Review Article

Inhibition of Mmp-9 Expression Through Nf-Kβ by Natural Compounds as a Possible Therapeutic Adjuvant Strategy in Breast Cancer: A Systematic Review

ABSTRACT

MMP-9 expression may be induced at the transcriptional level in response to different agents. Due to its fundamental role in cancer pathogenesis, the control of MMP expression, especially MMP-9, is the possible target of future adjuvant therapies that seek to reduce the development of metastases and angiogenesis in women with breast cancer. Therefore, the aim of this study was to seek in the literature available in digital databases evidence of extracts/or natural compounds that have potential therapeutic capacity to inhibit MMP-9 expression. Extracts and/or natural compounds identified in this review play a significant role in the inhibition of MMP-9 expression via NF-kβ, and may act on the prevention of metastases from primary breast tumors. The majority of the studies found have shown that natural products are capable of suppressing the migration and invasion of breast cancer cells, thus inhibiting the formation of in vitro metastases. Further studies are warranted to understand the potential mechanisms of breast cancer metastasis from signaling cascades intrinsic to the tumor. Lastly, the NF-kβ, followed by Mitogen Activated Protein Kinases / Activator protein 1 (MAPK / AP-1) were the major pathways affected by the extracts and / or compounds studied. These pathways are directly linked to the expression MMP-9.

Keywords: Inhibitor, Matrix Metalloproteinase, Metastasis, Breast Cancer.

1. INTRODUCTION

The development of therapies based on mechanisms for human cancer treatment was announced as a fruit of three decades of remarkable progress in research on mechanisms of cancer pathogenesis. Most target drugs, that have been recently developed, were deliberately directed towards specific molecular markers involved in one way or another in the capacitation of specific resources which confer adaptive capacity for tumor progression [1].

Malignant cells may invade tissues through extracellular matrix degradation by the action of matrix metalloproteinases (MMPs). MMPs are associated with the invasion of tumor cells through the basement membrane and stroma with increasing tumor angiogenesis and metastases. Therefore, this group of enzymes play a major role in primary tumor growth, angiogenesis and basement membrane degradation, favoring the development of metastasis and tumor promotion [2].

Positive MMP-9 expression is a significant predictive factor in breast cancer patients. It is potentially a useful biomarker for the prediction of clinical prognosis [3]. Yousef et al. [4] showed that MMP-9 overexpression is intimately associated with high histologic grade breast cancer, including triple-negative and HER2-positive molecular subtypes. Increased levels of MMP-9 expression are also associated with the emergence of lymph node metastases, a reduced time interval for recurrence and a shorter survival after relapse. Finally, the same authors suggest that the differential expression of MMP-9 contributes to breast cancer heterogeneity and is a fundamental feature of the "molecular signature" of more aggressive subtypes of breast cancer.
MMP-9 expression may be induced at the transcriptional level in response to different agents, such as growth factors, interleukins, tumor necrosis factor (TNF-α) and xenobiotics. Due to its

fundamental role in cancer pathogenesis, the control of MMP expression, especially MMP-9, is the possible target of future adjuvant therapies that seek to reduce the development of metastases and increase angiogenesis in women with breast cancer. Therefore, the development of drugs that inhibit MMP-9 may be useful in human cancer treatment [5].

Therefore, the aim of this study was to seek in the literature available in digital databases evidence of extracts/or natural compounds that have potential therapeutic capacity to inhibit MMP-9 ex pression.

2. MATERIAL AND METHODS

A search in the PubMed, SciELO and LILACS databases was carried out, focused on published articles that contained quantitative studies on the suppression of matrix metalloproteinase 9 in breast cancer. The search was limited to the English language. Only articles published in the last five years were included in this review, since during this period most studies investigating the effect of different substances on gene expression were performed. Search MeSH terms were: "matrix metalloproteinase 9" AND expression AND cancer AND "breast cancer" AND MMP9.

Inclusion criteria were the following: a) studies published in English, b) studies in which in vitro MMP-9 gene expression was evaluated, c) studies investigating a correlation between extracts and/or natural compounds with the capacity to inhibit MMP-9 expression, d) studies investigated the suppressive effect of MMP9, induced by extracts and/or natural compounds on the invasive and/or migratory capacity breast cancer lineages.

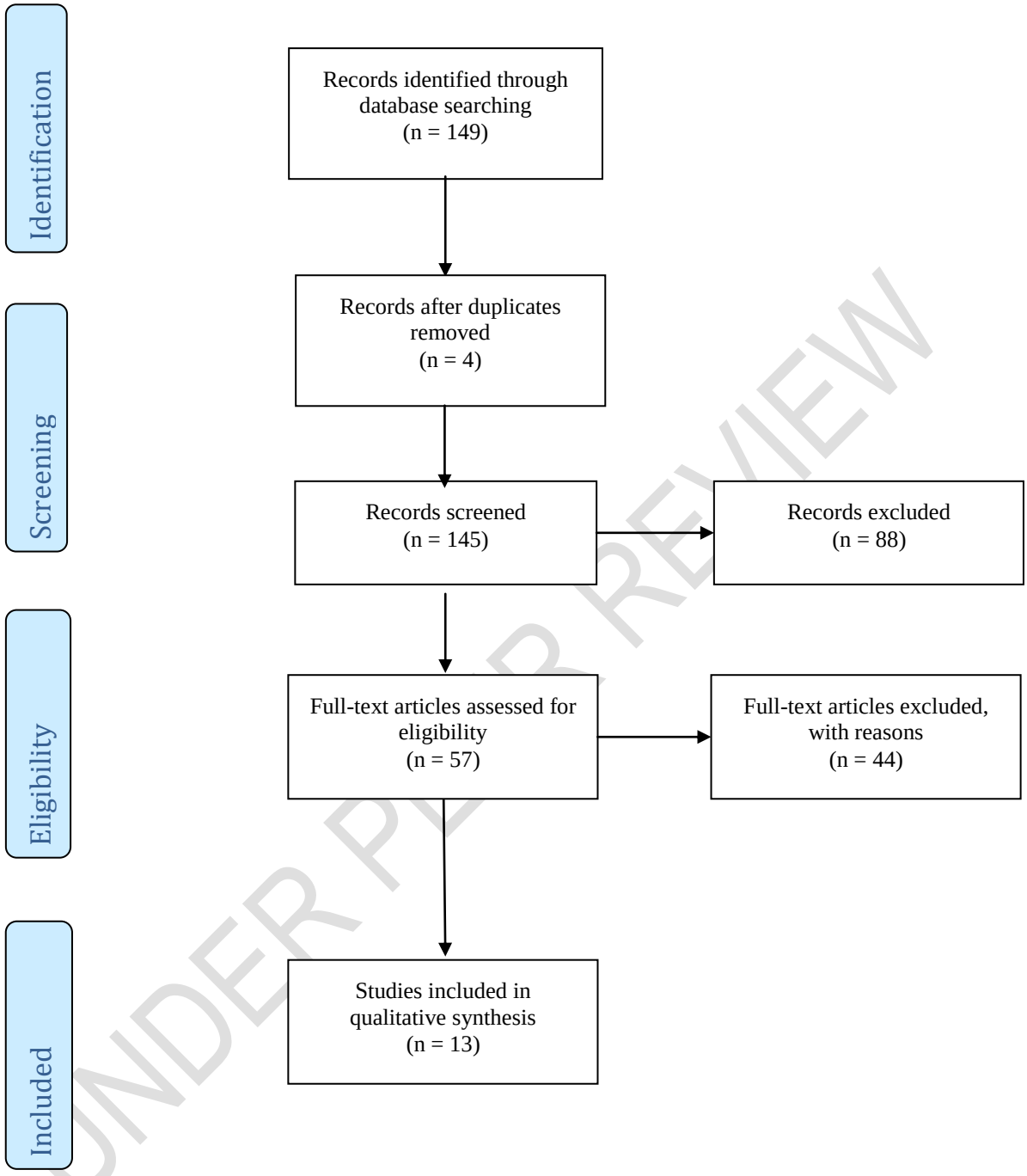
To broaden the scope of the search, the reference lists of all studies were inspected by two experienced authors. Studies were excluded if they were irrelevant, not within the aim of the search, duplicated publications, articles with only abstracts available, editorials, comments and letters to the editor.

3. RESULTS AND DISCUSSION

Of the 149 titles identified in databases following the use of key-words, only 57 fulfilled the inclusion criteria. Of these, 45 articles were excluded, and 4 were duplicated, 6 were considered irrelevant studies by reviewers, 2 articles were not available in full-text or were duplicated, 14 reported the clinical significance of suppression and/or overexpression, 4 involved suppressor drugs of MMP9, 3 evaluated food compounds, 8 investigated MMP9 overexpression and 3 were review. Thus, only 13 studies were used in the review (Figure 1).

The main lineages used in in vitro studies were MCF-7 (7 articles) and MDA-MB-231 (7 articles). However, ZR-75-30 and 4T1 lineages were also used in only one article each.
Concerning techniques used to evaluate the effect of extracts and/or natural compounds on MMP-9 expression and the invasion/migration potential of cell cultures, all articles used Western blotting and 10 articles used Gelatin zymography concomitantly. Migration and/or invasion assays were performed in 12 out of 13 articles evaluated. The assay of expression using reverse transcription polymerase chain reaction (RT-PCR) from RNA extraction of culture cells was only used in 7 articles.

Figure 1. Search flow diagram and selection



All articles that were found used assays as MTT (12 articles) or XTT (1 article) to evaluate cell viability and the cytotoxic potential of extracts and/or compounds tested. Regarding the inhibiting pathway of MMP-9 expression, in the studies evaluated, the majority found the Nuclear Factor Kappa B (NF-kβ) pathway (8 articles), followed by Mitogen Activated Protein Kinases / Activator protein 1 (MAPK/AP-1) (family of kinase protein converted from NF-kβ activation) (3 articles) as the main pathways affected by the extracts and/or compounds studied. These pathways are directly linked to MMP-9 expression. Therefore, a reduction in its expression also implies in the reduction of MMP-9 activity.

A summary of the studies used may be found in Table 1, where the compounds evaluated may also be observed.

Table 1. Study summary

Author / Year	Substance	Objective	Conclusion
Kim *et al*. 2013	Sulfuretin (*Rhusverniciflua Stokes*) (RVS)	Evaluate the potential activity of sulfuretin against the invasion of cells induced by TPA and MMP-9 the expression in MCF-7 cells, and the related molecular mechanisms were investigated.	Sulfuretin is a potent inhibitor of MMP-9 expression induced by TPA, by blockade of NF-kB signaling pathway in breast carcinoma cells. Sulfuretin also suppresses the invasion of cancer cells stimulated with TPA through inhibition of MMP-9 expression.
Noh *et al.* 2013	Guggulsterone (cis or trans) (*Commiphora mukul*)	Investigate the inhibitory effects of guggulsterone isomers (cis or trans) in MMP-9 expression induced by 12-O-tetradecanoilf o-bol-13 acetate (TPA).	Guggulsterone isomers negatively modulate MMP-9 expression induced by TPA in MCF-7 cells and invasion of tumor cells through specific suppression (cis-guggulsterone regulates the IKK/ NF-kB pathway and trans-guggulsterone regulates MAPK / AP-1) activation.
Mi *et al.* 2014	Celastrol (*Tripterygium wilfordii* Hook F.)	Investigate the pathways involved in the inhibition of anti-apoptotic gene expression induced by TNF-α and invasion in MDA-MB-231 cells of human breast cancer by celastrol.	Celastrol exhibits effective antitumor properties, inhibiting the proliferation of cancer cells and inducing apoptosis. Furthermore, there is evidence that celastrol may inhibit the invasion of breast cancer cells through a reduction in MMP-9 expression.
Kim *et al.* 2014 (a)	Decursin (*Angelica gigas* Nakai)	Examine the potential effects of Decursin on cellular invasion induced by TPA and on MMP-9 expression in MCF-7 cells	Decursin inhibited the invasion induced by TPA when reducing MMP-9 activation mainly through PKCα, MAPK and NF-kB pathways in MCF-7 cell activation
Kim *et al*. 2014 (b)	Supercritical Extracts of (*Citrus Hassaku*) Pericarp (SEPS)f	Investigate the potential of SEPS as anticancer agents and their antimetastatic activities and mechanisms of reduction of chemokine receptors CXCR4 and MMP-9 in MDA-MB-231human breast carcinoma cells.	SEPS may reduce the expression of CXCR4 and MMP-9 through the suppression of NF-kB signaling pathways, which makes it a potentially effective blocker of metastasis and tumor cell invasion.
Li *et al*. 2014	Ginsenoside Rg1	Investigate the effects of ginsenoside Rg1 on invasion and migration induced by PMA in MCF-7 cells	The results suggest that ginsenoside Rg1 inhibits MMP-9 activity induced by PMA through NF-κβ to suppress the migration and invasion of breast cancer cells
Zheng *et al.* 2014	Extracts of (*Momordica cochinchinensis*) seeds (ESMCs)	Investigate the effect *Momordica cochinchinensis* seeds on the migration and invasion of human breast cancer cells ZR-75-30, and its effects on enzymatic degradation of extracellular matrices	ESMC was capable of inhibiting the adhesion, migration and invasion of breast cancer cells (ZR-75-30), by attenuation of the activity and expression of MMP-2 and MMP-9.

Pei *et al.* 2015	Plantamajoside (PMS) - (Herbal extract *Plantaginis*)	Investigate the proliferation, migration and invasion of human breast cancer cell line MDA-MB-231 and rat breast cancer cell line 4T1 in response to treatment with the inhibitor PMS.	PMS restricted tumor growth significantly and also demonstrated an effect on the inhibition of MMP9 and MMP2 activity.
Jiang *et al.* 2016	Lunasin	Evaluate the possible inhibitory effects of lunasin on growth, migration, invasion and degradation of extracellular matrix of breast cancer cells.	Lunasin inhibited cell proliferation, migration, invasion and activity and expression of MMP-2 and MMP-9 in breast cancer cells, possibly exerting its inhibitory effect through suppression of FAK / Akt / ERK and NF-κB signaling pathways mediated by integrin.
Park *et al*. 2016	Pomolic acid (PA) (*Euscaphis japonica*)	Determine the molecular mechanism by which PA inhibits the migratory and invasive abilities of highly metastatic MDA-MB-231 cells induced by EGF.	PA inhibits cellular migration, invasion and motility of highly metastatic breast cancer cells MDA-MB-231 by inhibiting MMP-9 expression and FAK phosphorilation through inhibition of NF-κB / ERK mediated by EGFR / signaling pathways mTOR. PA may inhibit the expression induced by EGF of MMP-9 and phosphorilation of FAK in MDA-MB-231 cells by inhibition of PI3K / Akt / mTOR signaling pathways
Chung *et al.* 2017	Metanolic (MOD) and butanolic (BOD) (*Oldenlandia diffusa*) extract	Investigate the effects of aqueous extract MOD an BOD on the growth and death of human breast cancer cell line MCF-7.	MOD and BOD suppress the invasion stimulated by PMA of MCF-7 cells through inhibition of MMP-9 expression and induce apoptotic cellular death.
Kunte and Desai 2017	C-phycocyanin Extract (C-PC Extract) (*Spirulina platensis*)	Demonstrate the selective inhibitory effect of dC-PC extract in two distinct classes MMPs (MMP-1 and two gelatinases (MMP-2 and MMP-9)) at the level of enzymatic expression. and mRNA.	C-PC extract had significant inhibitory activity against human gelatinase, selectively inhibiting MMP-2 and MMP-9, without inducing any cellular toxicity. C-PC extract remained ineffective for MMP-1 and TIMP-1.
Lou *et al.* 2017	Arctigenin (*Arctium lappa L*.)	Investigate the antimetastatic effect of arctigenin in human breast cancer cells.	Arctigenin suppressed cancer cell metastasis MDA-MB-231 by downregulation of MMP-2, MMP-9 and heparanase.

IKK, complex inhibitor kappa kinase; TNF-α, tumor necrosis factors alpha *; PKC-α, protein kinase C alpha; CXCR4, C-X-C chemokine receptor type 4; MMP-2, matrix metalloproteinase-2; FAK, focal adhesion kinase; AKT, serine/threonine kinase; ERK, extracellular signal–regulated kinases; EGFR, epidermal growth factor receptor ; EGF, epidermal growth factor; PI3K, phosphoinositide 3-kinase; MMP-1, matrix metalloproteinase-1; TIMP-1, tissue inhibitors of metalloproteinases-1.*

Breast cancer is one of the most commonly encountered malignancies with an unfavorable prognosis [6]. Mortality due to distant metastasis is increased in this type of tumor [7]. In the majority of cases, the main cause of death is metastasis of malignant tumor cells and not the primary solid tumor [8]. Due to the great impact on the population, the prognosis and specific treatment for breast cancer need to be explored [2]. Unfortunately, the only anti-metastatic therapies in clinical practice, including anthracyclins, taxanes and trastuzumab, have limited efficacy. Therefore, it is imperative to find effective medication in cancer metastases, that has the capacity to inhibit migration, invasion and proteolytic degradation of the extracellular matrix (ECM) [9].

The growing interest in new bioactive compounds derived from natural sources opened new windows in the field of biotechnology and Medicine [10]. The main focus of studies involved in the development of effective strategies against invasion is the use of natural bioactive agents in MCF-7 cells [11]. Natural products are a very important source that provide promising clues for the development of new cancer treatment, due to results of potentially low toxicity and potential efficacy [12].

Effective agents involved in the combat against invasion have demonstrated a capacity to reduce MMP-9 expression [13]. It has been suggested that the regulation of MMP-9 expression is a possible approach to the development of anti-metastatic drugs [10]. Therefore, an inhibitory effect on MMP-9 expression is important in an experimental model therapy of tumor metastasis [2].
As in vitro model in cancer research, the MCF-7 cell line has been commonly used during the last 4 decades in studies of molecular profile, proliferation, migration, invasion and angiogenesis. Among human breast cancer cells, MCF-7 cells have limited migration due to positive expression of estrogen-α (ERα) receptors, therefore they are used as a typical model of non-invasive and non-progressive breast cancer. In contrast, MDA-MB-231 cells are frequently used as a model of invasion and progression, since they are ERα-negative [2].

In vitro and in vivo models of tumor metastasis were developed to test diverse experimental therapies. To develop invasive capacity in lineages such as MCF-7 and MDA-MB-231, the cells need to be stimulated by agents such as Epidermal Growth Factor (EGF) and 12-O-Tetradecanoylphorbol-13-acetate (TPA). These methodologies permitted the identification of new agents with anti-invasive potential and their possible inhibitory pathways of activity. NF-kβ is the main agent [14-18].

The NF-kβ family of transcription factors are key regulators of immune response, inflammation and cancer. Research has demonstrated that NF-kβ signaling pathways are intimately related to cancer metastasis, suggesting that the inhibition of NF-kβ activity disturbs the metastatic potential of breast epithelial cells in model systems. Interestingly, NF-kβ is induced by more than 150 different extracellular stimuli. In a similar manner, this gene has the capacity to promote expression in more than 150 target genes. Among possible targets regulated by NF-kβ, are genes linked to cell motility, invasion and metastasis, including genes that encode MMPs [19-20].

The promotion of extracellular matrix degradation is directly induced by NF-kβ when activating the expression of genes such as urokinase-type plasminogen activator (uPA), a matrix metalloproteinase 9 (MMP-9) and CXCR4 chemokine receptor [21]. The appeal to develop a target drug, that may inhibit NF-kβ activity and consequently the subproducts of genes involved in cancer progression is still currently an ongoing debate. It is fundamental to abort the capacity of tumor migration and metastases by attacking specific points that fail to generate great chemoresistance [22].

Primary tumor cells or metastases resistant to cytotoxicity of chemotherapy agents and ionizing radiation may limit the efficacy of other adjuvant therapies in breast cancer treatment. Chemoresistance associated with adjuvant therapy suggests that early use of adjuvant therapy may be fruitless, if not damaging in tumors that exhibit high NF-kβ activity. Nevertheless, since the inhibition of NF-kβ increases tumor cell sensitivity to apoptosis induced by chemotherapy agents and irradiation, the simultaneous use of NF-kβ antagonists may be advantageous [23].

4. CONCLUSION

Extracts and/or natural compounds identified in this review play a significant role in the inhibition of MMP-9 expression via NF-kβ, and may act on the prevention of metastases from primary breast tumors. The majority of the studies found have shown that natural products are capable of suppressing the migration and invasion of breast cancer cells, thus inhibiting the formation of in vitro metastases. Further studies are warranted to understand the potential mechanisms of breast cancer metastasis from signaling cascades intrinsic to the tumor. These molecular and cellular mechanisms include a reduction in MMP expression, interference with VEGF signaling, modulation of Epithelium Mesenchymal Transition (EMT) regulators, inhibition of the expression of NF-kβ, mTOR and other mechanisms. It is unlikely that natural compounds are anti-metastatic agents when used individually. However, their use as adjuvants of chemotherapy drugs may help prevent the progression of breast cancer metastasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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