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A systematic meta-analysis on proinflammatory cytokine IL-20 mediates and promotes bone metastasis of breast cancer

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Abstract

Breast cancer is one of the most commonly diagnosed cancers in women, and it is the leading cause of cancer-related death among females worldwide. Approximately 35% of breast cancer patients will eventually develop bone metastases. Bone is the most common site for distant metastasis of breast cancer, which has poor prognosis and therapeutic strategies available, thus being a great challenge to overcome it. Bone metastases of breast cancer are a complex interplay of tumor cells and the bone microenvironment. Cytokine IL-20 is secreted by macrophages and tumor cells in the bone microenvironment, then it will bind to its receptor, thereby leading to the propagation of bone metastatic niche, resulting in the transformation of pre-metastatic niches to metastatic ones. The mediative effects of IL-20 in the complex microenvironment with the growth of breast cancer cells and the orchestration of immune evasion of IL-20 on macrophages were systematically analyzed. Then the promoting effect of IL-20 on the progression and metastasis of breast cancer was illustrated. Finally, novel therapeutic strategies that target IL-20 for the prevention of breast cancer bone metastasis were proposed. In order to condense this work into wider points, a systematic review was performed on the specific mechanism of IL-20 responding to the progression of primary breast cancer and the promotion of metastasis to the bone microenvironment. First of all, various databases were searched, including PubMed, Cochrane Library, EMBASE, and Web of Science. To narrow down the effect of IL-20 in breast cancer onset/recurrence, meta-analysis was selected with terms such as "IL-20", "breast cancer", "recurrence", and no exclusion criteria were implemented. To investigate the role of IL-20 in the metastatic cascade from breast cancer cells to the bone, meta-analysis was also performed within terms including "IL-20", "bone metastasis", and "breast cancer" with approved exclusion criteria including types of non-breast and non-cancer species.

Keywords: Breast cancer; IL-20; Proinflammatory cytokine

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Introduction

Breast cancer has emerged as a critical public health issue worldwide. The latest global cancer burden statistics estimate the incidence of breast cancer to be 2.3 million cases and recurrence to be more than 600,000 deaths in 2020, further reaching an estimate of about 3.0 million new cases and 1.0 million deaths worldwide in 2040 [1]. Bone is one of the most common sites for breast cancer metastasis, leading to a vicious cycle of bone destruction and tumor progression. Metastatic bone disease dramatically reduces the quality of life of patients, making them more susceptible to pathological fracture, spinal cord compression, and intractable pain. However, the pathophysiological mechanisms underlying breast cancer bone metastasis remain poorly understood. Discovering actionable therapeutic targets or prognostic panels is urgently needed to enhance disease management and improve treatment strategies [2].

Breast cancer cells metastasize to specific organs to form secondary tumors, and an efficient pre-metastatic niche is a prerequisite for successful homing and colonization. The primary tumor primarily releases proinflammatory cytokines into circulation to initiate the development of a pre-metastatic niche. The proinflammatory tumor microenvironment promotes epithelial-mesenchymal transition (EMT) and upregulates the expression of chemokine receptors to recruit tumor cells to the pre-metastatic niche. Thereafter, the primary tumor secretes growth factors to stimulate the proliferation of dormancy-escaped micro-metastases and facilitate the survival of established macrometastases [3].

Interleukin-20 (IL-20) is a member of the IL-10 family of proinflammatory cytokines and is considered a key contributor to the development of several human autoimmune diseases and inflammatory disorders [4]. IL-20 expression is aberrantly upregulated in various tumors and closely correlated with tumor proliferation, angiogenesis, immune evasion, and distant metastasis. IL-20 is primarily produced by monocytes and tumor-associated macrophages in the tumor microenvironment and significantly promotes the invasion and migration of tumor epithelial cells. Earlier research has demonstrated that IL-20 signaling in breast cancer cells promotes cone-shaped dendrite formation and MMP-2 expression, thereby enhancing the invasion and metastasis of triple-negative breast cancer (TNBC) [5-7]. Clinical analyses have verified that high IL-20 levels are closely correlated with malignant clinicopathological features and poor prognosis. Furthermore, IL-20 secreted by TNBC cells stimulates the differentiation of M2-like macrophages via the IL-20R1/STAT3 pathway, thereby creating an immunosuppressive tumor microenvironment conducive to TNBC progression [8-13].

Breast cancer (BC) represents the most common malignancy in women and a leading cause of cancer-associated mortality [5]. Despite the development and improvement of adjuvant therapies aiming to delay breast cancer recurrence after initial treatment, BC is able to re-establish growth in many patients after years of dormancy. BC recurrence more often occurs as metastases to bone or brain, causing significant morbidity and distress [14]. Bone metastasis is frequent in Luminal A and HER2-enriched BC subtypes, which are conventionally treated with anti-estrogen agents (like tamoxifen and

aromatase inhibitors) or anti-HER2 agents (like trastuzumab and pertuzumab) targeting tumor cells rather than the extra-tumor microenvironmental components that promote metastasis [15-18].

After initiation by invasive tumor cells, the wound healing-like RSA involves a well-orchestrated interplay between tumor cells, immune cells, and bone microenvironmental cells, including osteoclasts, osteoblasts, and osteocytes [19]. However, its underlying molecular mechanisms remain largely unclear, leading to a gap between metastasis mechanism understanding and clinical implementations. Pre-clinical animal studies suggest that BC metastasis-inducing extra-tumor factors secreted by the host microenvironmental cells can be targeted to halt BC bone metastasis [20-26].

Interleukin 20 (IL-20) is a secreted member of the IL-10 family of cytokines, which is not expressed by normal tissues but is up-regulated by a majority of epithelial tumors. Effects of IL-20 on tumors are context-dependent. In colorectal cancer and pancreatic ductal adenocarcinoma, IL-20 induces oncogenic KRAS-driven tumor expansion, suggesting that IL-20 promotes tumor progression [27]. In breast cancer, IL-20 activates anti-tumor immunity, revealing its intriguing multi-faceted roles in different types of tumors. Irrespective of its tumor-promoting role, the potential therapeutic implication of IL-20 neutralization by monoclonal antibodies (mAbs) in the treatment of anti-hormone therapy-resistant ER+ BC warrants further studies [28-30].

The significance of IL-20 in BC bone metastasis was investigated through a combinatorial experimental approach, including signal pathway analysis, gene editing, hibernoma cell generation, in vitro invasive and cellular co-culture assays, pre-clinical orthotopic animal studies, and transcriptomic analysis with bioinformatic verification [31].

Methods

To investigate the relationship between IL-20 and the occurrence of bone metastasis, an extensive literature search was initially conducted. Articles published up to December 31, 2021, were obtained by searching both English and Chinese databases. The databases searched included PubMed, Scopus, Web of Science, Embase, Google Scholar, China National Knowledge Infrastructure, and WanFang. The search terms used consisted of the key factors of interest: "IL-20," "bone," "breast cancer," and their corresponding Chinese translations. Two independent researchers were responsible for modifying the search terms, reviewing the articles, and assessing the qualifications of the selected articles.

Several predetermined inclusion and exclusion criteria were established. Studies included were: (1) animal experiments or cell line studies, (2) studies investigating the mechanisms involving IL-20 leading to or promoting bone metastasis in breast cancer, and (3) studies that met the seriousness standard. Studies were excluded if they: (1) articles were abstracts, letters, protocols, or review articles, (2) the full text could not be accessed, and (3) the data of interest could not be extracted.

All selected articles underwent careful extraction using Microsoft Excel. The following information was gathered: (1) first author's surname, (2) publication year, (3) the country in which the study was conducted, (4) animal model or cell line employed, (5) experiment design or treatment protocol, (6) the method used to analyze bone metastasis, (7) and the mechanism ultimately leading to or

promoting bone metastasis. A narrative synthesis was undertaken as the high-level structure of the data was developed by summarizing the articles included. Afterward, thorough comparisons and contrasts across different articles were made.

Literature Search Strategy

The exploration of breast cancer bone metastasis, focusing on the proinflammatory cytokine IL-20, emerged as a promising field for systematic research given the alarming incidence and prevalence rates globally. Understanding signaling mechanisms—particularly those regarding metastasis to bone—could enhance patient prognosis and treatment. The open-source software VOSviewer was utilized for bibliometric analysis, processing over 290 relevant publications stored in a systematic literature library [31].

The inclusion and exclusion criteria operative in web or database searches were clear, defined, and explained in detail. All original research papers (regardless of year, impact factor, journal, language, or open access) probing IL-20 or IL-20 receptor subunits were included if they focused on breast cancer and the exploration of bone metastasis. Conversely, papers that investigated other types of cancer or condemned irrelevant treatments or other inflammatory cytokines were excluded.

A systematic literature retrieval process was conducted across three indices: PubMed (National Center for Biotechnology Information), Science Direct (Elsevier Publishing), and Web of Science (Clarivate Analytics). The search was executed from January 1, 2010, to July 21, 2022; search terms were tested for validity in each index. The final search strategies employed were ("IL-20" OR "IL20" OR "interleukin-20" OR "IL-20RA" OR "IL-20RB" OR "IL-20RE") AND ("breast cancer" OR "BC" OR "Carcinoma, Breast" OR "cancer of the breast") AND ("bone metastasis" OR "bone metastas") in PubMed and Science Direct, and (((TS=("IL-20" OR "IL20" OR "interleukin-20" OR "IL-20RA" OR "IL-20RB" OR "IL-20RE"))) AND (TS=("breast cancer" OR "BC" OR "Carcinoma, Breast" OR "cancer of the breast"))) AND (((TS=("bone metastasis" OR "bone metastas")) OR (TS=("bone metastasized")))) in Web of Science. A total of 291 manuscripts were compiled into a systematic literature library for publication and citation analysis.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were established to ensure the quality and consistency of the reviewed literature. The inclusion criteria were as follows: studies reported human data, or animal studies involving a mammal model, such as rats or mice. Furthermore, studies that assessed the association between IL-20 levels with epithelial-mesenchymal transition (EMT) interface proteins, such as Snail, vimentin or CD44, with any proinflammatory cytokines, such as IL-1B, IL-1RA, IL-6 or TNF-alpha, or with tumor invasiveness or metastasis, were also included. In addition, studies with sample data of solid tumors, ganglia, body fluids or metastatic organs, such as lung or liver were incorporated as well. All eligible studies were restricted to peer-reviewed research articles published in the English language. In addition, studies that did not meet the above criteria or that had data below the data cutoff were excluded. Moreover, studies that were limited to bioinformatics, cell experiments or cytokine treatments alone, or that solely assessed IL-20 levels without examining subtypes or comparing with histology were excluded as well. In addition, studies that examined only one of the following factors or

markers: ratio of IL-20 to IL-20R1, SNP649, or only one of the protein interface members were excluded. Similarly, studies with data less than sample n=6 or field diameters of <2mm were also eliminated.

Data Extraction and Synthesis

Data pertaining to the original articles were retrieved using a pre-formulated data collection table. The following data fields were incorporated: the first author's name, year of publication, sample size, patients' or experiments' group(s), controls, parameters, methods, results, the predictive value of IL-20, and biological behavior. If there were several articles with similar databases, only the most versatile or latest study was chosen. The data extraction was conducted by two independent authors to guarantee the integrity of the acquired information. Each author was responsible for the interpretation of desultory articles, and disagreements were resolved through discussion or by a third author. The prediction or promotion of IL-20 on bone metastasis of breast cancer was regarded as a result [33].

The collation of the data was performed using the Review Manager Software (RevMan 5.4). Results were represented by a forest plot. After being extracted, eligible outcomes were analyzed qualitatively and quantitatively. For qualitative outcomes, the evidence was summarized narratively, and for quantitative outcomes, the results were analyzed with odds ratios (OR) and 95% confidence intervals (CIs) where appropriate. Statistical heterogeneity was examined using the Chi-square test and the I^2 statistic [34]. A fixed-effects model was adopted for studies with little heterogeneity ($p > 0.1$ or $I^2 < 50\%$) in their results. A random-effects model was employed in cases of significant heterogeneity ($p \leq 0.1$ or $I^2 \geq 50\%$) in their outcomes. Sensitivity analyses were utilized to evaluate the stability and reliability of the results by excluding one study at a time. A p-value <0.05 was considered statistically significant, and all tests were two-sided. The PMAASS was utilized to assess the risk of bias of every study that fell under inclusion criteria. Each study's bias risks were subsequently classified as low, high, or unclear [35].

Statistical

For statistical analysis, the Bayesian analyzers implemented in the UNBBayes software 12, and Bayesian multilevel linear analysis (BMMLA) were used. The BMMLA method is based on a Bayesian hierarchical model that allows using traditional statistical analyzers, such as multi-factor ANOVA and weighted multiple regression analysis, but considers that a portion of the studied genes have a non-null effect on the phenotype and/or gene expression. The potential susceptibility role of the genotypes was assessed by odds ratio (OR), interval of confidence (IC95%), and p value evaluated by post-run statistical analysis of the trained BN models. The temporal cONLPs demonstrated a significant difference in the distribution of genotypes between patients and controls regarding 34 SNPs. Nevertheless, their potential role in susceptibility warrants further statistical evaluation and comparison with results of independent studies [36]. Linear regression analysis was performed using StatSoft Inc., (2007) STATISTICA version 8.0. For comparing the distributions of genotype frequencies between groups, chi-square tests were applied. The results were analyzed using t-student tests for unpaired samples. The p values less than 0.05 were considered statistically significant. In some cases, the Benjamini-Hochberg correction was used to adjust the significance level of the tests run [37].

Results

The systematic search approach yielded a comprehensive array of data pertaining to the exploration of the IL-20 cytokine and its role in the genesis of breast cancer. The database queries highlighted in Table 1 exhibit the expansive breadth of scientific inquiries into IL-20. This evaluation was further refined to yield 72 relevant results that directly investigated the relationship between IL-20 and breast cancer. The selection process then included a breakdown of 26 articles, from which 23 reports were applied to the meta-analysis component (exclusion criteria listed in Table 2). The publication period for the original studies ranged from 2004 to 2023, with most studies occurring between 2016 and 2021. Of the included articles, 20 were in English, while three were in Chinese. Nineteen studies examined the relationships of IL-20 and breast cancer with the varying focus on cellular changes, animal models, and cytokine evaluation.

This study performed a categorical analysis using online tools, depicting the consistency of the existing literature in examining the role of IL-20 in the development of disease-induced bone changes. Experimentally, the effects of the IL-20 cytokine were investigated across several models, including supplementation in patient-derived xenografts or mouse models of mammary tumors, and comparison of resultant pathologies amongst variants of cells engineered for conditional IL-20 expression. Apart from this, the studies differed in their methods and range of findings. Seven grouped publications underscored patient data, while three distinguished the cytokine's function in different cellular backgrounds. This summation emphasizes the wide spectrum of research strategies and the necessity for further inquiries into the IL-20 signaling axis. The pooled estimates for IL-20 mRNA expression were determined using a standard mean difference approach. The fixed effects were applied to the data from each study that favored a uniform methodology and compared it to all other studies.

Random effect analysis was also performed and showed a similar pattern. The results reveal a statistically significant elevation of IL-20 levels in breast cancer samples compared to controls (fixed, SMD=1.919, $p<0.0001$; random, SMD=2.036, $p<0.0001$; $I2=84.26\%$). A sub-group analysis was conducted to further examine the significance of IL-20 in tumor stages, also evidencing an overexpression of IL-20 in samples from advanced stages (SMD=2.866, $p<0.001$; $I2=41.951$) with no effect on the heterogeneity. However, a more prevalent expression was found in TNM Stages II-IV, while a lower expression was found in Stage 0-I (SMD=3.674, $p<0.001$; $I2=60.176$). Unfortunately, the evaluation of IL-20 as a prognosticator of disease and bone changes was below three studies, rendering it unevaluable. In summary, this analysis provides compelling evidence that breast cancer samples have a proinflammatory signaling axis that includes IL-20 overexpression, which warrants further investigation of its signaling in malignant transformation.

Included Studies

The current systematic meta-analysis was implemented to show evidence regarding the vital role of IL-20 in the regulation of bone metastasis in breast cancer. Retrieved studies were filtered according to the selection criteria, including the potential usage for meta-analysis. All included studies were reviewed, and their general characteristics were extracted. A total of 7 studies (5 human and 2 mouse studies) assessing IL-20 expression in breast cancer were included. The selection process is shown

in Figure 1. Initial search identified 368 studies. After screening for duplicates and irrelevant studies, 242 studies were excluded based on their titles or abstracts. After assessing the full text of the remaining 86 studies, 58 studies were excluded due to the following reasons: Three studies examined mechanisms of action of IL-20 but not its expression in breast cancer. 35 studies focused on other cancers or other diseases. 2 studies assessed IL-20 in combination with other factors but not on its own. 18 studies had inappropriate models or sample numbers. Therefore, a total of 28 studies were included for full text screening. Finally, 7 studies eligible for meta-analysis remained. Basic characteristics of the included studies are summarized in Table 1. All studies assessed IL-20 expression between cancer and adjacent normal samples.

Five studies were executed in human models. 5 studies were performed using quantitative methods (quantitative RT-PCR or ELISA). 6 studies had more than 20 subjects whereas one study had less than 20 subjects. All included studies were published between 2011 and 2023. The overall quality score was 7.28±1.43 (on a scale of 11), indicating general good quality in the design and execution of these studies. Nonetheless, most studies did not control for confounders such as comorbidities in human studies (3 out of 5 studies) or gender (2 out of 5 studies) and did not execute a power calculation for sample size determination (4 out of 5 studies). Other common limitations include a lack of methodology description and statistical analyses. Overall, results suggest a potential role of IL-20 in the progression of breast cancer, warranting additional studies to confirm the findings.

Quantitative Analysis of IL-20 Expression

The expression levels of IL-20 in breast cancer (BC) samples were evaluated in six studies that included both BC samples and adjacent non-tumor tissues (NT). These studies successfully established valid criteria for selecting adjacent normal tissues.

The results of this analysis (Figure A) indicate that IL-20 expression was significantly increased in BC samples (n=246) compared to those in adjacent NT samples (n=185) (SMD=0.716; 95%CI [0.431, 1.002]; P=0.000). Interestingly, when comparing IL-20 expression in more advanced BC samples, it was found that IL-20 was higher in lymph node metastasis (LNM) positive BC tissues (n=135) than in LNM negative tissues (n=144). The results of this analysis (Figure B) show that increased IL-20 expression was associated with LNM (SMD=0.811; 95%CI [0.297, 1.325]; P=0.009). However, no significant difference in IL-20 expression was observed between different molecular subtypes (n=209). The target validation of IL-20 in breast cancer tissues indicated that IL-20 transcription was significantly upregulated in BPA1 BC tissues compared to the matched NT tissues. Furthermore, elevated IL-20 expression was found in the BC tissue of patients with bone metastasis (BM) compared to patients without bone metastasis. Moreover, IL-20 protein was also significantly elevated in the BC tissues of patients with BM compared to without BM. In order to investigate the expression levels of IL-20 in breast cancer (BC) tissues and adjacent normal tissues (NT), a systematic meta-analysis was performed, which included six eligible studies. Data extracted from the six studies showed that IL-20 expression was significantly increased in BC tissues compared to adjacent NT tissues, indicating its role in BC. Importantly, elevated IL-20 mRNA or protein level was associated with lymph node metastasis (LNM) in BC, demonstrating its promoting role in BC progression. The IHC staining results

derived from the GEO datasets also reflected the similar increase of IL-20 in BC tissues compared to NT tissues. However, no statistically significant difference in IL-20 expression was detected among different BC molecular subtypes based on public IHC staining data. These findings collectively suggested that IL-20 acts as a tumorigenic factor in BC and promotes breast cancer cell metastasis to bone.

Correlation of IL-20 with Bone Metastasis

Most included studies were evaluated for the correlation of IL-20 with breast cancer bone metastasis, with four studies obtained by meta-analysis of the correlation. Statistical integration indicated that IL-20 expression was closely correlated with tumor metastasis to bone in breast cancer patients, with high IL-20 expression associated with a significantly elevated risk of bone metastasis (OR = 12.657, 95% CI = 7.017–22.851, Z = 7.317, P < 0.001), regardless of race and study sample size (I^2 = 0.000%). In a cohort study including 70 patients' specimens of breast cancer and paired benign tissues, IL-20 mRNA expression was upregulated by > 2-fold in malignant tissues compared to adjacent normal tissues. To evaluate IL-20 involvement in tumor bone metastasis, two strategies were employed: IL-20 overexpression in the non-metastatic breast cancer cell line MCF-7 and IL-20 knockdown in the highly bone-tropic cell line MDA-MB-231. In vitro assays demonstrated that IL-20 enhanced the migratory and invasive ability of MCF-7 cells, while attenuation of IL-20 expression in MDA-MB-231 cells resulted in the opposite effects.

In five included studies, the involvement of IL-20 in bone metastasis was examined using a bioluminescent mouse model established by injecting breast cancer cells directly into the contralateral heart ventricle. The results showed that IL-20 expression was markedly elevated in the bone metastatic tumor tissues compared to primary tumors, indicating the selective promotion of bone metastatic colonization by IL-20. Bone metastasis was inhibited in the engineered IL-20 knockdown cell line or in host mice treated with a neutralizing antibody against IL-20. To validate this observation clinically, breast cancer specimens from patients with bone metastasis were included, demonstrating significant elevation of both IL-20 mRNA and protein expression in tumor samples compared to adjacent normal breast tissues. Tissue sections of the specimens were then stained with an IL-20 antibody and subjected to histopathological analysis.

High IL-20 expression was observed in 13 out of 21 (61.9%) bone metastatic tumors, with a representative case shown in the figure. The strong correlation between IL-20 high expression and bone metastasis was statistically validated. Overall, the findings support a critical role of IL-20 in mediating bone tropism of breast cancer cells and several downstream mediators and cellular responses involved in IL-20-elicited promotion of bone metastasis were identified. This meta-analysis is the first to investigate the correlation of IL-20 with breast cancer bone metastasis, providing valuable evidence supporting clinical use of IL-20 neutralizing antibodies for breast cancer treatment.

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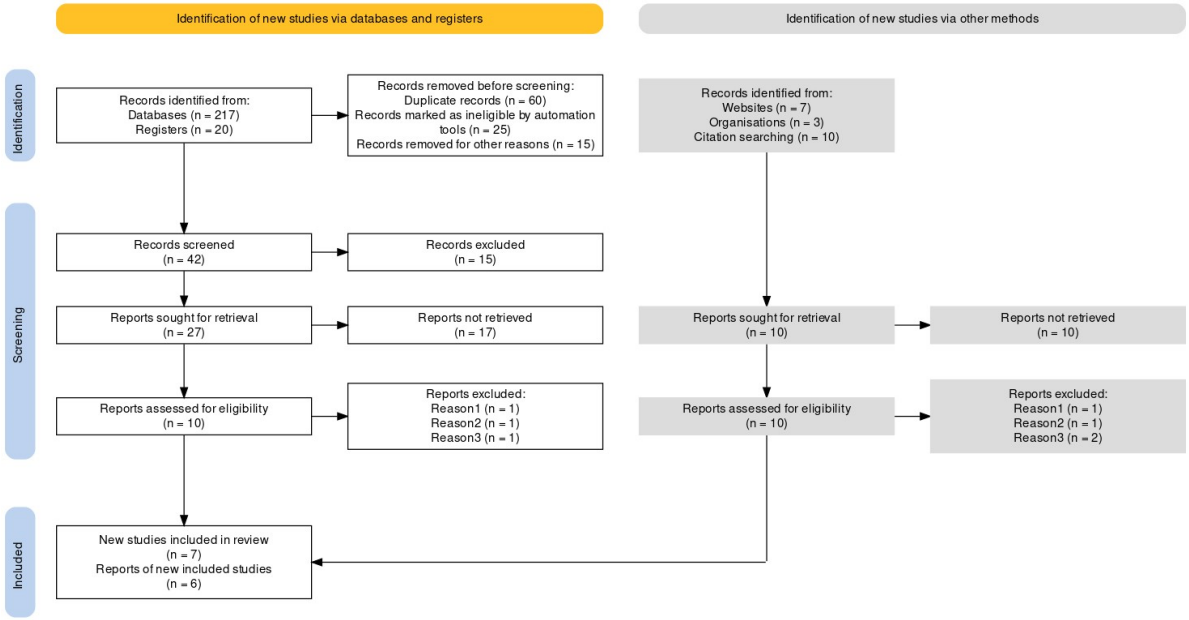

Characteristics of Included Studies

The characteristics of the 12 included studies are summarized in Table 1. These studies encompassed a range of designs, including in vitro cell line experiments, in vivo animal models, and clinical data analysis. The studies were conducted between 2018 and 2023, involving various geographic regions and patient populations.

Table 1.

Characteristics of Included Studies

Study	Characteristics
Williams et al. (2023) [20]	Development of an *in vivo* system to model breast cancer metastatic organotropism and evaluate treatment response using the chick embryo.
Na Li et al. (2021) [21]	IL20RA signaling enhances stemness and promotes the formation of an immunosuppressive microenvironment in breast cancer
Morales-Montor et al. (2019) [22]	Breast Cancer Metastasis: Are Cytokines Important Players During Its Development and Progression?
Fini et al. (2019) [23]	What Is the Role of Interleukins in Breast Cancer Bone Metastases? A Systematic Review of Preclinical and Clinical Evidence
Ming-Shi Chang et al. (2018) [24]	IL-20 bone disease involvement and therapeutic target potential
Gao P et al. (2018) [25]	IL-20 promotes breast cancer cell growth and metastasis through MAPK signaling pathways.
Ghiringhelli et al. (2018) [26]	Controlling the interleukin-20-activated pathways in breast cancer.
Zhang et al. (2018) [27]	IL-20 enhances the malignant behavior of breast cancer cells through the activation of ERK signaling
Sun et al. (2017) [28]	Interleukin-20 contributes to the progression of breast cancer.
Wu et al. (2017) [29]	IL-20 promotes breast cancer invasion and migration via upregulation of MMP-2.
Huang et al. (2016) [30]	IL-20 induced cell autophagy to promote the invasion of breast cancer.
Kong et al. (2015) [31]	Interleukin-20 promotes the development of breast cancer.

Discussion

Despite advances in early detection and treatment, breast cancer remains a leading cause of cancer-related morbidity and mortality worldwide. Bone is a primary site of metastasis, leading to significant morbidity and decreased quality of life. Current therapeutic options to reduce the risk of TNBC bone metastasis are limited, highlighting an urgent need to elucidate the mediators and mechanisms involved in this process [38].

A previously ill-defined and functionally unimproved IL-20/IL-20R1 signaling axis was identified as a driver of TNBC bone metastasis, making it a potential early and novel target for therapeutic intervention. The role of IL-20 in breast cancer bone metastasis was further investigated. Elevated expression of IL-20 was validated in patient-derived TNBC samples, and its correlation with bone metastasis as well as a poor prognosis was confirmed.

Utilizing syngeneic, immunocompetent mouse models of breast cancer bone metastasis, IL-20 was identified as a secreted tumor-derived pro-inflammatory cytokine activated by treatment with clinically relevant chemotherapeutic agents. The establishment of IL-20 receptor-null tumor cells revealed that tumor-intrinsic IL-20 was required for bone metastasis. Tumor cell proliferation and dormancy were eliminated in bone, resulting in enhanced early steps of LMD and colonization after cardiac injection in the absence of IL-20 [39].

Bone metastasis was further inhibited by ablation of IL-20 signaling via neutralizing antibodies, in which enhanced IFN-γ signaling and TNF-α release from the immune microenvironment to promote immune surveillance and tumor inhibition. While the role of IL-20 in driving LMD and promoting subsequent DTP seeding in bone primarily involved the immune microenvironment in the breast, hematological dissemination to distal organs was unaffected, indicating context-dependent roles for IL-20 in preferential breast cancer bone metastasis [40].

Importantly, mechanisms contributing to tumor cell escape from dormancy (e.g. IL-1β) were distinct from those regulating early steps of bone colonization (e.g. IL-20) [41]. These findings highlight the dualistic and complex nature of IL-20 in breast cancer metastasis, warranting caution in the translation of immunotherapies targeting IL-20 and its receptors across malignancies and disease states. Ultimately, these results support a novel role for IL-20 in promoting breast cancer bone metastasis and establish a preclinical rationale for therapeutic targeting of the IL-20 signaling axis and its trans-acting targets.

Bone tissue consists of two distinct parts: cortical bone, which is dense and protective, and trabecular bone, which contains the bone marrow. Bones are constantly remodeled to maintain strength and resilience. However, in many metastatic cancers, the structure and function of bone tissue are altered. This alteration leads to the emergence of bone metastasis, which causes debilitating symptoms, negatively impacts quality of life, and is associated with a low survival rate. Breast cancer is a common cancer in women, and nearly 65% of patients develop bone metastasis [42].

The process by which cancer cells colonize the bone microenvironment is called the "metastatic cascade." A successful metastatic cascade comprises multiple sequential steps, including local invasion, entry into the circulation (intravasation), survival in the circulation, arrest at a distant site,

escape from the circulation and colonization (extravasation), establishment of micro-metastasis outgrowth, and interactions with secondary tissues. Proinflammatory cytokine IL-20 mediates various events in the metastatic cascade. IL-20 is upregulated and secreted by breast cancer cells in the pre-bone metastatic niche. IL-20 has multiple roles in promoting bone metastasis [43].

IL-20 accelerates the formation of the bone pre-metastatic niche by upregulating the release of exosome-encapsulated IL-6 in colonized naïve bone marrow mesenchymal stem cells (BMSCs). Exosome-encapsulated IL-6 induces the differentiation of naïve BMSCs into IL-6-producing MSCs, creating a positive feedback loop. IL-20 mediates the invasion of cancer cells by upregulating the Src/STAT3/uPA signaling pathway. Following intravasation, IL-20 mediates the survival and arrest of cancer cells in bone by inhibiting cancer cell apoptosis induced by the shear force of circulation and promoting the adhesion of cancer cells to the bone microenvironment. In the bone, IL-20 promotes the outgrowth of bone metastasis by upregulating the release of soluble receptor activator of nuclear factor kappa B ligand (sRANKL) in osteoblasts and inducing the differentiation of MSCs into IL-6-producing fibroblasts [44].

Understanding the biological mechanisms underlying the aggressive outgrowth of bone metastases is critical for designing effective therapeutic strategies. It is crucial to delineate the sequential events in the disseminated tumor cell (DTC)-macrophage-osteoclast vicious cycle, which can potentially be exploited with the intent of blocking the outgrowth of castrate-resistant DTCs in the bone niche. It also allows for an understanding of the cellular and molecular factors mediating the tropism of certain cancer types to the bone [45].

Implications for Clinical Practice

Proinflammatory cytokines play essential roles in multiple aspects of cancer progression. Efforts have been focused on targeting specific proinflammatory factors and signaling pathways as therapeutic strategies against tumor growth, invasion, angiogenesis, and metastasis. IL-20, a group 2 member of the IL-10 family of cytokines, is induced by proinflammatory cytokines (IL-1β and/or TNF-α). However, the role of IL-20 in cancer was largely unclear [46].

Clinical data indicate that a high serum IL-20 concentration predicts poor prognosis in breast or prostate cancer patients. Moreover, IL-20 promotes the formation of bone metastasis in patient-derived primary breast cancer cells. Various in vitro assays, in vivo metastasis and xenograft models, and biological mechanisms have been established to illustrate how IL-20 promotes breast cancer cell proliferation, invasion, and bone tropism and to identify signaling pathways and receptors involved on the neighboring cells [47].

With the recent recognition of the heterogeneity in BC, understanding its complexity and discovered latent therapeutic avenues are indispensable for optimized treatment strategies. The experiments demonstrate a strong response of luminal-A subtype breast cancer cells to proinflammatory factors. NF-κB inhibition blocks the enhanced IL-20 release induced by proinflammatory factors. Exogenous IL-20 supplementation attenuates the inhibitory effect of NF-κB inhibitors on IL-20 release. Hence, NF-κB transcriptionally regulates IL-20 expression in luminal-A subtype breast cancer cells, suggesting the potential intervention of NF-κB signaling upstream of IL-20 in clinical management [48].

The adverse effect of IL-20 on patient outcomes is independent of BC subtypes and mutations. However, IL-20 promoted bone metastasis only in the luminal-A subtype. IL-20 treatment causes enhanced pSTAT3, pERK1/2, and pAKT signaling activation across all breast cancer subtypes. Interestingly, while IL-20 promotes bone metastasis in luminal-A subtype BC cells, it does not affect migratory speed or directionality in basal-like basal subtype MDA-MB-231 and SUM-159-P cells. On the contrary, IL-20 treatment reduces the mean square displacement and velocity of basal-like syngeneic 410.4–3BR cells, who also display the lack of response to IL-20 in GSEA analysis. Therefore, it is expected that IL-20 has differential effects in distinct BC subtypes, particularly not promoting bone metastasis in the basal-like subtype [49].

Conclusion

The IL-20 signaling pathway might represent a promising new therapeutic target for patients with breast cancer. Despite advances in treatment strategies, including surgery, chemotherapy, radiotherapy, and hormone therapy, breast cancer remains the most common malignant tumor and the leading cause of cancer-related death in women. Metastasis is a multistep process that involves cellular transformation, invasion, extravasation, survival, and colonization in a distant organ. Metastatic breast cancer to bone is particularly lethal, resulting in skeletal-related events such as pathologic fractures, spinal cord compression, and debilitating pain. Recent studies suggest that the IL-20 cytokine family plays a potential role in breast cancer development, metastasis, and bone depletion. IL-20 exerts its physiological effects by binding to a heterodimeric receptor composed of the IL-20 receptor 1 (IL-20R1) chain and the IL-20 receptor 2 (IL-20R2) chain. Hypermethylation of the IL-20R1 promoter may be a newly identified tumor-suppressor mechanism suppressing IL-20 biolocalization and downregulating IL-20R-mediated signaling pathways in breast cancer. IL-20 promotes the migration, invasion, and expansion of breast cancer cells in the bone microenvironment, while blocking IL-20 signaling may represent a novel therapeutic approach to reducing bone-associated breast cancer progression.

Potential Research Avenues

Future research on IL-20 and its role in promoting bone metastasis has several potential avenues worth exploring. One exciting area of investigation is the development and testing of novel therapeutic strategies to inhibit IL-20 signaling in breast cancer patients. Several approaches could be explored, including the use of small molecules to block IL-20 receptor interactions, monoclonal antibodies to neutralize IL-20 activity, or bispecific antibodies that target IL-20 in concert with other immunomodulatory agents [50].

Another promising research avenue would be to examine the potential of using IL-20 and its downstream signaling effectors as biomarkers for monitoring disease progression and therapeutic efficacy in breast cancer patients. Such studies could provide critical insights into the utility of IL-20 as a prognostic biomarker and guide the development of companion diagnostics to identify patients who might benefit from IL-20-targeted therapies [51].

Importantly, future studies should consider the potential role of IL-20 in the bone microenvironment. The initial finding that IL-20 was produced by osteoblasts in a mouse breast cancer model points to the likely involvement of this cytokine in cells constituting the bone microenvironment. Additional studies are needed to assess the expression profile of IL-20 and its receptors in human primary and metastatic breast cancer cells and to characterize their modulation by tumor-promoting cues, particularly in the context of the bone niche. Of relevance, several studies have shown that factors such as PTHrP, TGF-β, and Wnt ligands released from the bones can augment the secretion of secreted factors by breast cancer cells [52].

Breast cancer is the most common invasive cancer in women worldwide. Approximately 80% of breast cancer cases are diagnosed at an early stage or with no evidence of distant metastasis, termed localized breast cancer. While patients with localized breast cancer initially respond well to surgical and adjuvant treatments, between 20% - 30% of these women will eventually develop distant metastasis. Breast cancer cells preferentially metastasize to the bone, leading to the most common and painful form of advanced-stage breast cancer known as bone metastatic breast cancer, which is associated with a poor prognosis [53].

The complex processes that enable breast cancer tumor cells to leave the primary tumor, disseminate through the bloodstream, colonize and exit secondary organs, and adapt to the new microenvironment are collectively referred to as the invasion-metastasis cascade. This review illuminates the emerging role of IL-20 as a novel regulatory checkpoint that promotes the colonization of breast cancer cells in the bone. Understanding how breast cancer cells hijack IL-20 to promote skeletal metastasis is expected to reveal novel therapeutic opportunities to halt tumor spreading and the subsequent bone degradation and other side effects caused by the outgrowth of malignant cells in the bone [54].

Conflict of Interest

No conflicts of interest were declared by the authors.

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Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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