspects which seem to be common key features of cancer and bone remodelling.

Common traits between cancer cells and osteoclasts

Cancer cells may show hormonal, neuronal, metabolic and immunological interactions, require mesenchymal coupling for their growth, have extreme matrix-dissolving capacities by secreting metalloproteinases, are endowed with extreme plasticity and are sensitive to antirheumatics, bisphosphanates and polyphenols. These typical features also apply to osteoclasts as we will demonstrate in this review. A wide diversity of molecules and signalling pathways regulating adhesion, matrix alteration, neoangiogenesis, motility, chemotaxis, immune signalling, and multidrug resistance proteins, are traits we detect in cancer cells as well as in preosteoclasts. Several publications describe the osteomimetic properties of metastasizing prostate and breast cancer cells. [3-5] To our knowledge, the finding that primary cancer cells already show a large spectrum of different osteoclast aspects – with scaffold and proliferation as exceptions - has not so far been reviewed and investigated.

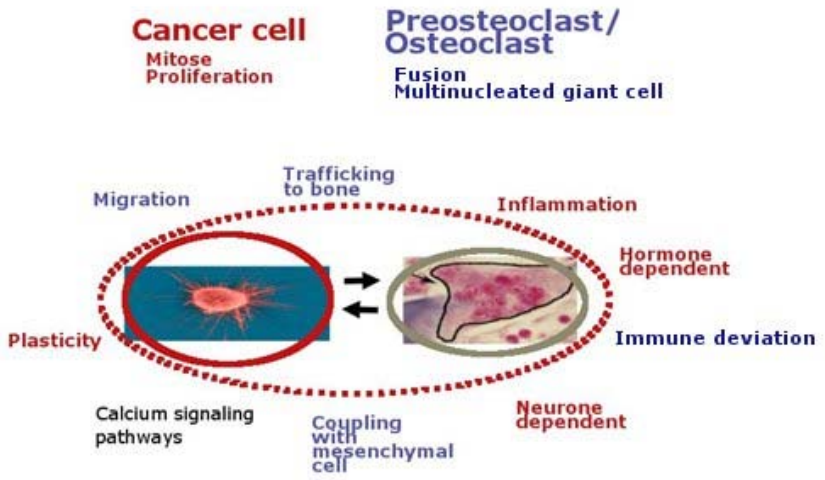

Figure 1 Common traits – around the oval circle – and divergent features – under the cell names – between a cancer cell and a preosteoclast/osteoclast

Migration

Trafficking to the bone

The migration of cancer cell, and of preosteoclast, are very reminiscent of each other. [6] Cancer cells and osteoclasts show the same extraordinary and unusual ability to migrate and transmigrate through cell layers and tissues in order to reach the skeleton. Although nearly all cancers may frequently metastasize to the bone, the commonest malignant tumors have a special predilection for the skeleton. In particular, cancers of breast, prostate, thyroid, kidney, rectum, uterus, cervix, bladder and lung frequently metastasize to the skeleton. The epithelial cancer cell transforms itself into a mesenchymal-like cell and gains extreme capacities to migrate through layers and tissues in order to reach its target. [7] When becoming committed to the osteoclast lineage, monocytes in the peripheral blood, and perhaps in inflamed areas, traffick to the bone in the same way, but there fuse with their equals. [6]

The trafficking of cancer cells and osteoclasts to bone involves many factors, among them chemo-attractants such as SDF-1(CXL-12) /CXCR4 axis, MCP-1, transcription factors such as NFkappaB and STAT3, cytokines such as RANK/RANKL and Il-6, hormones such as osteocalcin, oestrogen and androgens, further neurotransmitters, alphaVbeta3 integrins/ osteopontin, and calcium signalling pathways. [6]

 ISSN 1925-4067 E-ISSN 1925-4075

Migrating and transmigrating properties

Osteoclast precursors, like cancer cells, are able to cross the endothelial layer of blood capillaries and other basement membranes. Both cell types show mesenchymal as well as amoeboid migration. [7] In cancer cells as in osteoclasts, dynamic actin remodelling appears to be one major factor endowing these cells with their migrating property. During transmigration, integrin alphaVbeta3 is expressed around actin patches in cancer cells as in osteoclast precursors. [6] Matrix metalloproteinases (MMPs), secreted at the podosomes site, are required for the degradation of the extracellular matrix; and this contributes to the invasiveness of cancer cells. The same function facilitates the migration of osteoclasts through barriers, to form pits in the bone. [8]

The roles of the Chemokine receptor CXCR4 - chemokine SDF-1 (CXCL12) axis in cancer cells and in osteogenesis

The chemokine receptor-chemokine axis CXCR4/SDF-1 plays multiple roles in tumor pathogenesis [9] as well as in osteogenesis. [8] The tumor cells crosstalk with tumor-associated fibroblasts as do osteoclasts with osteoblasts via the chemokine axis CXCR4/SDF-1.

CXCR4/SDF-1 signalling is active in all cancer cells studied including rhabdomyosarcomas, prostate, kidney, melanoma, ovarian, endometrial cancer cells, multiple myeloma, plasma cells, nasopharyngeal carcinoma and brain tumors. [10,11] CXCL12 (SDF-1) is released by cancer-associated fibroblasts under a variety of conditions and promotes the malignancy of tumor cells. [12] The chemokine is important for the growth, angiogenesis, survival and trafficking to bone of malignant cells. [13] HER2 enhances the expression of CXCR4 in breast cancer cells. [14] It contributes to immune-suppressive networks within the tumor environment. [15]

Circulating osteoclast precursors react via their chemokine receptor CXCR4 to its ligand SDF1 produced by bone endothelium, bone marrow stromal cells and especially by osteoblasts. SDF1 stimulates matrix metalloproteinase-9 activity in the osteoclast precursors and enhances their directional migration through the collagen. It further initiates their differentiation into mature osteoclasts in cooperation with RANKL and other cytokines.

Matrix dissolving properties (tissue invasion and metastases): MMPs and Cathepsin K

MMPs and cathepsin are excreted by a variety of cells, among them cancer cells and osteoclasts. As proteases both possess matrix-resolving properties. These proteases are expressed in nearly all tumors including inflammatory cells contributing to tumorgenesis, and stimulate tumor growth, invasion, migration and metastasis. [16-19]

MMP-9 and MMP-2 secretion plays an important role during migration of the preosteoclast, and is necessary for the initiation of bone pit resorption. Especially during differentiation into osteoclast, MMP-9 excretion is increased. [8]

Cathepsins are members of the lysomal cysteine proteases family. Cathepsin K is overexpressed by cancer cells and cancer-associated fibroblasts. [20] Cancer patients with adenocarcinomas and squamous cell carcinomas of the lung, and carcinomas of breast and prostate, with high cathepsin K expression, showed a reduced overall survival. Stromal cathepsin K expression was significantly higher in invasive cancer compared with in-situ carcinomas, and thus correlated with higher tumor stage. In some cancer models, and in breast cancer biopsies, cathepsin K could not be detected in the neoplastic cells, but was found in the tumor's stromal cells. [18]

Cathepsin K is highly expressed in osteoclasts and plays an essential role in bone remodelling as shown by the osteopetrotic phenotype of cathepsin-deficient mice. [18] Overexpression of cathepsin K in osteoclasts results in enhanced bone resorption.

Neurogenesis and neuronal dependency

Bone remodelling, like most other homeostatic functions, but also carcinogenesis, cancer progression and metastasizing capacities, is subject to sympathetic influence. 70% to 90% of breast, colon, gastric, lung, ovarian, nasopharyngeal and prostate cancer tissues express beta2-adrenergic receptors. The migration of breast, prostate, ovary and colon carcinoma cells is enhanced by the stress-related neurotransmitter norepinephrine, and this effect can be inhibited by the beta-blocker propranolol. [21]

Both osteoblasts and osteoclastic cells are equipped with adrenergic receptors and neuropeptid receptors. [22] Sympathetic neurons in the hypothalamus and bone control osteoblast activity in bone formation, and osteoclast activity in bone resorption via beta2-adrenergic receptors. [23] Beta-adrenergic agonists, and adrenaline and isoprenaline, modulate osteoclastogenesis. Increased sympathetic nervous system activity leads to increased bone resorption through beta2-adrenergic receptors. [23]

Osteoblastic and osteoclastic cells on the one hand, and cancer cells on the other, constitutively express diffusible axon guidance molecules such as netrins and neurotrophins, which are known to function as chemoattractants for growing nerve fibres. [24,25]

Hormone dependence

Osteoclasts and many cancer cells are hormone responsive. Steroid hormones and other hormones such as osteocalcin, calcitonin and IGF-1 are crucial regulators of cancer disease as well as bone remodelling.

Oestrogens and androgens

Cancer growth and malignancy on the one hand, and bone remodelling on the other, are influenced by hormones e.g. sex steroids such as oestrogens and androgens.

In breast cancer, oestrogen-receptor status is a key factor for prognosis and treatment. Breast cancer cells also can express androgen receptors and this seems to be connected with tumor metastasis. [26] Cancer sites other than the breast express the oestrogen steroid receptors as well, demonstrated specifically in cancers of lung, oesophagus, stomach, colon, gynaecological organs, testes and in glioma. In lung cancers it has been shown that the membrane oestrogen receptors can transactivate the EGFR. [27] Increased serum oestrogen is associated with poorer survival among male and female lung-cancer patients. [28]

The androgen steroid receptor plays an important role in the course and treatment of prostate cancer.

Sex steroids are important for the growth and maintenance of both the female and the male skeleton. [29] Experiments using mice with inactivated sex steroid receptors demonstrated that activation of the oestrogen receptor alpha expressed by osteoclasts, and activation of the androgen receptor, both result in stimulatory effects on both the cortical and trabecular bone mass in males. Oestrogens and androgens exert pro-apoptotic effects on osteoclasts, but anti-apoptotic effects on osteoblasts and osteocytes, and decrease the number of remodelling cycles by attenuating the birthrate of osteoclasts and osteoblasts from their respective progenitors. Loss of oestrogen leads to increased rate of remodelling, and tilts the balance between bone resorption and formation in favour of the former.

Tamoxifen inhibits differentiation of osteoclasts and proliferation of cancer cells. [30]

IGF-1

IGF-1 (Insulin-like growth factor 1) is a polypeptide and plays a key role in bone remodelling as well as in cancer progression.

IGF-1 is implicated in the proliferation of many types of cancers such as pancreas, prostate, mammary, and colorectal carcinoma. Mouse models where circulating IGF-1 levels are reduced, while tissue expression of IGF-1 is normal, show lower risk for the development of colon and breast cancer and metastases when compared to control mice[31]. Patients with Laron syndrome (inactive GH receptor resulting in IGF-1 deficiency) seem to be protected from cancer[32,33]. In in-vitro tests, upon withdrawal of IGF-1 from the medium, cancer cells revert to an epithelial morphology. IGF-1 plays an important role in EMT, whereby epithelial cancer cells gain motility and the propensity to migrate. [34,35]

IGF-1 is an important regulator of the activity between preosteoclasts and mature osteoblasts through the regulation of RANKL and RANK expression between osteoclasts and osteoblasts. [36] IGF-1 deficiency has been found to impair osteoclastogenesis, resulting in decreased bone resorption. [37] It plays a paracrine role in the recruitment of circulating preosteoclasts from the vascular compartment into the bone tissue. IGF-1 receptors are expressed by osteoclast precursors. Through binding with their IGF-1 receptors, IGF-1 induces the migration of preosteoclasts.

 ISSN 1925-4067 E-ISSN 1925-4075

Calcium signalling

Osteopontin/CD44/integrin alphaVbeta3

Osteopontin is produced by cancer cells, in the same way that it is produced by osteoblasts and osteoclasts. Osteopontin is one of the major noncollagenous bone matrix proteins and is overexpressed by various cancers. [38] It binds to osteoclasts or cancer cells via CD44 or/and integrin alphaVbeta3. Among other things, its signalling plays an important role in the motility of both cancer cells and osteoclasts.

Osteopontin-integrin alphaVbeta3 signalling mediates CD44/MMP-9 complex formation on the cell surface and enhances survival in gastrointestinal and other cancer cells. [38,39] It is involved in MMP-9 secretion and migrating motility. Thus the expression level of osteopontin correlates with the metastatic potential of several cancer tumors.

In bone remodelling, osteopontin together with receptor alphaVbeta3, promotes cell adhesion of osteoclasts and chemotaxis during bone resorption. Osteoclasts deficient in osteopontin were found to be hypomotile, and exhibited decreased capacity for bone resorption *in vitro*.

Calcitonin-Calcitonin Receptor axis

Calcitonin is a small neuropeptide hormone produced in the thyreoidea, and counteracts the activity of parathyroid hormone. Both hormones regulate calcium and phosphate metabolism in the organism. Calcitonin is secreted in response to hypercalcaemia. The calcitonin receptor, along with TRAP, are specific osteoclast markers. [40] Calcitonin is not expressed by macrophages. [41,42] Besides the role of calcitonin in bone remodelling through inhibiting the bone resorbing activity of osteoclasts, it exerts decisive effects on cancer as demonstrated with prostate and breast cancer cells. In animal studies, calcitonin inhibited invasion of breast cancer cells and suppressed ERK1/2 phosphorylation. [43] However, in prostate cancer cells it seems to exert an opposite effect. It is produced by prostate cancer cells to a certain level for autocrine and paracrine stimulation of growth and invasiveness. [44]

Parathyroid hormone-related peptides (PTHrP)

PTHrP is an ubiquitously expressed protein playing important roles in various signalling pathways of cells and, among other things, is involved in epithelial-mesenchymal interaction during the formation of the mammary gland. In bone remodelling it acts between osteoclasts and osteoblasts, and under pathological conditions it is an essential growth factor for various cancers.

During tumor growth and invasion a number of growth and also angiogenic factors - such as interleukins, tumor-derived growth factor beta, platelet-derived growth factor and vascular endothelial-derived growth factor - modulate its expression. [45] Parathyroid hormone-related peptides are the responsible factor for hypercalcaemia associated with malignancy. [46] Most malignant human tumors express this hormone abundantly, however only in some tumors does this result in hypercalcaemia. [45] PHTrP might be related to heterotopic ossification associated with malignancies. [47]

PTHrP also functions as a bone cytokine to control the bone mass. [48] Osteoblasts produce PTHrP very early during their differentiation, and activate PTHrPR1 of mature osteoblasts to produce RANKL. This growth factor exerts a paracrine role in osteoclast formation. [49] A direct effect of PTHrP on osteoclasts cannot be excluded either, as osteoclasts express the PTHR-1. Whether osteoclasts or their precursors also express PTHrP acting in an autocrine manner, has so far not been clearly demonstrated. In giant cell granulomas the preosteoclasts express PTHrP as well as PTHR1. [50]

Osteocalcin

Osteocalcin is a small peptid hormone, which plays an important role in the remodelling of bone as well as in cancer progression.

Osteocalcin has been detected in prostate, breast, lung and pancreas carcinoma as well melanoma. Osteocalcin expressed in pancreatic cancer cell lines was found to enhance cell growth and invasion through autocrine and paracrine mechanisms. [51] Most studies on osteocalcin's role in cancer involved prostate and breast cancer. Osteocalcin RNA is highly expressed in most prostate cancer cells regardless of the tumor's metastatic status. [52] Elevated osteocalcin protein levels in the serum are found in metastatic prostate and breast cancer compared to non-metastatic patients. [52,53]

Osteocalcin is synthesized and secreted by normal maturing osteoblasts and acts as a bone hormone. Osteocalcin when carboxylated by vitamin K ensures the outfall of calciumphosphate in the bone while preventing it elsewhere in the organism. It regulates the dynamics of new bone formation and bone resorption by interaction with vitamin D and by influencing the differentiation of osteoblasts. As a chemoattractant it plays an important role in the migration of osteoclast precursors to the bone surface. [54] In a further function, osteocalcin acts as a hormone on insulin secretion and utilization. [55]

Coupling between cancer cells and mesenchymal cells, and between osteoclasts and mesenchymal cells

Both cancer cells and osteoclasts are coupled to mesenchymal cells and need them for their growth, differentiation and survival. Cancer cells are coupled to the mesenchymal tumor-associated fibroblast, and similarly osteoclasts are spliced to mesenchymal osteoblast.

In the tumor, the cancer cells are constantly interacting with tumor-associated stromal cells. The latter are not just bystanders in the tumor region, but contribute to tumor progression and metastasizing capacity. [12] The cancer cell releases factors that enhance the ability of the fibroblast to secrete a variety of tumor-promoting chemokines. Chemokines are involved in these processes: chiefly the CXCL12-CXCR4 axis, [9] Rankl-Rank, ephrinB2-EphB4, PTHrP-type 1- PTH receptor.

The importance of cancer-associated fibroblasts in epithelial carcinogenesis has been established in combination experiments. When immortalized nontumorigenic human prostate epithelial cells were mixed with fibroblasts from human prostate carcinomas grafted to immune-deficient animals, the epithelial cells developed into large carcinomas. In contrast, mixing the epithelial cells with fibroblasts from a normal prostate gland did not result in carcinomas. [56]

Parts of the stromal cells originate in the host's bone marrow. Primary tumors are able to encourage mobilization and recruitment of fibroblast precursors from the bone marrow, indicating that such tumors interact with the bone marrow in order to promote their own proliferation.

A similar situation exists between the preosteoclast and the osteoblast. They likewise communicate via these chemokines, through cell-cell contact, diffusible paracrine factors and cell-bone matrix interaction. [49] The osteoclasts are dependent on this crosstalk for their differentiation. [49]

RANK-RANKL

RANK-RANKL belongs as a cytokine system to the family of tumor-necrosis factors involved in osteoclastogenesis and cancer progression.

Analysis of surgical biopsy specimens showed the expression of RANKL, RANK and OPG in primary carcinomas, specifically lung, breast, head and neck, colorectum, kidney, thyreoidea and liver. The median percentage of RANKL-expressing cells was 60% in primary tumors and metastases, with no statistically significant difference between the two groups. [57] This study highlights the activity and function of the RANKL-RANK system not only in bone metastasis management but also in primary tumors. The expression of RANKL is associated with epithelial to mesenchymal transition as shown in human prostate cancer cell lines. Besides their own production of RANKL, cancer cells – like osteoclasts - can stimulate osteoblasts to secrete RANKL.

RANK is the essential signalling receptor for the osteoclast-differentiation factor in osteoclastogenesis. The binding of RANKL, expressed by osteoblast, with RANK receptor expressed by immature osteoclasts, stimulates their differentiation into multinucleated mature osteoclasts. [49]

Inflammatory context

Constitutive activation of NFkappaB and Stat3

Two major transcription factors, namely NFkappaB and STAT3, play a major role in transmitting inflammatory cytokine signals to the nucleus. Constitutive NFkappaB and STAT3 activation has been observed in about 40% of the

 ISSN 1925-4067 E-ISSN 1925-4075

tissue specimens of various cancer sites. The transcription factors may be involved in tumor initiation, angiogenesis and invasiveness. [58,59]

STAT3 and NFkappaB are also a prerequisite for the maturation of preosteoclasts into osteoclasts, and consequently the inhibition of NFkappaB activity blocks the differentiation of preosteoclasts into osteoclasts. NFkappaB induces the RANKL expression of osteoblasts. [60] The constitutive activation of NFkappaB is also a prerequisite for MMPs secretion and thus for the bone-resolving capacities of osteoclasts. [61] STAT3 activation is required in this context as well. [62]

MCP-1

Besides its production by macrophages, fibroblasts and endothelial cells, monocyte chemoattractant protein 1 (MCP-1 or CCL2) is secreted by both cancer cells and osteoclasts. MCP-1 plays a critical role in the recruitment and activation of monocytes to sites of inflammation, injury and cancer as well as in bone remodelling. [63] In the latter it is involved in the receptor activator of NF-kappa ligand-induced fusion of preosteoclasts and enhances osteoclastogenesis. [64]
In several cancers, MCP-1 has been shown to be an important factor for tumor growth and migration. [63]

Sensitivity to antirheumatics, steroids, bisphosphonates and polyphenols

Both cancer cells and osteoclasts are inhibited by antirheumatics, vitamin D, [65,66] bisphosphonates and polyphenols. The antirheumatic and analgetic substance aspirin inhibits the transformation of a benign tumor into malignant one. In-vitro studies with various antirheumatics show that they inhibit growth and proliferation of cancer cells. [67]

Bisphosphonates not only inhibit osteoclastogenesis, but also show direct anti-tumor effects on cancer cells. [68]

Numerous in-vitro and some in-vivo studies demonstrate that polyphenols, a substance class found in various plants, show direct antitumoral activity [69] as well as inhibition of osteoclastogenesis. [70]

Glycolysis

Proliferating cancer cells and preosteoclasts in differentiation show an increased level of glycolysis. Local hypoxia stimulates the aggressiveness of cancer cells and activates osteoclastogenesis, both under the control of HIFalpha1. Intermittant hypoxia specifically increases cancer cell migration, spontaneous metastasis formation, and osteoclastic activity.

Tumors generally show a shift in their metabolism from oxidative phosphorylation to glycolysis and thus exhibit increased glycolysis for ATP generation. [71] Cancers frequently rely less on mitochondria and obtain as much as 50% of their ATP by metabolizing glucose directly to lactic acid, even in the presence of oxygen. [72] Increased glycolysis induces acidification of the local environment, limiting proliferation and inducing cell death through necrosis and apoptosis. [73]

The role of glycolysis in osteoclast precursors and osteoclasts has not been studied to the same extent as in cancer cells. Preosteoclasts show a metabolic switch to accelerated glycolysis and mitochondrial respiration during RANKL-stimulated osteoclast differentiation. [71] Various studies were done with monocytes. As monocytes can transform into osteoclasts and may represent potential osteoclast precursors, we include them here. In particular, monocytes, and among them preosteoclasts, migrate through areas widely varying in oxygen tensions. Activated monocytes increase their energy gain through glycolysis even in the presence of enough oxygen, thus anticipating hypoxia and therefore better adaptation to low oxygen conditions in the tissue potentially arising in inflammation or ischaemia. [74] This results in prolonged survival under hypoxia in comparison to other cells and this is most likely due to increased glycolysis. [74]

Immune deviation

A still unresolved issue surrounding tumor formation involves the role of immune deviation. The hypothesis is that solid tumors suppress detection by various arms of the immune system, thereby evading eradication. For example cancer cells may paralyze immune cells by secreting TGF-beta or other immunosuppressive factors like interleukine-10. [75] On the other hand certain immune inflammatory process may even enhance tumor progression. [75]

Intensive interactions also take place between the immune system and osteoclasts. Bone cells are subject to the influence of immune cells. Activated T-cells enhance osteoclastic acitivity and their differentiation. This may lead to osteoporosis. On the other hand osteoclasts may induce immune suppression through the same cytokines as secreted by cancer cells

e.g. TFG-beta and interleukin-10 as shown in-vitro and in animal models. [76-79]Osteoclast can recruit T-cells and regulate them in a feedback loop. [77]

DISCUSSION

Most researchers try to understand the pathogenesis of cancer by investigating single cellular mechanisms or communication pathways within or among the cells in the context of a genetic or mutational concept. Here we choose another approach and offer a comparison of conceptual structures by compiling these single cancer findings on the one hand, and corresponding features of osteoclasts and osteoblasts on the other. This comparison shows that at the cellular and signalling pathway level there are more shared phenotypical aspects between cancer cells and preosteoclasts than between cancer cells and epithelial cells. Cancer cells seem to be more strongly related to preosteoclasts than to epithelial cells. The comparison can lead to various hypotheses to explain these exterritorial traits of cancer cells. It

Table 1 Examples of preosteoclast traits expressed by cancer cells

Common traits between cancer cells and preosteoclasts/osteoclasts	Common signalling pathways in cancer and in bone remodelling	Reference (c* and o*) and comments/references
Amoeboid or mesenchymal single-cell motilities; Migration, transmigration	Actin cytoskeleton; dynamic actin remodelling; RANK-RANKL; SDF-1/CXCR4; NFkappaB; Rac and Rho GTPase	[7]^{c} [80]^{o}
Trafficking to bone	SDF-1/CXCR4	[81]^{c} [15] ^{c} [13]^{c}[82]^{o}
Chemotaxis, chemokines, chemokine receptors	SDF-1/CXCR4; CCL9/MIP-1/CCR1	[81]^{c} [11]^{c} [82]^{o}
Angiogenesis	VEGFs angiogenic factors	[83]$^{o\ c}$
Neuronal dependence	Alpha2 adrenergig signalling	[21]^{c} [22]^{o} [23]^{o}
Neurogenesis	Neurothrophin and neurotrophin receptors; VEGFs	[25]^{c} [24]^{o}
Coupling and interaction with mesenchymal cells	RANK-RANKL; with fibroblast, myofibroblast; osteoblasts	[9]^{c} [12]^{c} [56]^{c} [49]^{o}
Growth factors	EGF/EGFR; HGF/Met; IGF-1/ IGF-1R	[32]^{c} [84]^{c} [33]^{o} [37]^{o}
Adhesion	Integrins alphaVbeta3	[39]^{c} [6]^{o}
Matrix-dissolving properties, aggressiveness	MMPs, cathepsins	[18]^{c} [8]^{o} [16]^{o}
Overexpressed signalling pathways of proliferation and anti-apoptosis	Ras/ERK; AKT; MAPK; Runx2	[35]^{c} [85]$^{c\ o}$ [86]^{o}[87]^{o}
Hormonal dependence	Steroid homones, oestrogens/ androgens	[27]^{c} Olivo-Marston, 2010 #37]^{c} [26]^{c} [30]^{o} [88]^{o} [
Glycolysis	IGF-1	[73]^{c} [31]^{c} [72]^{c} [71]^{o} [74]^{o}
Mesenchymal-like differentiation/plasticity	EMT; MET	[34]^{c} [89]^{o}
Scaffold	Cytokeratin; vimentin; actin	[90]$^{c\ o}$ [91]$^{c\ o}$ [92]$^{c\ o}$
Inflammatory signalling pathways	NFkappaB, STAT3	[57]^{c} [81]^{c} [59]^{c} [40]^{o} [60]^{o} [64]^{o} [62]^{o} [61]^{o}
Calcium signalling	Osteocalcin, calcitonin, Parathyroid hormon-like peptide; osteopontin	[52]^{c} [43]^{c} [51]^{c} [55]^{o} [46]$^{o\ c}$ [50]^{o}
Immune deviation	Treg cells, Il-10, TGF-beta, Suppression of Th1 activity	[75]^{c} [76]^{o} [77] ^{o} [78] ^{o} [79] ^{o}.
Sensitivity/inhibition	Antirheumatics, vitamin D, bisphosphonates, polyphenols, Tamoxifen, tyrosine kinase-inhibitors	[67]^{c} [69]^{c} [65]^{c} [68]^{c} [66]^{o} [70]^{o}

* ^{O} concerns osteoclasts, ^{C} concerns cancer

suggests a link between tumor cells and bone remodelling cells. Before we speculate about the possible causative factors for this connection, we will discuss the various common features and their specifity.

Concerning the phenotypic aspects of cancer development and bone remodelling we had to make a selection of features for this comparison. We chose noteworthy ones which can be classified as belonging to the key features of both cancer and osteoclasts. In some cases selection was undertaken arbitrarily. How specific are these features for both types of cells? There are probably no isolated features applicable only to these two cell types and not found elsewhere in other cells and tissues, but some are more prominent and are encountered in the same specific context in the cancer site as in the bone environment. This is true of the following aspects: the coupling of cancer cells with mesenchymal/stromal cells on the one hand and of osteoclast with mesenchymal osteoblasts on the other, the migrating and transmigrating capacities of cancer cells and preosteoclasts, their matrix-dissolving functions, their trafficking to bone (especially lung-,

prostate-, breast- and renal cell carcinoma), the paracrine stimulatory effects of cancer cells on osteoclasts and *vice versa*, specific calcium signalling pathways, e. g. calcitonin-calcitonin receptor axis, sensitivity to antirheumatics and bisphosphonates, immune deviation and extreme plasticity of both cell lines.

The calcium signalling pathways seem to play an important role in cancer as well in bone remodelling. Vitamin D, osteocalcin, calcitonin, osteopontin and parathyroid hormone-related peptide are reviewed in this study. Various studies hint at their decisive role not just in bone metastases, but also in carcinogenesis and cancer procession.

Cancer cells and osteoclasts are inhibited by the same therapeutic substances. Antitumoral substances inhibit osteoclastogenesis, and antiosteoclastic substances such as bisphosphonates and antirheumatics inhibit cancer cells and may prevent carcinogenesis. Does this apply to physiological substances as well? Does the enhancement of bone-mass formation by a physiological substance such as calcitonin also imply an antitumoral effect? The studies are contradictory in this regard. In breast cancer calcitonin shows antitumoral activity, whereas in prostate cancer it enhances the malignity. Whether this can be explained by the different bone metastasis action – breast cancer cells act in a more bonelytic, and prostate cancer in a more boneblastic manner - must be left open.

The rest of the reviewed features are found more ubiquitously. This applies to the IGF-IGF-1-receptor axis, constitutive NFkappaB/Stat3 activation, neurogenesis and neuronal and hormonal dependency. Myeloid-derived inflammatory cells are characterized by these properties, whereas epithelial cells are not. Thus the inflammatory features are characteristic for the whole lineage of myeloid-derived cells and consequently likewise substantiate our conceptual approach.

So far we have not referred to the differences between the two cell types. Cancer cells proliferate and preosteoclasts fuse with each other to become mature multinucleated osteoclasts. In this aspect, the two cell types function in an opposite way. We may ask whether there is a common basis for these two opposite behaviours. The reduced membrane potential may be a prerequisite for fusion of preosteoclasts [93] as well as for proliferation of cancer cells. [94] Could it be that initiation of reduced membrane potential is directed into continuous proliferation in the case of cancer because the potential preosteoclast cannot fuse at the epithelial site, but intends to do so?

When the epithelium is wounded or otherwise disturbed, e.g. by hypoxia, inflammatory cells such as monocytes - and among them a fraction of potential preosteoclasts - are attracted to this site. [95] The role of these potential preosteoclasts is unknown in this context and probably has to do with tissue repair. When their function is completed they may apoptose or re-enter circulation and perhaps migrate to the bone to fuse with their equals. As mature osteoclasts they remodel the bone in connection with osteoblasts. Compared to an inflammatory region, a cancer site attracts these specific monocytes even more. [96] Why do the cancers attract potential osteoclasts to such an extent? Furthermore, they attract mesenchymal cells from the bone marrow as well, thus creating here the connecting environment between myeloid and mesenchymal cells, as is the case in the bone where both cell types remodel the bone.

Cancer cells show a cytokeratin scaffold, whereas preosteoclasts show a mesenchymal-like one. However, cancer cells can transform their scaffold into vimentin, a process named EMT (epithelial-mesenchymal transition). The opposite transformation is possible as well, the mesenchymal-epithelial transition. Do the preosteoclasts have the same plasticity? There are very few publications on this issue, but they hint in this direction. Hematopoietic lineage-committed bone marrow cells, to which the progenitors of preosteoclasts belong among others, can transdifferentiate into epithelial cells[89]. We may further ask if cancer cells can transform themselves into osteoclasts. This is not the case. Cancer cells may however become multinucleated. When the prostate cancer cell line PC-3 and its xenograft in mice were exposed to polyethylene glycol, multinucleated cells were induced; however they later disappeared through apoptosis. The author suggests that the prostate cancer cells or their progenitor cells transformed into multinucleated giant cells and then apoptosed. [97] In a more polymerized form, porous polyethylene has biomaterial properties that can be fixed to bone. Polyethylene-wear particles from joint implants induce the formation of foreign giant cells and osteoclast precursors within the granuloma, whereas bone particles of the same size and under the same conditions induce osteoclast differentiation. [42] Polyethylene with its biomaterial properties exerts fusogenic properties on monocyte-lineage cells as well as on cancer cells. In another in-vitro study breast cancer cell lines showed bone resorbing activity, but they did not transform themselves into multinucleated osteoclasts in these experiments. [98]

In rare cases carcinomas may develop as a conjunction of pleomorphic and osteoclastic cells in the so-called giant cell carcinoma. Its incidence, e.g. in the pancreas is indicated from 2.1% to 12.8% in the literature. Its epithelial origin is discussed. [99]

Not all cancer cells metastasize to the bone. Cancer of the skin and of the gastro-intestinal tract, except rectum, seldom metastasize to the bone. One determinant of the site of metastasis is blood flow from the primary site. The liver may be a filter for carcinomas of the gastrointestinal tract, which seldom metastasize into the bone. It is not otherwise known why these primary cancer sites seldom metastasize to the skeleton.

Cancer cell stimulate the differentiation of preosteoclasts into osteoclasts, and their migration to the site of bone metastasis. [80,100] If the cancer cells are considered as cells with many preosteoclast features and functions, this stimulation can be interpreted as a paracrine, or quasi-autocrine enhancement. They stimulate the cell line of which, according to our interpretation, they may form a part.

How cancer cells or their progenitors acquire some preosteoclastic traits or *vice-versa*, or how potential preosteoclasts may adopt cancer properties and traits, is obscure. Formulated as a simplified question, we can ask: Are cancer cells preosteoclasts disguised as epithelial cells, and converted from fusion with each other to proliferation? Proposed hypotheses to explain some aspects of these findings are: fusion of preosteoclasts with epithelial stem cells, hybridization, DNA transfer, the plasticity of stem cell of the preosteoclastic lineage, or metaplasia. [101] The data reviewed in this paper tends to support the hypothesis that the plasticity of stem cells of preosteoclastic lineage may be implicated in carcinogenesis. There are various studies suggesting that tumor-initiating cells may be responsible for carcinogenesis and the maintenance of tumor growth. [102] In most studies the tumor-initiating cells were identified as being hematopoietic stem cells and thus - among others - progenitors of the preosteoclastic lineage. [103] These cells would have all the genetic prerequisites to differentiate into cancer cells with preosteoclastic traits. There is a second reason why we suggest that cancer cells emerge from the preosteoclastic lineage due to plasticity and the corresponding microenvironment. We already demonstrated that in the inner ear carcinogenesis does not arise although epithelial cells, lining the inner wall of the cochlea, are capable of proliferation and inflammatory processes. The unique feature in this area is however, that haematopoietic lineage can not develop into preosteoclastic and osteoclastic cells due to inhibitory decoy receptor osteoprotegerin present in this area suggesting that this missing process may prevent carcinogenesis in the inner ear. [2]

We did not review the common intracellular pathways specifically activated in preosteoclasts and cancer cells and we did not investigate in detail how the energy metabolism, especially lipid metabolism, is directed by bone cells and cancer cells in the same direction. This will be reserved for future publications.

This review may stimulate a new oncology research direction in future. It may also raise the question as to whether any antiosteoporotic measure - be it physical, nutritional or therapeutic -, might imply an antitumor activity. These concrete questions warrant further studies on the connection between bone remodeling and cancer.

Conflicting Interest: The author declares no conflict of interest.

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