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Title: Molecular basis of the effect of MMP-9 on the prostate cancer metastasis, a review

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Abstract: Introduction
Prostate Cancer (PCa) is the second most common cancer in men especially after 50 years old. The metastasis of said cancer involves a rise in terms of morbidity, metastasizing 90% of the occasions on bone. Metalloproteinases (MMPs) are involved in the process of bone formation and they are postulated to be involved in the process of metastasizing, in particular MMP-9.
This work is justified taking into account the scientific interest of the subject and the quality of the literature sources used. The PCa generates a high morbidity and
mortality in men, especially due to the process of metastasis, resulting in an impact to health and socio economic level.
Methods
This search was performed selecting articles published from 2003 to 2013. Items were selected and valued according to the Cochrane criteria (2011).
Results and Discussion
The selected articles (14) demonstrate the involvement of MMP-9 as a modulator of bone metastatic lesions either of osteoblast, osteoclast and/or mixed origin as well as
the recognition of the major mechanisms and/or molecules involved in the regulation of expression gene of MMP-9 and finally establishing the MMP-9 as a therapeutic
target for possible future drug development.

HIGHLIGHTS

- Summary of the last knowledge about the effect of MMP-9 on the prostate bone metastasis
- MMPs are postulated to be involved in bone metastases, in particular MMP-9
- Many articles support MMP-9 as a therapeutic objective but others do not agree
- MMP-9 is as an essential factor in the genesis and development of bone metastasis of PCa
- MMP-9 has a key influence on bone osteoblastic and osteoclastic activity

Molecular basis of the effect of MMP-9 on the prostate bone metastasis, a review

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Abstract

Prostate Cancer (PCa) is the second most common cancer in men especially after 50 years old. The metastasis of said cancer involves a rise in terms of morbidity, metastasizing 90% of the occasions on bone. Metalloproteinases (MMPs) are involved in the process of bone formation and they are postulated to be involved in the process of metastasizing, in particular MMP-9. This work is justified taking into account the scientific interest of the subject and the quality of the literature sources used. PCa generates a high morbidity and mortality in men, especially due to the process of metastasis, resulting in impacts to health and socio economic level. This search was performed selecting articles published from 2003 to 2017. Items were selected and valued according to the Cochrane criteria (2011). The selected articles (17) demonstrate the involvement of MMP-9 as a modulator of bone metastatic lesions either of osteoblast, osteoclast and/or mixed origin as well as the recognition of the major mechanisms and/or molecules involved in the regulation of expression gene of MMP-9 and finally establishing the MMP-9 as a therapeutic target for possible future drug development. Finally, this study evidences MMP-9 as an essential factor for the activation of the chain of the different MMPs and consequently in the genesis and development of bone metastasis of PCa due to its influence on bone osteoblastic and osteoclastic activity.

Keywords: metalloproteinases, MMP-9, cancer, prostate, bone, metastasis, therapeutic targets.

INTRODUCTION

Prostate cancer (PCa) is the second more frequent type of cancer in males. In Spain there are over 25,000 diagnoses per year, which suppose about 21% of male malignant tumors. This cancer is more frequent in people older than 50 years; especially between 70-80 years. Considering the incidence, that corresponds to 57 new cases/(100,000 inhabitants.year) [1].

The mortality is about 10%, standing at the third cause of death from cancer in men. Furthermore, based on the age onset, the manifestation age, population aging and life expectancy at birth (in Galician men is 78.3 years) estimated additional cancer cases could be around 650,000 at 2025. Around 65% of patients survive more than 5 years (15 dead/100,000 population.year) which tends to decrease [2].

PCa generates high direct and indirect costs. The latter include those arising from the disability caused by the cancer, particularly in bone metastases and the effect on the informal caregivers (burnout) [3].

PCa metastasis decreases significantly the patient life quality due to the organic and psychological effects, anxiety and depression that are involved [3]. It is, therefore, a key element in the morbidity and mortality as 90% of cancer patients die of metastases [3]. In PCa, the morbidity is associated with bone metastasis [3]; an important aspect considering that over 90% of metastases take place in bone [4] and in 50% of cases it constitutes the initial clinical manifestation [3].

Consequently, cancer and, specifically, prostate cancer is a priority line in public calls for projects supported by regional, national or international organizations such as "Acción Estratégica de salud en el marco del Plan Nacional de Investigación Científica, Desarrollo e Investigación Tecnológica 2008-11" and an important element in the various programs that make up the plan [5]. Priority actions extend to prevention,

promotion and delivery of health services in the public health system. One example is the Comprehensive Health Plan implemented by the "Junta de Andalucía" [6].

The best-known proteases involved in PCa invasiveness processes belong to the plasminogen activation system (Plg), which leads to the formation of serine protease plasminase and various matrix metalloproteinases (MMP), which include collagenases, gelatinases and stromelins.

Matrix Metalloproteinases (MMPs) are zinc-dependent neutral endopeptidases, capable of degrading key components of the extracellular matrix (ECM) and they are involved in several physiological and pathological processes [7]. Furthermore, they are not only involved in the process of bone formation, including epiphyseal [8], but there is increasing evidence of their role in the extravasation of tumor cells during metastasis, particularly in PCa bone metastasis. These enzymes are secreted or located on the cell surface and their substrates are extracellular proteins, some of which are associated with basal membrane and stroma invasion, blood vessel penetration and metastasis [9]. Therefore, they are of high interest as therapeutic targets [4].

Among the existing MMPs, it seems that MMP-9 plays a significant role in PCa bone metastasis by two mechanisms. The first mechanism acts on the ECM and the activity of bone cells, while the second would have a direct action on the tumor cells themselves [4].

Several clinical assays have confirmed a correlation between MMP-9 expression, disease progression and clinical outcome in patients with various types of tumors [10]. It is widely believed that invasion is facilitated by a form of MMP-9 that acts outside the cell to degrade ECM components or adjacent to the surface of the invasive cell. MMP-9 is capable of degrading ECM and basal membrane constituents such as type IV collagen, fibronectin and laminin [10]. MMP-9 can also activate several

latent proteinases and is also known to activate angiogenic factors or cytokine receptors, allowing the process of invasion and metastasis [10].

Considering the link between PCa metastasis and morbidity noted above, understanding the mechanisms by which MMP-9 promotes metastasis has a preventive and therapeutic interest. According to the reported above, the present review will discuss the molecular basis of the effect of MMP-9 in prostate bone metastasis.

MATERIAL AND METHODS

Search strategy

This review was developed by consulting the next databases/search motors: Medline, SciELO and ProQuest. They were searched for publications from January 2003 to May 2017 and there were included articles written in English, Spanish, French, Galician and/or Portuguese languages.

To begin this work, in a preliminary search to put the work into context, there were identified articles dealing with incidence, prevalence, mortality and health results related with PCa metastasis with the terms "cancer", "prostate", "bone" and "mmp 9". Besides, to identify the literature about the molecular basis, there were included terms about the production of metastatic lesions like osteolytic/osteoclastic and osteogenic/osteoblastic lesions, about the genetic regulation of MMP-9 and about the use of MMP-9 as therapeutic target.

After the preliminary search, the search was delimited by using the next combination of terms:

- From English edition databases, "cancer", "prostate", "bone", "mmp 9" "osteoclastic", "osteoclast", "osteoblastic", "osteoblast", "osteolytic", "osteogenic", "molecular machinery", "Runx2", "activin A", "androgenic", "OPG", "RANK",

"RANKL", "therapeutic target", "genistein", "zoledronic acid", "docetaxel" and "bifosphonate", interrelated with the Boolean "AND" or "and".

- From Spanish content databases, the same combination of terms was used but in this case translated into Spanish ("cáncer", "próstata", "hueso", "mmp 9", "osteoblástico", "osteoblasto", "osteolítico", "osteoclástico", "osteoclasto", "osteogénico", "maquinaria molecular", "Runx 2", "activina A", "androgénico", "OPG", "RANK", "RANKL", "diana terapéutica", "Docetaxel", "ácido zoledrónico", "genisteína" and "bifosfonatos" interrelated with the Boolean "y".

The MeSH terms *(Medical Subject Headings)* were: MMP-9, animals, humans, male, tumor, osteogenic/blastic, osteoclastic/lytic.

Assessment criteria for articles

This review was based in Cochrane assessment criteria *[11]*:

Type of studies by type of participants

- Studies "in vivo": with experimentation animals
- Studies "in vitro": with cell lines and cultures
- Clinical trials: with human participants

Intervention type

- MMP-9 analyses regarding the development of bone metastatic lesions of PCa.
- Establishment of the factors and/or mechanisms that determine the MMP-9 expression in prostate bone metastasis.
- Establishment of the MMP-9 as therapeutic target in prostate bone metastases.

Outcome measures

The studies were evaluated in terms of:

- Primary outcomes:
    - The MMP-9 generates:
        - Osteoclastic/lytic lesions.
        - Osteoblastic/genic lesions.
    - MMP-9 as a therapeutic target.
        - New drugs: zoledronic acid, Docetaxel, Biphosphonates (BFs)
        - Dietetic components: Genistein
- Secondary outcomes:
    - The more frequent factors that determine the regulation of the genetic MMP-9 expression: Runx 2; Activin A; androgenic activity; molecular mechanisms: OPG/RANK/RANKL/MMP-9.

RESULTS

Search strategy results and assessment criteria results

Articles excluded

Into **ProQuest** database [that included: ASFA: Aquatic Sciences and Fisheries Abstracts, ERIC, Linguistics and Language Behavior Abstracts (LLBA), MLA International Bibliography, PILOTS (Published International Literature OnTraumatic Stress), ProQuest Dissertations and Theses AandI and PsycINFO)] 40 studies were found that had to be excluded because they included incomplete results data; 38 did not study the relation between prostatic cells and bone metastases and 2 did not study the genesis of bone lesions.

Into **Medline** database 44 items were found, but 23 of them were excluded due to duplicates or triplicates in the database. Therefore, 23 articles remained. Subsequently,

4 were removed after applying the assessment criteria because: 1 did not include the genesis of metastatic lesions from PCa; 1 did not include contrasted evidences from the relation of MMP-9 with the studied subject and 1 studied one drug that is not included in this work.

Included studies

From **Medline** database, 17 articles were selected that satisfied the assessment criteria (Figure 1).

From **SciELO** database, 1 article was included as a selected article for this analysis.

Among the selected articles, there is one that is repeated in two databases, **Medline and SciELO**; this corresponds to Zhao et al. [2012].

Outcome measures results

Primary outcomes

- 11 articles evaluated the effect of the MMP-9 in the prostate bone metastasis.
    - 90% postulate that the lesions are osteolytic.
    - 10% postulate that the lesions are maybe osteogenic or mixed.
- 6 articles verified the existence of therapeutic targets which change and/or inhibit MMP-9 action and/or its synthesis.

Secondary outcomes

- 9 articles studied the effect from the primary factors/mechanisms that determine MMP-9 expression.
    - 75% analyze Runx 2 effect.

- 12.5% analyze Runx 2 and molecular machinery OPG/RANK/RANKL/MMP-9 effect.
- 12.5% analyze Activin A effect.

Results by objectives

Table 1 shows the different studies associated with the objectives of this work.

Below, the results are detailed by objectives:

"Assessment of modulation of the development of bone metastatic PCa lesions mediated by MMP -9"

Tumor growth, invasion and metastasis require cell proliferation, proteolytic digestion of the extracellular matrix (ECM), cell migration across basement membranes in the circulation, extravasation and growth in metastatic sites. MMPs contribute to the metastatic process and they also may promote tumor growth by increasing the bioavailability of growth factors in the ECM [12, 13].

Osteoblasts are intimately involved in the regulation of osteoclast differentiation via RANKL and OPG expression. So, sometimes a single mechanism of metastasis cannot be considered as responsible, whereas some authors suggest that the mechanism by which mixed lesions occur is not yet defined [8].

"The action of MMP -9 in bone extracellular matrix"

Different studies postulate that increased levels of MMP-9, specifically in the later stages or advanced stages of PCa, is a key factor triggering events post bone metastases, including osteolysis [14, 15].

Within the process of metastasis, degradation of the extracellular matrix is a key element. The MMP-9 degrades ECM, specifically the type IV collagen, promoting cell migration. [14, 16, 17].

These metastases may adopt different patterns of bone formation/destruction, depending on the profile adopted by the bone remodeling process: osteoblastic, osteolytic or mixed. In the last case, in the sequence of normal unmineralized bone, bone matrix is first degraded by osteoblasts and/or bone cells secreting coating MMPs and, thereafter, osteoclasts migrate into areas of bone remodeling, sticking to the exposed mineralized matrix and solubilizing it using the cysteine proteases. [4].

"The action of MMP-9 in bone homeostatic physiological activity"

"Rate Modulation of the development of metastatic lesions of osteoclastic/lytic character"

As for the relationship between the osteoclast activity and MMP-9, this review found that MMP-9 is highly expressed in osteoclasts and monocytes (osteoclast precursors) as well as in the multinuclear osteoclasts that reabsorb bone. This enrichment of MMP-9 in these cells is particularly evident in the front of ossification [4, 18, 19, 20].

Moreover, it is suggested that the increased activity of MMP-9 in tissue is due to a transient wave that is intended to promote osteoclast recruitment and favor their lytic activity [4]. Furthermore, it is believed that MMP-9 promotes tumor growth in the bone microenvironment and, therefore, this increase is related to osteoclast activity and that this activity is the main responsible for bone resorption, which is the trigger of the denominated "vicious cycle" or "vicious circle" [19]. This circle consists on bone metastatic PCa cells which alter the bone remodeling process accelerating it, because these cells cause an increase of growth factors and cytokines, which in turn stimulate tumor growth in chain [19].

Finally, it is observed that osteolysis is important for physical expansion of tumor cells in the bone marrow and that the degradation of the ECM provides calcium and growth factors derived from marrow for cell proliferation and differentiation of osteoblasts, which will enable the aforementioned circle [4].

"Rate Modulation of the development of metastatic lesions of osteoblastic/gene character"

Among the reviewed studies, only one finds that there is a direct relationship between the activity of MMP-9 and blast-metastatic lesions. Here, Bruni-Cardoso et al. [19] show that while osteoblasts express several MMPs, including MMP-9, there is a lack of studies verifying the impact of MMPs derived osteoblasts or other cell sources with osteoblastic activity in the pathological context of bone metastases [19, 20].

The same authors suggest that osteoblasts are an important source of MMP-9 in the bone microenvironment. However, given the interdependence between osteoblasts and osteoclasts, the relationship between MMP-9 and osteogenic changes should be determined [19]. Other authors [8] suggest that the increased expression of OPG and osteopontin can lead to an inhibition of the activity of osteoclasts, resulting in a change in bone remodeling towards osteoblast activity and bone mineralization.

"Rate Modulation of the development of mixed lesions"

It has been observed that the "vicious circle" described above may result in mixed lesions with large areas of bone destruction (osteolytic) and formation (osteogenic) specifically related to the main bone cells such as osteoclasts and osteoblasts, respectively [19].

"The effect of the main factors and/or mechanisms that affect the expression of MMP -9 "

"The effect of Runx 2"

Runx2 and MMP-9 are key regulators of bone growth plate maturation and formation. The genes corresponding to both proteins are characteristic markers of breast and prostate cancer cells that cause bone metastases [21].

As it was already mentioned, Runx2 is a protein previously known for its major regulatory roles in chondroosteoblastic lineage and it is emerging as a prometastatic transcription factor which can control various aspects of metastasis [21]. It is expressed in androgen-independent PC-3 cells and is a key regulator of the events associated with bone metastases of prostate cancer by promoting the activation of target genes including metastatic VEGF, osteopontin, MMPs, and survivin. It is also suggested that Runx2-mediated pathway may be involved in osteoblastic bone lesions properties [8].

Runx 2 is involved in transactivation of the promoter of the MMP-9 in osteoblasts and no-bone cells. Data shown that Runx2 overexpression in prostate cells increase significantly the endogenous level of MMP-9, while decreasing Runx 2 also decreases MMP-9, and concluding that Runx2 is a positive regulator of genes associated with metastasis and osteolytic disease, inducing MMP-9 overtranscription [21, 22] .

"The effect of Activin A"

Activin A is a cytokine which is related to the expression of MMPs, including MMP-9, as it is believed that this multifunctional cytokine is implicated in the regulation of osteoblastic activity, osteoclast differentiation and modulation of

intracellular MMP-9 in bone metastatic cells. This is demonstrated by the fact that increasing levels of Activin A also increase levels of MMP-9 [20].

"The effect of androgenic mechanisms"

Prostate cancers are generally dependent on androgens and, therefore, androgen antagonists are used as hormonal treatment in PCa [23]. This treatment however, produces a brief clinical response in most patients, ultimately failing. Most patients develop recurrences, which have been called androgen-resistant or hormone refractory PCa. Androgens play essential roles in the development, progression and metastasis of PCa by modulating the expression of proteins as adenomatous polyposis coli (APC), E-cadherin, GSK-3β, phospho-GSK-3β Ser9, NF-k B p50, Slug, N-cadherin, β-catenin, vimentin, MMP-9, Snail, and phospho-RSK1 Thr359/Ser363. AR has been reported to directly interact with β-catenin and GSK-3 β. It is not clear if the direct binding between AR and β-catenin or GSK-3β plays any role in the regulation of EMT marker proteins in PC-3 cells. [24]

The influence of androgenic factors in regulating expression of the MMP-9 is controversial because some studies indicate an inverse relationship between MMP-9 and androgens, but one study suggests that probably androgenic factors can stimulate the secretion of MMP-9 [14, 24]. Miyamoto et al. [23] sustain that androgen deprivation results in activation/overexpression of Akt, COX-2, and MMP-9 in androgen-sensitive PCa cells. This suggests that androgen deprivation in clinical settings may activate the Akt, COX-2, and MMP-9 pathways in prostate cancer, increasing cell growth and promoting a transition to an androgen-independent state. These same authors suggest androgen deprivation in combination with inhibition of the Akt, COX-2, and MMP-9

pathways as a treatment which could delay the androgen-independent transition and with better effects than hormonal therapy alone.

"The effect of the molecular cascade OPG / RANK / RANKL"

It is known that cancer cells spread to the bone and make use of the local cytokines machinery to stimulate osteoclasts, which results in a process of bone resorption and growth of prostate cancer cells in bone [17].

Prostate cancer cells make use of different signaling pathways using molecules such as: integrin $\alpha v\beta 3$, Rho A, OPG, CD44, RANK, RANKL and Runx 2. They all have an effect on the activation of MMP-9 and, consequently, entail an increase in osteoclast mediated bone resorption, causing osteolytic lesions and promoting invasion and migration of tumor cells [16].

The molecular machinery of the most important cytokine involved in bone metastasis of prostate cancer cells is OPG/RANK/RANKL/MMP-9 that plays an important role in bone remodeling. RANKL is expressed by osteoblasts and is necessary and sufficient to induce osteoclastogenesis [8, 17, 20]. RANKL is expressed by RANK receptor, present on the surface of osteoclast precursors and mature osteoclasts and involves the induction of osteoclast formation and activation, resulting in the expression of MMP-9, thus causing osteoblastic/lytic metastatic bone lesions [17, 20, 25].

Finally Rho A is considered a trigger, that it is to say, a signaling molecule for activating different protein promoters, in this case the activation of the molecular machinery mentioned above [16].

"Therapeutic targets aimed at modifying and/or inhibiting the action and/or synthesis of MMP-9"

About the use of MMP-9 as a therapeutic target to treat/prevent bone metastasis of PCa, it is controversial because some studies postulate that the use of drugs that inhibit the function of MMPs is not yet specific and it is necessary to conduct further studies to act more specifically [4, 21].

Furthermore, other studies describe the action of another drug which itself may have inhibitory effects on the expression of MMP-9 and consequently on the genesis of bone metastases. Those drugs are zoledronic acid [26], bisphosphonates [16], docetaxel [17] and soy products such as Genistein [25], among others.

Finally, one study indicates that the use as a tumor marker of MMP-9 is not recommended because it is not more specific than the well-known PSA marker [27].

DISCUSSION

This review shows that MMPs, including MMP-9, influence the genesis of bone metastatic lesions derived from PCa. This is due to the physiological process of bone remodeling, which is altered leading to a "vicious cycle" [19, 20]. This cycle, causing injuries of osteoclast/blastic origin, could be expected taking into account that normal bone matrix is first degraded by osteoblasts and then by osteoclasts [4, 20].

Tumor growth, invasion and metastasis require that tumor cells proliferate in the metastatic niche and thus the degradation of ECM. This degradation is carried out mainly by MMPs, including MMP-9, which acts specifically on the type IV collagen [14, 16, 17]. The levels of MMP-9 are particularly increased in advanced stages, thus promoting bone metastasis [13-15].

Due to the key role that bone remodeling has on the establishment of bone metastases, the majority of studies were focused on the genesis of osteoclastic lesions,

especially using the PC3 model [16-18, 20-22]. This is because osteoclasts and monocytes have especially expressed MMP-9 [4, 18-21], while this MMP has a call effect in order to recruit more osteoclasts and thus promote the lysis of the niche to be colonized by metastatic PCa cells [4]. Therefore, tumor growth in the bone microenvironment is promoted together with the "vicious circle" related to bone resorption that results from osteoclast activity [19, 20].

Other studies also postulate the origin of metastatic lesions in bone associated with osteoblastic activity, since there is an interdependence between osteoclasts and osteoblasts [19, 20] because the former can be inhibited by expression of OPG giving way to the osteogenic cells, osteoblast activity and bone mineralization [8].

It is possible that some studies are especially focused on osteoclast activity because they possibly have not located osteoblastic activity, since the PC3 cell line does not develop this activity in contrast to other lines as those derived from preclinical models LNCaP-derived C4-2B, LuCaP 23.1 LAPC9 MDA-PCa-2b, 22Rv1 VCAP and other that develop them [8]. Thus, it would be necessary to develop models with these lines to finally verify the influence of osteoblastic activity, as to the origin of metastatic bone lesions.

Regarding the regulation of gene expression of MMP-9, some studies show the influence of Runx 2 on the expression of the same protein [8, 19, 20], both involved in the regulation of the growth plate and maturation of bone, thus related to osteoblast activity [8, 21].

A directly proportional relationship between Activin A and MMP-9 was also found [18].

Moreover, numerous studies developed from PC3 cell lines postulate that androgen activity does not influence the expression of MMPs. Even, some works claim the possible existence of an inverse relationship between MMP-9 and autocrine activity. Other studies postulate that there is a relationship between MMP-9 and autocrine activity which in this case is a direct relationship between the two [14, 23]. Thus, this type of regulation is still controversial, so it could be interesting to develop new research projects with cell lines different to PC3 in order to show the relationship between the autocrine activity and expression of MMP-9.

Furthermore, it has been observed that there are different pathways particularly triggered by the Rho A trigger [16] and that the most important in terms of regulation of expression of MMP-9 molecular machinery is OPG/RANK/RANKL/MMP-9. RANK and RANKL play an important role in the regulation of osteoclasts and osteoblasts, respectively, and both express MMP-9 in situations where osteoblastic or osteolytic activity may predominate. [8, 16, 17, 20].

To finish the discussion about gene expression of MMP-9, some studios reported that all MMPs are interdependent among them, suggesting that it is necessary the activation of one for the successive activation of the other [19, 20, 23].

There has been much postulation about the use of MMP-9 as a therapeutic target. This was due to its use for metastasis inhibition drugs was not specific on the MMP, but was unspecific and with potential development of multiple side effects. Even so, this study shows that drugs such as zoledronic acid, bisphosphonates, docetaxel and soy derivatives like genistein can be act therapeutically on the MMP-9 [4, 16, 17, 21, 25, 26]. However, it must be taken into account that their effects would be indirect and

probably exerted on all the metalloproteinases. The direct inhibitors of MMP-9 have not yet proven effective on clinical trials.

It would be valuable to conduct further studies to inhibit the expression of MMP-9 in cases of PCa bone metastases as in Asian countries because such men suffer less PCa than Europeans and this may be due to the consumption of soy containing genistein. This compound may be a protective factor against the development of cancer that act by inhibiting various potentially carcinogenic molecules, as in this case, the MMP-9 [24].

Finally, as to the therapeutic use of MMP-9, it can serve as a target of inhibition to slow down mechanisms inducing bone metastases of prostate cancer, but studies have indicated that MMP-9 is not a better prostate tumor marker than PSA. Therefore the use of MMP-9 as a tumor marker for PCa still needs more evaluation [27].

CONCLUSIONS

Cancer is a chronic disease with a very high and broad associated morbidity and mortality. Currently there is an intense work to address it from all variants of therapeutics, prevention and health promotion. This work focused on the therapeutic modality on the molecular basis of bone metastasis of PCa, in particular in MMP-9, finding many articles that support its condition as a therapeutic objective but others that do not agree, thus existing some controversies. One should think that there is still a long way in the progress and research on MMP-9. Finally, this study evidences MMP-9 as an essential factor for the activation of the chain of the different MMPs and consequently in the genesis and development of bone metastasis of PCa due to its influence on bone osteoblastic and osteoclastic activity.

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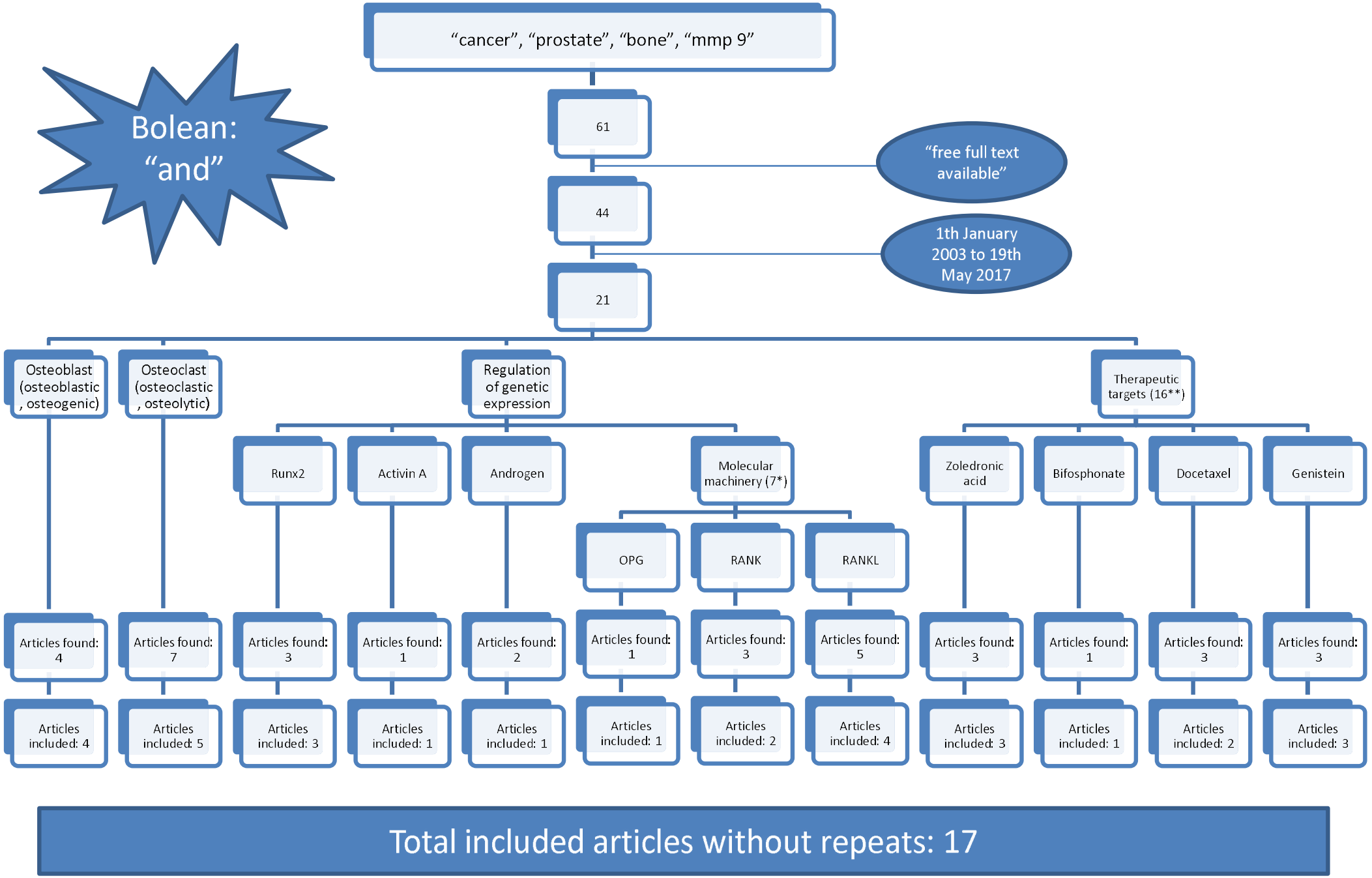
Figure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61
"free full text available"
44
1th January 2003 to 19th May 2017
21
Osteoblast (osteoblastic, osteogenic)
Osteoclast (osteoclastic, osteolytic)
Regulation of genetic expression
Therapeutic targets (16**)
Runx2
Activin A
Androgen
Molecular machinery (7*)
Zoledronic acid
Bifosphonate
Docetaxel
Genistein
OPG
RANK
RANKL
Articles found: 4
Articles found: 7
Articles found: 3
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Articles included: 1
Articles included: 1
Articles included: 2
Articles included: 4
Articles included: 3
Articles included: 1
Articles included: 2
Articles included: 3
Total included articles without repeats: 17

Caption to figure

Figure 1. Search strategy within PubMed database. The articles removed, included and the final number of articles used are indicated. *All these articles were included. **7 of these articles were included.

Table 1

Author and year	Type of Study	Sample	Measures	Results	Relationship with objectives
Li et al. 2004	"In Vivo"	PC3 cell line. Genetically Modified Mice	- Treatment with genistein. - Quantification of genistein in plasma. - Analysis of the expression of the genetic profile. - PCR. - Analysis of MMP-9	Dietary genistein may inhibit bone metastasis from prostate cancer by regulating genes related to metastasis. Genistein may therefore be a promising agent for the prevention and / or treatment of prostate cancer.	2
Dong et al. 2005	"In vivo"	PC3 cell line. Genetically modified mice	- Zymography of gelatin. - ELISA technique. - Immunostaining of PC3 tumors. - PCR. - Measurement of cellular activity. - Study of RNA interference. - Statistical analysis: GraphPad InStat.	The genesis of MMP-9-mediated osteoclastic lesions is asserted and that the development of specific inhibitors of MMP-9 can be represented as a potential therapeutic target in bone metastasis.	1, 3
Pratap et al. 2005	"In vitro"	Human and mouse cell lines (PC3)	- PCR - Immunoassay. - Western analysis. - Zymography of gelatin. - Measurement of osteogenic expression. - ChIP assay (Chromatin immunoprecipitation) - Electro-karyotype. - Cell invasiveness test	Runx2 is involved in the genetic regulation of numerous molecules and in turn contributes to the metastatic properties of cancer cells and their activity in the bone microenvironment	1, 2, 3
Leto et al. 2006	Clinic assay	30 patients with PCa or BCa 42 controls (12 women and 30 men)	- Bone scintigraphy, bone Rx. Serum chemistry, complete blood cell counts, evaluation of serum CA15.3 and PSA serum markers and of bone alkaline phosphatase, c-telopeptide and parathyroid hormone. - Symptomatic response to treatment: visual scale analogue scale (VAS) - Measurement of Cath B, MMP2, MMP9 and uPA. ELISA technique. - Statistics: Medcalc	ZA administration may provide bone pain relief in patients with diffuse bone metastases and confirm a possible implication of matrix proteinases in the formation of bone metastases but not in the pathogenesis of metastatic bone pain	1
Li et al. 2006	"In vivo"	PC3 cell lines and cell growth inhibitors. Genetically Modified Mice	- Analysis of the gene expression profile. - PCR. - Western analysis. - Analysis of the molecules OPG, RANK, RANKL, MMP-9. - Analysis of osteoclastic activity / differentiation. - Cell invasiveness.	The observed potentiation of the antitumor activity of docetaxel by genistein in the SCID-human model of experimental bone metastasis could be mediated by regulation of OPG / RANK / RANKL / MMP-9, resulting in inhibition of bone resorption Osteoclastic and bone metastases of PCa. It is concluded that genistein could be a promising non-toxic agent to improve the outcome of treatment of metastatic prostate cancer with docetaxel.	1, 2, 3
Salminen et al. 2006	"In Vitro"	84 patients; 49 without bone metastases and 35 with bone metastases	- Statistical analysis: SAS System for Windows (Version 9.1, SAS Institute Inc., Cary, NC, USA) - Biochemical Samples and Markers (ALP, PSA, MMP-2, MMP-9, TIMP-1, TRACP 5b [tartrate-resistant acid phosphatase isoform 5b])	Of the three new markers tested, only TRACP 5b was found to be predictive of survival in PCa with bone metastasis. Therefore, MMP-2 and -9 are not recommended for other studies in this context.	3
Desai et al. 2007	"In Vitro"	Genetically Modified PC3 Cell Lines	- Immunoassay - Biotinylation and immunoblot analysis. - Zymography of gelatin. - Wound closure test - Cell proliferation. - Statistical analysis: ANOVA	The various steps involved in the signaling pathway for the regulation of MMP-9 activation among which CD44 is found are potential therapeutic targets. The bisphosphonates act on Rho attenuating it and in turn decreases the interaction CD44 and MMP-9	1, 2, 3

Author and year	Type of Study	Sample	Measures	Results	Relationship with objectives
Incorvaia et al. 2007	Clinic assay	Blood donor patients; 79 with cancer and 57 healthy patients	- Enzyme immunoassay. - Measurement of Activin A and MMP-2 and 9. - Measurement of serum PSA and CA15.3 - Statistics: Medcalc. - Use of Helsinki Code.	Activin A, MMP-2 and MMP-9 may be considered as possible therapeutic targets in the treatment of metastatic bone disease. However, its usefulness as additional markers for bone metastasis has not yet been defined.	1,2
Hara et al. 2008	"In Vitro"	MDAP cell line Ca 2b.	- Invasiveness test. - Western analysis. - Zymography of gelatin. - Analysis of androgenic inhibition / overexpression. - Fractionation of the highly invasive population. - Statistical analysis: "student t" and "Dunnett's test"	- They support the use of adjuvant hormone therapy and the future development of more potent therapy through androgenic block.	1, 2
Feng Xin et al. 2009	"In vitro"	Sa052 and U20S cell lines	- Zymography of gelatin. - Western analysis. - PCR. - Statistics: manual processing (statistical professional)	Risedronate may reduce the invasiveness of studied cells by action on MMP-2 and MMP-9 as these two key molecules are involved in the invasion process.	1
Baniwal et al. 2010	"In vivo"	C4-2B, LNCaP, 22RV1 and PC3 cells Genetically Modified Mice	- PCR - Lentivirus production and infection - Luciferase assays - Measurement and analysis of high-throughput gene expression - Zymography of gelatin. - Measurement of invasion and proliferation. - Measurement of the cell cycle. - Western transfer analysis. - RT-qPCR - Statistical analysis: ANOVA.	The effects of Runx2 on C4-2B / Rx2dox cells, as well as similar observations performed using LNPCa, 22Rv1 and PC3 cells, highlight several mechanisms by which Runx2 promotes the metastatic phenotype of PCa cells, including Invasion of tissues, targeting of bone and induction of high bone turnover. Therefore, Runx2 is an attractive target for the development of new diagnostic, prognostic and therapeutic approaches for the management of PCa.	2
Bruni-Cardoso et al. 2010	"In Vivo"	Genetically Modified Mice	- Techniques of image to observe response in cranial cap. - Immunohistochemistry, cytochemistry and histomorphometry. - Determination of osteoclastic and osteogenic activity. - Statistical analysis: ANOVA.	The osteoclast response derived by the mediation of MMP-9 causes impacts on tumor growth in the bone microenvironment because it contributes to angiogenesis by inducing osteolytic or osteogenic changes.	1
Faccini et al. 2010	Clinic assay	22 patients between 43-80 years old (average 73)	- Clinic history, physical examination, Blood cell count, serum biochemistry, urine tests, serum PSA, ECG, echocardiogram with evaluation of FEVI, Chest Rx., CT scan, abdominal or pelvic ECO, bone scintigraphy, Bone Rx. Serum cytokine: Il-8, VEGF, MMP-2, MMP-9. ELISA technique - Statistics: ANOVA - Use of the Helsinki Code and request for informed consent	Combination of Docetaxel and zoledronic acid is feasible for its population sample and activity is promising	3
Al Nakouzi et al. 2012	"In Vivo"	IGR-CaP1 cell line and genetically modified mice.	- Transduction and cellular invasiveness. - Luminescence imaging techniques. - Micro X-ray and computed tomography. - Photon emission analyzed by computerized tomography. - Immunoassay. - PCR	This study has found the mediation of metastatic bone lesions of the osteoblastic and osteogenic PCa given that the used cell line develops both types of PCa.	1, 2

Author and year	Type of Study	Sample	Measures	Results	Relationship with objectives
Luo J. et al. 2013	“In Vitro” “In Vivo”	LNCaP, C4-2, C81 and CWR22Rv1 cell lines Mice	- Cell invasion assay - Cytokine array - RNA extraction and quantitative real-time PCR analysis - Western blot analysis - Histology and immunohistochemistry - Luciferase assay - In vivo BM-MSCs recruitment assay - In vivo metastasis studies - Student’s t-test	The BM-MSCs-mediated increased metastatic ability of PCa cells can be due to the PCa stem cell increase via alteration of the CCL5-AR signaling pathway.	1
Huo C. et al. 2015	“In Vitro”	PC-3 cells	- Transwell Migration Assay - Transwell Invasion Assay - Western Blotting Analysis - Wound Healing Assay - Gelatin Zymography assay	The re-expressing androgen suppresses migration and invasion of PC-3 cells via regulation of EMT marker proteins and MMP activity.	2
Shin J.E. et al. 2015	”In Vitro”	Murine bone marrow macrophages (BMMs)	- Cell Viability Assay - Osteoclast Formation Assay - Gelatin Zymography and Cathepsin K Assay - Pit Formation Assay. - Cell Invasion Assay - RNA Isolation and Quantitative Real-Time RT-PCR - Student’s t-test	Saikosaponins A and D inhibited RANKL-mediated osteoclast formation in BMMs and osteoclast-induced bone resorption by reducing the levels of MMP-2, MMP-9, and cathepsin K at non-cytotoxic concentration. In addition, saikosaponins A and D inhibited TGF-β-induced cell invasion and PTHrP mRNA expression in MDA-MB-231 metastatic human breast cancer cells.	1, 2

Caption to table

Table 1. Analysis of the content of the included articles and association with the objectives of the present work.