Investigating the Effects of Indirect Coculture of Human Mesenchymal Stem Cells on the Migration of Breast Cancer Cells: A Systematic Review and Meta-Analysis

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ABSTRACT

PURPOSE: Breast cancer is the most diagnosed cancer and the leading cause of cancer death in women globally, and mesenchymal stem cells have been widely implicated in tumour progression. This systematic review and meta-analysis seeks to identify and summarise existing literature on the effects of human mesenchymal stem cells (hMSCs) on the migration of breast cancer cells (BCCs) in vitro, to determine the direction of this relationship according to existing research and to identify the directions for future research.

METHODS: A systematic literature search was conducting using a collection of databases, using the following search terms: in vitro AND mesenchymal stem cells AND breast cancer. Only studies that investigated the effects of human, unmodified MSCs on the migration of human, unmodified BCCs in vitro were included. Standardised mean differences (SMDs) were calculated to determine pooled effect sizes.

RESULTS: This meta-analysis demonstrates that hMSCs (different sources combined) increase the migration of both MDA-MB-231 and MCF-7 cell lines in vitro (SMD = 1.84, $P = .03$ and SMD = 2.69, $P < .00001$, respectively). Importantly, the individual effects of hMSCs from different sources were also analysed and demonstrated that MSCs derived from human adipose tissue increase BCC migration (SMD = 1.34, $P = .0002$) and those derived from umbilical cord increased both MDA-MB-231 and MCF-7 migration (SMD = 3.93, $P < .00001$ and SMD = 3.01, $P < .00001$, respectively).

CONCLUSIONS: To our knowledge, this is the first systematic review and meta-analysis investigating and summarising the effects of hMSCs from different sources on the migration of BCCs, in vitro.

KEYWORDS: Breast cancer, migration, mesenchymal stem cells, in vitro, systematic review and meta-analysis

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Introduction

Cancer incidence and mortality are growing rapidly worldwide. Breast cancer is the most diagnosed cancer and leading cause of cancer-related death in women. In 2020, breast cancer accounted for 11.8% of all new cancer cases in the United Kingdom in both sexes and 25.5% of new cases in women.[1] Despite important advances in the treatment of breast cancer, metastatic dissemination of the disease continues to have a poor prognosis and remains fatal.[2]

Cancer metastasis is a multifaceted process, typically involving the cancer cell undergoing a sequence of steps which must be completed successfully, before reaching its secondary destination. Chambers et al[3] split the process of metastases into 5 steps: (1) epithelial to mesenchymal transition (EMT), (2) intravasation, (3) survival and maintenance of cells in circulation, (4) extravasation, followed by (5) seeding and colonisation. The primary step (EMT) is a process by which epithelial cells that usually adhere to the basement membrane undergo a series of biochemical changes, subsequently acquiring mesenchymal properties that enable them to successfully invade surrounding tissues.[4] An EMT programme has been suggested as the critical mechanism for the acquisition of mesenchymal phenotypes by epithelial cancer cells, and various signalling factors can contribute to the progression of EMT within the tumour microenvironment (TME).

The TME is understood to be made up of tumour cells and various nonmalignant stromal and immune cells, and the cellular interactions between these subpopulations within the TME are critical to the pathophysiology of cancer. Mesenchymal stem cells (MSCs) are a population of multipotent stromal cells that are found within most tumours, and these cells have been implicated in the progression of tumour growth and metastasis.[5] They are known to migrate towards inflammatory sites, and as tumours have been described as 'wounds that never heal',[6] MSCs have been shown to home towards tumours with high affinity, incorporating themselves into the tumours on arrival.[7] Once in situ, they communicate with cancer cells and surrounding stromal cells, and these dynamic interactions, together with the surrounding extracellular matrix, form an extremely complex TME.[7] Despite an increasing body of literature researching the effect MSCs have on cancer cells and tumour progression, discordance on their overall effects continues to exist, with research demonstrating that MSCs display both tumour-promoting and tumour-suppressive effects.[5,8-10] The underlying mechanisms that give rise to these divergent effects remain unknown: this could be due to the different sources of

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MSCs, the dose of MSCs, or the in vitro model being used, along with many other factors. Further research is required to better understand the conflicting body of research that exists.

Mesenchymal stem cells can be isolated from several different sources,[11] including from bone marrow, adipose tissue, and the umbilical cord. For cells to be classed as MSCs, they must be plastic adherent, express specific cluster of differentiation markers (CD)105, CD73, and CD90, and lack expression of CD45, CD34, CD14/CD11b, CD79α/CD19, and human leukocyte antigen class II by ⩾95% and ⩽2% of the cell population, respectively. Finally, they must be able to differentiate into osteoblasts, chondroblasts, or adipocytes.[12] Despite all MSCs from various sources meeting these 3 classifications, other characteristics can vary between them. For example, bone marrow–derived MSCs (BM-MSCs) have been reported to have the greater differentiation capabilities compared with adipose tissue–derived MSCs (AD-MSCs); however, umbilical cord–derived MSCs (UC-MSCs) have a greater proliferation capacity than BM-MSCs and are reported not to senesce over serial passages.[13]

The purpose of this systematic review and meta-analysis is to identify and summarise the existing literature investigating the effect of human MSCs (hMSCs) on the migration of breast cancer cells (BCCs) in vitro, to provide direction and magnitude of effect sizes for the interaction of various subcategories of BCC types and MSCs from different sources.

Method

Search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and the Cochrane Handbook for Systematic Reviews of Interventions[14] were used as guidelines; this systematic review and meta-analysis adheres to the PRISMA standard and checklist. A systematic literature review was conducted using the PubMed (PubMed Central and MEDLINE), SPORTDiscus, and EBSCO host databases using the following search terms: in vitro AND mesenchymal stem cells AND breast cancer. Articles were assessed for eligibility manually by 1 reviewer (M-JB) using predefined data fields and were selected based on predefined inclusion criteria (Appendix 1). Article titles and abstracts were assessed initially to identify relevant papers. The full abstract screening tool can be found in Appendix 1. Inclusion criteria required articles or conference papers to be written in English between 1995 and July 2021, investigating the effects of BCCs or MSCs on MSC or BCC migration in vitro. Restrictions were placed on the source of BCCs and MSCs so that only studies using human, unmodified MSCs and BCCs were included (Figure 1).

Data extraction/retrieval

All articles were independently reviewed, and relevant information was extracted. Specifically, mean values for control and intervention studies, along with standard deviations and n numbers, were obtained from papers where possible. If the data needed to conduct appropriate analyses were not available within the text, authors were contacted to request the data. After 6 weeks, where data were not available or received, data were extracted from the published graphs within the articles using the WebPlotDigitizer tool.[15] This was the case for all but one paper by Dittmer et al[16] included in this review. In the instance where this was not possible due to quality of graphics, the article was excluded. To enable comparisons to be made and data to be collated, we used the most commonly reported outcome measure for the assays evaluated. For further information, please refer to result figure legends and Table 1.

Risk of bias assessment

Using the National Toxicology Program's Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool for Human and Animal Studies[27] combined with criteria from Hirsch and Schildknecht's 'In Vitro Research Reproducibility: Keeping Up High Standards',[28] a risk of bias (RoB) protocol was collated. Risk of bias was then assessed independently by 3 different assessors at the study level, reducing the likelihood that assessments would be influenced by a single person's biases. The following domains were assessed: (1) randomisation, (2) allocation concealment, (3) participation selection, (4) experimental conditions, (5) blinding during study, (6) incomplete data, (7) exposure characterisation, (8) outcome assessment, (9) reporting, and (10) other. The in vitro aspects of the included studies were the focus of this RoB assessment. Any inconsistencies were resolved by consensus. This RoB assessment allowed for appropriate caution to be taken when interpreting any results from the meta-analysis.

Data handling and details of meta-analytical method

Data were extracted as detailed above and subsequently grouped into the following categories to determine effect sizes: studies investigating effects of (1) hMSCs on MDA-MB-231 cells, (2) hMSCs on MCF-7 cells, (3) cellular factors (cellular factors defined as conditioned media, extracellular vesicles [exosomes], cell lysates, or homogenates.) from hMSCs on BCC migration (both BCC lines), (4) transwell coculture (transwell coculture defined as indirect coculture of BCCs and hMSCs using transwell inserts.) of hMSCs and BCCs on BCC migration, (5) hAD-MSCs on BCC migration, (6) hUC-MSCs on BCC migration, and finally, (7) hBM-MSCs on BCC migration. Forest plots in the 'Results' section show which manuscripts were grouped into each of the categories. The programme Review Manager 5[29] was used to conduct the statistical analyses, compute effect sizes, and produce all forest plots. The inverse variance method of study weighting and the random effects model of analysis were used. Standardised mean difference (SMD) was the chosen effect measure. The variance of the distribution of between-study variance and true effect

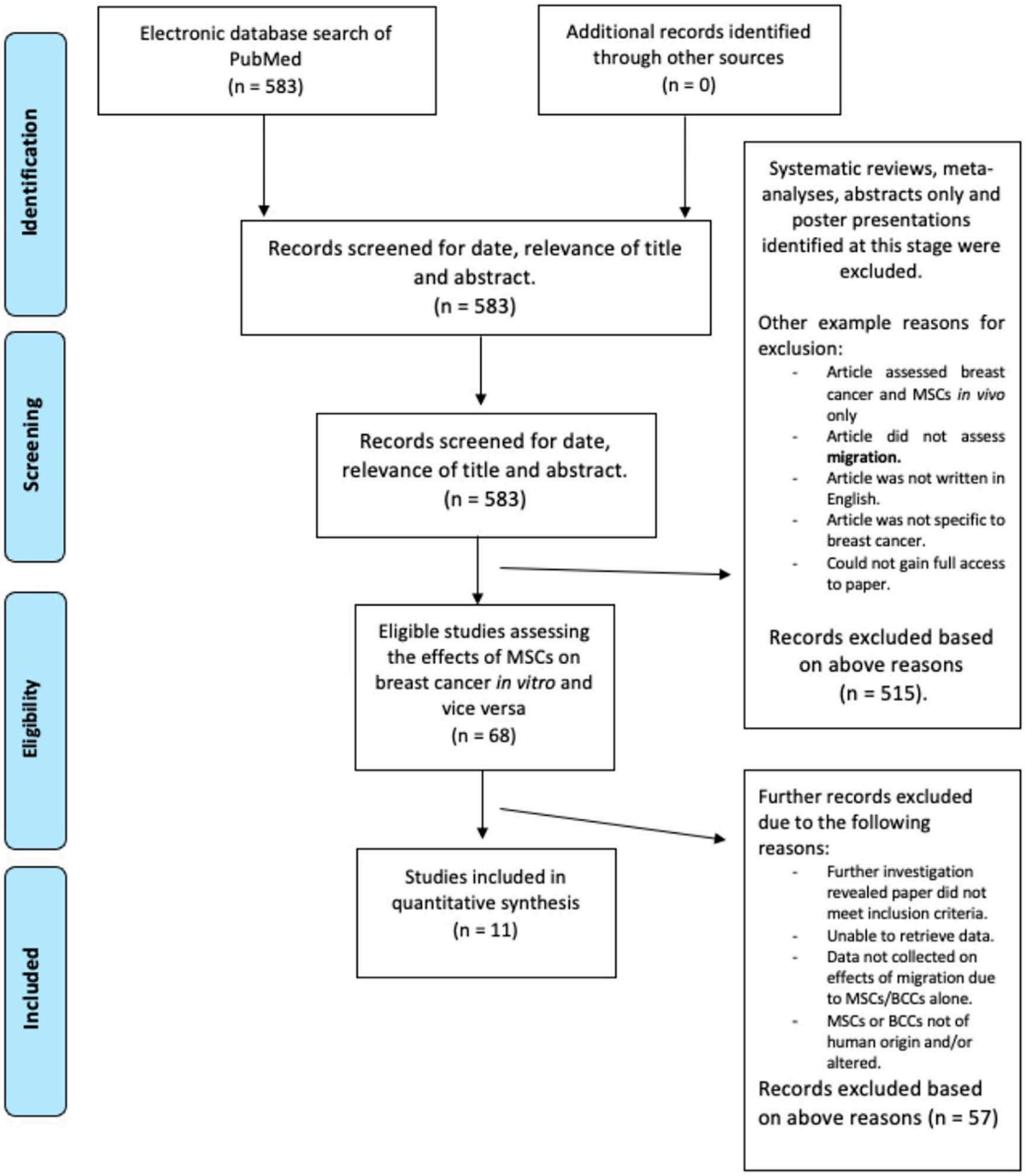

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) flow diagram presenting selection process. BCCs indicates breast cancer cells; MSC, mesenchymal stem cells.

sizes (heterogeneity, I^2; Tau^2) were automatically calculated. I^2 was used to describe the percentage of variability in estimates of effect size that is due to heterogeneity, rather than sampling error or chance. Confidence intervals (CIs; 95%) were calculated and values of $P \leqslant .05$ were considered significant.

Results

Study selection

Five hundred and eighty-three papers were identified using the selected search terms. After papers were excluded because of the selected inclusion criteria (see Appendix 1), 17 papers remained. The necessary data to conduct appropriate statistical analyses were only successfully obtained for 11 of these papers, and these were subsequently included in the quantitative analysis. The other 6 papers either did not report all necessary data (n = 4) or the measured outcome directionality was opposite to the other included studies (n = 2), meaning the data could not be included without distorting the analyses of the data, and a separate analysis was not possible due to the low number of studies. Therefore, these studies were excluded from the quantitative analyses. Figure 1 shows the PRISMA flowchart detailing the search strategy and subsequent screening, along with the rationale for exclusions at the various stages. Information on the source of hMSCs, BCC line used, main experimental methods, and conclusions is tabulated in Table 1.

Table 1. Descriptive summary of all papers included in meta-analysis (provided in alphabetical order of the first named author).

DATE	AUTHOR	BREAST CANCER CELL LINE	MESENCHYMAL STEM CELL SOURCE	SUMMARY OF MODEL AND METHODS	CONCLUSION
2009	Dittmer A et al[16]	MDA-MB-231 and MCF-7	hBM-MSCs	Migratory activity of BM-MSCs when cultured with or without BCCs using a transwell assay, or with or without BCC-CM. Migratory activity of BCCs when cultured with or without BM-MSCs using a transwell assay, or with or without BM-MSC-CM.	hBM-MSC migratory activity was increased when cultured with MDA-MB-231 cells or conditioned media from these cells. Migratory activity of MCF-7 also increased when cultured with or without hBM-MSCs in a transwell system.
2018	Alshareeda et al[17]	MDA-MB-231	hCV-MSCs	Migration of MDA-MB-231s measured after coculture with high (1:3) or low (1:1) doses of CV-MSCs using transwell assay.	Migration of treated MDAs significantly decreased when treated with either high or low doses of hCV-MSCs when compared with controls.
2019	Chen et al[18]	MCF-7	hAD-MSCs	Migration of AD-MSCs measured after coculture with or without MCF-7 cells using transwell assay or using MCF-7 conditioned media.	Migratory activity of hAD-MSCs significantly increased when cocultured indirectly with MCF-7 in a transwell assay and using MCF-7 conditioned media.
2010	Rhodes L et al[19]	MCF-7	hBM-MSCs	Migration of MCF-7N cells	Migration of MCF-7N cells increased significantly when cultured with hBM-MSCs
2012	Gauthaman et al[20]	MDA-MB-231	hWJ-MSCs	Migration of MDA-MB-231 measured after culture with conditioned media or cell lysate from hWJ-MSCs.	Migration of MDA-MB-231 cells decreased significantly when cultured with conditioned media or cell lysate from hWJ-MSCs.
2017	Koellensperger et al[21]	MDA-MB-231 and MCF-7	hAD-MSCs	Optical density of migrated BCCs measured after coculture with or without hAD-MSCs in a transwell assay.	Migration of BCCs significantly increased when cultured with or without hAD-MSCs in a transwell assay.
2015	Li et al[22]	MDA-MB-231 and MCF-7	hUC-MSCs	Number of migrated cells and % ratio of wound closure measured of BCCs when cultured with or without conditioned media from hUC-MSCs.	Migration of BCCs increased significantly when cultured with conditioned media from hUC-MSCs.
2009	Molloy et al[23]	MDA-MB-231	hBM-MSCs	Migration of MDA-MB-231 cells after culture with or without conditioned media from hBM-MSCs.	Migration of MDA-MB-231 cells increased significantly when cultured with conditioned media from hBM-MSCs in a transwell assay.
2018	Wu S et al[24]	MCF-7	hAD-MSCs	Migration of BCCs measured using wound closure assay after coculture with or without hAD-MSCs.	Migration of MCF-7 cells increased significantly after coculture with hAD-MSCs compared with control.
2013	Zhang C et al[25]	MCF-7	hBC-MSCs	Migration of MCF-7 measured using wound closure assay after coculture with or without hBC-MSC conditioned media.	Migration of MCF-7 cells increased significantly when cultured with hBC-MSC conditioned media compared with the control.
2019	Zhou et al[26]	MDA-MB-231 and MCF-7	hUC-MSC-EVs	Migration of MDA-MB-231 and MCF-7 measured using transwell and wound closure assays with or without different concentrations of hUC-MSC-EVs.	Migration of MDA-MB-231 and MCF-7 cells increased significantly when cultured with medium containing hUC-MSC-EVs.

Abbreviations: BCC-CM, breast cancer cell-conditioned medium; BM-MSC-CM, bone marrow mesenchymal stem cell–derived conditioned media; hAD-MSCs, human adipose tissue–derived mesenchymal stem cells; hBM-MSCs, human bone marrow–derived MSCs; hCV-MSCs, human chorionic villi–derived MSCs; hUC-MSC-EVs, extracellular vesicles from human umbilical cord–derived mesenchymal stem cells; hUC-MSCs, human umbilical cord–derived MSCs; hWJ-MSCs, human Wharton jelly–derived MSCs.

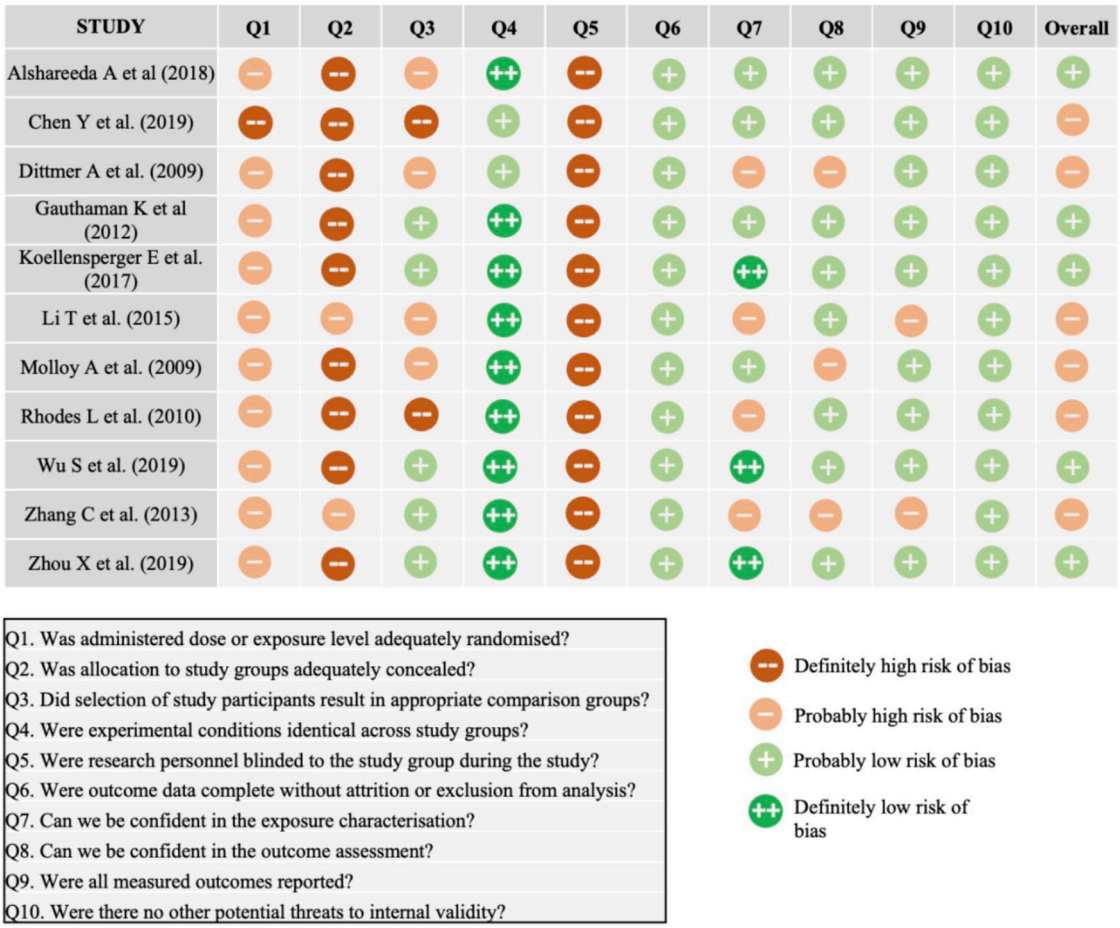

Figure 2. Risk of bias assessment results.

Risk of bias

The RoB assessment determined 6 of the 11 papers included in the meta-analysis to have a 'probably high' RoB overall. The 'overall' score was given to papers based on a numerical system (definitely high risk = 1, probably high risk = 2, probably low risk = 3, and definitely low risk = 4) by calculating the average bias rating across the 10 questions. Five of the 11 papers had an overall 'probably low' RoB rating. All papers were allocated a 'definitely high risk of bias' score for Q5 (Were research personnel blinded to the study group during the study?). All papers were rated either 'probably' or 'definitely' high RoB for Q1 (Was administered dose or exposure level adequately randomised?) and Q2 (Was allocation to study groups adequately concealed?). See Figure 2 for the assessment results.

Human mesenchymal stem cells increase MDA-MB-231 migration

Sixteen individual experiments from 6 separate papers were included in this analysis.[17,20-23,26] All studies investigated the effect of hMSCs on the migration of the invasive MDA-MB-231 cell line using either cellular factors from hMSCs or coculture via transwell. Four of the individual experiments gave negative SMDs and 11 gave positive SMDs (Figure 3). The overall random effects model pooled SMD was 1.84 (95% CI: 0.19 to 3.50; Z score = 2.19, $P = .03$) suggesting that overall, hMSCs from different sources appear to increase the migratory activity of MDA-MB-231s in vitro at a statistically significant level. I^2 was 68% suggesting relatively high heterogeneity between studies, supporting the use of the random effects model of analysis.

Human mesenchymal stem cells increase MCF-7 migration

Seventeen individual experiments from 7 different papers were included in this analysis.[16,19,21,22,24-26] All studies were investigating the effect of hMSCs on the migration of the noninvasive MCF-7 cell line. All studies gave a positive SMD and the overall pooled SMD was 2.69 (95% CI: 1.89 to 3.50, Z score = 6.57, $P < .00001$) suggesting there is an extremely statistically significant effect of hMSCs on MCF-7 migration; all studies found that hMSCs increased migratory activity of MCF-7 cells compared with their relative controls (Figure 4). I^2 was 9%.

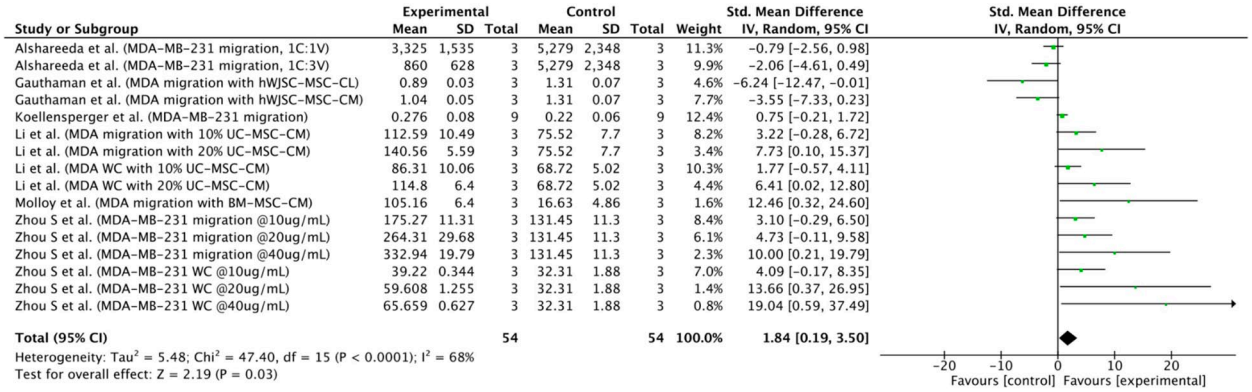

Figure 3. Pooled effect of hMSCs on the migration of MDA-MB-231 cells in vitro. Heterogeneity between studies was detected using Tau^2. The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 1.84 (95% CI: 0.19 to 3.50; *Z* score = 2.19, *P* = .03) suggesting that hMSCs increase the migration of MDA-MB-231 in vitro. Methods: Alshareeda et al[17] – MDA-MB-231 cultured with or without hCV-MSCs. Gauthaman et al[20] – MDA-MB-231 cultured with hWJSC-CM, hWJSC-CL, or control media. Migration measured using optical density of migrated cells. Koellensperger et al[21] – MDA-MB-231 cells cultured with or without hAD-MSCs. OD of migrated cells measured. Li et al[22] – MDA-MB-231 cultured with or without hUC-MSC-CM at varying concentrations. Migration measured via the number of migrated cells measured in the transwell system and % ratio of closure in scratch wound assay. Molloy et al[23] – MDA-MB-231 cultured with or without hBM-MSC-CM. The number of migrated cells measured using the transwell system. Zhou et al[26] – MDA-MB-231 cultured with varying concentrations of hUC-MSC-EVs. Migration measured using the transwell system and scratch wound assay. CI indicates confidence interval; hAD-MSCs, human adipose tissue–derived mesenchymal stem cells; hBM-MSC-CM, human bone marrow mesenchymal stem cell–derived conditioned media; hCV-MSCs, human chorionic villi–derived mesenchymal stem cells; hUC-MSC-CM, human umbilical cord mesenchymal stem cell–derived conditioned media; hUC-MSC-EVs, extracellular vesicles from human umbilical cord–derived mesenchymal stem cells; hWJSC-CM or CL, human Wharton jelly mesenchymal stem cell–derived conditioned media or cell lysate; OD, optical density; SMD, standardised mean difference.

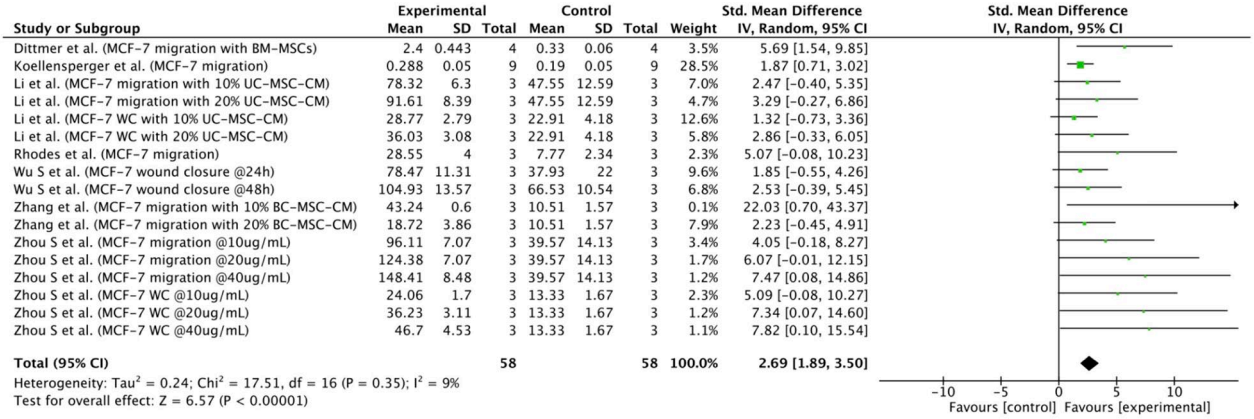

Figure 4. Pooled effect of hMSCs on the migration of MCF-7 cells in vitro. Heterogeneity between studies was detected using Tau^2. The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 2.69 (95% CI: 1.89 to 3.50, *Z* score = 6.57, *P* < .00001) suggesting there is an extremely statistically significant effect of hMSCs on MCF-7 migration; all studies found that hMSCs increased migratory activity of MCF-7 cells compared with their relative controls. Methods: Dittmer et al[16] – MCF-7 cells cultured with or without hBM-MSCs. Migratory activity and area of scratched site were measured. Koellensperger et al[21] – MCF-7 cells cultured with or without hAD-MSCs. Migration measured using OD of migrated cells. Li et al[22] – MCF-7 cells cultured with or without hUC-MSC-CM at varying concentrations. Migration measured via the number of migrated cells measured in the transwell system and % ratio of closure in scratch wound assay. Wu et al[24] – MCF-7 cells cultured with or without hAD-MSCs. Migration measured using scratch wound assay. Zhang et al[25] – MCF-7 cells cultured with or without hBM-MSC-CM at different concentrations. Migration measured by scratch wound assay. Zhou et al[26] – MCF-7 cells cultured with varying concentrations of hUC-MSC-EVs. Migration measured using the transwell system and scratch wound assay. CI indicates confidence interval; hAD-MSCs, human adipose tissue–derived mesenchymal stem cells; hBM-MSC-CM, human bone marrow mesenchymal stem cell–derived conditioned media; hBM-MSCs, human bone marrow–derived mesenchymal stem cells; hUC-MSC-CM, human umbilical cord mesenchymal stem cell–derived conditioned media; hUC-MSC-EVs, extracellular vesicles from human umbilical cord–derived mesenchymal stem cells; SMD, standardised mean difference.

Cellular factors from hMSCs increase BCC migration in vitro

Twenty-one individual experiments from 3 papers were included in this analysis.[20,22,26] All studies investigated the effect of cellular factors from hMSCs on MDA-MB-231 and MCF-7 migration. Two experiments gave negative SMDs, and the remaining gave positive SMDs. This analysis gave an overall pooled SMD of 3.23 (95% CI: 1.84 to 4.61, *Z* score = 4.56, *P* < .00001), suggesting the cellular factors from hMSCs increased invasive and noninvasive BCC migration in vitro, and this was at a statistically significant level (Figure 5).

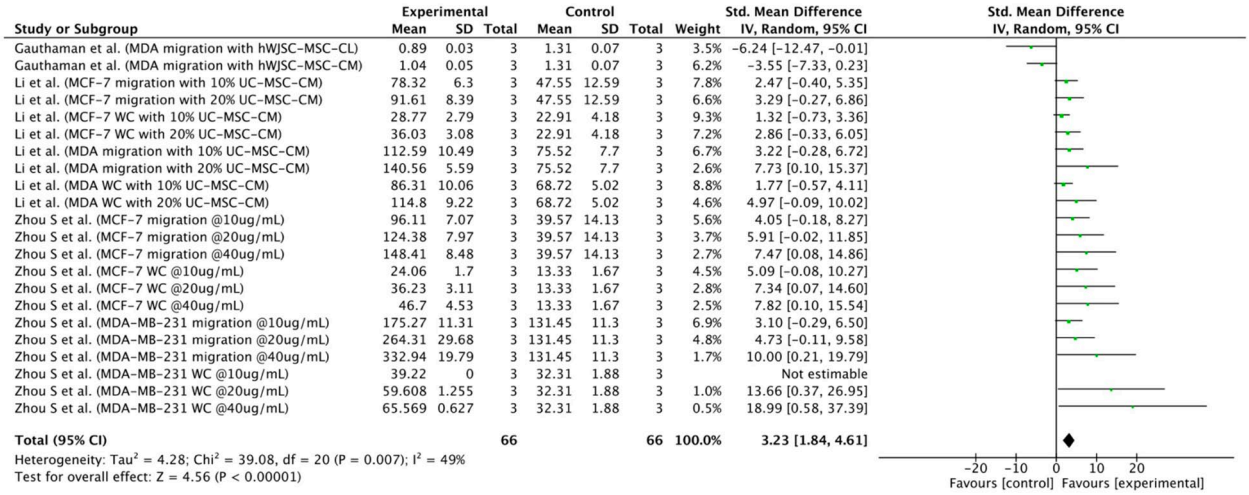

Figure 5. Pooled effect of cellular factors from hMSCs on the migration of MDA-MB-231 and MCF-7 cells in vitro. Heterogeneity between studies was detected using Tau^2. The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 3.23 (95% CI: 1.84 to 4.61, *Z* score = 4.56, *P* < .00001), suggesting there is an extremely statistically significant effect of cellular factors from hMSCs on breast cancer cell migration in vitro. Methods: Gauthaman et al[20] – MDA-MB-231 cultured with hWJSC-CM, hWJSC-CL, or control media. Migration measured using optical density of migrated cells. Li et al[22] – MCF-7 and MDA-MB-231 cells cultured with or without hUC-MSC-CM at varying concentrations. Migration measured via the number of migrated cells measured in the transwell system and % ratio of closure in scratch wound assay. Zhou et al[26] – MCF-7 and MDA-MB-231 cells cultured with varying concentrations of hUC-MSC-EVs. Migration measured using the transwell system and scratch wound assay. CI indicates confidence interval; hUC-MSC-CM, human umbilical cord mesenchymal stem cell–derived conditioned media; hUC-MSC-EVs, extracellular vesicles from human umbilical cord–derived mesenchymal stem cells; hWJSC-CM or CL, human Wharton jelly mesenchymal stem cell–derived conditioned media or cell lysate; SMD, standardised mean difference.

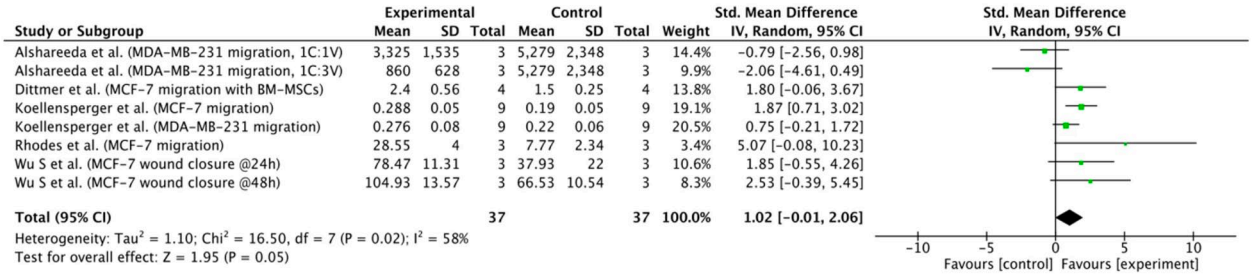

Figure 6. Pooled effect of indirect coculture via transwell with hMSCs on the migration of MDA-MB-231 and MCF-7 cells in vitro. Heterogeneity between studies was detected using Tau^2. The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 1.02 (95% CI: −0.01 to 2.06, *Z* score = 1.95, *P* = .05) suggesting there is a statistically significant effect of hMSCs on BCC migration when cocultured via transwell systems in vitro. Migration increases when BCCs are cocultured with hMSCs in a transwell system. Methods: Alshareeda et al[17] – MDA-MB-231 cultured with or without different concentrations of hCV-MSCs. Dittmer et al[16] – MCF-7 cells cultured with or without hBM-MSCs. Migratory activity and area of scratched site were measured. Koellensperger et al[21] – MCF-7 and MDA-MB-231 cells cultured with or without hAD-MSCs. Migration measured using OD of migrated cells. Rhodes et al[19] – MCF-7 cells cultured with or without hBM-MSCs. Migration measured by the number of migrated cells. Wu et al[24] – MCF-7 cells cultured with or without hAD-MSCs. Migration measured using scratch wound assay. BCCs indicates breast cancer cells; CI, confidence interval; hAD-MSCs, human adipose tissue–derived mesenchymal stem cells; hBM-MSCs, human bone marrow–derived mesenchymal stem cells; hCV-MSCs, human chorionic villi–derived mesenchymal stem cells; OD, optical density; SMD, standardised mean difference.

Transwell coculture with hMSCs increases BCC migration in vitro

Five different papers were included in this analysis; a total of 8 individual experiments from these studies were used.[16,17,19,21,24] These papers investigated the effects of hMSCs on MDA-MB-231 and MCF-7 migration using transwell coculture. Two experiments gave negative SMDs, 6 gave positive SMDs. The overall pooled SMD for this analysis was 1.02 (95% CI: −0.01 to 2.06, *Z* score = 1.95, *P* = .05) which suggests that overall, when hMSCs were cocultured in transwells with BCCs, there was an increase in both invasive and noninvasive BCC migration at a statistically significant level (Figure 6).

Human adipose tissue–derived MSCs increase BCC migration in vitro

Koellensperger et al[21] and Wu et al[24] could be included in this analysis, but there were 4 separate experiments contained within. All experiments were investigating the effects of hAD-MSCs on BCC migration. Three of the 4 experiments used the MCF-7 cell line; only 1 used the MDA-MB-231 cell line.

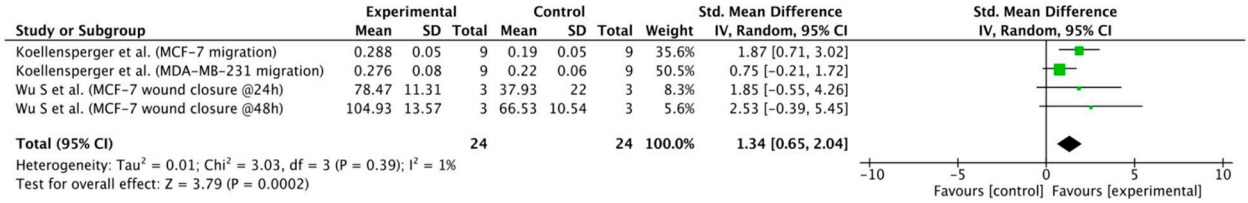

Figure 7. Pooled effect of hAD-MSCs on the migration of MDA-MB-231 and MCF-7 cells in vitro. Heterogeneity between studies was detected using Tau². The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 1.34 (95% CI: 0.65 to 2.04, *Z* score = 3.79, *P* = .002) suggesting hAD-MSCs increased BCC migration at a statistically significant level. Methods: Koellensperger et al[21] – MCF-7 and MDA-MB-231 cells cultured with or without hAD-MSCs. Migration measured using OD of migrated cells. Wu et al[24] – MCF-7 cells cultured with or without hAD-MSCs. Migration measured using scratch wound assay. BCC indicates breast cancer cell; CI, confidence interval; hAD-MSCs, human adipose tissue–derived mesenchymal stem cells; OD, optical density; SMD, standardised mean difference.

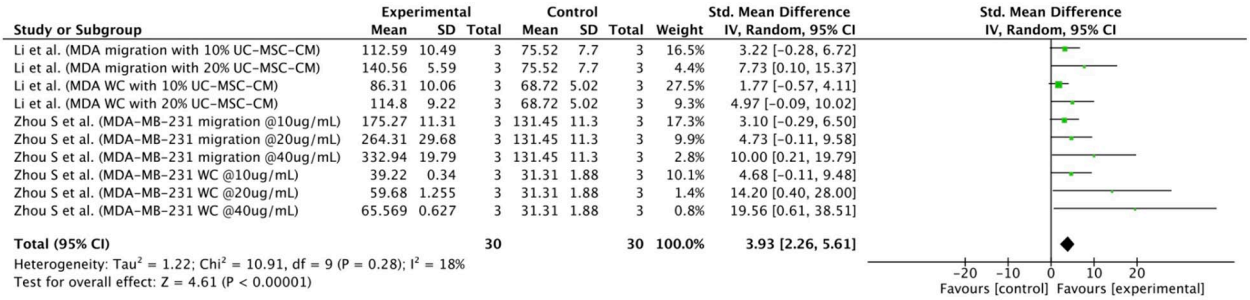

Figure 8. Pooled effect of hUC-MSCs on the migration of MDA-MB-231 in vitro. Heterogeneity between studies was detected using Tau². The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 3.93 (95% CI: 2.26 to 5.61, *Z* score = 4.61, *P* ⩽ .00001) suggesting hUC-MSCs increased MDA-MB-231 migration at an extremely statistically significant level. Methods: Li et al[22] – MCF-7 and MDA-MB-231 cells cultured with or without hUC-MSC-CM at varying concentrations. Migration measured via the number of migrated cells measured in the transwell system and % ratio of closure in scratch wound assay. Zhou et al[26] – MCF-7 and MDA-MB-231 cells cultured with varying concentrations of hUC-MSC-EVs. Migration measured using the transwell system and scratch wound assay. CI indicates confidence interval; hUC-MSC-CM, human umbilical cord–derived mesenchymal stem cell conditioned media; hUC-MSCs, human umbilical cord–derived mesenchymal stem cells; hUC-MSCs-EVs, extracellular vesicles from human umbilical cord–derived mesenchymal stem cells; SMD, standardised mean difference.

The overall pooled SMD for this analysis was calculated as 1.34 (95% CI: 0.65 to 2.04, *Z* score = 3.79, *P* = .0002). This suggests that overall, hAD-MSCs significantly increase the migration of both the noninvasive cell line MCF-7 and the invasive cell line MDA-MB-231, in vitro (Figure 7).

Human umbilical cord–derived MSCs increase MDA-MB-231 migration in vitro

Ten individual experiments from 2 papers[22,26] were included in this analysis. All experiments investigated the effect of hUC-MSCs on MDA-MB-231 migration at varying cellular concentrations of hUC-MSCs. The overall pooled SMD for this analysis was 3.93 (95% CI: 2.26 to 5.61, *Z* score = 4.61, *P* < .00001) suggesting a highly statistically significant ability of hUC-MSCs to increase the migration of MDA-MB-231 cells, in vitro (Figure 8).

Human umbilical cord–derived MSCs increase MCF-7 migration in vitro

Ten individual experiments from 2 papers[22,26] were included in this analysis, and each experiment was investigating the effects of hUC-MSCs at varying concentrations on MCF-7 migration in vitro. The overall pooled SMD for this analysis was 3.01 (95% CI: 1.83 to 4.20, *Z* score = 4.98, *P* < .00001) suggesting that hUC-MSCs significantly increased the migration of MCF-7 cells in vitro (Figure 9). I^2 was calculated as 0% in this analysis.

Human bone marrow–derived MSCs increase BCC migration in vitro

Three studies[16,19,23] used hBM-MSCs in their experiments investigating their effect on MCF-7 and MDA-MB-231 migration and were therefore included in this analysis. All 3 studies gave positive SMDs, and the overall pooled SMD was 3.92 (95% CI: −0.22 to 8.06, *Z* score = 1.85, *P* = .06). See Figure 10. This suggests that as a positive SMD was calculated, hBM-MSCs may increase the migration of BCCs in vitro, but this effect was not statistically significant.

Discussion

Results from this systematic review and meta-analysis demonstrate that hMSCs from various sources have a statistically significant effect on the migration of MDA-MB-231 cells in vitro;

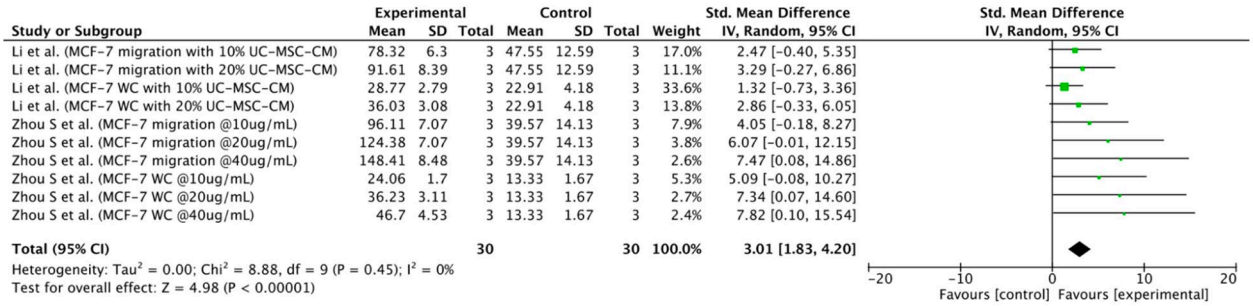

Figure 9. Pooled effect of hUC-MSCs on the migration of MCF-7 cells in vitro. Heterogeneity between studies was detected using Tau². The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 3.01 (95% CI: 1.83 to 4.20, *Z* score = 4.98, $P \leq .00001$) suggesting hUC-MSCs increased MCF-7 migration at an extremely statistically significant level. Methods: Li et al[22] – MCF-7 and MDA-MB-231 cells cultured with or without hUC-MSC-CM at varying concentrations. Migration measured via the number of migrated cells measured in the transwell system and % ratio of closure in scratch wound assay. Zhou et al[26] – MCF-7 and MDA-MB-231 cells cultured with varying concentrations of hUC-MSC-EVs. Migration measured using the transwell system and scratch wound assay. CI indicates confidence interval; hUC-MSC-CM, human umbilical cord–derived mesenchymal stem cell conditioned media; hUC-MSCs, human umbilical cord–derived mesenchymal stem cells; hUC-MSCs-EVs, extracellular vesicles from human umbilical cord–derived mesenchymal stem cells; SMD, standardised mean difference.

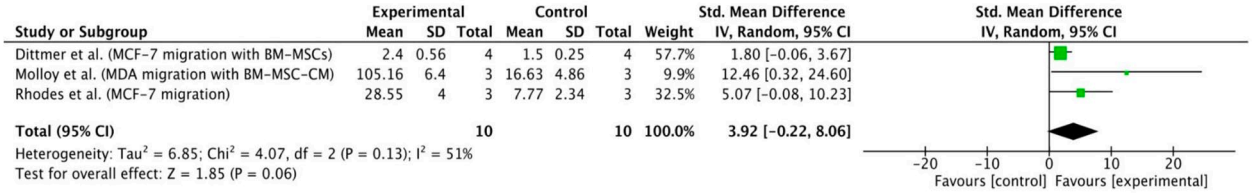

Figure 10. Pooled effect of hBM-MSCs on the migration of MDA-MB-231 and MCF-7 cells in vitro. Heterogeneity between studies was detected using Tau². The inverse variance meta-analytical method was used. The random effects model gave an overall SMD of 3.92 (95% CI: −0.22 to 8.06, *Z* score = 1.85, *P* = .06) suggesting hBM-MSCs increased BCC migration, but this increase was not statistically significant. Methods: Dittmer et al[16] – MCF-7 cells cultured with or without hBM-MSCs. Migratory activity and area of scratched site were measured. Molloy et al[23] – MDA-MB-231 cultured with or without hBM-MSC-CM. The number of migrated cells measured using the transwell system. Rhodes et al[19] – MCF-7 cells cultured with or without hBM-MSCs. Migration measured by the number of migrated cells. BM-MSCs indicates human bone marrow–derived mesenchymal stem cells; CI, confidence interval; hBM-MSC-CM, human bone marrow–derived mesenchymal stem cell conditioned media; SMD, standardised mean difference.

the overall random effects model pooled SMD was 1.84 (95% CI: 0.19 to 3.50; *Z* score = 2.19, *P* = .03), suggesting hMSCs can increase the migratory activity of the MDA-MB-231 cell line in vitro. Second, hMSCs from various sources also have an extremely statistically significant effect on the migratory activity of the 'non-invasive' BCC line MCF-7, in vitro (SMD = 2.69, 95% CI: 1.89 to 3.50, *Z* score = 6.57, $P < .00001$); MCF-7 migratory activity increases when cultured with hMSCs compared with control conditions. We found that the overall pooled SMD for the effect of cellular factors from hMSCs on BCC migration was 3.23 (95% CI: 1.84 to 4.61, *Z* score = 4.56, $P < .00001$). Cellular factors from hMSCs appeared to increase both invasive (MDA-MB-231) and noninvasive (MCF-7) BCC migratory activity in vitro, to a statistically significant level.

Cellular factors excreted by hMSCs

Human mesenchymal stem cells could be compared with 'trophic factories' due to their ability to secrete large numbers of molecules into the local environment,[30] and many researchers consider the paracrine and trophic properties of hMSCs to be their primary means of therapeutic potential. Of importance, the hMSC secretome includes interleukin-6 (IL-6), chemokine ligand 5 (CCL5), endothelial growth factor, hepatocyte growth factor (HGF), platelet-derived growth factor, and transforming growth factor beta (TGF-β),[18] molecules that are all linked to the induction of EMT and implicated in breast cancer progression in various ways. For example, IL-6 expression induces phosphorylation of signal transducer and activator of transcription 3 (STAT3), which regulates the expression of EMT-associated genes such as SNAI1 (SNAIL) and TWIST1.[31] Transforming growth factor beta is considered a 'master switch' of the EMT process, regulating EMT through Smad and non-Smad-mediated pathways that regulate and inhibit the transcription of epithelial genes and activate transcription factors such as Notch and SNAIL and phoshatidylinositol-3 kinase/protein kinase B (P13K/Akt) and the mitogen-activated protein kinase pathways.[32]

Transforming growth factor beta 1 excreted by hAD-MSCs was found to regulate EMT in MCF-7 cells by targeting the

zinc finger E-box binding homeobox/miR-200 (ZEB/miR-200) regulatory loop.[33] ZEB1 and ZEB2 are important transcription factors involved in the regulation of EMT; they repress epithelial gene expression, and the miR-200 family inhibits the expression of ZEB1 and ZEB2 in epithelial cells,[34] thus inhibiting them from inducing EMT in epithelial cells. Bracken et al[34] found that ZEB1/2 and miR-200 expression levels in MCF-7 changed after coculture with hAD-MSCs; ZEB1 and ZEB2 were significantly upregulated, whereas miR-200 levels were downregulated. When an anti-TGF-β1 antibody was introduced, ZEB1/2 expression in MCF-7 decreased and miR-200b and miR-200c were upregulated, providing strong evidence that hMSC-secreted TGF-β1 contributes to the regulation of EMT. Second, they provided evidence that showed culture of MCF-7 cells with hAD-MSCs induced an autocrine TGF-β1 signalling that would subsequently maintain and enforce a mesenchymal state. Endogenous TGF-β1 levels in MCF-7 cells gradually increased with coculture and MCF-7 cells in a stable mesenchymal state were actively secreting TGF-β1. Finally, they also demonstrated the inhibition of TGF-β receptor 1 led to a time-dependent decrease in ZEB mRNA levels, increased expression of miR-200, and an increase in hallmark epithelial features such as increased expression of E-cadherin. This pathway is just 1 of the ways that cellular factors secreted by hMSCs can potentially influence EMT of epithelial cells.

The CCL5 (RANTES) is an inflammatory cytokine expressed by hMSCs that is also widely documented to be involved in tumour progression.[35,36] A 2-way interaction exists between hMSCs and BCCs, as it has been shown that BCCs stimulate de novo secretion of CCL5 from hMSCs,[37] which in turn acts in a paracrine fashion on the BCCs, ultimately enhancing their invasion and metastasis. When cocultured together, Karnoub et al[37] found that the level of CCL5 increased 60-fold, compared with BCC cultures alone. Importantly, they confirmed the major source of the increase to be the hMSCs in the coculture. They also confirmed that the receptor for this cytokine (CCR5) is expressed by MDA-MB-231s and not by MSCs, and when inhibiting CCR5 expression in MDA-MB-231 cells, the ability of MSCs to enhance the metastatic capabilities of MDA-MB-231s was reduced. This CCL5-CCR5 paracrine interaction is another critical mechanism behind the increased metastasis of BCCs induced by hMSCs and a potential therapeutic target for preventing breast cancer metastasis. Human mesenchymal stem cell secretion of CCL5 has also been linked to the induced expression of programmed cell death ligand 1 (PD-L1) in BCCs. Recent evidence showed that an increased expression of PD-L1 on low PD-L1-expressing BCCs was observed after coculture in hMSC-CM for 72 hours.[38] The PD-L1 is known to influence several internal mechanisms in cancer cells, including migration and invasion, and studies have shown that silencing of PD-L1 by PD-L1-siRNA in tumour cells inhibited the migration of BCCs.[39]

The inflammatory cytokine IL-6 has been implicated in the progression of breast cancer in many ways, including via the activation of various signal transduction pathways such as JAK/STAT3, RAS/ERK, and P13K/AKT signalling cascades. Specifically, IL-6 is produced in significant amounts by hMSCs[18,40,41] and hMSC-secreted IL-6 has been evidenced to sustain BCC migration by activation of the above signalling pathways[41] – T47D and MCF-7 cell migration increased significantly after coculture with hMSC-CM, and this effect was inhibited when an anti-IL-6 antibody was added. Interleukin-6 can trigger the increased malignancy of BCCs via the activation of Hippo-Yes-associated protein (YAP) signals, where knockdown of YAP can attenuate IL-6-induced increased migration and invasion of BCCs.[42]

Human mesenchymal stem cell source

This meta-analysis also provides evidence that different sources of hMSCs exert different effects on BCC migration. We concluded that hAD-MSCs and hUC-MSCs demonstrate the ability to significantly increase BCC migration when cocultured. However, the effect of hBM-MSCs on BCC migration was not significant according to the data assessed (SMD = 3.92, $P = .06$). It is well documented that although similar, MSCs derived from different sources possess different characteristics, including proliferation and differentiation capacities.[13] Human adipose tissue–derived mesenchymal stem cells are much more abundant and more easily isolated than hBM-MSCs, and it is reported that the immunosuppressive effects of hAD-MSCs are greater than those of hBM-MSCs,[43] and these correlate with different cytokine profiles.[44] A review article on the properties of MSCs from different sources concluded that foetal and hAD-MSCs are better for immunomodulatory function than hBM-MSCs.[45] One study found that hMSCs derived from breast cancer tissue enhanced the proliferation of the noninvasive MCF-7 cell line more potently than hMSCs derived from bone marrow, and that MSCs derived from breast cancer tumours also secreted significantly more IL-6 compared with hBM-MSCs.[46]

In this meta-analysis, the only studies that found that MSCs did not increase BCC migration were studies that used MSCs derived from birth-derived tissues (human chorionic villi–derived mesenchymal stem cells [hCV-MSCs] and human Wharton jelly mesenchymal stem cell–derived conditioned media [hWJSC-CM]).[17,20] Another study that was excluded from the meta-analysis after we failed to obtain the data needed for statistical analyses[47] also found that hUC-MSCs inhibited the migration of both noninvasive MCF-7 and invasive MDA-MB-231 cells. Interestingly, however, Li et al,[22] who also used hUC-MSCs, reported a significant positive

increase of both MCF-7 and MDA-MB-231 migration after coculture with hUC-MSCs. Importantly, our analyses show that hUC-MSCs can increase the migration of both the aggressive, triple-negative cell line MDA-MB-231s and the nonaggressive MCF-7 cell line; however, hCV-MSCs and human Wharton jelly–derived MSCs (hWJ-MSCs) appear to inhibit increases in BCC migration according to the data from papers included in this meta-analysis.

Research reports that IL-6 expression was greater in hBM-MSCs than in hWJ-MSCs when cultured in normal conditions;[48] as IL-6 can trigger the increased migration of BCCs, this could explain the findings that hWJ-MSCs did not increase BCC migration, providing possible explanations to the results found in this meta-analysis relating to birth tissue–derived MSCs. The expression of PD-L1 was also found to be significantly higher in hBM-MSCs compared with hAD-MSCs. Human Wharton jelly–derived MSCs also have a significantly lower population doubling time compared with hBM-MSCs, and the secretion of HGF by hWJ-MSCs was 3 times higher than that of hAD-MSCs and 9 times higher than that of hBM-MSCs,[48] further demonstrating the vast differences in characteristics between MSC sources. Further extensive research is needed to continue to unpick the complex relationship between hMSC source and the effect they have on BCCs.

In our meta-analysis, we concluded that the effect of hBM-MSCs on the migration of BCCs was not statistically significant from the data analysed. We cannot ignore the possibility that the low number of studies providing data for the hBM-MSC subgroup analysis may have influenced this statistical analysis. Despite BM-MSCs being 1 of the most popular and extensively investigated sources,[13] only a small number of studies using hBM-MSCs met our inclusion criteria. Low n numbers significantly reduce the power behind statistical analyses and therefore increase the risk of false-positive or false-negative results. Researchers should consider performing a priori sample size calculations to ensure meaningful results are obtained, particularly when performing in vitro studies as sample sizes are often small.

Limitations

Each study included in this meta-analysis investigated the effect of hMSCs on BCC migration using indirect methods of coculture, via conditioned media or transwell assays. Existing research shows that direct and indirect interactions between hMSCs and cancer cells may exert different effects on cell migration.[49,50] More research investigating the effects of cell-mediated effects of hMSCs on BCC migration is required to further understand the relationship and therapeutic opportunities that these interactions may present.

Despite identifying numerous papers that were eligible to be included in this meta-analysis, not all could be included in the analysis due to various issues with data retrieval. Attempts were made to obtain all data necessary, but this was not possible for all papers, and they were subsequently removed from the meta-analysis. Differences in directionality of reporting also meant that papers were removed from the meta-analysis despite providing all data. For example, several papers reported the changes in the open area of scratch wound assays after a period, where most papers reported the closed area/rate of wound closure, and we therefore used the most commonly reported outcome measure for the assays evaluated to make appropriate comparisons. It may be useful for researchers in the field to attempt to develop a consensus for reporting results or use methods that mean results are comparable.

As mentioned briefly already, the low n numbers in in vitro studies can reduce the power of statistical analyses. When completing subgroup analyses, n numbers were reduced further, and this was not helped by the issues with data retrieval from some papers. In addition, an analysis that investigated the effect that BCCs had on hMSC migration would be highly appropriate and beneficial in this context, but too few studies reported results on this relationship. Finally, it is important to note that whole studies may have been missing from the review because of publication bias where papers were not published, cited, or may have been incorrectly indexed, meaning they were not returned in the databases searched.

Finally, only 2 BCC lines (a triple-negative BCC line and a hormone receptor-positive cell line) used in the studies were included in this systematic review. Future research should investigate the effects of hMSCs of other BCC lines with different molecular and histological subtypes to further our understanding.

Conclusions

When pooled together, the investigations on the effects of hMSCs on BCCs in vitro that were identified in this meta-analysis concluded that hMSCs have a statistically significant effect on increasing BCC migration in vitro. The individual effect of hMSCs from different sources on BCCs in vitro was also summarised; hUC-MSCs and hAD-MSCs increase BCC migration in vitro, to statistically significant levels. Human bone marrow–derived MSCs appear to increase BCC migration in vitro, but this effect was not statistically significant. Further research is required to continue to collect data on the effects of various sources of hMSCs on breast cancer progression, but importantly, this meta-analysis provides evidence of the pro-metastatic effect of hMSCs on BCCs in vitro.

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

All authors provided consent for the publication of this article.

Author contributions

Marie-Juliet Brown: Conceptualisation; Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing – original draft; Writing – review & editing.
Mhairi A Morris: Conceptualisation; Supervision; Writing – review & editing.
Elizabeth C Akam: Conceptualisation; Supervision; Writing – review & editing.

Acknowledgements

None.

Availability of Data and Materials

The datasets generated during and/or analysed during this study are available from the corresponding author on reasonable request.

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Appendix 1: Abstract screening tool and inclusion criteria

Is the article written in English? YES/NO
Is the article written between 1995 and 2021? YES/NO
Is the article a systematic review or meta-analysis? YES/NO
Is the article title and abstract relevant to the topic: The effects of human mesenchymal stem cells (hMSCs) on breast cancer in vitro or vice versa? YES/NO
Is the article available in full text? YES/NO
Is the article investigating specifically the effects of hMSCs on breast cancer only? YES/NO
Is the article investigating the effects of hMSCs on breast cancer using in vitro models? YES/NO
Is the article investigating the effects of hMSCs on breast cancer migration and vice versa? YES/NO
Are the cells used human, unmodified MSCs and breast cancer cells? YES/NO